

National Environmental Science Programme

Field Manuals for Marine Sampling to Monitor Australian Waters

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List of Acronyms & Abbreviations

AMP	Australian Marine Park
AODN	Australian Ocean Data Network
AUV	Autonomous Underwater Vehicle
BRUV	Baited Remote Underwater Video
Chl-a	Chlorophyll-a
DOV	Diver-Operated Video
GA	Geoscience Australia
IMOS	Integrated Marine Observing System
LOI	Loss on Ignition
MaRS	Marine Sediments Database
MBES	Multibeam Echosounder
MPA	Marine Protected Area
NESP	National Environmental Science Programme
OTU	Operational Taxonomic Unit
QA	Quality Assurance
QC	Quality Control
ROV	Remotely Operated Vehicle
RUV	Remote Underwater Video
SOP	Standard Operating Procedure
TOC	Total Organic Carbon
USBL	Ultra-Short Baseline
UVC	Underwater Visual Census

Executive Summary

Australia has one of the world's largest marine estates that includes many vulnerable habitats and a high biodiversity, with many endemic species crossing a wide latitudinal range. The marine estate is used by a variety of industries including fishing, oil & gas, and shipping, in addition to traditional, cultural, scientific and recreational uses. The Commonwealth government has recently established the Australian Marine Parks (AMPs), the largest network of marine protected areas in the world, complementing existing networks in State and Territory waters.

Monitoring the impacts of these uses on the marine environment is a massive shared responsibility that can only be achieved by making the best use of all the information that is collected. Australia now has a number of significant long-term marine monitoring and observing programs, as well as a national ocean data network. Without some common and agreed standards, much of the information collected will not be comparable with other areas or sectors. This may reduce its value to regional and national management, while the individual project or survey may lose the opportunity to interpret results in a regional or national context.

We have therefore developed a suite of field manuals for the acquisition of marine benthic (i.e. seafloor) data from a variety of frequently-used sampling platforms so that data can become directly comparable in time and through space, thus supporting nationally relevant monitoring in Australian waters and the development of a monitoring program for the AMP network. This objective integrates with one of the eight high-level priorities identified by the National Marine Science Plan (2015-25): the establishment of national baselines and long-term monitoring.

Due to the large geographic area, diverse flora and fauna, and range of environmental conditions represented by the Australian marine estate, a single method of sampling is neither practical nor desirable. For this reason, we present a standard operating procedure (SOP) for each of six key marine benthic sampling platforms that were identified based on their frequency of use in previous sampling and monitoring programs, as well as a pilot pelagic sampling platform included due to its similarity with benthic BRUVs:

- Multibeam sonar (MBES) provides bathymetry and backscatter data that are used to map the seafloor.
- Autonomous Underwater Vehicles (AUVs) acquire high-resolution continuous imagery of the seafloor and its associated habitats and organisms.
- Benthic Baited Remote Underwater Video (BRUV) systems acquire video of demersal fish attracted to a baited camera system dropped to the seafloor.
- Pelagic BRUVs acquire video of pelagic fish and other fauna that are attracted to a baited camera system suspended in the water column. This platform is included as an emergent sampling method for pelagic ecosystems.
- Towed cameras acquire video or still imagery of the seafloor and its associated habitats and organisms.
- Grabs and box corers collect sediment samples that can be analysed for biological, geochemical, or sedimentological variables.
- Sleds and trawls collect benthic or demersal fauna near the seafloor.

The main challenge in the development of these manuals was to find a balance between being overly prescriptive (such that people prefer to follow their own protocol and ignore the manuals) and overly flexible (such that data is not consistent and therefore not comparable). A collaborative approach was paramount to addressing this concern. Ultimately, over 70 individuals from over 30 organisations contributed to the field manual package. By engaging researchers, managers, and



technicians from multiple agencies with a variety of experience, sea time, and subject matter expertise, we strove to ensure the field manuals represented the broader marine science community of Australia. This not only improved the content but also increased the potential for adoption across multiple agencies and monitoring programs.

Future work is based on the understanding that SOPs should be periodically checked and revised, lest they become superseded or obsolete. Resources are available to develop a Version 2 of this field manual package, due for completion in late 2018, following additional community consultation and input. As part of this version, a long-term plan for managing the field manuals will be developed, including maintenance, version control, and the scoping of further SOPs as new sampling platforms are ready for use in monitoring programs.





National Environmental Science Programme

1. INTRODUCTION

Rachel Przeslawski and Scott Foster



1.1 Background

Australia has one of the world's largest marine estates that includes many vulnerable habitats and a high biodiversity, with many endemic species crossing a wide latitudinal range. The marine estate is used by a variety of industries including fishing, oil & gas, and shipping, in addition to traditional, cultural, scientific and recreational uses. The Commonwealth government has recently established the Australian Marine Parks (AMPs), the largest network of marine protected areas in the world, complementing existing networks in State and Territory waters (Cochrane 2016).

Monitoring the impacts of these uses on the marine environment is a massive shared responsibility that can only be achieved by making the best use of all the information that is collected. It now has a number of significant long-term marine monitoring and observing programs (Table 1.1), as well as a national ocean data network (aodn.org.au). Without some common and agreed standards, much of the information collected will not be comparable with other areas or sectors. This may reduce its value to regional and national management, while the individual project or survey may lose the opportunity to interpret results in a regional or national context.

Australia is now uniquely placed to develop standardised national approaches to monitor the marine environment. This objective integrates with one of the eight high-level priorities identified by the National Marine Science Plan (2015-25): the establishment of national baselines and long-term monitoring. This will also contribute to the effective coordination across the marine science and observing community (including industry and citizen scientists). Such coordination has been recognised as integral to a governance system for sustained and effective monitoring in Australia's marine environment (Hayes et al. 2015) and yet was identified as currently highly variable and frequently inadequate in the 2016 State of the Environment Report (Evans et al. 2017). In order to facilitate objective and robust conclusions about the status and trends of the marine ecosystems, it is crucial that sampling methods are as consistent as possible while still allowing for practical differences among equipment, vessels, and weather conditions. This need for consistent methodology in marine monitoring has been identified in several reports on regional and national marine monitoring frameworks (Hedge et al. 2013, Bowden et al. 2015, Hayes et al. 2015), and its contribution to supporting a blue economy is also recognised (Golden et al. 2017).

Although many biological monitoring programs focus on single elements of the marine environment (e.g. Wraith et al. 2013), several large-scale marine monitoring programs that include multiple areas are currently under development or implementation in Australian waters. Table 1.1 lists some of these programs, as well as the associated indicators to be measured or sampling platforms if specified. Standardised marine monitoring has been done successfully in Australian waters for shallow waters (e.g. underwater visual census in Reef Life Survey) and pelagic animals (e.g. acoustic tagging in IMOS Animal Tracking Facility), but it has yet to be developed, implemented, and adopted at a national scale for most other biological sampling platforms (but see IMOS AUV Facility in Table 1.1).

Table 1.1 Large-scale biological or ecological monitoring programs currently operating or under development in Australia as of Dec 2017. UVC = underwater visual census, DOV = diver-operated video, ROV = remotely operated vehicle, AUV = autonomous underwater vehicle, BRUV = baited remote underwater video, MBES = multibeam echosounder.

Version 1

		Program	Region	Indicator	Sampling Platforms	Example Reference
		Continuous Plankton Recorder (CPR)	Global	Plankton assemblages, colour index	CPR	Hosie et al. 2003
		IMOS Animal Tracking Facility	National	Marine megafauna movement	Acoustic telemetry, satellite tracking	Taylor et al. 2017
gic		IMOS Ships of Opportunity	National	Temperature, salinity, water column backscatter, biochemistry	Bathythermograph, echosounder, biogeochemical and meteorological sensors	Alory et al. 2007
Pelaç		IMOS National Reference Stations	National	Nutrients, microbes, phytoplankton, zooplankton, environmental factors	Moored sensors, water sampling	Sloyan and O'Kane 2015
		RIMREP	GBR	Various	Various (TBC)	GBRMPA 2015
		Marine Integrated Monitoring Program	NSW	Various (TBC)	Aerial imagery, UVC, BRUVs, AUVs, towed imagery, grabs, DOVs, ROVs	NSW Government 2017
		WAMSI estuary science program	WA	Various (TBC)	Various (TBC)	Thomson et al. 2017
	ersal	Reef Life Survey	Global	Demersal fish and benthic invertebrate assemblages	UVC	Stuart-Smith et al. 2017
	/ Deme	Long-Term Monitoring Program (AIMS)	GBR and NW Australia	Fish and benthic invertebrate assemblage, coral health and cover	UVC, DOV, Towed imagery	De'ath et al. 2012
:	thic /	IMOS AUV Facility	National	Benthic invertebrate assemblages	AUV	Perkins et al. 2017
	Ben	VIC Signs of Healthy Parks monitoring program	VIC	Various	UVC, drone/UAV, AUV, BRUVS, ROV, towed video, aerial photography	Parks Victoria's Technical Series
		WA marine monitoring program	WA	Various	Various	Dept Biodiv Conserv Attractions 2017
		NESP field manual package*	National	Various	MBES, AUV, BRUV, Towed camera, Sled/trawls, Grab/corer	Current study

* Primarily benthic and demersal platforms, but also includes emergent pelagic method (Pelagic BRUVs)



Marine Sampling Field Manuals for Monitoring Australia's Commonwealth Waters

Due to the large geographic area, diverse flora and fauna, and range of environmental conditions represented by the Australian Marine estate, a single method of sampling is neither practical nor desirable (Bouchet et al. 2018, Przeslawski et al. 2018). For this reason, we present a standard approach for each of six key marine benthic sampling platforms that were identified based on frequency of use in previous open water sampling and monitoring programs: Multibeam sonar (MBES), Autonomous Underwater Vehicles (AUVs), benthic Baited Remote Underwater Video (BRUVs), towed video, grabs and box cores, and sleds and trawls. Each of these platforms targets a discrete data type (bathymetry, imagery, biological and sediment samples) in particular environments (consolidated, unconsolidated substrates) (Table 1.2), with specific advantages (Table 1.3). In addition, we provide a field manual for pelagic BRUVs as a concept sampling method in pelagic ecosystems due to its similarity to benthic BRUVs. Importantly, the inclusion of these sampling platforms in the current version is not an assessment of their value but instead an indication of their frequency of use and suitability for national monitoring (e.g. established methods, dedicated users, integration with existing national programs).

One of the main challenges in assessing marine biodiversity is the lack of standardised approaches for monitoring it (Duffy et al. 2013, Teixeira et al. 2016). As such, the overarching goal of these field manuals is to reduce the bias and variance in data from differences in sampling procedures, thereby ensuring that patterns in data are due to patterns in the community rather than patterns of how or when the community was sampled. If the measured ecological variable and the variation in sampling techniques are confounded, it is challenging if not impossible to objectively determine if observed changes are due to real ecological change or sampling technique. If variability is sufficiently high, real changes that would trigger appropriate management may not be detected in time, if at all. Importantly, many state marine monitoring programs use their own standard operating protocols (SOPs) relevant for wetland, estuarine, embayment, or intertidal habitats (Table 1.1). The current package of field manuals is not meant to replace them, but rather to complement them for deeper waters and national monitoring purposes. At the same time, we hope that individual state marine monitoring programs will also identify opportunities to adjust current practices to increase national consistency and that the SOPs will provide an opportunity for industry and industry consultants to contribute to national monitoring through standardising their ongoing activities. To that end, marine managers from all states and territories in Australia were engaged in the process of developing these field manuals. This ensured that methods were similar whenever possible and differences were clearly explained in relation to marine monitoring in Commonwealth waters.



Table 1.2: Summary of prioritised benthic sampling platforms and their acquisition targets

	Data Type	Data Target	Spatial coverage	Environment	Chapter
MBES	Bathymetry, backscatter	Seafloor	Continuous	All	3
AUV	Imagery	Epifauna	Continuous	All	4
BRUV	Imagery	Demersal fish	Point (qualitative)	All	5
Towed	Imagery	Epifauna	Transect	All	7
Grab/Boxcore	Biological and sediment samples	Macrofauna, infauna	Point	Unconsolidated substrate	8
Sled/Trawl	Biological and sediment samples	Megafauna, epifauna	Transect (qualitative)	Consolidated substrate	9

Table 1.3: Advantages of prioritised benthic sampling platforms.

	MBES	AUV	BRUV	Towed	Grab/Boxcore	Sled/Trawl
Continuous (i.e. grid) broad-scale spatial coverage	Х					
Continuous (i.e. grid) fine-scale spatial coverage		Х				
Non-extractive	Х	Х	Х	Х		
Able to revisit exact sites (repeatability)	Х	Х				
Able to sample over variety of environments	Х	Х	Х	Х		
Species-level identifications ¹					Х	Х
Genetic, morphological etc analysis possible					Х	Х
Behaviour observed			Х	Х		
Cryptofauna included					Х	Х
Quantitative	Х	Х	Х	Х	Х	
Concurrent physical and biological data		Х		Х	Х	
Minimal technical expertise			Х	Х	Х	Х
Vessel flexibility			Х	Х	Х	

¹ Refers to identifications able to be made with unknown or cryptic species (i.e. well-known, distinctive species can be identified via imagery)



1.2 Scope

This field manual package aims to provide a standardised national methodology for the acquisition of marine data from a prioritised set of frequently-used sampling platforms (below diver depths) so that data is directly comparable in time and through space. This will then facilitate national monitoring programs in Australian open waters and contribute to the design of an ongoing monitoring program for AMPs. The long-term goal is to produce a set of manuals that is applicable to a broad range of users and to be prescriptive enough that all data are collected without unnecessary technical variation.

We generally limit these platforms to benthic biological sampling, with a few exceptions (e.g. pelagic BRUVs included as a proof-of-concept due to its similarity to benthic BRUVs; water column, sedimentology, and geochemistry data included for comprehensiveness related to the relevant platform). These Marine Sampling Field Manuals focus on data acquisition and post-processing including data management, particularly as applied to marine monitoring. Standardisation of sampling design is important and is addressed accordingly in Chapter 2. Data analysis and reporting are generally not included in the field manuals, although we direct users to useful methods or resources within each field manual.

For each field manual, a scope specific to that particular gear and data type is presented in a separate section. Overall, these field manuals are meant to cover basics and important considerations, with agency- and gear-specific SOPs supplemented as needed by individual researchers. Detailed and gear-specific SOPs are outside the scope of this field manual package due to the large number of existing SOPs and the variety of gear currently employed by researchers. In this first version of the field manuals, it is impractical that researchers would agree on detailed SOPs (and associated gear). Rather, we have developed these field manuals to find consensus about as many issues as possible, while noting the differences. These differences can then be assessed in the future (e.g. they may not correspond to large amounts of variation in data), and addressed if need be. Wherever possible, however, we have mandated or recommended specifications (e.g. imagery resolution) that should be used in future equipment upgrades or purchases.

This field manual package does not describe the decision to use a particular sampling platform, supporting previous recognition that a top-down, one-size-fits-all approach to monitoring is unlikely to be effective in systems with large environmental variability (Fancy et al. 2009). Ultimately, the decision to use particular marine sampling platforms depends on a variety of factors, including depth (e.g. reef vs slope), substrate (e.g. hard vs soft), purpose (e.g. voyage of discovery vs impact assessment), and resources (e.g. minimal expertise vs technologically complex). For a more detailed review of each sampling platform, as well as a comparative assessment among them, we refer readers to our companion report (Przeslawski et al. 2018). After the decision to use an appropriate sampling platform has been made, using the appropriate field manuals will help ensure that the collected data can be compared with data collected previously and in the future, thus contributing to national marine monitoring and reporting.

1.3 Format

In order to maximise uptake, methods in each field manual are usually presented as simple steps. All steps listed are considered essential unless they are clearly marked with brackets and italics as recommended (i.e. Use netsonde or bottom contact sensor to ensure sled or trawl is suitably deployed along the seafloor [*Recommended*])

Version 1

The field manual package is designed to be separated into its component chapters representing discrete sampling platforms, as needed. For this reason, the package can be downloaded in its entirety as a single pdf, or as standalone chapters representing discrete field manuals (Figure 1.1). References will be listed accordingly at the end of each chapter.

Field Manual Package



Figure 1.1 The structure of the NESP field manual package (version 1) with numbers indicating respective chapters

1.4 Development of Field Manuals

The main challenge in the development of these manuals was to find a balance between being overly prescriptive (such that people prefer to follow their own protocol and ignore the manuals) and overly flexible (such that data is not consistent and therefore not comparable). A collaborative approach was therefore paramount to their development.

Ultimately, over 70 individuals from over 30 organisations contributed to the field manual package. By engaging researchers, managers, and technicians from multiple agencies with a variety of experience, sea time, and subject matter expertise, we strove to ensure the field manuals represented the broader marine science community of Australia. This not only improved the content but also increased the potential for adoption of the SOPs across multiple agencies and monitoring programs. Input from additional stakeholders will be actively sought during the 2018 outreach program.





Figure 1.2 Collaborative network that developed the marine sampling field manuals. Working group members are listed in Appendix A as authors or collaborators.

The process used to develop each field manual included in this package is shown in Figure 1.3 and described below:

- For each field manual, a working group was formed in which known users of the given sampling platform were invited. To be as inclusive as possible, we also extended more general invitations through email lists (e.g. Australian Coral Reef Society, Australian Marine Science Association (AMSA), NESP) and presentations (e.g. AMSA 2017 conference). Each working group was led by a coordinator(s) to develop content. Coordinators were identified as experts in their particular sampling platform and took on the role of lead author(s) for their respective field manual (Figure 1.2).
- 2. Content was developed by the coordinators based on meetings with the working group and associated input, including existing SOPs.
- 3. A draft field manual was distributed to the working group as a strawman for further discussion and refinement.
- 4. A complete field manual was submitted for internal review and approval by the editors, NESP, Geoscience Australia, and IMOS.
- 5. A complete field manual was submitted to an external reviewer who was not previously associated with the project.
- 6. A final revised field manual package was released as Version 1 on the Ocean Best Practice Repository (<u>www.oceanbestpractices.net</u>) and the website (<u>www.nespmarine.edu.au</u>).
- 7. Feedback was solicited through a questionnaire, particularly geared towards field testers.
- 8. Content of field manuals was revised based on feedback and new developments (e.g. data discoverability and accessibility). This will form the next version of the field manual package.





Figure 1.3 Flow chart showing the iterative process used in the development of this field manual package (version 1, orange and green), as well as subsequent future versions (orange only).

1.5 Contributors

All individuals that contributed to this field manual package are listed in Appendix A, with the following categories assigned based on their level of contribution:

- *Editors* oversaw production of the entire field manual package, ensuring consistent scope, style, and formatting throughout.
- Lead authors led working groups associated with discrete chapters or sampling platforms.
- Authors helped write chapters or provided crucial information to do so.
- Contributors participated in working group discussions.
- *Reviewers* provided assessments of draft chapters.
- Field testers provided input based on their actual use of a manual in the field.

1.6 Universal Protocols

In this section, we generally describe some of the protocols that span all sampling platforms. Further detail on each of these is also provided in each chapter, as it is specifically relevant to a given sampling platform.

1.6.1 Sampling design

There are several overarching issues related to sampling design across all marine sampling platforms (e.g. randomisation, efficient designs, and uncertainty). We strongly encourage users of any field manual contained in this package to read Chapter 2 to familiarise themselves with these issues.



1.6.2 Permits

Prior to undertaking any marine survey, researchers are responsible for ensuring appropriate applications for permission are lodged, with subsequent relevant approvals obtained and documented. A list of potential permissioning documents relevant to marine sampling in Commonwealth waters are listed in Appendix B.

1.6.3 Risk Assessments

Risk assessments not only help quantify potential risks associated with planning and field activities, they can help make fieldwork safer and reduce costs. They may also be a requirement for some organisations. It is recommended that a risk assessment is completed during the survey planning phase and again prior to the commencement of fieldwork for any of the sampling platforms included in this manual:

- <u>Planning risk assessment</u>. The assessment during the planning phase identifies risks and mitigation strategies associated with attaining appropriate equipment, staff, finances and other resources. In addition, it should include potential reasons survey objectives may not be met. This provides an opportunity to develop contingency plans and prioritise objectives.
- <u>Fieldwork risk assessment</u>. This assessment identifies risks associated with onboard activities, including safety hazards, equipment damage or loss, inclement weather, and any other aspect that may compromise budget, survey objectives, or crew health and safety. There will be some overlap with the risks identified in the planning phase, but this risk assessment should explicitly address onboard risks. This provides an opportunity to ensure the survey is compliant with workplace health and safety issues, as well as optimising the potential for successful data acquisition.

1.6.4 Quality assurance and control

These field manuals define quality assurance (QA) as measures adopted before and during data acquisition, while quality control (QC) are measures adopted after data acquisition. Specifically QA represents the processes necessary to support the generation of high quality data and QC represents the follow-on steps that support the delivery of high-quality data, requiring both automation and human intervention. The documentation of the QA/QC process is arguably just as important as data acquisition itself. The QA/QC process can affect data analysis and interpretation (e.g. observer bias in marine imagery in Durden et al. 2016b), and it is thus an integral part of standardisation to facilitate comparisons between datasets (Lara-Lopez et al. 2017). The appropriate methods for QA/QC depends on the data type (e.g. multibeam, underwater imagery, biological specimen). As such, further details on QA/QC are included in each field manual.

1.6.5 Data discoverability and accessibility

All marine metadata and data should be publicly released so that it is discoverable and accessible to the public, unless circumstances require otherwise (e.g. confidentiality clause or embargo for commercial work). Even in situations when data cannot be shared, the metadata should be made available so that future surveys are based on informed decisions about existing sampling locations. Refer to Stocks et al. (2016) for further information on appropriate information management including useful advice on data quality control and data sharing. The appropriate methods for release of marine data depend on the data type (e.g. multibeam, underwater imagery, biological



specimen). As such, further details on data management (including accessibility and discoverability) are included in each field manual.

Data can be licensed with the Creative Commons BY license which attributes the author but allows for free use of the data, including commercial applications. Some agencies may prefer restriction on commercial applications based on their data in which case Creative Commons BY-NC should be used.

1.6.6 Post-survey report

A post-survey report is highly recommended within a year of survey completion. Such a report is valuable documentation of the survey objectives, methods, and preliminary results. It is especially important because it is a single resource describing the multiple methods and data often acquired from a given survey, and it provides overarching context to a survey that is not found in the associated metadata or data. Many agencies have their own post-survey report template, and we have also included one with suggested headings and content in Appendix C for reference.

1.7 Maintenance of Field Manuals

Keeping up with technological advances to ensure uniformity of data acquisition across multiple agencies over time is a challenge for some platforms, particularly those that are based on rapidly advancing technology (e.g. AUV, MBES). In order to ensure that field manuals include relevant advances, they should be periodically checked and revised, lest they become superseded or obsolete.

Resources are available to develop a Version 2 of this field manual package, due for completion in late 2018, following additional community consultation and input. As part of this version, a long-term plan for managing the field manuals will be developed, including maintenance, version control, and the scoping of further SOPs as new sampling platforms are ready for use in monitoring programs. Potential future SOPs include marine plastics and genomics. Shallow reef census methods are not included presently but a separate Marine Hub project has assessed the major national diver-based reef monitoring programs (Stuart-Smith et.al., 2017).

1.8 Outreach

After the release of the current field manual package (version 1), efforts will be focussed on outreach to increase the adoption of the field manuals by the broader marine science community, as well as industry, regulators, and policymakers. This will be done initially through conference presentations and face-to-face meetings, with follow-up meetings and questionnaires to gauge the success of adoption. Feedback on the current version, as well as suggestions for future versions and field manuals can be given here: www.surveymonkey.com/r/CQKC688.

In addition, there is a need to establish institutional uptake of the field manuals, rather than just individual uptake. This will ensure the continuity and long-term applicability of the SOPs even if advocating individuals leave an agency. Ultimately, institutional uptake will maximise the comparability of datasets from various surveys, thus increasing the amount of comparable data able to be applied to national products and syntheses. Efforts are currently underway to establish a high-level oversight committee to develop and implement actions needed for this.



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2. STATISTICAL CONSIDERATIONS FOR MONITORING AND SAMPLING

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2.1 Introduction

A rigorous scientific process is essential to forming sound conclusions that can inform evidencebased decision-making. This process starts with defining a research question, assessing what level of information is needed and then critically assessing how that information should be obtained (see Table 2.1 and Hayes et al., submitted). Evidence can be obtained from a variety of sources, ranging from expert opinion, through ad-hoc data collection, then well-designed observational surveys, and finally to randomised controlled experiments. Well-designed experiments/surveys that are targeted to the research question, however, are also generally more expensive than expert opinion, which is a source of information that may be adequate in some certain situations (see Leek and Peng, 2015). Table 2.1 provides a brief overview of the hierarchy of research questions and the types of data that are appropriate to answer them.

Research Type	Description	Example Question	Complexity
Descriptive associations	Summaries of observed data	What is happening within our <i>sample?</i>	Simple
Exploratory	Identify trends and relationships <i>within the sample</i> ¹	What correlates with reef die-back in the sample?	
Inferential	Extending the patterns in the sample to the <i>population</i> from which the sample was taken	What is the status of species X in a marine protected area?	
Predictive	Predict the values at unsampled locations based on sampled data	What assemblage is likely to be found in this location?	
Causal	Identify the reason for a particular association	Are the implemented MPAs having an effect?	Complex

Table 2.1 Different types of research questions (adapted from Leek and Peng, 2015)

¹There is no way to tell if the *sample's* associations are the same as the *population's*

Observational data from well-designed marine surveys are able to inform all research types and are sometimes the only source of adequate information (Table 2.1). The exception is for causal inference (attributing impacts to specific causes), where randomised controlled experiments are often needed. However, in that case there are usually other limiting factors whose discussion is beyond the scope of this manual (see Hayes et al., submitted). Causal questions require special attention and are usually more demanding in terms of the resources needed to answer them. Thus, we focus on (marine) observational surveys, and in particular the design of surveys. Wil the topics discussed in this section are relevant to investigating causal relationships, other considerations would also be required to be addressed before undertaking research (we do not deal with those here). For more information on the evidence hierarchy, and a more thorough description of the different design types for marine ecology, see Hayes et al. (submitted).

A key concern in this scientific process is ensuring that survey data are trustworthy and fit-forpurpose (i.e. can answer the research question). To this end, it is important that surveys and monitoring programs are designed and implemented in such a way that the resulting data are: (i) appropriate for the research question under consideration; (ii) are representative of the population under investigation so that (for example) the sample mean is generalisable to the population mean; and (iii) information rich so that uncertainty around inferences is reduced as much as survey



budgets will allow. We focus here on survey designs that will help ensure environmental monitoring programs deliver data with these characteristics.

2.1.1 Scope

This chapter will not follow the usual presentation for statistical design in ecology. Rather, we will focus on what we believe to be most important aspects from a practical (and management) viewpoint. We do not intend it to be like a 'text-book' and explicitly do not include formulae or descriptions of tangental details. Readers will want to look elsewhere for such detail (Urquhart and Kincaid, 1999; Gitzen *et al.*, 2012; Thompson, 2012, are a good start, although there are many). We hope to only introduce the relevant concepts and stress that these are the things that should be thought about by all researchers involved with survey planning. In particular, we discuss: (i) randomisation, (ii) efficiency of design, (iii) uncertainty reduction, (iv) sampling in space and time, and (v) specifics for different gear types. This all leads to an illustrative example design, using the *MBHdesign* R-package. (available from CRAN, <u>https://cran.r-project.org/package=MBHdesign</u>). For those readers interested in acronyms: *MBHdesign* are outlined throughout this chapter.

2.2 Randomisation

In all areas of science (and where statistical methods are applied), representative samples are typically achieved by randomisation (e.g. Thompson, 2012; Smith et al., 2017; Tillé and Wilhelm, submitted). Randomisation ensures that the information contained in the sample is generalisable to the population that it was obtained from (Fisher, 1925). Simply using some sort of random sampling ensures that many types of research questions are answerable (see Table 2.1). The alternative, which is unfortunately still relatively common in marine ecology, is to select sites based on other (non-random) properties. These properties could include their convenience to sample, or what a researcher expects to find. This is called 'ad-hoc', 'opportunistic', 'haphazard', 'judgemental' or 'convenience' sampling. While at first glance this approach appears to be efficient, it in fact removes the ability to answer any questions about the population as a whole, which limits questions to those involving the specific sample only: descriptive and exploratory questions (unless non-testable assumptions are made). The reader is referred to Smith et al. (2017) for a recent discussion of this topic in ecology.

The implication here is immediate and clear – **researchers should randomise** the sampling process if they expect that the patterns observed in the sample to hold in the population. No researcher should routinely perform haphazard sampling. Of course, there may be situations where a particular location appears so interesting that it could be *appended* to a randomised survey design, but its data can only be included into the analysis with additional (strong) assumptions and/or complexities in analysis approaches. The randomisation process is particularly important for monitoring programs where data from multiple surveys (through time and/or space) are combined.

An important side-effect of randomisation is that a researcher must specify what the statistical population under study is. Formally, for surveying geographic areas, the population is a collection of potential survey locations from which a random sample is taken, often called a *sample frame* in the literature. The formal specification of the sample frame is important as it gives the extent to which the results are legitimately generalisable. A sample frame may be delimited by some combination of: spatial extent, depth, habitat type, season and the type of sample that the selected gear can adequately collect. Generalisation beyond the sample frame are identical to those within it. It is best to try and avoid these assumptions by expanding the sample frame prior to undertaking the survey.



2.3 Efficient Designs

Simple randomisation – randomly scattering sampling locations through space – is not necessarily an efficient approach, and in many circumstances a large number of samples are necessary to obtain acceptably precise estimates of population parameters (e.g. Tillé and Wilhelm, submitted). This is one of the reasons that haphazard sampling can initially although mistakenly appear quite attractive, however there are ways to address this inefficiency, and to generate designs that require fewer samples and resources. Researchers have proposed statistically valid restrictions on the randomisation process, and research in environmental sciences has ultimately led to spatially balanced designs (Stevens and Olsen, 2004; Dobbie et al., 2008; Grafström et al., 2012; Grafström, 2012; Grafström and Tillé, 2013; Grafström, 2013; Robertson et al., 2013; Brown et al., 2015; Foster et al., 2017; Tillé and Wilhelm, submitted), with similar ideas known as 'spatial coverage designs' (Royle and Nychka, 1998; Brus et al., 1999, 2006; Minasny and McBratney, 2006; Walvoort et al., 2010) and 'even sampling designs' (Chen et al., 2012). A spatially balanced design can be seen as an extreme form of stratification (Stevens and Olsen, 2004) that aims to reduce the frequency of placing samples close to each other (relative to simple randomisations). This process improves efficiency by reducing the amount of spatial auto- correlation between data implying that each sample is providing as much unique information as possible (Grafström and Tillé, 2013). Additionally, spatially balanced designs are more efficient than other types of randomised designs as they tend to increase balance on many environmental variables (also known as covariates), where the populations covariate mean is equal to the samples covariate mean (Grafström, 2013). This is more than just stratifying for important environmental gradients, as that process does not ensure balance unless explicitly accounted for. Even if balance is sought in stratification, the simple randomisation process within strata lacks efficiency, can complicate analyses, and can be wasteful of 'degrees of freedom' in the analysis (reducing analytical power). In summary, spatially balanced designs are used to enhance efficiency so that the greatest amount of information is obtained from the any given number of sample locations (compared to other forms of randomisation).

Some researchers will know spatially balanced designs as 'GRTS' (for generalized random tessellation stratified; Stevens and Olsen, 2004), but GRTS is just one type of spatially balanced design. It is a good design approach and it is the prime reason that spatially balanced designs are gaining popularity. However, it is not the most spatially balanced design, which implies that it is also not the most efficient (Grafström et al., 2012; Robertson et al., 2013; Foster et al., 2017). Between the various spatially-balanced design types, the differences in relative performance are minor. Computational methods for GRTS, via the *spsurvey* R-package (Kincaid and Olsen, 2016), in our experience can be cumbersome, time-consuming and in some ways inflexible. The inflexibility stems from sampling only in two dimensions. Experienced GRTS users can legitimately continue using it, as the efficiency cost is not large, and they have already overcome many of the more cumbersome aspects. However, we recommend that new users, and some more discerning users, start with *MBHdesign*.

While we focus here on spatial balance, many (but not all) of the algorithms for producing spatial balance can be employed to sampling more than just 2-dimensional space. In particular, the algorithms implemented in *MBHdesign* are equally applicable to space-time scenarios and even space-depth-time ones (where a 3-dimensional volume, such as a water mass, is sampled over time). In fact, the algorithms scale well with dimensions, and there is no limiting dimensionality, except what is practical in the application.

The efficiencies of spatially balanced designs can be further improved by increasing the probability of selecting sampling locations where the sampling variable is thought to have greater variance (e.g. Godambe and Joshi, 1965; Brewer et al., 1988; Chambers, 2011; Grafström and Tillé, 2013). This is achieved by altering the so-called inclusion probabilities of each potential sampling location. Inclusion probabilities specify the chance of each site being randomly chosen to be part of the



survey and they can be chosen on the basis of data from a pilot study or from other sources (e.g. literature on similar species and/or regions). An inclusion probability near zero will imply that the site will almost never be sampled, whereas a site with inclusion probability of one will be chosen much more often. The inclusion probabilities are prescribed by the survey designer to indicate where the sampling effort should be placed (see Grafström and Tillé, 2013, for more information on how to perform this task). In ecology, where biological variables often have an increasing mean-variance relationship (e.g. through Taylor's power law; Taylor, 1961), this equates to increasing inclusion probabilities in locations where the population being sampled is expected to have high abundance. If no prior knowledge exists, such as from previous surveys or a pilot study, then the inclusion probabilities should be equal.

Altering inclusion probabilities requires the identification of one or more measured covariates (available at time of design) that can be used to guide the variation in inclusion probabilities. It also is beneficial only in situations where the inclusion probabilities are related to the sampling variable. When inclusion probabilities *do not* have this relationship, then this will cause a *loss* of efficiency (lower precision) than equal inclusion probabilities. We caution against using too many covariates in the design stage and point out that equal inclusion probabilities is a conservative approach. In fact, fewer covariates is better in many ways. The simple reason is that if they are used to define the design then they must also be used in the analysis (as the design is conditional on these covariates), see Gelman et al. (2013) and Foster et al. (2017) for discussion. This means that precious 'degrees of freedom' must then be used to estimate potentially non-helpful parameters, which has the effect of increasing analysis complexity and reducing the discrimination ability of the analysis. So, the survey designer must weigh up the anticipated reduction in variation due to incorporating the covariate against the necessity to use more terms in the model.

The concepts of stratification and altered inclusion probabilities are almost, but not quite, identical in situations where stratification is applicable. However, at the cost of being conceptually more sophisticated, the inclusion probability concept is more general and more flexible. The reasoning for the equivalence is that the inclusion probabilities can be designed to match the stratification, so that *on average* the specified number of survey sites is chosen within each strata, but this is not guaranteed for every randomised design. Contrastingly, all stratified designs will have the specified number of survey sites within each strata. To us, this is not a large difference and the benefit of being able to spatially balance the design is likely to lead to bigger benefits. We therefore recommend altering inclusion probabilities with spatial-balance in preference to formal stratification. However, stratification is not a bad option and is more efficient than simple randomisation (when the stratification is meaningful). We note that the *spdesign* software that implements GRTS allows for stratification *and* spatial balance by balancing within each spatially-contiguous strata.

When planning marine monitoring programs, the ability to incorporate any existing sites will often be advantageous. In the NESP Marine Biodiversity Hub, methodology was developed to incorporate these *legacy* sites into a spatially balanced design. Legacy sites (or historical, reference or sentinel sites) are those sites that have been sampled in the past and the researcher wants to re-visit them as part of the upcoming survey. Readers are referred to Foster et al. (2017) for details. Briefly however, spatial-balance is achieved by adjusting inclusion probabilities (within the proximity of legacy sites) downwards so that new samples are less likely to be placed near legacy sites.

2.3.1 Software

There are many pieces of software that will generate spatially-balanced designs, most of which are based on different algorithms. For monitoring the marine environment, we developed a specific software – the R-package *MBHdesign*. It is intended to be easy to use and tailored to common situations in marine ecology². It also has the ability to make designs spatially balanced around existing legacy sites, see Foster et al. (2017). We will use *MBHdesign* in the example to follow.

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2.4 Uncertainty, Precision, and Power

It is important to consider how to reduce the uncertainty (and increase precision) in statistical analyses of survey/monitoring data. Practically, there are two components to this: 1) increasing the information content in the dataset; and 2) reducing the noise in the collection process. Performing an efficient survey/monitoring design, such as a spatially balanced design, is aimed at increasing information content in the dataset (by trying to make each sample represent as large a portion of the sample frame as possible). More information implies that the signal in the data can be clarified with more ease. Noise reduction comes from using measurement protocols that are designed to be repeatable (so that two measurements on the same sample will generate very similar observations). See the gear specific chapters in this field manual package for detailed advice on reducing measurement noise. For some novel measurement platforms, measurement/scoring techniques are still being assessed and these updates should be incorporated where possible. Examples of this process are Perkins et al. (2016) for scoring AUV images and Schobernd et al. (2014) for scoring BRUV deployments. We stress though, that whilst noise reduction is important, it is not the only consideration and that particular care should be taken to maintain protocols within already established monitoring programs, or calibrate new protocols with old. In addition to reducing 'noise', it will ensure that, for example, time-series do not get 'broken' and that data are directly comparable in time and space without unfortunate confounding due to a change in sampling methodology.

Most Chapters in this field manual package are variations on the noise-reduction theme as they provide a foundation for reducing variation between and within surveys. In particular, if adhered to, they will help minimise, or possibly even eliminate, inherent systematic variation (bias) between different surveys or within a monitoring program. This will have the effect of increasing the utility of combining data from different surveys (as there will be minimised bias between the two sets). We have unfortunately come across too many long-term studies that could not be used to estimate trends in the target species because of inconsistencies in sampling design and implementation (Hosack and Lawrence, 2013).

Any approach to reducing variance in the sample statistics should be welcomed whole-heartedly, so long as there is no introduction of confounding between it and any spatial/temporal signals or other important trends. This includes processes to minimise measurement variation (e.g. non-uniform gear deployment, faulty measurement equipment, poor laboratory practices) and data entry errors. In most circumstances however, measurement variation is likely to be relatively small compared to the variation in the ecological processes that are being sampled. Understanding this means that exorbitant amounts of time should not be placed in perfecting each measurement – especially not if the cost of perfection is a substantial reduction in the number of samples taken. Often a much richer sample is obtained (in terms of signal to noise) by taking more, slightly noisier, samples than fewer precise ones. Unfortunately, we are aware of no rules-of-thumb to guide researchers with this issue. However, we do note that standard errors decrease with the square root of sample size and increase linearly with residual standard deviation. The same argument suggests that one should avoid taking excessive sub-samples.

Some design experts advise that a power analysis be performed before any survey effort is undertaken. Recall that a power analysis calculates the probability that the survey will be able to detect a difference if there actually is one (a true positive). This is undoubtedly a good thing to do when there is a clear hypothesis to be tested and a clear effect size to be detected. However, this is not always the case. It has been observed that power analyses are often performed without great thought, leading to (perhaps) overly large stipulated sample sizes (e.g. Mapstone, 1995); probably larger than any reasonable budget will allow. The arguments outlined in Mapstone (1995) are, to us, quite compelling as they make a researcher undertaking a power analysis think critically about the relative environmental/economic/political costs of making a poor decision. Sometimes it will be more



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important to guard against making a false-negative (type II) errors than false-positive (type I). Such a situation could occur if the cost of falsely declaring significance is larger than that of falsely declaring *non*-significance (e.g. declaring impact may result in closure of a factory or imposing fishing quotas). This is quite contrary to many applications of hypothesis testing in other areas of science. If a power analysis is undertaken, then there is some general advice that we offer to marine ecologists. First, don't blindly follow text-book recipes for power analyses. They make some strong assumptions that are unlikely to be met in ecology (e.g. normality of observations, independence of observations, and constancy of variance in space and/or time). Second, be prepared to do a lot of homework about the sizes of the components of variation that you are likely to observe: "How much overdispersion is there in your study region?" "Is there any spatial autocorrelation likely?" "What analysis methods are intended to be used?"

It is our opinion that a very useful, and often not too difficult, method for assessing power is to use simulation. There have recently been attempts to provide simplified R-based tools for this process (Green and MacLeod, 2016, for mixed models), and these show promise. The simulation approach consists of a small number of steps: 1) simulate some data under the alternative hypothesis (incorporating the effect that is being considered), 2) analyse the data and see if there is a significant effect, and 3) repeat steps 1) and 2) many times. The proportion of analyses (of simulated data) that produce a significant analysis will give one minus the power of the test. This approach has been used in many places, including the marine realm (Foster et al., 2014, Perkins et al., 2017). It is not the only piece of information that can come from the simulation though. In particular, it can be used to support the evaluation of how sample size and study design impacts more general monitoring objectives (e.g., the ability to estimate parameters in a model or predict future data).

2.5 Spatio-Temporal Sampling

Sampling in space is a task that requires plenty of thought, as demonstrated by the previous sections. Sampling in space *and* time (i.e. monitoring) requires even more thought as there are even more options. Generally, if one wants to sample repeatedly then the focus will be (at least partly) on trends though time. It is commonly established in the survey literature, that the uncertainty around temporal signals is reduced by repeatedly visiting the same sites (e.g. Urquhart and Kincaid, 1999). This comes at a cost though – less sites are sampled and therefore the sample may not be as representative of the population as it could be. Extreme cases in marine sampling are when the sampling gear actively alters the population size (through extractive sampling) or its habitat (for example removal of epibenthic structure). In these cases, repeatedly sampling the same sites will not reflect the trends in the population.

Intuition tells us that, unless sampling is destructive, then you should revisit at *least some* of the sites. This is due to the reduction in variation in the temporal signal (the site-to-site variability is removed). The proportion of sites to be revisited, and the pattern of revisits (e.g. rotating panel, fixed panel, and so on – see McDonald, 2003), will depend upon the temporal (and spatial) variability of the biota under consideration (see Perkins et al., 2017, and references therein). Legacy sites can, and should, be incorporated into a temporal monitoring program. Our advice is to try and make sure that some legacy sites get sampled during each revisit for new sites. This has the effect of ensuring 'a link back to the legacy site time-series' for each revisit. If the biota change rapidly, even at the same spatial location, then there is little point revisiting sites. This is especially so for monitoring programs with substantial time between revisits. In summary, think carefully about the relative importance of the temporal signal versus the generality. This will reflect the number of revisits to perform. Special consideration should be given to the spatial and temporal variances – if the biota exhibit a high temporal variance, then repeats will not reduce uncertainty substantially.



2.6 Gear-Specific Considerations

Some gear types need special consideration as they naturally force the survey designer into different modes of thinking. To our mind, the biggest distinction in sampling gears for marine biota, for design considerations at least, is whether the gear collects a single observation from each deployment (e.g. a grab) or whether it collects many (e.g. an AUV). There is some grey area here: we class BRUVs as point collection methods and we class trawls also as point source methods. BRUVs can be thought of as a single spatial point but with potentially many *temporal* observations. Trawls integrate locations along a transect by means of combining the catch in a cod-end.

When the spatial scale of the sample-frame is geographically large, in relation to the transect size (e.g. AUV) or field-of-view (e.g. BRUV), then all these methods can be treated as point collection and standard survey principles apply. However, when the sample frame is geographically small in relation to the size of the area sampled by the sampling gear, then the position of the observation within the sampling unit becomes important as biota from two separate samples may be spatially close. The only design advice in the literature for the gear types considered in this field manual package, that we are aware of, is to try and space samples well apart in space (Foster et al., 2014). However, proposed Marine Biodiversity Hub research (for 2018) aims to provide greater utility around this. Developed methods will be implemented into the R-package *MBHdesign*.

There are more considerations when designing a transect-based survey. Chiefly, one needs to consider how long the transects are and in what direction the transects should be performed. Our intuition tells us that, logistics aside, the length of the transect should be dependent on the spatial properties of the biota being surveyed. Biota with large spatial autocorrelation should be sampled with many short transects, whereas biota with short spatial autocorrelation could be sampled with longer transects. See Foster et al. (2014) for an example of identifying length and direction of spatial autocorrelation from image-based transect data. Of course, it may be cheaper to deploy the sampling platform for longer and then simply sub-sample or account for the autocorrelation within an analysis model, but the reasoning will still provide advantages. In any situation, care needs to be taken in the analysis to account for this autocorrelation (see next paragraph for further elaboration). The direction of the transects might be gear dependent - for example it may be 'safer' to take transects down-slope or across-slope. However, irrespective of the restrictions on direction the design should aim to cover the study area as evenly as possible. Image based transects have further considerations – how much effort to place in scoring each image versus how much effort to place in scoring more images. Perkins et al. (2016) suggests that this too depends on the spatial properties of the biota under consideration and suggests apportioning effort according to these properties.

When designing temporal surveys, it is important to consider if you can actually perform replicates with enough geographical accuracy to be useful. If the exact transect cannot be repeated then there is a confounding of temporal and spatial variation, and if the spatial patterns are quickly changing then the temporal uncertainty will also be inflated (Perkins et al., 2017). This is particularly concerning for gear types that are located only by the location of the deployment vessel. Even for accurately re-deployable gears the spatial repeatability is sometimes not sufficient (Perkins et al., 2017).

Whilst this chapter is about statistical *design*, we feel it important to mention statistical *analysis* of survey data, especially that resulting from transect-based sampling platforms. These produce data that are spatially close to each other, often very close. This naturally raises concerns about spatial autocorrelation and its impact on an analysis. Our advice for these platforms is to use geostatistical models (e.g. Diggle and Ribeiro, 2007; Banerjee et al., 2004). These naturally account for the spatial dependence between observations and adjust measures of uncertainty accordingly. This is not an easy approach and involves a steep learning curve for many practitioners. However, it does



circumvent the unfortunate (and dangerous) consequence of falsely considering that there is less uncertainty in the data than there actually is, which is effectively what happens when one assumes that geographically close observations are independent. Again, subsetting the individual observations within a transect is likely to have some beneficial effect on mitigating autocorrelation (e.g. Mitchell et al., 2017). However, doing so presupposes that the range of the autocorrelation is less than the distance between the subsetted observations.

2.7 Multibeam as Foundation Data

Multibeam data that covers an entire area (sampling frame) is a real boon for designing efficient surveys. It enables the survey design team to produce a design that picks out the major sources of variation in the ecosystem (typically depth and hard substrate), which can then be used to alter inclusion probabilities. To use it one must consider how the multibeam data might be related to the variance in the target biota being sampled; it is reasonable to spend greater survey effort on hard substrate to reduce uncertainty. For example, sponge abundance will have higher variance on hard bottom than on soft bottom and so a sponge survey should disproportionally target hard bottom. Once these areas have been identified, then the inclusion probabilities for those regions can be increased, which will increase the chance of sampling hard substrate but maintaining the ability to infer to the sampling frame. This is the intuition in the approach that was used in Lawrence et al. (2015).

Although our recommendation is to map the survey area using multibeam prior to designing biological surveys, it is not always possible. One alternative approach, which tries to leverage as much multibeam information as possible, is to stage the sampling: perform a limited amount of multibeam mapping and work within those limited areas. Done smartly, like in Lawrence et al. (2015) this approach can still offer good estimates of biota. However, it is not without difficulties (principally in the analysis stage) and these complications could be, in some cases, overly limiting.

2.8 Case Study: Surveying a Marine Park in Tasmania

To illustrate some of the technical aspects of the design process, we plan a survey design for the Governor Island Marine Reserve off Bicheno on the East Coast of Tasmania. The marine protected area (MPA) is geographically complex with boundaries governed by natural land formations. The depth profile of the MPA is decreasing away from the land-based boundary, and there is less 'shallow' regions in the MPA than 'deep' ones.

We will present three designs. The first is a plain (vanilla) spatial design where all sites within the MPA are equally likely to be sampled. The second design intentionally samples shallow sites more often as these sites are likely to be more hetereogeneous and diverse than their deeper water counterparts. The third type of design is when there are legacy (reference) sites in the area that should be resampled as it is considered important to create a time-series for this MPA. The spatial balance should then account for the locations of these legacy sites when finding the new sites. For more details on how to perform this third type of design, please see the *MBHdesign* vignette (by loading the *MBHdesign* package into R and typing vignette('MBHdesign'). Another good place to look is the paper describing the method: Foster et al. (2017). The inclusion of legacy sites in this example is somewhat artificial, as we have to first choose the legacy sites to incorporate. However, we hope that the process is illustrative nevertheless.

The example here is performed in R, an open source statistical platform. Importantly, there are other free and licensed software and programming languages that can also be used, depending on



your proficiency and what is available to you. Some of the code may, at first glance, look a little daunting. Well, that's R for you. Most of the lines written here are for plotting purposes and for reading in data. Since this is a document, we have taken some care with how the plots appear. This produces pretty(er) pictures but it also produces longer and more detailed code. Users should feel free to use the code below as a template, but please don't blindly do so without thinking if the actions of the code is appropriate for your data. If you do re-use code, then please run checks to see if the code has done what you think that it ought to.

If you are new to R, then you could try to get an introduction by one of the many online tutorials (e.g. https://cran.r-project.org/doc/manuals/R-intro.html). That particular one is likely to be like R helpfiles (helpful but takes time) and it could be quite *dense*. Another option is the excellent book Venables and Ripley (2002), which introduces you to R *and* gives a good introduction to some types of data analysis. Other recommended introductions to R include: Crawley (2007); de Vries and Meys (2015). However, these are just suggestions, you should shop-around until you find a reference/tutorial that is at-your-level and in no time at all you will be reading in data, analysing it, plotting it, and summarising results.

2.9 Set Up R to Generate Design

To start we have to set up R for generating designs. This should not be onerous in this case. The most difficult thing is in setting up the data file in the first instance (usually through a GIS). Here we have used an .asc file as this is relatively easy to read into R. This file is included in the field manual package, along with the R code to create the output below.

This document was created using the R-package *knitr* (Xie, 2014). It is a wonderful tool, but like any tool it requires interpretation. Most notable here is that the R-code is placed in a grey box, to enable readers to highlight the code versus the document text. Within the code sections, anything that comes after a '#' symbol is a comment that is not interpreted by R (most of these are a brown colour). Bold dark blue words are function names. Dark blue words are argument names. Green is for text and light blue for numbers.

##if you don't have MBHdesign installed, please do so using
install.packages("MBHdesign")

#Load required packages
Library(MBHdesign) #For spatial sampling
Library(fields) #for lots of things, but for plotting in this example
Library(sp) #for reading the ascii file of cropped depths for the MPA

#Set a seed for reproducability
set.seed(666)

```
#Read in depth as a asc file containing long, lat and depth
#This path/file only exists on the first author's system
# you will need to change it if running this code
#the projection will need to be changed for each region too
#bth.orig.grid <- read.asciigrid("./ExampleGovIsland/gov_bth.asc", proj4string =</pre>
```

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```
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```

```
CRS("+proj=utm +zone=55 +datum=WGS84"))
bth.orig.grid <- read.asciigrid("gov_bth.asc", proj4string = CRS("+proj=utm +zone=55</pre>
+datum=WGS84"))
#convert to a data.frame for ease
DepthMat <- as.matrix( bth.orig.grid)</pre>
bth.orig.grid <- as.data.frame(</pre>
  cbind( coordinates( bth.orig.grid), as.numeric( DepthMat)))
colnames( bth.orig.grid) <- c("Easting", "Northing", "Depth")</pre>
bth.orig.grid <- bth.orig.grid[order( bth.orig.grid$Northing,</pre>
  bth.orig.grid$Easting),]
#Setting up plotting for now and later
uniqueEast <- unique( bth.orig.grid$Easting)</pre>
uniqueNorth <- unique( bth.orig.grid$Northing)</pre>
ELims <- range( na.exclude( bth.orig.grid)$Easting)</pre>
NLims <- range( na.excLude( bth.orig.grid)$Northing)</pre>
#Fix up ordering issue
DepthMat <- DepthMat[, rev(1:ncol(DepthMat))]</pre>
#plot it to see what we are dealing with.
image.plot( uniqueEast, uniqueNorth, DepthMat,
    xlab="Easting", ylab="Northing", main="Governor Island MPA",
    legend.lab="Depth (m)", asp=1, ylim=NLims, xlim=ELims,
    col=rev(tim.colors()))
```

Governor Island MPA



Figure 2.1: Map of Governor Island study region with depths. Note the non-regular shape and the non-uniformity of the regions depth profile.

2.9.1 Generate a spatially balanced design

Generating a spatially balanced design within the MPA is quite straight-forward using *MBHdesign*. Here we do it for 30 sampling sites spread throughout the MPA (Figure 2.1). Note that designs will vary from one realisation to the next, unless the seed it fixed (like we did in the previous subsection). Try it a few times, if you like, and see what happens between the realisations. Note that *on average* (over all realisations) the spatially balanced designs will have good spatial coverage.




35 5364000 30 5363800 - 25 Northing E Depth 20 5363600 15 10 5363400 5 608800 609200 609000 609400 609600 Easting

Spatially Balanced Sample

Figure 2.2: A uniform inclusion probability sample for Governor Island



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2.9.2 Preference shallow environments

The equal inclusion probability design (Figure 2.2) assumes that all sites are equally advantageous to sample. Previously, we mentioned that this may not be an efficient approach to sampling. In particular, it can be advantageous to over-sample sites/regions that have greater variability. In the Governor Island MPA, this corresponds to the shallower depths as these typically are more heterogeneous and biodiverse on the east coast of Tasmania. We can design a survey with this in mind by increasing the probability that shallow sites will be sampled (i.e. by increasing their inclusion probabilities). This has the obvious effect of also decreasing the probability that deeper sites will be sampled (Figure 2.3). The code below shows how this can be done. It is a little more involved, but most of the complexity comes from detail. The approach is simple though: 1) find the empirical distribution of depths in the MPA; 2) define the inclusion probabilities based on this empirical distribution; and 3) sample according to those inclusion probabilities. We will sample a few more sites (n = 100), just to make the effect of the depth adjustment clear.

par(mfrow=c(1,3), mar=rep(4,4)) n <- 100 #The number of 'depth bins' to spread sampling effort over. nbins <- 4 #force the breaks so R doesn't use 'pretty' breaks <- seq(from=min(bth.orig.grid\$Depth, na.rm=TRUE), to=max(bth.orig.grid\$Depth, na.rm=TRUE), length=nbins+1) #Find sensible depth bins using pre-packaged code tmpHist <- hist(bth.orig.grid\$Depth, breaks=breaks, plot=FALSE) #Find the inclusion probability for each 'stratum' tmpHist\$inclProbs <- (n/(nbins)) / tmpHist\$counts #Matching up locations to probabilties tmpHist\$ID <- findInterval(bth.orig.grid\$Depth, tmpHist\$breaks)</pre> #A container for the design design <- data.frame(siteID=1:nrow(bth.orig.grid), Easting=bth.orig.grid\$Easting, Northing=bth.orig.grid\$Northing, Depth=bth.orig.grid\$Depth, inclProb=tmpHist\$inclProbs[tmpHist\$ID]) #Plot the depths and the inclusion probabilties with (design, plot (Depth, inclProb, main="Inclusion Probabilities", ylab="Inclusion Probabilities", xlab="Depth (m)", pch=20, cex=1.4)) #Plot the inclusion probabilities in space with(design, image.plot(uniqueEast, uniqueNorth, matrix(inclProb, nrow=length(uniqueEast), byrow=FALSE), xlab="", ylab="", main="Inclusion Probability", asp=1, vlim=NLims, xlim=ELims)) #Take the Sample using the inclusion probabilities samp <- quasiSamp(n=n, dimension=2,</pre> potential.sites = design[,c("Easting","Northing")], inclusion.probs=design\$inclProb, nSampsToConsider=100*n) #Plot the design with(design, image.plot(uniqueEast, uniqueNorth, DepthMat,



xlab="", ylab="", main="Spatially-Balanced Sample", asp=1, ylim=NLims, xlim=ELims, col=rev(tim.coLors()))) points(samp[,c("Easting","Northing")], pch=20, cex=2) write.csv(design, file="design.csv", row.names=FALSE)



Figure 2.3: (Left panel) The empirical distribution of the 4 different depth bins. (Middle panel) The spatial distribution of the depth bins. (Right panel) A non-uniform spatially balanced sample, with inclusion probabilities based on the distribution of depths throughout the region. Shallow sites have been over-represented in the sample.

2.9.3 Incorporate legacy sites

Here, for edification purposes, we provide an illustration of how to design a spatially-balanced survey that accounts for the locations of legacy sites, which are those sites that we wish to include in the survey. The most likely reason for including legacy sites is that they have been sampled before, hopefully as part of a previous randomisation process. Various names exist for legacy sites, including 'reference sites', and perhaps even 'sentinel sites' in some situations.

In our example, we first generate legacy sites and then generate more sites around them. To provide a little extra spice to the design we try to mimic the learning process: the n = 6 legacy sites are chosen with uniform probabilities (as we would do when there is no information about the area) and then the n = 15 new sites are chosen with a depth gradient altering the inclusion probabilities (Figure 2.4). This example therefore incorporates elements of the previous two examples.

```
#set up the plotting structure
par( mfrow=c(2,2), mar=c(3,3,3,3))
#number of samples
n_1 <- 6
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```



```
##Take the sample for the legacy sites.
#Here they are a spatially balanced sample but in practice
# they would be supplied from a previous randomisation process
samp_legacy <- quasiSamp( n=n_1, dimension=2,</pre>
    potential.sites = bth.orig.grid[,c("Easting","Northing")],
    inclusion.probs=!is.na( bth.orig.grid$Depth))
#plot the legacy sites
with( bth.orig.grid, image.plot( uniqueEast, uniqueNorth, DepthMat,
    xlab="Easting", ylab="Northing", main="Legacy Sites",
    legend.lab="Depth (m)", asp=1, ylim=NLims, xlim=ELims,
    col=rev(tim.colors()), legend.mar=8.1))
points( samp_legacy[,c("Easting","Northing")], pch=17, cex=2)
#plot the depth-based inclusion probabilities
# scale first to sum to n=15
n <- 15
design$inclProb <- n * design$inclProb / sum( design$inclProb, na.rm=TRUE)</pre>
with( design,
    image.plot( uniqueEast, uniqueNorth,
        matrix( inclProb, nrow=length( uniqueEast)),
        xlab="", ylab="", main="Inclusion Probability", asp=1,
        ylim=NLims, xlim=ELims, legend.mar=8.1))
##Depth-based inclusion probabilities
#Alter the inclusion probabilities for the next sample
# inclusion probs taken from previous example
p2 <- alterInclProbs( legacy.sites=as.matrix(</pre>
    samp_legacy[,c("Easting","Northing")]),
    potential.sites=bth.orig.grid[,c("Easting","Northing")],
    inclusion.probs=design$inclProb)
#plot the altered inclusion probabilities
with( design,
    image.plot( uniqueEast, uniqueNorth,
      matrix( p2, nrow=length( uniqueEast)), ylim=NLims, xlim=ELims,
      xlab="", ylab="", main="Altered Inclusion Probability", asp=1, legend.mar=8.1))
##Take the new sample, spatially balanced around the legacy sites
samp <- quasiSamp( n=n, dimension=2,</pre>
    potential.sites = design[,c("Easting","Northing")],
    inclusion.probs=p2, nSampsToConsider=100*n)
#plot legacy sites and new sample sites.
with( design, plot( Easting, Northing,
    col=c('white', grey(0.9))[1+!is.na(inclProb)], ylim=NLims, xlim=ELims,
    xlab="", ylab="", main="Combined Sample Locations", asp=1))
points( samp_legacy[,c("Easting","Northing")], pch=17, cex=2, col='red')
points( samp[,c("Easting","Northing")], pch=20, cex=2)
Legend( "bottomleft", c("Legacy Sites", "New Sites"), pch=c(17,20), pt.cex=2,
    col=c('red','black'), bty='n')
```







Figure 2.4: A spatially balanced design for Governor Island that incorporates legacy sites and has depth-varying inclusion probabilities (shallow sites are over-represented).

2.9.4 Case study summary

We have now seen how to generate three different kinds of designs: 1) a spatially balanced design with equal inclusion probabilities for when little is known about the sources of variation of the system; 2) a spatially balanced design with unequal inclusion probabilities for when we think we know where the locations with higher variance are likely to be; and 3) a spatially balanced design for when we have legacy sites that we want to take a repeat sample.



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If future researchers wish to re-survey the area at some point in the future, then they have a choice to make: (I) Do they wish to revisit the same sites (to get a good temporal estimate)? (ii) Do they choose a new set of sites (to get a good spatial estimate)? Or, (iii) Do they assume that the temporal change is not important and include the previous survey as part of the sample? The last scenario would be performed efficiently by using the original sample locations as legacy sites and spatially balance the new sample locations around those (as was done in the example). It will usually be sensible to combine these objectives by repeating some (not all) of the samples but choosing some new locations as well.

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² Feedback on both these claims is welcome, as are suggested improvements. Please do so through the survey <u>www.surveymonkey.com/r/CQKC688</u>.





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3. SEAFLOOR MAPPING FIELD MANUAL FOR MULTIBEAM SONAR

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3.1 Platform Description

Swath mapping systems use acoustic technology to collect data on the bathymetry (topography) and the backscatter (impedance) of the seafloor (Figure 3.1). These systems can either be mounted on a ship; autonomous underwater vehicle; remotely operated vehicle or a remote surface vehicle. They work by transmitting a sound pulse, called a ping, through a transducer at a specific frequency (or a range of frequencies simultaneously). This same ping is then recorded through a receiver placed very close to the transducer. The elapsed time that the ping takes to reach the seafloor and return to the receiver is used to measure the depth of the water. Certain attributes of the shape of the sound-wave are used to infer characteristics about the seafloor (geomorphology). Typical multibeam echo sounder (MBES) data products include bathymetry (seafloor depth) as well as backscatter intensity, which can provide a metric for seafloor "hardness" and will indicate the substrate type (Figure 3.1 a-d).

MBES have become one of the standard tools for geophysical surveying and mapping of the seafloor and have been used for a variety of scientific, safety at sea (hydrographic and military operations) and industrial applications. MBES can produce a spatially continuous acoustic image of the surface of the seafloor by generating a "swath" or "fan" of continuous data points, increasing the resolution of the resulting surfaces. This has revolutionized our ability to understand physical processes occurring at the seafloor, and the composition and distribution of substrate, which has in turn significantly improved our knowledge of seafloor ecosystems (McArthur et al. 2010, Lucieer and Lamarche 2011, Porter-Smith et al. 2012). Mapping of bathymetric morphology will delineate geological features that have relief (using the changes in seafloor depth information), however in regions where the relief is smaller than the minimum mapping unit (resolution of the grid cell is larger than the feature of interest) backscatter data can be used to assess the boundaries of the geology or sediment structure.

Australia's marine estate spans an incredible range of water depths; from the coast to 6000m+. Water depth has a very large influence on the acoustic survey acquisition, as it will dictate the resolution of the data (i.e., number of pings per unit area which will dictate the minimum pixel size) and the efficiency for surveying using MBES acoustics (i.e., swath width). While practices for employing the equipment have developed rapidly over the last few decades, there are a number of specific and common issues that need to be considered and detailed in a national standard operating field manual. This document has been developed in collaboration with Australia's *National Multibeam Guideline* written by the National Seabed Mapping Coordination working group which includes over 40 representatives from government departments, scientific institutions, universities and industry (see inset box).

During the development of this manual, a broader assessment of multibeam survey standards by a national seabed mapping coordination working group was started. This program will provide guidelines for national standards of acquisition on and off the shelf, and improved data interoperability and access. This national working group guideline aims to be relevant for a wide range of purposes such as hydrographic mapping, marine infrastructure installation and planning, and baseline habitat mapping. It provides a more detailed description of the technical considerations of acquisition and international surveying standards, including details of operational procedures. Further details can be found in the *National Multibeam Guideline* to be available on www.ausseabed.gov.au by mid-2018.

In order to avoid duplication of details, this field manual will provide a procedure for specific planning, acquisition and processing steps relevant to marine monitoring. Where applicable, it will



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refer to the *National Multibeam Guideline* for further details of operational steps. It will also provide further specific details of pre- and post-surveying considerations required for marine monitoring activities when planning swath mapping surveys. This will include surveys required for both broad scale mapping to inform the development of habitat maps, and those being conducted as a component of monitoring. Further details of marine sampling platforms used to ground truth acoustic data, and to monitor of ecological indicators are presented in the accompanying NESP field manuals (Chapters 4-9).



Figure 3.1. a) Multibeam transducer mounted on the hull of a ship in the (b) gondola. c) Multibeam acoustic bathymetry image c) coincident backscatter image and d) interpreted geomorphology map. (reference: Watson et al., 2017).

3.2 Scope

This manual refers to the use of multibeam or interferometric echosounders (referred herein as just multibeam or MBES) to conduct surveys of seafloor bathymetry and backscatter that can be used to derive maps of geomorphic features and habitats. It does not mandate use of a specific multibeam acoustic system (either an interferometric³ or beamforming⁴ multibeam). The examples given herein refer to Kongsberg systems merely as an exemplar of the procedure to be conducted. Similarities can be drawn from these examples to any particular MBES system being employed on the survey.

There are a number of multibeam echosounders that have been commonly used for surveying in Australian waters that would be suited to marine monitoring activities. It is important that the surveyor be mindful that there are differences in the way bathymetric measurements are made from both interferometric and beamforming multibeam echosounders, and these influence the scale and resolution of features being detected and the fidelity of the acoustic measurements (which is important for monitoring). The main difference is namely due to beam formers measuring range for each of a set of angles, and interferometers measuring angle for each of a set of ranges (Table 5). We have outlined the standard methods that are relevant to any of these systems to provide a framework to create a nationally consistent multibeam data archive for Australian marine and coastal waters.



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This field manual details the specific planning, acquisition, processing and reporting considerations that are required to meet the seafloor surveying needs of monitoring programs. Although MBES can be used for water column data collection, these data are outside the scope of this standard operating procedural document in its current version (Version 0.1). This document provides guidance to organisations responsible for permitting and supporting research programs (e.g. Parks Australia) to collect multibeam data for monitoring programs (e.g. government research agencies, universities) to ensure consistency in acquisition and processing of multibeam acoustic data. This will increase the chance that data and spatial data products from different organisations and swath systems can be combined and reused into the future and become a valuable data asset for national research objectives; ongoing monitoring and planning. This manual is subset by four main phases of a seabed mapping survey as outlined in Figure 3.2:

- 1. Data acquisition;
- 2. Data processing;
- 3. Benthic classification (data interpretation), and
- 4. Accuracy assessment and reporting (including metadata management of spatial data products).



Figure 3.2. Workflow from MBES survey design to spatial data products and reporting



Table 3.1. Comparison of bathymetric systems (reference: Bathyswath.com)

Parameter / Function	Interferometric Multibeam	Beamforming Multibeam	Notes
Number of depth measurements	6000+	60-120	Depends on range
Range vs. water depth	10 - 20	3-5	Beam former footprint becomes unacceptably large at far range.
Amplification / processing channels	4-5	60 +	In a harsh environment, simplicity is important
Outboard transducer electronics	Passive	Active	The outboard component of an interferometer is extremely robust, and cheaper to replace if damage does occur
Outboard transducer size and weight	350x160x60mm 5 kg (air)	120x190x450mm 16 kg (air)	Dimensions for a common portable beam former. Many beam formers are much larger.
Horizontal resolution at range	Good	Poor	Beam former footprint becomes unacceptably large at far range.
Angular coverage	260°(including 20° overlap)	90°- 180° (or beyond 180° using a dual head system)	
Co-incident sidescan	True	Partial	An interferometer collects amplitude in the

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			same way as its bathymetry: as a time- series.
Profile data density	Increases with reducing grazing angle	Decreases with reducing grazing angle	Higher complete profile data confidence with an interferometer.
Ability to resolve several targets at the same range	No	Yes	
Ability to resolve several targets at the same angle	Yes	No	
Profile data density	Increases with reducing grazing angle	Decreases with reducing grazing angle	In the first 5 m of horizontal range, a beam former collects slightly more depth samples. Beyond that, an interferometer collects many more.
Capacity to acquire water column information?	Yes	Yes	Interferometric systems can identify targets in the water column but are unable to characterise them accurately due to lack of beam forming angles to locate the target.



3.3 Multibeam Acoustics for Marine Monitoring

The use of MBES for mapping and monitoring marine habitats has experienced a rapid increase since 2000 (Figure 3.3), and there is now a wealth of knowledge from which we can synthesise a 'best practices' document.



Figure 3.3. Annual total of peer reviewed papers featuring multibeam mapping for seafloor survey (Web of Science 2017-search words "multibeam seafloor habitat survey").

The **objectives** of multibeam acoustic surveys conducted by mapping programs are to collect seafloor data to identify, delineate and map biogenic, anthropogenic and geological features. This objective requires particular data to be collected that can a) chart the water depths creating a high resolution bathymetric map at an appropriate resolution in regards to the target habitat or feature and b) be able to differentiate boundaries between different substrate and/or habitat types.

To meet these objectives, there are two particular needs for mapping and surveying that can be defined as either baseline surveys or monitoring surveys (see Table 2). MBES can be used for both survey types, however, they have different acquisition and post-processing standards. A **baseline survey** is for exploratory purposes where data will be collected in a 'single pass operation'. This data is used to map the distribution of marine habitats at a particular spatial scale, and provide information necessary for more targeted field surveys using such tools as towed video, AUVs and stereo baited remote underwater video stations (BRUVs) (Lucieer et al. 2013, Monk et al. 2016). In contrast, a **monitoring survey** may have already identified target habitats or features (such as rocky outcrops) from previous broad scale or other hydrographic data that are to be monitored to assess change in distribution and extent (Rattray et al. 2009, McGonigle et al. 2010). This type of survey will require acoustic data to be collected at a higher resolution and with a greater degree of positional accuracy. Mapping for baseline survey and monitoring surveys will be dealt with separately throughout the manual, with their differences and the requirements that need to be considered to meet the aims of each survey type outlined in Figure 3.4.





Figure 3.4. Decision tree for seabed classification survey design (adapted from Anderson et al. (2007)).



Table 3.2 Standard Operating Procedures identified according to survey purpose: Baseline or Monitoring

Specification	NESP Baseline	NESP Monitoring
Purpose	 Used to identify seafloor habitats and potential biodiversity hotspots. Used for discovery purposes in regions that have had no baseline mapping conducted. 	 Used to ensure spatio-temporal assessment of the seabed and habitat. The survey accuracy standard is very high to ensure reproducibility over time. Used for repeat mapping and for targeting key habitats for monitoring purposes.
Pre survey preparation	 The coverage of the area to be surveyed (bounding box) with the datum and coordinate system clearly identified. Establishment of line spacing Determination of the system offsets and calibration area (patch test) area to be conducted as soon as practical and after system is completely set up ready for survey. The location and scheduling of the Sound Velocity Profiles 	 In addition to baseline survey specifications: Synthesis of all pre-existing survey data into survey region database Identification of locations of seafloor targets to be monitored Establishment of line spacing with min of 60% overlap.
Installation Offsets	Provide Mobilisation Calibration Reports and logs	Provide Mobilisation Calibration Reports and logs
Data Logging	 Bathy: Mandated Seabed Backscatter: Mandated Water column backscatter: Recommended (if available) 	 Bathy: Mandated Seabed Backscatter: Mandated Water column backscatter: Mandated (if available)
Acquisition setting	Mode: Equidistant mode where system allowsMinimise setting changes to optimise backscatter	• same
Sound Velocity Profiles	 Min of 1 per day, but should be monitored. If sound speed at the transducer varies by > 2m/s another SVP should be collected 	 Min of 2 per day (beginning and end of survey), but should be monitored. If sound speed at the transducer varies by > 1m/s another SVP should be collected
Geodetic Parameters	• GDA2020. Horizontal accuracy: 5m + 5% of water depth. Vertical accuracy: 1% water depth	• GDA2020 Horizontal accuracy: absolute positioning to be at< 2 or less. Vertical accuracy: < 1m
Survey Speed	 Recommended 6 knots (or at survey speed appropriate to capture resolution required) 	 Recommended 5 knots (or at survey speed appropriate to capture resolution required)
Mapping Coverage	• 100% Coverage with 30% overlap between survey lines of data with an 80% confidence level.	• 100% coverage with 60% overlap between survey lines of data with an 80% confidence level.
Resolution	• 1 m resolution in < 50m depth ; 5% of depth beyond 50 m	• 1 m resolution



ine Sampling Field Manua	Is for Monitoring Australia's Commonwealth Waters Version 1	_
Tides and GPS Tide	• Record GPS tides. All soundings shall be reduced to the ellipsoid.	• Record GPS tides. All soundings shall be reduced to the ellipsoid.
Point data attribution	• All data should be attributed with its uncertainty estimate at the 80% confidence level for both position and, if relevant, depth.	• All data should be attributed with its uncertainty estimate at the 95% confidence level for both position and, if relevant, depth.
Metadata and Reports	• Metadata report shall include heading and data deliverables outlined in this manual. Calibration Report and Report of Survey.	 Metadata report shall include heading and data deliverables outlined in this manual. Calibration Report and Report of Survey.
Archiving	 Australian Online Data Network (AODN) data portal. National MBES Data Centre 	Australian Online Data Network (AODN) data portal.National MBES Data Centre
Purpose	 Used to identify seafloor habitats and potential biodiversity hotspots. Used for discovery purposes in regions that have had no baseline mapping conducted. 	 Used to ensure spatio-temporal assessment of the seabed and habitat. The standard is very high to ensure reproducibility over time. Used for repeat mapping and for targeting key habitats for monitoring purposes.
Pre survey preparation	 The coverage of the area to be surveyed (bounding box) with the datum and coordinate system clearly identified. Establishment of line spacing Determination of the system calibration (patch test) area to be conducted as soon as practical and after system is completely set up ready for survey. The location and scheduling of the Sound Velocity Profiles 	 In addition to baseline survey specifications: Synthesis of all pre-existing survey data into survey region file Identification of seafloor targets for monitoring Establishment of line spacing with min of 60% overlap.
Installation Offsets	Provide Mobilisation Calibration Reports and logs	Provide Mobilisation Calibration Reports and logs
Data Logging	 Bathy: Mandated Seabed Backscatter: Mandated Water column backscatter: Recommended 	 Bathy: Mandated Seabed Backscatter: Mandated Water column backscatter: Mandated
Sound Velocity Profiles	 Min of 1 per day, but should be monitored. If sound speed at the transducer varies by > 2m/s another SVP should be collected 	 Min of 2 per day (beginning and end of survey), but should be monitored. If sound speed at the transducer varies by > 2m/s another SVP should be collected
Geodetic Parameters	• 84ITRF with epoch reference. Horizontal accuracy: 5m + 5% of water depth. Vertical accuracy: 1% water depth	 84ITRF with epoch reference Horizontal accuracy: absolute positioning to be at< 21 m or less. Vertical accuracy: < 1m
Survey Speed	• 6 knots	• 5 knots



Marine Sampling Field Manua	Is for Monitoring Australia's Commonwealth Waters Version 1	
Mapping Coverage	• 100% Coverage with 30% overlap between survey lines	• 100% coverage with 100% overlap between survey lines
Resolution	• 1 m resolution in < 50m depth ; 5% of depth beyond 50 m	 1 m resolution (may require AUV or towed body in water depths >200 m)
Tides and GPS Tide	• Record GPS tides. All soundings shall be reduced to the ellipsoid.	Record GPS tides. All soundings shall be reduced to the ellipsoid.
Point data attribution	• All data should be attributed with its uncertainty estimate at the 80% confidence level for both position and, if relevant, depth.	 All data should be attributed with its uncertainty estimate at the 95% confidence level for both position and, if relevant, depth.
Metadata and Reports	 Metadata report shall include heading and data deliverables outlined in this manual. Calibration Report and Report of Survey. 	 Metadata report shall include heading and data deliverables outlined in this manual. Calibration Report and Report of Survey.
Archiving	 Australian Oecan Data Network (AODN) data portal. National Data Centre 	 Australian Ocean Data Network (AODN) data portal. National Data Centre



3.4 **Pre-Survey Preparations**

There are a number of important factors that first need to be considered in order to ensure relevant areas are surveyed, time and costs can be accurately estimated, appropriate vessels and acquisition gear is used, and previous survey data is considered.

Firstly, it is important that all spatial data for the survey region must be sourced to gain a preliminary understanding of the seabed as this will influence several survey considerations. This information can be used to create a *survey plan*, which would include a summary of the following components:

- The coverage of the area to be surveyed (bounding box) with the datum and coordinate system clearly identified,
- Planned survey lines (direction and acquisition order of the survey lines),
- System calibration survey lines (patch test),
- Seafloor topography (features of interest) and slope, and
- The location and frequency of the Sound Velocity Profile (SVP).

Background spatial data might include electronic nautical charts from the Australian Hydrographic Office (AHO), aerial photos (in shallow water regions), LiDAR or satellite derived bathymetry data. It may also include previous maps of seabed habitats generated from single beam acoustic surveys or maps of sediment distribution from broad scale seafloor grab or dredge surveys. Information on seabed habitats can also be collected by analysing the distribution of other activities conducted within the survey region (for example, ancillary research such as fisheries surveys may be an indicator of habitat type).

The survey plan will aim to establish the range of water depths and seafloor complexity across the survey region. The range of water depths will define how many survey lines need to be conducted to ensure sufficient overlap between the acoustic swaths to ensure 100 % seafloor coverage (refer to Chapter 2 where coverage may relate to selected sampling sites). Where the water depth is relatively constant (such as on the outer continental shelf), the survey plan may provide adequate structure for accurate planning. In shallower waters, where the depths may change rapidly (or are unknown to the resolution of national satellite derived products) a comprehensive plan of survey lines may not be useful, as they will need to be modified as the bathymetric data is collected. In this case, a defined survey area boundary (polygon) with an initial survey line for calibration may be sufficient.

An essential component of the survey-planning phase is the need to obtain the relevant permits that may apply for sediment data collection which is common for MBES data validation, especially when conducted within marine parks. See Appendix B for a list of potential permits needed.

Following the establishment of the survey plan the logistical preparations for data acquisition can be conducted. These are outlined in the following sections and recorded in the vessel or field logbook over the duration of the survey and made available in the final reporting documentation.



3.5 Data Acquisition

3.5.1 Installation offsets

The spatial relationship between all of the sensors in an MBES system (GPS, transducer, motion reference unit etc.) with the vessel's frame of reference is paramount to obtaining high resolution and accurate data. The vessel's Central Reference Point (CRP) is determined upon each *new* installation of a MBES system and to best suit the vessels balance, and installation criteria (if the MBES is hull or pole mounted) (Edward and Martin 2015). The CRP is defined, as an example, within the Kongsberg operating systems Seapath software along with the installation offsets for the Global Navigation Satellite System and Motion Reference Unit. The offset of the MBES transducer is defined within the Kongsberg Seafloor Information System (SIS) software. Where possible the CRP should be defined at the MBES transducer directly. All installation offsets are required to be recorded and detailed within the survey report and processing log of the supplied raw data files.

To ensure that the depth charted is the true depth and not depth under keel, the vessel draft must be taken into consideration during data acquisition (likely at the start and end of a survey) to account for changes in draft due to foe example (fuel usage) although this will depend on the model of the vessel used for survey. Although this manual recommends that depths be provided in relation to the ellipsoid, to enable other users to reduce data to chart datum, vessel draft should be measured at the start and end of surveys and dynamic draft taken into consideration with measurements of the waterline conducted regularly. The vessel draft is recorded during a survey in the vessel log and/or entered in the acquisition system. For further information see section 2.4 of Australia's Multibeam Guideline (Version 0.1).

3.5.2 Data logging

During a survey with a MBES system there are a number of data products that should be recorded. These include:

- 1. Raw data: Always log raw proprietary format for all type of data (multibeam echosounder or ancillary systems). Raw positional data and motion datagrams are to be recorded at a rate of 1Hz and 100Hz respectively. These datagrams are logged to the raw sonar file.
- Raw sonar data are recorded in the native/proprietary format of the multibeam system used (e.g. *.all for Kongsberg, *.s7k for Reson) and the ancillary data. Log complete backscatter i.e. beam intensity (RI and snippets or equivalent. Files are recorded for a duration of 30 minutes for shallow systems (< 150 m) and 120 minutes for deep systems (> 150 m) to account for computer processing speed.
- 3. Water column data [Recommended, if available]: Water column datagrams are logged to a separate file in proprietary format (e.g. *.wcd for Kongsberg). These files can take up a large amount of storage space (~ 10 times raw bathymetry), and the surveyor must ensure necessary disc space prior to collection.
- 4. File naming convention: It is important that the surveyor adhere to a consistent and acceptable naming convention that links to the metadata of the raw data format. Raw sonar files in proprietary format recorded by an acquisition software (e.g. SIS for Kongsberg) have the following naming convention.
 [Name, wavymmdd, bhmmss, Vasselname, system Extension] [Survey line, year, month, day.

[Nnnn_yyyymmdd_hhmmss_Vesselname_system.Extension] [Survey line, year, month, day, hour, minute, second, vessel name, multibeam acoustic system. proprietary format extension (e.g.all for Kongsberg and s7k for Reson]. Survey lines are organised by Julian



day within the processing software but are acquired sequentially as line IDs throughout the survey. Where two separate systems are being operated at the same time on the same vessel, they can be distinguished by the system name of the MBES in the file name. If a new survey is to be created within the acquisition software during a survey the line number should be reset to the last number used +1.

- 5. Filters and settings: Both noise and spike filters should be monitored during the survey to ensure the data quality and integrity is maintained over the course of the survey. Beam spacing mode should be set to equidistant. It is very important that the pulse length should not be changed at any time during the survey so that all data are standardised.
- 6. Delayed heave: delayed heave datagrams are recorded by the acquisition computer and logged to files in the proprietary format.

3.5.3 Sound velocity profiles

A sound velocity profile (SVP) measures the speed of sound in water at different vertical levels in the water column and this data can be used to accurately form the beam of the sound. Some multibeam systems have a SVP sensor built onto the head of the transducer but for others that do not, it is important that a SVP sensor be deployed to collect this information.

Why are sound velocity profiles so important?

A MBES system emits a sound pulse in an arc out from the transducer. As the sounds contacts the seabed, it is reflected/backscattered back towards the transducer and received. Each backscattered pulse from each individual seabed point can be considered a discrete beam. The speed of the beam through the water column is governed by the water temperature and density. Because the water column, in most cases, is not evenly mixed, the speed of the pulse changes at different levels in the water column. At each change in speed, refraction or 'bending of the pulse path' occurs, unless the angle of incidence is equal to 90 degrees, as with a single beam echosounder. Refraction can happen many times throughout the pulse's path through the water column. Therefore, to enable best ray tracing possible and consequently depth conversion of each soundings, details of the water column sound profile are essential. Depending on the location of the survey and the conditions over the area (sea state, mixing regime, thermal layering etc.) of the survey, sound velocity profiles (SVP) should be conducted at the appropriate intervals or location. The SVP can be determined using one of the following four methods:

- Direct observation via deployment of a SVP measuring device (e.g. Valeport monitor)
- Calculation of SVP through deployment of an eXpendable Bathy Thermograph (XBT)
- Calculation of SVP using CTD (Conductivity/Temperature/Depth) data and applying the UNESCO formula (https://www.usna.edu/Users/physics/ejtuchol/documents/SP411/Chapter4.pdf) or;
- Calculation of SVP from Sea Surface Temperature and Climatology using SVP builder software (Sinquin et al. 2016).

How are SVPs applied to multibeam surveys?

A sound velocity profile (SVP) must be taken *within* the survey area at least once at the beginning of the survey and once at the end for monitoring surveys. In some areas, multiple SVPs should be taken. For example, profiles will vary due to freshwater inflows from rivers or currents from areas with different salinity e.g. proximity to an estuary. Surface sound speed variation may also be strongly affected also by solar warming. If variations can be expected, where and when the SVPs are to be taken must be carefully planned, and the survey line schedule adjusted to consider this.



Sound speed data is used in the following ways:

- correction for the fact that the transducer staves are the wrong spacing in wavelengths;
- correcting for the change in total sound path length because of the speed of sound variation, but ignoring refraction and;
- correcting for both refraction and sound path length.

Path length correction uses the speed of sound to determine the sonar path length from the time the ping is transmitted to the time it is received. The average speed of sound within the speed of sound profile is used for this, measured from the depth of the transducers to the depth of the seabed.

3.5.4 Geodetic parameters

The datum parameters entered into the acquisition software will use the Global Navigation Satellite System (GNSS) datum for example WGS84 (Table 3.2). Any datum shifts will need to be applied at the post processing stage. The use of differential GPS as a positioning system is required for all on-shelf and off-shelf multibeam acoustic data collection as we aim to resolve an absolute positional accuracy greater than 1 m. All positioning data should be provided as track plots (in x, y format) to enable interpretation of the vessel transits.

3.5.5 Survey speed

The speed of the vessel will have a direct impact on the density of soundings reaching the seafloor, the quality of the data (in the return signal) and to some degree determine the resolution of the final raster datasets (as it dictates the distance along track between pings). Depending on the type of vessel employed for the surveying, the survey speed must be kept constant and between 5-6 knots (11 - 14 km/h). The distance between pings along the track of the vessel is determined by the pulse repetition frequency (PRF) and ship speed; the faster the vessel, the fewer pulses ensonify the seafloor per distance along track. Aeration problems (bubble sweep) is a function of sea state but also of the heading with respect to the wave direction and the vessel speed. Aeration problems reduce the signal or the quality of the signal at the transducer head.

It is strongly advised that the surveyor creates a record of aeration problems versus sea state with respect to heading and vessel speed. This record will be helpful in ensuring that the survey is performed efficiently with a minimum of line rejections and corresponding reruns and infills. This should be recorded in the field log book.

3.5.6 Line spacing

Line spacing is the distance between adjacent survey lines. The best spacing between survey lines is determined by a combination of horizontal range limit (sonar coverage from one transducer) expected at that depth of water and the accuracy required from the survey (either baseline mapping or monitoring). The horizontal range expected depends on the water depth as well as the sea state, seabed type and the sonar frequency. If the surveyor is using two transducer heads, the total swath width from the port edge to the starboard edge is twice this range.

The horizontal range is limited by two factors: grazing angle and spreading loss. The grazing angle limit is related to the angle that the sound "beam" makes with the seabed. At the grazing angle limit, the sound makes a very small angle with the seabed. Most of the sound at this point is reflected away and the signal scattered back from the seabed is too small to be detected.



Due to this loss of signal on the outer beams of the swath, some overlap in swath is required. A minimum of 30% overlap should take into account line keeping errors and where sea state is calm and create a 100% coverage of the seafloor. The type of survey being undertaken will determine the overlap with the highest quality requiring 100% overlap and the lowest quality requiring 30% overlap (Table 3.2).

The seafloor topography and the slope (gradient of the slope) is an important consideration for planning the survey lines. For MBES data collection, it is strongly advised to <u>run the lines parallel to</u> the seabed contours (along the slope, not up or down the slope). This is beneficial for keeping the coverage reasonably constant along the survey lines (as the swath width will vary with depth). It is also beneficial because less acoustic energy is reflected towards the transducer from steep slopes, causing poorer detections and the possibility of false detections in the sidelobes⁵. If survey lines must run up and down the slope, a reduction of vessel speed or reduction in swath width may be required to allow for the echo sounder to track the bottom continuously. Planned lines must be activated in this instance to ensure that gaps are not created between the survey lines as the swath coverage is reduced coming into shallow water and additional lines may need to be added.

For surveys where backscatter information is critical, the overlapping area should be increased (from 60% to 100%) to compensate for the high variability of individual backscatter intensities on the edges of the outer beam (Gavrilov & Parnum, 2010). For surveys where backscatter information is considered a secondary product, it is recommended that the overlapping be kept as minimal as practical (30% overlap).

3.5.7 Pulse length

The pulse length affects the amount of the transmitted acoustic energy into the water and the vertical resolution of the observed depth. Increasing pulse length enhances penetration through the water column but reduces vertical resolution. Kongsberg systems have limited, pre-defined options for pulse length which may be synonymous with other software packages. Therefore, the selection used may compromise the quality of backscatter data in order to meet the objectives of the survey.

Pulse length and sampling frequency must be considered as related to backscatter data. The sampling frequency of the system must be considered in order to hold the Nyquist-Shannon sampling theorem⁶. This enables the analogue signal to be reconstructed from the digital data (e.g. Kongsberg EM3002 systems recommended minimum pulse length of 100µsec or greater).

3.5.8 Tides and GPS tides

MBES data shall be corrected in real time for draft and tide variations as well as attitude input (roll, pitch, yaw and latency) via the vessel's Motion Reference Unit (MRU). All soundings shall be reduced to an ellipsoid with a minimum depth accuracy of 0.2% relative to water depth.

3.5.9 Data type

Bathymetry and backscatter datasets shall be processed and plotted onboard to monitor the coverage and data quality. This will allow for additional acoustic to be collected prior to the finalisation of the survey, in the event of data gaps between survey lines for example. Processing will be carried out to create full coverage bathymetric maps with contours, slope values and backscatter images. Onboard processed products shall include the following:



Bathymetry

- All raw bathymetric data is to be provided in proprietary format.
- Processed data is to be provided as a point cloud text file (or .csv) in UTM and depth (with depth value as negative) with uncertainties attached to each sounding.
- Processed data shall also be provided as gridded data in formats csv, ARC GIS Grid. ESRI ASCII *.asc format in UTM format.
- The requirement for gridding interval of MBES data is 1 m or better in shallow water (<100 m), 5 m in deeper water (100 200 m) and 10 m off the shelf (>200 m).
- Bathymetric charts shall be displayed with the smallest contour interval representative of the seafloor morphology.
- Any smoothing of contour lines is to be kept to a minimum.
- As a QA product, two images of the gridded processed bathymetry data should be provided with sun-illumination from two orthogonal directions and 5 times exaggeration

Backscatter

- All raw backscatter data is to be logged in proprietary format.
- Processed data is to be provided as a text file (or .csv) showing latitude, longitude and intensity (dB) (or provided in a format that is able to be converted to comma delimited csv files).
- Processed backscatter data is preferred as xyz ASCII comma delimited (XY in UTM zones; z in dB with 2 decimal places), and/or ESRI ASCII *.asc (values in dB) (Buchanan et al. 2013).

3.6 Data Processing

The data acquisition parameters are established to ensure that the data are fit for the purposes of benthic habitat mapping (i.e. baseline) and monitoring of Australia's waters. The post processing parameters and techniques, on the other hand, are generally optimised for a targeted purpose (and differ for a baseline or monitoring survey). There are a number of software packages available for processing MBES data and many of the software are in commercial proprietary to the specific multibeam system used for acquisition.

Regardless of the data processing software that is used (e.g. CARIS), the goal is for the minimum final products to be released at the completion of a field survey (summarised in Table 3.3):

- Bathymetric surface as both an x,z,y point surface and a raster x,y,z to the appropriate resolution requested (Table 3.2);
- Vessel transect log map to show the position of the vessel survey lines within the region;
- Map showing the location of field validation data (e.g. point map of where sediment grabs or video transects have been conducted etc) [Recommended];
- Digital terrain models with hill-shading of the bathymetry from two orthogonal direction and 5 time exaggeration to easily identify artefacts of the dataset remaining, but also to identify key geomorphological features (including slope map) [Recommended];
- Backscatter mosaic (both raw and processed) in geotiff format in the optimal resolution from the snippet and at 1 m from the average beam values;



- Water column backscatter (display of water column acoustic anomalies and x,y,z location of features detected in the water column) [*Recommended, if available*]; and
- 3D perspective videos of significant findings or seabed features (abrupt changes in relief, shipwrecks, canyon head steps etc.) [Recommended].

3.6.1 Bathymetric data processing

Uncertainty related to the bathymetric (depth) measurements can be quantified and incorporated into a statistical model to derive the total propagated uncertainty (TPU) of the resulting bathymetric surfaces. A number of factors will influence this uncertainty including: draft setting of the transducer, incorrect sound velocity profiles, spatial variation in the sound velocity, temporal variation in the sound velocity, instrumental uncertainty (internal precision of the MBES unit) and motion (incorrect heave, pitch and roll corrections), settlement and squat of the vessel in the water and incorrect tidal corrections to name a few.

Where possible the CUBE (Combined Uncertainty and Bathymetry Estimator) should be used to calculate the TPU for the bathymetric surface as a measure of uncertainty in the survey. CUBE uses soundings and their associated uncertainty estimates as input and through spatial and uncertainty weighting, while also relying on the very high data density of multibeam data sets, outputs a bathymetry gridded surface and its associated uncertainty (error) surface. In addition, it tracks the statistical hypotheses for each depth point, and where there is more than one estimate, makes an attempt to determine which the most likely value is. This makes it a very powerful tool for identifying and removing outliers in the data. Once these have been removed from the data, CUBE is rerun to generate the final bathymetry and uncertainty surfaces. See "CUBE Bathymetric data Processing and Analysis (CHS February 2012)". The uncertainty surface is a quantification of the survey quality, which can be compared against specifications and used as input to the metadata for the survey (CHS 2013).

3.6.2 Backscatter data processing

A final compensated Geotiff mosaic of the acoustic backscatter for the survey region should be generated. We refer to the Lurton, X.; Lamarche, G. (2015) Backscatter measurements by seafloor-mapping sonars. Guidelines and Recommendations. Geohab Report. 150p, for optimal processing procedures for MBES backscatter image generation.

3.7 Data Interpretation

MBES bathymetric data will be processed to characterise and classify the seafloor in terms relevant to the distribution of benthic habitats and to help in the understanding of the spatial and temporal distribution of marine habitats. The combination of topography (bathymetry) and textural surfaces (backscatter) provide an excellent reference dataset for research and management of Australian marine seafloor habitats.

Geomorphological analysis can be used to classify the multibeam bathymetry data and define the extents of particular habitat types such as seagrass beds, rocky reef, and sand plains. We recommend the use of the national standardised benthic habitat classification nomenclature as documented by Seamap Australia (Butler et al. 2017). Importantly, this classification system includes other established and developing national classification schema such as CATAMI (Althaus et al. 2015) and Geoscience Australia's *Classification and Glossary of Seabed Geomorphology*.



The backscatter Geotiff can be interpreted into a sediment distribution and habitat map using one of two automated segmentation methods:

- 1. Image-based segmentation (e.g., using e-Cognition (<u>www.ecognition.com</u>)) where the image is segmented into regions of similar backscatter characteristics and using the bathymetric data to identify these boundaries and transition zone. These segments are then classified as surface features, backscatter intensity patterns of sediment/habitat distribution etc.
- 2. Signal based segmentation (e.g., using ENVI (<u>www.esriaustralia.com.au/envi</u>) where changes in the backscatter intensity, with increasing grazing angle from nadir, are analysed to classify the data.



Table 3.3 Expected data deliverables for a baseline mapping or monitoring survey to accompany metadata reporting

Deliverable item	Comment
Raw sonar data	Raw sonar data in native format as created directly from the native acquisition system of the multibeam system used. e.g. *.all for Kongsberg EM series, *.s7k for newer version of Reson SeaBat or *.xtf for the older one Data format: native format as produced by the acquisition system, except for the *.xtf Datagram: all logged automatically for Kongsberg EM series. For Reson SeaBat , datagrams with the following IDs are required: 1003, 1012, 1013, 7000, 7001, 7002, 7004, 7006, 7005, 7007, 7012, 7022, 7028, 7200, 7504 The water column data, recorded as separate files, for both Kongsberg and Reson are only required on special request in survey planning. For all other multibeam systems, it is required that raw data include SV profile, attitude, navigation, heading, raw bathymetry, raw backscatter per beam and if available raw backscatter in time series i.e. the equivalent seabed image or spippet style
Processed sonar data	Processed multibeam bathymetry data, including processed multibeam backscatter data, if requested Preferred format: Caris HIPS & SIPS project structure including processed bathymetry surface (see processed bathymetry grids below) (*.csar and XYZ) and time series-generated backscatter mosaic (*.csar) in Fieldsheets subfolder, processed line data & geobar in HDCS_Data subfolder, tide data used (*.tid) in Tide folder, individual sound velocity profiles (*.csv) used together with additional information on time and location of the cast in SVP subfolder. Backscatter mosaic and geobar are only required on request Alternative format: Processed line: SAIC GSF (*.gsf) if no other alternative
True Heave	Delayed, processed heave saved independently from raw sonar file, logged in 600-720 minutes period Data format: Applanix ATH or equivalent (Caris compatible)
Processed	Processed multibeam bathymetry surface grid
bathymetry grids	Data format: CSAR and xyz ASCII comma delimited (XY in specified UTM; z in negative metre at 2 decimal places) and/or ESRI ASCII *.asc (values in meter).
Processed	Processed multibeam time series-generated backscatter mosaic
backscatter mosaic	Data format: xyz ASCII comma delimited (XY in specified UTM; z in dB at 2 decimal places) and/or ESRI ASCII *.asc (values in dB).
Tide	Tide data used for tide correction (date, time and depth(m.mm)/pressure (dBar) Data format: Caris tide *.tid or ASCII *.csv
Sound velocity profile	Sound velocity casts used in SIS or equivalent acquisition system together Data format: ASCII *.csv
Log file (SVP cast)	SVP cast info (date, time, depth of cast and seafloor, location and line applied to) Data format: ASCII text
TPU/ CUBE related information	 XYZ of MRU to Transducers XYZ of NAV to Transducers Transducers mounting angles (if not horizontal) Type of Navigation system Type of MRU system Sign conventions used to calculate XYZ (Down positive etc) Data format: ASCII text



3.8 Data Release

At the time of writing this manual, there is not currently a complete repository for multibeam data collected in Australian waters, although several agencies house some multibeam data (e.g. AHO, GA, IMOS, CSIRO), and several portals promote its accessibility and visualisation (e.g. <u>seamapaustralia.org</u>). Initiatives are underway for a single repository to be linked to appropriate visualisation platforms, and this is expected to be addressed in Version 2 of this field manual.

In the meantime following the steps listed below will ensure timely release of data and maximise data discoverability:

- Create metadata record(s) describing the survey and data collection (for both raw and QA/QC data products). Minimum metadata requirements for multibeam data include the following:
 - Title of the survey region (e.g., AMP name and ID) and, if not a well-established region, its geographic boundary;
 - Surveyor's name and company;
 - Start and end dates of the survey;
 - Vessel name, type of vessel and MBES unit used, details regarding the positioning system, acquisition software, and operation parameters;
 - The number of lines recorded and corresponding number of kilometres; and
 - Summary of the main survey results (water depths, observed tidal range, sonar features of interest- anomalies, unusual targets etc.).
- 2. Publish metadata record(s) to the <u>Australian Ocean Data Network (AODN) catalogue</u> as soon as possible after metadata has been QC-d. This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the <u>AODN Data</u> <u>Submission Tool at https://metadataentry.aodn.org.au/submit</u>. Note that user registration is required, but this is free and immediate.
- 3. Generate interactive map imagery of the following derived data layers:
 - Location map with limits of the survey area;
 - Bathymetric map showing the depths, slope and bathymetric hill shading results;
 - Backscatter data map showing boundaries between habitat features;
 - Location of auxiliary data sampling (point features of sediment grabs) or transect lines of video surveys; and
 - Map showing the track plot of the vessel position, indicating the region of the patch test calibration.
- Upload raw multibeam data files and all field logs generated during the survey to a secure, publicly accessible online repository (<u>contact AODN</u> if you require assistance in locating a suitable repository).



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- 5. Add links to the location of raw data and derived map imagery to the previously published metadata record. Metadata accompanied by map imagery as described above may be additionally showcased through the <u>Australian Ocean Data Network portal</u>.
- 6. Produce a technical or post-survey report documenting the purpose of the survey, sampling locations, sampling equipment specifications etc. Provide links to this report in all associated metadata records [*Recommended*].

3.9 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 3 (field manual for MBES) is below:

Version Number	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019

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³ An interferometric multibeam measures the angle of the incoming sound wave fronts in a time sequence of samples. Slant range is obtained from the time of the sample and speed of sound.

⁴ A beamforming multibeam mathematically forms a set of "beams", and detects the range to the seabed in each beam.

⁵ The sidelobes are smaller beams that are away from the main beam. These sidelobes represent energy received in undesired directions which can never be completely eliminated.

⁶ In the field of digital signal processing, the sampling theorem is a fundamental bridge between continuous-time signals (often called "analog signals") and discrete-time signals (often called "digital signals"). It establishes a sufficient condition for a sample rate that permits a discrete sequence of samples to capture all the information from a continuous-time signal of finite bandwidth.





National Environmental Science Programme

4. MARINE SAMPLING FIELD MANUAL FOR AUVS (AUTONOMOUS UNDERWATER VEHICLES)

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4.1 Platform Description

Autonomous Underwater Vehicles (AUVs) are untethered robotic platforms that operate independently to complete pre-determined surveys. The endurance of AUVs typically range from hours to several days (Huvenne et al. 2018). However, with the rapid development of battery technology long-period deployments ranging from weeks to months are now possible (Furlong et al. 2012; Hobson et al. 2012). Maximum operational depths range from a few hundred metres for the smaller vehicles (Wynn et al. 2014) to over 6000 m for larger units (Huvenne et al. 2009).

Huvenne et al. (2018) classify AUVs as either "cruising" or "hovering" vehicles (Figure 4.1). Cruising AUVs are traditionally torpedo-shaped, driven by a single propeller at speeds up to 2 ms⁻¹, and are optimised to cover large distances along pre-designed survey tracks (Wynn et al. 2014). These cruising AUVs are usually not well suited to photographically surveying high-relief seabed terrain due their lack of vertical agility. Traditionally, cruising AUVs are the main type of AUVs used in the commercial world, with prominent scientific examples including the Autosub series from the National Oceanography Centre (UK), the AsterX and IdefiX from French Research Institute for Exploitation of the Sea (IFREMER; France) and the Dorado series from Monterey Bay Aquarium Research Institute (USA) (Furlong et al. 2012; Rigaud 2007). By contrast, hovering AUVs are equipped with several propellers, which facilitate multi-directional manoeuvrability capabilities, similar to a remotely operated vehicle (ROV). Hovering AUVs are designed for precision operations, slow motion surveys (e.g. seabed photography) and work in distinctly 3-dimensional terrains, such as around high-relief reefs (Williams et al. 2012). Among the best-known scientific examples of hovering AUVs are ABE and Sentry from Woods Hole Oceanographic Institute (USA) (e.g. Tivey et al. 1998; Wagner et al. 2013) and Sirius from Australian Centre for Field Robotics (Australia) (e.g. Bewley et al. 2015; Williams et al. 2016; Williams et al. 2012).

Depending on the size of an AUV they can be equipped with a range of sensors such as conductivity, temperature, depth, acoustic doppler current profilers, chemical sensors, photo cameras, sonars, magnetometers and gravimeters (Connelly et al. 2012; Sumner et al. 2013; Williams et al. 2010). Importantly, on-board battery capacity is the primary limitation to the number of sensors and survey duration for AUVs. Furthermore, AUVs are currently not yet equipped for extensive physical sampling of seabed or fauna, although sampling of the water column can be achieved (Pennington et al. 2016). Overall, AUVs are more suited for survey operations, acquiring sensor data along pre-programmed transects, while ROVs are optimal for high-resolution, highly detailed and interactive work, including high-definition video surveying and physical sampling. An extensive review of the use and capabilities of AUVs for geological research was recently published by Wynn et al. (2014). There is, however, no equivalent review discussing the capabilities of AUVs for ecological research (but see section 3.3 in Wynn et al. 2014; Durden et al. 2016).

This document focuses on hover class AUVs can control their position and heading at very low speeds, which makes them suitable for operations over rough terrain while maintaining an appropriate altitude for imaging small scale targets. When equipped with navigational sensors such as GPS, Ultra Short Baseline Acoustic Positioning System (USBL), acoustic doppler profiler, and forward-looking obstacle avoidance sonar, hover class AUVs enable precise tracking along the pre-programmed routes. These characteristics make them particularly suited to collecting highly detailed sonar and optical images over high-relief seabed terrain, which can be geo-referenced with high precision. These can then be stitched together into photomosaics to focus on large features or specific details on the seafloor.

While most of the well-known AUVs used in scientific research are custom built, technological developments over the last five years have seen a number of ready-built, commercial units becoming available, with examples such as the cruising <u>lver</u> and hovering <u>Subsea 7</u> AUVs. The release of these units into the market will likely increase the uptake of AUVs for scientific research.





Figure 4.1 Examples of AUV classes. Left: an example of the cruising class AUV *Nupiri muka* operated by the University of Tasmania (photo credit: Damien Guihen). Right: an example of the hovering class AUV *Sirius* operated by Australian Centre for Field Robotics for Integrated Marine Observing System (Photo credit: Asher Flatt).

4.2 Scope

The primary aim of this field manual is to establish a consistent approach to marine benthic sampling using AUVs and facilitate statistically sound comparisons between studies. This manual will focus on hover class AUVs designed to survey the seabed due to their proven use in marine benthic monitoring compared to other marine imagery platforms (described in next section of this chapter). It will not consider cruising class AUVs. The scope of the manual is to cover everything required from equipment, pre-survey preparation, field procedures and post-survey procedure for using hover class AUVs to photographically survey seabed assemblages found on Australia's continental shelf regions. Deep-sea environments are currently excluded from this field manual as we do not currently have an AUV in Australia capable of image-based surveys at these depths. Although it should be noted that AUV-based photographic surveys of the deep-sea benthos have been successfully undertaken internationally (e.g. Morris et al. 2014; 2016; Milligan et al. 2016).

4.3 AUVs in Marine Monitoring

Application of AUVs for monitoring benthic marine ecosystems has experienced a rapid increase over the past two decades. Researchers have used hover class AUVs in monitoring the impacts of invasive species (Ling et al. 2016; Perkins et al. 2015), for ecosystem-based fisheries management (Smale et al. 2012), assessing population trends in demersal fishes (Clarke et al. 2009; Seiler et al. 2012), mapping of benthic habitats (Lucieer et al. 2013), examining diversity in reef communities (Bridge et al. 2011; James et al. 2017; Monk et al. 2016), changes in structural complexity of coral reefs (Ferrari et al. 2016a, b), and mapping the spatial and depth extent of kelp forests (Marzinelli et al. 2015).

Compared to other marine imagery platforms (e.g. towed systems), hover class AUVs have several strengths applicable to marine monitoring:

• They navigate precisely defined flight paths and the geolocation of individual images along this path. The geolocation of imagery and flight paths allows relatively precise repeat transects to be conducted, and also for the imagery to be used to ground-truth multibeam sonar (Lucieer et al. 2013) as well as for modelling the environmental factors driving species' distributions (Hill et al. 2014).



- The time-gain it provides over an ROV. This particularly the case if the AUV system can be left alone (i.e. that are truly autonomous).
- An AUV will follow the set path, will not slow down or divert for something pretty, exciting or scary in the water: something that tends to happen to the humans when piloting an ROV.
- They generate spatially accurate photomosaics and finescale digital elevation models. Multibeam data which is often available with accurate georeferencing can provide important information regarding habitat types and structural complexity but is often limited to cell resolutions of 50 cm to 5 m. Finescale digital elevation models from AUV photomosaics can be done at 1-10cm cell resolution, thus enabling extremely detailed structural information to be extracted (Ferrari et al. 2016a,b). Additionally, and perhaps more importantly, the benefits of using AUV to provide digital elevation models is that the AUVs also provide colour information (via the photomosaics), which is crucial for species identification and the evaluation condition (e.g. live vs. dead coral).

The manner that data is extracted from imagery (i.e. image annotation) is context-dependent and ranges from the simple scoring of presence-absence of indicator organisms or habitats within individual images (e.g. Perkins et al. 2016) to automated habitat classification that uses sophisticated algorithms (e.g. Friedman et al. 2011). Random point count is one of the commonly employed approaches in the quantification of the cover of benthic habitats or organisms (e.g. James et al. 2017; Monk et al. 2016; Perkins et al. 2016). Whilst pattern recognition annotation has the potential to substantially speed up the image scoring process, it is not a point yet where it is accurate enough to replace manual point-counts. Accordingly, this manual will focus on point-count annotation approaches.

4.4 **Pre-Survey Preparations**

<u>Ensure all permits, safety plans and approvals have been obtained.</u> Any research undertaken within Australian Marine Parks (AMPs) requires a research permit issued from Parks Australia. See Appendix B for a list of potential permits needed.

Define question/aim of project.

<u>Confirm sampling design</u> is statistically sound with adequate spatial coverage and replication, and addresses the initial question/aim. This is generally achieved through the use of an explicit randomization procedure to ensure that independent replicates are obtained (Foster et al. 2017; Smith et al. 2017). See Chapter 2 for further details on sampling design.

<u>Select appropriate transect design for AUV deployment.</u> Two AUV transect designs are recommended for marine monitoring: 1) broad grids and 2) dense grids. Foster et al. (2014) evaluated a number of broad grid designs and determined that a grid consisting of three long parallel transects (each generally covering a total of 2000-4000 m) was generally the most optimal design for monitoring purposes (Figure 4.2). The dense grid transects are used to get a complete coverage photomosaic that covers a 25 x 25 m scale (Figure 4.2). Combinations of both within a survey can be applied if required (e.g. Morris et al. 2016). Essentially, broad grids cover more ground but are less repeatable, whereas dense grids are more repeatable but less general (essentially you get more information about less).

The decision to which transect design is most appropriate is driven by the question being addressed, as well as the environment, available time and logistics of AUV deployment and retrieval. For example, in the deeper regions (> 100m) within the AMPs that are exposed to strong currents, dense grids are not recommended for temporal monitoring purposes because the challenges with maintaining physical position in these conditions make it difficult to successfully



repeat the same 25 x 25 m grid. This ultimately results in limited temporal overlap between sampling points over time (Figure 4.3). Where inference is the primary objective of the study it is recommended that broad grids are used to increase sampling power (Chapter 2). Conversely, if the physical structure of the seafloor or biota (e.g. corals; Ferrari et al. 2016a) are the focus then dense grids are best suited.

Broad grids are generally used in mid-outer continental shelf Tasmanian waters as result of strong currents. Conversely in Western Australia, the patchy nature of inshore reefs, coupled with a lack of shelf slope to encompass a wide depth range along broad grid designs meant that dense grids surveys undertaken within each of a replicate number of patch reef systems and depths was the most pragmatic solution. In southern Queensland, dense grids were the primary method used due to the initial process-based research focus, however, the missions are time intensive, as is post processing and analysis, and could readily be modified to a broad grid design in the future to simplify analysis. In NSW a combination of both broad and dense grids has been conducted at most sites over several time periods, although more recent surveys in the Sydney region have just used broad grids.

<u>Stereo-cameras must be pre- or post-calibrated</u> in shallow water using the techniques similar to those outlined in Boutros et al. (2015).

<u>Decide on appropriate navigational systems (e.g. USBL)</u>. Accurately geo-referenced imagery is crucial to the success of any AUV deployment, and appropriate effort must be given to this during the survey planning phase.

<u>Ensure appropriate software is installed</u> on onboard laptops (e.g. AUV navigation software platform, GIS, etc), and potential users are familiar with it so that the AUV can be tracked and its mission success monitored while underway.



Figure 4.2 Examples of AUV transect designs over multibeam mapped reef features. Left: stand-alone 25 x 25 m dense grid transect. Middle: stand-alone broad grid. Right: combination of broad grid with a dense grid imbedded. Note with this design broad grid transects are usually shorter due to the time required to complete both grid types.





Figure 4.3 Example of spatial mismatch between sample time points for a 25x25 m grid in a high current/wave action environment. Note the limited overlap between all three sampling points.

4.5 Field Procedures

4.5.1 Onboard sample acquisition

<u>Complete an on-site briefing</u>. Prior to deployment, a deployment briefing should always be completed to ensure the operation can be completed safely. Always take a precautionary approach to risks associated with vehicle deployment. See Chapter 1 for further information about risk assessments.

<u>Set up and test AUV system.</u> Allow sufficient time during survey mobilisation to undertake system checks, calibrations and testing of equipment and account for unforeseen problems; in most cases it will be possible to complete all system setup and tests within half a day. The conduct of pre-start checks should be noted in the trip log and any test failures specifically recorded for later-reference. Detailed settings for each component should be made using relevant operations manuals (e.g. USBL operations manual etc.).

On-deck tests should include, but not limited to, the following checks:

- on-board data storage
- on-board power
- cameras
- strobe lighting
- · iridium beacon, RF and emergency strobes
- propellers
- all blanking plugs are installed
- · correct and new corrodible link attached emergence ascent drop weight
- crane and associated shackles are working order
- check all seals/o-rings and blanking plugs are good working order
- check all surface communications

Wet testing should include checks of the following:

• USBL and internal navigation (e.g. compass and avoidance sonar)

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- cameras and strobes
- through-water communications

Acoustic tracking setup

- Set position of GPS receiver. Differential GPS is mandatory for repeat site monitoring.
- Deploy USBL transceiver (e.g. pole or vessel mounted).
- Measure offsets of USBL transceiver head to GPS receiver and put offsets into navigation system.

Conduct AUV transects

Pre- deployment

- Transects should only be undertaken in areas where the substratum is known/mapped (often in the form of multibeam mapping) as to avoid entrapment and potential loss of AUV. Do not deploy blind, as this increases the risk of equipment loss and damage, as well as unnecessary impact on potentially vulnerable ecosystems.
- Once final transect locations have been determined, provide the locations of the transects (usually in ESRI shapefile format) and associated multibeam maps (in geotif format) to the AUV engineers responsible for uploading missions. Cross-check the uploaded transect corresponds to the correct area on the geotif (i.e. ensure the geographic coordinates are defined for all spatial data).
- The flight elevation of AUV should be set and maintained at ~ 2m from the seafloor to facilitate a consistent field of view. General sampling methodology can be found in Williams et al. (2012). Although this needs to be informed by 'survey question', camera type and performance, illumination type and output power, etc.
- Prepare for AUV launch and recovery on deck, and ensure only essential personnel participate in its preparation and deployment.
- Place USBL transceiver in water and ensure functionality.
- Correctly insert the deployment release pin.

AUV deployment and retrieval

- 1. Disconnect any power or data cables, ensuring any blanking plugs are fitted prior to deployment.
- 2. Install sacrificial ballast weights. Ensure that there is sufficient time allocated to transect when selecting corrodible link.
- 3. Vessel master must ensure the vessel is positioned at the start of the transect start location.
- 4. Following the signal to deploy from the vessel Master, use the crane and/or A-Frame to lift and guide the AUV from the deck into the water.
- 5. Minimise the time taken from when the AUV is let out of reach, to when it is lowered in the water, so as to reduce potential swing and impact against the vessel.
- 6. Using appropriate software (see Pre-Survey Preparations), monitor the AUVs progress to the seabed and start of transect location. Note the start time of transect using a timer as this will be used to determine when the sacrificial weight will be automatically released (if fitted) in the case of an emergency.
- 7. Confirm data is being recorded where possible (e.g. recording indicators, hard drive operating).
- 8. Ask the vessel's Master to follow the AUV during transects, to maintain USBL communication and AUV tracking.



- 9. Monitor weather forecast conditions prior to and during deployment to maintain safe working environment. Consider aborting operations if local weather and forecast conditions are marginal.
- 10. When the transect is complete or if the transect is being aborted, advise the vessel Master of the intention to retrieve the AUV.
- 11. Watch for the AUV to resurface, ensuring only required personnel are near open transom. Avoid approaching the AUV looking into the sun as this increases the risks of collision.
- 12. Use grapple hook to connect the lift line to the AUV for retrieval. At least three personnel should be present with hooks to avoid the AUV colliding with vessel [Recommended].
- 13. Shut down the AUV and connect relevant power or data cables.
- 14. Remove the sacrificial ballast weights.
- 15. For the last transect of the day, wash down the AUV with freshwater, unplug the USBL and turn off emergency beacons.
- 16. Raise the USBL transducer (if pole mounted) before moving vessel to next location.

Procedures for seabed entanglement or loss of communications with AUV

Potential entanglement of the AUV is always a possibility. The following procedures should be followed upon entanglement:

- 1. Log the last known position of the AUV.
- 2. Send an abort code to AUV to manually end the transect.
- 3. If the AUV appears entangled (i.e. not moving), a mini remotely operated vehicle (ROV) should be used to locate and retrieved the unit. If the AUV is trapped under a ledge/cave, or ensnared in fishing line or kelp, the automatic release of the sacrificial weights may cause issues with recovery of the unit. Under such circumstances it is recommended that a ROV is deployed to recover the AUV.
- 4. If the AUV is fitted with a sacrificial dump weight, which automatically releases after a user defined period, it may surface on its own. Once it's on the surface, use the fitted iridium beacon, RF, GPS and emergency strobes to locate unit.
- 5. Ensure that you check AUV thoroughly for damage before redeployment.

Completion of operations

Prior to any vessel movement or engine start-up, operators should check the following:

- All equipment is clear of the water, including the USBL transducer pole.
- AUV is shut down.
- All gear is safely stowed.
- All power and data cables are connected.
- An "All Clear to Move" command is given to vessel Master when the AUV team is satisfied it is OK for the vessel to move on.



4.5.2 Onboard data processing and storage

- 6. Once the AUV transect is complete, it is good practice to download associated raw imagery and associated positional data. Imagery and associated positional data should be checked to ensure no failures have occurred, including but not limited to the following:
- Miss-timing between image capture and strobes (i.e. dark/black imagery)
- Failure of one of the stereo cameras
- Failure of positional logging
- 2. Name data files according to established conventions. File naming conventions are important for ensuring both efficient and effective management of field data and its integration into appropriate data management repositories. It is important to note that these conventions will differ among agencies and academic institutions.
- 3. Ensure accurate recording of metadata. Metadata is a descriptive data source comprised of information that may be used to process the images or information therein Durden et al. (2016). While it is important to follow agency specific protocols for capturing metadata, it is also essential that metadata is sufficient enough in detail to satisfy conformance checks for subsequent data release via AODN. Minimum data for each transect should contain as follows:
 - Campaign (i.e. Survey identifier)
 - Station/event number
 - Platform
 - Latitude and longitude (WGS 1984 in decimal degrees with a minimum of 6 decimal places [Recommend])
 - Altitude
 - Depth
 - Time and date stamp
 - AUV orientation (roll, pitch, heading)
 - Precision details (e.g. type of navigation system used and its associated errors)
 - Data provenance
- 4. Backup data. This is necessary to ensure all data collected in the field is safely returned and securely backed-up at host facilities, prior to quality control and public release. Onboard copies of data should be made as soon as practical following acquisition. When operating external to a network, it is recommended that all data be backed up on a RAID or a NAS that contain built-in storage redundancy in case of hard-drive failure. A duplicate copy of all data onto external hard drives for transportation back to host facilities is *[Recommended]*.

4.6 **Post-Survey Procedures**

4.6.1 Data processing

A general workflow for data processing methodology can be found in Williams et al. (2012). Key requirements for raw image processing and positional data are as follows:

- It is recommended that at least one of the stereo images is in colour and enhanced following similar procedures as outlined by Bryson et al. (2016).
- All stereo images should be georectified following Williams et al. (2012). If not stereo then processing routines can be found in Morris et al. (2014).

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 Positional data should be post-processed using Simultaneous Localisation and Mapping (SLAM) as demonstrated in Barkby et al. (2009) and Palomer et al. (2013)

4.6.2 Data annotation

Scoring of individual images can be done using a number of annotation software tools. Examples include, Transect measure, Coral Point Count, CoralNet and Squidle+. For national consistency Squidle+ (<u>http://squidle.greybits.com.au</u>) is recommended as it is free and allows for different approaches in image subsampling, which appears to influence inferences from data (Monk et al. unpublished data), as well as stratified and random point count distribution on images. It also automatically imports the collected AUV data once it is uploaded to the AODN making it ready for analysis, and has tools for exploring survey data as well as analysis. In addition, it supports multiple annotation schemes, and will provide consistency through translation between schemes, which is an important point that differentiates Squidle+.

There are three approaches recommended for annotating georeferenced imagery from AUVs:

- Annotation of individual images
- Annotation of photomosaics
- Extracting structural complexity from orthomosaics

Annotation of individual images or photomosaics can be undertaken using three methods:

<u>Full assemblage scoring of imagery</u> across space and time. It is important to note that this is
a time-consuming process, requiring a lot of replicate images to be scored to enable
sufficient power to detect biologically meaningful change as most morphospecies are < 10 %
cover within images. This approach appears to be good for delineating bioregional and
cross-shelf patterns at a morphospecies (Monk, et al. unpublished data) and CATAMI
(Althaus et al. 2015) level (James et al. 2017; Monk et al. 2016). This approach will no doubt
be effective in choosing initial suite of indicators for national level monitoring and reporting.

As a general guideline, and dependant on the survey question, we recommend that 25 random points per image from at least 50 images per transect leg are a good starting point for recording most morphospecies present within images (based on Perkins et al. 2016). It is important to note that the properties of the organism themselves will also influence the number of points/images to score. Obviously morphospecies that are less abundant require more effort, but also the 'clumpiness' of species will affect the scoring effort needed (Perkins et al. 2016). Van Rein et al. (2011) and Perkins et al. (2016) suggest that, while a higher number of points per image can increase the detection rate of more organisms within an image, increasing the number of scored images using fewer points is likely have a similar (or greater) effect. Ideally, increasing both the number of images scored and the number of points scored within an image would result in greater power (Roelfsema et al. 2006), but preference is usually for increasing the number of images (Perkins et al. 2016). Unfortunately, the adoption of this approach is likely to result in substantial increases in processing time and thus cost.

 <u>Targeted scoring of indicators or proxies</u> (such as grouping fine level morphospecies into broader level CATAMI classes; Monk et al. unpublished data). This approach has been shown to work very well at an indicator morphospecies level for detecting change at a regional level (Perkins et al. 2017) as well as for detecting invasive species trends (Ling et al. 2016; Perkins et al. 2015). More recently this approach has been extended to mobile species, such as fish (Seiler et al. 2012) and lobster (Bessell et al. unpublished data). Care needs to be taken if length data (using photogrammetry or structure from motion) is extracted from stereo pairs from Sirius data as both Seiler et al. (2012) and Bessell et al.



(unpublished data) found precision can be poor for mobile species if camera separation is inadequate (see Boutros et al. 2015)

Since this approach requires substantially less effort to score each image, more images (i.e. often all images) can be scored and, thus, increased statistical power. The drawback is that narrower understanding of the environment is produced.

 <u>Automated analysis of imagery</u> potentially provides a cost-effective alternative to annotating imagery from AUVs. It is important to note that automated imagery analysis is a relatively new, and largely developmental, way of annotating images. Despite this some studies suggest that coral and macroalgae can be reliably identified using automated image analysis (Table 7).

The last approach to annotating AUV imagery involves the extraction of 3D structural information from stereo images using structure from motion techniques outlined in Ferrari et al. (2016) and Pizarro et al. (2017). This approach works particularly well too for sessile species to track changes in growth form through time at a 25 x 25 m scale (Ferrari et al. 2016).

Table 4.1 A brief summary of methods for automated benthic image classification. The number of classes and the main taxa included in the respective studies are also shown.

Authors	Classes	Main Species
Marcos et al. (2005)	3	Corals
Stokes & Deane (2009)	18	Corals, Macroalgae
Pizarro et al. (2008)	8	Corals, Macroalgae
Beijbom et al. (2012)	9	Corals, Macroalgae
Denuelle & Dunbabin (2010)	2	Kelp
Bewley et al. (2012)	19	Corals, Algae and Kelp
Bewley et al. (2014)	19	Corals, Algae and Kelp
Beijbom et al. (2016)	10	Corals, Macroalgae
Mahmood et al.(2016a)	9	Corals, Macroalgae
Mahmood et al. (2016b)	2	Corals, Macroalgae

4.6.3 Data curation and quality control

A national AUV steering group has been set up to oversee a nationally coordinated AUV benthic monitoring program which is supported by the Integrated Marine Observing System (IMOS) (Table 4.2). Any new AUV deployments should be discussed with this steering group to ensure that, wherever possible, they can be integrated within the national program [*Recommended*].

Table 4.2 Key contacts in national AUV steering group as of Jan 2018

Name	State	Organisation
Neville Barrett*	Tasmania	IMAS
Craig Johnson	Tasmania	IMAS
Peter Steinberg	New South Wales	SIMS
Alan Jordan	New South Wales	NSW DPI
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Version 1

Stefan Williams	New South Wales	USyd
Gary Kendrick	Western Australia	UWA
Russ Babcock	Western Australia	CSIRO
Paul Van Ruth	South Australia	SARDI
Hugh Sweatman	Queensland	AIMS
Tom Bridge	Queensland	JCU/QLD Museum
Daniel Ierodiaconou	Victoria	Deakin
* Chair		

Data quality control at both the collection and annotation stage is critical. Most importantly, the annotation schema needs to be consistent between studies. Morphospecies and associated CATAMI parent classes be used *[Recommended]*. An initial morphospecies catalogue for southeastern shelf waters is currently held and maintained at the Institute for Marine and Antarctic Studies (IMAS) (contact Dr Neville Barrett or Dr Jacquomo Monk).

Other annotation schema are available, and can be applied. In such situations where an alternative schema are used to annotate AUV imagery, it must be able to be mapped to CATAMI so that comparisons can be made with previous studies or between regions. Translations between schema can be readily applied within Squidle+. The quality control of all annotations undertaken by novice scores should be assessed against an experienced analyst (e.g. using confusion matrices; Figure 4.4). Logically, it is important to correct any discrepancies between annotators. This can be done by re-examining the images to ensure an agreement can be reached between annotators. Alternatively, if an agreement cannot be reached, then the miss-classified morphospecies could be potentially grouped into a higher level CATAMI class.





Figure 4.4 Confusion matrix showing the CATAMI classes scored by novice 1 (AW) and experienced (JH) for 30 co-scored images. Black outlined boxes indicate consistent classification between scorers, the percent of all points scored as any particular class are is shown in each box and colour coded. Blue outlined boxes indicate sponge, bryozoan/hydroid and substratum respectively moving from left to right across the image.

4.6.4 Data release

<u>Squidle+</u> is a centralised online platform for standardised analysis and annotation of georeferenced imagery and video. Many national marine observing programs (for example IMOS through the Australian Ocean Data Network (AODN) or the Marine Geoscience Data System (MGDS) in the USA) routinely store imagery data online in an openly accessible location. Squidle+ operates based on flexible distributed data storage facilities (i.e. imagery can be stored anywhere in an openly accessible online location) to reduce data duplication and inconsistencies, and provides a flexible annotation system with the capability to translate between different annotation schemes.



Following the steps listed below will ensure the timely release of imagery and associated annotation data in a standardised, highly discoverable format.

- 1. Create a metadata record describing the data collection. Provide as much detail as possible on the deployment (either directly in the metadata record itself, or in the form of attached field sheets as .csv, .txt or similar). Details of minimum metadata requirements are provided in Onboard Data Processing and Storage section above.
- 2. Publish metadata record(s) to the <u>Australian Ocean Data Network (AODN) catalogue</u> as soon as possible after metadata has been QC-d. This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the <u>AODN Data Submission</u> <u>Tool</u>. Note that user registration is required, but this is free and immediate.

Lodging metadata with AODN in advance of annotation data being available is an important step in documenting the methods and location of acquired imagery and enhancing future discoverability of the data.

- 3. Upload raw imagery from the survey to a secure, publicly accessible online repository (<u>contact</u> <u>AODN</u> if you require assistance in locating a suitable repository).
- 4. Create a Squidle + campaign as soon as possible after imagery is uploaded, choose the most appropriate annotation schema, and commence annotation of imagery.
- 5. Add links to the location of the Squidle+ campaign to the previously published metadata record. You may also wish to attach or link a copy of the annotation data directly to the record.
- 6. Produce a technical or post-survey report documenting the purpose of the survey, sampling design, sampling locations, sampling equipment specifications, annotation schema (e.g. morphospecies, CATAMI, etc.), whether the survey was assemblage-based or targeted towards key (morpho)species, number of points, interval between images (e.g. every 50th image), and any challenges or limitations encountered. Provide links to this report in all associated metadata. See Appendix C for a suitable template [*Recommended*].

4.6.5 Data analysis

The breadth of research questions precludes any detailed advice on the analysis of data from AUV transects. However, one common attribute of the image-based data that will have to be contented with for all analyses is spatial proximity. The closeness of images, within and sometimes between transects, means that image data are unlikely to be independent (due to spatial autocorrelation). Yet, this is an assumption that many statistical methods rely upon. The failure to meet this assumption means that the inferences from the statistical analysis may be: (i) over-confident, e.g. having a p-value that is too small; (ii) biased, i.e. the estimates do not reflect the truth; (iii) both, or; (iv) no effect. Obviously, the fourth category is what a researcher hopes for, but it is improbable and must be validated. However, if it is known that the study organism exhibit particularly low autocorrelation then the analysis need not consider it explicitly.

Methods to analyse data, accounting for autocorrelation are available. These include geostatistical models (see Foster et al. 2014 for AUV-based examples). However, in certain situations subsampling images will help (see Mitchell et al. 2017 for a marine based example), but not necessarily alleviate completely. Further, if the study is for a broad area, where transects are small and are well-separated, then amalgamating data to transect level may also be appropriate.



4.6.6 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 4 (field manual for AUVs) is below:

Version Number	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
1.1	Link to Squidle+ corrected	March 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019

4.7 References

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5. MARINE SAMPLING FIELD MANUAL FOR BENTHIC STEREO BRUVS (BAITED REMOTE UNDERWATER VIDEO)

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5.1 Platform Description

Stereo-baited remote underwater video (stereo-BRUV) systems consist of two video cameras inside waterproof housings, attached to a base-bar and encased within a frame with some form of baited container in front of the cameras (Figure 5.1, Figure 5.2; Cappo et al. 2007). Benthic stereo-BRUVs are lowered to the seafloor and are left recording for a set duration. The video footage can then be used to assess the recorded fish assemblages and associated habitats. Stereo-BRUVs are becoming widely adopted as a non-extractive technique for sampling the relative abundance and size structure of fish assemblages (Cappo et al. 2004, 2007, Watson et al. 2009, Langlois et al. 2010, 2012, Hill et al. 2014, Whitmarsh et al. 2017).

5.1.1 Comparison of stereo-BRUV with other sampling methods

Importantly, baited video and stereo-BRUV have been found to be comparable to other commonly used ecological and fisheries dependent sampling methods. Willis et al. (2000) demonstrated that spatial variation in abundance estimates from baited video were comparable to variation in fisheries catch rates, and less confounded by behavioural biases potentially experienced by diver based visual methods (i.e. UVC). Subsequent studies have demonstrated that stereo-BRUVs overcome certain behavioural biases associated with Underwater Visual Census (UVC) techniques (Colton & Swearer 2010, Lowry et al. 2012), however UVC will typically record greater species diversity whereas baited video will record greater diversity and abundance of target species. Across latitudinal gradients, Langlois et al. (2010) demonstrated that measures of species richness/diversity obtained by baited video and diver based methods were comparable. Importantly for studies of the impacts of fishing pressure, biomass distribution and ecosystem dynamics, the size composition of targeted species sampled by stereo-BRUVs has been found to be comparable to line (Langlois et al. 2012) and trap (Langlois et al. 2015) fisheries.

5.1.2 Advantages of stereo-BRUV

As a non-extractive technique, stereo-BRUV have little impact on the ecosystem being studied, making this an ideal sampling platform to use in marine protected areas. The use of stereo-BRUVs also overcomes some of the biases associated with Underwater Visual Census (UVC) techniques (Colton & Swearer 2010, Lowry et al. 2012). Remote video eliminates the need for scuba diving, providing a strong safety advantage, while reducing the risk of incorrect fish identifications and inter-observer variability through recording a permanent and reviewable record. Furthermore, video techniques can access depths that are off-limits to divers and produce highly accurate length measurements (Harvey et al. 2001). The use of bait can increase the relative abundance and diversity of fishes observed, particularly species of interest to fisheries, without precluding the sampling of prey or herbivorous fish species (Lowry et al. 2012, Hardinge et al. 2013). Multiple stereo-BRUVs can be deployed in the field consecutively, making efficient use of researcher and boat time (Cappo et al. 2007, Langlois et al. 2010, Hill et al. 2014, Whitmarsh et al. 2017). This allows for the possibility of large spatial coverage and high replication even during short field campaigns.

5.1.3 Limitations of stereo-BRUV

The extent of the limitations and possible biases of stereo-BRUVs have been discussed in various studies (trophic biases, Goetze et al. 2015, bait biases, Langlois et al. 2015, behavioural biases, Coghlan et al. 2017). In addition, their suitability is decreased in habitats where the field of view is likely to be obscured (e.g. tall kelp habitats, very high relief reefs or low-visibility, highly turbid waters), similar to underwater visual censuses (UVCs). Nevertheless, BRUV technology is relatively Page | 83



simple and easy to deploy, providing consistent sampling of the benthic fish community and an index of abundance and diversity.

Overestimates of abundance can occur through double counting fish. This occurs when the same individual/s are viewed at different time points throughout a deployment. To overcome this, counts of the maximum number (MaxN) of individuals of any one species seen over the recording period have been used (Cappo et al. 2007, Harvey et al. 2007). In a monitoring context, comparative studies have suggested that the use of MaxN may be "hyper-stable" when fish abundance is high due to saturation of the field of view (Schobernd et al. 2013) and have suggested alternative metrics (e.g. MeanCount). However, MaxN is the most widely accepted metric in Australia and internationally, and provides an established option for standardisation between sampling programs.

In addition, the variation in the distance the bait plume travels, the responses of different fish species to the bait plume and the distances they will travel to get to the bait are unknown (Harvey et al. 2007). For these reasons, estimates of individual species abundance from BRUVs are currently limited to measures of relative abundance rather than density (Cappo et al. 2007). The use of MaxN also results in conservative estimates of the relative abundance and biomass of fish. Limitations have also been acknowledged for cryptobenthic and site-attached species that are often underrepresented using video-based methods (Holmes et al. 2013). While BRUVs are considered unsuitable for estimating density, they are a powerful and cost-effective method for detecting spatial and temporal changes in the relative abundance and lengths of fish assemblages (Watson et al. 2009, Harvey et al. 2013, Hill et al. 2014, Malcolm et al. 2015).

Importantly, for sampling in deeper water habitats, the depth limitation of using roped stereo-BRUVs will depend on local conditions and will typically vary with water current conditions (e.g. ~1500 m, Zintzen et al. 2012). Non-roped stereo-BRUV systems have been developed internationally (Merritt et al. 2011) and in Australia (Marouchos et al. 2011) but have not yet been widely applied. In areas with strong currents, even in depths of ~60 m, the water resistance can act on the rope catenary to pull BRUV systems over, and the potential for this increases with depth. An associated limitation can include the surface floats being pulled underneath the surface until the current slows. Options for remotely deployed deepwater BRUVs using a sequence of bait release and monitoring over a 24-hour period, before the BRUV is released to the surface are still in development mode but have been trialled in the Flinders AMP (https://www.csiro.au/en/Research/OandA/Areas/Marine-technologies/Hi-tech-ocean-observing/DeepBRUVS).



5.1.4 Definition of terms

Sample

- Single observational unit (e.g. a single BRUV deployment).
- Sample/OpCode is interchangeable.

Method

• Sampling method, e.g. stereo-BRUV (stereo baited remote underwater video).

Campaign

- Discrete set (temporal and spatial) of Samples.
- Uses the same sampling and image analysis methods.
- CampaignID is a unique identifier for a Campaign made up of YYYY-MM_Project.name_Method (* is used to denote a CampaignID throughout this guide).

Project

- Contains one to multiple Campaigns with a shared purpose/objective (e.g. monitoring of a certain Marine Park, a bioregional study).
- Project is a unique identifier and the name should be carefully chosen (e.g. "MarineParkMonitoring" is not a good Project name but "Houtman Abrolhos Reef Observation Areas long-term monitoring" is a great Project name).



Figure 5.1 Left A: typical stereo baited remote underwater video (stereo-BRUV) and Left B: schematic of typical deployment setup of a stereo-BRUV unit sitting upright on the substrata with a rope leading to two buoys on the surface (Source: T. Simmonds/AIMS). Right: A photograph of a typical stereo-BRUV with the dimensions of the frame.





Figure 5.2 a) Deploying a stereo-BRUV from side of vessel. Note that this is a heavy-weight stereo-BRUV setup (Photo: C. Wellingtion/DPIRD). b) Deploying a stereo-BRUV through trawl door on a large vessel. c) Retrieving a standard stereo-BRUV. d) Retrieving a heavy-weight stereo-BRUV off large vessel.

5.2 Scope

This benthic stereo-BRUVs Field Manual includes gear designed to acquire imagery of demersal fish assemblages and their habitat within the field of view. A separate manual will address sampling pelagic fish assemblages using BRUVs (Chapter 6). This field manual covers everything required from equipment, pre-survey preparation, field procedures, post-survey procedures and data management for using benthic BRUVs to sample and monitor fish assemblages. The aim is to develop a consistent approach to using this field equipment and allow statistically sound comparisons between studies. Stereo-BRUVs are recommended, over mono-BRUVs, when monitoring demersal fish assemblages. Stereo-BRUVs consist of two cameras strategically and accurately placed on a frame that enable lengths and distance measurements to be made through the use of specialised software. These data are crucial to help monitor changes in fish assemblages over time. Therefore, the following standard operating procedures are written based on the use of stereo video.

5.3 Stereo-BRUVs in Marine Monitoring

A range of tethered and remote video methods, with roped and unroped designs, have historically been used to sample fish assemblages (see Mallet & Pelletier 2014). The use of BRUVs in scientific research has greatly increased over the past decade (Figure 5.3; Whitmarsh et al. 2017). This is in part due to the cost-efficiency and statistical power typically achieved for a wide range of trophic fish groups (Langlois et al. 2010) which has been recognised as an important metric for the investigation of ecosystem processes, the effects of fishing, and comparisons with fisheries-dependent data sets (Rochet & Trenkel 2003, Langlois et al. 2012). In Australia, benthic stereo-BRUVs have been used

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to successfully monitor spatial and temporal changes in benthic fish communities and their habitat structure (Figure 5.4; Cappo et al. 2004, Langlois et al. 2006, 2010, Harvey et al. 2013, Hill et al. 2014, Whitmarsh et al. 2017). There has been a steady increase in the use of stereo video over mono video systems, as equipment costs have fallen and the utility of length information for ecosystem studies has become apparent (Langlois et al. 2015). Stereo-BRUVs provide a non-extractive method for quantitatively assessing fish assemblages without the need for divers with the added benefit of having a permanent record if data are lost or identifications need to be checked. Many studies have compared the use of BRUVs with other 'traditional' methods such as diver transects, diver operated video (DOV), towed video or netting (Cappo et al. 2004, Watson et al. 2009, Colton & Swearer 2010, Langlois et al. 2010, Lowry et al. 2012, Goetze et al. 2015, Logan et al. 2017). In general, stereo-BRUVs recorded comparable species richness, greater abundance of targeted species with comparable size composition to fisheries dependent methos and provide the most cost effective method for sampling fish assemblages across a broad depth range (Langlois et al. 2010).

Sampling with stereo-BRUVs provides data for:

- Understanding anthropogenic impacts (fishing, climate change, oil and gas exploration, artificial reefs).
- Assessing changes in fish assemblage diversity, relative abundance, population size structure and growth.
- Exploring fish behaviour, including interactions between species.
- Determining the relationship between fish assemblages and their associated habitat structure.
- Assessing changes in fish assemblages and size structure across a depth gradient.

The following standard operating procedure provides a widely accepted protocol for the use of benthic stereo-BRUVs and will facilitate comparability of data from different surveys among space and time.





Figure 5.3 a) The The frequency of BRUVS studies published by year until 18/07/2016. b) The continent or geographical realm in which each study was conducted. c) The habitat type in which BRUVS were deployed for the 161 studies assessed. The 'Multiple' category was used where more than one habitat type was studied and included some of the other habitat categories listed (except for pelagic and deep-water), as well as some included in the 'Other' category, such as bare sand. 'Deep-water ([100 m)' habitats included shelf slope, soft sediments and hard substrates. d) The setup type used within each study, classified as either single (with one forward facing camera) or stereo (two cameras positioned to be able to determine fish measurements)(Source: Whitmarsh et al. 2017).

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Figure 5.4 Examples of the fish assemblages observed using benthic stereo-BRUVs on reef and near reef sediments in 80-100 m of water in the Hunter CMR (Photos: J Williams NSW DPI). a) An example of mado (*Atypichthys strigatus*), ocean leatherjacket (*Nelusetta ayraudi*), and eastern rock lobster (*Sagmariasus verreauxi*). b) An example of Port Jackson shark (*Heterodontus portusjacksoni*) and silver sweep (*Scorpis lineolata*). c) An example of a school of nannygai (Centroberyx affinis) and an eastern wirrah (*Acanthistius ocellatus*). d) A conger eel (*Conger verreauxi*) and a school of nannygai (*Centroberyx affinis*). e) An example of a school of pearl perch (*Glaucosoma scapulare*), mado (*Atypichthys strigatus*), strigatus), and Port Jackson shark (*Heterodontus portusjacksoni*). f) An example of a teraglin (*Atractoscion aequidens*).



5.4 Equipment

Equipment must be appropriately set up to ensure as much consistency as possible among surveys and facilitate gear replacement if necessary. The key components for a benthic stereo-BRUV include the following:

- Per stereo-BRUV unit:
 - 2 x cameras (with batteries and memory cards). Cameras capable of operating in low-light conditions are recommended (e.g. Canon HF G40 ~\$1500). Cheaper action cameras (e.g. GoPro) are typically not adequate for low-light conditions.
 - 2 x camera housings (with o-rings)
 - Frame with weights
 - Bait arm with bait bag/container (reinforced if needed)
 - o Bait
 - Synchronizing device (i.e. clapper board or synchronizing diode)
 - Lighting (If required, for example if sampling in depths >60m. Light colour choice is important and blue light is recommended (Fitzpatrick et al. 2013))
 - Additional weights (if sampling in high currents or at depths of >40 m)
 - Sensors (e.g. temperature, current profilers)
 - Spare parts kits (O-rings and silicone grease etc)
 - Spare cameras (note need to recalibrate if cameras are replaced, which can be done post survey)
 - Spare bait bags/bait arms
 - Deployment / retrieval rig:
 - Rope (1.5:1 rope length to depth ratio)
 - Marker buoys
 - Winch (or pot hauler)
 - Protective gloves and helmet
 - Towel/Cloths
- Other
 - GPS
 - Site maps with coordinates of sites
 - Hard drives
 - Laptop(s) with charger(s)
 - Powerboards and extension leads
 - Data sheets
 - Permits
 - Spare batteries and memory cards
 - Grapnel, extra weight and rope for BRUV recovery

5.5 **Pre-survey planning**

<u>Confirm sampling design is statistically sound and feasible</u> with existing resources. Sampling design is crucial to ensuring that there is adequate replication and spatial independence to ensure a statistically sound study. Therefore, it is important that a statistician is consulted prior to beginning any sampling. Chapter 2 of this field manual package provides details of sampling design considerations, as well as example code and data for implementing a spatially-balanced design, as outlined in Foster et al. (2017). Specific sampling considerations pertaining to stereo-BRUVs include:



- Concurrent stereo-BRUVs should be separated by a minimum of 200-500 m to avoid bait plume overlap and animals moving between cameras.
- Deployments should be conducted at least 1 hour after sunrise and 1 hour before sunset to both improve visibility and remove the effect of crepuscular behaviour.
- Optimal soak time for comparisons with other studies is 60 mins. However, 30 min deployments may increase level of replication without sacrificing statistical power for reef-affiliated species accumulation curves (Harasti et al. 2015).

The time of <u>fish biologists or taxonomists should be included as line items in budgets</u> to ensure that all footage can meet appropriate QA/QC checks and species can be correctly identified. Care must be taken to ensure that a consistent nomenclature is used, with <u>FishBase</u>, the <u>World Register of Marine Species</u> (WoRMS) and the <u>Codes for Australian Aquatic Biota</u> (CAAB) being popular, authoritative sources of taxonomic information. Undescribed or unnamed species (e.g. defined operational taxonomic units) must also be meticulously documented to maximise consistent nomenclature among surveys and research groups. Archives of reference images from previous sampling campaigns have been established by numerous agencies across Australia and can serve as a useful benchmark for problematic sightings, which are kept up to date with recent taxonomic changes.

<u>Consideration must be given to the location of stereo-BRUVs during deployment.</u> Instruments should not be deployed inside shipping lanes, near fishing gear, or wherever they are likely to constitute or become a navigational hazard. At a minimum, deployment and retrieval locations should be recorded, with vessel location monitored at regular time intervals as a back-up. It should also be noted that deploying stereo-BRUVs on high relief reef or reef with tall algae can be very difficult or impossible. Potential entanglements with wildlife such as humpback whales also need consideration in some locations during certain times of the year, with interactions and encounters increasing as whale populations recover. Although this doesn't preclude the use of stereo-BRUVs, it can limit how they are deployed and attended.

<u>Ensure all permits, safety plans and approvals have been obtained.</u> Any research undertaken within Australian Marine Parks (AMPs) requires a research permit issued from Parks Australia. Other potential permits and approvals that may be required include; animal ethics, safety plans. Risk assessments and state specific research permits. See Appendix B for a list of potential permits required at the Commonwealth level.

<u>Obtain sufficient data storage and backups</u>, including hard drives to copy and backup memory card from each camera (*2TB hard drives or greater recommended*). Ensure each hard drive is formatted and labelled appropriately. NOTE: You will need to allow for two copies of every deployment, one working and one backup. A single 60-minute video is currently around 8 GB if you are using 30 frames per second (FPS). Ensure sufficient memory cards for cameras are packed (*one 64 GB per camera plus spares is suggested*). Use high speed for greater downloading speeds. Number the memory cards to allow easy identification in the field. Ensure the downloading laptop is operational; laptops with multiple USB 3 ports are recommended for greater download speed and the ability to download footage from multiple memory cards at the same time. Planning to backup each hard drive in the field is essential. This can be done using either single hard drives, a faster solid state hard drive, a RAID hard drive system, cloudbase, or server-based data storage if cellular coverage is available. This will avoid data loss due to hardware failure that can occur.

<u>Test appropriate lights and additional sensors</u>. If using lights or sensors (temperature, light/PAR, current, etc), check they are working and fully charged and have chargers, spare parts, and the required equipment for downloading data whilst in the field or upon return to the lab. The Hobo Page | 91



Pendant temperature and light data loggers (UA-002-08) have been used with reliable results. The Marotte HS drag-tilt current meters (James Cook University; www.marinegeophysics.com.au/products/) can be fixed to the rear of the BRUV to move freely in the water column and record water temperature, current speed, and current direction.

<u>Select and check appropriate camera settings</u> are the same across all cameras (e.g. frame rate, video resolution, field of view mode, zoom, anti-shock sensors etc). Prior to any fieldwork, cameras should be checked to ensure they are serviced, cleaned, and calibrated (see below; note if a camera is moved or removed from its base plate it will need calibrating). It is important to note that small action cams (such as GoPro) do not perform particularly well in low-light conditions, especially with illuminated blue lights. If using such cameras, it is recommended that trials are undertaken prior to the sampling campaign. Clean and inspect housings for damage, check and replace o-rings if needed and lubricate with silicone grease. Ensure that housings are shipped with covers or protected in some way. This will prevent damage to the housing sealing surfaces and face plates.

<u>Order sufficient quantities of bait well ahead of time</u>. Due to differences in local supply, it is difficult to recommend a standardised baitfish. As a general rule a locally sourced, sardine-type, soft-fleshed, oily bait is recommended. This also reduces the likelihood of potential translocation of disease. Many BRUV studies from Australia have used pilchards (*Sardinops* spp.) as they are readily available, long lasting, and provide consistent bait size between field trips and studies (Dorman et al. 2012). Sourcing bait locally from factory discards (e.g. fish heads, tails and guts) is an attractive alternative for reducing costs and the ecological footprint of sampling. Allow at least 1 kg per planned BRUV deployment (recommended). When ordering, allow 20 % extra for repeating failed deployments.

Decide on the preparation and presentation of bait and consumables. Most studies use crushed or chopped bait presented in either a mesh bag or perforated PVC tubes. Bait arms should be angled towards the seabed and ideally in contact with the seabed so that the bait bag is not flapping in the current and so potentially disrupting fishes' natural inclination to be attracted to the bait (Cappo. pers. comm.). Ensure there are plenty of spare bait arms and bait bags or tubes. Bait arms may need to be reinforced with fibreglass rods if available or doubling up of PVC tubes. Having a number of rolls of duct tape and bags of cables ties is strongly recommended for running repairs.

<u>Check ropes, bridles, floats/buoys</u> for damage and ensure ropes are of sufficient length for the water depth that you are operating in (1.5:1 rope length to depth ratio). Float and rope configuration can also impact on deployment success. It is recommended that local trap fishers (e.g. lobster fishers) should be consulted on appropriate rope and float arrangement (Figure 5.5). Highly quality pot rope is recommended (e.g. New Zealand or Australian made). Check that there are a sufficient number and size of marker buoys and that they are coloured to make them visible at sea. Buoys should be marked with 'Research' and have each permit number. Make up spare ropes and floats in case gear is damaged or lost or damaged. Ensure sufficient weights are available for use at greater depths and in high currents. Typically double the weight is required at the front of the stereo-BRUV systems when deploying in deep water, but this arrangement will depend on local conditions and the frame design.





Figure 5.5 Example of top float (F) arrangement for using stereo-BRUVs.

<u>Check spare parts kit, make sure tools are oiled and in working order</u>. Spare parts are crucial for repairs and troubleshooting BRUVs in the field.

<u>Sampling gear specifications should always be fully documented</u> to achieve maximum transparency and comparability. This includes documenting the camera model, camera height above seafloor, camera separation, camera angle, camera field of view, underwater light lumens and colour, bait arm length and bait holder type.

5.5.1 Calibrating stereo-BRUVS

Stereo-BRUVS require regular calibration to ensure accurate measurements. The calibration process takes into account the base separation, camera angle and lens distortion all of which are unique to each camera, hosing and mount. Hence, each BRUV must be calibrated separately. Stereo-BRUVs should be calibrated using a 3D cube following recommendation by (Boutros et al. 2015)

It is ideal to calibrate each BRUV before and after each field campaign. This provides a backup in the event a camera moves or gets damaged during fieldwork. If cameras are swapped in the field due to damage or some other issue, the new cameras will require post-field calibration.

SeaGIS (<u>https://www.seagis.com.au</u>) have long been the primary provider of third-party calibration hardware and software, although alternative open-source packages have also begun to emerge, including the MATLAB Calibration Toolbox (<u>http://www.vision.caltech.edu/bouguetj/calib_doc/</u>) or the StereoMorph R package (<u>https://cran.r-project.org/package=StereoMorph</u>). For accurate and reliable stereo-calibration, SeaGIS software and calibration hardware is recommended.



5.5.2 Pre-survey checklist

Task	Description/comments
Fish biologists and taxonomists engaged or identified	
Adequate benthic stereo-BRUV sampling design (see chapter 2)	
Deployment protocol determined, including methods for locating/tracking gear	
Appropriate permits obtained and printed copies made (on waterproof paper if necessary)	
Coordinates of sampling sites calculated and checked for safety hazards.	
Bait ordered in adequate quantities	
Camera settings checked and cameras calibrated	
Data storage needs identified and hardware purchased accordingly	
Metadata sheet prepared	
Gear shipment arranged	

5.6 Field Procedures

5.6.1 Arrival on site

- 1. Unpack and set up stereo-BRUV units. Check for any breakages that may have occurred during transportations.
- 2. Attach bait bags to bait arms ensuring there are sufficient spares.
- 3. Check synchronizing device (such as diode batteries).
- 4. Defrost bait for first day of sampling.
- 5. All cameras and equipment should be carefully checked to ensure setting or switches haven't moved during transportation.
- 6. Check camera batteries are charged, memory cards are formatted and that everything is labelled.
- 7. Discuss deployment plan and safety with the team and ensure the skipper has the coordinates for all sites.

5.6.2 Deployment

- 1. Fill bait bags with ~1 kg of crushed or chopped bait.
- 2. Check camera settings.
- 3. Check data sheet is ready (note site, camera numbers and memory card numbers).
- 4. Move the BRUV frame to a secure and safe position.

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- 5. Attach lights and sensors if required.
- 6. Turn cameras on, check there is battery and storage space available.
- 7. Film data sheet or information board so that the site/location is identifiable at the beginning of the video.
- 8. Insert cameras into housings, check that the housing is dry and that there is no sand, hair or other objects obstructing the o-rings, and ensure there is a good seal and the clips are tight. Use shark clips to lock if necessary.
- 9. Attach ropes and buoys. Ensure the rope is free, coiled, and facing the correct direction to uncoil without hindrance. Ensure there is a 1:1.5 depth to rope (10 m of water = 15 m of rope). If there is a strong current you may need longer rope. It is also highly advised that there are several small surface floats followed by a large surface float.
- 10. Attach diode, or use clapper board, or alternative device to synchronise videos.
- 11. Attach bait arm.
- 12. With two people, lift the BRUV into position onto the gunnel or at the door of the vessel.
- 13. Push or throw the frame so that is clears the side of the boat.

Important: If possible the skipper should keep the vessel directly above the site until the stereo-BRUV reaches the bottom and the crew gives the all clear to depart, i.e. all ropes clear of the boat. If the boat moves off the site before the cameras reach the bottom they will likely be pulled/tip over.

To ensure it the BRUV lands upright in shallow water deployments (i.e. <40 m), tug the unit when it first hits the water to correct the horizontal orientation, then let it sink quickly until it reaches ~1-2 m from the seafloor (ask the skipper for the depth then count out rope lengths as you lower the BRUV to do this), then give it a good yank to make sure it is upright again and lower slowly for the remaining 1-2 m. You should also be able to feel if the BRUV lands well through the rope i.e. one jolt suggest a good landing compared to multiple when it hits bottom then keeps tumbling. A drop camera attached to the stereo-BRUV frame with a quick release system can also be used to ensure the stereo-BRUV lands upright and has a clear field of view. If the stereo-BRUV has fallen over or obstructed then you can simply lift it 1-2m and try lowering again. For deeper deployments, and in high current environments, weights should be added to ensure the frames do not drag or flip. It is recommended that when operating in depth >40 m, and using SeaGis BRUV frames, that two weights are added to the front first then one to the back if necessary. Also, if operating at depth and in high currents that you may need to feed the buoys and rope out in a broad circle around the sample site prior to dropping the BRUV in the water. This reduces the OHS risk associated with long ropes.

- 14. On the data sheet, note the exact time of deployment and depth off the depth sounder, include comments where necessary e.g. issues, weather conditions.
- 15. Mark a GPS waypoint and log the GPS coordinates of the deployment on the recording sheet.
- 16. Once all stereo-BRUVs are deployed it is important to move away from where stereo-BRUVs are set to avoid impacts of vessel noise on fish assemblages.

5.6.3 Retrieval

1. It is currently recommended that stereo-BRUV deployments are made for a minimum of 60 minutes to allow for comparisons with other studies. Therefore, the first stereo-BRUV can be retrieved after a minimum of 60 minutes from deployment.





- 2. The skipper should manoeuvre the vessel alongside the floats heading upwind or current towards the stereo-BRUV. A crew member will either gaff or grapple the rope near the floats and quickly hand haul in the slack rope.
- 3. The skipper should then manoeuvre the vessel directly above the stereo-BRUV. Once above the stereo-BRUV, the rope should be placed in the pot hauler or winch if available or pulled by hand.
- 4. It is important that the stereo-BRUV is not hauled until the vessel is directly above. This is to minimise the risk of snagging the rope or stereo-BRUV and to minimise damage to the habitat.
- 5. As the stereo-BRUV comes off the bottom the skipper should then manoeuvre the boat downwind or current to assist retrieval.
- 6. Only a crew member who is trained in using the pot hauler or winch, or under supervision by a trained crew member, should winch the stereo-BRUV.
- 7. The second crew member should help coil the rope that will aid in a quick redeployment.
- 8. Once the stereo-BRUV comes into view and is close to the boat, inform the skipper and slow the winch to ensure the bait and diode and facing away from the vessel.
- 9. The stereo-BRUV should be winched onto the deck or gunnel and carefully lowered down.
- 10. Dry the seals around the housing with a towel and carefully remove the cameras (if conducting surveys over multiple days, the O-rings will require cleaning and re-greasing with silicone at regular intervals, ideally daily).
- 11. Stop cameras recording, and turn them off. Store cameras in a dry, safe place until next deployment. If possible, turn off lights to conserve battery.
- 12. Remove memory cards and store.
- 13. If required charge or change camera batteries.
- 14. Either setup the stereo-BRUV for redeployment or secure on deck.

5.6.4 Retrieval of snagged or lost BRUV

In the event that a BRUV becomes snagged on the bottom the following procedure should be followed:

- 1. Stop retrieval.
- 2. Reposition vessel in opposite direction to initial attempt and recommence retrieval.
- 3. Repeat as necessary altering retrieval direction each time. Caution is needed as it is important to not allow rope to become worn either because of fouling on reef or at the pot hauler.
- 4. Some types of stereo-BRUV frames either bend or break at sacrificial pins (if fitted).

In the event that a stereo-BRUV is lost (rope cut or the current drags the camera system) the following procedure should be followed:

- Attempt to grapple camera frame or rope. This can be challenging in deep water and will take time. A good technique for grappling in deep water is to attach weights every ~10 m on grapple line. Deploy the grapple line so that you encircle the stereo BRUV location. Weighting the grapple line ensures a higher chance of entangling lost stereo BRUV or its rope. Retrieve grapple and repeat as necessary.
- 2. If this fails an alternative approach is to locate lost stereo-BRUV using drop camera system or ROV if available. A depth sounder could also be used to rope and locate floats if submerged.

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- 3. Lower the drop camera on the grapple rope to locate stereo-BRUV.
- 4. Winch as usual.
- 5. If the stereo BRUV system is not retrieved within 60 minutes, mark its exact location with GPS and/or anchored rope with buoys. Mobilize dive team (if in shallow enough water) or a ROV (for deeper waters) if available.
- 6. Notify Parks Australia of lost equipment if operating in an AMP.

5.6.5 Fieldwork data management

Data management and quality control is crucial for monitoring and comparisons between studies within AMPs. Following simple steps and using easily understandable and transferable metadata (Table 10) will enable simple harmonisation between studies.

- Store used cards separately from unused cards to avoid confusion. If storing all memory cards to download, ensure they are clearly labelled and stored in a waterproof container. Memory cards should not be re-used or reformatted until data has been downloaded and a backup created.
- 2. If downloading occurs in the field it is important that all hard drives are clearly labelled in a way that can be discerned from the file name. For example: using the date, study name, and hard drive number, "176022_Groote_Island_stereo-BRUV_HD1".
- 3. Files should also be labelled in a way that can be discerned from the filename. For example, with site_year_month_day_study_cam1_cam2_L (folders on hard drives should follow a naming convention so that programs like "Bulk Rename Utility" can be easily used to rename all files with OpCode and camera number in the correct format).
- 4. Field metadata sheets should be transcribed/backed-up into a database or Excel spreadsheet which should be saved and backed up daily.

5.7 Post-Survey Procedures

5.7.1 Data management

Large amounts of data are created from BRUVS with large video files, field data sheets, and software output. It is therefore important to consistently label folders and files to easily locate data and to simplify analysis. We also recommend documenting the file naming and folder structure in a post-survey report (Appendix C).

5.7.2 Processing video footage

Fish annotations

It was recently recognised by the national BRUVs steering group that, where possible, species composition, abundance and length data for all species should be recorded. It is recommended that every fish within a MaxN frame should be measured. However, fish that occur in large schools, and are of similar size, can be attributed to binned length measurement using the Number field associated with each length in EventMeasure-Stereo (see below). It is important to document the range from camera as this is likely to change between regions/ecosystems. This information is included in the standard outputs of EventMeasure-Stereo and is imported by default into

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GlobalArchive (see Section 5.7.4). Fish that occur in large schools can be attributed to binned length measurement using the Number field associated with each length in EventMeasure-Stereo.

There are several software packages available, but it is important the output from the analysis of data is in the same or similar formats to facilitate comparison of data between campaigns, studies, and organisations. The most commonly used annotation software is EventMeasure-Stereo from SeaGIS (<u>https://www.seagis.com.au</u>). If afforded then the EventMeasure-Stereo software is recommended, unless your organisation already has an alternative established stereo-video annotation workflow (e.g. AIMS). The essential information produced by such annotation software includes three main outputs:

- Point information
- Length measurements
- 3-D point information

Point information is typically used to calculate MaxN values, while length and 3D point information is used to calculate length and biomass metrics. EventMeasure-Stereo has established queries built-in to produce typical metrics over a user defined period within the footage. In addition, EventMeasure-Stereo annotation datasets held within GlobalArchive (<u>http://globalarchive.org/</u>) can be queried in a similar fashion to produce such metrics (see the manual for <u>GlobalArchive</u>).While there are a number of relative abundance metrics available, MaxN (maximum number of individuals for given species counted within the field of view at the same time) is the most widely accepted (Cappo et al. 2007, Harvey et al. 2007).

Type of fish length (e.g fork length or total length for fish and disc length for rays) should be clearly indicated as part of the adequate annotation information for each Campaign.

Habitat classification from field of view

Scoring of habitat information from the field of view is a relatively quick process and can provide extra information about habitat type. Classification of benthic composition and relief should be recorded from still image grabs for each deployment (e.g percent cover of benthos types) (Recommended). Collecting this information as continuous variables will enable regression approaches to be used to investigate the influence of habitat within the field of view on the fish assemblage. To enable comparisons between studies it is important that researchers use comparable classification schemes. Recent studies (McLean et al. 2016, Collins et al. 2017) have adopted the CATAMI classification scheme (Althaus et al. 2013) in a systemised approach to scoring habitat composition and relief from forward facing imagery using TransectMeasure from SeaGIS (<u>https://www.seagis.com.au</u>). This approach and standardised annotation schema have been documented in an open-access <u>GitHub repository</u> (Langlois 2017).

5.7.3 Quality control and data curation

Quality control and data curation are vital, but are potentially time consuming. These time considerations (and associated costs) should be considered during the survey planning stages.

All data corrections should be made within the original annotation files (i.e. within EventMEasure) to ensure data consistency over time. Four complementary approaches for QAQC of data are recommended:

• Analysts should first be adequately trained by completing deployments for which a species composition and density are known to which they can be compared.

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- Once the first annotation for a deployment is completed, a different analyst should view each MaxN annotation to double check the species ID and abundance estimates.
- Footage from any previously unrecorded (i.e. range or depth extensions) or unidentifiable species should be sent to the project taxonomist for formal ID. It is important to send footage clip rather than still images.
- R workflows are provided in a <u>GitHub repository</u> to enable comparison with regional species lists and likely minimum and maximum sizes for each species (Langlois et al. 2017).

It cannot be stressed enough that any corrections should be made to the annotation files before data is exported to GlobalArchive or other repositories (i.e. only QC-d annotations should be publicly released).

A national BRUV steering group has been set up to oversee a nationally coordinated BRUV monitoring program (Table 5.1). Any new BRUV deployments should be discussed with this steering group to ensure that, where possible, they can be integrated within the national program (*Recommended*).

Name	State	Organisation		
Euan Harvey*	Western Australia	Curtin		
Tim Langlois	Western Australia	UWA		
Neville Barrett	Tasmania	IMAS		
Jacquomo Monk	Tasmania/Victoria	IMAS		
Alan Jordan	New South Wales	NSW DPI		
Hamish Malcolm	New South Wales	NSW DPI		
Daniel Ierodiaconou	Victoria	Deakin		
Charlie Huveneers	South Australia	Flinders University		
Leanne Currey	Queensland	AIMS		

Table 5.1 Key contacts in national BRUV steering group, as of Jan 2018.

* Chair

5.7.4 Data release

GlobalArchive (<u>www.globalarchive.org</u>) is a centralised repository for stereo- and single-camera fish image annotation data, in particular from Baited Remote Underwater stereo-Video (stereo-BRUVs) and Diver Operated stereo-Video (stereo-DOVs). A user manual for GlobalArchive is available in an open-access <u>GitHub repository</u>. Metadata should be made publicly available via GlobalArchive as soon as possible after survey completion and data QA/QC and validation. This should include positional data, as well as the purpose of the sampling campaign, the survey design, all sampling locations, equipment specifications, and any challenges or limitations encountered. Annotations can

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also be uploaded once complete. Spatial metadata from GlobalArchive data will in the future be harvested by the Australian Ocean Data Network, and the metadata will accordingly be available on their national portal. Until this is done, metadata should be published on both GlobalArchive and AODN to ensure data discoverability [*Recommended*].

There is currently no national repository for BRUV imagery so we recommend following agencyspecific protocols to ensure public release. A national marine imagery repository (including for BRUV imagery) will be scoped in 2018 and updates provided in Version 2 of this field manual.

Following the steps listed below will ensure the timely release of video and associated annotation data in a standardised, highly discoverable format.

- Immediate post-trip reporting should be completed by creating a metadata record documenting the purpose of the BRUV sampling campaign, the survey design, all sampling locations, equipment specifications, and any challenges or limitations encountered. This can be done far in advance of annotation (scoring) of raw video which is time-consuming and often does not occur for some time following completion of sampling.
- 2. Publish metadata record to the <u>Australian Ocean Data Network (AODN) catalogue</u> as soon as possible after metadata has been QC-d. This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the <u>AODN Data Submission</u> <u>Tool</u>. Note that user registration is required, but this is free and immediate.

Lodging metadata with AODN in advance of annotation data being available is an important step in documenting the BRUV campaign and enhancing future discoverability of the data.

- 3. Annotate video (fish counts and length) using EventMeasure or similar software.
- 4. Upload annotation data and any associated calibration, taxa and habitat data to GlobalArchive.
- 5. Upload raw video data to a secure, publicly accessible online repository (<u>contact AODN</u> if you require assistance in locating a suitable repository for large video collections).
- 6. Add links to GlobalArchive campaign and raw video storage location to previously published metadata record. You may also wish to attach or link a copy of the annotation data directly to the published metadata record.
- 7. Produce a technical or post-survey report documenting the purpose of the survey, sampling design, sampling locations, sampling equipment specifications, annotation schema, and any challenges or limitations encountered. Provide links to this report in all associated metadata. See Appendix C [Recommended]

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5.8 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 5 (field manual for Benthic BRUVs) is below:

Version Number	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019



Table 5.2 Example metadata sheet for benthic stereo-BRUV fieldwork. Left and right memory card numbers must be recorded for each camera pair.

Date	Site	BRUV#	Cam. Left #	Cam. Right#	Time in	Location in	Time out	Depth	Comments
2017-10-25	SITE-A	15	12	10	08:00	(115.12E; 32.54S)	10:15	95m	



5.9 References

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6. MARINE SAMPLING FIELD MANUAL FOR PELAGIC STEREO BRUVS (BAITED REMOTE UNDERWATER VIDEOS)

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Photograph: Pelagic stereo-BRUV deployed in French Polynesia. Credits: Manu San Felix, National Geographic Society (2014).

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6.1 Platform Description

Underwater videography has become a staple of observational studies in both tropical and temperate environments, where the technique offers a robust, non-invasive, and affordable means of monitoring marine species *in situ* (Mallet and Pelletier 2014) (Chapters 4, 5, 7). Initially pioneered for applications in the abyssal zone (Priede et al. 1994), benthic BRUVs (see Chapter 5) have been extensively used in shallow, inshore environments (e.g. McLean et al. 2011, Langlois et al. 2012, Zintzen et al. 2012, Oh et al. 2017, Juhel et al. In press).

However, a growing international commitment to expand the world's marine protected area coverage in recent years (Pala 2013) has motivated efforts to adapt BRUVs to pelagic, open ocean habitats away from coasts (Bouchet and Meeuwig 2015). Multiple research groups and organisations (Table 6.2) have concurrently developed several pelagic BRUV designs (Figure 6.1), most of which share similar elements, namely (i) one (monocular) or a pair (stereo) of cameras in appropriate underwater housings, (ii) a base frame on which the camera(s) is/are mounted, (iii) an attractant, usually olfactory in the form of bait, (iv) a synchronisation device (e.g. diode, clapperboard) and (iv) a suspension system (consisting of weights, ropes, and floats).

Pelagic BRUVs retain all the qualities that have made camera-based sampling a flexible and effective approach to non-destructive marine monitoring, as:

- They are suitable in areas where fishing or other extractive activities are prohibited.
- They are straightforward and relatively quick to operate.
- They have little direct impact on wildlife and ecosystems, other than through bait use.
- They present a safety advantage over diver-based methods and overcome some of their limitations and biases (e.g. depth and time constraints, avoidance behaviour in fishes).
- They produce accurate body length measurements when configured in stereo.
- They yield a permanent archive of high-definition footage.
- They generate quantitative data, while also documenting behaviour.
- They are viable in a range of depths, underwater terrains and ocean conditions.

Importantly, the use of one or more attractants substantially increases the likelihood that nearby animals enter the field of view of the cameras for digital capture (Rees et al. 2015). Extensive collective experience in the deployment of pelagic BRUVs across a range of habitats, climates, and conditions indicates that the instruments are capable of detecting a large suite of taxonomic groups (including many of interest to fisheries), from teleost fishes to elasmobranchs, marine mammals, molluscs, crustaceans, and reptiles (Figure 6.2).

In spite of their performance, pelagic BRUVs suffer from a number of limitations, many of which apply equally to demersal videography, including:

- Footage quality is affected by high turbidity and low visibility.
- Correct identification of some species can be difficult for small, shy or morphologically similar species and individuals.
- Bait dispersal is a complex, dynamic process likely to fluctuate spatio-temporally. Quantifying the size of the effective area being sampled and its variation remains an unresolved challenge.

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- Bait elicits diverse animal behavioural responses whose strength, timing and duration often relate to many unknown parameters (e.g. olfactory performance, prey search strategy, human presence etc.).
- Numerous species may also respond to non-olfactory cues in ways that have seldom been quantified (but see Rees et al. 2015).
- The nature and magnitude of observation biases arising from the presence of conspecifics (and other species) are largely unknown (Dunlop et al. 2014, Coghlan et al. 2017).
- Counts of wildlife on BRUVs reflect measures of *relative* rather than *absolute* abundance and can be biased, e.g. by screen saturation (Lowry et al. 2011, Schobernd et al. 2013).
- Detection/attraction probabilities likely vary by time of day, habitat, bathome, and species.
- Zero-inflation is common and may undermine the statistical power needed to identify patterns and changes in pelagic communities (Santana-Garcon et al. 2014b).
- Benthic "species contamination" can occur wherever the ratio between suspension and seabed depths approaches one (e.g. pelagic BRUVs suspended at 10 m in a total of 15 m of water) (Letessier et al. 2013b).

Further discussion of some of these caveats can be found in Bouchet and Meeuwig (2015), Santana-Garcon et al. (2014b) and Espinoza et al. (2014), among many others.

6.2 Scope

This manual relates to gear designed to acquire digital video imagery of macro-organisms living in the ocean's water column, from small zooplankton (Letessier et al. 2013a) to marine megavertebrates (Letessier et al. 2014). A sister chapter on benthic BRUVs is included in the field package and addresses sampling protocols for demersal fish and shark assemblages (Chapter 5). The document aims to span everything from pre-survey planning to equipment preparation, field procedures, and on-board data acquisition to guarantee the efficient and correct use of pelagic BRUVs as monitoring tools in Australian Marine Parks (AMPs) and other Commonwealth waters. Such information is critical for supporting the development of consistent, concise, transparent and standardised guidelines in the collection and processing of pelagic BRUV data that can allow statistically robust comparisons between studies, sites, projects, and institutions.

Here, we consider both mono- and stereo-BRUVs⁷. While the latter can be calibrated to allow measurements of individuals' body lengths and animal positions in three-dimensional space (Letessier et al. 2015), the former seems to remain a more prevalent approach in the literature due to lower costs and personnel/labour requirements (Whitmarsh et al. 2017). It is worth noting that other imagery-based methods such as mid-water towed video transects (Riegl et al. 2001), in-trawl cameras (Underwood et al. 2014), drop cameras (Friedlander et al. 2014), infrared thermography (Zitterbart et al. 2013), unmanned aerial vehicles (Kiszka et al. 2016), or diver operated videos (Goetze et al. 2015) are also available for monitoring pelagic environments and wildlife. These would each warrant a field manual in their own right (Mallet and Pelletier 2014), and are thus not included here (for further information, see Bouchet et al. 2017).



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6.3 Pelagic BRUVs in Marine Monitoring

The need for pelagic monitoring programmes is becoming increasingly urgent as the diversity and abundance of pelagic species decline and the pressure to meet global conservation targets rises (Letessier et al. 2017). While pelagic baited video techniques remain in their infancy, they show promise as efficient and affordable tools for monitoring wildlife communities and characterising biodiversity patterns at a range of spatial and temporal scales. For instance, Letessier et al. (2013b) and Heagney et al. (2007) were able to detect regional differences in the structure of pelagic fish assemblages, whilst Santana-Garcon et al. (2014b) reported changes in species diversity with water depth. Pelagic BRUVs may therefore be useful for providing rapid assessments of the effects of spatial closures. Although neither Heagney et al. (2007) nor Santana-Garcon et al. (2014c) found significant differences in species composition and relative abundance between fished and protected areas within their respective study sites, their data represent valuable baselines for future surveys. Knowledge of pelagic species distributions and habitat preferences are also critical to successful management, and pelagic BRUVs can yield geo-referenced data with sufficient replication to support the development of predictive statistical models (Bouchet and Meeuwig 2015). Lastly, pelagic BRUVs allow cost-effective observations of behaviour in free-ranging animals that might otherwise be difficult to obtain outside laboratory settings (Santana-Garcon et al. 2014a, Kempster et al. 2016, Ryan et al. 2018). Many aspects of the behaviour and basic biological requirements of pelagic fishes remain largely unknown, and pelagic BRUVs can thus be a powerful way of filling these knowledge gaps, for example by documenting biologically important areas like spawning (Fukuba et al. 2015) and nursery grounds (A. Forrest, unpublished data).

In brief, BRUV sampling (and by extension pelagic BRUV sampling) generates quantitative, monitoring-relevant data on:

- The extent and magnitude of anthropogenic impacts (e.g. fishing, climate change, oil and gas exploration, novel ecosystems such as man-made structures).
- Temporal and spatial variability in the relative diversity, abundance, and size structure of fish assemblages (when used in stereo).
- Behaviour observed in situ.
- Species-habitat relationships.

For a detailed overview of observational methods used in the spatial monitoring of fishes, with notes on baited videography, see Murphy and Jenkins (2010) and Mallet and Pelletier (2014). Struthers et al. (2015) offer additional insights into the value and limitations of action camera technology for field studies and education/outreach.

6.4 Equipment

It is crucial that equipment be appropriately set up to ensure maximum consistency among surveys and to facilitate gear replacement where/when necessary. Key components for a pelagic BRUV are listed in Table 6.3.

Equipment configurations can vary among terrains, bathomes and as a function of study objectives (Figure 6.1). For instance, Santana-Garcon et al. (2014b)'s design is remarkably stable compared to Letessier et al. (2013b) but is constrained by the need to moor, which Bouchet and Meeuwig (2015)'s design bypasses. Likewise, bait arm length is usually variable, and may be reduced under turbid conditions to optimise species identification capacity.







Figure 6.1 Examples of possible deployment configurations for pelagic BRUV sampling. Schematics extracted from or as used in (A) Santana-Garcon et al. (2014b), (B) Schifiliti et al. (2014) and Kempster et al. (2016), (C) Letessier et al. (2013b). Cameras can be either forward-facing (A, C) or downward-facing (B). The anchored design shown in C was adapted in Bouchet and Meeuwig (2015) to let BRUV units drift freely.



6.5 **Pre-Survey Preparations**

6.5.1 Methodology

<u>A statistically robust sampling design must be chosen</u>, allowing for adequate spatial/temporal coverage and replication whilst meeting the overall survey objectives, given available equipment and vessel time. Santana-Garcon et al. (2014b) recommend a minimum of 8 replicates per experimental treatment in warm-temperate and tropical coastal environments, although this may be dependent on the geographic distribution and abundance of species. The final design should be communicated to all personnel before the survey to maximise clarity and efficiency during field operations. As a rule, pelagic BRUVs should be deployed a minimum of 200-500 m apart to reduce the likelihood of bait plume overlap and inter-camera animal movements (Santana-Garcon et al. 2014b, Bouchet and Meeuwig 2015), but further field testing is required to determine if this separation is sufficient to consistently guarantee independence between replicates when sampling large, mobile vertebrate species. See Chapter 2 for additional details.

The timing and duration ("soak time") of BRUV deployments should be determined. Deployments conducted 30-60 min after sunrise and before sunset should abate the effects of differential crepuscular behaviour in fishes (Axenrot et al. 2004, Potts 2009). If BRUVs are only one part of a larger research programme, it is important to think carefully about the timing of BRUV operations, as bait use may bias subsequent observations at that same site (e.g. if diver surveys were to follow). Optimal soaking time is likely to vary across habitats and represent a practical compromise between increasing sample size and making the best use of available vessel time in light of the target level of replication. Previous studies have reported soaking times of 45 min (Rees et al. 2015), 120-135 min (Letessier et al. 2013b, Santana-Garcon et al. 2014c), 165 min (Bouchet and Meeuwig 2015), or 180 min (Santana-Garcon et al. 2014b). Santana-Garcon et al. (2014b) suggested a soak time of 120 min. In cool-temperate waters, Bouchet and Meeuwig (2015)'s species accumulation curves failed to plateau after 3 hours. Although some attempts have been made to develop a range of plausible bait plume dispersal models (e.g. Olsen & Laevastu 1983; Sainte-Marie & Hargrave 1987), further on bait diffusion in the mid-water is needed to confirm the minimum distance that should be allowed between deployments, estimate the effective sampling area in a range of conditions, and better understand the dynamics bait flushing across different levels of fish activity. Lastly, careful thought must be given to the choice of suspension depth, as different assemblages may vary along depth gradients away from the surface (Santana-Garcon et al. 2014b).

<u>Consideration must be given to the location of BRUVs during deployment.</u> Instruments should not be deployed where there is a risk of entanglement (e.g. near fishing gear) or where they are likely to constitute or become a navigational hazard (e.g. inside shipping lanes, where trawlers are operating). At a minimum, deployment and retrieval locations should be recorded, with vessel location monitored at regular time intervals as a back-up. GPS loggers can be mounted on flag poles or buoys when deploying free-drifting BRUVs and are advised for capturing the exact spatial trajectories of the units (Bouchet and Meeuwig 2015). VHF radio beacons are also recommended to avoid gear loss in adverse weather conditions. Geofencing technology could be used (as it has been with fish aggregation devices) should the user need to be alerted when BRUVs exit a predefined area.

<u>Appropriate approvals must be obtained.</u> All research activities within Australian Marine Parks are to be undertaken under permit, and most institutions will also require Animal Ethics approval, even if the proposed methods are non-invasive. All institutional health and safety requirements must also



be satisfied (e.g. travel risk assessment, volunteer insurance proposal). See Appendix B for a list of potential permits required at the Commonwealth level).

<u>Appropriate camera settings must be selected</u> (e.g. frame rate, video resolution, field of view mode, action cams vs camcorders, see Table 6.1) in light of their performance relative to the study goals and market availability. Correct date/time settings are particularly crucial for file management during subsequent analyses. When using GoPro cameras, note that standard and dive housings are rated to 40 m and 60 m respectively. Special backdoors must be also fitted if battery packs are considered. All equipment must be carefully checked prior to deployment, including that cameras have been serviced, cleaned, and calibrated (if using stereo-BRUVs). Spares (batteries, memory cards, cameras, Table 6.3) are essential as a contingency plan against equipment failure/damage/loss or adaptive changes in the sampling plan (e.g. additional deployments).

Table 6.1 Example camera settings for a pelagic BRUVs. Values reflect the use of GoPro Hero3 cameras. Options may differ in other camera models.

Settings	Value				
Camera					
Resolution	1080				
Frame Rate	25 fps				
Field of View	Medium				
Capture					
Upside Down	Up				
Spot Meter	Off				
Looping Video	Off				
Set up					
Default Mode at Power Up	Video (default)				
One Button	Off				
NTSC/PAL	PAL				
Onscreen Display	ON				
Camera Status Lights	2				
Sound Indicator	Off				
Manual Power Off	Manual				

Bait must be ordered ahead of time in sufficient quantities. Sourcing bait locally from factory discards (e.g. fish heads, tails and guts) is an attractive option for reducing costs and the ecological footprint of sampling. For some applications, bait balls comprising minced fish, oil, and/or meal, may also be appropriate, though care should be taken to standardise bait mixtures across deployments. Between 800g-3kg of bait is generally adequate for deployments of up 3 hours (Letessier et al. 2013b, Santana-Garcon et al. 2014b), though having extra supplies (e.g. 20%) may be useful if extra/longer deployments can/must be undertaken. Ultimately, the choice of bait quantity should be informed by consideration of the desired soaking time, expected flushing rate, and likely level of fish activity. Sufficient freezer space must be made available on-board accordingly. Debate is still ongoing over the most efficient way to prepare bait, although crushed/slurried mixtures seem more likely to disperse well into the water column. Presentation is also important, with wire mesh baskets (Santana-Garcon et al. 2014b) and perforated PVC tubes (Bouchet and Meeuwig 2015) being two popular options, despite the lack of comparative studies of their relative efficiencies. Critically, recent research demonstrates that bait alone may be a biased/poor attractant for pelagic fishes, and that consideration should be given to combinations of multiple attractants associated with sight, sound, and scent to help generate more effective abundance estimates for some species (Rees et al. 2015).



<u>Rig set up should reflect the chosen BRUV design</u>, and may need to be adapted in response to vessel constraints (e.g. available deck space). It is critical to check that the correct amount of weight, length of ropes, number of buoys etc. are available before the survey begins (Figure 6.1). Spare units and parts are essential in all circumstances.

<u>Sampling gear specifications should always be fully documented</u> to achieve maximum transparency and comparability. Over a third of studies fail to report on basic methodological choices (Whitmarsh et al. 2017), including rigging plans, camera orientation, spacing, convergence angle, field of view, inter-BRUV distances, soak time, bait choice and quantity, bait preparation technique, bait dispenser type, suspension depth, deployment configuration (Figure 6.1), number of replicates, among others.

<u>Data storage needs must be anticipated.</u> 2TB portable hard drives will typically provide enough storage space for 100 hours of high-resolution video footage, though this may vary by camera model/make. Equally important is making sure that enough power boards, adapters, USB hubs, data cables, etc. are purchased, and can be configured safely for use at sea, so that data offload and backup following each deployment can occur. Planning for double copies of each hard drive and for offline storage on institutional servers is highly recommended to avoid data loss in the event of hardware failure.

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6.5.2 Pre-survey checklist

Task	Description/comments
Sampling design chosen and coordinates of sampling sites calculated and checked for safety hazards	
Pelagic BRUV design and configuration determined	
Deployment protocol determined, including methods for locating/tracking gear	
Appropriate permits obtained and printed copies made (on waterproof paper if necessary)	
Bait (and/or other attractants) ordered in adequate quantities	
Camera settings determined, and cameras calibrated as appropriate	
Data storage needs identified and hardware purchased accordingly	
Metadata sheet prepared	
Gear shipment arranged	



6.6 Field Procedures

A visual summary of the key steps to follow when deploying pelagic BRUVs is shown in Figure 6.3.

6.6.1 Calibrations

Stereo-BRUVs require calibration to ensure accurate length measurements. Calibration frequency will ultimately depend on the hardware used and recommendations from the manufacturer. Calibrations are best carried out prior to surveying and commonly take place in enclosed pool environments. Additional post-survey calibrations are also advantageous, particularly following long sampling campaigns where the risk of camera displacement during operation or transport is higher. The calibration process takes into account the base separation, camera angle and lens distortion, all of which are unique to each BRUV (Harvey and Shortis 1998), meaning that individual units must hence be calibrated separately, and cameras should not be swapped between units. In addition, if a camera is damaged or knocked out of position during field work, calibrations will need to be repeated post-survey. While some studies show that purpose-built three-dimensional calibration cubes yield maximum accuracy (Boutros et al. 2015), recent evidence suggests that planar checkerboards may be equally accurate, at a fraction of the cost (Delacy et al. 2017). Where possible, carrying out 'mock deployments' of a single unit may be useful to ensure the BRUV units sit correctly and consistently in the water column.

<u>SeaGIS</u> have long been the primary provider of third-party calibration hardware and software, yet alternative open-source packages have now also begun to emerge, including the <u>MATLAB</u> <u>Calibration Toolbox</u> or the <u>StereoMorph</u> R package (Olsen and Westneat 2015, Díaz-Gil et al. 2017).

6.6.2 Arrival on site

- 1. Unpack equipment and check for any damage that may have occurred during transport.
- 2. Check that all camera settings are correct (Table 6.1), batteries are full and memory cards formatted.
- 3. If not already done, number each individual camera and memory card using a permanent marker, and make a note of which card is used in which camera on the data sheet. It may be useful to also number batteries and battery extension packs, to facilitate the troubleshooting of any hardware malfunctions.
- 4. Lubricate the cameras' O-rings and check them for cuts or nicks. Replace damaged O-rings as appropriate.
- 5. Set up pelagic BRUV unit(s) (see Table 6.4 for an example). Attach bait containers to bait arms and securely stack/stow equipment on deck.
- 6. Discuss deployment and safety plans with captain/crew/team and deliver a copy of sampling site coordinates to the skipper.

6.6.3 Deployment

- 1. Take bait out of the freezer before sampling and place it in a rubber bin (empty or filled with seawater) to allow it to thaw. This can be done anywhere between 1 and 12 hours beforehand. Note that in tropical countries, bait loses texture and quality if thawed too early. It is also generally easier and cleaner to crush half-frozen bait than bait that has thawed fully.
- 2. When on route to the drop location, rigs can be laid out in order with the first rig to be deployed closest to the stern (along with corresponding lines if a winch is not being used).

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- 3. Prepare bait (e.g. mince, slice or crush) and fill bait bags/canisters with desired weight.
- 4. Seal bait canister (e.g. tighten screw caps) and store upright in a plastic container until use.
- 5. Check that metadata sheets are ready (see Table 6.5). These sheets should be printed on waterproof paper before leaving for the expedition. Fill in drop numbers, camera numbers and memory card numbers when preparing cameras for the day's work. Follow this in the field and fill in the other information as available.
- 6. Attach lights and sensors, if available.
- 7. If using a VHF transmitter, remove the magnet and note the device's frequency, checking it is working correctly and a signal can be heard/detected. Place it in a small pelican case attached to the flag buoy, along with one GPS logger (turned on by holding down the middle button) and close tightly.
- 8. Insert cameras into housings and check that the housings are dry and sand-/hair-free, without any other objects obstructing the O-rings to ensure a good seal.
- 9. Turn the cameras on (e.g. for GoPros, by pressing the front button until the red light starts flashing and the timer starts), check there is battery and storage space available.
- 10. Place the data sheet (or Magnadoodle/slate/white board/paper sheet) showing drop number, date, rig number and location in front of each camera and in the centre over the bait arm so that it is clearly seen in the fields of view of both cameras. Verbal logs are an alternative/complementary option, as modern cameras are usually sufficiently sensitive to record spoken instructions/information.
- 11. Attach a diode to the bait arm if using stereo-BRUVs. If a diode is not available, clap slowly 3-4 times in front of the cameras (using a clapperboard or bare hands) over the bait arm in clear view to allow synchronisation during video analysis.
- 12. Attach the flag pole, one cluster of buoys and the first of the rigs to be deployed to the end of the first longline via double action clips. Ensure the rope is free, coiled, and facing the correct direction to un-coil without hindrance
- 13. At the captain's go-ahead (i.e. vessel in position and stationary), drop the flagpole into the water.
- 14. Once the flagpole is clear, push or throw the first rig so that it clears the side of the boat, ensuring all lines are clear of feet and untangled. Drop the cluster of buoys over first, followed by the rig ensuring not to drop the rig on any of the other lines in the water. This works best if one person handles the buoys and another the rig. Note that this sequence differs slightly for moored BRUVs, which require the ballast/anchor to be dropped first, followed by the rig and the floats in this order.
- 15. Mark a GPS waypoint when the unit is deployed and record both deployment time and site coordinates on the data sheet, which will have been pre-populated with location, rig number, camera numbers, memory card numbers etc. Include comments where necessary e.g. issues, weather conditions.
- 16. For single-rig designs, travel to the next site. For multi-rig designs, repeat until all units are in the water, making sure the captain moves forward slowly to pay out the lines.

6.6.4 Retrieval

- 1. Manoeuvre the vessel alongside the flag/grappling buoy, heading upwind of the current towards the BRUV.
- 2. Either gaff or grapple the rope joining to flag buoy to the first cluster of buoys.

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- 3. Haul the line in and retrieve the flag buoy, taking care not to knock the tension wires on the stern of the boat. Remove and store the VHF transmitter and GPS logger when convenient. Wear gloves when hauling and coiling. Pelagic BRUVs are relatively light so manual handling is generally possible, however use a winch or pot hauler if available and warranted.
- 4. Unclip buoys and coil rope to facilitate future deployments.
- 5. Turn off the cameras, rinse them with freshwater, dry the seals around the housings with a towel and carefully remove the cameras from their housings when convenient. If conducting surveys over multiple days, it is good practice to clean and re-grease the O-rings with silicone at regular intervals.
- 6. Store the rig and buoys out of the way.
- 7. Repeat until all units are retrieved.
- 8. Remove memory cards.
- 9. If required, charge or change camera batteries.

10. Either setup the equipment for redeployment or securely stow on deck.

6.7 **Post-Survey Procedures**

Data management and quality assurance/control are crucial for monitoring and comparisons between studies within a given area. Following simple steps and using easily understandable and transferable metadata (see Table 6.5) will enable efficient harmonisation between studies.

6.7.1 Data management

Store used cards separately from unused cards.

- 1. Download the video data onto a portable hard-drive using a card reader or equivalent.
- 2. Save the files from each camera in a separate folder named using the unique site/drop identifier and L for left side or R for right side (e.g. CH001L).
- 3. Use multiple laptops or extra card readers to speed up the process.
- 4. During downloads, check that the videos are of good quality and note any interesting species etc. If any issue occurred with a camera, rig etc. attempt to rectify the issue before the next day's sampling.
- 5. At the end of each day, make a backup of the day's videos to two hard-drives stored in separate locations.
- 6. Transcribe the data from the data sheets into an expedition spreadsheet updated and backed up daily. The spreadsheet should also include the hard drive number where each sample is saved.

<u>Note:</u> It is important that all hard drives be clearly labelled – e.g. with the date, project name, contents and hard drive number. Ideally, files should also be labelled according to a standardised and unambiguous naming convention. All memory cards should be stored in waterproof containers. They should not be re-used or reformatted until data has been download and a backup created.

Pelagic BRUVs typically generate large volumes of data, including video imagery, field data sheets and software outputs. Consistently labelling folders and files is therefore essential to easily locating information and simplifying analyses. An example folder name is "176022_Groote_Island_stereo-BRUV_HD1", which concatenates the deployment date, study location/name, and hard drive



number. Similarly, an appropriate file name could reflect the following structure: OpCode_year_month_day_study_cam1_cam2_L (folders on hard drives should follow a naming convention so that programs like Bulk Rename Utility can be easily used to rename all files with OpCode and camera number in the correct format). Template folder/file structures and further details on data management and quality control are provided in Chapter 5.

At this stage, there are no online video file storage databases, however the <u>GlobalArchive</u> platform has been created to store metadata (see Section 6.7.4). Refer to the software's website for instructions on metadata and data recording instructions.

6.7.2 Quality control

Quality assurance/quality control (QAQC) is an equally vital but potentially time-consuming undertaking for organisations and individual researchers. Following straightforward steps and using easily understandable and transferable metadata will enable harmonisation between studies.

It is important that any data corrections are made within the original annotation files to ensure consistency over time. Four complementary QAQC approaches are recommended:

- Analysts should first be adequately trained by processing videos for which species composition and density are known, and to which their results can be compared.
- Once the first annotation (fish counts and lengths) for a deployment is completed, a different analyst should view each MaxN annotation to double-check the species ID and abundance estimates.
- Footage from any previously unrecorded (i.e. range or depth extensions) or unidentifiable species should be sent to the project taxonomist for formal ID. It is important to send footage clip rather than still images.

R workflows are provided in a GitHub repository to enable comparison with regional species lists and likely minimum and maximum sizes for each species (Langlois 2017).

Importantly, any corrections should be made to the annotation files before data is exported to GlobalArchive or other repositories.

6.7.3 Video processing

Trained analysts/fish biologists/taxonomists must be engaged to ensure that all footage can be appropriately processed and species can be correctly identified. Care must be taken to ensure that a consistent nomenclature is used, with FishBase, the World Register of Marine Species (WoRMS) and the Codes for Australian Aquatic Biota (CAAB) being popular, authoritative sources of taxonomic information. Undescribed or unnamed species (e.g. defined operational taxonomic units, OTUs) must also be meticulously documented. Archives of reference images from previous sampling campaigns have been established by numerous agencies across Australia and can serve as a useful benchmark for problematic sightings. The Collaborative and Annotation Tools for <u>Analysis of Marine Imagery and Video (CATAMI) Project</u> offers a framework for the cataloguing, annotation, classification and analysis of underwater imagery (Althaus et al. 2015).

A number of software tools are currently available for image analysis, with <u>SeaGIS EventMeasure</u> being arguably the most widespread but also the costliest. Advanced packages such as <u>Image-Pro</u><u>Plus</u>, <u>SigmaScan</u>, or simpler programmes such as <u>ScreenCalipers</u> can also be used to make measurements calibrated by scale bars. The <u>StereoMorph</u> R package (Olsen and Westneat 2015) is an open-source alternative that additionally allows the reconstruction of 3D objects. Irrespective of the approach chosen, it is critical that any output be produced in a format comparable to other studies to facilitate comparison of data between campaigns and organisations.

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Overestimates of abundance can occur as a result of double counting, for instance when the same individual/s is/are viewed at different time points throughout a deployment. To overcome this challenge, counts of the maximum number (MaxN) of individuals of any one species seen over the recording period have been used. In a monitoring context, comparative studies have suggested that the use of MaxN may be "hyper-stable" (i.e. underrepresents the magnitude of changes in true abundance) when fish abundance is high due to saturation of the field of view (Schobernd et al. 2013) and have suggested alternative metrics (e.g. MeanCount). However, MaxN remains the most widely accepted metric, and provides the best option for standardisation between sampling programs.

The essential information produced by annotation software should include three main outputs:

- Point information
- Length measurements
- 3-D point information

Point information is typically used to calculate MaxN values, while length and 3D point information is used to calculate length and biomass metrics. EventMeasure-Stereo has established queries built-in that produce a number of chosen metrics over a user defined period within the footage. In addition, EventMeasure-Stereo annotation datasets held within GlobalArchive can be queried in a similar fashion to produce such metrics. While there are a number of relative abundance metrics available, MaxN is the most widely accepted (Harvey et al. 2007).

The type of fish length measured (e.g. fork length or total length for fish and disc length for rays) should be clearly indicated as part of the annotation information for each sampling campaign.

6.7.4 Data release

<u>GlobalArchive</u> is a centralised repository for stereo- and single-camera fish image annotation data, in particular for Baited Remote Underwater stereo-Video (stereo-BRUVs) and Diver Operated stereo-Video (stereo-DOVs). A user manual for GlobalArchive is available in an open-access <u>GitHub repository</u>. Metadata should be made publicly available via GlobalArchive as soon as possible after survey completion and data QA/QC and validation. This should include positional data, as well as the purpose of the sampling campaign, the survey design, all sampling locations, equipment specifications, and any challenges or limitations encountered. Annotations can also be uploaded once complete. Spatial metadata from GlobalArchive data will in the future be harvested by the Australian Ocean Data Network, and the metadata will accordingly be available on their national portal. Until this is done, metadata should be published on both GlobalArchive and AODN to ensure data discoverability [*Recommended*].

There is currently no national repository for BRUV imagery so we recommend following agencyspecific protocols to ensure public release. A national marine imagery repository (including for BRUV imagery) will be scoped in 2018 and updates provided in Version 2 of this field manual.

Following the steps listed below will ensure the timely release of BRUV imagery and associated annotation data in a standardised, discoverable format.

 Immediate post-trip reporting should be completed by creating a metadata record documenting the purpose of the BRUV sampling campaign, the survey design, sampling locations, equipment specifications, and any challenges or limitations encountered. This can be done far in advance of annotation (scoring) of raw video which is time-consuming and often does not occur for some time following completion of sampling.



- Publish metadata record to the <u>Australian Ocean Data Network (AODN) catalogue</u> as soon as possible after metadata has been quality controlled (see section 6.7.2). This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the <u>AODN Data Submission</u> <u>Tool</u>. Note that user registration is required, but this is free and immediate.

Lodging metadata with the AODN prior to making annotation data available is an important step in documenting the BRUV campaign and enhancing future discoverability of the data.

- 3. Annotate video (fish counts and length) using EventMeasure or similar software.
- 4. Upload annotation data and any associated calibration, taxa and habitat data to GlobalArchive.
- 5. Upload raw video data to a secure, publicly accessible online repository (<u>contact AODN</u> if you require assistance in locating a suitable repository for large video collections).
- 6. Add links to GlobalArchive campaign and raw video storage location to previously published metadata record. You may also wish to attach or link a copy of the annotation data directly to the published metadata record.
- 7. Produce a technical or post-survey report documenting the purpose of the survey, sampling design, sampling locations, sampling equipment specifications, annotation protocol, and any challenges or limitations encountered. Provide links to this report in all associated metadata. See Appendix C [Recommended].

6.8 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 6 (field manual for Pelagic BRUVs) is below:

Version Number	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019

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Figure 6.2 Example species observed on pelagic BRUVs. (A) Bryde's whale Balaenoptera brydei, (B) Manta ray Manta birostris, (C) Dusky dolphin Lagenorhynchus obscurus, (D) Whale shark Rhincodon typus, (E) Dolphin fish Coryphaena hippurus, (F) Atlantic horse mackerel Trachurus trachurus, (G) Blue shark Prionace glauca, (H) Shortfin mako shark Isurus oxyrinchus, (I) Sea snake Hydrophiidae sp., (J) Green turtle Chelonia mydas, (K) Krill Euphausia sp., (L) Loggerhead turtle Caretta caretta, (M) Atlantic spotted dolphin Stenella frontalis, (N) Longfin yellowtail Seriola rivoliana, (O) Sub-Antarctic fur seal Arctocephalus tropicalis, (P) Yellowfin tuna Thunnus albacares, (Q) Pilot fish Naucrates ductor, (R) Blue marlin Makaira nigricans, and (S) Unicorn leatherjacket Aluterus monoceros.



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Figure 6.3 Images from key steps involved in the use of pelagic BRUVs for marine monitoring. (A) Using a calibration cube in an enclosed pool environment. (B) Once set up, the gear can be easily stacked and stowed on deck. (C) Example of a GoPro camera turned on before deployment. (D) Relevant metadata can be recorded on waterproof paper. (E) Pelagic BRUVs are versatile and can be deployed manually from a variety of platforms ranging in size from small rigid inflatables to large research vessels. (F) Maintaining visual contact with the gear is key to avoiding equipment loss. Should the deployment vessel need to leave the site (e.g. to support additional activities), a VHF transmitter can be used to re-locate the gear. (G) Flags and brightly coloured buoys help locate the equipment for recovery. (H) Videos are typically downloaded and backed up at the end of each sampling day. (I) Processing and analysis of the imagery occur in a computer lab post-survey.

Table 6.2 Summary of pelagic video systems used in marine monitoring. Orientation refers to the angle of the camera(s), and can be either horizontal (forward-facing) or vertical (downward-facing). Deployments can be conducted with instruments either moored to the seafloor ('anchored'), linked to a vessel via a coaxial cable or similar ('tethered), or free drifting (as individual units or in a longline configuration). NSW: New South Wales. WA: Western Australia. Due to differences in local supply, it is difficult to identify a standardised type of baitfish. As a rule, small pelagic species with soft, oily flesh are usually recommended. For instance, sardines/pilchards (*Sardinops sagax*) have been a staple of BRUV research in Australia and New Zealand, as evidence suggests they result in consistent numbers of fish among samples (less variation), exhibit higher mean abundance among sites and are more persistent (i.e. longer time to depletion) (Dorman et al. 2012). MW = mid-water. P = pelagic. S = Stereo.

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Authors	Location	Stereo	Orientation	Method	Attractant	Attractant type	Bait type	Instrument name
Heagney <i>et al</i> . (2007)	Lord Howe Island (NSW, Australia)	×	Horizontal	Anchored	~	Olfactory (dead bait)	Mixture of minced pilchards, bread and tuna oil (8:1:1), combined in matrix of vegetable meal (falafel) [100g]	MW BRUVs
Letessier <i>et al.</i> (2013)	Shark Bay (WA, Australia)	~	Horizontal	Anchored	~	Olfactory (dead bait)	Pilchards, squid, and combination (slurry, 1:1)	MW camera rigs
Rees <i>et al.</i> (2015)	Jervis Bay (NSW, Australia)	×	Horizontal	Anchored	~	Olfactory, visual, acoustic	Visual: Spearfishing 'swivel flasher'. <u>Acoustic:</u> Playback recording of bait fish. <u>Olfactory:</u> Mixture of white bread and pilchards.	MW RUVs
Scott <i>et al.</i> (2015)	Sydney Harbour (Australia)	×	Horizontal	Anchored	✓	Olfactory (dead bait)	Mixture of minced pilchards, bread, and tuna oil, in an (8:1:1) [100g]	P BRUV
Bouchet & Meeuwig (2015)	Perth Canyon (WA, Australia)	✓	Horizontal	Drifting	✓	Olfactory (dead bait)	Crushed pilchard heads, guts and tails [2-3kg]	PS BRUVs



Santana et al. (2014b) Coral Bay \checkmark Olfactory (dead Pilchards [800g] \checkmark Horizontal Anchored PS BRUVs (WA. Australia) bait) Santana et al. (2014a) Ningaloo Reef \checkmark Horizontal Anchored \checkmark Olfactory (dead Mullets (cut in halves) [1kg] PS BRUVs (WA, Australia) bait) Olfactory (dead Crushed pilchards [800g] Santana et al. (2014c) Western Australia \checkmark Horizontal Anchored \checkmark PS BRUVs (several bait) locations) Olfactory (dead Santana et al. (2014d) Houtman \checkmark Horizontal Anchored \checkmark Crushed pilchards [800g] PS BRUVs Abrolhos Is. (WA, bait) Australia) Kempster et al. (2016) Mossel Bav \checkmark \checkmark Olfactory (dead Sardines and fish heads [0.5kg] RemORA Vertical Tethered (South Africa) bait) Ryan et al. (2018) Mossel Bav \checkmark Vertical Tethered \checkmark Olfactory (dead Crushed sardines [0.5kg] N/A (South Africa) bait) Ningaloo Reef N/A Schifiliti et al. (2014) \checkmark Vertical Tethered \checkmark Olfactory (dead RemORA (WA. Australia) bait) \checkmark Olfactory (dead Vargas et al. (2016) Surf-Australian east x Horizontal Drifting Chopped pilchards and squid coast (several BRUVs bait) [500g] locations) Fukuba et al. (2015) Mariana Trench Drifting Olfactory (live Vertical \checkmark Live matured eels Una-Cam x (Western North bait) Pacific)

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Table 6.3 Example packing list. The list reflects the equipment needed to deploy pelagic BRUVs in an adaptation of Bouchet and Meeuwig (2015)'s protocol, whereby 3-5 camera units are tethered to each other on a longline (ca. 250 m) and drift with prevailing currents.

Item description	Quantity
BRUV units	
Rig frames	As required
Rig uprights + lynch pins (stainless steel ~ 5cm) + shackles	1 / rig + spares
Bait arms (stainless steel, 1.8m)	1 / rig + spares
Dumbbells (rubberised 2.5kg)	2 / rig + spares
Bait canisters (PVC tubes ~ 50cm)	1 / rig + spares
Rope (8mm or thicker – silver rope preferable for hauling)	1 / longline
Rubber rope bin	10m / rig / flag buoy + 200m / longline + spare
Double action clips (stainless steel ~10cm)	2 / rig + spares
Shark clips for bait arms (~10cm) + longlines (~7cm)	2 / rig + 1 / longline + spares
Buoys (orange, soft plastic, approx. 300mm x 400mm)	3 / rig
Sub-surface buoys	1-2 / rig
Flag buoys	1 / longline
Bait (pilchards/mulies/bonito whole fish frozen)	~1kg / drop + spare
GPS loggers and VHF transmitter	4
CAMERA EQUIPMENT	
Cameras (e.g. GoPro Hero 3+ Silver)	2 / rig / drop + spares
Camera battery extension packs (e.g. GoPro Battery BacPac)	1 / camera + spares
Spare internal camera batteries	10
Memory cards (e.g. micro SD 64GB)	1 / camera + spares
Camera housings	2 / rig +spares
DATA RECORDING	
Laptops (HP Probook 450 G2 + power cable)	2
Hard drives (2TB Seagate portable hard drives)	~1 / 100 hours of footage + spares
Magnadoodle / slate / white board and marker / pen and	1
Power adapters + power boards	~4
USB hubs	8
USB2 cables	50
SD card adapters	3
Clipboard	1
Waterproof paper (for datasheets) + pencils	1 ream + 1 box
Handheld GPS	1
GENERAL	
Toolbox	1
Socket set	1
Power drill and charger (battery operated)	1
Hot knife (for cutting and sealing rope)	1
Gloves (full fingered sailing gloves for hauling)	1 pair / person
Safety boots	1 pair / person
Air compressor hose and nozzle	1
Tupperware tubs (to store cameras in the field)	2 boxes
Dry bag (to store cameras in wet conditions)	1
Nuts and bolts (Phillips head stainless steel bolts with nylon locking nuts 3/16" x 25mm)	2 / rig + spares
Screwdriver set (assorted flathead and Phillips head)	1
Hex (Allen) key set	1
Wrench set (150mm, 200mm and 250mm adjustable)	1
Spanner set (14mm and 10mm for BRUVS)	1
Wire cutters	1
Cable ties (assorted, for repairs etc.)	500
Packing tape (e.g. duct tape)	10 rolls

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Plastic packing film	1 large roll
Laminated packing labels (premade for shipping out and back)	3 / item

Table 6.4 Example instructions for setting up a pelagic BRUV. Note that BRUV components are often made of stainless steel to prevent rusting in the marine environment. All replacement parts (e.g. spare bolts, nuts etc.) must therefore also be marine grade stainless (316).

Order	Action				
Rigs					
Step 1	Attach camera housings to the mounts on the crossbar using a stainless steel nylon locking nut and bolt (Phillips head 3/16" approx. 25mm). Ensure they are tightly in place and will not move if bumped. Do not remove after attachment to ensure calibration accuracy.				
Step 2	Place the upright through the hole in the centre of the rig and secure with locking nut.				
Step 3	Weight rigs by placing 2 x 2.5kg dumbbell weights (rubber coated preferable) on the base of the vertical pole in the centre and secure with a stainless steel lynch pin.				
Step 4	Place the loop of the 10m rig line into the shackle on the top of the rig upright, and ensure the shackle is done up tight (use mousing wire to ensure the shackle does not come loose with the movement of the rig in situ).				
Step 5	Fix the bait arm in place with a shark clip.				
Bait canisters					
Step 6	Take a ~50 cm length of PVC pipe, glue a cap on one end and a screw cap on the other. Once dry, use a power drill to drill small ~1-2 cm holes in the end without the screw cap and one large hole all the way through in the centre to allow the bait arm to fit through. Drill small holes in the cap at the holey end and cable tie a dive weight to the inside of the canister.				
Lines					
Step 7	Equip each rig with 10 m of rope. Note: The length of rope can be adapted depending on the suspension depth relevant to the project.				
Step 8	At one end of the loop, make a small (~15cm) eye by splicing the rope back on itself. This end will be attached to the rig upright.				
Step 9	At the other end, pass the line through the eyelet of a double action clip and splice it back on to itself to create a loop with the clip on the end. This will be attached to the longlines and				
Step 10	Close to the top of this line (~2 m down), tie on a short length of shock cord (~1 m), to create a D-shape with the shock cord making the short side. At the top of this tie using a small length of line to attach a small buoy.				
Step 11	Cut four 200 m lines for each set of 5 rigs (or 9 for sets of 10) to act as the long lines				
Step 12	Spice small loops at the ends of each of these lines (~15 cm).				
Step 13	Store on a winch clipped together with shark clips to make one line. If a winch is not available coil the lines into separate nelly or rubber rope bins, keeping the ends free and easily accessible for deployment.				
Buoys					
Step 14	Inflate buoys using a compressor and needle.				
Step 15	Take a length of line (1.5-2 m works well) and thread through the eyelets of three buoys and splice it back on to itself, leaving about 1 m free.				
Step 16	Pass the free end through the eyelet of a double action clip and splice it back on itself to create a small loop with the clip on the end. You should be left with a loop with the three buoys and a 1m length with a clip at the end. Note: Smaller sub-surface buoys can also be added to the suspension line and will generally help stabilise the rig, thereby facilitating species identification and length measurements.				
Step 17	To deflate the buoys at the end of the expedition, simply unscrew the bung (some are flat head and some are Phillips head).				
Step 18	Inflation and deflation of buoys should be considered for storage and mobilisation (i.e. whether an air compressor is on board the work vessel and what attachment is required).				
Cameras					
Step 19	Two [insert model name, e.g. GoPro Hero 3] are required per rig plus spares. The night before field work is conducted, ensure that the camera settings are correct (Table 6.3) and that the cameras and battery packs are charged (power-boards, USB hubs and USB2 cords are the easiest way to do this) NOTE: adapters may be required if working overseas.				



Step 20	Once the cameras and battery packs are charged, store them in a Tupperware container lined with a layer of foam padding above and below (or a specifically fitted Pelican case) to keep them from moving around in transit.						
Step 21	Store in a cool dry place until needed.						
Step 22	Camera housings should be attached to rigs permanently. Consider the depth ranges being sampled and choose housing types accordingly. Standard GoPro housings are rated to 40 m while dive housings are rated to 60 m. This may vary amongst manufacturers and brands. External battery packs must be used to ensure that the cameras run for the required time.						
Flag/GPS/VHF buoy							
Step 23	Assemble using socket and spanner sets.						
Step 24	Make sure tension wires are tight.						
Step 25	Splice a 10 m length of line to the buoy, pass the opposite end through the eyelet of a double action clip and splice back on itself to fix in place.						



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Table 6.5 Example metadata sheet for pelagic stereo-BRUV fieldwork. Left and right memory card numbers must be recorded for each camera pair.

Date	ID	Rig	Left cam	Left card	Right cam	Right card	Time in	Location in	Time out	Location out	Comments (e.g. wildlife, behaviour, habitat etc.)
2017-10-25	SITE-A	15	12	05	10	02	08:00	(115.1252E; 32.5437S)	10:15	(115.2411E; 32.5008S)	Seabird aggregation observed near deployment site



6.9 References

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⁷ Mono-BRUVs consist of a single camera usually mounted directly behind or above the bait arm (Whitmarsh *et al.* 2017). Stereo-BRUVS consist of two cameras mounted at specific angles (ca. 7-8 degrees) either side of the bait arm.





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7. MARINE SAMPLING FIELD MANUAL FOR TOWED UNDERWATER CAMERA SYSTEMS

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Left image courtesy of the Marine National Facility. Right image courtesy of the Australian Institute of Marine Science

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7.1 Platform Description

Towed underwater camera systems, of various configurations, have been used since the turn of the 20th century to acquire video and photographic still images of the seafloor (Bicknell et al. 2016) They are deployed on a cable from a surface vessel, have no propulsion mechanisms, and generally have forward-looking obligue and/or downward-looking cameras that either record images which are stored and subsequently downloaded, or transmit data directly to the surface in real-time via a coaxial or fibre optic cable (Bowden and Jones 2016, Durden et al. 2016a). Towed underwater cameras not only augment data from collected specimens (Chapter 8, 9); they also provide an important non-invasive sampling alternative where extractive methods are either unnecessary or unsuitable, such as in sensitive deep-sea habitats (e.g. Althaus et al. 2009, Williams et al. 2015, Sherlock et al. 2016), or for repeated sampling in marine reserves (e.g. Lawrence et al. 2015). Towed platforms also have the added advantage of providing cost-effective permanent data capture along transects that can be up to several kilometers in length and can be used to traverse highly heterogeneous seafloor topography (Shortis et al. 2007, Sheehan et al. 2016). The quality of imagery acquired by towed systems depends largely on sea conditions and water clarity, both of which may vary considerably depending on geographic location, season of sampling and extent of tidal influence. In depths greater than around 30 m, lighting and camera specifications become increasingly important to image quality. The quality and versatility of equipment and the maintenance of a consistent flying altitude above the seabed are also critical factors affecting image quality and usability.

Conventional underwater still photography and video imagery were initially applied by marine ecologists to collect basic qualitative data (e.g. simple visual assessment of seabed conditions to assess habitat type or dominant species), or often low-accuracy quantitative data estimated through the use of parallel lasers to define the scale of the images (see Harvey et al. 2002, Shortis et al. 2008, Durden et al. 2016a). Recent technological advancements have emerged that permit collection of high-resolution benthic imagery using versatile multifunctional towed platforms carrying a variety of camera systems (e.g. stereo-image measurement systems) and a range of other sensors (e.g. high-resolution multibeam and side-scan sonars, motion sensors, conductivity temperature and depth sensors, and subsea acoustic positioning systems) (Kocak et al., 2008, Rattray et al. 2014, Bowden and Jones 2016, Durden et al. 2016a, Logan et al. 2017). This technology, coupled with advances in camera resolution, positional accuracy, digital data processing and visualisation techniques, has enabled more quantitative and spatially-referenced studies of the seafloor. Calibrated stereo-imaging in particular has facilitated more reliable length measurements of mobile species, such as epibenthic invertebrates and demersal fish, and more accurate estimates of biomass and population distributions (Harvey et al. 2002, Shortis et al. 2009). Towed underwater imaging systems can be applied to acquire baseline data, evaluate benthic diversity, map benthic habitats, identify vulnerable communities, assess changes in biota, and support spatial and ecological modelling/monitoring.

7.2 Scope

As still and video cameras can be mounted to tow bodies in a variety of ways (Figure 7.2, Table 7.1), this field manual does not mandate specific gear types. Rather, it provides recommendations for future updates or replacement of existing platforms. It targets the suite of towed camera platforms currently being used to acquire quantitative imagery of benthic habitats in Australian waters, and seeks to standardise monitoring efforts by recommending standard operating procedures (SOPs) for survey planning, field acquisition and post-survey data processing, description, and storage for public accessibility (Figure 7.1). The primary aim of this field manual is



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to establish a consistent approach to marine benthic sampling using towed camera systems which will facilitate statistically sound compilation between studies. Note that hybrid towed systems and other video-based monitoring platforms (e.g. dropped video cameras, or video and still cameras mounted on sleds or trawls) that are commonly used to gather qualitative sample data (e.g. general animal behaviour) fall outside the scope of this manual.



Figure 7.1: Workflow for towed camera image acquisition and processing. Purple represents onboard methods, while blue represents post-survey methods.

7.3 Towed Underwater Cameras in Marine Monitoring

Standardised methods of survey design, data collection, analysis and reporting are essential to monitoring both the status and change in Australia's vast benthic marine environment. Efficient management of a given area requires first establishing a baseline of the key biota, and then regularly monitoring their status to detect changes over time. Changes to the diversity and abundance of benthic organisms and communities are commonly used ecological metrics in marine imagery because epibenthos is considered to be functionally important and sensitive to human activities (Williams et al. 2015). Although repeated presence-absence surveys for occupancy estimation or changes in benthic community composition can be achieved using towed camera systems, returning to a precise geographical location for a particular monitoring purpose (e.g. Bridge et al. 2014, Ferrari et al. 2016, Pizarro et al. 2017) requires an alternate sampling platform entirely (e.g. AUV in Chapter 4). However, despite known biases and limitations (e.g. Jones et al. 2009, Katsanevakis et al. 2012, Durden et al. 2016a, Durden et al. 2016b), towed camera systems are anticipated to play an important role in future monitoring strategies, and have been identified as one of the sampling methods capable of monitoring the indicators associated with shelf reef systems (Hayes et al. 2015).

The application of towed underwater camera systems to environmental monitoring involves several key steps. These include survey design (Chapter 2), pre-survey preparations, field implementation (e.g. image acquisition and onboard data storage and description), and post-survey procedures (e.g. processing of imagery for data extraction, image annotation, statistical analyses of extracted data and data release). A brief overview of these fundamental steps is provided below.



7.4 **Pre-Survey Preparations**

<u>Ensure all permits, safety plans and approvals have been obtained.</u> Any research undertaken within AMPs requires a research permit issued from Parks Australia. See Appendix B for a list of potential permits that may be required.

<u>Confirm sampling design meets survey objectives</u>, is achievable with planned equipment and time, and has been communicated to all key scientists and managers. Generally, the sampling design in an ecological study should be statistically sound with adequate spatial coverage and replication, and it should use an explicit randomization procedure to ensure that independent replicates are obtained (Durden et al. 2016a). Increasing sample size where possible will also help to better inform models, and increase the study's robustness (Mitchell et al. 2017). See Chapter 2 for further details on sampling design.

<u>Define the sampling area</u> to be surveyed in terms of space and time and identify any categorical constraints that may need to be imposed (e.g. acceptance of only those images captured within an altitude range of 2–4 m above the seabed) (Durden et al. 2016a).

Determine sampling unit (what to quantify within an image) and sample size (number of images, <u>number of transects</u>) to sample the habitat of interest. A complication in the determination of sample size in image-based studies using towed camera systems is variability in the physical size represented by respective images as the camera-to-subject distance often varies (Durden et al. 2016a).

<u>Determine appropriate imagery system</u> based on metric to be quantified. For seafloor imagery, some of the most important operational factors for the design of a platform and its deployment are depth, bottom topography, duration and spatial extent of survey, current speed, altitude control, turbidity and surface sea conditions (Barker et al. 1999). The specific configuration of equipment will depend on the scientific objectives of the survey and the type of data required. For example, high-definition video is commonly used to assess the spatial distribution, abundance and behaviour of benthic epifauna, and is also well-suited to identifying the spatial extent of substratum types and biological habitats (Bowden and Jones 2016). High-resolution images from stereo-cameras on the other hand are necessary for detailed species identification and precise sizing of individual organisms and quantifying specific seabed features (see Dunlop et al. 2015, Durden et al. 2016a, Sheehan et al. 2016).

Determine appropriate camera orientation. Camera orientation for towed systems is a critical parameter for quantitative interpretation of imagery (Bowden and Jones 2016). Images captured perpendicular (i.e. downward-facing) to the seabed are commonly used for spatial benthic ecological studies of sessile organisms, and substratum or seabed composition (Durden et al. 2016a). Whereas, images captured at oblique angles tend to be used for studies of motile fauna, such as demersal fish, as the image frame captures a greater area of seabed (or a larger volume of the water column) (see Bowden and Jones 2016, Durden et al. 2016a). Oblique camera orientation typically introduces inherent gradients of both lens-to-subject distance and illumination intensity, while a vertical orientation generally provides more even illumination and uniform subject-to-camera distance (Bowden and Jones 2016). These properties make vertical (i.e. downward-facing) orientated images more optimal for quantitative analyses of benthic substrata and sessile or sedentary biota . We recommend combining high-definition oblique video with high-resolution downward-facing camera/s, as this makes full use of both the descriptive potential of oblique-facing video (N.B, stereo-video required for examining fish metrics) and the potential for accurate quantitative analyses from vertical images, as well as reducing the risk of collision with seabed



obstacles (Bowden and Jones 2016). Downward-facing camera/s, coupled with accurate geographic positioning (e.g. USBL, motion sensor) can facilitate mosaicking of images similar to that achievable with AUV platforms.

<u>Particular care should be taken when selecting platform and optics</u>, especially when developing a long-term ecological monitoring program. For example, it is not recommended to change the gear specifications over the monitoring period if the purpose of the study is to detect change over space and time (Sheehan et al. 2016).

<u>Ensure accurate geo-referencing (position, position, position!).</u> The geographic position and orientation of the camera(s) at the time of image capture is *critical* for ensuring accurate geo-referencing of an image (and the objects within it). This geographic position must be integrated with other sensor data to develop habitat maps or interpolations (see below). It is also critical for relating the sampled area to environmental co-variates extracted from hydro-acoustic (Mitchell et al. 2017) and other platform sensors (Shortis et al. 2007).

<u>Ensure synchronisation of time stamps.</u> The time standard (typically UTC) for a given survey needs to be pre-determined and strictly adhered to. Synchronisation of time stamps across all systems (e.g. USBL and other platform sensors, PC time(s), ship navigation, video and still camera systems) is *critical* for ensuring accurate geo-referencing of images. Time accuracy to three decimal places is optimal.

<u>Determine real-time annotation protocols, if desired.</u> Although real-time annotation is not required for this field manual, it is recognised that this is an established practice for many individuals and agencies. If a real-time imagery feed is available, follow agency-specific protocols for onboard annotation. At the least, a qualitative description can be written for each station, thus ensuring some information is immediately available for post-survey reporting and to guide subsequent analysis (see Appendix C) [Recommended].

<u>Stereo-cameras should be pre- or post-calibrated</u> in shallow water using the techniques outlined in Shortis and Harvey (2009). Typical requirements of a multi-station, self-calibration network include multiple convergent photographs, camera roll at each location and a 3D target array (see Shortis et al. 2009). If housings or mounts are changed or damaged during deployment, re-calibraton is required.

<u>Paired calibrated lasers should be used if not using stereo-cameras</u>, with a known separation distance used as a reference for scaling objects. This can enhance the performance of 2-D and 3-D imaging systems/reconstructions (Caimi et al. 2008) and align video and stills by time.

<u>Consider potential spatial and temporal errors</u> that may result from the choice of towed camera system and how these errors may potentially affect habitat mapping and modelling of data (e.g. Monk et al. 2012, Rattray et al. 2014). It is important to take into account errors from vessel motion (i.e. heave, pitch, roll and yaw), USBL beacon positioning, GPS, and measurement inaccuracies resulting from the application of stereo-camera calibrations carried out in shallow water to imagery gathered at greater depths (see Shortis et al. 2009). It is also important to ensure that the recording frequency of sensor data is matched to the intended use of the sensor data – e.g. pitch recorded at 1s intervals may not be sufficient to correct for changes in the field of view in a video as the camera is towed.

<u>Consider locational uncertainty in occurrence data.</u> To generate realistic predictions, species distribution models require accurate geo-referencing of occurrence data with environmental variables (Mitchell et al. 2017). Although some high-performing, fine-scale models can be generated from data containing locational uncertainty, interpreting their predictions can be misleading if the



predictions are interpreted at scales similar to the spatial errors (Mitchell et al. 2017). See Foster et al. (2012) and Stoklasa et al. (2015) for a more statistical view of this issue in an ecological context.

<u>Consider onboard data formats and establish workflow for data transfer and battery charging</u> prior to survey commencement. This field manual does not mandate particular data formats as these may differ depending on the choice of annotation software and process for specific extensions. For example, video data may require transcoding into web-viewable format (e.g. H264). Common formats include .mp4 and .avi for video data and .jpeg, and .tiff for still imagery. Several video containers (e.g. Quicktime) allow embedding of timecode and/or closed caption tracks into the video file and are frame-accurate during playback. Where possible such formats are preferable. The H264 codec is suboptimal for high speed transects so original video file copies should be kept for reference during analysis. In some instances, saving information in raw format may be necessary for the purpose of post-processing. Files may also need to be compressed for public accessibility. Regardless of data formats, it is essential to establish a workflow for data transfer and battery charging prior to survey commencement.

<u>Consider the metadata required for subsequent data post processing, storage and release</u>, such as the video or image location, camera attributes, date, time, altitude, angle of acceptance, motion of towed platform (i.e. heave, pitch, roll and yaw) and the precision required of each (Durden et al. 2016a). Consider size, location and access of final imagery and video datasets and where these will be archived. Metadata must be adequate enough to satisfy conformance checks for data release via open access data portals such as the Australian Ocean Data Network (AODN http://imos.org.au/facilities/aodn/aodn-submit-data/).

<u>Consider how metadata will link to media type.</u> The most effective way to link visual imagery with metadata is by incorporation into a spatially enabled relational database (Bowden and Jones 2016), using the synchronised time stamps and GIS position for linking imagery and sensor data. Important considerations include:

- Archived filenames should include Platform, Date and Start-Time (PlatformYYYYMMDDHHMMSStextstring)
- If possible we recommend writing image metadata into EXIF fields embedded in the digital image file to ensure metadata is not separated from images
- Geotagging video imagery is less established but various options exist including: i) Embedding position, date and time on the imagery itself suggest using an inconspicuous location within the field of view; ii) Utilizing the video audio track or closed-caption track to record position date and time using a geostamping device, iii) Proprietary video recording and playback equipment and /or software that associates position metadata with recorded video files (e.g. Streampix <u>https://www.norpix.com/products/streampix/modules/gps.php;</u> GeoDVR <u>https://www.remotegeo.com/mission/marine/subsea-rover</u>); and iv) Embedding UTC timecode into the video media file (e.g. Quicktime .imov files recorded by AJA KiPro devices can have timecode generated and embedded by a GPS-timecode generator)

7.5 Field Procedures

The steps below are comprehensive for the entire workflow of towed camera systems. In many cases, there will be a designated specialist or team to perform some of these steps. Indeed, for heavy Deep-Tow and complex systems (e.g. JAMSTEC's deep-tow systems), most, if not all of these steps may be managed by external technicians and engineers. In this case, it is the



researcher's responsibility to ensure that the externally managed workflow is comprehensive and addresses the steps as described in this field manual. This is best done in Pre-Survey Preparations.

7.5.1 Pre-deployment

Risk Assessment

Complete an on-site Workplace Health and Safety risk assessment following agency-specific protocols. A risk assessment should always be completed prior to deploying equipment to ensure the operation can be completed safely. Always adopt a precautionary approach.

Set up and testing

Allow sufficient time during survey mobilisation to undertake system checks, calibrations and testing of equipment and account for unforeseen problems. In most cases it will be possible to complete all system tests and checks within a few hours to half a day. The conduct of pre-start checks should be noted in the trip log and any test failures specifically recorded for later-reference. Detailed settings for each component should be made using relevant operations manuals (e.g. USBL operations manual etc.).

On-deck dry tests should include, but are not limited to, the following checks:

- On-board storage;
- On-board power;
- Cameras, including a review of image quality (colour chart test);
- Lights and strobes;
- Seals/o-rings;
- Recording devices;
- File copy times for offline recording devices (e.g. GoPro);
- Winch operation;
- Sea fastening;
- Surface communications; and
- X-Y-Z co-ordinates from the tether termination to the imaging chip of each camera, altimeter, depth sensor/CTD and transponder.

Wet testing should include checks of the following:

- Power;
- Cameras, including a review of image quality;
- Acoustic tracking system (USBL) and any internal navigation; and
- Lighting and strobes.

Acoustic tracking setup

- Set position of GPS receiver. Differential GPS is recommended as a minimum and is mandatory for repeat site monitoring.
- Deploy acoustic tracking transceiver (e.g. pole, flange or vessel mounted).
- Measure offsets of USBL transceiver head to GPS receiver and put offsets into navigation system.



• Ensure accurate vessel dimensions are obtained and entered into the vessel plan repository of the navigation software.

Stills camera time calibration

- Calibrate the stills camera and video feed from GPS in the video overlay relative to UTC time
- Ensure all sensor logging systems, cameras, computers have been synchronised to UTC time
- Time coding calibration should be applied at the commencement of a survey and checked for consistency at least once a day while the survey is in progress
- Ensure recording media/storage device is working correctly and review imagery/video

Pre-deployment checks

- 1. Ensure all personnel understand their roles by conducting an appropriate toolbox talk, incorporating risk assessment and appropriate PPE to be worn. See Chapter 1 for further information about risk assessments.
- 2. Confirm with vessel master that GPS tracks for the proposed deployments are accurate and the order of transect sampling is clearly communicated.
- 3. Discuss the desired target location and the feasibility of deploying at that location. Main items to take into account are:
 - Terrain. To minimise the risk of a deployment almost all tows will be conducted on either a flat or downward sloping seafloor. This will reduce the chance of the camera hooking up and allow for the platform to fly out into deeper water if there is a winch failure. Consider if there are any large ridges, boulders, drop-offs, etc. along the proposed tow route as with minimal forward vision, 10 m or less, there is not a large margin for avoidance.
 - Weather/sea state. When the camera is flying along the ocean floor, the ship will need to travel at ~ 0.5-1ms⁻¹. This can limit the manoeuvrability of the ship and depending on the direction of the prevailing wind and swell, is not always possible on a particular heading. As the sea-state and swell can affect the ships manoeuvrability when travelling at low speeds it is essential to regularly check the weather forecast to ensure the sea state is acceptable and the platform can be safely deployed and retrieved.
 - Depth. Be aware of the depth limitations of the towed body and the wire that the platform is deployed on.
- 4. The vessel Master must approve each deployment and communicate with crew prior to launch.
- 5. Prepare tow body on deck and ensure only essential personnel participate in its preparation and deployment.
- Check for correct operation of cameras and lights (check explicitly for miss-timing between image capture and strobe firing) and winch including watertight seals, power requirements, hydraulic power and hoses, time synchronisation (PC, USBL, camera systems) and recording media.
- 7. If necessary, attach the USBL beacon to the frame and check that it is operational.
- 8. Perform laser alignments as per manufacturer's procedure.

- 9. Inspect the platform for any deterioration in cables and cable ties, ensure frame nuts and bolts are tight and all equipment mounts are secure.
- 10. Ensure all connection to pressure housings and equipment are tight and secure.
- 11. Ensure winch clutch or load relief mechanism is adjusted to the correct tension prior to initial deployment.
- 12. Once all instruments are confirmed working, handclap within an overlapping field of view of all cameras.
- 13. Inform the bridge and deck you are ready to deploy and wait for confirmation from the bridge that the ship is at deployment speed and is approaching the start of the survey line.
- 14. Ensure the nominated winch driver is in the operations room with a functional and fully charged winch remote control, set to the specified channel.

7.5.2 Deployment

- 1. Run the towed body termination through the large block on the centre of the A-Frame and make sure there are no twists in the wire.
- 2. Following the signal to deploy from the vessel Master, use the winch and A-Frame to lift and guide the tow body from the deck into the water as the vessel begins tracking towards the start of transect line.
- 3. Minimise the time taken from when the tow body is let out of reach, to when it is lowered in the water, so as to reduce potential swing and impact against the vessel.
- 4. Deploy the platform into the water.
- 5. Check for cable loops or problems at the surface while the tow body is being lowered into the water before losing sight of the platform below the waterline.
- 6. Once in the water, lower the camera to an appropriate depth where system can be checked, turn everything on, including the lasers, and check that all is functional.
- 7. Check the USBL is receiving and the ship and platform are indicated on the bathymetry overlay.
- 8. Confirm that the USBL data is being logged.
- 9. There are several factors that affect how much wire out is required for the towed camera system to reach a target depth. These include: vessel speed through the water, payout/haul in speed, and cable diameter, package drag and weight. Determine the appropriate wireout ratio specific to the vessel and its speed, noting that ocean currents can affect this ratio.
- 10. Continually monitor the descent rate at separate intervals, checking the ratio of wire out to depth. This can impact on when the platform will actually reach the required depth and the location this will be. If the ratio is too high, there is the possibility of not reaching the required depth before passing over the target area. If the ratio is too low, the platform will reach the required depth well before the target area. The platforms descent rate and estimate touchdown location needs to be continually monitored for a successful tow.



- 11. To mitigate any positional errors, it is important to carefully monitor the ship speed and deployment rate to an appropriate ratio. If having reached the seafloor too early, try to resist speeding up the ship. This will cause the platform to rise when speeding up and fall uncontrollably when slowing down again.
- 12. Continue descent to a pre-determined height above the seafloor (e.g. 2–3m) and try to maintain this height throughout the tow using the winch remote control. Note: hauling in cable onto the winch or paying out cable has an immediate effect on the camera platform height above the seafloor; however, the degree of change on height above bottom is in relation to the cable angle, which is determined by the ships speed and current.
- 13. Confirm still photos are being taken and video feeds are being recorded where possible (e.g. recording indicators, hard drive operating).
- 14. Confirm timecode being embedded is GPS-time accurate.
- 15. If employing real-time annotation, record the time and position of the camera on the seafloor (See Pre-Survey Preparations).
- 16. While maintaining a consistent flying altitude above the seabed, the co-pilot needs to continually check the camera feeds to ensure all footage is being recorded and anticipate the need to come up on the winch so as to avoid approaching obstacles and minimise the chance of a seabed hook-up, and review.
- 17. Monitor sea conditions during deployment to maintain safe working environment.
- 18. Consider aborting operations if sea conditions are marginal, visibility is poor or any fault develops that may interfere with the towed camera system operation.

7.5.3 Retrieval

- 1. Continue deployment until advised by the watch leader/chief scientist that enough footage has been recorded.
- 2. When the survey line is complete or if the transect is being aborted, advise Vessel Master of intention to retrieve the tow body.
- 3. When close to the surface ask the officer on watch to confirm the ship is on the best heading for retrieval and hand over operational control to the deck crew.
- 4. Watch for approach of tow body near surface ensuring only required personnel near open transom.
- 5. If possible, turn off lasers before reaching ocean surface and turn off lights just below sea level.
- 6. Use winch and A-Frame to guide tow body back onto deck with smooth winch and A-Frame control inputs.
- 7. Ensure crew grab hold of tow body as soon as safe to do so when the tow body leaves the water, so it can be guided safely away forward of the transom and lowered to the deck.
- 8. Once clear of the water, stop all recordings, and turn all cameras, sensors and power off.



- 9. Rinse towed platform frame and all camera/sensors with fresh water.
- 10. If attached, remove USBL beacon and recharge.
- 11. Check and rename video footage, still camera photos and log files and complete Metadata Information sheet.

7.5.4 Seabed hook-up procedures

Hook-up of the tow body is always a possibility with the ideal altitude for capturing quality still images close to the seabed. The following procedures should minimise the potential of a hook-up occurring and lower the potential of damage to the tow body or total loss:

- 1. Communication link between tow camera winch station and bridge should be maintained at all times (e.g. VHF or intercom).
- 2. Bridge should monitor video feed from tow body while undertaking tows
- 3. At first sign of a hook-up (e.g. video image stationary over seabed), ensure forward speed of vessel is backed off to reduce tensile load on cable.
- 4. With crew monitoring position of the cable and directing the Vessel Master with regard to the position of the cable, the vessel is to maneuverer back to a point directly over the hook-up point to see if the tow body can be freed.
- 5. Cable tension should be taken up by the winch to ensure no loose cable enters the vessel propellers.
- 6. If the initial retrieval attempt from overhead fails, various points of the compass should be tested by the vessel to pull the tow body off the seafloor, using only the winch to ensure enough cable remains.
- 7. If all options for retrieval have been exhausted the cable must be cut at the shortest possible point and the position recorded with GPS.
- 8. A substitute tow body and cable would need to be prepared for continuance of survey operations.

7.5.5 Operation completion

Prior to any vessel movement or engine start-up, operators should check the following:

- All equipment is clear of the water, including acoustic tracking equipment;
- All gear is safely stowed and powered down where appropriate;
- Any servicing that requires the vessel to be stationary is completed;
- When the towed camera team is satisfied it is OK for the vessel to move on, an "All Clear to Move" command should be given to Vessel Master; and
- Data collected from previous tows should be checked for integrity prior to deploying the towed system on further tows.

7.5.6 Onboard data processing and storage

<u>Consider navigation, data logging, real-time quality control, and display.</u> A range of specialized marine image annotation tools have been developed worldwide to facilitate real-time underwater image analysis (reviewed in Gomes-Pereira et al. 2016). These tools generally consist of a graphical user interface, with a video player or image browser that recognizes a specific time code or image code, allowing events to be logged in a time-stamped (and/or geo-referenced) manner . Examples include: Adelie, Customizable Observation Video imagE Record (COVER), Frame-Grabber, Ocean Floor Observation Protocol (OFOP), SeaScribe/Seatube, Video Annotation & Reference System (VARS), VideoNavigator, Jason Virtual Control Van (web browser logger on a ships network allowing for digitally logging comments and observations during capture), CampodLogger . These software packages integrate data associated with video collection, the simplest being the position coordinates of the video recording platform, with more advanced packages allowing the input and display of data from multiple sensors or multiple annotators via intranet or internet .

<u>Name data files according to established conventions.</u> File naming conventions are important for ensuring both efficient and effective management of field data and its integration into appropriate data management repositories. It is important to note that these conventions will differ among agencies and academic institutions. For example, CSIRO uses: Survey code_operation#_UTCTime(hhmmss) (potentially Date time: YYYYMMDD-hhmmss)

Ensure accurate recording of metadata. Metadata is a descriptive data source comprised of information that may be used to process the images or information therein (Durden et al. 2016a). While it is important to follow agency specific protocols for capturing metadata, it is also essential that metadata is of sufficient detail to satisfy conformance checks for subsequent data release via AODN (See Table 7.2 for sample metadata sheet). Metadata should also contain survey-specific information such as camera specifications and imagery file naming protocol, as well as product lineage. Minimum data for each image/frame capture should include georeferenced information, as well as any other related *sensor information* and (where appropriate) *real-time characterisation* details:

- Campaign (i.e. Survey identifier)
- Station/event number
- Platform
- Latitude and longitude (WGS 1984 in decimal degrees [Recommended])
- Altitude
- Depth
- Time and date stamp
- Platform and/or vessel motion (roll, pitch, heave)
- Metadata from other sensor data (see example below, CSIRO data file headers)
- Precision details (e.g. type of navigation system used and its associated errors)
- Data provenance

<u>Quality control</u>. Once the towed camera transect is complete, it is good practise to download associated raw imagery and positional data. Imagery and associated position data should be checked to ensure no failures have occurred, including but not limited to the following:

- Mis-timing between image capture and strobes (i.e. dark/black imagery)
- Failure of camera/s
- Failure of positional logging

<u>Backup data.</u> This is necessary to ensure all data collected in the field are safely returned and securely backed-up at host facilities, prior to final quality control and public release. Onboard copies of data should be made as soon as practically possible following acquisition. It is recommended that all data be backed up on a RAID or a NAS that contains built-in storage


redundancy in case of hard-drive failure. A duplicate copy of all data onto external hard drives or LTO tapes for transportation back to host facilities is *[Recommended]*.

7.6 **Post-survey procedures**

7.6.1 Data processing

Image/video post-processing, selection and annotation method and detail will depend on the objectives of the survey/project. If documented properly using adequate metadata, imagery can be analysed, processed and annotated in a number of different ways to achieve different purposes.

A general workflow for data processing methodology can be found in Williams et al. (2012a). If constructing photomosaics from imagery, key requirements for raw image processing and positional data are as follows:

- It is recommended that at least one of the stereo images is in colour and enhanced following similar procedures as outlined by Shortis and Harvey (2009) and Bryson et al. (2016).
- All stereo images should georectified following Williams et al. (2012b).
- Positional data should be post-processed using Simultaneous Localisation and Mapping (SLAM) as demonstrated in (Barkby et al. 2009) and (Palomer et al. 2013).

7.6.2 Annotation framework

Scoring of individual images can be done using a number of annotation software tools. Examples include, Transect measure, Coral Point Count, CoralNet and Squidle+. For national consistency Squidle+ is recommended as it allows for different approaches to subsample images, which appears to influence inferences from data, as well as stratified and random point count distribution on images. It also automatically imports the collected towed camera data once it is uploaded to the AODN making it ready for analysis, and has tools for exploring survey data as well as analyses. In addition, it supports multiple annotation schemes, and will provide consistency through translation between schemes, which is an important point that differentiates Squidle+.

There are two main approaches recommended for annotating georeferenced imagery from towed camera systems:

- Annotation of individual images/frame grabs (real-time or post-acquisition)
- Annotation of photomosaics

Annotation of individual images or photomosaics can be undertaken using two methods:

 <u>Full assemblage scoring of imagery</u> across space and time. It is important to note that this is a time consuming process, requiring a lot of replicate images to be scored to enable sufficient power to detect biologically meaningful change as most morphospecies are < 10 % cover within images. This approach appears to be good for delineating bioregional and cross-shelf patterns at a morphospecies and CATAMI (Althaus et al. 2015) level (Monk et al. 2016, James et al. 2017). This approach would be effective in choosing an initial suite of indicators for national level monitoring and reporting.

As a general guideline for full assemblage scoring, we recommend that 25 random points per image from at least 50 images per transect leg are a good starting point for recording most morphospecies present within images (based on Perkins et al. 2016). It is important to note that the properties of the organism themselves will also influence the number of points/images to score. Obviously morphospecies that are less abundant require more effort,



but also the 'clumpiness' of species will affect the scoring effort needed (Perkins et al. 2016). Van Rein et al. (2011) and Perkins et al. (2016) suggest that, while a higher number of points per image can increase the detection rate of more organisms within an image, increasing the number of scored images using fewer points is likely have a similar (or greater) effect. Ideally, increasing both the number of images scored and the number of points scored within an image would result in greater power (Roelfsema et al. 2006), but preference is usually for increasing the number of images (Perkins et al. 2016). Unfortunately, the adoption of this approach is likely to result in substantial increases in processing time and thus cost.

2. <u>Targeted scoring of indicators or proxies</u> (such as grouping fine level morphospecies into broader level CATAMI classes). This approach has been shown to work very well at an indicator morphospecies level for detecting change at a regional level (Perkins et al. 2017) as well as for detecting invasive species trends (Perkins et al. 2015, Ling et al. 2016). More recently this approach has been extended to mobile species, such as fish (Seiler et al. 2012) and lobster (Bessell et al., unpublished data). Care needs to be taken if length data (using photogrammetry or structure from motion) is extracted from stereo pairs as Seiler et al.(2012) found precision can be poor for mobile species if camera separation is inadequate (see Boutros et al. 2015).

Since this approach requires substantially less effort to score each image, more images (i.e. often all images) can be scored, thus increasing statistical power. The drawback is that a narrower understanding of the environment may result.

7.6.3 Data curation and quality control

Data quality control at both the collection and annotation stage is critical. Most importantly, the annotation schema needs to be consistent between studies. Where possible morphospecies and associated CATAMI parent classes should be used *[Recommended]*. Clearly, other annotation schemas are available and can be applied. Where an alternative schema is used to annotate towed camera imagery, it is most important that it can be mapped to CATAMI so that comparisons can be made with previous studies or between regions. Translations between schema can be readily applied within Squidle +. The quality control of all annotations undertaken by novice scorers should be assessed against an experienced analyst (e.g. using confusion matrices; see Figure 4.4 in Chapter 4). Logically, it is important to correct any discrepancies between annotators. This can be done by re-examining the images to ensure an agreement can be reached between annotators. Alternatively, if an agreement cannot be reached, then the miss-classified morphospecies could be potentially grouped into a higher level CATAMI class.

7.6.4 Data release

<u>Squidle+</u> is a centralised online platform for standardised analysis and annotation of georeferenced imagery and video. Many national marine observing programs (for example IMOS through the Australian Ocean Data Network (AODN), or the Marine Geoscience Data System (MGDS) in the USA) routinely store imagery data online in an openly accessible location. Squidle + operates based on flexible distributed data storage facilities (ie imagery can be stored anywhere in an openly accessible online location) to reduce data duplication and inconsistencies, and provides a flexible annotation system with the capability to translate between different annotation schemes.

Following the steps listed below will ensure the timely release of imagery and associated annotation data in a standardised, highly discoverable format.



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- Create a metadata record describing the data collection. Provide as much detail as possible on the deployment (either directly in the metadata record itself, or in the form of attached field sheets as .csv, .txt or similar). Details of minimum metadata requirements are provided in Onboard Data Storage section above. Publish metadata record(s) to the <u>Australian Ocean Data</u> <u>Network (AODN) catalogue</u> as soon as possible after metadata has been QC-d. This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the <u>AODN Data Submission</u> <u>Tool</u>. Note that user registration is required, but this is free and immediate.

Lodging metadata with AODN in advance of annotation data being available is an important step in documenting the methods and location of acquired imagery and enhancing future discoverability of the data.

- 2. Upload raw imagery from the survey to a secure, publicly accessible online repository (<u>contact</u> <u>AODN</u> if you require assistance in locating a suitable repository).
- 3. Create a <u>Squidle+</u> campaign as soon as possible after imagery is uploaded, choose the most appropriate annotation schema, and commence annotation of imagery.
- 4. Add links to the location of the Squidle+ campaign to the previously published metadata record. You may also wish to attach or link a copy of the annotation data directly to the record.
- 5. Produce a technical or post-survey report documenting the purpose of the survey, sampling design, sampling locations, sampling equipment specifications, annotation schema (e.g. morphospecies, CATAMI, etc.), and any challenges or limitations encountered. Provide links to this report in all associated metadata [Recommended]

7.6.5 Data analysis

The breadth of research questions precludes any detailed advice on the analysis of data from underwater towed camera transects. However, one common attribute of the image-based data that will have to be contended with for all analyses is spatial proximity. The closeness of images, within and sometimes between transects, means that image data are unlikely to be independent (due to spatial autocorrelation). Yet, this is an assumption that most statistical methods rely upon. The failure to meet this assumption means that the inferences from the statistical analysis may be: (i) over-confident, e.g. having a p-value that is too small; (ii) biased, i.e. the estimates do not reflect the truth; (iii) both, or; (iv) no effect. Obviously, the fourth category is what a researcher hopes for, but it is improbable and must be validated. However, if it is known that the study organism exhibit particularly low autocorrelation at the scales of interest then the analysis need not consider it explicitly.

Methods to analyse data, accounting for autocorrelation are available. These include geostatistical models (see Foster et al. 2012 for an AUV-based example) and other models that incorporate dependence (e.g. Foster et al. 2009). However, in certain situations subsampling images will help (e.g. Mitchell et al. 2017 for a marine based example), but not necessarily alleviate it completely. Further, if the study is for a broad area, where transects are small and are well-separated, then amalgamating data to transect level may also be appropriate. The potential for observer bias, vignetting, and intra and inter station variability should also be carefully considered.



7.7 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 7 (field manual for towed camera) is below:

Version no	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
1.1	Link to Squidle+ corrected	March 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019



Figure 7.2 Types of towed camera systems deployed in Australian waters. a) MNFs Deep Towed Camera platform; b) and c) AIMS towed camera platform being deployed off RV Solander; d) towed camera platform being trialled by Geoscience Australia off RV Southern Surveyor; e) and f) Deakin University towed video system.



Table 7.1: Types of towed camera systems deployed in Australian waters and their main characteristics. Note this list is not comprehensive. See reviews on towed cameras and perspectives in visual imagining for information about gear deployed elsewhere in the world (Durden et al. 2016a).

Towed Platform	Dimensions (W x H x L)	Weight (kg)	Max depth (m)	Camera system (video) & orientation	Camera system (stills) & orientation	Illumination	Laser(s)	Sensors	Suitable terrain	Example Reference
AIMS Towvid	~ 400 mm x 350 mm x 600 mm	~ 15 (Towed body only)	150	SD video forward facing Additional forward facing GoPro (HD) (optional)	12MP downward stills	Keldan 8M 8000 lumen floodlights (video) Inon D2000 strobe (still camera) synced to camera hotshoe by LED trigger and optic slave cable	In development		All, but steep inclines are best surveyed downslope; rugged terrain in low visibility is also risky.	(Nichol et al. 2013)
MNF Deep Tow		490	2500	Canon C300 high definition <u>video</u> <u>camera</u> paired with a Hitachi – HV- D30P <u>Look Ahead</u> <u>Camera</u> (8°) 1 x Watec 1/3" WAT231S with Avenir TTSG0234 lens	Digital Stills System Canon 1DX stills camera with a 18mm lens set at an oblique angle 8.3 Megapixel Lens Canon EFS10-22 F3.5-4.5 USM set to ~ 12 mm Strobes – dual Canon 580EX – ETTL mode Flash sync – Canon STE2 transmitter	2 x Deep Sea Power and Light – Deep Multi Sealites 250W each	2 x Laserex 10 mW (red) 16-laser array unit for stereo video calibration A pair of lasers with a known separation distance (10cm) is used as a reference for scaling objects and aligning video and stills in time.	Pressure: Druck PTX1400, range 0-250 Bar absolute Platform Pitch/Roll: Crossbow Dual Axis CXTA02 Tilt sensor Fluorometer: Seatech Serial No 100S Compass:Honeywell HMR3100 Altimeter: Datasonic/Benthos, PSA900 CTD: Falmouth Scientific 2" MicroCTD Serial #1468M Serial Interface: Quatech 4 port Serial Device Server	The Deep Towed Camera can only be deployed on a downhill/flat gradient and travelling towards deeper/open water to mitigate against winch failures	(Shortis et al. 2007, Sherlock et al. 2016)



Marine Sampling Field Manuals for Monitoring Australia's Commonwealth Waters

Version 1

					Cameras (50°) 2 x 1/3" 3CCD Hitachi HV- D30P with Fujinon TF2.8DA-8			Position: Sonardyne Super Sub Mini 7970 using channel H6 Sonardyne USBL Ver5.15C Transceiver # 1151 GPS: Vessel differential corrected Ashtech GGA,VTG		
NSW OEH	1100 L x 900 H x 500 W	15	200	Forward looking xx video camera at 30 degrees through Fibre Optic Cable; camera spec?	Downward looking stills Canon xx	Seagis LEDs + 2 Keldan	A pair of lasers with downward looking camera	Pressure, Camera Temperature, Applanix POS MV providing 100 Hz Roll/Pitch/Yaw and positioning (G2 GNSS), sounder depth, camera angle from horizontal, USBL 1500	All but relatively steep terrain – always planned downslope; usually <100m water depth, turbidity, wind waves and strong currents in nearshore limiting factor – small vessel ops	(Jordan et al. 2010)
Deakin	400mm*600m m x 300mm	20	120	SD video oblique facing Additional oblique facing STEREO HD GoPro with 400mm base bar	12MP downward stills with strobe	Video ray lights for oblique view and strobe for down facing imagery		HOBO Pendant temperature/light data loggers (UA-002-08) recorded mean light (lum/ft ²) and temperature (°C) at ten-second intervals for the duration of each deployment		(Logan et al. 2017)



Table 7.2: Sample field datasheet to record metadata (i.e. deployment or event data) from each towed camera deployment.

	Gear ir	n watei	r	Gear o	n bott	om		Tow speed	Wire out (length) ¹	Wire out (angle) ¹	Gear off bottom				Gear o	out of w	/ater	Notes
Tow ID	Long	Lat	Time	Long	Lat	Depth	Time				Long	Lat	Depth	Time	Long	Lat	Time	



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8. MARINE SAMPLING FIELD MANUAL FOR BENTHIC SLEDS AND BOTTOM TRAWLS

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8.1 Platform Description

Benthic sleds (also called sledges) and bottom trawls both use nets to collect organisms while they are towed across the seafloor. While trawls use free nets with doors to spread the net, sleds use frames and runners to protect and anchor the net (Eleftheriou and Mcintyre 2005). Benthic sleds target sessile or sedentary macrofauna and megafauna with some designs able to be deployed over rugged terrain, while bottom trawls are typically more successful in collecting demersal or mobile fauna and are deployed over smooth terrain or soft sediments.

There is no one type of sled or trawl suitable for all habitats and depths, and selection of the most suitable type depends on scientific objectives, previous knowledge, targeted fauna, environment, depth, and vessel capabilities (Clark et al. 2016, Kaiser and Brenke 2016). Acquired data are usually semi-quantitative (Table 2.1 in Schiaparelli et al. 2016a) due to inconsistencies in gear path. swept area, and movement (e.g. sled skipping along seafloor), as well as taxa targeted by the gear (e.g. avoidance by highly mobile megafauna, herding effect in some fish). Imagery of the seafloor will help enormously with sled choice and deployment techniques. Imagery and geospatial positioning can be obtained with available technology and can aid in the success of each deployment. In the absence of imagery, bathymetry can also provide a good indication of gear suitability. The use of multiple types of sleds and trawls may be most appropriate for surveys trying to quantify overall biodiversity in a given location (Williams and Bax 2001, Clark and Roberts 2008), while a single sled or trawl may be more efficient for quantifying species in a particular location or habitat for monitoring purposes (Przeslawski et al. 2015). For these reasons, this manual does not mandate specific gear types, although sled and trawl types historically used in Australian waters are listed in Table 8.1 to help facilitate decisions regarding equipment for a given marine survey. Nevertheless, for monitoring purposes, it is preferable to maintain consistent gear in time and space, and we therefore recommend this where possible.

8.2 Scope

This Sled and Trawl Field Manual includes gear designed to sample organisms on the seafloor, excluding microbes and meiofauna (see chapters in Eleftheriou and Mcintyre 2005, Danovaro 2010 for such methods). General steps are outlined in and described in detail in the sections below.

Pipe dredges, rock dredges and other such gear are not included because biological collections by these are incidental. Similarly, commercial dredges are not considered because they have a narrow taxonomic focus (e.g. scallop dredge) and are not suitable for general monitoring purposes. Fish traps and similar gear are not included because they apply to shallow waters or reef-associated species and often use bait. This Field Manual does not target endobionts or burrowing species (e.g. animals living within sponges, rocks, corals) due to the excessive amount of time needed to process such animals (Coggan et al. 2005) and their limited use in a national monitoring program. Although some sleds are designed to sample small macrofauna and infauna (e.g. Brenke 2005), for the purposes of this field manual, we include only larger macrofauna and megafauna. Smaller taxa are targeted in the Grab and Boxcore Field Manual. If researchers opt to use a sled to sample smaller fauna, we recommend combining *Pre-survey Planning* and *Onboard Sample Acquisition* sections from this field manual with *Onboard Sample Processing* from the Grab and Box Corer Field Manual (Chapter 9).



8.3 Sleds and Trawls in Marine Monitoring

Sleds and trawls can be used to successfully monitor changes in benthic communities over time (Billett et al. 2001). However, they are becoming less popular for this purpose due to their destructive sampling, difficulty in revisiting exact sites, and sampling variability due to species and size selectivity. In addition, more quantitative underwater imagery technologies continue to develop and become more accessible.

Instead, sleds and trawls are now most likely to be applied in the first stage to a monitoring program as baseline data to inform imagery annotations by providing species inventories or biodiversity assessments (Przeslawski et al. 2015), particularly as related to new, endemic, or cryptic taxa. This is essential for environments and regions in which extractive sampling is the only means to examine and identify many species in complex ecosystems. The specimens themselves are used to inform taxonomic studies, ascertain species distributions, and as a source of genetic (DNA) data and isotope data. Thus their application is similar to grabs and boxcores, but sleds and trawls sample a large transect rather than a point. Therefore, they may be more suitable to assess macrofaunal biodiversity in the deep sea where light is low, species have few colour cues, abundances may be low, and deployment times are high.

8.4 Equipment

Equipment must be appropriately set-up to ensure as much consistency as possible among surveys and also to facilitate gear replacement if necessary. Equipment configurations can vary among substrate types. For example, in abyssal plains, wider skids on a beam trawl reduce sinking into mud. Table 8.1 lists the specifications, where available, of benthic sleds and trawls deployed in Australian waters.

The key components for a bottom trawl include the following, all of which should be documented and photographed:

- Sampling gear
 - Net (full net plans, including mesh types and sizes)
 - Floatation system (headline floatation plan, size, number, and position of floats)
 - Groundrope (groundrope composition, length, details of all components)
- Rigging plans
 - Sweep and bridle size and lengths
 - Layback of the headline (if any)
- Deployment procedures
 - Warp-to-depth ratios for amount of trawl wire
 - o Standard electronics to be used, and acceptable values of certain measurements
 - Required towing speed

The key components for a benthic sled include:

- Sampling gear
 - Net (full net plans, including mesh types and sizes)
 - Frame (full frame plan, including dimensions and weight, chafing mat)
 - Buoys (size, number, position)
 - Mouth dimensions
- Rigging plans
 - Bridle size and lengths
 - Weak links
- Deployment procedures
 - o Estimated amount of trawl wire

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- Standard electronics to be used, and acceptable values of certain measurements
- Required towing speed

8.5 **Pre-Survey Preparations**

<u>Identify a chief biologist or ecologist</u> who will be responsible for making decisions related to samples onboard, particularly regarding prioritisation of samples during onboard processing. This will be particularly helpful during busy periods with large hauls or multiple back-to-back tows. If 24-hour operations are planned, a second-in-charge will be needed as well.

<u>Confirm sampling design</u> meets survey objectives, is achievable with planned equipment and time, and has been communicated to all key scientists and managers. See Chapter 2 for further details on sampling design.

Consideration must be given to the <u>location of the trawl or sled during deployment</u>. Ultra-short baseline acoustic technology (USBL) is recommended to identify the true location of the sled/trawl during bottom contact (Schlacher et al. 2007), particularly in deep waters where the sled/trawl may be kilometres away from the vessel during a tow (Clark and Stewart 2016). If a USBL is unavailable in deep waters, the angle and length of wire payed out should be recorded so that sled/trawl location can be trigonometrically estimated (Milroy 2016). Station record forms should record gear location wherever possible, with vessel location recorded as a back-up.

Consideration must be given to the <u>stability of the trawl or sled during deployment</u>. Ideally, a Netsonde or bottom contact sensor will be used to indicate when the gear is lifting off the seafloor so that speed can be reduced or more wire payed out or retracted. With trawls, door-spread or wingend sensors are also useful to ensure consistency of gear set-up and performance. If these are unavailable, strict attention must be paid to the winch wire and constant adjustments performed or a self-tensioning winch used to ensure continuous bottom contact (Clark et al. 2016).

During the planning phases, <u>taxonomists and museum curators must be engaged</u> to ensure that samples will be appropriately identified and voucher specimens are lodged at national repositories (i.e. museums). They can also advise on the likely species selectivity of the proposed gear for certain taxa. Preferably, taxonomists will participate in marine surveys in which case they can identify much of their respective groups onboard (Zintzen et al. 2011). The appropriate taxonomic resolution at which specimens will be identified should also be determined. Species-level identification may be appropriate for voyages of discovery (Poore et al. 2015), while family level may be suited for measuring relationships with environmental covariates (Hirst 2006). For many surveys, identifications will only target selected groups (e.g. sponges in Przeslawski et al. 2015). This should be decided in the pre-survey planning stage, not *a posteriori* (i.e. during or after the survey). Importantly, non-target specimens should still be retained for museum lodgement if possible, in order to facilitate identification in the future if resources or priorities allow.

<u>The purposes of biological samples must be determined.</u> For monitoring purposes, samples of each target species or operational taxonomic unit (OTU) must be collected for taxonomic identifications. Further objectives specific to a given survey or project may also include samples for genetic or biochemical analyses for particular groups. Protocols for these samples (including preservation as per point below) must be developed prior to the start of the survey.

The <u>level of onboard searching and sorting</u> should be decided during the planning phase where there is sufficient information to inform discussion of likely catch rates. Onboard searching refers to the time spent looking through non-biogenic material to find biota, while onboard sorting refers to the taxonomic level to which biota are identified. Both will be determined by the key survey objectives, onboard taxonomic expertise, and available time and space. It is important that search Page | **156**

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effort is not adjusted between deployments as this is a source of variation. Onboard sorting may vary among groups (i.e. many fish may get sorted to species while invertebrates stay in coarse groups). At a minimum, samples should be sorted onboard by phylum to ensure correct preservation and assist dissemination post-voyage, but samples should also be able to readily be subdivided for many phyla (e.g., Cnidaria, Arthropoda, Echinodermata). Taxonomists are far more likely to be willing to engage in post-survey identifications where the sample has been sorted to an appropriate level onboard

<u>Decide on preservation methods</u>. This should be done in consultation with curators, taxonomists, molecular biologists, and biochemists that will be involved in using the samples. See Coggan et al. (2005) and Schiaparelli et al. (2016b) for information about appropriate preservatives for a range of taxa and purposes (e.g., species identification and description, genetic analysis, biochemical analysis), noting the variation between taxa.

<u>Ensure adequate risk assessments are undertaken</u> regarding safety and use of chemical onboard (i.e. ethanol, formalin). This should include where appropriate onboard storage for chemicals, as well as personal protective gear and ventilation.

<u>Determine if specialists are needed for gear use.</u> Many nets and sleds require experience to prepare, deploy and retrieve. The details below are not targeted for any one particular equipment or system, and we recommend engaging an experienced crew who have previously deployed similar devices.

<u>Obtain appropriate permits</u> that may apply for collection (Appendix B). Ideally, all surveys using sled, trawls or dredges will have a permit for biological collection, even if target samples are rocks and sediments. This will ensure incidental biological specimens do not get discarded overboard. Current regulations require permits for biological material being deposited in registered institutions. For Commonwealth waters, these include

- 1) Australian Fisheries Management Authority "Application for Scientific Permit"
- 2) Parks Australia: "Application for a permit to access biological resources in Commonwealth areas"
- 3) Parks Australia: "Application to Conduct Research Activities Within Commonwealth Marine Reserves"

Collection ethics approval may also be required from the research institution. In addition, more focussed permits including animal ethics may be needed for particular taxa (e.g. fish and cephalopods). Permits must be considered not just for collecting activities, but also for shipping and storage (e.g., biosecurity containment facilities). For example scleractinians, antipathrians, and some fishes are regulated under the Convention on International Trade in Endangered Species, and there may be restrictions on shipping these taxa to museums or other repositories (especially overseas institutions) without a permit.

Document the specifications of all sampling gear to be used, including photographs (see Equipment). Specifications that should be documented include gear size and configuration (mesh, floats, ground ropes, frame, spread between trawl doors), rigging plans (bridle, headline layback), and deployment needs (wire length estimated, required towing speed, netsonde or USBL methods). This can assist with estimating location and area of the seafloor sampled, as well as providing crucial information for comparisons with other surveys. Where possible, the gear set-up and specifications should be standardised across all surveys using the same equipment.

<u>Decide on procedures for very large hauls</u>. Sub-sampling or a focus on key taxonomic groups may save time needed for other survey operations (e.g. multibeam mapping) or objectives (e.g. biodiversity characterisation in different location) (Shimadzu and Darnell 2015). Alternatively, coarse

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level estimation of abundances could occur based on visual estimates or case counts. Such procedures must be decided before gear deployment and remain consistent for a given survey, and in all cases, representatives of all taxa should be collected and appropriately preserved. If time permits, pilot deployments can help determine the efficiency of the gear, deployment times, suitability of terrain, catch sizes over distances, and processing times.

<u>Organise shipment of samples from vessel to repository (e.g. museum).</u> If samples are frozen and are not too bulky, it may be most cost-effective to have individuals transport them on aircraft in which case airline requirements should be considered. If samples are in ethanol or formalin, transport of dangerous goods must be organised. Planning for shipment of samples well in advance of the survey will expedite demobilisation and ensures sample integrity. The destination museum can likely provide advice on shipping methods and regulations. See Schiaparelli et al. (2016b) for shipping advice.



8.5.1 Pre-survey checklist

Task	Description/comments
Identify onboard chief ecologist/biologist	
Confirm sampling design meets necessary criteria	
Engage taxonomists and curators	
Determine onboard sorting level	
Determine preservation methods	
Complete necessary risk assessments	
Identify specialists needed for gear configuration and deployment	
Data storage needs identified and hardware purchased accordingly	
Decide on methods for locating gear during deployment	
Decide on methods to assess gear stability during deployment	
Obtain appropriate permits	
Document gear specifications	
Determine procedures for large hauls	
Organise shipment of samples	



8.6 Field Procedures

A visual summary of the key steps to follow when deploying benthic sleds or bottom trawls is shown in Figure 8.1.

8.6.1 Onboard sample acquisition

- 1. Use acoustic data or underwater imagery to confirm areas to sample with the appropriate benthic gear (Schlacher et al. 2007, Williams et al. 2010). Do not deploy blind, as this increases the risk of equipment loss and damage, as well as unnecessary impact on potentially vulnerable ecosystems.
- 2. Brief crew and sorting staff on potential venomous or otherwise dangerous catch (i.e. cone shell, blue-ringed octopus, some fishes, corals, sponges).
- 3. Ensure the gear is set-up and deployment parameters and procedures are as documented in the gear-specific protocols.
- 4. Use netsonde or bottom contact sensor to ensure sled or trawl is suitably deployed along the seafloor [*Recommended*]
- 5. Use USBL System to ensure accurate positioning (Schlacher et al. 2007, Williams et al. 2015) [*Recommended*]
- 6. Mark sled runners or trawl groundline with waterproof pencil or paint to gauge success of seafloor deployment. Also check for polishing on the bobbins or runners. *[Recommended]*
- 7. Record all metadata related to a given tow, specified in Table 8.2.
- 8. For rugged slopes (e.g. seamounts), ensure appropriate gear is used and tow downslope to reduce snags.
- 9. Maintain speed that is appropriate for the gear and seafloor terrain. Epibenthic sleds and most beam and Agassiz trawls should be towed at 1–2 knots to maintain bottom contact, while faster speeds of 3–3.5 knots are appropriate for otter trawls and other gear dependent on speed to maintain net spreading. See Clark et al. 2016 and Kaiser and Brenke 2016 for details.
- 10. Tow into the swell, tide, current and/or wind so that vessel speed and steerage can be better controlled.
- 11. We are unable to set a standard required tow distance (i.e. bottom time) for monitoring purposes because tow distance is highly dependent on gear type and seafloor environment. However, within a given survey, tow distance for each sled or trawl should be standardised to assess relative abundances. It should also be recorded in the metadata (Table 8.2). If the same sled is used on multiple surveys in similar environments, the tow distance should remain the same so that spatiotemporal comparisons can be made. For benthic sleds deployed along the continental shelf over mixed terrain, a tow distance of ~100 m is recommended. Longer tows will be needed (commonly 300 m) in deep waters due to lower density of macro- and megafauna. Information from multibeam data (see point 1) can help inform tow duration decisions.



- 12. Assess success of deployment. If there is significant damage to gear, signs of minimal bottom contact, or ripped nets, this should be recorded in the metadata (Table 8.2). The catch from such deployments should be considered only for presences-only analyses, species inventories or biological analyses but should not be used for quantitative comparisons with other tows. In such situations, gear configuration should also be checked after recovery to ensure its correct specification for the next deployment (see point 3).
- 13. When the sled or trawl is lifted from the water, follow gear- and vessel-specific protocols for safe release of the catch onto the deck or sorting table.
- 14. Record biomass of entire catch using electronics from winch system or onboard scale [Recommended]
- 15. Photograph entire catch with station identification placard and make notes of catch composition (e.g. lots of mud or rocks) in metadata sheet (Table 8.2).
- 16. Remove all animals from the entire net, including the fore-parts of nets and sleds and not just the codend where most of the catch should have been collected. As soon as practical, begin onboard processing of the samples (next section).
- 17. Clean sled of all material and prepare for next deployment.

8.6.2 Onboard sample processing

- 1. For very large catches, implement sub-sampling protocol if applicable (see Pre-Survey Preparations).
- 2. Consider retaining material on ice or in an ice slurry while awaiting sorting to ensure material remains in best condition to assist accurate and consistent identification.
- 3. Separate large easily visible taxa into sorting trays by coarse groups: fish, sponges, soft corals, echinoderms, molluscs, ascidians, bryozoans, annelids, other. Weigh each group. Discard severely damaged organisms and non-biogenic material, unless otherwise needed. It can be useful to record the weights, descriptions, and images of rock, coral rubble and other non-biogenic material as this gives useful information on substrate type. Add a label to each sorting tray with Tow ID so as to avoid confusion when multiple tows are being processed.
- 4. Follow Animal Ethics procedures to euthanize animals where applicable
- 5. Place fragile organisms in seawater in the sorting trays. Use chilled seawater for deep-sea and polar samples to minimise sample degradation during sorting time.
- 6. Transfer groups to the sorting station, if not already there. See Coggan et al. (2005) for practical advice on setting up a sorting station.
- 7. Based on previous decisions about onboard level of sorting (Section 8.5), progressively sort organisms into finer taxonomic groups, as much as time or expertise allow, with OTU (operational taxonomic unit) or species representing the finest taxonomic level.



- 8. Weigh, count, and photograph each of the final groups, including a scale bar and unique identifying sample number. Refer to Schiaparelli et al. 2016 for suggestions on specimen photography.
- 9. Record data against a unique station identifier for the data base and keep a label with the same unique identifier with the specimen(s) (Table 8.3). At this stage identify specimens (or subset of specimens) for analyses purposes (whole specimens for taxonomy/ isotopes/genetics etc.) or where appropriate (and pre-determined in plan) take tissue samples for analyses (genetics isotopes etc.) If there are large numbers of the same species or OTU, only a sub-set may need to be preserved for museum collections; this should be established during Pre-Survey Planning in consultation with taxonomists or curators. In this case, record the total number collected as well as the number in the collection container.
- 10. If applicable relax and fix specimens according to survey objectives and taxonomists' preferences (e.g. samples for genetic analysis should not be fixed in formalin).
- 11. Preserve specimen according to methods decided in Pre-Survey Preparations, and place into container. See Rees (2009) and Schiaparelli et al. (2016b) for comprehensive description of fixatives and preservatives used for marine invertebrates.
- 12. Place solvent-hardy label with unique identifier in each sample container. It is not sufficient to label only the outside of the container, as this can easily rub off. See Box 15.6 in Schiaparelli et al. 2016 for suitable label characteristics.
- 13. Place container in large sealable container (i.e. lidded drum) with other samples preserved using the same chemicals (e.g. ethanol) or method (e.g. freezing). It saves time in post-survey sample distribution if taxa are grouped together in containers rather than by station.

8.6.3 Onboard sample storage

- 1. Store large labelled drum onboard in the freezer or in an approved storage area for hazardous chemicals.
- 2. Transcribe metadata from Tables 8.2 and 8.3 into digital format as soon as possible to minimise the build-up of data entry. This must be done onboard preferably during the same shift because it provides a back-up and an immediate check of the record, as well as facilitating timely metadata release.
- 3. Check the data entry is correct by cross-checking field sheets with database. This is best done by the person who didn't enter the data [*Recommended*].
- 4. During demobilisation, ensure samples and drums are properly labelled and closed, and implement shipping according to decision made during pre-survey planning.



8.7 **Post-survey procedures**

8.7.1 Sample curation

- 1. Lodge all specimens in an internationally recognised and routinely maintained specimen collection (e.g. museum) for curation and public accessibility [*Recommended*].
- 2. If all specimens are unable to be lodged at a museum due to lack of resources or need for destructive analyses (e.g. biochemical analyses), voucher specimens must be lodged (i.e. at least one animal per OTU).

8.7.2 Data release

Traditionally, data related to biological specimens have been delivered as presence-only taxonomic identifications. These are often managed by individual museum scientists or curators and subsequently harvested by the Atlas of Living Australia (ALA) and the Ocean Biogeographic Information System (OBIS). These portals do not yet include absences or information related to sampling effort, thus reducing the applicability of such database to monitoring purposes.

There are current initiatives underway that aim to incorporate species presence data to more ecologically relevant applications. For example, OBIS International manages a project called OBIS-ENV-DATA that extends data structures to allow linking species data to other related data (environmental, images, sampling effort) (De Pooter et al. 2017). In the meantime, the steps listed below will ensure appropriate and timely release of both metadata and data:

- Create a metadata record describing the data collection. Provide as much detail as possible on the collection/deployment (either directly in the metadata record itself, or in the form of attached field sheets as .csv, .txt or similar). This should include sampling locations and dates, equipment used, level of sorting applied, etc. All collection/deployment information must be QC-d before inclusion.
- 2. Publish metadata record(s) to the Australian Ocean Data Network (AODN) catalogue as soon as possible after metadata has been QC-d. This can be done in one of two ways:
 - a. If metadata from your agency is regularly harvested by the AODN, follow agencyspecific protocols for metadata and data release.
 - b. Otherwise, metadata records can be created and submitted via the <u>AODN Data</u> <u>Submission Tool</u>. Note that this tool requires user registration, but this is free and immediate.

This step provides immediate documentation of the methods and location of the collection of biological material. This stage may also include links to field reports or data sheets.

- 3. Produce a technical or post-survey report documenting the purpose of the survey, survey design, sampling locations, sampling equipment specifications, and any challenges or limitations encountered (Appendix C). Provide links to this report in all associated metadata records [Recommended]
- 4. Complete the species identifications and associated abundance or biomass for targeted groups identified. This can take quite some time, depending on sample size and available resources. It is not unusual for taxonomic identifications to lag years behind survey completion, but this should not delay publication of initial metadata and deployment information. Care must be taken Page | 163



to ensure consistent nomenclature is used and documented for undescribed or unnamed species (e.g. defined Operational Taxonomic Units, OTUs). Ideally photographic catalogues of OTUs are established such that subsequent surveys may use consistent OTU classification, thereby ensuring comparability of data between surveys.

- 5. QC the data. This includes checking for spelling errors, missing data, consistent nomenclature and use of OTUs, and confirmation that outliers are not data entry errors (e.g. 100 individuals really were collected, not just 10).
- 6. Attach or link the full data spreadsheet (including absences and abundances/biomass) to the metadata record previously created and published to the AODN. This will ensure public discoverability and accessibility of the complete data, including absences.

8.8 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 8 (field manual for sleds and trawls) is below:

Version Number	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019





Figure 8.1 Images from key steps involved in the use of benthic sleds and bottom trawls for marine monitoring: a) a modified WHOI sled with attached pipe dredges, b) seafloor imagery from towed video and bathymetric grids, c) lowering the AIMS benthic sled, d) sorting animals on the back deck, e) photographing specimens in ship laboratory, f) securely sealed containers to ship animals to museums

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Table 8.1 Types of benthic sleds and trawls deployed in Australian waters and their associated characteristics. See reviews on benthic sleds and trawls for information about gear deployed elsewhere in the world (Clark et al. 2016, Kaiser and Brenke 2016).

Туре	Dimensions (mouth, h x w)	Weight	Target taxa	Cod end	Other features	Suitable terrain	Ref
Sherman (CSIRO- SEBS) sled	600 X 1200 mm	860 kg (excluding modifications from Lewis 2009)	Benthic invertebrates and fish	Polyethylene twine, 3.2 m long, 25 mm mesh	Reinforced frame, weak link chains, chaffing mat, net sonde, optional infaunal or 1 mm net	Seamount, rugged terrain, hard substrates	(Lewis 1999, 2009)
Rainer sled	2900 mm width	590 kg	Benthic invertebrates	25 mm stretch mesh	Sled divided into epibenthic and infaunal halves	Various shelf substrates	(Bax et al. 1999)
AIMS sled	1500 x 1000 mm		Large benthic invertebrates	45 mm stretch diamond		Various shelf substrates	(Colquhoun et al. 2007)
SARDI sled	600 x 1800		Sessile and sedentary epibenthos	50 mm mesh		Soft sediment shelf ecosystems	(Ward et al. 2006)
NIWA seamount sled	1130 x 380	400 kg	Sessile and sedentary epibenthos	28 mm	Reinforced frame, weak link chains, location beacon, anti- chafing net, smaller model available (250 kg)	Seamount, rugged terrain, hard substrates	(Clark and Stewart 2016)
Brenke Sledge (MNF)	1.3 m wide, 1.24 m high		Benthic macrofauna	0.5 mm mesh	Dual nets, nodule exclusion mesh, insulated cod end	Smooth terrain	(Brenke 2005)
MAPS sled					Concurrent planktobenthic and		(Przeslawski and McArthur 2009)



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					benthic sampling, tri- layered net		
Scaled down Woods Hole	300 mm					Estuaries	(Hirst 2004)
CSIRO beam trawl	500 x 4000 mm			25 mm mesh	Tickler chains, triple tow bridle, chaffing mat, pivot points	Flat to low relief terrain, soft substrates	(Lewis 2010)
Orange roughy trawl (ORH)	26 m x 6.5 m	3 t in water	Large mobile fauna	Various depending on cod-end fitted (40 mm common)	Small attached cone nets to sample small animals, otter boards, heavy duty high ground gear	Rough bottom, including seamounts	(Clark et al. 2016)
Full-wing bottom trawl	28 m x 3.5 m	3 t in water	Mobile fauna, demersal and benthic species	Various depending on cod-end fitted (40 mm common)	Otter boards	Smooth terrain	(Clark and Roberts 2008)
NORFANZ beam trawl	300 x 4000 mm		Slower-moving demersal fish, benthic invertebrate mega-fauna	10 mm	Chaffing mat	Smooth terrain	(Clark and Roberts 2008)
Florida flyer shrimp trawl			Mobile fauna, demersal and benthic species			Smooth terrain	(Wassenberg et al. 1997)
McKenna market trawl (CSIRO)	19m wide, 5 m high		Mobile fauna, demersal and benthic species	15 mm	Weighted bottom line, floats hold up the upper line, doors keep the net	Smooth terrain	SEF voyages, NWS voyages, <i>RV</i> <i>Investigator</i> deep- sea



	Gear ii	n wate	r	Gear	on bot	ttom		Tow speed	Wire out (length) ⁸	Wire out (angle) ⁸	Gear o	off bott	om		Gear out of water			Total catch biomass ⁹	Notes ¹⁰
Tow ID	Long	Lat	Time	Long	Lat	Depth	Time ¹¹				Long	Lat	Depth	Time ¹¹	Long	Lat	Time ¹¹		
GENI (e.g.	ENERAL GEAR NOTES																		

Table 8.2 Sample field datasheet to record metadata (i.e. deployment or event data) from each sled or trawl haul. Waterproof paper and pen/pencil is required.

⁸ Record the length and angle of wire payed out during seafloor contact. This is required if deep water survey with no USBL; otherwise recommended.
⁹ Include units (e.g. kilograms)
¹⁰ Record person entering data, spread of trawl doors if applicable
¹¹ UTC timezone



Table 8.3 Sample field datasheet to record metadata from each sorted biological sample. Waterproof paper and pen/pencil is required.

Tow ID	Sample ID	Phylum	Class	Order	Family	Genus, Species / Common Name	Weight	Abundance	Preservative / Quantity	Photos	Notes



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9. MARINE SAMPLING FIELD MANUAL FOR GRABS AND BOX CORERS

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9.1 Platform Description

Grabs and box corers both use receptacles to collect sediment after they are dropped to the seafloor. While the scooping motion of grabs disrupts unconsolidated sediment to various degrees, box corers return largely undisturbed samples of the sediment strata (Eleftheriou and Mcintyre 2005). Grabs and box corers target surface sediment and associated porewater and fauna. They are typically deployed over sandy or muddy substrates, although some grabs can collect gravel or cobbles.

There is no single type of grab or box corer suitable for all environments, and selection of the most suitable type depends on the biological or physical target, substrate, depth, and vessel capabilities (Narayanaswamy et al. 2016). Acquired data can be quantitative (e.g. volumetric or mass specific) or semi-quantitative due to inconsistencies in sample volume and sediment disruption due to bow waves or other gear effects (Blomqvist 1991). For these reasons, this manual does not mandate specific gear types. There are numerous references to help facilitate decisions regarding grab and box corer equipment for a given marine survey (Riddle 1989, Eleftheriou and Moore 2005, Danovaro 2010, Narayanaswamy et al. 2016). Nevertheless, for monitoring purposes, it is preferable to maintain consistent gear through time and space, and we therefore recommend this where possible.

9.2 Scope

This Grab and Box Corer Field Manual includes gear designed to sample unconsolidated sediment and organisms on the seafloor. General steps are outlined in Figure 9.1and described in detail in the sections below.

The samples collected by grabs and box corers can be used to derive a range of physical, chemical, and biological parameters (Eleftheriou and Mcintyre 2005), and each of these parameters requires a particular method to process and analyse the sample (Danovaro 2010). In the interest of developing a standard protocol for marine monitoring that is readily accessible to multiple users among various disciplines, this field manual includes only a sub-set of these variables (Table 9.2). These variables were chosen because they can be used by multiple disciplines, are relatively easy to undertake, require minimal specialised equipment or chemicals, and are applicable to ecological indicators in marine monitoring (Hayes et al. 2015). Importantly, the protocol detailed here does not preclude other parameters from being investigated; rather it provides an achievable standard for acquiring fundamental data for monitoring that can be expanded as required to meet additional objectives on a given survey.

This field manual does not include methods for sediment contaminant monitoring, as this is considered more applicable to coastal environments and is comprehensively covered elsewhere (Simpson et al. 2005). As activities develop (e.g. deep-sea mining) the scope may be expanded in future field manual versions to encompass sediment contaminant monitoring.

Other equipment able to sample sediment is not included in this field manual due to difficulties deploying in deeper waters (e.g. suction samplers) orlimited applicability to biological sampling (e.g. gravity, piston, vibrocores) (Eleftheriou and Moore 2005). In addition, multicorers are not explicitly included because small sample volume may preclude the collection of representative biological communities without aggregation (Williams et al. 2017), although we note that multicorer samples can be aggregated and processed as described in this manual. Although they are able to quantify infaunal activity, sedimentology, and biogeochemistry, sediment profile imaging (SPI) is also excluded from this field manual due to the vast differences in equipment requirements and data processing (Aller et al. 2001, Germano et al. 2011).

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Although larger grabs and box corers can sample larger macrofauna and megafauna, including epifauna, for the purposes of this field manual, we focus on smaller macrofauna, including infauna. Epifauna are targeted in the Sled and Trawl Field Manual. If researchers opt to use a grab or box corer to sample epifauna, we recommend combining Pre-Survey Preparations and Onboard Sample Acquisition from this Field Manual with Onboard Sample Processing from the Sled and Trawl Field Manual (Chapter 8). Meiofauna are not included in the current version of this field manual, and in 2018 we will scope the feasibility of their inclusion in future versions.

9.3 Grabs and Box Corers in Marine Monitoring

Grabs and box corers have been used successfully to monitor changes in benthic environments over time (Maurer et al. 1993, Ruso et al. 2007, Frid 2011, Clare et al. 2015), although the challenges revisiting sites mean that multiple samples across a representative area of a given habitat type may be necessary to detect trends (Morrisey et al. 1992, Rogers et al. 2008, Spencer et al. 2011). In addition, repeated sampling using grabs and corers in the same area may result in disturbance and associated artefacts (Skilleter 1996). Grabs and corers can also provide species inventories or biodiversity assessments which can then be applied to a monitoring program as baseline data or to inform the interpretation of imagery (Przeslawski et al. 2013). In this way, they are similar to sleds and trawls, but grabs and corers sample a much smaller spatial area (< 1 m^2 , often considered a point location) rather than the hundreds of square metres often traversed by a sled. This characteristic needs to be considered in environments of low faunal abundance (e.g. some deep sea areas) or high heterogeneity.

9.4 Equipment

Equipment must be appropriately set-up to ensure as much consistency as possible among surveys and to facilitate gear replacement if necessary. The overarching goal of appropriately choosing and setting up equipment is to sample as much of the sediment as possible with minimal disruption, within the limitations of the given equipment. It is recommended that a survey include at least two gear types to sample sediments, one targeted for finer sediment (muds) and the other targeted for sands and coarser sediments (gravel). Researchers should ensure appropriate statistical tests are performed to test for potential confounding factor of gear type on biological variables.

The key components for a grab include the following, all of which should be documented:

- Type of grab, including firing mechanism (e.g. Van Veen, Smith-McIntyre, Shipek)
- Weight of grab
- Bucket (shape, maximum volume)
- Maximum penetration into the substrate
- Trap door to allow examination of sample volume upon recovery and to allow sediment sampling from the relative undisturbed centre. All grab designs can have this feature, but not all manufacturers include it.
- Additional weights (by providing an option for extra attached weights to a grab or corer, equipment functionality can be optimised among more habitat types)
- Standard electronics to be used (e.g. camera, USBL)

The key components for a corer include the following, all of which should be documented: Page | 174

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- Type of corer (e.g. box, multicorer)
- Weight of corer
- Maximum sample volume
- Additional weights (by providing an option for extra attached weights to a grab or corer, equipment functionality can be optimised among more habitat types)
- Standard electronics to be used (e.g. camera, USBL)

Grabs and box corers can also be fitted with other sampling platforms and sensors. A mounted video camera can add valuable information about the *in situ* appearance of the seabed that is sampled, as well as an indication of the performance of the gear (Blomqvist 1991). Similarly, conductivity-temperature-depth (CTD) meters and other sensors provide information about the surrounding environment, while a pinger (i.e. near-bottom echosounder) provides information to the operator about distance to the seafloor which can be very important for controlling the final operation near the seafloor (Narayanaswamy et al. 2016).

9.5 **Pre-Survey Preparations**

<u>Identify a chief scientist</u> who will be responsible for making onboard decisions related to samples, particularly regarding prioritisation of samples during onboard processing. This will be particularly helpful during busy periods with multiple back-to-back deployments. For 24-hour operations, a second-in-charge must also be identified.

<u>Confirm sampling design</u> meets survey objectives, is achievable with planned equipment and time, and has been communicated to all key scientists and managers. See Chapter 2 for further details on sampling design.

Address fine-scale variation and the need for replication in survey sampling design. Although replication should be considered in sampling design among all platforms (Chapter 2), it is particularly important for grabs and box corers due to the large variation in biological and environmental variables across metres to centimetres that may preclude the detection of changes over time (Rogers et al. 2008). Each box core or grab deployment should be treated as a discrete sample (i.e. sub-dividing sample is not replication). In addition, the type and size of bedforms present should be considered in the assessment of replicates. For example, a grab may drop on the crest of a sand wave, thereby returning a sample that is not representative of the broader seafloor. We recommend at least three replicate deployments be undertaken at each station (e.g. Long and Poiner 1994) to enable the quantification of fine-scale variation. When this is not be feasible (e.g. in deeper waters with long deployment times, priority to maximise spatial extent of sampling area), replicates should be collected from a sub-set of stations (e.g. Przeslawski et al. 2013) or appropriate geostatistical methods must be used to estimate grab-to-grab variance (Diggle and Ribeiro 2007).

The most <u>appropriate grab or box corer must be identified</u> to suit the vessel, environment, and scientific objectives (Rumohr 1999). Although this Field Manual does not require equipment that preserves the integrity of sediment samples (e.g. multicorer), the use of such equipment may be necessary if a marine survey has scientific aims complementary to the monitoring program (e.g. characterising infauna or geochemical variables through the vertical sediment profile Eleftheriou and Mcintyre 2005). The results of some sedimentological and geochemical analyses are sensitive to the manner in which the original samples are collected, handled and stored. Ideally, marine sediment collection for the assessment of sedimentology and biogeochemistry should be carried out avoiding any unnecessary manipulation of the sample that could preclude identification of the surface layers. In order to concurrently acquire the fundamental data identified in this Field Manual Page | **175**

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(biology, sedimentology, biogeochemistry), the chosen grab or box corer should sample an area of the seafloor at least 0.1 m^2 and be able to penetrate 5-10 cm into the sediment (Rumohr 1999, Bale and Kenny 2005). To maintain consistency between sites and repeat surveys, only the top 2 cm should be sampled for sedimentology and biogeochemistry; if the sample is disturbed such that the top 2 cm cannot be identified, we recommend redeploying the gear.

Consideration must be given to the <u>location of the grab or corer during deployment</u>. For deep waters where the gear may be hundreds of metres away from the vessel during sample collection, an ultrashort baseline (USBL) is recommended up to 6000 m to identify the true location (Narayanaswamy et al. 2016). If a USBL is unavailable, the angle and length of wire payed out should be recorded so that gear location can be trigonometrically estimated (Milroy 2016).

During the planning phases, <u>taxonomists and museum curators must be engaged</u> to ensure that all biological specimens are appropriately identified and lodged at national repositories (i.e. museums). The appropriate taxonomic resolution at which specimens will be identified should also be determined. Species-level identification may be appropriate for voyages of discovery (Przeslawski et al. 2013), while family level identifications can be reliable measures of response to environmental gradients (Olsgard et al. 1998, Thompson et al. 2003, Wlodarska-Kowalczuk and Kedra 2007).

Similarly, <u>contractors or collaborators for sedimentological and geochemical analyses must be</u> <u>engaged</u> if in-house capability is not available, including cost and funding sources for such analyses. Geoscience Australia can be contacted at <u>marine@ga.gov.au</u> regarding grain-size, carbonate and loss on ignition (LOI) analyses to confirm capability and timing.

Decide on sediment storage and biological specimen preservation or fixation methods. Sediment samples will need to be refrigerated (for sedimentology) or frozen (for biogeochemistry) while biological specimens will need to be preserved. Depending on the collaborating taxonomists and project objectives, larger or fragile biological specimens may be preserved separately (e.g. ophiuroids) or in a different preservative (e.g. formalin to retain morphological integrity of soft-bodied animals). In addition, staining may be used to aide sorting, although this may hinder species-level identifications. Choice of fixatives, preservatives and stains must be done in consultation with taxonomists, molecular biologists, and biochemists that will be involved in using the samples. See Coggan et al. (2005) and Schiaparelli et al. (2016b) for information about appropriate preservatives for a range of purposes (species identification and description, genetic analysis, biochemical analysis).

<u>Ensure adequate risk assessments are undertaken</u> regarding safety and use of chemical onboard (i.e. ethanol, formalin). This should include where appropriate onboard storage for chemicals, as well personal protective gear and ventilation.

<u>Obtain appropriate permits</u> that may apply to collect specimens. Ideally, all surveys using grabs or corers will have a permit for biological collection. If target samples are sediments for physical analyses (e.g. geology survey), biota will still be collected as part of the sample. Without appropriate permits, biological material simply gets discarded overboard. Permits must be considered not just for collecting activities, but also for sample transport to receiving institutions. For example, scleractinian corals are regulated under the Convention on International Trade in Endangered Species, and there may be restrictions on shipping these taxa to museums or other repositories (especially those overseas) without a permit. See Appendix B for a list of possible permits needed for sampling in Commonwealth waters.

<u>Document the specifications of all sampling gear</u> to be used. This includes gear size and configuration (dimensions, weight) and deployment needs (wire length estimated, USBL methods),



as well as sampling area, maximum volume, and maximum digging depth. This information must be included in survey metadata.

<u>Determine if specialists are needed for gear use.</u> Many grabs and box corers require experience to safely prepare, deploy and retrieve. The details below are not targeted for any one particular equipment or system, and we recommend engaging an experienced crew who have previously deployed similar devices. Adequate risk assessment of gear use must be undertaken prior to deployment, with all gear operators thoroughly briefed.

<u>Establish a standardized winching process</u> suitable for the chosen gear, as this is critical to maintenance of sample quality. For example, most gear should involve a complete stop and slow lowering for the last few metres. This will reduce the shock wave and associated loss of surface material and reduce the likelihood of raising of the sampler before closure is completed (Rumohr 1999).

<u>Design</u> workspaces and workflows for sedimentology, biogeochemistry, and biological subsampling. Each collected sediment sample must be sub-sampled because each discipline requires particular methods and preservatives that may interfere with the other. For example, the decomposition of infaunal animals affects organic content and other biogeochemical parameters, but biological preservatives will interfere with many geochemical analyses (Bale and Kenny 2005).

<u>Organise shipment of samples from vessel to repository.</u> If only of a small size, refrigerated and frozen sediment samples may be more cost-effective to be transported by passengers on aircraft in which case airline requirements should be considered. Samples in ethanol or formalin are considered dangerous goods, and associated transport must be arranged. Planning for shipment of samples well in advance of the survey will expedite demobilisation and ensures sample integrity.



9.5.1 Pre-survey checklist

Task	Description/comments
Identify onboard chief scientist	
Confirm sampling design meets necessary criteria (e.g. replicates)	
Identify most appropriate grab(s) or corer(s) to be used	
Engage taxonomists, curators and contractors. Cost activities	
Storage and preservation methods determined. Risk assessment done.	
Method(s) decided for locating gear during deployment	
Appropriate permits obtained	
Document gear specification and establish winch protocols	
Determine if specialists needed for deployment	
Design workspaces and workflows	
Organise shipment of samples	


9.6 Field Procedures

9.6.1 Onboard sample acquisition

- 1. Use multibeam data or underwater imagery to confirm appropriate areas to sample (soft vs hard substrate) and to identify the most appropriate equipment based on fine or coarse sediments.
- 2. Use USBL System to ensure accurate positioning (Schlacher et al. 2007, Williams et al. 2015) [recommended, especially in deep waters]
- 3. Record all metadata related to each sample station, specified in Table 9.3..
- 4. Deploy the grab or corer according to gear-specific protocols.
- 5. When the equipment is lifted from the water, follow gear-specific protocols for its safe return to deck and access to the sample. Special care may be needed in rough conditions to ensure the sample is not spilled.
- 6. Assess the success of deployment and record the proportion of grab or corer filled (Table 9.3).
- 7.). If there is significant damage to gear, gear closure failure, sample spillage or scant sample return, the sample should not be used in quantitative comparisons with other deployments. If possible, repeat a deployment at that location. Scant sample is defined as being at least 50% empty.
- 8. Record general observations, particularly evidence of anoxic or reduced sediments (i.e. black/green colour, sulphur smell).
- 9. Photograph the entire sample with station identification placard.
- 10. As soon as practical, begin onboard processing of the sample for sedimentology, biology, and biogeochemistry (next sections, Figure 9.1).
- 11. After all samples have been removed (next sections, Figure 9.1), thoroughly wash gear to prevent cross-contamination. Set up gear for next deployment or safely stow if operations have ceased for the day.





Figure 9.1 Workflow for onboard sample acquisition and processing from grabs and box cores.

9.6.2 Onboard sample processing & storage

- 1. For most equipment, the sedimentology and geochemical sub-samples can be accessed while the sample remains in the grab or corer, thus minimising disturbance. The biological sub-sample can be processed after these sub-samples have been removed.
- Pass any excess water from the sampling gear over a 500 µm sieve; for a box core this will likely need to be done with a siphon. Process the material retained on the sieve, refer to biological steps below.
- 3. Undertake geochemical, sedimentological, and biological processing steps below for each sediment sample collected.
- 4. After samples are processed, transcribe the metadata from Table 9.3 into digital format. This can be done in the evening or during other shipboard operations, but it should be done onboard because it provides an immediate back-up, allows for correction of obvious errors, and facilitates timely metadata release.
- 5. During demobilisation, ensure samples and drums are properly closed and implement shipping according to decision made during pre-survey planning.

Sedimentology (texture, colour and composition)

The following procedures are to be used to obtain sediment samples for quantification of commonly analysed metrics related to grain size and carbonate content (Nichol et al. 2013).

1. Using a spatula or spoon, scrape surface sediment to a maximum depth of approximately 2 cm. Collect a maximum of 300 g wet weight (or three tablespoons) in a plastic zip-lock bag. Leave any visible living organisms for biological steps below, but retain shell material.



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Describe the entire sediment sample using a visual assessment. First estimate the dominant constituent as Mud, Sand or Gravel. Do this by estimating the proportion of Gravel as %, and then the relative ratios of Mud to Sand. Gravel is > 2 mm diameter, including any shell fragments, coral, rhodoliths or rocks. Sand is < 2 mm and > 0.063 mm diameter. Mud is < 0.063 mm diameter.

The following description will assist a visual and tactile assessment:

- <u>Sand</u> Individual grains can be readily seen and felt. When moist, sand will form a cast that crumbles when touched.
- <u>Muddy sand</u> Sand grains are visible but the sample contains enough mud (silt and clay) to make it somewhat coherent. Will form a cast when moist that can bear careful handling without breaking.
- <u>Mixed sediments</u> Even mixture of sand and mud. Has a gritty feel, but smooth overall and slightly plastic. Will form a cast when moist that can bear firm handling without breaking.
- <u>Sandy mud</u> Overall fine texture, slightly gritty to feel that can form a thin ribbon when rolled. Will form a cast when moist that can bear robust handling without breaking.
- <u>Mud</u> Uniformly fine texture, sticky and with very slight gritty feel if silt is present. Will form a long flexible ribbon when rolled.
- 3. Assign a Simplified Folk Textural Class to the sample, based on the estimated mud, sand, and gravel proportions (Figure 9.2, Table 9.1).







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%	Sand :	Simplified Folk	
Gravel	Mud Ratio	Class	
>80	>9:1	Coarse sediment	
>5, <80	<9:1	Mixed sediments	
<5	>4:1	Sand and muddy	
		sand	
<5	<4:1	Mud and sandy mu	d

Table 9.1: Simplified Modified Folk Textural classes for visual classification of seabed sediments

- 4. Assign a colour to the <u>whole</u> sample using a Munsell colour chart, noting the Munsell code (colour, value, chroma) <u>and colour name [*Recommended*]</u>.
- 5. Estimate whether the sample is comprised of dominantly (>50%) carbonate material, non-carbonate (i.e. lithics), or mixed.
- 6. Note the presence of other materials, such as whole shells, articulated bivalves, shell fragments, corals, wood or lithics and record the relative abundance as: Trace (just noticeable); Few (noticeable); Common (very noticeable); Abundant (little else noticeable).

Record the above properties with all available metadata (Table 9.3), as in the example below:

- Sand and muddy sand
- 7.5 YR 7/6 (reddish yellow)
- Carbonate dominant
- Trace of volcanic rock fragments
- 7. Photograph the sediment sample with a label, scale and Munsell colour chart [Recommended].
- 8. Double bag the sample. Label clearly on the surface of the bags, as well as on aluminium tags or waterproof paper placed between the bags. Refrigerate.

Biogeochemistry (chlorophyll-a, organic matter content, redox)

These geochemical analyses are based on the assumption that the sediment surface is relatively intact and the surface sediments can be identified. If this is not the case, it is recommended only organic matter content is assessed, with information on sediment mixing recorded in the comments section of the metadata sheets (Table 9.3). The following procedures are to be used to obtain geochemical samples for quantification of commonly analysed metrics related to chlorophyll-a (Danovaro 2010), organic matter content (Heiri et al. 2001, Wang et al. 2011), and redox (Danovaro 2010, Edgar et al. 2010). For all biogeochemical samples, record the geochemical samples taken on a station form with all available metadata (Table 9.3).

<u>Redox</u>

- Use a suitable redox probe consisting of a portable pH/Eh meter, redox electrode (with shaft >15 cm long, preferably as thin as possible, with Platinum indicator electrode) and a reference electrode (double junction silver/silver chloride).
- Use Zobell solution as a reference to calibrate the redox electrode. The solution (0.003M potassium ferricyanide, 0.003M potassium ferrocyanide, and 0.1M potassium chloride) has an Eh value of +430 mV at 25°C.



- Carefully insert the redox electrode into the intact sediment surface as soon as possible after collection at depth intervals of 1 cm from the surface to 10-20 cm (depending of depth of sediment).
- 4. Record the Eh readings (in mV) when the meter readings stabilise at each depth.

This method provides a rough indication of the levels of oxygen in the substrate. This information is crucial to assess the interstitial conditions of the sediment as affected by burrowing organisms or anthropogenic factors. Measured in millivolt, often reported as Eh (hydrogen standard electrode) the redox potential has a low-definition significance because of the multi-factors interacting in producing it, and as such is semi-quantitative. Generally positive values are associated with well-oxygenated sediments, whereas highly negative values (<-200 mV) are typical of suboxic or anoxic conditions (Danovaro 2010).

Chlorophyll-a & phaeophytin

- Using a spatula or spoon, scrape the surface sediment to a maximum depth of 2 cm. Collect ~ 100 g wet sediment (~ two tablespoons).
- 2. Remove any visible living or soft-bodied organisms for biological steps below, but retain shell gravel.
- 3. Place a sub-sample of wet sediment into a 50 mL plastic vial for chl-*a* analysis. Chl-*a* degrades in sunlight so this step should be performed quickly and out of direct sunlight if possible.
- 4. Wrap in foil and store frozen at -20°C in the dark until post-survey analysis of chl-*a*. Ensure sufficient head-space in the vial or bag to allow for the expansion of sample when frozen. Note that analysis should be performed within 4 weeks of collection, although use of ultracold freezers extends storage times.

Organic matter content

- 1. Place another sub-sample of wet sediment into a 50 mL plastic vial or small zip-lock bag for post-survey analysis of organic matter content.
- 2. Homogenise this sample, and store frozen at -20°C until analysis of organic matter content, generally within 3 months of collection. If liquid nitrogen is available, samples should be snap frozen and stored in a dewar following appropriate protocols.

Biology (infauna and macrofauna)

- After supernatant water has been passed through a sieve and sedimentology and geochemistry steps have been performed (< 5 tablespoons of sediment removed, see above), transfer the remaining sample from grab or corer to an elutriating bin. If additional survey objectives require data on sediment depth (see Pre-Survey Preparations), each sediment layer should be in a separate nally bin.
- 2. Weigh the whole sample using an onboard scale. Record in metadata sheet (Table 9.3).
- 3. Rinse the grab or corer thoroughly to avoid contaminating the next sample collected.
- 4. Elutriate the sample by running moderately flowing seawater into the elutriating bin and gently agitating the sediment to release light-bodied animals into the water. The water should flow from the bin through an outlet under which the sieve is placed (next step). To avoid damage to animals during elutriation, avoid directing water from the deck hose at the sieve, separate fragile visible animals, and remove rocks and shells (these can be saved as part of the heavy fraction if desired, Step 12). Elutriation should be performed until water runs clear, ideally the same amount of time among all sample sites. For coarse-grained



sediments, this may only be ~5 minutes, but for deep-sea ooze this may be far longer due to stickiness of the sediment which makes elutriating a challenge.

- 5. Stacked sieving is an alternative to elutriation and can provide immediate data related to invertebrate size distribution and biomass (Edgar 1990), although this method is not suitable for coarse-grained sediments that are retained on the sieve and subsequently require much time to sort from organisms. If a researcher elects this option, stack larger sieves (e.g. 1000 μm) on top of smaller ones (e.g. 500 μm), add small amounts of sample to top sieve and gently flush through with seawater. Skip to Step 12.
- 6. Retain macrofauna by allowing water to flow onto a 500 μm sieve. This size was chosen, as it has already been used in AMPs (Nichol et al. 2013, Przeslawski et al. 2013) as well as successful international monitoring of soft sediment communities (Frid 2011). It is a compromise between the 1 mm recommended by other protocols (Rumohr 1999) and the time and effort needed to process specimens using 300 μm or smaller. If individual survey objectives require a finer mesh size (e.g. 100 or 300 μm) or comparison with datasets from larger mesh size (e.g. 1000 μm), layer the sieves and process samples separately so that the recommended standard of 500 μm is still followed and data are comparable.
- 7. Sort the heavy fraction by hand and remove any live animals that do not float during elutriation (e.g. molluscs, hermit crabs, animals attached to rocks) (i.e. heavy fraction specimens).
- 8. Material retained on the sieve should be flushed off using seawater in a squirt bottle directed from the underside of the sieve into a funnel and sample container. It is important to minimize the amount of water used in this step to ensure adequate preservative concentration. If a large amount of seawater is used for flushing, the sample can be sieved and flushed again.
- 9. Preserve elutriated and heavy fraction specimens according to methods decided in 'Presurvey Planning' in sample container. If there is a large volume of material, use multiple sample containers to ensure enough preservative in each container. See Rees (2009) and Schiaparelli et al. (2016b) for comprehensive description of fixatives and preservatives used for marine invertebrates. Larger organisms may be preserved separately (e.g. polychaetes may be relaxed in MgCl and fixed in formalin).
- 10. Place a solvent-hardy label in each sample container with sample and station number, date, location and vessel/collector. This information is essential for quality control in processing and archiving of specimens. It is not sufficient to label only the outside of the container, as this can easily rub off. See Box 15.6 in Schiaparelli et al. 2016 for suitable label characteristics.
- 11. Place the sample container in a large sealable container (i.e lidded drum) double-lined with a durable plastic bag with other samples preserved using the same chemicals (e.g. ethanol). Label the drum with survey details and the type of chemical fixative/preservative inside. Since samples from the same grab may end up in different drums due to different preservatives, it is imperative to have a good record-keeping system.
- 12. After placing samples within the inner bag of the drum, back fill between the bags with an appropriate amount of spill kit (eg vermiculite or absorbent kitty litter). In this way the contained specimens are compliant with handling (triple bagged) for road transport of Dangerous Goods. [Recommended]



13. Store large drum onboard in an approved storage area for hazardous chemicals.

9.7 **Post-Survey Procedures**

9.7.1 Sample curation and submission for analysis

Sedimentology

Sedimentology samples can be transported as refrigerated freight in a fully sealed, rigid container (e.g. esky) to Geoscience Australia for laboratory measurement. Alternatively, researchers may transport samples to their own labs if performing analyses in-house. Regardless of whether the sample is analysed by GA or elsewhere, data should still be submitted to the national sediment data repository (Marine Sediments Database (MarS)) (Section 9.7.2). Analytical methods include wet sieve separation into mud, sand and gravel fractions, laser granulometry of mud and sand fractions, and acid digestion of carbonate content for the bulk or mud and coarse fractions. Other methods are also available for those with their own expertise and equipment (e.g. calcimeter method in Kennedy and Woods 2013).

If lodging samples at GA for analysis, the following metadata is required prior to receipt of sediment samples:

- Survey metadata including: survey name, survey number, survey vessel, start and end date of survey, latitude and longitude of survey bounding area, name of chief investigator
- Sample location for every sample listed in decimal degrees to at least five decimal places
- Sample water depth for every sample listed
- Sample ID follows a standard naming convention (see example attached)
- Sample bags are labelled clearly with the sample ID (as above)
- Sample condition is as when collected (i.e. wet, disaggregated, excess water drained)

Biogeochemistry

Geochemical analysis of sediment samples should be conducted by the organisation undertaking the survey. Alternatively, sample analysis should be outsourced to Geoscience Australia (Loss on Ignition analysis, as described below) or commercial laboratories or collaborators (chl-*a* analysis).

Total organic matter content

Total organic matter content of marine sediments is determined by Loss on Ignition (LOI). Note that LOI is not the same as total organic carbon (TOC) (Schumacher 2002). Parameters such as temperature and combustion time vary among individual researchers, and there is no universally adopted standard. Here we choose parameters based on a compromise appropriate to a diverse range of environments (Heiri et al. 2001, Wang et al. 2011). We strongly recommend that researchers use these guidelines to ensure data from different surveys can be compared. The general recommended steps for LOI to contribute to a national standardised dataset are:

- 1. Homogenise wet sample (1-2 g dry weight).
- 2. Place sample into a pre-weighed crucible.
- 3. Oven dry for 24 h at 105°.
- 4. Reweigh crucible and dry sediment.
- 5. Place crucible in muffle furnace and combust at 550°C for 4 h.

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6. Weigh crucible and combusted sediment.

The water content is the difference between the wet and dry sediment weights and is expressed as a percentage of the initial sediment weight. The total organic matter content is obtained as the difference between the dry and combusted sediment weights and is expressed as a percentage of the sediment dry weight.

Chlorophyll-a & phaeophytin

Chlorophyll-*a* is the principal pigment in plants and is a biomass indicator of aquatic micro-algae which support food webs in the sea, and phaeopigments (e.g. phaeophytin) are the degraded non-photosynthetic products of chlorophyll (e.g. Bax et al. 2001). The ratio between them indicates the "freshness" of the organic matter. Note that samples can be freeze-dried first and this may increase extraction efficiency but also increases risk of chlorophyll degradation over time. For the purposes of this field manual, we recommend using wet material; this will ensure comparability among datasets. The general steps for chl-*a* analysis are:

- 1. Place approx. 5 g wet sediment into centrifuge tube.
- 2. Add 10 mL acetone (90% saturated with MgCO₃)
- 3. Mix rigorously (with glass rod or vortex mixer)
- 4. Place in ultrasonic bath for 30 minutes under dark conditions (Note: other methods can be used, e.g. shaker)
- 5. Centrifuge sample (>1500 g for 5 minutes) and decant extract.
- 6. Use a spectrophotometer to measure absorbance at 665 and 750 nm.
- 7. Acidify extract with 2 drops of 0.1 N HCl, mix and rest for 60 s.
- 8. Measure absorbance again at 665 and 750 nm.
- 9. Claculated the concentrations of corrected chl-*a* and phaeophytin using the equations of Lorenzen (1967).

<u>Redox</u>

Redox measurements are provided onboard with a probe and there are thus no post-survey procedures required, other than to QC data.

Biology

- 1. All animals from a given grab or box core should be sorted into separate small containers based on phylum or class to facilitate taxonomic identifications (arthropod, annelid, mollusc, echinoderm, other). This can be done onboard if time permits, but consideration must be given to working under a microscope on a moving vessel. Sorting can usually be done by a non-expert, with only a few groups posing potential challenges (Figure 9.4). Containers should be filled with 10% formalin or 70% ethanol (as per Pre-Survey Preparations) and labelled appropriately with solvent-proof paper.
- In order to test for potential bias due to differences in sorting efficiency among people, randomly selected samples should be re-sorted by a different person. Removal of 95% or more of the organisms during the sorting process is acceptable; otherwise, re-sorting may be necessary (Simpson et al. 2005) [recommended when multiple people are involved in Step 1]



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- 3. Within each sorted phylum, count individuals and identify organisms to a taxonomic resolution that enables data production in a timely manner. Identifications can be done by the organisation that collected the samples, museum taxonomists, geneticists, or external private consultants. Care must be taken to ensure consistent nomenclature is used for undescribed or unnamed species (e.g. defined operational taxonomic units, OTUs).
- 4. Lodge all specimens in an internationally recognised specimen collection (e.g. museum) for curation and public accessibility [*Recommended*].
- 5. If all specimens are unable to be lodged at a museum due to lack of resources or the need for destructive analyses (e.g. biochemical extractions), then a voucher collection should be produced (i.e at least one animal per OTU). This voucher collection can be held temporarily by the agency undertaking the survey if there are other surveys planned in the region to aide in subsequent identification. Ultimately, this voucher collection should be lodged in an internationally recognised specimen collection (e.g. museum).

9.7.2 Data Release

Produce a technical or post-survey report documenting the purpose of the survey, survey design, sampling locations, sampling equipment specifications, and any challenges or limitations encountered. See Appendix C for a sample template. Provide links to this report in all associated metadata [Recommended].

Sedimentology

For samples submitted to GA for sedimentological analysis, sedimentology data will be publically available in the national Marine Sediments database (MarS, <u>http://dbforms.ga.gov.au/pls/www/npm.mars.search</u>) following lab analysis and QC checks as part

of GA's internal workflow processes. This database includes sediments from estuaries, coasts, shelf, and the deep-sea.

For samples from which sedimentological analysis were done elsewhere, please submit the data to <u>marine@ga.gov.au</u>, along with required metadata (Section 9.7.1).

Biogeochemistry

Submit all geochemical sample metadata and analysis results to GA including:

- Reduced sediments (Y/N)
- Total organic matter content (%)
- Chl *a* (ug g⁻¹ dry sediment)

The easiest way to do this is to add two columns to Table 9.3 for LOI and chl-*a* data and submit this to marine@ga.gov.au.

Biology

All biological data should be publicly released, unless circumstances require otherwise (e.g. confidentiality clause or embargo for commercial work). Even in situations when data cannot be shared, the metadata and deployment information should be made available (Steps 1-2 below).



Poor scientific data management and lack of data sharing has been shown to hamper scientific progress (Stocks et al. 2016).

Traditionally, data related to biological specimens in biodiversity surveys have been delivered as presence-only taxonomic identifications. These are often managed by individual museum scientists or curators and subsequently harvested by the Atlas of Living Australia (ALA) and the Ocean Biogeographic Information System (OBIS). These portals do not include absences or information related to sampling effort, thus reducing the applicability of such data to monitoring purposes.

There are current initiatives underway that aim to incorporate species presence data to more ecologically relevant applications. For example, OBIS International manages a project called OBIS-ENV-DATA that extends data structures to allow linking species data to other related data (environmental, images, sampling effort) (De Pooter et al. 2017). In the meantime, the steps listed below will ensure appropriate and timely release of both metadata and data:

- Create a metadata record describing the data collection. Provide as much detail as possible on the collection/deployment (either directly in the metadata record itself, or in the form of attached field sheets as .csv, .txt or similar). This should include sampling locations and dates, equipment used, level of sorting applied, etc. All collection/deployment information must be QC-d before inclusion.
- Publish metadata record(s) to the <u>Australian Ocean Data Network (AODN) catalogue</u> as soon as possible after metadata has gone through the QC process. This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the <u>AODN Data Submission</u> <u>Tool</u>. Note that this tool requires user registration, but this is free and immediate.

This step provides immediate documentation of the methods and location of the collection of biological material. This stage may also include links to field reports or data sheets.

- 3. Complete the species identifications and associated abundance for targeted groups identified. This can take quite some time, depending on sample size and available resources. It is not unusual for taxonomic identifications to lag years behind survey completion, but this should not delay publication of initial metadata and deployment information. Care must be taken to ensure consistent nomenclature is used and documented for undescribed or unnamed species (e.g. defined Operational Taxonomic Units, OTUs). Ideally catalogues of OTUs are established such that subsequent surveys may use consistent OTU classification, thereby ensuring comparability of data between surveys.
- 4. QC the data. This includes checking for spelling errors, missing data, consistent nomenclature and use of OTUs, and confirmation that outliers are not data entry errors (e.g. 100 individuals really were collected, not just 10).
- 5. Attach or link the full data spreadsheet (including absences and abundances/biomass) to the metadata record previously created and published to the AODN.



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9.8 Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual will be updated in 2018 as Version 2. Updates will reflect user feedback and new developments (e.g. data discoverability and accessibility). Version 2 will also detail subsequent version control and maintenance.

The version control for Chapter 9 (field manual for grabs and box corers) is below:

Version Number	Description	Date
0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
2	Relevant updates, including Data Release sections based on NESP, AODN, IMOS, GA, and CSIRO projects	Early 2019



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Figure 9.3 Images from key steps involved in the use of grabs or box cores for marine monitoring: a) recording metadata during gear deployment, b) Retrieval of a Smith-McIntyre grab, c) transferring sample for sedimentological analysis from grab to storage bag, d) elutriating sediment over a sieve, e) a bucket of infaunal samples preserved in ethanol, f) cumaceans sorted under the microscope from elutriated infaunal samples.

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Table 9.2 List of potential measurements from grabs and corers, including whether they are included in this field manual.

	Parameter	Description	Included in field manual
Sedimentology	Sediment texture	A measure of the proportions of mud, sand and gravel size fractions within a sample	Y
	Mean grain size	A summary statistical measure of the size of sediment grains by using effective spherical diameter (ESD)	Y
	Kurtosis	A summary statistical measure of the range of grain size within a sample, ranging from platykurtic (wide range) to leptokurtic (narrow range)	N
	Skewness	A summary statistical measure of the size and direction of the tail in a sediment size frequency distribution, ranging from negative skewness (coarse-tailed) to positive skewness (fine-tailed)	Ν
	Carbonate	A measure of the proportion of a sample comprising calcium carbonate material	Y
	Mass physical properties	A measure of bulk or dry density, water content, porosity, or permeability	N
Biogeochemistry	Organic matter content	A measure of the total organic matter content, organic carbon, or organic phosphorus	Y
	Contaminants	Concentrations of various pollutants including heavy metals, PAHs, PCBs, etc	N
	Pigment	Quantification of chlorophyll-a, phaeophytin and other byproducts of photosynthesis	Y
	Bioavailable organic matter	Quantification of carbohydrates, proteins and lipids	Ν
	Redox balance	Quantification of the Eh of sediments, providing an indication of anaerobic conditions and diagenesis	Y
	Sediment respiration	Quantification of the release of CO ₂ from sediments over time	Ν
	Porewater chemistry	Chemical characterisation of water between sediment grains	Ν
Biology	Microbes	Abundance, biomass, or composition of viruses, bacteria and other prokaryotes, protists	Ν
	Meiofauna	Abundance, biomass, or composition of metazoan meiofauna	Ν
	Macrofauna	Abundance, biomass, or composition of macrofauna	Υ
	Megafauna	Abundance, biomass, or composition of megafauna	Ν



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Table 9.3 Sample field datasheet to record metadata from each grab or corer deployment. Waterproof paper and pen/pencil is required.

		Gear on bottom			Wire out (length, angle) ¹	Recovery (%)	Sample weight	Photo (Y/N)	Sample taken (Y/N), Sample ID number		Qualitative data and other comments				
Gear ID	Date	Long	Lat	Depth	Time					Bio	Sed	Geoc h	Biology	Sed (Folk, Munsell, carbonate/lithic, other materials)	Geoch (anoxic sediments)
GR01	1/1/17	152.444	-24.675	20 m	19:28	25 m, 0°	75	7 kg	Y	Y 100 1	Y 1002	Y 1003	Large worm preserve d separate ly	sG (Sandy Gravel) 7.5 YR 7/6 (red yellow) Carbonate dominant Trace of volcanic rocks	Patches of sediment are black with sulfur smell

¹ Recording the length and angle of wire payed out during seafloor contact is required if the survey is in deep water with no USBL; otherwise this is just recommend



Figure 9.4 A brief description of taxa that can be challenging to identify but are often encountered when sorting organisms from elutriated sediment samples.

- a) **Scaphopods (molluscs).** These are curved shells with a larger and smaller hole on each end.
- b) *Aplacophorans (molluscs).* These are often confused with worms but are actually molluscs covered with spicules that can make them look furry.
- c) *Foraminiferans (protists).* These can be mistaken for gastropod shells and can be particularly common in deep-sea sediments. This field manual does not target forams so their inclusion in sample processing is not required (image from Wikimedia).
- d) *Crinoids (echinoderms).* The small animals or their dropped arms can superficially resemble polychaete worms.
- e) Hermit crabs (crustaceans). These can be mistakenly sorted as molluscs because the crab has retreated into its shell and is barely visible.
- f) Ophiuroid arms (echinoderms). These can often be confused with polychaetes, but you'll never see a head. There is no need to save ophiuroid arms unless the central disk is present.
- **g)** Ostracods (crustaceans). Ostracods can be mistaken for bivalves, but they are small shrimp-like animals encased in two shells. You can often see their legs protruding from the shell.





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APPENDIX A: COLLABORATORS

List of all people who collaborated on the field manual package. Gray text denotes TBC.

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National MBES group	Guideline	Various	Contributor	MBES
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* An abridged version of the grab field manual was developed for the AHO for sedimentology, excluding geochemical and biological data.



APPENDIX B: PERMISSIONS

List of permissioning documents relevant to marine sampling in the Commonwealth waters (defined as 3 nm to the EEZ 200 nm and extended continental shelf). This list is a guide only, and certainty should be sought from responsible agencies. DoEE = Department of Environment and Energy. Compiled by Melissa Fellows, Dec 2017.

Activity	Sample type	Jurisdiction	Responsible agency	Legislation/Treaty/ Documents	Requirements for approval	Link
Research and monitoring	All activities	Australian Marine Parks	DoEE	Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act)	Authorisation is required for all zones	https://parksaustralia.gov.au/marine/
	Activities that could have a significant impact on a matter of national environmental significance	Within EEZ and on or in the continental shelf beyond 200nm	DoEE	Australia Marine Park Management Plans EPBC Act	EPBC Act referral	http://www.environment.gov.au/protection/environment-assessments\ http://www.environment.gov.au/epbc/what- is-protected
Sampling	Biological Samples	EEZ (3-200nm)	Department Agriculture and Water Resources	Biosecurity Act 2015	No importation required if preserved by storage in a sealed container with 70% alcohol or 10% formalin or Minimum 2% glutaraldehyde or plastinated curable polymers and labelled	https://bicon.agriculture.gov.au/BiconWeb4 .0/ImportConditions/Questions/EvaluateCa se?elementID=0000086465&elementVersi onID=201
		Waters and seabed of the EEZ and the continental shelf	DoEE	Environment Protection and Biodiversity Conservation Regulations 2000 Part 8A	Otherwise refer to BICON for importation requirements	https://bicon.agriculture.gov.au/BiconWeb4 .0/ImportConditions/Search http://www.environment.gov.au/topics/scien ce-and-research/australias-biological- resources/permits
	Sediment			Biosecurity Act 2015	Import requirements for samples collected beyond	https://bicon.agriculture.gov.au/BiconWeb4 .0/ImportConditions/Search
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					200 nm	
Interactions with Cetaceans	Seismic and other acoustic equipment	3nm to EEZ (200nm)	DoEE	EPBC Act Policy Statement 2.1	EPBC Referral and comply with Policy Statement 2.1	http://www.environment.gov.au/resource/e pbc-act-policy-statement-21-interaction- between-offshore-seismic-exploration-and- whales
	Whale and Dolphin watching	3nm to EEZ (200nm)	DoEE	Environment Protection and Biodiversity Conservation Regulations 2000 EPBC Regulations' Australian National Guidelines for Whale and Dolphin Watching 2005 Whale and Dolphin Watching Guidelines	Comply with EPBC Regulations	http://www.environment.gov.au/marine/publ ications/australian-national-guidelines- whale-and-dolphin-watching-2017
	Aircraft, helicopters and drones	3nm to EEZ (200nm)	DoEE	EPBC Regulations Whale and Dolphin Watching Guidelines	Comply with EPBC Regulations Permits required to operate a drone in close proximity to a whale or dolphin. Refer to Whale and Dolphin Watching Guidelines for allowable operating distances	http://www.environment.gov.au/system/files /resources/7f15bfc1-ed3d-40b6-a177- c81349028ef6/files/aust-national- guidelines-whale-dolphin-watching- 2017.pdf
	Vessel interaction	3nm to EEZ (200nm)	DoEE	EPBC Act EPBC Regulations (part 8)	Report death, injury, stranding or entanglement of whales and dolphins to DoEE Specific requirements for vessels	
	Study of cetaceans: take, keep, move, interfere with (harass, chase, herd, tag, mark or brand) and to possess or treat (divide cut up, extract any product from)	Australian Whale Sanctuary 3nm to the EEZ (200nm) And in waters beyond for Australian residents	DoEE	EPBC Act	Research permits for research actions that contribute significantly to the conservation of cetaceans	http://www.environment.gov.au/marine/mar ine-species/cetaceans/research-permits



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Interaction with Heritage	Historic Ship wrecks	Waters above the Australian continental shelf	DoEE	Historic Shipwrecks Act 1976	Ship wrecks and relics older than 75 years are protected. Some ship wrecks lie within protected zones. Permits required to enter a protected zone for some activities.	http://www.environment.gov.au/heritage/his toric-shipwrecks
Offshore petroleum and greenhouse gas exploration	Geophysical, geotechnical, seismic, drilling.	3nm seawards to the outer limits of the continental shelf.	National Offshore Petroleum Title Administrator NOPTA	Offshore Petroleum and Greenhouse Gas Storage Act 2006 (OPGGSA) Offshore Petroleum and Greenhouse Gas Storage (Resource Management and Administration) Regulations 2011	Title required to undertake activity.	http://www.nopta.gov.au/ http://www.nopta.gov.au/guidelines-and- factsheets/offshore-petroleum- guidelines.html
		3nm seawards to the outer limits of the continental shelf.	National Offshore Petroleum Safety Environment NOPSEMA	Offshore Petroleum and Greenhouse Gas Storage Act 2006 Offshore Petroleum and Greenhouse Gas Storage (Environment) Regulations 2009	Accepted Environment Plan in place, includes EPBC Act requirements.	https://www.nopsema.gov.au/environmenta I-management/assessment- process/environment-plans
Installations	Installations, in contact directly or by cable or similar device with the seabed for 30 continuous days or one or more period during the 60 days that sum to 40 days.	3nms seaward to EEZ or outer limits of the continental shelf		Sea Installations Act 1987	Permitting system no longer applies, however maritime safety, customs, immigration and quarantine matters continue. Safety zone of 500m may apply.	http://www.environment.gov.au/topics/mari ne/marine-pollution/sea-dumping/sea- installations
Restricted vessel movement and moored scientific equipment that			Australian Hydrographic Service AHS		Notice to mariners 2-3 weeks prior to survey commences.	http://www.hydro.gov.au/n2m/about- notices.htm datacentre@hydro.gov.au,rccaus@amsa.g ov.au
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create navigation hazards			Australian Marine Safety AMSA		Vessel to RCC to update NAVAREA X alerts	https://www.amsa.gov.au/safety- navigation/navigation-systems/maritime- safety-information-database natuticaladvice@amsa.gov.au rccaus@amsa.gov.au
Research in the Great Barrier Reef Marine Park GBRMP	Research, except for limited impact research.	GBRMP	Great Barrier Reef Marine Park Authority GBRMPA	Great Barrier Reef Marine Park Act 1975 EPBC Act	Limited impact research may be conducted under a letter of authority issued by an accredited educational or research institutions All other research requires permission	http://www.gbrmpa.gov.au/zoning-permits- and-plans/permits http://www.gbrmpa.gov.au/zoning-permits- and-plans/permits/research-permissions
Research around infrastructure, cables and pipelines	Disturbance of the seafloor and strong acoustic disturbance (seismic)	Cables – Australian continental shelf Pipelines – 3 nm to 200 nm and extended continental shelf	Cables Australian Communications and Media Authority ACMA Pipelines National Offshore Petroleum Titles Administrator NOPTA	Telecommuncations Act 1997 International Cable Protection Committee (ICPC) recommendations	500m safety zone Liability for damage to cables Spatial pipeline data	https://www.acma.gov.au/Industry/Telco/Inf rastructure/Submarine-cabling-and- protection-zones/submarine- telecommunications-cables-submarine- cable-zones-i-acma https://www.submarinecablemap.com/ https://www.submarinecablemap.com/ https://www.iscpc.org/ https://www.iscpc.org/ https://www.iscpc.org/ http://www.nopta.gov.au
Sea dumping	Deliberate dumping of wastes at sea	EEZ	DoEE GBRMPA	Environment Protection (Sea Dumping) Act 1981 London Convention, 1972/96	Permits for large scale dumping required	http://www.environment.gov.au/marine/mar ine-pollution/sea-dumping



APPENDIX C: RECOMMENDED POST-SURVEY REPORT TEMPLATE FOR SAMPLING IN AUSTRALIAN MARINE PARKS

<List of agencies involved>

AUSTRALIAN MARINE PARK BASELINE AND MONITORING SURVEY

POST SURVEY REPORT

<insert Marine Park name>

<month year>

<insert image(s)>

Authors and affiliations [Pick the date]

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EXECUTIVE SUMMARY

Guidance note: Provide a short summary of the post survey report, including:

- survey name and ID, vessel, survey location and dates of survey;
- participating agencies and institutions;
- brief description of AMP and study area, including regional context;
- high-level survey objectives that link to Parks Australia research priorities and information needs (e.g. "...to build the baseline inventory of seabed habitats in xxxx marine park....");
- specific survey objectives, including science questions and/or hypotheses being addressed/tested;
- key results including summary statistics for data types acquired (e.g. km² seabed bathymetry and backscatter coverage; line km of towed video/AUV; number of hours of baited underwater video deployment; number of physical seabed samples etc)
- preliminary interpretations of survey results at high level and in terms of habitats, biodiversity, trends, responses to pressures, etc
- highlights of new science discoveries (new species, seabed features previously unknown, etc)



INTRODUCTION

Background and Rationale for Survey

Guidance note: Narrative that provides the context and drivers for the survey in terms of scientific questions/issues being addressed and links to the research priorities and information needs of key stakeholders. Briefly introduce the marine park that the survey was conducted within.

Australian Marine Park Context

Guidance note: Overview of management plan that applies to the particular marine park that was covered by the survey, including identification of conservation values (physical, biological, oceanographic), pressures, key ecological features and biologically important areas that intersect the survey area. Include relevant maps, and reference monitoring plan and objectives if one exists.

Aims and Objectives

Guidance note: List of overarching aims of survey and specific objectives, including scientific questions and/or hypotheses being addressed



SURVEY AREA

Location & Description

Guidance note: Description of the survey area in terms of general physiographic, oceanographic and biogeographic setting. Identify the marine planning region and the marine park the survey was undertaken within. Provide a description of the seabed characteristics, oceanography and biological communities, as they are known and/or understood for the particular marine park, including previous studies (referenced). Identify knowledge gaps for the particular marine park.

Survey Grids

Guidance note: Identify the specific areas within the marine park where data acquisition was undertaken. This could be presented as grids, transects and points; or a combination of these. Include relevant maps.



SURVEY DESIGN AND SCHEDULE

General Information

Guidance note: Describe the approach to survey design as linked to survey objectives and research questions. For example, the survey may have applied a spatially balanced randomised method for pre-selection of sampling sites; or a survey that is weighted towards sampling at certain depth intervals (transects), or across particular habitats.

Survey Design

Guidance note: Present details of areas targeted for mapping, sampling stations/transects.

Survey Timetable

Guidance note: Tabulated schedule of events as they occurred during the survey. Optional (could go in Appendix).



METHODS AND DATA COLLECTED

Seabed mapping (multibeam sonar bathymetry and backscatter; sub-bottom profiles; side-scan sonar)

Guidance note: Brief description of instruments used to undertake seabed mapping (e.g. XYZ 300 kHz dual-head multibeam sonar) and statistics for the area mapped. Statistics should include km², line kilometres, bathymetric range and acoustic reflectance (backscatter) range for multibeam sonar and depths of penetration for sub-bottom profiles. Include summary tables and maps that show navigation tracks and spatial coverage in the context of the marine park boundary and zones. Also include summary of basic processing steps completed for multibeam, backscatter, sub-bottom and side-scan data)

Seabed sampling (grab samples, cores, other)

Guidance note: Brief description of sampling instrument(s) used and seabed samples collected, including number and bathymetric range. Include a summary table that lists samples collected per site (station), and maps showing sample locations. Include a summary of planned analytical methods (e.g. identification of infauna by expert taxonomist) and lodgement of samples (e.g. sediment samples lodged at GA, infauna lodged at Museum of Victoria).

Seabed observations (towed video, AUV, BRUV)

Guidance note: Brief description of imagery systems used for seabed observations and number, duration and bathymetric range. Supported by a summary table that lists data collected (line km), and maps showing navigation tracks. Include a summary of planned image processing (e.g. Simultaneous Location Algorithm Mapping to develop photomosaics) and annotation (e.g. point count using CATAMI classification in Squidle+) methods.

Pelagic observations (BRUV, visual sightings)

Guidance note: Description of pelagic observations, including number and duration. Include a summary table and maps showing sample locations. Include a summary of planned annotation methods (e.g. use EventMeasure to extract size and MaxN data from video).

Oceanographic measurements (underway, moorings, glider)

Guidance note: Description of oceanographic observations, including number and duration. Include a summary table that lists samples collected per site (station), and maps showing sample locations and navigation tracks. Include a summary of planned post-processing and analysis methods.



RESULTS AND PRELIMINARY INTERPRETATIONS

Seabed Features

Geomorphic features

Sub-seabed structure

Guidance note: Description of seabed geomorphic features as identified from processed multibeam sonar and backscatter data. Features should be classified using standardised terms (e.g. Geoscience Australia glossary of seabed features, in prep.). Include summary statistic on these features (e.g. depth range, area, slope gradients, acoustic reflectance range) as preliminary measurements/assessments. If sub-bottom profiles were collected, include a description of representative transects that illustrate sub-seabed structure of key habitats (e.g. sediment veneer over reef; evidence for sedimentary infilling of depressions/scours; evidence for active bedform migration). Include representative examples of bathymetry grids produced from multibeam data. Relate new findings to previous research if possible. Specify where metadata and data can be accessed.

Seabed Biological Communities

Epifaunal Communities

Infaunal Communities

Guidance note: Description of seabed biological communities as determined by direct sampling and/or imagery. Present in the context of seabed bathymetry and backscatter by overlay onto survey maps. Include summary statistics as recorded during the survey (e.g. depth range, percent cover, area, linear distance) as preliminary measurements/assessments. If specimens were collected, include summary statistics of number of specimens collected, general lifeforms and preliminary identifications. Include example imagery if acquired during the survey. Relate new findings to previous research if possible. Specify where metadata and data can be accessed including DOIs if available.

Pelagic Fauna

Guidance note: Description of pelagic biological communities as mapped by direct sampling and/or imagery. Present in the context of seabed bathymetry and backscatter by overlay onto survey maps. Include example imagery, summary statistics as recorded during the survey (e.g. depth range of observed individuals/schools, number of individuals observed), and preliminary identifications. Relate new findings to previous research if possible. Specify where metadata and data can be accessed, including DOIs if available.



Oceanographic Data

Guidance note: Description of oceanographic data collected. Include general spatial patterns in currents/temperature/salinity/turbidity and summary statistics as recorded during the survey (e.g. trends in CTD profiles, presence of stratified layers, ADCP current patterns). Relate new findings to previous research if possible. Specify where metadata and data can be accessed including DOIs if available.

New Discoveries

Guidance note: Identify and highlight any new discoveries from the survey that serve to add to the knowledge base of the marine park. For example, first-time mapping of particular seabed features; detection of change in habitat and/or biological communities; new marine fauna and flora discovered etc. Specify where metadata and data can be accessed including DOIs if available.

FUTURE WORK

Guidance note: Description of planned, proposed or potential analyses (including future surveys) that will maximise the value of the datasets collected, and contribute to the evidence base to support monitoring and performance assessments of the particular marine park.

Identify science products that can be used to promote the awareness and public interest in this particular marine park, and in marine science in general.

REFERENCES

As appropriate



ATTACHMENT 1 – DAILY LOG OF SURVEY ACTIVITIES

Guidance note: Narrative of daily activities, including key events, decisions and progressive description of survey progress against aims and objectives.

ATTACHMENT 2 – PERSONNEL ON BOARD

Guidance note: Personnel list, including roles performed during the survey (e.g. Survey Leader/Chief Scientist; Multibeam sonar acquisition/processing; Towed-video operator...etc)

Scientific Personnel

Ship Crew

ATTACHMENT 3 – SAMPLES LIST

Guidance note: Tabulated list(s) of all physical samples collected and any descriptions recorded during the survey (following Standard Operating Procedures for various data types). As a minimum, sample lists to include:

- Sample ID (following a standard naming convention);
- Sample type (e.g. sediment, biological
- Gear type (grab, core, sled, towvid etc)
- Sample location (latitude, longitude, decimal degrees to 6 d.p)
 - Recorded as one set of co-ordinates for point observations/samples
 - Recorded as start-of-line (sol) and end-of-line (eol) co-ordinates for transects
- Date of collection (yyyymmdd)
- Date of collection (Julian Day)
- Time of collection (UTC)
 - o Recorded as an 'event time' for point observations/samples
 - Recorded as start-of-line (sol) and end-of-line (eol) time for transects
 - Recorded as start-of-deployment and end-of-deployment for instrument/mooring deployments (e.g. BUVs)
- Water depth (m, to 2 d.p)
 - Recorded as an single depth for point observations/samples
 - Recorded as water depth at start-of-line and at end-of-line for transects
- Repository where sample has been lodged
- Comments/Descriptions



ATTACHMENT 4 – LICENCES AND PERMITS

Guidance note: Copies of Permits obtained to undertake work in the particular marine park, including one or both of the following:

Permit to Undertake Research in a Commonwealth Marine Park

Permit to Access Biological Resources in a Commonwealth Marine Area





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