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researchonline@jcu.edu.au

Advancing Elasmobranch Research

**Advancing Elasmobranch Research: New Technology Applications for Expanding Spatial and
Temporal Scales of Traditional Methods**

Submitted by

Martina Lonati

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Nature of assistance	Contribution	Contributor
Intellectual support/data supply	Supervisors	
	Study design	Andrew Chin, Adam Barnett, Stacy Bierwagen, Jodie Rummer
	Theoretical background	Andrew Chin, Adam Barnett, Stacy Bierwagen
	Editorial assistance	Adam Barnett, Stacy Bierwagen, Andrew Chin
	Permits and animal ethics	Adam Barnett, Stacy Bierwagen
	Field/analysis method	Adam Barnett, Stacy Bierwagen
	Data supply	Adam Barnett, Stacy Bierwagen
	Training	Stacy Bierwagen
	Collaborators	
	Study design	Mohammad Jahanbakht, Eric Fisher
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	Permits and animal ethics	Eric Fisher
	Field/analysis method	Jonathan Smart
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Thesis Abstract

Elasmobranchs are a diverse group of marine vertebrates, living in all marine and some freshwater ecosystems, from shallow estuaries to deep open water. Unlike teleost fish, elasmobranchs are generally less abundant, with many species being highly migratory, rare, or cryptic. Due to extensive overfishing and habitat degradation, global abundance of sharks has declined of 71% since 1970 and the latest assessment from the International Union for the Conservation of Nature (IUCN) Red List Assessment classifies 31% of sharks and ray species as threatened with extinction. Declines in shark and ray abundance may influence marine ecosystems in ways that are hard to predict, mostly due to the limited knowledge of elasmobranchs ecological role as predators. Collecting representative data on the occurrence and abundance of marine predators is arguably the biggest challenge for effective conservation of these species. However, reliable data is crucial for understanding the ecological roles of predator species, and in turn how predator conservation can be instrumental for better management of marine resources. Various methods, including traditional catches, underwater visual surveys, underwater photography, remote video systems, and drones, have been employed, each with inherent limitations in their capacity to detect species and provide meaningful data across spatial and temporal scales. The advent of high-resolution underwater photography, affordable high-performance cameras, aerial and underwater drones, and artificial intelligence, new technologies have made their way into elasmobranch research. This project provides two examples on how to incorporate new technologies into traditional methods to expand spatial and temporal scales on which the data is collected.

Artificial intelligence (AI) was applied to photographic identification (photo ID) of epaulette sharks (*Hemiscyllium ocellatum*) to evaluate morphological stability at early life stages and changes over time in adults. The study developed an accurate AI model for individual identification that does not require high-performance computing or extensive photographic databases. This is significant for processing long-term, large databases without specialized computing resources. The study also found that neonate and juvenile epaulette sharks have unreliable morphology for long-term ID, highlighting the importance of considering life stages when building photo ID databases.

As a pilot study, ROVs were tested for elasmobranch surveys at three locations and compared against underwater visual surveys (UVS), baited remote underwater video systems

(BRUVS), and catch methods. ROVs were evaluated for their effectiveness from stationary platforms and tenders, during nighttime, and in deep waters. The findings indicate that observation-class ROVs perform comparatively to underwater surveys on scuba, suggesting they are viable for UVS in deep waters and nocturnal conditions where scuba diving is not feasible. Additionally, ROVs can be used as a mobile transect video method to complement BRUVS and catch surveys. When compared to BRUVS, ROVs detected less species but sighted the similar or higher number of elasmobranchs when both methods were standardised by effort. When compared to catches, the CPUE of ROV timed swims was higher than catches. Additionally, species composition of ROV surveys was strikingly different than catches. Practical suggestions and best practices for ROV use were also provided to improve the technology and the way that it can be effectively used to survey elasmobranchs.

With the two case studies presented in **Chapter 3** and **4**, this thesis investigates the application of new methods and technologies for traditional elasmobranch surveys. In the specific case of photo ID, the study highlighted how AI can be used not only to support the sorting of photographs, but also to test whether a species satisfies the photo ID requirements for successful re-sightings through time. In terms of ROVs effectiveness for elasmobranchs UVS, the pilot study in **Chapter 4** demonstrates how a new technology can practically be tested against traditional methods, highlighting the advantages and strengths of that specific technology. The results indicate that, despite notable trade-offs in troubleshooting and advancing new techniques, technological advancements can significantly address the spatial and temporal limitations of current survey methods, thereby improving the accuracy and scope of elasmobranch research.

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Chapter 1: General Introduction

Determining the abundance of animals and the patterns of abundance in time and space is one of the central themes in ecology. For instance, it is not always possible to observe and count all animals in a population. For that reason, sampling methods focus on capturing a representative portion of the population (Greenwood, 1996). Some of the most common methods are captures within sampling units (quadrats), mark-recapture, transects, and distance methods (Harvey, 2008). Studying marine animals presents additional challenges, as much of the ocean remains inaccessible, and scuba divers are constrained by limited underwater time. These difficulties are further compounded when the target species are elusive, cryptic, or naturally rare traits commonly observed in higher trophic level predators and mesopredators, both in marine and terrestrial ecosystems (Dedman et al., 2024). For example, some large African cat species are difficult to observe, yet night-time video traps have successfully captured previously undocumented behaviours (Kane, Morin, & Kelly, 2015; Polistar et al., 2014). However, such technologies are not easily transferable to the marine environment, particularly for deep-sea predators inhabiting dark, remote, and difficult-to-access habitats. Night-time underwater cameras, for instance, require artificial lighting, which can alter animal behaviour. Additionally, while terrestrial camera traps are often deployed within known territories, many elasmobranchs are non-territorial and capable of traveling vast distances within a single day (Guzman et al., 2018). These are only some of the examples illustrating the inherent challenges of effectively sampling elasmobranch populations.

The occurrence and abundance of elasmobranchs has traditionally been recorded using a combination of various methods, including fisheries data, observer programs, fisheries-independent surveys, and specialised techniques developed for hypothesis-driven research projects. Each of these survey approaches has its benefits and drawbacks. Some of the most readily available data come from historical fishing and observers' records, especially for pelagic elasmobranch species, which are naturally sparse and challenging to detect through sporadic and brief survey efforts. Although such data are valuable for covering large temporal scales, fisheries data are subject to numerous biases that can lead to over- or under-estimations of true elasmobranch abundance and species occurrence (Braccini et al., 2021).

While research-driven methods are often more reliable than fisheries data, they can be limited in terms of their spatiotemporal scales (Braccini et al., 2021). For example, underwater surveys on scuba have been traditionally one of the most used methods but are limited to shallow and clear

waters (Jessop et al., 2022). Baited remote underwater video stations (BRUVS) can be used for deep water surveys, are effective, and easy to deploy, but the use of bait and the stationary nature of this video method may affect estimates of *in situ* abundance (Harvey et al., 2013; McLean et al., 2015; Sherman et al., 2020). Traditional capture-mark-recapture (CMR) studies are widely used for population assessments, but this method can be challenging for elasmobranchs due to their low abundance, resulting in a low likelihood of recapture (Dudgeon et al., 2015; Winton et al., 2023). With new technologies, some of the traditional methods have been improved, and new methods are emerging. For example, close kin mark recapture (CMR) can now be used to improve stock assessments and population estimates (Bravington et al., 2016), eDNA methods are improving our ability to detect the occurrence of rare and protected species (Green et al., 2024; Simpfendorfer et al., 2016). Underwater, high-resolution, multi-cameras and spherical cameras have improved accuracy in estimates of occurrence and abundance for BRUVS (Kilfoil et al., 2017; Whitmarsh et al., 2018). Underwater drones have extended the depth at which elasmobranchs can be surveyed (Sward et al., 2019). Furthermore, many different applications of artificial intelligence (AI) have contributed to streamlining data processing, ultimately broadening the spatial and temporal scale of traditional methods (Conservation X Labs, 2024).

One of the most widely used techniques for studying elasmobranchs has been photographic identification (Pierce, 2019). This method is a non-invasive CMR method and has been successfully used for several different elasmobranch species, especially for whale sharks (*Rhincodon typus*), white sharks (*Carcharodon carcharias*) and manta rays (*Mobula spp.*) (Araujo et al., 2019; Armstrong et al., 2020; Domeier & Nasby-Lucas, 2007). Only species that present distinguishable, persistent, and individually unique markings can be studied with photo ID (Marshall & Pierce, 2012). For instance, body scars are unreliable for long-term photo identification, as elasmobranchs heal quickly, causing scars to fade or disappear over time. (Chin et al., 2015; Marshall & Pierce, 2012). Markings from birth are assumed to remain constant through adulthood. Manta rays and whale sharks, the two most studied species with photo ID, are assumed to have stable adult markings (Town et al., 2013), although the rarity of neonates and juveniles complicates testing the persistence of morphology in early life stages. On the other hand, some elasmobranch species, like the Indo-Pacific leopard sharks (*Stegostoma fasciatum*), tiger sharks (*Galeocerdo cuvier*), and epaulette sharks (*Hemiscyllium ocellatum*), undergo significant morphological changes as they mature. When employing photo ID as a non-invasive CMR method for species that undergo morphological changes over time, ideally supplementary methods should be applied to track these changes and ensure accurate re-

identification (Bansemer & Bennett, 2008). Finally, processing the extensive photograph databases collected for photo ID projects can be time-consuming. For this reason, artificial intelligence (AI) is now broadly applied to assist the sorting task and searching task (Christin et al., 2019; Petso et al., 2021).

Another widely used, standardised, survey method for elasmobranchs is underwater visual surveys (UVS) (Ward-Paige, 2010). UVS are relatively cost-effective and are easy to implement (Chin & Pecl, 2018). However, most data on elasmobranchs collected via UVS originates from tropical and temperate shallow, clear water marine ecosystems during daytime. This limitation arises because divers are restricted to depths shallower than 30 meters and often cannot conduct dives at night (Fitzpatrick et al., 2013). Consequently, the window for data collection is quite narrow, potentially leading to unrepresentative estimates of abundance and occurrence. Remote video systems, such as baited remote underwater video stations (BRUVS) and unmanned underwater vehicles (UUVs), such as remotely operated vehicles (ROVs), on the other hand can be deployed for nocturnal surveys, at depth and in unsafe environments (Butcher et al., 2021; Harvey et al., 2012; Roi et al., 2007). ROVs have shown occurrence of elasmobranchs in deep water, often while surveying artificial deep-water structures for fish communities (Sward et al., 2019). These studies mostly happened opportunistically as videos from commercial ROVs were made available to researchers (Macreadie et al., 2018). Comparative studies have evaluated the performance of ROVs against traditional methods by examining species diversity, total abundance detected, and the behavioural reactions of various species to the ROVs (Andaloro et al., 2013; Laidig et al., 2013; Trenkel et al., 2004; Wetz et al., 2020). Overall, ROVs are comparable to divers' surveys, but the effectiveness of this method seems to be mostly contextual, depending on the species, habitats, and survey technique used. ROV surveys often lack standardization, which hinders consistent performance evaluation (Sward et al., 2019). Furthermore, few ROV surveys specifically target elasmobranchs, possibly because ROVs are thought to elicit avoidant behaviour (McLean et al., 2017), or because it is thought that elasmobranchs would remain outside of the limited field of view (Kilfoil et al., 2021). However, targeted adjustments and customizations may be effective and have yet to be tested. For example, adding a bait bag to a remote underwater video station (RUVS) maximises the occurrence of larger predators, making BRUVS one of the most effective methods for elasmobranch surveys (Harvey et al., 2021; Colton et al., 2010, Pimentel et al., 2020). Therefore, the effectiveness of ROVs for surveying elasmobranchs specifically needs to be tested before they can be consistently applied as an alternative to UVS.

Thesis Outline and Aims

Elasmobranch species are highly diverse and can be mobile, widely distributed, rare, and cryptic, making them challenging to survey with methods traditionally used for teleost fish. The current knowledge available for elasmobranchs heavily depends on the survey methods used to gather data on their abundance and occurrence (Brooks et al., 2011; Colton & Swearer, 2010; Santana-Garcon et al., 2014), as well as the timing and location of these surveys (Braccini et al., 2021). Each survey method comes with its own biases and limitations, which, combined with the circumstances under which data is collected, often result in data that reflects a specific time and place rather than providing a comprehensive understanding of the species. For this reason, it is important to review current methods, identify their strengths, and test whether some of the newest technologies available can improve spatial and temporal limitations (Butcher et al., 2021; Christin et al., 2019; Pearce et al., 2021). Therefore, the primary aims of this thesis are to first review and compare the available methods for surveying occurrence and estimating abundance for elasmobranchs. Then focus on new technologies that may advance camera-based methods.

Chapter 2 will describe the current methods used for estimating the abundance and occurrence of elasmobranchs, comparing their effectiveness, and highlighting the limitations of each method in terms of spatial and temporal coverage. This chapter will thoroughly review the two primary methods used in subsequent chapters, considering emerging technologies and their potential to enhance the use of these methods for elasmobranch research.

In Chapter 3, epaulette sharks (*Hemiscyllium ocellatum*) will be used to develop a systematic protocol to test the effectiveness of photo ID for species that have not yet been surveyed using this method. The protocol will outline the process of testing the uniqueness and stability of patterns across ontogeny and time. A deep neural network model will be trained on individuals' unique patterns across three life stages: neonates, juveniles, and adults. The model will be tasked with successfully identifying individuals from all life stages and detecting changes in patterns over two years by comparing time-consecutive photographs of adult epaulette sharks. The aim is to develop an artificial intelligence approach that is easily customizable for different species, user-friendly for various computational capabilities, and applicable to both small and large databases of photographs. Additionally, the artificial intelligence model developed in this chapter will help validate one of the most critical requirements for long-term photo ID studies: the stability of patterns over time for large, multi-year databases. This readily available protocol will support projects globally, including those in

remote locations with limited computational capabilities, by offering a standardized method for photo ID. This will broaden the scope of this method globally, enhancing its applicability and utility in elasmobranch research.

In Chapter 4, I will evaluate ROVs as an alternative to underwater visual surveys (UVS) for surveying elasmobranchs at depth and during the night. The study will be conducted at three different locations across the Great Barrier Reef and the Coral Sea. ROVs will be piloted at various times of the day and night, at depths ranging from 3 to 90 meters, across diverse marine habitats and weather conditions. The aim is to pilot this technology and explore its potential specifically for elasmobranch surveys. Tests will include different light types, survey strategies, multiple camera setups, the use of bait, and the deployment of a stationary platform as a base or tender. To understand its potential applications alongside traditional survey methods, ROV surveys will be conducted alongside UVS, BRUVS, and catches, with each method tested at different location.

Chapter 2: Methods for Estimating Abundance and Occurrence of Sharks and Rays

Abstract

Accurate estimates of species abundance and occurrence are fundamental to ecology and essential for effective conservation and marine resource management. However, obtaining reliable estimates can be challenging, particularly for higher-trophic marine predators, which are often difficult to survey. Elasmobranch populations have experienced unprecedented declines due to overexploitation and habitat degradation, yet current research and conservation efforts remain insufficient to mitigate these losses effectively. Fisheries surveys and catch-based methods have traditionally been used to assess abundance and occurrence, while genetic techniques now provide insights into population health and the presence of rare species. The advent of modern underwater camera systems has led to the widespread adoption of baited remote underwater video systems (BRUVS), while scuba-based underwater surveys continue to be among the most commonly used and accessible methods for studying elasmobranchs. More recently, drones have expanded the spatial and temporal scope of marine surveys, offering new opportunities for data collection. While traditional survey methods remain valuable, the urgent conservation status of elasmobranchs demands more efficient and innovative approaches to data collection and analysis. These advancements must be rapidly integrated into conservation strategies and effectively communicated to policymakers, conservation agencies, and management bodies. Although new technologies are readily adopted in industry, they are often underutilized in research. Leveraging and adapting emerging technologies for elasmobranch studies could help address critical knowledge gaps and enhance conservation efforts.

Introduction

Understanding species distributions, stock structure, and abundance estimates is crucial for the sustainable management of natural resources and the conservation of threatened species (Duarte et al., 2020). The knowledge of species occurrence and abundance is grounded in the collection of data on species occurrence and abundance (Hyde et al., 2022; Queiroz et al., 2019). Estimating absolute abundance is challenging, as counting every individual in a population is rarely feasible (Greenwood, 1996). This is only possible in exceptional cases, such as when all fish in a lake are captured and counted or when locally restricted, easily observed terrestrial animals can be surveyed (Harvey, 2008). However, these conditions are virtually absent for wide-ranging, non-air-breathing marine species. (Dulvy & Yan, 2020). The methods and technologies developed to count rare

terrestrial animals are often under-performing for underwater species, simply due to the added challenge of the underwater, marine environment (Przeslawski & Christenhusz, 2022; Sward et al., 2019). Consequently, scientists rely on approximated indices of abundance rather than absolute abundance (Pope et al., 2010; Seber, 1973). Collecting data on occurrence and abundance indices is particularly challenging for highly migratory, deep-water, cryptic, or naturally less abundant species (Braccini et al., 2021).

This challenge is especially evident in the Chondrichthyes (sharks, rays, and chimeras), a class of widely distributed marine vertebrates found in diverse ecosystems at all latitudes, including ocean depths of up to 4500 meters (Ebert et al., 2021; Last et al., 2016). Elasmobranchs play varied roles in ecosystems depending on species, habitat, life stage, prey availability, niche partitioning, and behavioral interactions with other species (Carrier et al., 2022; Heithaus et al., 2010). In addition to their high diversity, elasmobranchs tend to be less abundant than teleost, often rare or cryptic, difficult to observe in their natural habitats, and highly migratory (Carrier et al., 2022). As a result, no single method can effectively detect occurrence and estimate their abundance across all species.

Traditionally, commercial fisheries catch data, or research fishing surveys have been the main methods used to gather occurrence and abundance data for elasmobranchs (Bird et al., 2020; Mucientes et al., 2009; Stevens et al., 2000). Estimates of abundance are based on number of individuals caught, fishing effort, and/or capture-mark-recapture (CMR) studies (Harry et al., 2011; Musick & Bonfil, 2005). However, catch rates can be low for highly migratory species, leading to reduced statistical power (Winton et al., 2023). These techniques can also be considered stressful and invasive, with the possibility of post-release mortality for some species (Martins, 2017). Advances in technology and a focus on species conservation have led to a shift towards non-invasive methods or enhancing traditional capture surveys (Barnett et al., 2010; Harvey et al., 2018). Effective non-extractive methods for estimating elasmobranch abundance and occurrence include UVS, BRUVS and RUVS (baited and non-baited), aerial and underwater drones, photographic identification, and eDNA techniques (Harvey et al., 2018; Le Port et al., 2018; Pierce, 2019). Despite their effectiveness for many marine species, the cryptic and mobile nature of elasmobranchs often requires combining different methods and case-specific strategies (McCauley et al., 2012).

Collecting representative data for elasmobranchs remains challenging, resulting in coarse estimates of abundance and circumstantial evidence of occurrence (Braccini & Taylor, 2016; Dulvy et

al., 2017; Hyde et al., 2022). Research efforts must be optimized by selecting the most effective methods for the species and ecosystem of interest, while considering the spatial and temporal scales and limitations of each method (Heupel et al., 2019; Jorgensen et al., 2022). The aim of this Chapter is to 1) provide a concise overview of the methods used to survey occurrence and estimate elasmobranchs abundance; 2) Compare survey methods in relation to species and spatiotemporal implications 3) Discuss the role of new technologies in overcoming spatiotemporal limitations of traditional methods.

Survey Methods for Sharks and Rays

Fisheries-dependent and Fisheries-independent Surveys

Survey methods for detecting species occurrence and estimating abundance can be grouped into fisheries-dependent and fisheries-independent methods. Fisheries-dependent surveys are often preferred for species of fisheries interest because they leverage routine fishing operations, by collecting catch and effort data as part of fishing operations and stock assessments, without added costs (Hilborn & Walters, 2013; Maunder & Piner, 2014; Musick & Bonfil, 2005). Catches are analysed as the number or weight/biomass of the catch, standardized by fishing effort, e.g. number of hooks or net hours of fishing (Musick & Bonfil, 2005). Catch rates are then calculated as catch per unit of effort (CPUE) and used as a proxy of abundance (Seber, 1973).

An advantage of fisheries methods is the large spatial scale and time frames that can be monitored (Braccini et al., 2021). However, CPUE is often a reflection of fishery dynamics, more than population dynamics. For instance, fishing (and thus sampling) effort may be ambiguous or variable through time, fishing gear or gear configuration can change, and gear is often designed to target certain size class, and therefore only captures a section of the whole population (Brooks, 2013; Simpfendorfer et al., 2002). Although observer programs can help account for some of these biases, catches can also be under or over-reported (Booth et al., 2021; Watson & Pauly, 2001), and elasmobranchs are often not reported at the species level (Booth et al., 2019; Dulvy et al., 2021; Sherman, Simpfendorfer, et al., 2023). For instance, several species of carcharhinids have very different life history parameters, but can be very hard to discern, and have been lumped together and reported as whalers (Sherman, Digel, et al., 2023). Nevertheless, fisheries data is often the best available resource for estimating species occurrence and abundance, and CPUE still represents one of the best available parameters we have for monitoring population trends (Braccini et al., 2021; Dulvy et al., 2017).

Fisheries-independent surveys or “scientific surveys” allow some flexibility in the sampling strategy, and therefore can be designed to address specific research questions, providing independent abundance and occurrence data while minimizing biases (Braccini et al., 2019). At times, sampling mirrors the fishery in terms of gear, effort, and locations fished (Ward et al., 2004) while at other times effort, gear and/or location can be altered to target a particular life-stage/size class or specific habitats. Surveys are usually conducted outside routine fishing operations and will use the same gear configurations or variations on gear used in the fishery, aiming to collect data not captured in the fishery (Rago, 2005). Fishery-independent surveys can create more appropriate and reliable indices of abundance compared to fisheries stock assessments, especially when dealing with complex population dynamics (Grubbs et al., 2007; Peterson & Grubbs, 2023; Talwar et al., 2020). For example, including capture-mark-recapture (CMR) methods in fisheries-independent surveys can be a cost-effective way of gathering larger sample sizes for multiple target species. Recaptures can be insightful of species movement and of fisheries effort (Bird et al., 2020; Rago, 2005).

Scientific Surveys Methods

Scientific surveys are research driven sampling regimes specifically designed to address clear research questions and hypotheses. The sampling strategy/design can vary depending on the research question. Methods commonly used to collect indices of abundance and occurrence of marine species include capture-mark-recapture (Pine et al., 2003), photographic identification (Pierce, 2019), underwater video stations (baited such as BRUVS and non-baited, RUVS), underwater visual surveys (UVS), aerial and underwater drones, and genetic methods such as microsatellite DNA and eDNA (Bravington et al., 2016; Pearce 2021). A range of research techniques have also been used or combined to monitor occurrence and estimate abundance. For example, by acoustically tagging catches, it is possible to detect the occurrence of vulnerable life stages in areas close to fishing. BRUVs and eDNA have been combined effectively, resulting in more than 30% higher detection compared to each method alone (Stat et al., 2019). Underwater survey methods and BRUVs or RUVs have also been used together and have been effective in detecting species of elasmobranchs that tend to avoid divers (Colton & Swearer, 2010; Fernanda A. Rolim et al., 2022).

When it comes to choosing the most appropriate method, several factors come into play, including the aims of the study, spatial and temporal scales and implications, management and ethical considerations, and the species of interest. Generally, collecting estimates for highly migratory and

cryptic species is challenging, as indices of abundance and occurrence are highly dependent on spatial and temporal patterns of occurrence (Harvey, 2008).

Fishing surveys and tagging studies

Catch rate studies using traditional fishing methods, such as hook and line (set lines, longlines, rod and reel) or netting, are among the oldest techniques for studying shark abundance. These studies use fishing gear and time fished to calculate CPUE (catch per unit effort) indices, similarly to fisheries-based methods (Rago, 2005). Gear configurations are typically chosen to target specific species, with variables such as hook size and number, net mesh size, and net length adjusted accordingly. Set locations can vary, including bottom, midwater, or surface sets. Sampling designs may involve fixed sites fished periodically, stratified sampling, or a nested design where random sites are fished within fixed locations (Cameron et al., 2019; Harry et al., 2011; Simpfendorfer et al., 2002). CPUE studies can be labour-intensive, and different gear configurations might be needed to sample all elasmobranchs or cover various size ranges (Zhou, 2008).

Simple external tags have been traditionally used for capture-mark-recapture (CMR) studies to investigate the abundance and occurrence of threatened species and species of commercial interest (Dudgeon et al., 2015). Mark-recapture studies involve capturing animals, tagging them for future identification, and using the proportion of recaptured individuals along with demographic parameters to estimate population sizes, life history parameters, and movement (Kohler & Turner, 2001; Ovenden et al., 2018). While this method and its associated modelling approaches have been successful in ecology for decades, it is often limited for absolute abundance estimates for elasmobranchs (Buckland, 1980; Jolly, 1965; Pine et al., 2003). This is because many elasmobranch species are migratory and have open population dynamics, making it challenging to use CMR models, which are typically more effective for closed populations (Pollock, 2000). Even using open-population CMR for abundance estimates is particularly challenging for large, mobile shark species that move over extensive distances, as it is difficult to meet assumptions for CMR modelling, such as survival probability, likelihood of recapture, tags retention (Sollmann et al., 2013; Lettink & Armstrong, 2003). Accurate estimates typically require extensive data sets collected over many years with multiple recapture events (Bird et al., 2020; Field et al., 2012). However, low catch rates and recapture probabilities have traditionally been an issue for CMR studies of elasmobranchs (Winton et al., 2023), with recapture rates for most shark studies being around 5% (Kohler & Turner, 2001). Some solutions include combining CMR with commercial fishing operations to tag larger sample sizes and increase

recapture probabilities (Blaber et al., 2009; Braccini et al., 2019; Cartamil et al., 2011) or designing studies targeting known seasonal aggregations to boost sample sizes over short, intensive fishing periods (Barnett et al., 2010; Hewitt et al., 2010). Incorporating other techniques, such as acoustic and satellite tracking, can help overcome some temporal and spatial limitations of CMR and sample occurrence at day and night across multi-dimensional spatial scales (Andrzejaczek et al., 2022; Bouyoucos et al., 2020). For example, acoustic tagging can improve accuracy and precision of population models from CMR studies, as often tagged individuals can be present in the fishing area but are not recaptured, providing better estimates of survival and temporary emigration, as well as movement, behaviour, and habitat use (Dudgeon et al., 2015; Sollmann et al., 2013). Catch data can also be complemented with genetic estimates of population sizes, and genetic drift can validate population estimates of mark-re-capture studies (Andreotti et al., 2016). Even with the aid of acoustic tags and population genetics, some species and individuals remain difficult to capture, leading to incomplete data. Additionally, catches may be prohibited for protected species or within no-catch areas (Albano et al., 2021; Dickson et al., 2022).

Photographic identification

Since its first applications in the early 1970s, photographic identification (photo ID) in elasmobranchs has become more popular, effective, and precise, largely thanks to improvements in underwater cameras, video imaging software, and machine learning technologies for image recognition (Pierce et al., 2019). Photo ID is cost-effective and minimally invasive (Meenakshisundaram et al., 2021), with proven application in the field of elasmobranch population assessments, demographics, movement, and migration (Venables et al., 2019). Key to this technique is the presence of distinctive and unique natural markings or pigmentation on the skin of the animals, which is widely assumed to remain consistent through time and distinguishes individuals from one another (Marshall & Pierce, 2012). For instance, individual animals are distinguished based on their natural and unique patterns, and photographs are taken at every encounter, simulating a non-invasive CMR approach (Winton et al., 2023). Another advantage of the method is its potential spatial scale. When combined with a citizen science approach, photo ID can be used to collect large datasets on animal movements across oceans, which would be cost-prohibitive using other means such as electronic tagging. Photo ID can also record other factors that would not be captured by tagging such as entanglements with marine debris or fishing injuries and recovery (Chin et al., 2015; Chin & Pecl, 2018). These can have conservation implications, for example, fresh hooking injuries in a no-fishing zone can indicate illegal fishing activity. Considering the limited lifespan and tag shedding of traditional

external tags (Chin et al., 2013), photo ID can substantially extend the temporal scale of mark-recapture studies for species that are well suited to this methodology (Armstrong et al., 2020; Armstrong et al., 2019; McCoy et al., 2018). For instance, only species with distinguishable and permanent morphological traits can be studied with photo ID, and animals need to be reliably sighted and easily approached in relatively clear water (Pierce et al., 2019). These conditions substantially narrow the scope of this method to a few species and environments, excluding deeper study sites with low-visibility, highly mobile species that do not aggregate in easily accessible locations, and species of dull colouration, which is the case for most elasmobranchs.

Underwater visual surveys

Underwater visual surveys/census (UVS/UVC) are one of the most used and standardized traditional methods to survey elasmobranchs (Harvey et al., 2018; Wetz et al., 2020). Diver-based surveys, mostly belt transect and point count surveys, can provide estimates of the relative abundance and occurrence of elasmobranchs (McCauley et al., 2012). During belt transect surveys, divers record the species and length of all fish observed within a determined area extending each side of a fixed length transect line (Bohnsack & Bannerot, 1986). During point count surveys, the diver remains still, in the same position, and counts all fish within a predetermined radius for a given time (Brock, 1954). When performing timed swims, a diver swims for a determined time and counts fish along the way (Kimmel, 1985). The performance of different UVS method and their parameters is mostly dependent on the nature and behaviour of species (MacNeil et al., 2008). For instance, shy and cryptic species may avoid divers or remain at distance, while bold and more curious species may be more easily detected (Pais & Cabral, 2018).

For the most part, UVS tend to be non-instantaneous methods, meaning that new fish entering the survey area are counted even though they were not present at the beginning of the survey. Depending on the nature of the species, non-instantaneous count methods may overestimate abundance when fish repeatedly moved in and out of the survey area (Ward-Paige et al., 2010). Increasing survey speed to account for the tendency of overestimating fast-swimming species can lead to lower detectability of cryptic species (Pais & Cabral, 2018). For point count surveys, bias can be reduced for most species by increasing survey area (Prato et al., 2017), while increasing the length of a transect may worsen the overestimates for mobile species (Pais & Cabral, 2018). Because of the mobile nature of many elasmobranch species and the bias associated with the reaction to divers, estimates from UVS are often considered inaccurate (Vianna et al., 2014). Some species of

elasmobranchs tend to be avoidant of divers, depending on habitat and context. Studies have found that elasmobranch attraction or avoidance of divers can be dependent on several factors such as remoteness, fishing impacts, and provisioning activities (Dulvy & Yan, 2020; Moreno et al., 2021). To minimize disturbance, some studies have also documented that surveys completed using closed-circuit scuba equipment record significantly greater roving species diversity and abundance than surveys using conventional scuba equipment. To ensure accuracy and identify if certain species appear absent from surveys, it is advisable to complement UVS with data collected from underwater remote videos or catches (McCauley et al., 2012). However, it's important to consider the biases inherent in each method, and rather than deeming one method superior, combining both to provide a more comprehensive understanding estimate (Pais & Cabral, 2018).

Underwater videos

Stationary underwater videos are simply configured as an underwater camera mounted on a weighted frame that is designed to either sit on the benthos or float in the water column (Harvey et al., 2018; McLean et al., 2015; Murray et al., 2023). Remote underwater videos (RUV) are an affordable way to record elasmobranchs' occurrence and behaviour, without the limitations typical of underwater visual census, namely divers' effect on behaviour, safety concerns of diving operations (depth, time), and issues with misidentification of species. Un-baited RUVs are generally not appropriate when sampling elasmobranchs, as most species are highly mobile, most times occur in low numbers and therefore have a low likelihood of detection when not attracted by bait (Harvey et al., 2018). However, un-baited RUVs can be a viable survey method where animals are reliably encountered, such as areas of high abundance and aggregation sites (Anderson et al., 2011; Dudgeon et al., 2008) and have the advantage of recording passive behaviours as animals are not attracted or deterred by other stimuli (Rhodes et al., 2020).

Baited Underwater Video Systems/Stations (BRUVS) are a baited version of RUVs that rely on the attraction of predatory fish to increase the likelihood of detection. A canister containing bait is added to the frame, at the end of a 1-meter-long pole, in front of the camera (Cappo et al., 2000). BRUVs have quickly become the 'method of choice' for elasmobranchs, and they have been used to address a wide range of research questions, including abundance estimates, species occurrence, size estimates and biomass with stereo video (Harasti et al., 2017), stock assessments for commercially valuable species (Sheaves et al., 2020), behaviour (Lester et al., 2021; Shea et al., 2020; Sherman et al., 2020), species composition (Bruns & Henderson, 2020; Espinoza et al., 2020), fish species

association with depth and habitat (Brown et al., 2022; Ferrari et al., 2018), the performance of Marine Protected Areas (Dwyer et al., 2020; Goetze et al., 2024), abiotic and anthropogenic drivers of distribution (Goetze et al., 2018; Murray et al., 2023). Although the use of BRUVs for some research questions is debated, surveys employing this method have led to highly impactful studies for elasmobranch conservation (Simpfendorfer et al., 2023). For instance, although BRUVs are highly effective at detecting low abundance species such as elasmobranchs, the attractive effect of the bait (and different types of bait) may bias estimates of occurrence and abundance by attracting mobile species from different locations. The presence of the bait can also cause competitive interactions, resulting in larger fish close to the bait and smaller fish in the background or absent from the field of view (Hardinge et al., 2013). Currently, there is a lack of models to determine the precise area of effect of the bait plume, likely due to the complexity of open water marine ecosystems (Taylor et al., 2013). Additionally, as fish is likely to come in and out of the camera frame, there are metrics, such as MaxN, used to count individuals and aiming to correct for over-counting (Whitmarsh et al., 2016). However, these metrics may be underestimating the occurrence of elasmobranch species (Sherman et al., 2018). Since BRUVs are a valuable non-invasive survey technique, attempts have been made to address the challenges of this method, including the use of different metrics, spherical cameras for multiple viewpoints, and complementary methods (Kilfoil et al., 2017; Sheaves et al., 2020; Whitmarsh et al., 2018).

Underwater drones

Underwater drones (unmanned underwater vehicles) can be a safe, cost-efficient technological solution to the limitations of traditional underwater surveys performed by divers, while at the same time providing a mobile video alternative to stationary underwater video systems such as BRUVs (Kumar et al., 2023; Sward et al., 2019). The most common underwater drones used for elasmobranch surveys are remotely operated vehicles (ROVs), and autonomous underwater vehicles (AUVs) (Butcher et al., 2021).

Remotely operated vehicles

ROVs can be working-class, usually employed by offshore oil and gas industry operations, and observation-class, mostly small and commercially available. Observation-class ROVs are portable, relatively light, cost-effective, and easy-to-operate underwater drones that communicate to the surface via a tether, allowing for high manoeuvrability and a real-time connection to the operator

(Spanier et al., 1994; Sward et al., 2019). This feature is valuable for navigation across complex substratum and for exploratory missions, where the operator can make real-time changes to the survey strategy and alteration to the course in case of prohibiting circumstances (Butcher et al., 2021). The camera is used as a reference for piloting, but it also records and archives high-resolution videos that can then be repeatedly and methodically analysed (Sward et al., 2019). ROVs have been used for studies on fish species composition and rare species (Andaloro et al., 2013; Boavida et al., 2016), assessing species presence and abundance in deep water habitats (La Mesa et al., 2021; Norcross & Mueter, 1999; Sward et al., 2019; Trenkel et al., 2004), or habitats that can be potentially dangerous for divers to access (Todd et al., 2020), marine protected areas surveys, and general exploratory surveys, mostly using strip transects similar to divers UVCs (Sward et al., 2019). Studies using ROVs have highlighted some limitations, mostly related to technology performance and logistical challenges (McLean et al., 2015; Wetz et al., 2020). However, ROVs have shown potential in replacing divers' surveys where divers' safety can be at risk, such as extreme depths, low visibility environments, rough surface conditions in proximity to rocky shores and structures, and enclosed underwater spaces (e.g., wrecks, caves) (La Mesa et al., 2021).

Autonomous underwater vehicles

AUVs are self-reliant and designed to cover long distances, powered by fuel or battery cells, and guided by a navigation system (Butcher et al., 2021). AUVs are untethered and can follow a predetermined pathway underwater by progressively assessing their position in the water using GPS, a compass, a pressure sensor, and an altimeter (Kumar et al., 2023). Originally used for seafloor mapping, under-ice research, underwater archaeology, and military purposes (Miller et al., 2021), AUVs find their most effective application in elasmobranch research by actively tracking free-swimming sharks, following the acoustic signal, while recording water parameters and behaviour (Haulsee et al., 2015; Packard et al., 2013; Skomal et al., 2015).

Remotely piloted vehicles: eyes in the sky

Remotely piloted vehicles (RPVs such as aerial drones) are increasingly being used to track and count elasmobranchs in shallow and relatively clear waters (Bullock et al., 2024). Drones are a low-cost option that requires low training, and relatively easy fieldwork preparation (Dickson et al., 2022; Kiszka et al., 2016; Tucker et al., 2021). Drones are one the best methods to obtain behavioural information to complement abundance estimates. For example, stingray feeding behaviour in shallow habitats can be described and quantified (Crook et al., 2022). In turn predation on stingrays can also

be recorded (Lubitz et al., 2023). The main limitations are quite significant: 1) short battery life, 2) atmospheric conditions and 3) poor detectability of animals in unfavourable water states (Butcher et al., 2021; Dutton et al., 2019). Detection probability can be influenced by seabed type and morphology, water depth, proximity to estuarine environments, and tide/wave action (Benavides et al., 2019; Colefax et al., 2019; Hensel et al., 2018). Additionally, certain species are not suited to this method as they rarely occur close to the surface and in shallow environments. In terms of flying mechanics and stability, piloting becomes challenging above 15 knots of wind and, even a light rain could compromise the outcome of the mission (Butcher et al., 2021). In some areas there are also strict restrictions on drone operations for security, safety, and privacy, and operators may need costly certifications. In recent years developments in video resolution, the advent of applicable machine learning, and the improvements in flying mechanics, are promising developments in finding solutions to poor visibility issues (Colefax et al., 2019; Oleksyn et al., 2021) Drones have been somewhat revolutionary in wildlife research (Hardin & Hardin, 2010), and advancements in technology should see drones used in many more applications (Schiele et al., 2023).

Genetics and genomics

Molecular genetics has become affordable, practical, and easy since polymerase chain reaction (PCR) and nucleotide sequencing (Pearce et al., 2021; Saiki et al., 1988). Genomics has mostly substituted traditional genetics for research questions ranging from species composition, stock structure, philopatry, and population sizes (Andreotti et al., 2016; Dudgeon et al., 2012; Mourier et al., 2013). To study population structure, genetic differences between individuals are investigated based on markers within a species genome (Nikolic et al., 2023). The most common markers are mitochondrial markers within the mitochondrial genome (mtDNA), microsatellites, and single nucleotide polymorphisms within the nuclear genome (Dudgeon & Ovenden, 2015). MtDNA is maternally inherited and used for investigating sex-biased dispersal, phylogenetics, and localized population structure, but it is susceptible to genetic drift, changes in population dynamics, and demographic events (Heist, 2005). Microsatellite loci have been used for studies on population sizes, relatedness, and relationships, yet often only produce a relatively small number of markers (Mourier & Planes, 2013). In recent years, Single nucleotide polymorphisms (SNPs) have become the preferred genetic tool as reduced costs and high through-put sequencing can provide thousands of high-resolution markers (Dudgeon et al., 2012).

Population estimates with genetics.

Relatively new methods and advancements in genomics have seen several genetic techniques used to estimate population sizes of species (Nikolic et al., 2023; Pember et al., 2023). For instance, identifying genetically related individuals within a population can be used to infer the number of breeding adults (Ovenden et al., 2018). Linkage disequilibrium has been used to infer rates of inbreeding and consequently estimate effective population sizes, genetic structures, and demographic history (Dudgeon & Ovenden, 2015). Close-kin mark-recapture (CKMR) can be used to delineate stock structure and population size estimates (Rawding et al., 2014). Essentially, re-captures are classified as detections of related individuals, assuming that parent-offspring pairs can be detected, and that each juvenile has two parents. Consequently, the total adult population can be estimated (Bravington et al., 2016). However, a drawback of CKMR is the need for a large DNA sample size for a given species, and analysis can be expensive. Due to these limitations, there are only a few studies to date that have used CKMR, however, as the technique continues to evolve, it will likely become a key tool in ecology, conservation, and fisheries management (Delaval et al., 2023; Swenson et al., 2024).

Environmental DNA (eDNA)

Environmental DNA (eDNA) is a non-invasive way of sampling genetic material left behind as organisms move through their environment. In marine environments, it is sufficient to collect a sample of water and sift the sample through a filter that collects the genetic material. This method is relatively low-cost, and low effort, especially when compared to the traditional ways of estimating elasmobranch occurrence (Barnes & Turner, 2016; Bonfil et al., 2021; Boussarie et al., 2018). Yet, eDNA samples have a high risk of contamination, sample degradation, and face significant challenges when inferring abundance derived from samples. While it may be tempting to assume that eDNA quantities are positively correlated with biomass or abundance, eDNA quantities can be influenced by environmental factors (Terrasa et al., 2022). The quantity of eDNA in a water sample is also influenced by the nature of the water body, the physiology and behaviour of the organism that affects eDNA shedding rates, and eDNA degradation rates (Caren S. Goldberg et al., 2016). These confounding factors have led researchers to avoid attempting to estimate abundance using eDNA due to its 'elusive quantitative power' (Hsu et al., 2023). Although there are promising applications in the detection of rare and elusive marine species (Bonfil et al., 2021; Simpfendorfer et al., 2016), eDNA methods are relatively novel and still under development, and thus limitations still need to be addressed to improve accuracy and reliability in population genetics (Bakker et al., 2017; Sigsgaard et al., 2016; Thomsen et al., 2016).

Discussion

Selecting the appropriate research method involves a multi-step process that culminates in finding the optimal balance between effectiveness, affordability, and logistical feasibility. In evaluating effectiveness, it's important to consider the nature of the species targeted (Dulvy et al., 2017). This includes considering where the species is typically found, whether it is commonly observed by divers or predominantly caught by fisheries (Rhodes et al., 2020; Séguigne et al., 2023; Simpfendorfer et al., 2002; Talwar et al., 2020). Additionally, the species' conservation status plays a role: protected species may require non-invasive methods (Bansemer & Bennett, 2008; Setyawan et al., 2022). Although some shallow-water species can be observed with divers, the probability of encounter may be low, due to divers' avoidance, rarity, or cryptic behavior (MacNeil et al., 2008; Pais & Cabral, 2018). For instance, employing BRUVS to attract the species without direct diver interaction might be advantageous (Rolim et al., 2022). Using a combination of methods that include catch and non-catch methods can sometimes ensure more representative data of species occurrence (Barnett et al., 2022, Heagney et al., 2007; Rhodes et al., 2020).

Next, considerations arise regarding the scale of surveys based on available resources. Long-term surveys are preferable as they provide more reliable and representative abundance estimates and validate species occurrence in an area (Heupel & Simpfendorfer, 2005; Matich et al., 2020; Pratt et al., 2022). However, conducting long-term studies is expensive and poses logistical challenges. To expand their temporal and spatial scales research projects can collaborate with fisheries programs (Braccini et al., 2021; Mucientes et al., 2009; Shephard et al., 2019), engage in government-funded long-term monitoring efforts (Emslie et al., 2020), or involve citizen science initiatives (Chin & Pecl, 2018; Magson et al., 2022). Other types of scales include spatial exploration of deep waters and temporal variations between day and night. New technologies such as high-resolution underwater cameras, underwater and aerial drones, have already augmented traditional methods by extending spatial and temporal scales (Butcher et al., 2021). However, increasing these scales results in larger datasets that require extensive processing. In this case, new data processing technologies like artificial intelligence (AI) can play a pivotal role (Christin et al., 2019; Malde et al., 2020; Sheaves et al., 2020).

Table 2.1. Methods for surveying occurrence and abundance of elasmobranchs across temporal and spatial scales. Summary of key points.

Method	Species targeted	Temp. limitations	Spatial limitations	Pros	Cons
Catches (CPUE, logbooks, CMR)	Fishery target species. Bycatch species (limited to species that can be captured. E.g., not for protected no-catch species)	Survey time is equal to fishing time	The area surveyed is the area where fishing occurs.	Can be designed to closely replicate fisheries, sample at night or turbid waters. When using tags, behavior and movement can be always observed, vertically and horizontally.	Labor intensive. Bias can come from inaccurate logbook reporting (especially when gear and effort change over time).
Underwater surveys	Shallow species in clear water. Deeper surveys are possible but not practical	Mostly daytime due to logistic and safety limitations of night diving	Most diving is limited to 30 meters depth	Collection of data in real time, and no need for post-processing. Standardized and historically used worldwide.	Limited time in water, limited depths, difficulties with species ID in low visibility.
BRUVS/RUVS	All species. Competitive effect when bait is present; detection of bait plume can be species dependent	Short term due to short camera battery. Some extended (24hr) BRUVS have been trialed	BRUVS rely on bait plume for attraction. Currents and water movement transports bait plume influencing spatial capabilities. RUVS are limited to the area observed in the field of view	Can be used for species occurrence and relative abundance for deep waters and at nighttime. Can be used for stock assessments from sizes and biomass estimates.	Bait plume effects, competitive effects; underestimates of elasmobranchs with MaxN. Time spent post-processing
Aerial drones	Species that occur in shallow, coastal areas	Short term (battery). Mainly daytime	Coastal, shallow water	Expansive field of view; doesn't affect behavior; can reach sites that are hard to access by boat	Wind speed, weather, and turbid waters

Underwater drones	All species	Short term (battery)	ROVs are limited by cord length and complex substrate structure due to tether entanglement. AUVs are limited to the pre-determined survey area.	Can actively survey in deep water and at nighttime	Poor visibility hinders detection and species ID. Possible species avoidance. Observer-grade models don't perform well in currents. Long post-processing time.
Photo ID	Shallow clear water	Limited to aggregation sites for recaptures and therefore limited by seasonality (aggregations can be seasonal)	Limited to shallow waters and high-recapture areas (aggregation sites)	Global data bases can be created by citizen science project. This increases spatial and temporal scales. Divers can send pictures; record other factors.	Only few species can be effectively studied. Good visibility and picture quality is essential. AI might be necessary to streamline post-processing and image matching appropriate. Visibility
Genetics (pop. Genetics)	All species (capture is required)	None to be noted	May need to sample over a wide region or collect many samples	Tissue can be procured from fisheries and collaborators	Need large sample sizes.
Genetics (eDNA)	Rare and threatened species and species that cannot be detected by other studies	The life span of DNA in the water sample is limited	Presence of DNA in the water is determined by currents, location, water temperature (can vary between regions) and water body	Cheap and easy to test occurrence of a species.	Sample contamination. quantitative uses confounded by many variables; many aspects to still show proof of concept and/or standardize

Method Selection: Detection of Species Abundance and Occurrence

Catch methods are versatile and provide valuable data on species abundance and occurrence, particularly when long-term fisheries data is available and collaborative surveys can extend the spatial and temporal scale of data collection (Blaber et al., 2009; Simpfendorfer et al., 2002). When tagged and released, catch data can be used in Capture-Mark-Recapture (CMR) models to study populations and gather life history parameters (Latour, 2005). Although deploying satellite and acoustic tags adds financial and logistical challenges, it also allows for movement data to be integrated in CMR models (Dudgeon et al., 2015; Latour, 2005; Sollmann et al., 2013). However, these methods are limited to species that tolerate capture and regions where catches are permitted. Some elasmobranch species for example cannot be captured, as they are threatened with extinction, long-lived species that have slow-reproduction strategies (Armstrong et al., 2019, Bansemer et al., 2008). For these species, such as manta rays, whale sharks, and white sharks, photo identification (photo ID) serves as a viable, non-capture alternative for CMR models (Table 2.1; Andreotti et al., 2016; Araujo et al., 2014; Armstrong et al., 2020). Effective photo ID requires species to have unique, identifiable morphological traits and patterns, and it works best in aggregation sites to ensure high recapture probabilities. Therefore, this method is only effective for a select group of species (Marshall & Pierce, 2012), and assumptions of photo ID should be tested, when possible, with the use of other methods (Araujo et al., 2019; Bansemer & Bennett, 2008). BRUVs are particularly effective for estimating elasmobranch occurrence and abundance due to the bait's attraction effect. However, data from BRUVs can be significantly influenced by factors such as bait attraction, environmental conditions, and species-specific movement patterns and behaviors (Kilfoil et al., 2021). For instance, large migratory elasmobranchs may travel long distances following a BRUV's bait plume. This makes it challenging to accurately infer species-habitat associations or the effectiveness of area closures, as these species might come from other areas (Sheaves et al., 2020).

Differently from catches and BRUVS, which attract the animals to an area, underwater surveys are a mobile type of survey that relies on the encounter of the target species during a randomized series of transects. Not only divers surveys are a non-invasive alternative to catches but provide a mobile-survey perspective to complement the stationary nature of videos from BRUVS (Kilfoil et al., 2021). For instance, underwater visual surveys (UVS) are one of the most used survey methods for marine species in tropical and temperate ecosystems (Hill & Wilkinson, 2004), globally standardized, simple, and affordable. This makes UVS the preferred method for

surveys aiming to monitor populations and habitats over the long term, given its extensive standardization and widespread use across geographically diverse locations (O'Connor & Cullain, 2021; Ward-Paige et al., 2022; Ward-Paige et al., 2018). Additionally, UVS offers a straightforward approach to surveying biodiversity, providing initial estimates of species richness, and detecting significant changes, such as species disappearing over time (Ward-Paige, 2010). Additionally, the use of stereo-DOV (divers operated video), can help with detection biases and allow for biomass estimates from length measurements (Goetze et al., 2019). UVS are excellent for citizen science projects, allowing trained volunteers to contribute to elasmobranch observation (Table 2.1; Chin & Pecl, 2018; Ward-Paige et al., 2018). A pitfall of citizen science projects is that surveys are opportunistic resulting in presence-only estimates creating difficulty in modelling species distribution effectively. Though some techniques such as point process models (MAXENT) can better account for these distributions (Renner & Warton, 2013). When survey effort is substantially increased, the higher likelihood of detecting even rare and cryptic species can counterbalance low counts and enhance the robustness of estimates (Araujo et al., 2020; Chin, 2014).

Similarly to divers' surveys, underwater drones can provide a mobile, underwater video survey elasmobranchs encountered during the transect. Underwater drones are effective for fish surveys, showing promising results for elasmobranch species as well (Sward et al., 2019). A known type of underwater drone are remotely operated vehicles (ROVs), which can operate in inaccessible and hazardous environments, reaching depths of up to 4000 meters, thereby enhancing the capabilities for deep-water elasmobranch surveys (Table 2.1; Sward et al., 2019). Observational studies using work-class ROVs have led to the discovery of new fish species and have expanded the known spatial and depth ranges of other species (Macreadie et al., 2018; McLean et al., 2018). Similarly to underwater drones, aerial drones have become increasingly popular for surveying shallow water, coastal environments (Butcher et al., 2021). Aerial drones are a valuable method for observing undisturbed occurrence and behavior in these areas (Table 2.1), and they significantly increase the spatial scale of surveys with relatively limited effort and cost (Hensel et al., 2018; Kiszka et al., 2016; Skomal et al., 2015).

Finally, one of the most effective methods to detect occurrence of low-abundance species is environmental DNA (eDNA). EDNA is arguably the method with the highest "return on investment" (Terrasa et al., 2022). It only requires a sample of water, is completely non-invasive and can be insightful for detecting rare and cryptic species that may go completely undetected

in hard-to-access environments (Table 2.1; Simpfendorfer et al., 2016). Additionally, this method has been used for impactful species conservation work in the fisheries field (Green et al., 2024). However, this method involves unpredictability that hinders accurate abundance estimates (Boussarie et al., 2018; Terrasa et al., 2022).

Addressing Spatial and Temporal Limitations with Complementary Methods

Estimates of abundance are challenging for elasmobranchs due to the low-abundance, sparse distribution of many species in this taxon (Heupel et al., 2019; Dulvy et al. 2017). Although the choice of the survey method is often determined by available assets, financial, and logistical constraints, some methods are often regarded to be more effective when estimating abundance. For instance, methods that employ fisheries gear and techniques are often useful for estimates of abundance that will ultimately feed into population and stock assessments (Braccini et al., 2016; Rago, 2005). Additionally, the tissue, blood, and swab samples collected from catch surveys can provide insights into the size and state of the breeding population (Dudgeon & Ovenden, 2015; Luikart et al., 2010). Finally, when appropriately standardised, beach transects with aerial drones can be used to extrapolate counts to the level of relative abundance, providing a reliable and undisturbed sample for shallow water species (Hodgson et al., 2013; McIvor et al., 2022). When it comes to abundance estimates using BRUVS researchers have developed precise indices and accounted for potential biases, such as double counting, competitive interactions around the bait bag, limited field of view (Sherman et al., 2018; Sherman et al., 2020; Asher et al., 2019). UVS on the other hand have been extensively adopted for surveys, but data on elasmobranchs is often affected by the presence of divers in the water and the human-bias related to counting and identifying species (Colton et al., 2010). For these reasons, catch surveys paired with genetics and potentially improved using acoustic and satellite tagging are some of the most used methods for abundance estimates (Carrier et al., 2018). Drones, BRUVS, and UVS are valid methods that may be more effective as a complementary addition to catch surveys (Caceres et al., 2022; Santana-Garcon et al., 2014). However, it is important to consider circumstance when catches are prohibited in the area or for a protected species (Jabado et al., 2021; McKinney et al., 2017). In these cases, a combination of non-invasive methods such as drone surveys, BRUVS and photo ID, may be the best option to estimate reliable abundance.

To collect representative data on the occurrence of species and individuals in an area, catch surveys alone can be unreliable, and do not provide evidence of undisturbed occurrence

and behaviour (Rago, 2005). For this reason, unbaited underwater cameras, aerial drones, and eDNA are likely to be the methods that collect the most authentic occurrence and behaviour (Bullock et al., 2024; Bonfili et al., 2021). However, due to the nature of many elasmobranch species, relying on the random chance of encounters may need unrealistically extensive survey time to be able to collect large enough datasets. For this reason, baited underwater cameras like BRUVS have been more effective, as they attract elasmobranchs using a bait (Brooks et al., 2011; Cambra et al., 2021; Cheal et al., 2021). The occurrence on elasmobranchs on BRUVS however may not be fully representative of the presence of animals and species in the area. For instance, the scent of the bait may be transported far enough by ocean currents to be attracting species that are not usual visitors of an area (Dorman, Harvey, and Newman 2012; Hardinge et al., 2013). Overall, when estimating abundance and occurrence of elasmobranchs, multi-methods approach could result in more accurate and representative data compared to single methods alone (Barnett et al., 2022)

Using multiple survey methods is essential for mitigating biases and interpreting variability in abundance estimates across different elasmobranch species and habitats (Asher et al., 2019; McCauley et al., 2012). For instance, comparative studies have demonstrated substantial variation in abundance estimates between underwater video systems (UVS) and baited remote underwater video stations (BRUVS), highlighting the importance of methodological choice in capturing species diversity (Schramm et al., 2020). Catch surveys have been used as a complementary method alongside aerial drones, catching species that do not surface often and would be undetected by drones (Nykänen et al., 2018). BRUVs can also be used together with drones when sampling a large area that encompasses different depths and habitats (McIvor et al., 2022). Catch studies, including satellite and acoustic tagging, can be used to gather data on relative abundance and occurrence, alongside BRUVs to determine habitat use, residency, and complement species detection (Barnett et al., 2022). Complementary methods not only fill gaps in detection capabilities and accuracy of estimates, but also serve to test and address the inherent biases of the methods themselves. For example, the combination of baited and un-baited underwater video stations can offer an insight on the relative effect of the bait on the presence/absence of species (Rhodes et al., 2020). In the same way, telemetry methods can be used to supplement movement and survival variables withing CMR models that would otherwise lack robustness due to the mobile nature of many elasmobranch species (Sollmann et al., 2013). Finally, genetics methods are often used alongside catches to reinforce estimates of populations and stock structure (Andreotti et al., 2016; Delaval et al., 2023), and

eDNA is effective in detecting species that are difficult to capture or observe with traditional methods due to their rarity or tendency to avoid divers (Goldberg et al., 2016; Stat et al., 2019).

Conclusion

Several methods are available to collect data on occurrence and abundance of elasmobranch, with new ones continually being developed and tested. Each method carries its own set of strengths, limitations, and inherent biases, which must be carefully weighed by scientists when selecting the most suitable approach to address specific research questions. It's crucial to recognize that the effectiveness of a method is not solely determined by its technical application but also by the spatial and temporal design of the sampling program. Acknowledging and addressing these biases is fundamental to ensure accurate interpretation of the data, and ground truthing methods through multiple approaches enhances confidence in research findings. New technological applications can contribute to expand the spatial and temporal scales of current methods, and it is crucial to consider not just functionality but also factors like cost-effectiveness, efficiency, and suitability for various geographic and resource contexts.

Chapter 3: Novel Use of Deep Neural Networks on Photographic Identification of Epaulette Sharks (*Hemiscyllium ocellatum*) Across Life Stages.

Abstract

Photographic identification (photo ID) is an established method that is used to count animals and track individuals' movements. This method performs well with some species of elasmobranchs (i.e., sharks, skates, and rays) where individuals have distinctive skin patterns. However, the unique skin patterns used for ID must be stable through time to allow re-identification of individuals in future sampling events. More recently, Artificial Intelligence (AI) models have substantially decreased the labour-intensive process of matching photos in extensive photo ID libraries and increased the reliability of photo ID. Here, photo ID and AI are used for the first time to identify epaulette sharks (*Hemiscyllium ocellatum*) at different life stages for approximately two years. An AI model was developed to assess and compare the reliability of human-classified ID patterns in juvenile and neonate sharks. The model also tested the persistence of unique patterns in adult sharks. Results indicate that immature life stages are unreliable for pattern identification, using both human and AI approaches, due to the plasticity of these sub-adult growth forms. Mature sharks maintain their patterns through time and can be identified in AI models with approximately 86% accuracy. The approach outlined in this study has the potential of validating the stability of ID patterns through time; however, testing on wild populations and long-term datasets is needed. This study's novel deep neural network development strategy offers a streamlined and accessible framework for generating a reliable model from a small data set, without requiring high-performance computing. Since many photo ID studies commence with limited datasets and resources, this AI model presents practical solutions to such constraints. Overall, this approach has the potential to address challenges associated with long-term photo ID data sets and the application of AI for shark identification.

Introduction

In ecological research, identifying and counting individuals within a species is often the first step to understanding population dynamics. Long-term monitoring of aquatic species has traditionally used capture-mark-recapture (CMR) methods, which involve tagging or 'marking' of individual organisms to differentiate them within their population, facilitating subsequent recaptures over time and the reconstruction of re-encounter histories (Jolly, 1965; Musick & Bonfil, 2005; Pine et al., 2003). Data derived from CMR studies are then used to estimate population sizes and collect information on life history parameters, animal movement, and habitat use (Cameron et al., 2019; McCoy et al., 2018; Peterson & Grubbs, 2023). However, CMR

methods require the physical capture and handling of individuals, posing logistical challenges, potential danger for the animal and the researchers, and possibly disrupting natural behaviours (Bouyoucos et al., 2020; Pauli et al., 2010). Photographic identification (herein, photo ID) has emerged as a non-invasive alternative, gaining traction alongside technological advancements in media data acquisition and processing (Miele et al., 2021; Pierce, 2019). Photo ID originated in the 1970s (Myrberg & Gruber, 1974) and applies image-based biometrics (i.e., photographs). This method leverages unique and temporally stable biometric features similar to fingerprints, which are unique to an individual, thus helping identification (Jenrette et al., 2022).

Tracking the same individuals through time can be challenging in marine environments, particularly when targeting cryptic species that naturally occur in low abundance, spend most of their time at inaccessible depths, or constantly move across large distances (Harty et al., 2022; Jackson et al., 2006; Ramsey et al., 2019). The advent of underwater video technologies has assisted the increasing popularity and growing adoption of photo ID as a method for monitoring marine animals (Anderson & Goldman, 1996; Arzoumanian et al., 2005; Corcoran & Gruber, 1999; Hammond et al., 1990). This is particularly relevant for animals that are too large to be captured or for protected species where direct manipulations may be challenging. Amongst these species, there are several examples of elasmobranchs (i.e., sharks, skates, and rays) that have been studied using photo ID (Marshall & Pierce, 2012). Photo ID is arguably the standard method for monitoring white sharks (*Carcharodon carcharias*; Becerril-García et al., 2020; Micarelli et al., 2021; Schilds et al., 2019), whale sharks (*Rhincodon typus*; Araujo et al., 2019; Arzoumanian et al., 2005), grey nurse sharks (*Carcharias taurus*; Bansemer & Bennett, 2008), Indo-Pacific leopard sharks (*Stegostoma tigrinum*; Dudgeon et al., 2008) and manta rays (*Mobula alfredi* and *M. birostris*; Harty et al., 2022; Town et al., 2013). However, from the ~1200 species of elasmobranchs (IUCN SSC Shark Specialist Group, 2023), there are only a few examples of photo ID being used for other species, for example, blacktip reef sharks (*Carcharhinus melanopterus*; Mukharror et al., 2019), basking sharks (*Cetorhinus maximus*; Gore et al., 2016; Southwood, 2008), great hammerhead sharks (*Sphyrna mokarran*; Guttridge et al., 2017), nurse sharks (*Ginglymostoma cirratum*; Castro & Rosa, 2005), white spotted eagle rays (*Aetobatus narinari*; Cerutti et al., 2018), bull rays (*Aetomylaeus bovinus*; Moreno et al., 2021), and several species of skates (Benjamins et al., 2018). Generally, photo ID has gained acceptance and traction as a reliable method for studying elasmobranch population dynamics and ecology (Pierce, 2019). Yet the low number of species studied implies that not all species are suitable for photo ID (Marshall & Pierce, 2012).

Certain conditions and assumptions need to be satisfied to achieve reliable and accurate results in photo ID studies. One of the main conditions for photo ID is that morphological features used for identification must be both discernible and stable through time, and several species of elasmobranchs exhibit natural patterns conducive to ID (Armstrong et al., 2020; Arzoumanian et al., 2005; Harty et al., 2022). However, only a few studies have attempted to validate photo ID through multi-modal methodologies or by convergent evidence from different markers, including sex and external tags (Bansemer & Bennett, 2008; Dudgeon et al., 2008; Gubili et al., 2009; Winton et al., 2023). For example, white sharks are identified by the trailing edge of their dorsal fin, which may seem quite similar among individuals, but upon closer examination, it carries substantial individuality (Andreotti et al., 2014). When photo ID is used as a CMR method for long-term studies, the stability of patterns is often inferred via observational evidence, but this is rarely subjected to a systematic, longitudinal evaluation (Bègue et al., 2020; McCoy et al., 2018; Pratt et al., 2022; Winton et al., 2023). Additionally, in species that exhibit ontogenetic changes in their morphology, such as Indo-Pacific leopard sharks (*Stegostoma fasciatum*) and tiger sharks (*Galeocerdo cuvier*; Fu et al., 2016), the presence and persistence of patterns during early life stages constitute a species-specific variable that is not universally addressed, thereby constraining the applicability of photo ID to mature life stages for most species (Marshall & Pierce, 2012). Another essential consideration is the approachability of the species in their natural habitat. While contemporary underwater video technologies can access a broad range of environments and depths, optimal photograph quality is typically achieved at shallow depths and in clear waters (Deakos et al., 2011; Marshall et al., 2011; McCoy et al., 2018). Consistent or well-defined seasonal aggregations of certain species in known locations facilitate longitudinal, photographic documentation, thereby enhancing the reliability of re-sightings for population modelling (Changeux et al., 2020; Marshall & Pierce, 2012; Pratt et al., 2022).

Some elasmobranch species, such as manta rays, whale sharks, and white sharks, meet the assumptions and conditions for successful photo ID, resulting in large-scale, multi-location, long-term projects that compile extensive datasets of photographs through time (Araujo et al., 2017; Armstrong et al., 2019; Norman et al., 2017). For example, the Wild Book for whale sharks (McCoy et al., 2018) and the Manta Matcher (Town et al., 2013) are examples of open-access global datasets of photographs that can be collected by anyone, anywhere. To expedite processing times and reduce the potential for human error, many photo ID projects have implemented automated or semi-automated image analysis through artificial intelligence (AI; Carter et al., 2014; Miele et al., 2021; Weinstein, 2018). As an example, open access platforms

such as WildMe (<https://www.wildme.org/what-we-do.html>) have developed AI powered computer vision technologies for some of the most iconic elasmobranch species to facilitate individual recognition, such as Sharkbook and Manta Matcher (Conservation X Labs, 2024). These platforms are under continuous maintenance to develop new solutions and improve the speed, automation, and accuracy of identification.

In computer technologies, AI refers to a goal, machine learning is a technique, and deep learning is a tool, but these terms are used interchangeably in the literature. Machine learning refers to a type of algorithm that can autonomously identify patterns in data, even when data are nonlinear and complex, and can create predictive models (Christin et al., 2019). The application of machine learning in computer vision goes back to simple statistical modelling and shallow neural networks (Hu et al., 2012), but this technology has recently evolved into advanced, deep neural networks (DNN; Jahanbakht et al., 2022). Most photo ID projects on elasmobranchs have used shallow neural networks, heavily relying on researchers' expertise to perform feature extractions and labelling (Andreotti et al., 2018; Arzoumanian et al., 2005; Hughes & Burghardt, 2017; Town et al., 2013). The more advanced DNNs use a function approximator called a neural network that contains different modifiable parameters organised in layers of neurons. These layers can receive the data, process it (within the processing core), and give the result of the model (output layer). One of the most important advantages of DNNs in photo ID applications is the automated extraction of distinguishing features from an image. The feature extraction process takes place within the multiple layers of the processing core, where progressively more accurate outputs are given through a self-improving learning process (LeCun et al., 2015). This is achieved by customising one of the layers of the neural network for the image classification task. Specifically, the convolutional layer is used in Convolutional Neural Networks (CNNs) and is often adopted for computer vision as it can extract specific patterns to classify images. CNNs have achieved the best performances in photo ID studies of turtles, great apes, giant pandas, and giraffes, but major limitations arise with small and unlabelled training datasets, temporal changes in morphology, and re-identification (Christin et al., 2019; Miele et al., 2021). The automated re-identification of previously known individuals is a necessary feature of AI applied to photo ID. Currently, most models rely on an extensive library of photographs for training, and as a new individual is photographed, the model needs to be completely retrained (Schneider et al., 2022). Similarity comparison networks are a novel approach to addressing the issues of re-identifying individuals from small and unlabelled datasets (Miele et al., 2021), which is often the best available resource when studying wild populations of elasmobranchs.

The aim of this study was to develop a framework to incorporate novel AI approaches in photo ID projects for long-term monitoring of elasmobranchs. In photo ID studies, the species of choice needs to be easily distinguishable by its individually unique patterns. In this study, this requirement was fulfilled by choosing a species of elasmobranch, the epaulette shark (*Hemiscyllium ocellatum*), as the focal species and developing a photo ID protocol that incorporates both standard photo ID processes and innovative AI applications. Importantly, epaulette sharks change patterns as they mature from their neonatal stage, allowing this study to assess the implications of unstable patterns during early ontogeny (Ferreira et al., 2020). Focusing on a captive population of epaulette sharks, the model could be trained with photographs of known individuals, from all life stages, and at different times throughout the study. The same model was used to test if patterns change over time and whether immature life stages can reliably be identified from their patterns. The trial-and-error process in this study led to the final photo ID protocol and AI model, which represents an ideal trade-off between simplicity, flexibility, precision, and AI innovation.

Materials and Methods

Ethics

The care and use of experimental animals complied with the animal welfare laws determined by the Australian Code for the Use of Animals for Scientific Purposes, and the guidelines and policies as approved by the James Cook University Animal Ethics Committee (protocol A2826). The animals that were photographed in captivity were collected under the appropriate Great Barrier Reef Marine Park Authority (GBRMPA #G19/43380.1) and Queensland Fisheries (#200891) permits.

Photographing Sharks

Epaulette sharks have a distinctive and individually unique pattern that can be used to distinguish adult individuals from each other. On the other hand, newborn and juvenile individuals can be harder to identify, as their pattern is constantly changing as they mature (Figure 3.1) (Payne, 2012). However, to date, there has been no scientific study that systematically observes and annotates how the patterns change with growth, and all available information has only been from anecdotal observations in captive environments. The changes observed in the patterns of young epaulette sharks provided an opportunity to test the stability of patterns for non-mature individuals. In the current study, eight adults, four juveniles, and five neonates were photographed at the Marine and Aquaculture Research Facility Unit (MARFU) at

James Cook University. The sharks photographed in this study were maintained in a controlled environment and separated in different tanks, allowing researchers to easily distinguish known individuals with their respective ID number. Additionally, the controlled environment provided a convenient setting for repeated photographic documentation of known individuals (Bansemmer & Bennett, 2008). Also, the quality of photographs can be ensured, and unusable photographs can be re-taken. This is not always feasible with wild populations due to varying conditions, including low visibility, surface and underwater photography, and the use of different camera types. To account for that, photographs for this study were taken with different angles, light availability, and all sorts of devices were used, from phones to professional cameras. This approach provided the AI model with a sufficient degree of variation in the examples used for training. In turn, this would give the model a certain degree of flexibility in what type of photographs can be usable for training. An additional difference between working with a captive population and a wild population is that captive populations are often closely monitored, sharks are known by ID, and records of labelled photographs are often available. These favourable conditions provide the AI model with a good base of information to start training. On the other hand, training a model for wild population might be an ongoing and continuously updating process, as new individuals enter the population and need to be identified. To overcome the inconvenience of having to re-train the model every time a new individual is added, this model will implement a similarity network approach similar to the one used in past studies (Schneider et al., 2022).

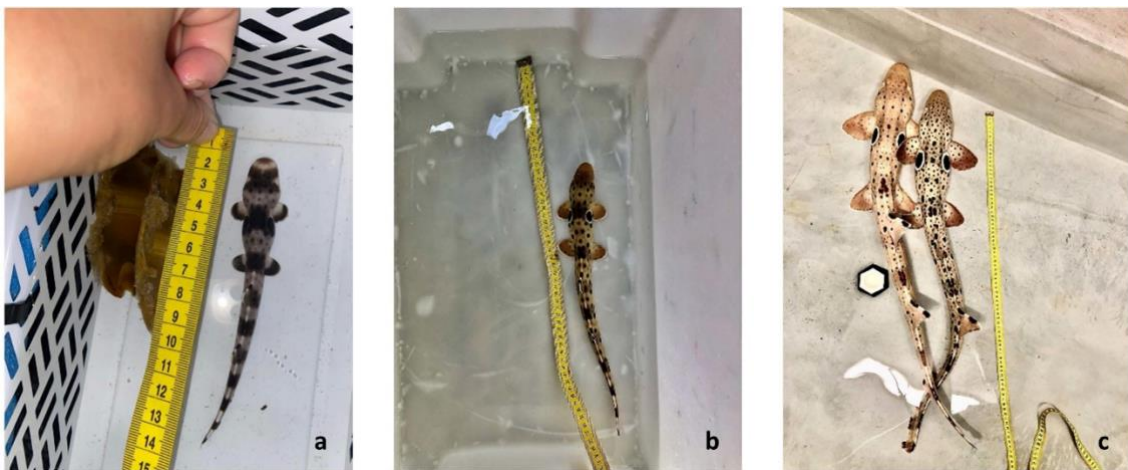


Figure 3.1. From the left, typical neonate (a), juvenile (b), and adult (c) epaulette sharks (*Hemiscyllium ocellatum*).

Dataset Organisation

Two series of photographs were taken: the 'Baseline' series and the 'Time' series. The Baseline series comprised photographs of all sharks, and each shark was photographed multiple times on the same day. These photographs were classified by ID into the 'Baseline dataset'. The purpose of this dataset was to have multiple photographs of the sharks to train and test the AI model. Additionally, because all photographs were collected on the same day, there were no confounding effects of morphological changes that could have occurred through time. The time series of photographs were classified in the 'Time dataset'. The time dataset consisted of temporally consecutive photographs of the same sharks and was employed to test the model's ability to compare photographs of the same individual over time to assess if morphological changes have occurred in the ID patterns over time. Each photograph in the Time dataset was labelled with the shark's ID and a time marker, such as T0, T1, T2, T3, (...), Tn. Time intervals between consecutive photographs of adult and juvenile sharks were not standardised (see Appendix A, Table A.1). This approach is relevant for the model's real-world applicability, given that re-sightings of wild elasmobranchs are unlikely to occur at regular intervals due to environmental factors. However, the time elapsed between consecutive photographs of neonate sharks was deliberately controlled to be no less than one week and no more than one month apart. For instance, significant pattern changes were expected for neonate epaulette sharks within the first year, post-hatch (Figure 3.2), and photographs were taken often and regularly to capture such changes.



Figure 3.2. A typical neonate growth and change in pattern morphology in approximately 6 months.

Photograph Enhancement and Masking

Expanding on the dataset preparation, both the Baseline and Time datasets underwent a cleaning process. This involved removing duplicates (identical photographs) and blurry/low-resolution photographs. Additionally, any photograph that did not display a dorsal orientation was excluded. Further, each of the retained photographs was masked using the free program, Gimp – GNU Image Manipulation Program (version 21.0; GIMP Development Team, 2019). This masking technique is further illustrated in Figure 3.3 (a and b), where the visual focus is solely on the shark's silhouette, effectively eliminating any background distractions. Cropping the image to exclude the background facilitates the task of the AI model and decreases the amount of computational power needed. Generally, when processing images with low computational power (when HPC are not available), it is best to pre-edit the images used for training and testing. Cropping away the background and flagging identification features is more labour intensive but dramatically reduces the need for large computational power. This step can be avoided if a HPC is available for image processing.

Skin Boundary Labelling

To accurately train deep learning models without overtraining, images were randomly grouped into batches of 16 images. The DNN's internal variables were gradually updated by these batches in an iterative process. High-Performance Computing (HPC) is often necessary when training with photographs of whole sharks, and in this case, to train an advanced DNN with batches of 16 high-resolution images (i.e., 5472×3648 pixels). However, such computing power was not available for this study, and images of whole sharks needed to be “down-sized. Specifically, four patches (i.e., body sections) from each shark image were subsampled (Figure 3.3 c, d, e, f). These patches include the head (*head*, Figure 3.3, c), pectoral/gills area (*pec* Figure 3.3, d), the dorsal area just ahead of the first dorsal fin (first dorsal front, *FDF*, Figure 3.3, e), and the dorsal area just past the first dorsal fin (first dorsal back, *FDB*, Figure 3.3, f).

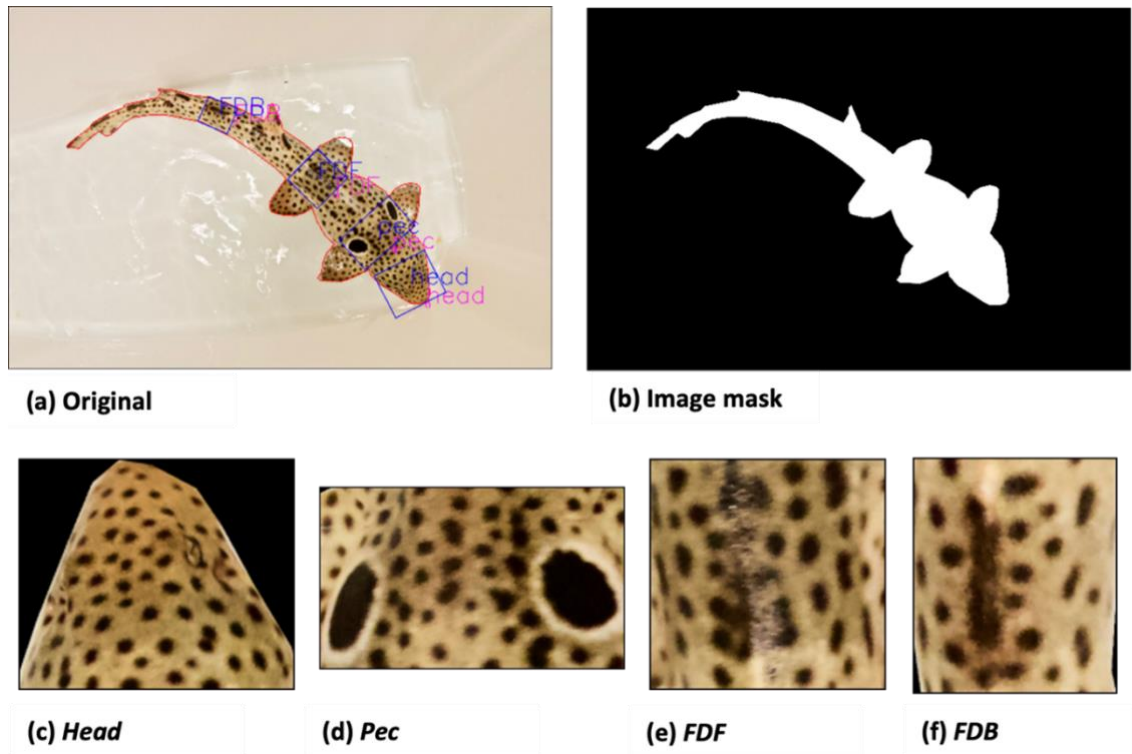


Figure 3.3. (a) Original image, with pink and purple labels indicating the position of body patches in relation to the whole body; (b) image mask; (c, d, e, f) four subsampled image patches (head, pec, FDF, and FDB) of image HO_100_B0P2. These patches are sections of the sharks' body: (c) the head, (d) the area around the gills and pectoral fins, (e) the dorsal area in front of the first dorsal fin, and (f) the dorsal area behind the first dorsal fin.

By cropping and resizing the original images into four patches of 380×380 pixels, the need for an HPC will be eliminated. These body sections were chosen based on the experience of researchers and volunteers working in the lab, where animals are routinely identified for measurements, feeding, and experiments. These 4 sections are thought to be the sections with most differences between individuals. The *roLabelImg* program (Cgvict, 2017) in Python (Van Rossum & Drake, 2009) was used to create 'boxes' around these body sections (Figure 3.3a): The *head* region starts from the tip of the shark's nose, and down to approximately 3/4 of the head length before the beginning of the pectoral region. The *pec* region starts from the top of the pectoral fin, behind the gills, to the base of the shark's black ocellus. The *FDF* region was designated as the heavily spotted region between the top of the pelvic fins to the base of the pelvic fins, found in front of the first dorsal fin. The *FDB* region was indicated by the densely spotted region between the base of the first dorsal fin and the front of the second dorsal fin. Four rotated rectangular boxes were drawn and tightened around the targeted skin patterns. Boxes were rotated to align with the direction of the vertebral line. Then, the front of each box was marked to indicate the direction toward the shark's head. This step was important to be

able to align the boxes in the correct order of the respective body sections. This collection of boundaries was saved and uploaded with the respective labelled photographs and masks.

Image Augmentation

In the training phase of the DNN, photographs in the training dataset were randomly augmented to virtually increase their count and to avoid over- and under-training. Each image is randomised with a series of flips (X-flip and Y-flip), rotations (90°, 180°, and 270°), Gaussian noise, Gaussian blur, gamma contrast, linear contrast, and other image manipulations. This image augmentation disturbs the RGB spectrum while keeping the sharks' skin patterns visually recognisable. This helps with training the model on shark skin patterns, instead of learning skin colours and colour contrasts (Figure 3.4).



Figure 3.4. An example of random image augmentations for HO_100_BOP1. (a) Original head, pec, FDF, and FDB patches of the shark, and (b) head, pec, FDF, and FDB with random augmentations.

Training and Testing

The AI model was initially trained and tested using the Baseline dataset. When developing AI models, available photographs are sub-grouped into training and testing dataset. This process is necessary to 'teach' the model with similarities and differences for distinguishing individuals. From the Baseline dataset, photographs of only 6 adults, 3 juveniles, and 4 neonates were used for training. It is important to note that not all the adults, juveniles, and neonates were shown during training. This process ensured that, when tested, the model looked for features within a new photograph, rather than remembering the previously seen the photographs themselves (Schneider et al., 2022). The remaining photographs not shown during

training were grouped in the testing dataset, together with photographs of the remaining 2 adult, 1 juvenile, and 1 neonate. Furthermore, the Time dataset was only used for the model testing. After the model had been trained on the basic task of distinguishing individual sharks, it got tested by presenting temporally consecutive photographs of the same shark from the Time dataset. The purpose of this additional step was to test whether the morphology of the same shark changes through time enough so that the shark was not as distinguishable as it would be if no changes had occurred.

Model Development

The DNN model used in this study is based on the EfficientNetB4, which is a CNN with optimum depth, width, and resolution scaling, introduced by Tan and Le (2019). Based on the benchmarking available in the Keras website (Keras, 2023), EfficientNet and EfficientNetV2 are the most accurate models that offer small to x-large architectures with 29 to 479 MB size ranges. Among them, EfficientNetB4 was chosen as its 75 MB architecture fits well into an Nvidia GeForce RTX 2080 GPU processor. To train the proposed model with the limited Baseline dataset (8 adults, 4 juveniles, and 5 neonates), a transfer learning strategy was employed. The EfficientNetB4 model was pre-trained with existing 14,197,122 images of 1,000 annotated objects in the ImageNet (Deng et al., 2021). The bottom layers of the pre-trained model were then retained, and the top layer replaced with a customized new top. During the training, all 17,673,823 pre-trained weights of the bottom layers were frozen, and only the 121,886 weights of the new top layer received training from the augmented images dataset.

The trained bottom layers were duplicated to form two identically frozen EfficientNetB4 models, each accepting an image patch at their inputs (Figure 3.5). The output of both models passed through an average pooling block to extract two image representations of the two input patches with a numerical vector that simply represents/encodes its relevant image. The two vectors were then concatenated into a single vector and passed through two subsequent dense layers. The first dense layer consisted of 32 fully connected neurons with Rectified Linear Unit (ReLU) activation function. The second dense layer comprised one neuron with sigmoid activation to output a single probability-like number between 0 and 1. This number is called similarity index, and it shows the probability of the two input images being the same shark (1) or not (0). The larger the value means a higher probability of two patches having similar skin patterns. The similarity index was adopted to identify single sharks by comparing the similarity indices returned during the analysis. Due to the limitation of only having 20 different sharks, the

model was asked ‘Are these two sharks similar?’ instead of ‘What is the ID of this shark?’. This approach has been referred to as the similarity network approach and has been tested on several species, with photographs from open-access datasets (Schneider et al., 2022).

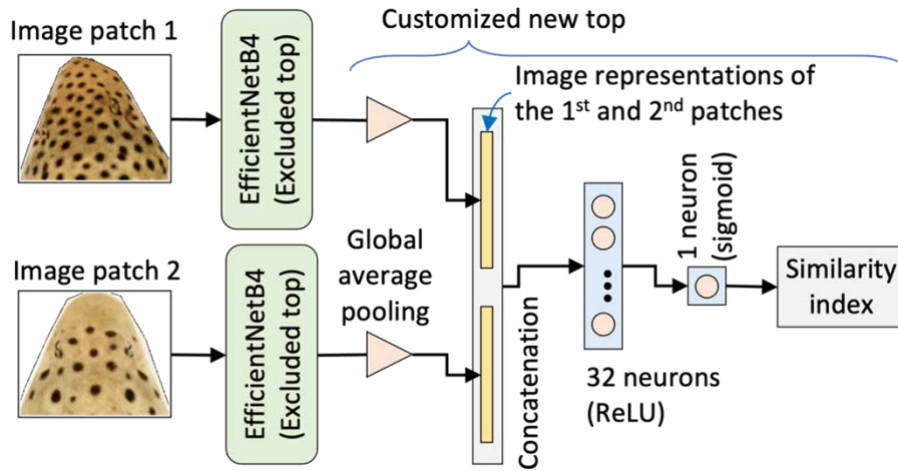


Figure 3.5. The proposed DNN, based on the EfficientNetB4 architecture, which receives two image patches, and returns their calculated similarity index.

Model Ensemble Process

Using the four different patch types in the image dataset (i.e., head, pec, FDF, and FDB), four independent AI models were trained. With a further innovative step, the four AI models were ensembled (e.g., a combination of multiple algorithms) into a single decision-making model. The chosen model was XGBoost ensemble, which has recently attracted more attention as an ensembling method (Jahanbakht et al., 2023). The four similarity indexes of the AI models were concatenated into X, which was then inputted into an XGBoost model and represented as a collection of M decision trees (Figure 3.6). The optimum value is automatically detected by the algorithm during the data fitting process (a.k.a., model training). Each decision tree i receives similarity indexes and returns $T_i(X, r_{(i-1)})$, where $r_{(i-1)}$ is the residual output from the previous tree. The overall output of the XGBoost regression ensemble was then calculated as follows (Jahanbakht et al., 2023).

$$Final\ similarity\ index = \sum_{i=1}^M a_i T_i(X, r_{i-1})|_{r_0=0}$$

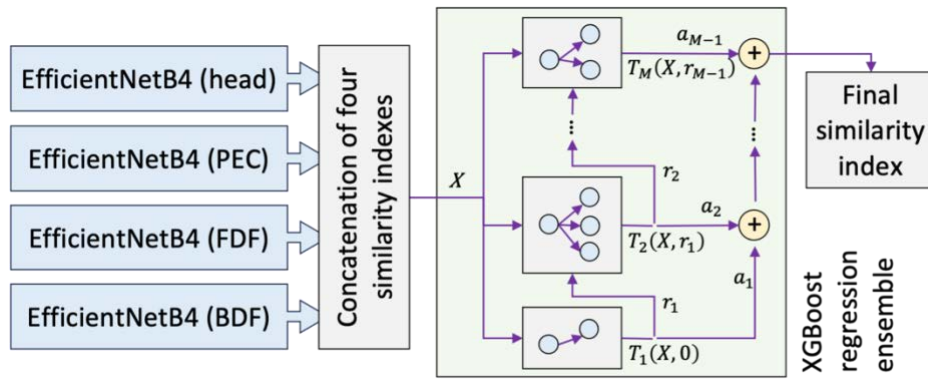


Figure 3.6. Merging the outputs of four independently trained DNN models of Figure 3.5 (on head, PEC, FDF, and BDF), into a single XGBoost regression ensemble.

To infer an XGBoost model, images of two sharks were needed (either the same or different individuals). The *head*, *pec*, *FDF*, and *FDB* patches were extracted for each image, and the pairs fed into their relevant EfficientNetB4 model. This process generated the calculated final similarity index at the output of the XGBoost model. Any value greater than 0.5 (on a 0-1 scale) means that the input two images belong to the same shark; otherwise, they belong to different sharks. The process was named ‘image-based evaluation’: one image of one shark is compared to another image of another shark. However, in this case, multiple images of the same shark are available, and a better-performing process can be implemented. If K_1 images are confidently taken from shark 1, and K_2 images are taken from shark 2, then $K_1 \times K_2$ is the number of different image pairs that can input the XGBoost model one by one. This resulted in $K_1 \times K_2$ different final similarity indexes that were averaged to a number between 0 and 1. In contrast to the previous image-based evaluation, this process is called ‘shark-based evaluation’.

Results

Dataset Organisation

The image-based evaluation method was employed, where the similarity score is the result of the comparison between one image of one shark with only one other image of a different shark. The accuracy for the EfficientNetB4 – *head/pec/FDF/FDB* ranged between 47.0% – 56.2% accuracy, with low precision below 10% (Table 3.1: all life stages, image-based). XGBoost showed a better performance (approximately 90% accuracy) as it combined all independent AI results into the final accurate decision on similarity or dissimilarity. Despite the accuracy increased by XGBoost, the precision was still lower than 10% (Table 1: all life stages, image-based). This suggests that, although the approach is correct, there might be issues with the distinguishability of individuals, possibly arising from having only one image per shark with which to train.

Table 3.1. Comparing the accuracy, precision, and Mean Absolute Error (MAE) performances of different DNN models on multiple combinations of the testing dataset.

Image data	Evaluation	Model	Accuracy	Precision	MAE
All life stages	Image-based	EfficientNetB4 - Head	47.00%	8.10%	0.53
		EfficientNetB4 - PEC	41.70%	8.20%	0.583
		EfficientNetB4 - FDF	44.90%	5.60%	0.551
		EfficientNetB4 - FDB	56.20%	7.10%	0.438
		XGBoost-all patches	90.40%	9.40%	0.096
All life stages	Shark-based	EfficientNetB4 - Head	46.20%	14.40%	0.538
		EfficientNetB4 - PEC	39.20%	14.80%	0.608
		EfficientNetB4 - FDF	37.40%	10.60%	0.626
		EfficientNetB4 - FDB	53.80%	13.30%	0.462
		XGBoost-all patches	90.10%	47.20%	0.099
Adult only	Image-based	EfficientNetB4 - Head	71.30%	31.20%	0.287
		EfficientNetB4 - PEC	75.00%	28.80%	0.25
		EfficientNetB4 - FDF	23.30%	14.60%	0.767
		EfficientNetB4 - FDB	65.40%	18.40%	0.346
		XGBoost-all patches	85.30%	45.50%	0.147
Adult only	Shark-based	EfficientNetB4 - Head	75.00%	47.10%	0.25
		EfficientNetB4 - PEC	75.00%	46.20%	0.25
		EfficientNetB4 - FDF	22.20%	22.20%	0.778
		EfficientNetB4 - FDB	40.40%	11.10%	0.596
		XGBoost-all patches	86.10%	~ 100%	0.139

To improve the precision of the models the shark-based evaluation was applied to the first version of the models in the previous section. The shark-based evaluation used the same datasets for training and testing but compared two groups of images (each group containing images of two different sharks), instead of one image per shark. Each group was previously classified to the correct ID in the training and testing datasets. All models increased precision by averaging over the multiple image pairs, with the *XGBoost* model as the most optimal with scores of 90% accuracy and 47.2% precision (Table 3.1: all life stages, shark-based).

Testing the Model: Can the Model Distinguish Individuals?

The Receiver Operating Characteristic (ROC) curves show the performance of the models when distinguishing individuals (Figure 3.7). By adopting the similarity learning approach, the model was tested with the question “Are these two sharks similar or dissimilar?” rather than the question “Who is this shark?”. This approach simplifies and streamlines the task by adopting an intuitive solution and asking to learn to distinguish individuals rather than learning their ID. Furthermore, this approach allows for any new animals to enter the population without having to re-train the model to include a new ID (Schneider et al., 2022). In Figure 3.7,

the area under the curve (AUC) values ranged from 0 to 1 and represent how well the model distinguishes two images.

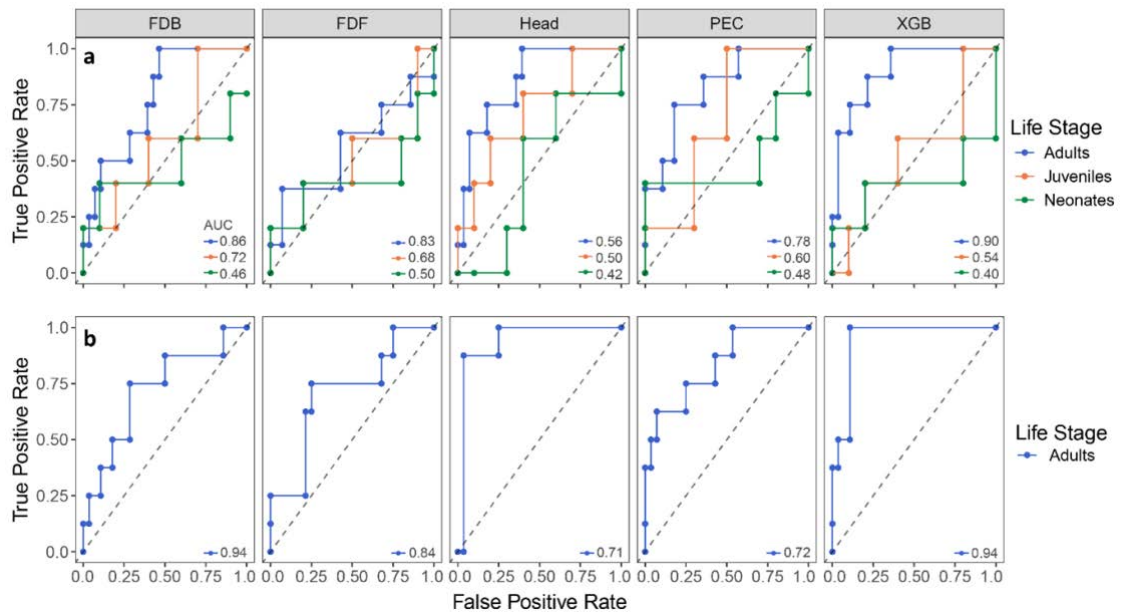


Figure 3.7. (a) Receiver Operating Characteristic curve for models trained on all life stages, and (b) on adults only. The area under the curve (AUC) represents the degree of separability between two classes (pairs of similar and dissimilar sharks in our case). The closer the value of AUC to 1, the better the model is at distinguishing two sharks from each other.

When testing models on pairs of neonate sharks, the AUC values ranged from 0.50 for the model focusing on the *pec* area, to 0.40 for the *XGBoost* model (Figure 3.7a, neonates). The low AUC values indicate that there is no model available that is effective enough to distinguish neonate sharks from each other. The inability of the model to distinguish immature life stages is consistent with the experience of researchers who were tasked with the ID of neonate sharks. After repetitive ID sessions and several hours of sorting photographs, researchers succeeded in matching the ID to the right individual, mainly referring to the *head* area. Similarly, researchers were challenged with the ID of juvenile sharks (Figure 3.7a, juveniles and neonates). Models for juveniles showed some AUC improvement over neonates, ranging from 0.72-0.50, but were still considered to be poor determinants for differences among sharks. Overall, no model could reliably distinguish immature sharks from each other.

Alternatively, models trained on all life stages and tested on adult sharks showed considerable promise in the identification of differences between adult individuals. The *head* model and the *XGBoost* model retained the strongest AUC values, 0.86 and 0.90, respectively

(Figure 3.7a, adults). The least accurate model was the *FDF* (0.56) model, followed by the *FDB* model (0.78) (Figure 3.7a, adults).

Training on Adults Only, Image-Based, and Shark-Based Methods

Because the model is not able to differentiate immature individuals, the training of the first version of the models could have been affected by the presence of images that cannot be distinguished by the model. The presence of neonates and juveniles in the Training datasets might have ‘confused’ the model in the training phase. For this reason, neonates and juveniles were removed from the Training dataset, and the second version of the models were trained on the adult sharks only. The precision increased from 9.4% of the previous image-based model to 45.4% when the model was trained on adults only (Table 3.1: adult only, image-based). However, the accuracy of the model decreased from 90.4% to 85.3% because of the smaller training dataset including only adult sharks. The best results overall were achieved when introducing the shark-based evaluation to the model trained on adult sharks, reaching 86% accuracy and nearly 100% precision (Table 3.1: adult only, shark-based). With this approach, the ROC curves for the *head* and *pec* models have an AUC value of 0.93 and 0.83 respectively, while the *XGBoost* model seems to have the best results and an AUC value of 0.94 (Figure 3.7b). Intuitively, because the training for this last set of models was done on adults only, the model can only be tested on adult sharks.

Results of the Temporal Analysis

Finally, with the same AI model design, the Time dataset was used to assess substantial changes in the morphology by using images of the same adult shark through time. Each time step (T1, T2, T3, and T4) was measured against baseline images of the same shark, which were taken after all the photographs for the Time dataset (See Appendix A, Table A.1). The analysis was limited to adult sharks, as the previous results have shown the AI model could not reliably distinguish juveniles and neonates from each other. The spot patterns of adult epaulette sharks are thought to be permanent, but the long-term stability of patterns was never tested for adults, juveniles, and neonates. Results from the deep learning model confirm that the pattern morphology of adult sharks remained stable through the duration of the study (approximately 21 months). The same model could be used with longer time intervals between photographs. Although historical database of photographs could be insightful of longer-term morphological changes, this study suggests that, once reached adult maturity, patterns can stabilise and remain the same through the lifetime of epaulette sharks. The effectiveness of the applied deep learning

model is most evident with larger, historic database of photographs. In the case of long-term database, this model could be applied across thousands of individuals and recognise small changes in morphology through time. Such changes would be represented by a small decrease in similarity score, indicating that the animal seems to be the same individual, but something has changed in its morphology. The best-performing *head*, *pec* and *XGBoost* models were trained on adults only (Figure 3.8a) and on all sharks (Figure 3.8b). Each model returned similarity indexes above 0.5, but as expected, the models trained on adults only had better performance. Similarity values above 0.5 suggest that spot patterns do not change significantly. In terms of the performance of the models, when trained on all age groups (Figure 3.8b), *pec* is a better indicator of age’s effect on skin pattern. When models are trained with adult sharks only (Figure 3.8a), the *head* area seems to be a better indicator of differences through time. *XGBoost* was determined to be a reasonable choice in both cases.

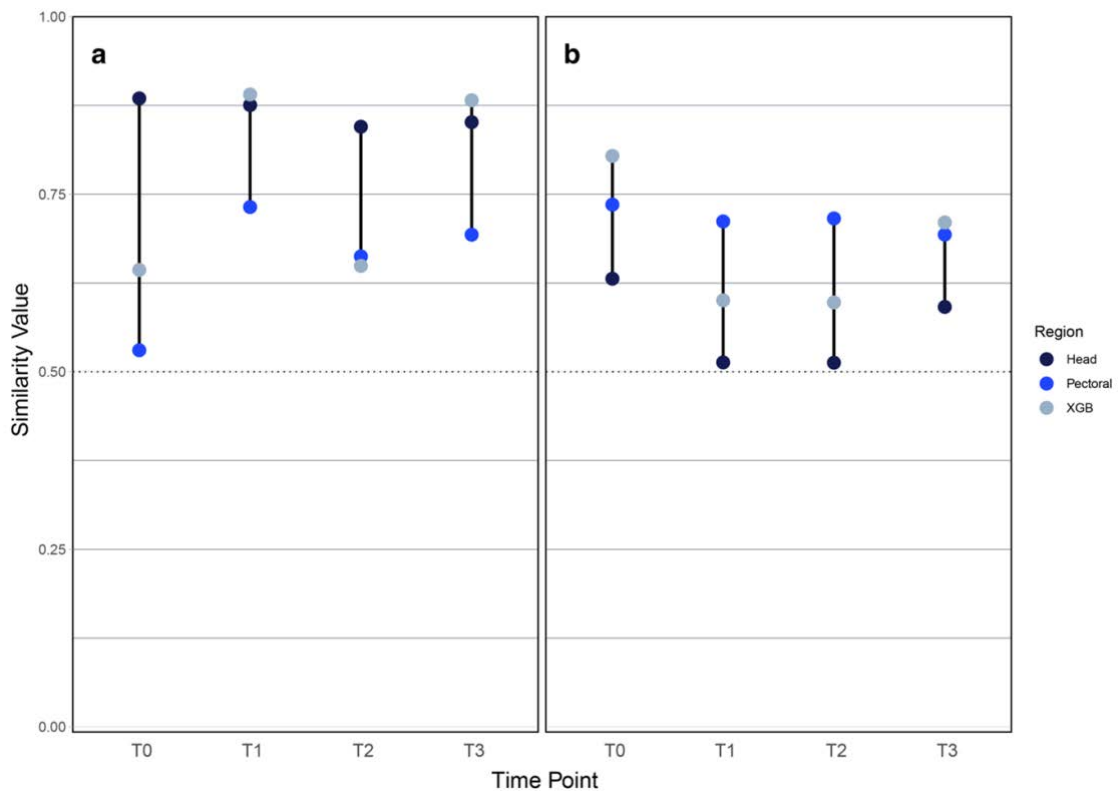


Figure 3.8. (a) Similarity indices of the image pairs of the same sharks through time (T0 to T3) for adult-only, and (b) all life stages. A 50% similarity threshold is represented by a dotted line, where any AI output above this line means that image pairs correctly belong to the same shark.

Discussion

This study is the first to develop a photo ID protocol for epaulette sharks, contributing to the small list of species that can be effectively studied using photo ID. The protocol outlined

in this study includes a series of innovative solutions in the field of applied AI to photo ID. First, the deep learning model was effectively trained on a small dataset of labelled images, the model was developed with a regular computer with standard computational power, and the model used the similarity network approach to ID individual sharks, overcoming the typical close-population assumption of many photo ID models. Furthermore, the protocol was used to assess the reliability of patterns for immature life stages, and to detect changes in morphology through time. Because of the small number of images required, the proposed protocol provides a cost- and time-effective tool to test the applicability of photo ID on new species of elasmobranchs and perhaps teleost fishes and cetaceans. The same method can be useful for species like manta rays and whale sharks that have been surveyed with photo ID for decades (Harty et al., 2022; McKinney et al., 2017). For instance, this new approach provides a tool to validate whether skin patterns change over long-term studies by using the similarity network approach on large databases of photographs collected through time. Long-term stability of ID patterns can also be confirmed by pairing photographs with genetic samples of individuals (Gubili et al., 2009), but genetic methods are not always logistically or financially possible. However, the reliable identification of individuals with morphology and the long-term stability of such morphological traits are fundamental requirements for population studies using photo ID as a non-invasive capture mark recapture method. The protocol proposed in this study represents an applicable set of solutions to photo ID and AI applications to animal identification and population studies.

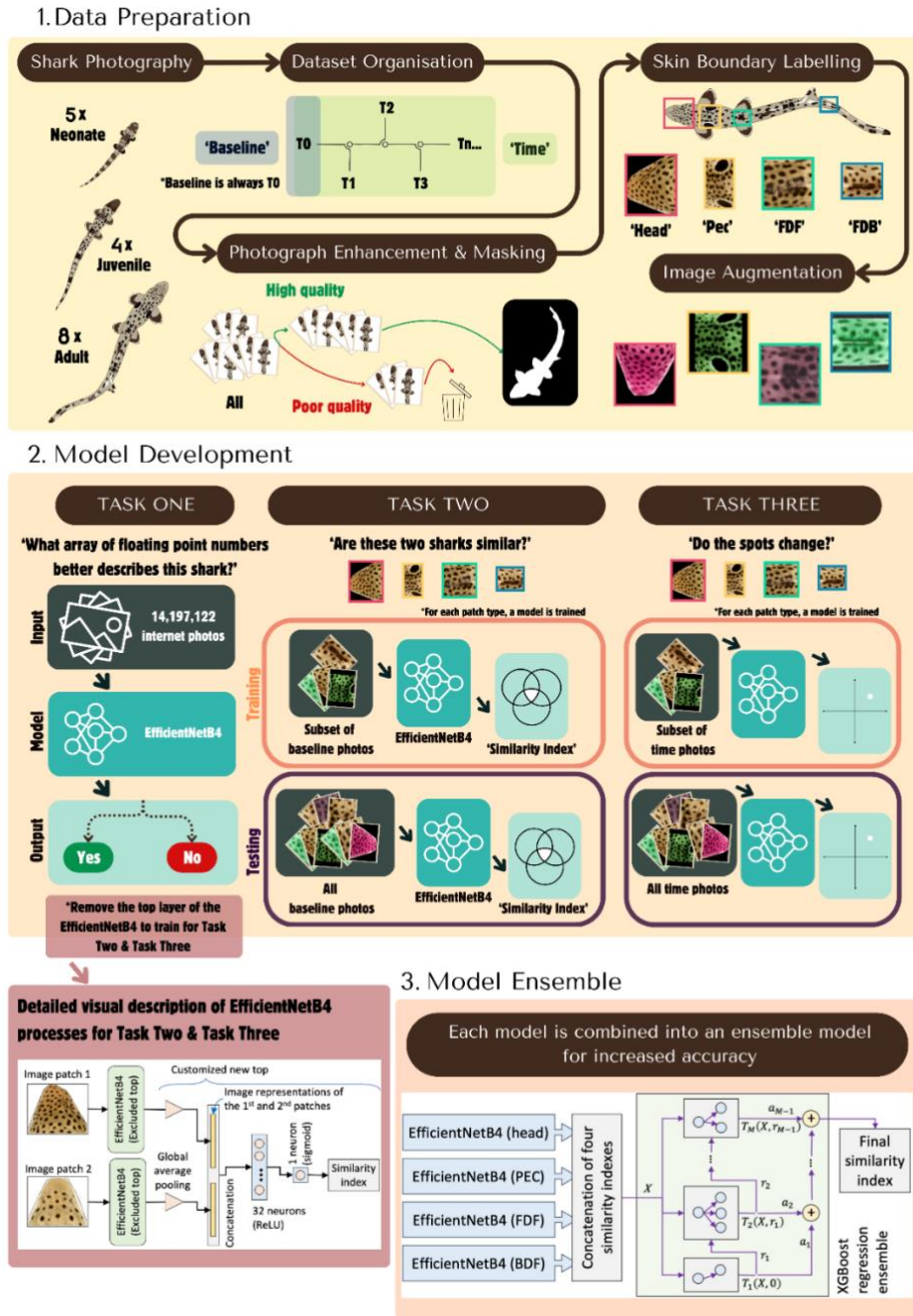


Figure 3.9. Flowchart that shows the protocol outlined in the study. From data preparation to the development and training of the models, with detailed sections on the *EfficientNetB4* and *XGBoost ensemble* models.

A Step-by-Step Approach to Elasmobranch Photo ID

This study presents a practical example to test photo identification for a species that hasn't been previously studied using this approach. This includes identifying features for ID, photographing sharks, labelling, and editing photographs, and setting up a semi-automated process to organise photographs by ID with high accuracy. Although current photo ID projects

use similar methods (Gómez-Vargas et al., 2023; Pierce, 2019; Schneider et al., 2022), only a few studies outline the step-by-step process of testing the method, compiling a database, and automating the ID process (Schneider et al., 2022). Additionally, only a few studies offer solutions that can be easily customised for different species and hypothesis (Gómez-Vargas et al., 2023). Here we present a flowchart (Figure 3.9) to visualise and exemplify the protocol, from photographs, to model development, to final similarity index classifying animals by ID.

Data Preparation: Collecting, Editing, and Organising Photographs.

1. To test if the species has unique identification features, photographs need to be collected first for a few individuals (Pierce, 2019). If available, photos of different life stages should be collected to test reliability of patterns across ontogeny. These photographs can be classified in the Baseline folder.
2. Existing photographs collected through time can be classified in the Time folder. This is used to test the stability of features through time (Marshall & Pierce, 2012; McCoy et al., 2018; McKinney et al., 2017). In this study, photographs of the captive animals were collected as standard research protocol. For wild populations the collection of photographs through time requires high residency and/ or known aggregation sites.
3. Photographs are prepared as described in the Methods section and visualised in the flowchart (Figure 9). Importantly, the skin boundary labelling step not only allows for easier and faster model development, but it can identify which sections of the body are most indicative for ID and which sections may be most affected by changes through time.

Model Development: Task one, Task two, and Task three

1. Due to the highly time-consuming task of sorting photographs, it is beneficial to develop an automated or semi-automated model for individual recognition, even with a small dataset of photographs and only a few individuals. For instance, once the backbone of the model is in place, improvements and new individuals can always be added, and the model can be easily re-trained when needed. This can save time, allow for re-allocation of resources, and increase productivity for researchers.
2. The task one presented in the flowchart shows the transfer learning approach, a fundamental step used to pre-train the model when only a small dataset of photographs is available. Task two represents the standard animal identification task, which is performed by using the similarity network approach and the *XGBoost ensemble* model

(see Methods sections for details on model development). Finally, the same approach is used for task three: “Do the spots change?”

As each species is unique, the protocol suggested in this study can be customised and further improved in its applications. The controlled environment of this study represents both a strength and a limitation. The known ID of individuals, the existing record of labelled photographs, and the quality of the photographs was an important factor to be able to develop the model. On the other hand, although the model was trained to work with a small dataset and with different options of photograph quality, the real-life success of this method needs to be tested by applying the protocol to a wild population of epaulette sharks.

Advancements in AI Applications for Photo ID

The application of AI through customised deep learning models is becoming increasingly important to process and analyse long-term datasets (Meekan et al., 2020; Schneider et al., 2022; Winton et al., 2023). Establishing a semi-automated classifier early into the stages of a project is most beneficial in saving time when later the large volume of photos makes visual identification more challenging. However, new photo ID projects face certain challenges in developing AI models to ID individuals from limited photographs. First, new projects, especially when working with wild populations, may have a limited number of photographs of the same animal for training the AI on ID features (Christin et al., 2019; Miele et al., 2021). Second, projects might not have access to powerful image classifiers or HPC technology, limiting the applications of AI technology. And finally, when new individuals are added to the population, models need to be fully re-trained (Schneider et al., 2022). The accuracy of the DNN model developed in this study was a stepwise process largely attributed to the efficacy of the similarity network method and XGBoost ensemble model, resulting in more than 85% accuracy and 100% precision when identifying individuals from a small database. With the similarity network approach, the model can be presented with new individuals, evaluating them as different from any shark it has previously learned, thereby identifying them as new sharks. Additionally, the comparison of similarity scores allows for the evaluation of morphological changes in a target species by comparing images from different time periods and analysing the resulting similarity scores. The similarity network approach is a crucial technological advancement for addressing the key requirement for long-term photo identification: patterns and morphology must remain stable over time for consistent re-identification of individuals (Ferreira et al., 2020; Marshall & Pierce, 2012). In addition to the similarity method, another innovative step of this project was the

XGBoost ensemble model, particularly for new photo ID projects that may lack access to high-performance computing (HPC). As a result, high-precision AI models can be accessible to smaller photo ID projects and feasible for smaller NGOs and community-lead initiatives, that often have limited resources (Chin & Pecl, 2018). The implementation of the XGBoost ensemble model, which integrates outputs from four independently trained models, each focusing on different cropped sections of the shark's body, significantly optimised the ability of the DNN to distinguish adult individuals with high accuracy. The XGBoost model can assess model performance on each body section and assembling the independent models by ranking their respective performances. For instance, models with better performance are indicative of which body sections are most distinct across individuals, a valuable insight for cases where photographs capture only parts of an animal's body (Andreotti et al., 2018; Armstrong et al., 2019; Pierce, 2019).

Using AI to Identify Morphology Changes Through Time

The best-performing version of the DNN model confirmed that adult epaulette sharks maintained their patterns throughout the study, as the model performance remained high when identifying individuals from consecutive photograph in time. Changes in patterns through time would have appeared as a substantial change in performance score for this task. Although the efficiency of this process is yet to be validated with wild populations, it is important to consider the effect that temporal changes in morphology could have in the training of an AI model. If changes in the ID patterns do occur the ease of re-identification through time might be affected (Pierce, 2019). Additionally, if morphology does change in time, and training datasets are assembled with temporally consecutive photographs, the training of the model might be affected, and the model might not be able to accurately learn the similarities between individuals. For this reason, the similarity network approach proposed in this study can be applied by comparing photographs of the same individual through time and testing the performance of the model as a proxy of morphological stability. Overall, training on multiple photographs taken at the same time is a better approach compared to training on temporally consecutive photographs. When possible, photographs taken at the same time offer multiple examples of different lighting and angles from which models can reliably learn from (Christin et al., 2019). While this is the best approach to learning features, there are other methods, such as one-shot learning, to train a model with only one photograph (Schneider et al., 2022). For adult individuals, patterns need to be distinguishable and persistent through time (Marshall & Pierce, 2012), and these assumptions need to be tested for juveniles and immature individuals, which

might have significantly different morphologies compared to adults (Bellodi et al., 2023; Fu et al., 2016).

When the identification protocol used on adult epaulette sharks was applied to neonates and juveniles, ID was much more challenging. Visually distinguishing epaulette sharks at early life stages was challenging, and therefore poor model performance across early ontogeny was expected (Christin et al., 2019). Consequently, photographs of juveniles and neonates had to be excluded from the training dataset. The exclusion of immature life stages from the training dataset raises the question of whether photo ID is suitable for other species that experience significant ontogenetic morphological changes during development (Ferreira et al., 2020; Marshall & Pierce, 2012). For instance, the Indo-Pacific leopard shark (*Stegostoma tigrinum*), also called zebra sharks due to their skin patterns during the juvenile stage (Dahl et al., 2019), may be a good candidate to test the reliability of patterns for individual juveniles. As photo ID can only be performed with reliable patterns, and species might experience significant changes from their juvenile to the adult stage, it is important to consider removing those stages to prevent poor model performance. The similarity approach could be used to compare photographs of the same juvenile taken over short intervals (e.g., every 2–3 days). While a juvenile may become unrecognizable after two weeks, images captured within a few days may still retain identifiable similarities. This would help determine which body areas are most likely to maintain consistent features over time, how the changes in these body areas occur, and how they could be potentially interpreted as a proxy of aging in juvenile sharks. However, achieving this requires a pilot study with consecutive photographs of juveniles of known identity.

Conclusion

Initially, the assessment of baseline conditions necessary for photo ID was conducted, followed by the development of an AI model to semi-automate the photograph sorting process. The resulting deep learning model represents a seamless integration of established methodologies and innovative solutions, effectively identifying adult sharks and assessing the stability of their patterns over time. This study introduces a novel approach to developing a deep learning by successfully navigating the challenges posed by small training image datasets and the absence of high-performance computing capabilities. The solutions outlined in this study are replicable, offering a framework that can be applied to test the temporal stability of identification features for other species. This is relevant to studies currently using photo ID or exploring its applicability to new species. The next phase is to test this method broader

application across wild populations of elasmobranch species. Tests should include both projects with long-term, large databases and projects focusing on species that change morphology over time.

Chapter 4: Testing the Effectiveness of Underwater Remotely Operated Vehicles for Surveying Elasmobranchs.

Abstract

Surveys of elasmobranchs at night, in low visibility, and in deep water present significant challenges for traditional survey methods. Underwater visual surveys (UVS) are limited to shallow water and daylight hours, and while Baited Underwater Video Stations (BRUVS) offer an alternative for non-diveable depths, the methods are different. BRUVS, as stationary systems, may detect different species and abundances than the mobile transect method of UVS. Remotely Operated Vehicles (ROVs) can be a solution for surveying elasmobranchs at temporal and spatial scales that have been largely unachievable. To evaluate the effectiveness of this technology, this study employs a small, observation-class ROVs (modified BlueROV2), which are accessible and comparatively affordable to a wide range of research applications. This study demonstrates how ROV technology can be optimized for elasmobranch surveys via application of various light and camera customizations and an iterative survey approach. The ROV performance is compared to three methods; UVS, BRUVS, and catch data, investigating overall counts and species richness detected across three locations along the Great Barrier Reef and the Coral Sea. ROVs proved comparable to UVS in terms of relative abundance and species composition. When compared to BRUVS, ROVs detected less species and lower abundance, except for some locations where ROVs had higher counts. Compared to catches, ROVs detected more elasmobranchs overall and a different species composition. This study suggests that elasmobranch research could benefit from ROV technology as a complementary tool for mobile transect surveys in conditions where diving not possible.

Introduction

Coral reef systems are characterized by high species diversity and complex habitats. Much of our current understanding of coral reef ecosystems is derived from data collected through underwater visual surveys (UVS) and remote video systems, such as Baited Remote Underwater Video Stations (BRUVS). However, these methods have some limitations. UVS are confined to depths shallower than 30 meters for standard SCUBA operations and are typically limited to daylight operations. This method is less suitable for studying elasmobranchs due to the sparse and avoidant nature of many of these species, which decreases the likelihood of detection by scuba divers (Jessop et al., 2022; Ward-Paige, 2010). In contrast, the attractive effect of the bait makes BRUVS effective at detecting elasmobranchs, even at depths beyond standard scuba diving operations (Muñoz & Burton, 2019; Osgood et al., 2019; Pimentel et al.,

2020). However, BRUVS are stationary, limiting observations of species diversity and abundance estimates to the field of view of the camera/s (Kilfoil et al., 2017). Additionally, bait can attract species that occur in different habitats to where the BRUV was placed, limiting capability of BRUVs to determine species-habitat associations and may bias data towards detecting larger, bolder species (Colton & Swearer, 2010; Harvey et al., 2007). Standard BRUV methods also require relatively flat or sloping topography to be reliably anchored to the substrate for the duration of the survey (Galbraith et al., 2022).

Records of reef-associated elasmobranchs in deep waters, beyond standard scientific diving depths, suggest that UVS may not provide reliable estimates of relative abundance or occurrence for species that migrate into mesophotic coral ecosystems (Asher et al., 2017; Galbraith et al., 2022; Papastamatiou et al., 2015). For example, while species, such as epaulette sharks (*Hemiscyllium ocellatum*) and wobbegong sharks (*Orectolobus spp.*), tend to remain in relatively shallow waters, other reef-associated species, including white tip reef sharks (*Triaenodon obesus*), silvertip sharks (*Carcharhinus albimarginatus*), grey reef sharks (*Carcharhinus albimarginatus*; Espinoza et al., 2015), bull sharks (*Carcharhinus leucas*), tiger sharks (*Galeocerdo cuvier*), whale sharks (*Rhincodon typus*), and hammerhead sharks (*Sphyrna spp.*; Royer et al., 2023), frequently migrate below 30 meters (Andrzejczek et al., 2022; Espinoza et al., 2014). Although some species only occasionally migrate deeper than 30 meters (Fitzpatrick et al., 2011), other species often spend significant time in deeper waters (Tickler et al., 2017). Although available data on deep water occurrence are primarily derived from telemetry studies, remote video methods such as BRUVS also can operate in deeper waters (McLean et al., 2015b; Pearce et al., 2023; Pinte et al., 2020). However, the use of a complementary mobile transect method could improve species detection (Galbraith et al., 2022). As a mobile underwater video system, ROVs offer a survey method that combines the advantages of UVS and BRUVS, allowing observations in deep waters where many elasmobranch species remain unobserved. Working-class ROVs are large, powerful multi-tasking machines, with long negatively buoyant tethers and operated by a team of trained personnel. This type of ROVs is commonly used by oil and gas operations for monitoring and maintaining underwater structures (Neha et al., 2024). Consequently, these ROVs collect substantial data on fish occurrence and abundance around these submerged structures to depths exceeding 4600 meters. (Macreadie et al., 2018; McLean et al., 2017; Tohill et al., 2024). Observation-class ROVs are smaller, commercially available, with shorter and more buoyant tethers, usually operated via user-friendly piloting software by two people (Andaloro et al., 2013; Baronio, 2012; Sward et al., 2019). Although this technology is evolving fast, observation-class ROVs can reach depths of

about 400 meters, depending on the model (Sward et al., 2019). ROVs sampling design can be aligned with UVS methods by replicating transect sampling designs used by divers, e.g. belt transects and timed swims (Wetz et al., 2020). Therefore, there is potential for data sets from both methods to be integrated into the one analysis, thereby including data from depths below 30 meters (Quattrini et al., 2017; Radziejewska et al., 2024).

Only a handful of studies have focused on the nocturnal ecology of elasmobranchs, yet many species appear to be either nocturnal or crepuscular (Carroll & Harvey-Carroll, 2023; Hammerschlag et al., 2017). Capture studies with fishing gear have documented higher catch rates at night (Chapman et al., 2007; Orbesen et al., 2017) and tracking studies have shown increased activity and changes in depth use at night for many elasmobranchs. For instance, vertical migration and increased activity has been associated with foraging at night (Andrews et al., 2009; Barnett et al., 2010; Fitzpatrick et al., 2011). Nocturnal non-catch surveys at night are less common, with most nocturnal studies using BRUVS to investigate occurrence, and species communities' composition (Harvey et al., 2012; Holzman & Genin, 2003; Torres et al., 2020). However, the white lights used for nocturnal BRUVS can affect fish occurrence and behaviour, potentially influencing bait attraction, competitive interactions, and predation (Fitzpatrick et al., 2013). Different-colour lights have been tested, including blue, red, and infrared lights, with red and infrared lights showing higher abundances of fish comparatively (Carazo et al., 2013; Lucena et al., 2021). However, red light may decrease visibility for ROV operations and decrease the ability of identify species (Birt et al., 2019). For all light colours, backscatter in the video becomes obstructive to the viewing range. Meanwhile, underwater transect surveys performed using SCUBA, such as UVS, are rarely used at night due to safety and logistical challenges (Bassett & Montgomery, 2011).

ROVs have been used to assess fish abundance, diversity, and behaviour in various environments, including natural habitats and artificial structures (Butcher et al., 2021; Macreadie et al., 2018; Wetz et al., 2020). Additionally, some studies have compared the performance of this method with other survey techniques (Andaloro et al., 2013; McLean et al., 2015a). Since the first use of ROVs for underwater surveys in 1996, this technology has greatly advanced, especially in terms of camera and light quality, components and ROV manoeuvrability (Sward et al., 2019). Imagery collected from industrial and professional use ROVs demonstrate high potential in collecting valuable species sightings through hours of recorded footage and images (Macreadie et al., 2018). For instance, industrial ROVs deployed from seafloor drilling well-head infrastructures have documented sightings of elasmobranch species that are

endangered or previously believed to be absent from the area (McLean et al., 2018; Moore & Gates, 2015). Other ROV applications show the benefit of observing deep-water organisms without the need for extractive surveys, such as deep-water trawling (Wiebe & Benfield, 2003). These applications highlight the potential of ROVs to extend observational effort beyond the limits of scuba divers and overcome some of the limitations and biases associated with UVS and BRUVs (Butcher et al., 2021). However, there are also documented challenges in ROV survey. Comparisons of ROVs and UVS to date have shown that ROVs can be less effective than UVS at documenting fish communities due to the restricted field of view, especially for cryptic species (Andaloro et al., 2013; Wetz et al., 2020). Additionally, ROVs may elicit avoidance behaviour in certain species, biasing counts, particularly when sightings are recorded by a single forward-facing camera (Lorance & Trenkel, 2006; Trenkel et al., 2004). Although limited, comparisons between ROVs and catch surveys are available, focusing on teleost fish and mostly reporting variable results depending on species and fishing gear (Sward et al., 2019). Some studies found ROVs to be as effective as fishing surveys in describing fish assemblages (Mapula et al., 2016), while a study comparing ROVs to trawls found that ROVs have generally higher CPUE (Norcross & Mueter, 1999). Comparative studies have provided insights into the use of this technology for teleost fish surveys, but elasmobranch specific surveys present their own unique challenges.

In this chapter, an iterative trial-and-error process is used to evaluate the performance of ROVs for surveying elasmobranchs. The aims of this study were to evaluate the performance of commercially available observation-class ROVs for elasmobranch surveys across depth gradients from 3 to 90 meters, across continuous day and night periods, and within various marine ecosystems. Experiments were performed in three different locations, focusing on overall detection of elasmobranchs and species richness obtained with ROVs to determine the performance of ROV technology in comparison to three widely used survey methods; UVS, BRUVS, and catch data.

Methods

Overall Survey Design

The surveys performed in this study were approved under the Marine Parks Permit G21/38062.1, and G22/46908.1, and complied with James Cook University Animal Ethics Committee (protocols A2899 and A2846). Surveys were Conducted at two locations within the Great Barrier Reef Marine Park and remote locations in the Coral Sea Marine Park (Table 4.1).

Table 4.1. Breakdown of each experiment details: location and habitat type, environmental variables relevant to the surveys, and specific details on the survey technique, time, and lights treatment.

	Location	Dates	Habitat type	Depth range	Visibility range	Methods comparison	Survey Length	Number of Sites	ROV Transect Type	Length	Survey Times	Light Treatments	ROV camera set-up
Experiment 1A	Moore Reef, Cairns (QLD)	May 2023	crescentic reef slope, crest, and flat.	4m – 15m	5m – 20m	ROV vs UVS (divers operated videos: DOVs)	9 days	4	Belt Transect survey	100m	Dawn, Day, Dusk	Red, White	4 Go Pros mounted on top (360 view) + pilot camera
Experiment 1B	Moore Reef, Cairns (QLD)	May 2023	crescentic reef slope, crest, and flat.	4m – 15m	2m– 6m	ROV day vs ROV night	9 days	4	Belt Transect survey	100	Nocturnal	Red, White	4 Go Pros mounted on top (360 view) + pilot camera
Experiment 2	Northwest Island (Capricorn Bunker Group, QLD)	Feb 2024	Sand lagoon around Northwest island	4m – 20m	2m – 15m	ROV vs catches (CPUE)	5 days	1	Drift Timed Swim	30 minutes (300m)	Dawn, Day, Dusk, Night (no catches at night)	White	4 Go Pros mounted on top (360 view) + pilot camera
Experiment 3	Coral Sea Marine Park (QLD)	4 voyages between February 2021 and February 2022	Variable reef types and reef aspects: inner and outer reefs, lagoon, crest, flat, slope, deep sandy habitats	2m -98m	5m – 30m	ROV vs BRUVS	81 days total	ROVs: 28, BRUVS: 18	Depth Bin Timed Swim	ROVs: 30 m (3 min), BRUVS: 1 hr.	Day	White	Pilot camera only

Blue ROV2 Configuration and Design

Blue ROV2 high-performance underwater vehicles from BlueRobotics were used in this study. ROVs typically consist of a main body (the ROV itself), a tether (the cable connecting the ROV to the pilot), a surface interface, a controller, and a computer (unless the controller includes an integrated screen and piloting software). For this study, the tether was 200 meters long and was either wrapped in a spool or organized in a larger tub, which facilitates quick deployment and retrieval of the ROV. Each study site featured a unique gear setup tailored to its specific requirements. Since tether drag affects maneuverability during operation, it is essential to determine the optimal method for efficiently managing the tether, especially when the ROV is deployed, piloted, and retrieved from a tender. The basic design of the Blue ROV2 is similar to work-class ROVs (Figure 4.1), featuring an open frame that houses the electronics and battery enclosures, an 8-thruster vector configuration, a high-definition onboard camera for piloting, two high-powered lumen Subsea lights, buoyancy foam, and ballast weights. For the Moore Reef and Northwest surveys, a platform was installed atop the ROV frame, featuring four GoPro cameras arranged to capture 360-degree video (Figure 4.2). Furthermore, four torches were fastened to the frame's base using custom 3D-printed holders secured with zip ties (Figure 4.1). This modification proved crucial for conducting night-time surveys, as the standard lights on the BlueROV2 are forward-facing and could not be fitted with

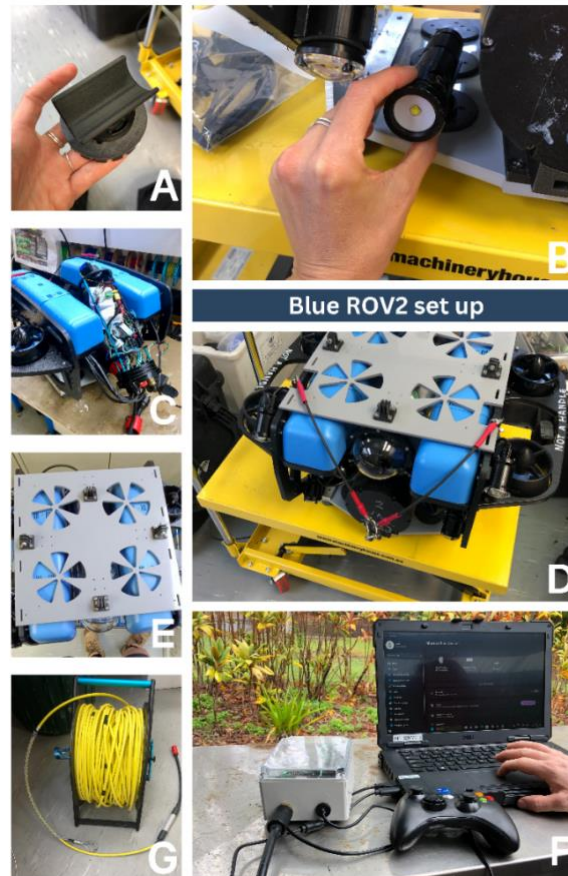


Figure 4.1. Blue ROV2 set up. Panels A and B show the 3D printed torches mounts and the location on the bottom frame of the ROV; panel C shows the open canister with the electronics housing; panel D and E show the top mounted frame with 4 attachments for the Go Pros; panel F shows the surface interface, controller, and laptop; panel G shows the tether spool.

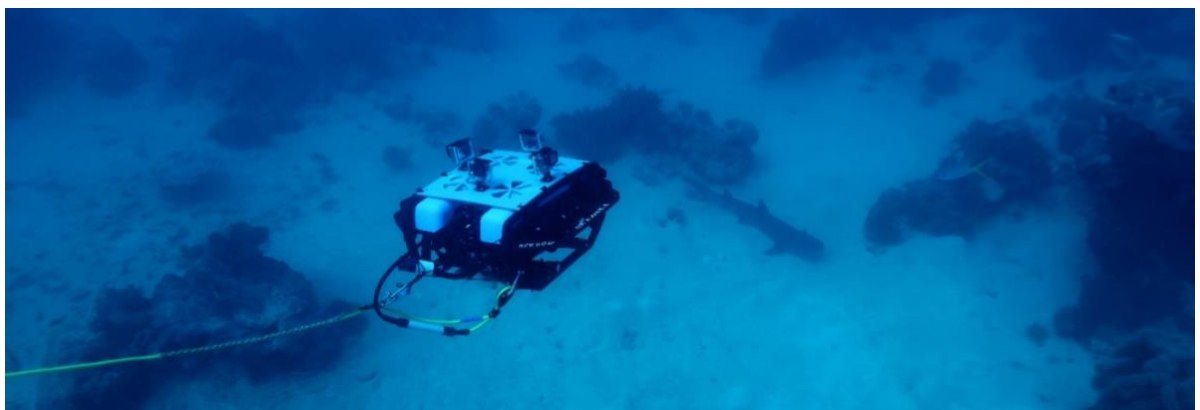


Figure 4.2. 360-degree view Go Pro set up on Blue ROV2 during transect survey.

Video Analysis

Videos from the four GoPro cameras of the ROV and the stereo-pair of the DOV were synchronised and composited into a single video with four/two frames using VidComp (SeaGIS, 2024b) (Figure 4.3). This step was essential to create a comprehensive 360-degree video, thereby reducing the likelihood of double-counting individuals circling the ROV. The composited videos were annotated using EventMeasure software (SeaGIS, 2024a), where sightings were recorded as point count measurements, meaning each instance of an individual observed in the composited frame were treated as a new count. Videos were processed over the full length and elasmobranchs observed within the transect were recorded and identified to the lowest possible taxonomic level. Visibility (variable between 5 – 20 meters during the day and 2 – 6 meters at night) was a limiting factor for recording sightings and assessing species correctly.

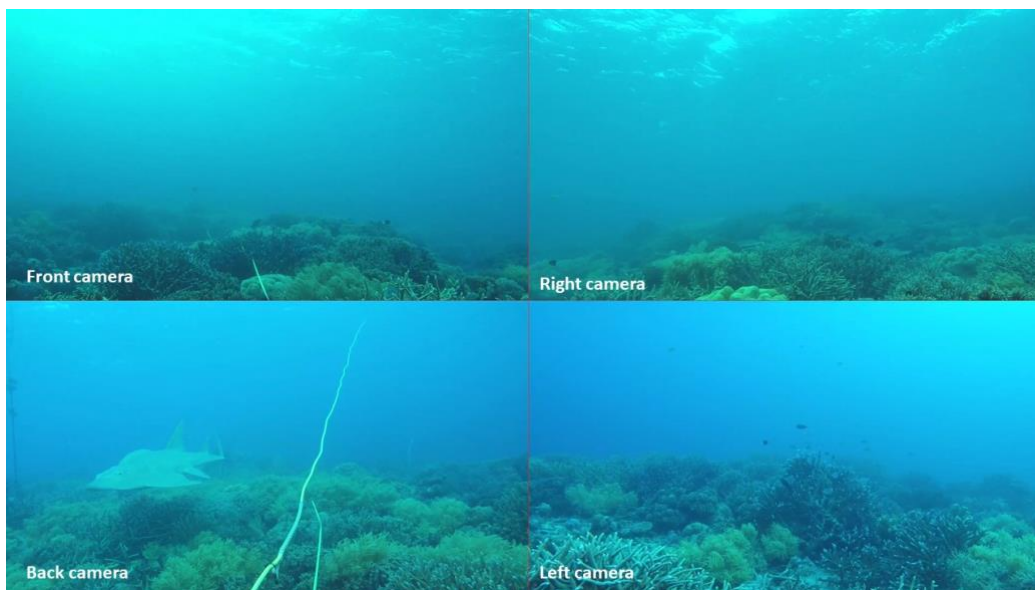


Figure 4.3. VidComp composite video for ROV annotation. All videos belong to the same transect and treatment. Each of the four sections show the view from one of the cameras. The back camera shows a bowmouth guitarfish approaching the ROV from behind.

Experiment 1A Moore Reef: ROV and UVS Comparison

Moore Reef is a crescentic reef located approximately 35 nautical miles west of Cairns (QLD, Australia). All fieldwork operations were carried out on the Reef Magic pontoon, located in the northern section of the reef (Figure 4.4). Four temporary fixed transects were deployed at this site to compare survey techniques. Transect 1 was positioned on the shallow slope and

flat area north of the pontoon and transect 2 on the deeper reef slope and crest to the south/southwest, transect 3 on the shallow reef flat to the west, and transect 4 on the outer reef crest running from northeast to southwest (Figure 4.4: Map).

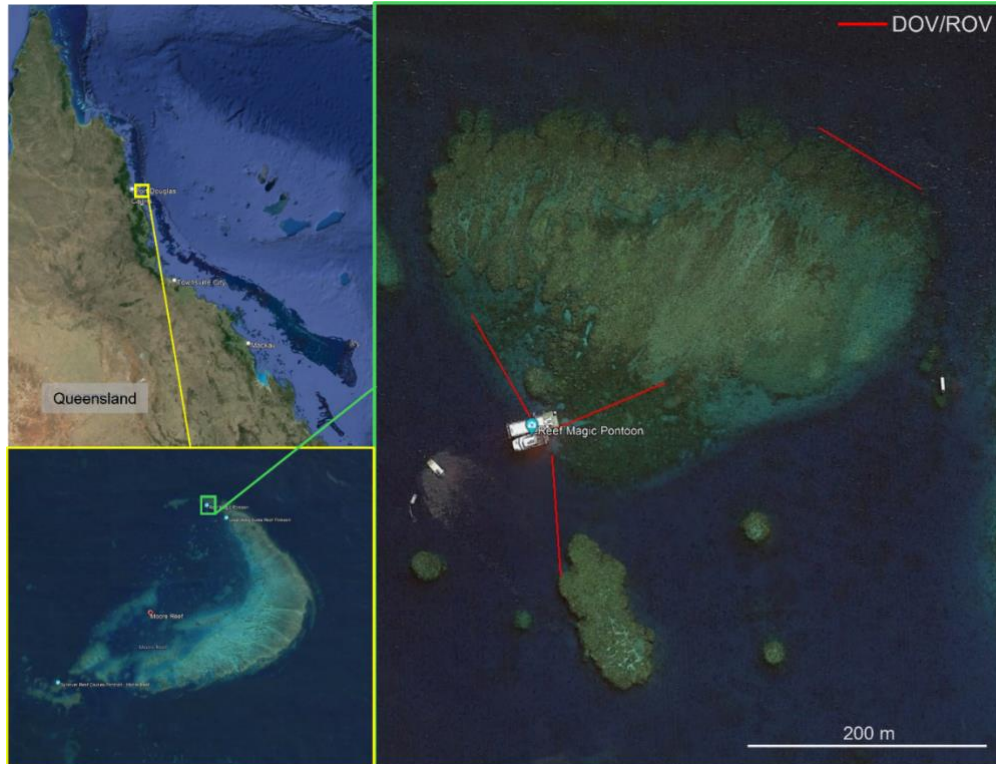


Figure 4.4. Moore Reef Experiment 1A and 1B locations. Red lines – approximate 100m locations of the ROV/DOV transects. Data sources: Google Earth Landsat Satellite Imagery.

Surveys at Moore Reef took place in May 2023 and the sampling design consisted of four transects across different reef gradients, namely the reef flat, crest, shallow slope, and deeper slope (Figure 4.4). One-hundred meter transects were pre-laid by divers at each reef site and remained at that location for four days. Transects 1 and 2 were initially deployed for the first four days, then moved to different locations (Transect 3 and 4) for the remainder of the study. The number of temporary fixed transects in the water at any time was constrained by the window of crepuscular light and time required to complete dawn and dusk surveys. Transects were performed at dawn, daylight (pm), dusk, and nighttime (nocturnal 1 and 2). For each transect, the ROV was piloted first along the transect using the ROV camera to guide the pilot, while the GoPro cameras recorded the surroundings. For dawn, daylight, and dusk surveys, divers performed the transect immediately following the ROV survey. While along the transect, divers carried a diver operated video (DOV) system, which consisted of an aluminum frame with

two forward facing camera housings mounted on a base bar at a slightly converging angle. The ROV transects were always performed first as ROV disturbance was assumed to be less than the disturbance from divers. For instance, although some studies have investigated the behavioral reaction of fish to ROVs (Laidig et al., 2013), it has been documented that divers can trigger mixed behavioral responses in some elasmobranch species (Baronio, 2012; Cubero-Pardo et al., 2011; Smith et al., 2010). Divers did not perform the transect after dusk, due to operational diving limitations.

Experiment 1B Moore Reef: Nocturnal ROV surveys

The ROVs light Experiment were then performed following daylight surveys. For dawn, dusk, and nocturnal surveys, the ROV was equipped with 4 torches facing in the direction of each of the Go Pro cameras (front, left, right, back). Each transect was surveyed once with red lights (red-light treatment) and once with white lights (white-light treatment). Based on previous studies (Carazo et al., 2013), red light should have less of an effect elasmobranchs behaviour than white light, so red-light transects were always performed first. This strategy was adopted to maximise the likelihood of encounters with the light treatment considered to be the most effective. To reduce external influence on the surveys, lights surrounding the pontoon were kept off during nocturnal operations. Nocturnal surveys were also tide-dependent with surveys performed as close as possible to slack tide. This minimised the effect of strong currents during the survey as constraining factor of ROV operation.

Experiment 2 North West Island: ROV, B-ROV, and Catch Data Comparison

Northwest Island is a carbonate reef island 40 nautical miles offshore from Gladstone (QLD, Australia). Northwest Island is the largest reef island of the Capricorn group, approximately 1850 meters in length and 850 meters in width (total of 119.8 hectares), oriented from east to west. Most of the substrate is sand, with sections of fringing reef surrounding the island (Batianoff et al., 2002). ROV surveys were conducted on the northern side of the island (Figure 4.5) during the day and night, over 5 days in February 2024, for a total of 48 transects and approximately 16 hours. This matched fishing effort in the same area only during daylight hours with 10 drumlines for a total of 460 hours of total fishing. Drumlines were set at the beginning of the trip, baited each morning, and bait was removed at sunset. Drumlines were checked every hour and catches were recorded, together with the drumline number, time of capture, bait type, and species.

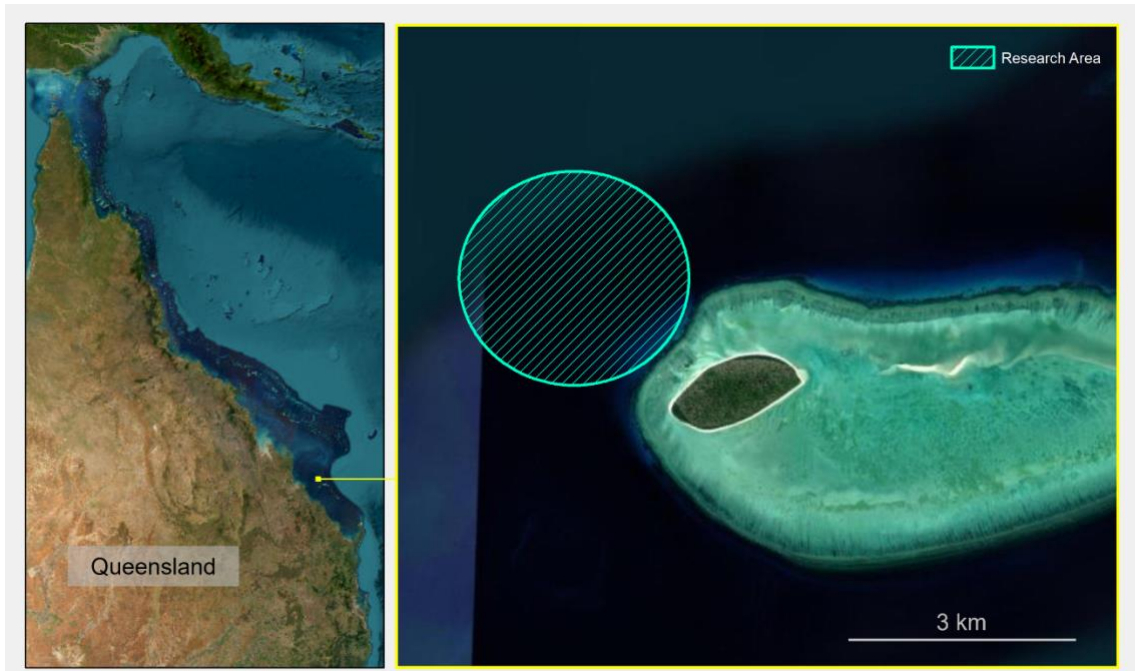


Figure 4.5. Northwest Island Experiment 2 location. Circled area represent location of drumline sets and ROV timed swims. Data sources: Google Earth Landsat Satellite Imagery.

The ROV was launched from a tender, piloted to the bottom, and positioned approximately 1-2 meters above the seafloor, depending on visibility and habitat composition. For example, sufficient distance from the seabed was maintained to minimize sediment disturbance by the thrusters. The ROV was then oriented mid-water, facing the direction of the current, with the pilot making only minor adjustments to depth and trim. At the surface, the tender driver followed the ROV's direction, with a tether operator allowing enough slack to reduce the effect, and the ROV operator piloting the ROV using the QGround Control program (Figure 4.6). This protocol allowed the ROV to be fully propelled by the current, conserving battery life and enabling longer surveys of 30 minutes. This time period was determined to be optimal as surveys were limited to battery life of the camera and ROV. For 2 out of 5 days of surveying the ROV was baited using whole mullet for each timed swim (B-ROV). The bait was attached to the ROV by using the front-extending gripper (Figure 4.7).

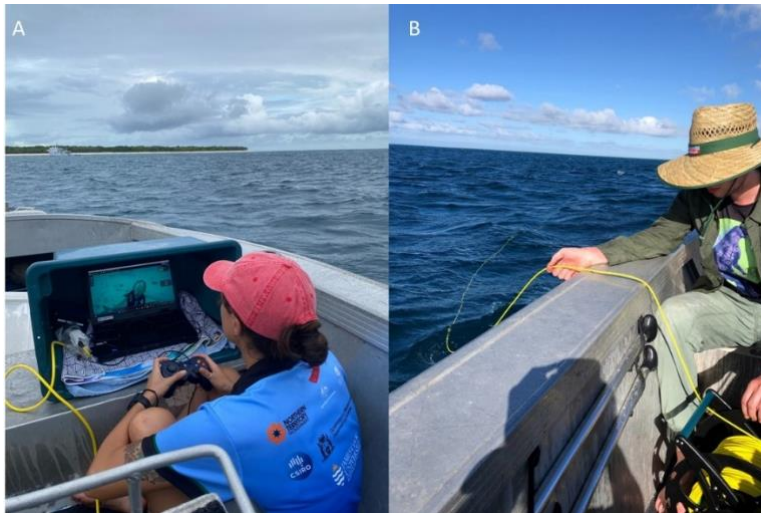


Figure 4.6. Boat set up for drift surveys. The ROV operator uses QGround Control to pilot the ROV along the survey (A), while a tether operator ensures that tether is reeled out properly allowing for sufficient slack to minimise drag (B).



Figure 4.7. View from the pilot camera of the ROV. Mechanical arm extended forward carrying a mullet bait.

Experiment 4 Coral Sea: BRUV and ROV Comparison

The Coral Sea Marine Park (CSMP) located off the north-east coast of Australia encompasses an area of 989,836 km² (Australian Marine Parks, 2024; Hoey et al., 2022) (Figure 4.8). Surveys took place during 4 voyages between February 2021 and February 2022 (Galbraith et al., 2022). Reefs and survey sites were chosen based on operational constraints and subject to current, sea and weather conditions. For lagoon surveys, sites were chosen using bathymetric charts to facilitate surveys at depths greater than 30 meters in these environments (Galbraith et al., 2022). ROV surveys were performed at 28 sites, across 15 reefs, for 81 days (Saumarez, Wreck, Kenn, Marion, Lihou, Flinders, Frederick, Holmes, Bougainville, Osprey, and Herald's Surprise Reefs, Chilcott, Diamond, and Willis Islets, and Herald Cays). BRUVs were dropped at 18 sites across 11 reefs (Saumarez, Wreck, Kenn, Marion, Lihou, Flinders (north and south), Holmes and Ashmore Reefs, Chilcott and Willis Islets, and Herald Cays) (Figure 4.8).

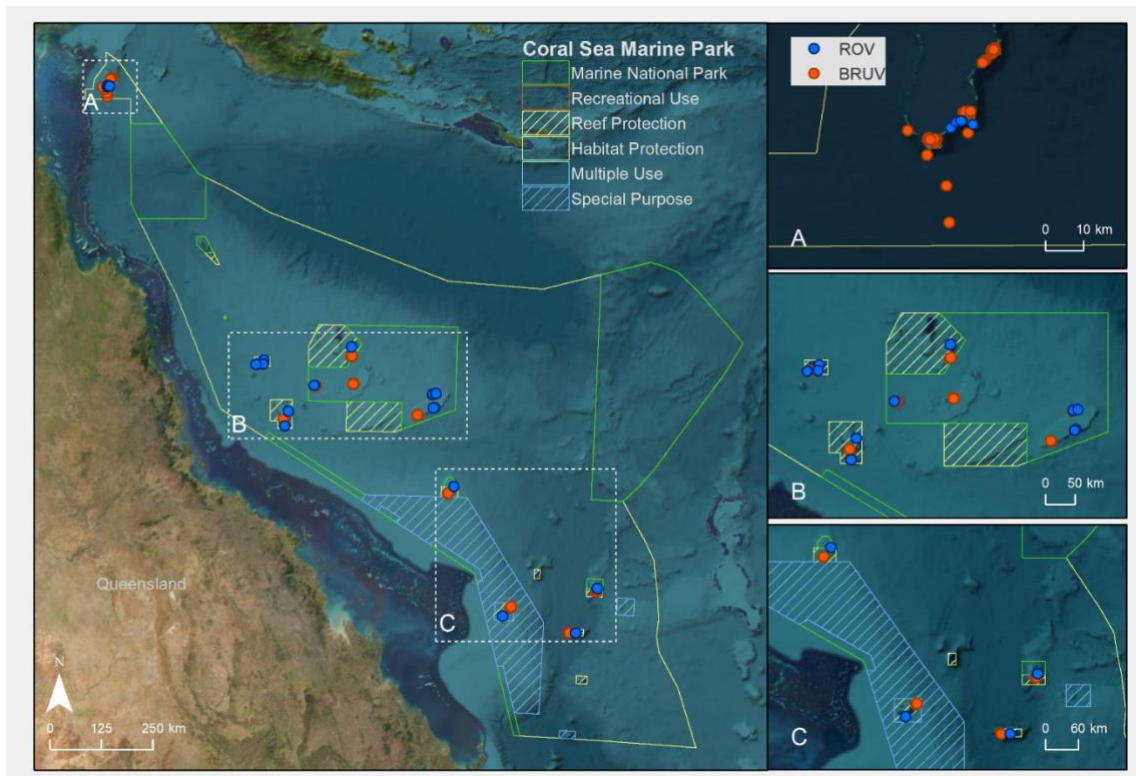


Figure 4.8. Map of the Experiment 3 Study Site within the Coral Sea marine park. Locations of the BRUV (orange sites) and ROV sites (blue). Data Sources: Australian Marine Parks Commonwealth Zoning and ESRI World Imagery Layers 2024.

ROV surveys were conducted from a tender deployed from the mother ship. For each survey, the ROV was piloted to the deepest point possible, according to the ROV maximum depth, topography, and sea conditions. Once settled at depth, the ROV hovered about 0.5 meters above the seafloor (or alongside vertical reef walls), conducting two timed transects at a consistent depth. Each transect covered 30m at a steady speed of 0.2 m/s. Transects within the same depth zone were spaced apart horizontally by 5-10 m. After completing the second transect, the ROV ascended by 5-10 m, conducting two more transects in the opposite direction at the new depth bin (i.e., just below the previous zone). This surveying pattern continued at 10 meters intervals until the final two transects were carried out in the uppermost 10 meters of the water column (Figure 4.9). BRUVs were assembled using a sturdy weighted frame, a waterproof housing for the camera, a bait arm, and a bait bag. Prior to deployment, each unit was provisioned with 1 kg of thawed pilchards (*Sardinops spp.*), crushed, and placed inside the bait bag. BRUV surveys were performed between 8:00 am and 4:00 pm each day from the main vessel. Each drop was spaced at least 500 meters apart and maintained for a minimum of one hour.

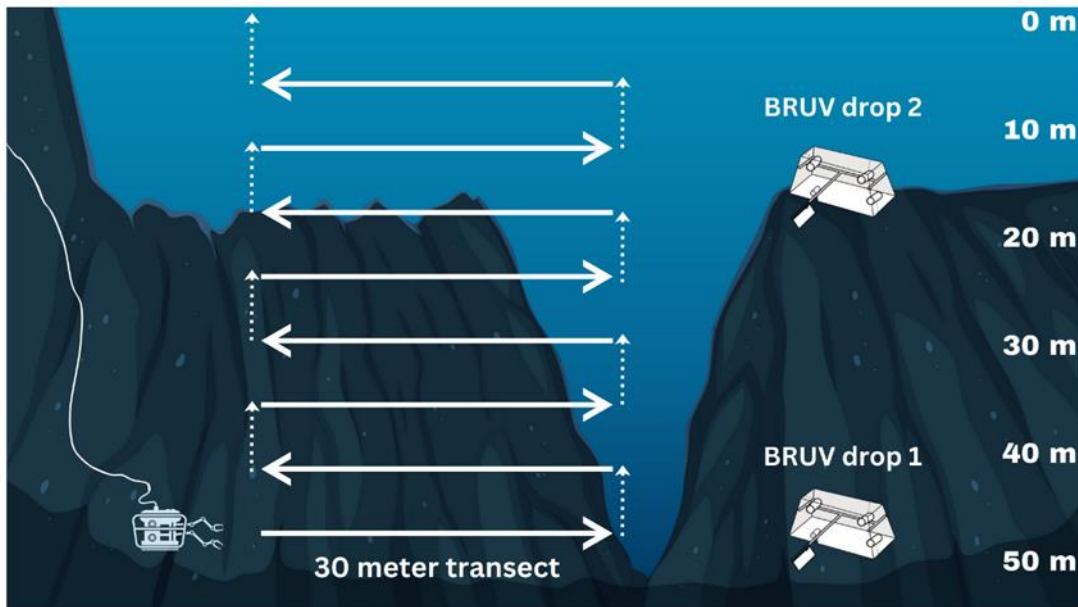


Figure 4.9. Figure adapted from Galbraith et al. (2022). ROV transect survey methodology alongside BRUV drops (for representation). Horizontal arrows represent the ROV transects and the vertical dashed arrows the 5-10 meters ascent before commencing a shallower transect on the opposite direction.

Data Analysis

Data analysis was conducted using R statistical software (version 4.3.1; R Core Development Team, 2023). The package '*tidyverse*' (version 1.3.1; Wickham et al., 2019) was used for data manipulation, visualisation, and analysis, including the '*dplyr*' package (version 1.1.4; Wickham et al., 2023). Descriptive statistics and model results were plotted using '*ggplot2*' package (version 3.5.1; Wickham, 2016). Generalised linear models (GLMs) were used for methods comparisons and fitted using the package '*glmmTMB*' package (version 1.1.9; Brooks et al., 2017). Model diagnostics were checked using the '*performance*' package (version 0.11.0; Lüdtke et al., 2021). For each of the four Experiments, elasmobranch species diversity and relative abundance between the different survey methods was examined as follows.

Moore Reef – Experiment 1A

During video annotation, each elasmobranch sighting, the start and end time of the transect, reef habitat and reef aspect, survey method, treatment (Red or white light), camera, and time of the day were recorded. Species composition was plotted for each method as the total count of individuals seen per species. To account for all observations, species counts were summed into a general elasmobranch count variable per transect. All transects were considered in the model, including transects with no elasmobranch sightings (zero counts).

To test if ROV transects detected a higher number of elasmobranchs, a GLM model with a Poisson distribution was fitted to the data. An aggregated species count was used as the dependent variable, and method (ROV or DOV) was modelled as independent variable [*glm (count ~ method, family = "Poisson")*]. Model diagnostics were checked for overdispersion, zero inflation, and visual diagnostics of residuals, such as homogeneity of variance, influential observations, and collinearity. A negative binomial family was also trialled but did not outperform a Poisson distribution given there was no overdispersion. The data was tested for zero inflation by comparing the ratio of observed to predicted zero, with a tolerance of 5%. This model was compared to a null model using a likelihood ratio test. Similarly, the probability of detecting a shark using either a DOV or ROV was tested using a GLM with a binomial distribution where shark sighting was treated as a binomial dependent variable with method as an independent variable [*glm (presence ~ method, family = "binomial")*].

Moore Reef – Experiment 1B

To test the effect of red and white light on the total count of elasmobranchs, a GLM with a Poisson distribution was fitted to the data with aggregated count of total individuals of all species as the dependent variable and treatment (red or white light) as the independent variable ([*glm (count ~ treatment, family = "Poisson")*]). Only nocturnal and crepuscular transects were considered in this analysis, and all UVS (DOV surveys) and transects with natural light were excluded. Model diagnostics and zero inflation were checked, and the model was compared to a null model, which determined that none of the factors were significant. To test whether the interaction between light and time of day was more significant than the light treatment alone, an interaction of time was added to the model variable ([*glm (count ~ treatment*Time_Day, family = "Poisson")*]).

Northwest – Experiment 2

Catch data was standardized by effort (catch per hook hours) to be able to compare with ROV and B-ROV sightings from videos (hours of survey). Species composition was plotted for each method as the total count of individuals seen per species. To analyse overall abundance, all observations were summed into a total species count variable. Data from catch surveys were compared to daylight ROV and B-ROV observations using a generalised linear model (GLM) with a negative binomial distribution. After checking model diagnostics and re-running the model with a Poisson distribution, the negative binomial distribution was determined to be better performing. All timed swims and drumlines sets were considered in the model to account for

time (effort), including zero count sets/transects. The total species count was used as the dependent variable and method (drumlines, ROV, and B-ROV) was modelled as independent variable. To account for differences in overall effort between methods, an offset variable was calculated as the soak time or survey time in hours for each drumline set and timed swim $[(glm.nb(count \sim method + offset(log(soak.time.hrs)))]$.

Coral Sea – Experiment 3

Both species occurrence and count data were compared between BRUVs, ROVs. The relative abundance of species was measured as the total count of individuals seen per species. Count data was in the form of single sightings/count values for the ROVs, and MaxN (the maximum number of individuals observed in a single video frame for each species in each video) values for BRUVS. To compare the BRUVS method with the abundance estimates from ROVs in this pilot study, MaxN was included as a metric of relative abundance. The MaxN values for BRUVS are collected during 1-hour recording at specific depth. ROVs transects, however, encompass several depths throughout a single transect. To be able to compare the two methods, the ROV sightings were grouped in 10-meter depth bins, so that each transect is divided in multiple depth bins. Additionally, the maximum number of sightings for each species, for each depth bin was calculated. Rather than calculating the mean count of sightings for each depth bin, the maximum number of sightings per species seemed to be more appropriate as a comparison to BRUVS MaxN. Consequently, MaxN from BRUVS at a specific depth, day, and site, could be compared to ROV transect sum for that corresponding depth bin, on the same day, and at the same site.

To test whether the mean number of elasmobranchs sighted per transect was significantly different for the two methods (ROV and BRUVS) a GLM with a Poisson distribution was fitted to the data with mean number of elasmobranchs as the dependent variable and method as the independent variable. An offset variable was also added to the model as the number of transects within a depth bin, multiplied by 3.2 minutes (the average time of an ROV transect), and divided by 60 (the time for all BRUVS surveys). Models were fitted only for reefs where ROV transects performed a minimum one hour of survey $[(glm(MaxN \sim Method + offset(log(N_time)))]$. Model diagnostics and zero inflation were checked, and the Poisson GLM model was shown to be better performing in this instance to a negative binomial GLM model. Additionally, transects shallower than 30 meters were filtered out of the data and the analysis was re-run to detect significant changes in overall abundance.

Results

Experiment 1A: ROV and UVS Comparison

At Moore Reef, the ROV surveyed 78 transects (20 transects at dawn, 10 transects in the daylight, 21 transects at dusk, and 27 transects at night), 8-10 minutes each transect. At the same time, divers performed a total of 31 transects with the DOV (12 at dawn, 10 during daylight, and 9 at dusk), averaging 10 to 60 minutes per 100 meter transects. ROV surveys recorded the majority of shark and ray sightings when compared to UVC (DOV surveys) during daylight and crepuscular transects, as no diving was allowed at night (Table 4.1). For both survey methods, the most observed species was the white tip reef shark (*Triaenodon obesus*), followed by the grey reef shark (*Carcharhinus amblyrhynchos*). Some species were only sighted by the ROV: lemon sharks (*Negaprion acutidens*), one bowmouth guitarfish (*Rhina ancylostoma*), and three non-identified individuals of the Carcharhinidae family (*Carcharhinus spp.*) (Figure 4.10).

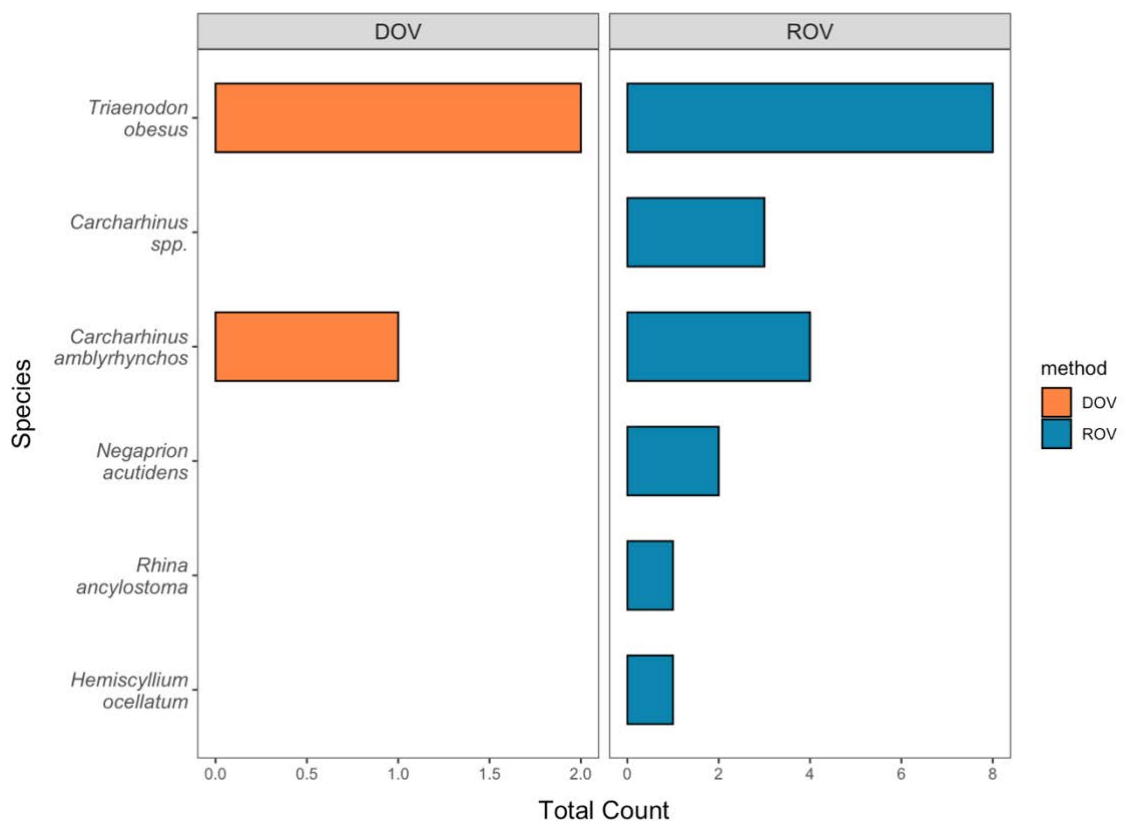


Figure 4.10. Total species sightings for each method (DOV and ROV) for all transects.

The forward facing ROV camera recorded the most sightings across all transects, times, and light treatments. This camera is used for piloting and following the transect, mostly looking down towards the bottom, and recording benthic species and species resting on the bottom, such as white tip reef sharks (Appendix A, table A2).

Methods Comparison (ROV and DOV): Elasmobranch Detections and Probability of Detection

Results from the Poisson GLM model suggest that ROVs did not detect a significantly higher number of elasmobranchs compared to the DOV surveys (UVS) (Table 4.2). Similarly, results from the presence/absence binomial GLM suggest that the probability of detecting elasmobranchs was not significantly higher with ROVs compared to DOVs (Table 4.3, Figure 4.11). As ROVs had the highest detection when considering all survey times, it is possible that the non-significance of the model is likely a result of excluding nocturnal observations from the model, as no diving was allowed at night. Although most sightings were recorded during dawn and dusk, omitting ROVs observation at night might be sufficient for showing no difference between the two methods in terms of observations per hour and probability of detection. An offset variable was also added to the model to investigate whether survey time could affect the model results.

Table 4.2. Summary of model coefficients from the GLM fitted with a Poisson distribution estimating mean number of elasmobranchs (dependent variable) for each method (ROV, DOV, independent variable).

Model	term	Estimate	Std. error	statistic	p. value
Poisson – overall counts	(Intercept)	0.097	0.577	-4.04	<0.005
	Method ROV	2.52	0.621	1.49	0.137
Binomial – probability of detection	(Intercept)	0.107	0.607	-3.68	<0.005
	Method ROV	2.89	0.664	1.55	0.121

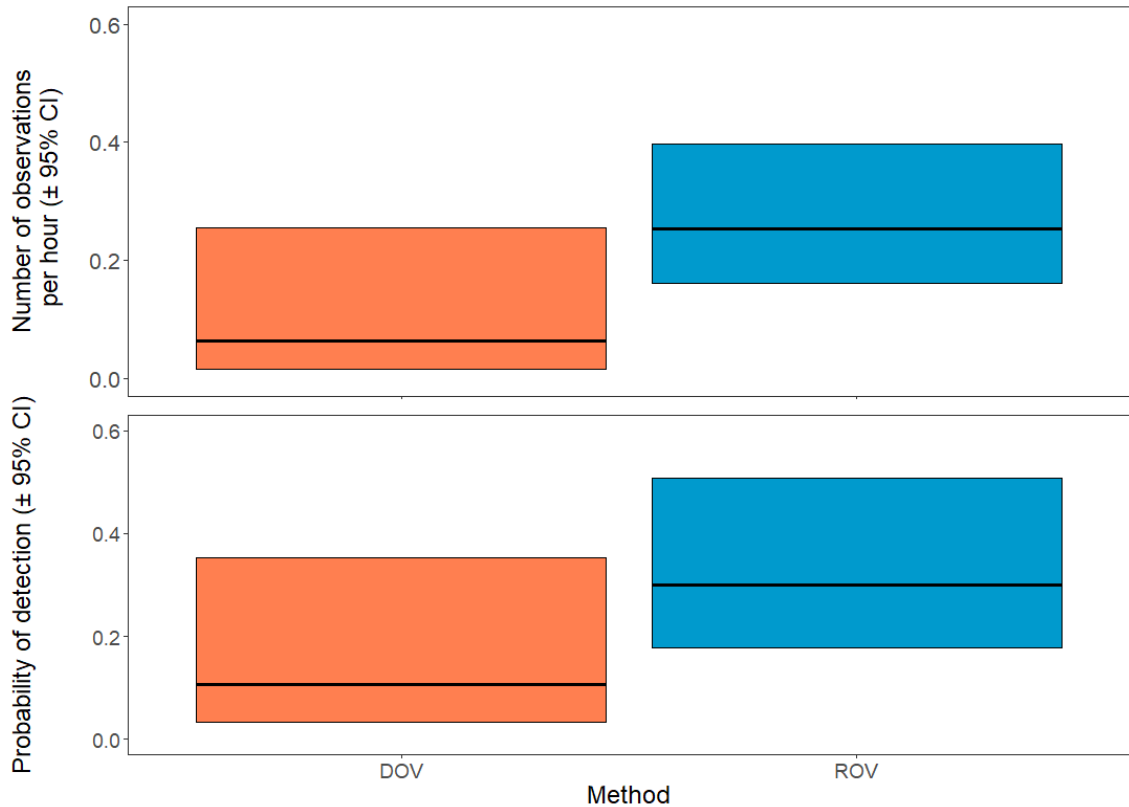


Figure 4.11. Method comparison between DOV and ROV. A) The mean number of sharks per hour estimated from the GLM with a Poisson distribution for different methods. The coloured crossbars indicate 95% confidence intervals B) The probability of detecting a shark using different methods from the GLM with a binomial distribution. The coloured crossbars indicate 95% confidence intervals.

Moore Reef Experiment 1B: Nocturnal ROV surveys

The highest species’ richness was sighted during crepuscular hours, with only 3 elasmobranchs seen during nocturnal hours, capturing one known nocturnal species (*H. ocellatum*; appendix A table A2). Comparatively red-light surveys observed double the number of elasmobranchs (Red = 10, White = 5) across Dusk, Nocturnal, and Dawn transects. However, The GLM model fitted with a Poisson distribution suggests that no significant difference was found between white and red-light observations (Table 4.3, p – value = 0.306). Similarly, The GLM model fitted with a binomial distribution (presence/absence) found no significant difference between white and red light counts (Table 4.5, Treatment White p – value = 0.344). When the time of survey, such as dawn, dusk, and night, was added as an interaction, the models suggested no significant differences when different lights are used at different times. Thus, the model indicates that there is no conclusive evidence to suggest that any specific light treatment is more effective in detecting elasmobranchs.

Table 4.3. Summary of model coefficients from the GLM fitted with a Poisson distribution estimating mean number of elasmobranchs (dependent variable) for each light treatment (Red, White, independent variable).

Model		term	estimate	Std. error	statistic	p. value
Poisson counts	– overall	(Intercept)	0.167	0.447	-4.01	<0.005
		Treatment Red	1.74	0.539	1.02	0.306
Binomial of detection	– probability	(Intercept)	0.357	0.368	-2.79	<0.005
		Treatment Red	0.560	0.613	-0.946	0.344

Northwest Island – Experiment 2

At Northwest Island, the ROV surveyed a total of 48 timed swims (4 surveys at dawn, 17 surveys in daylight, 3 transects at dusk, and 18 surveys at night), averaging 20 minutes per survey. The 10 drumlines at the study site were set each day at sunrise and un-baited at sunset. Catch per unit of effort (CPUE) was calculated as sighting over survey time (for ROV and B-ROV surveys) and catches over soak time for drumlines. ROV and B-ROV timed swims recorded approximately the same number of elasmobranch sightings as the catch surveys (ROV = 17, drumlines = 18), but the two methods recorded markedly different species. Drumlines caught mostly larger roving sharks, such as tiger sharks (*Galeodon cuvier*), great hammerhead sharks (*Sphyrna mokarran*), grey reef sharks (*Carcharhinus amblyrhynchos*), pigeye sharks (*Carcharhinus amboinensis*) and blacktip reef sharks (*Carcharhinus melanopterus*). On the other hand, ROVs and B-ROVs sighted mostly ray species, such as eagle rays (*Aetobatus narinari*), Kuhl's maskray (*Neotrygon kuhulii*), and white tip reef sharks (*T. obesus*). When comparing B-ROVs and ROVs, most observations were recorded when the bait was attached to the ROV (Figure 4.12).

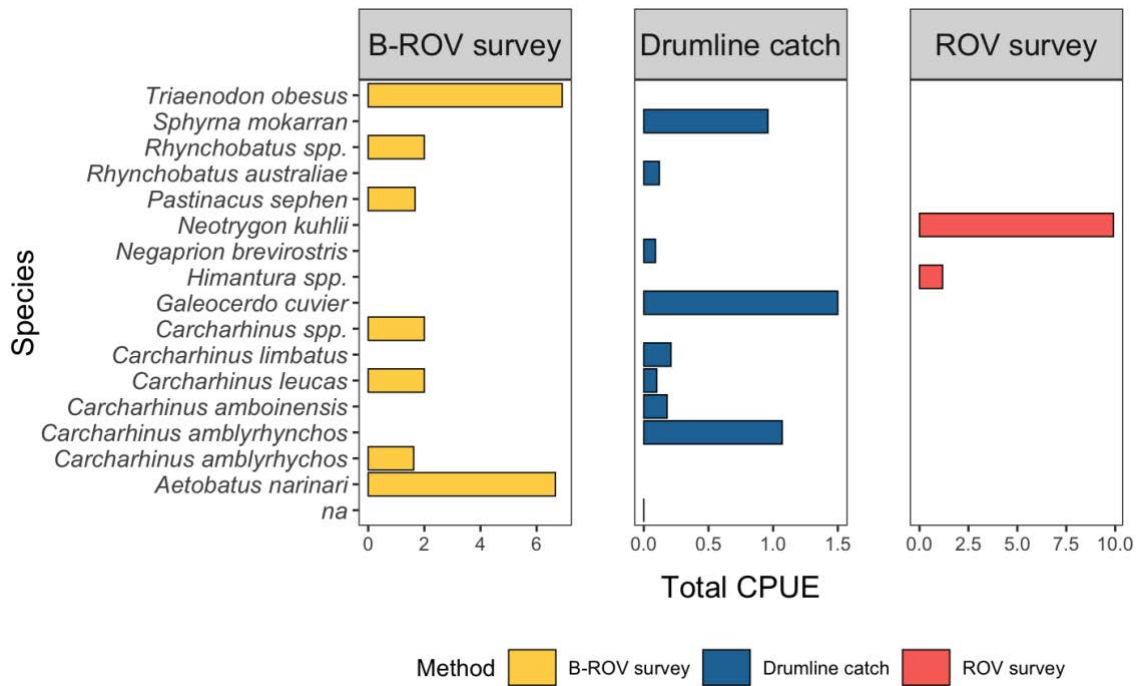


Figure 4.12. Total species sightings for each method (drumlines and ROVs) for all timed swims and drumlines sets and for all times of the day (dawn, day, dusk, night).

Nocturnal ROV surveys did not record any elasmobranch sightings in Experiment 2, likely due limited visibility. Therefore, nocturnal surveys were not considered further in this experiment. Interestingly, the two days during which the ROV was ‘baited’ with a mullet (B-ROV), most sightings were recorded, with CPUE values of 14.53 for day 4 and 8.34 for day 3 (Table 4.4). On the same days the drumlines had been baited with shark bait, but drumline CPUE values did not seem to increase substantially compared to mullet bait increase seen when the ROV had mullet bait (day 1 CPUE = 1.9, day 3 CPUE = 1.04; Table 4.4).

Table 4.4. Bait type for each method across 5 days and relative CPUE. Highest CPUE values for ROVs were recorded on day 4 and 3, when drumlines were baited with shark and the ROV was baited with mullet.

Day	Method	Bait type	CPUE
Day 1	ROV	no bait	2.3
	drumlines	Mullet	1.9
Day 2	ROV	no bait	3.6
	drumlines	Mullet	0.66
Day 3	ROV	Mullet	8.34
	drumlines	Shark	1.04
Day 4	ROV	Mullet	14.53
	drumlines	Shark	0.21
Day 5	ROV	Mullet	0
	drumlines	Mullet	0.3

Methods Comparison (ROV, B-ROV, and drumlines): Elasmobranch Detections

Over 460 hours in 5 days drumlines caught 18 sharks, while ROVs surveys accounted for a total 16 hours and sighted 17 elasmobranchs. Results of the GLM negative binomial model fitted to the data (with reference variable set as drumlines) show that ROV and B-ROVs surveys detected a significantly higher number of observations compared to drumline sets (Table 4.5, Figure 4.13). ROV surveys, without bait, predicted a mean of 2.31 sightings per hour \pm 1.16 SE. The B-ROV surveys significantly increased the predicted mean sightings to 7.35 per hour \pm 2.04 S.E., while the drumline sets had the lowest predicted mean sightings, at 0.19 per hour \pm 0.04 S.E.

Table 4.5. Summary of model coefficients from the GLM fitted with a negative binomial distribution estimating mean number of elasmobranchs (dependent variable) for each method (drumlines, ROV, B-ROV independent variable) standardised by effort (soak time/survey time).

Model	Term	Estimate	Standard error	Statistic	p. value
Negative binomial – MaxN per hour	(Intercept)	-3.238	0.236	13.737	<0.005
	Method ROV	2.518	0.553	4.554	<0.005
	Method B-ROV	3.674	0.364	10.091	<0.005

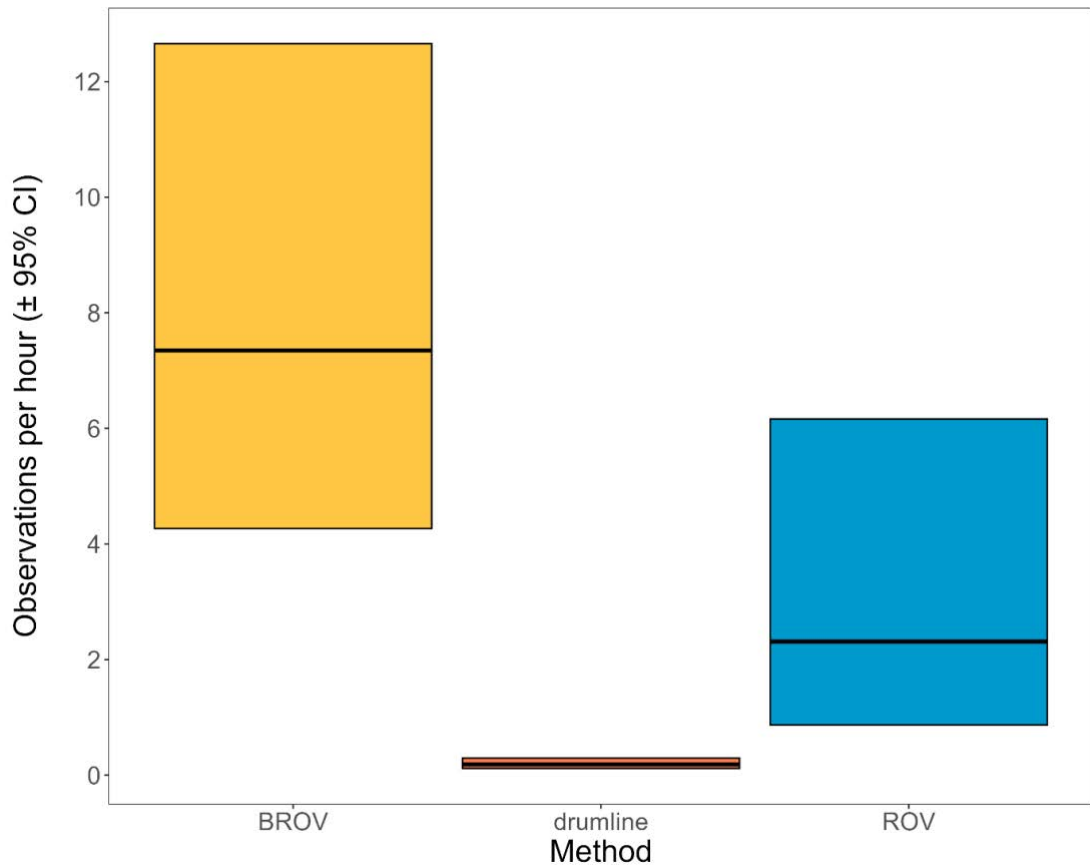


Figure 4.13. Method comparison between B-ROV (baited ROV), ROV, and drumlines. The mean number of sharks per hour estimated from the GLM with a negative binomial distribution for different methods. The coloured crossbars indicate 95% confidence intervals.

Coral Sea – Experiment 3

Overall, across all reefs visited and depths, BRUVs recorded higher species diversity than ROV surveys, and it was the only method to detect tiger shark (*Galeocerdo cuvier*), lemon shark (*Negaprion acutidens*), Kuhl’s maskray (*Neotrygon kuhlii*), and ornate wobbegong (*Orectolobus ornatus*). ROV surveys recorded species such as grey reef sharks (*C. amblyrhynchos*), silvertip sharks (*C. albimarginatus*), and white tip reef sharks (*T. obesus*) (Figure 14.4).

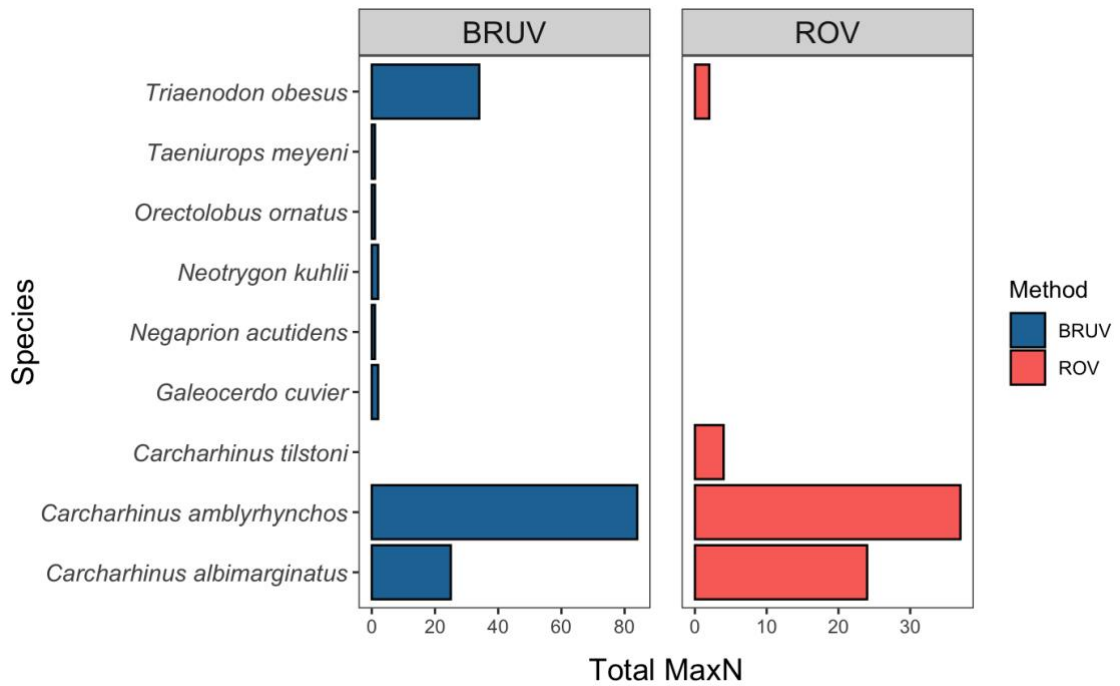


Figure 4.14. Total species sightings for each method (BRUVS and ROVs) for all timed swims and BRUV drops and for all survey sites.

In terms of total observations of all elasmobranch species, BRUVS recorded the highest overall number. However, when standardised by effort, ROVs detected more elasmobranchs in comparatively less hours at Ashmore, Flinders, and Willis reefs (Figure 4.15).

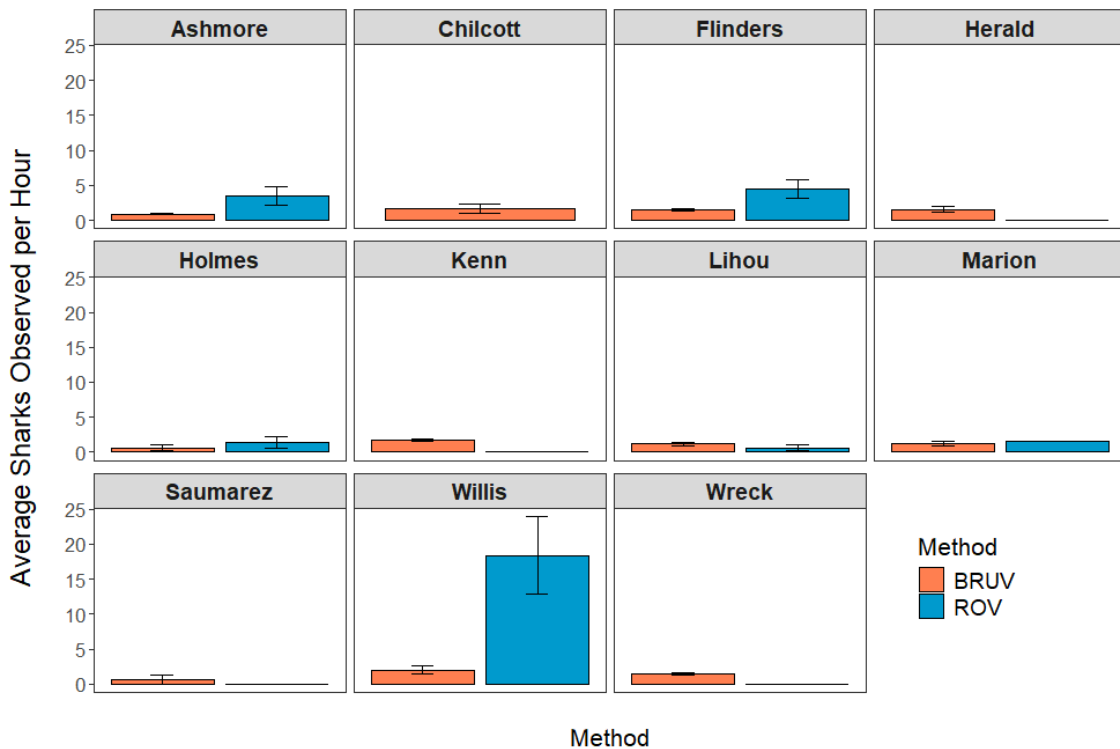


Figure 4.15. Average elasmobranchs observations per hour, for ROV and BRUVS across all reefs.

BRUVS and ROVs data were modelled using a GLM with a Poisson distribution and results were variable across reefs, with ROVs performing recording more elasmobranchs per hour at Ashmore, Flinders, and Willis (Table 4.6). These results indicate that for Ashmore and Willis reef, ROVs are estimated to see more elasmobranchs per hour on average during deep water surveys compared to BRUVS (Figure 4.16). Importantly, standardising sightings by effort allowed to gain perspective into the actual performance of these two methods.

Table 4.6. Summary of model coefficients for the GLMs fitted with a Poisson distribution estimating the mean number of elasmobranchs (dependent variable) per transect for each method (ROV and BRUV). Models fitted with time as an offset.

Reef	term	estimate	Std. error	statistic	p. value
Ashmore	(Intercept)	0.879	0.186	- 0.696	0.487
	Method ROV	3.79	0.399	3.34	<0.005
Holmes	(Intercept)	0.6	0.577	0.885	0.376
	Method ROV	1.58	0.764	0.601	0.548
Flinders	(Intercept)	1.50	0.218	1.86	0.063 <u>2</u>
	Method ROV	2.72	0.498	2.01	0.044 <u>5</u>
Lihou	(Intercept)	1.19	0.229	0.749	0.454
	Method ROV	0.390	0.743	-1.27	0.205
Willis	(Intercept)	2	0.316	2.19	0.0284
	Method ROV	7.88	0.384	5.37	<0.005

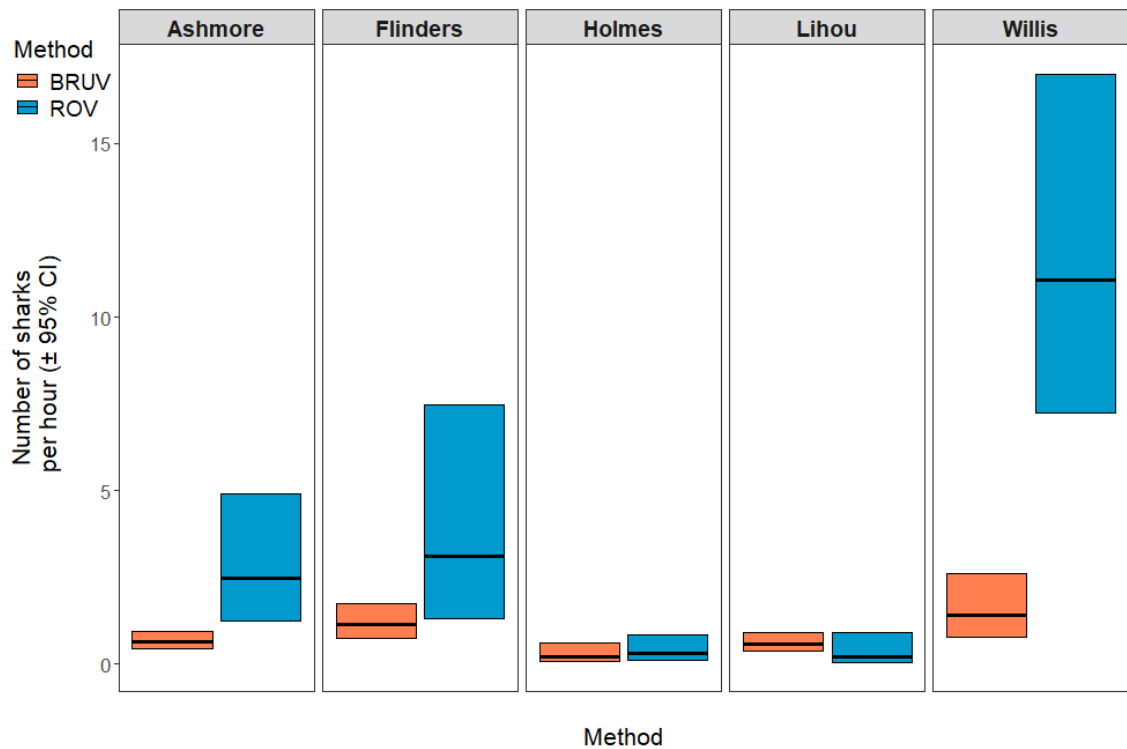


Figure 4.16. Method comparison between BRUVs and ROV across reefs. The mean number of sharks estimated from the GLM with a Poisson distribution for different methods. The coloured crossbars indicate 95% confidence intervals.

Discussion

This study was designed as a pilot to evaluate the effectiveness of observation-class ROVs in surveying elasmobranchs. The ROVs were tested across various times of the day and night, at depths ranging from 3 to 90 meters, and within different marine ecosystems throughout the Great Barrier Reef and the Coral Sea. Additionally, ROVs were compared against some of the most used methods for surveying elasmobranchs: UVS, BRUVS, and catches. The study was successful in testing the performance of ROVs, revealing both strengths and limitations. It also provided insights into the complementary role ROVs can play alongside UVS, BRUVS, and catches.

The main findings of this study are summarised in table 4.7. First, ROVs were comparable to UVS in terms of the relative abundance of elasmobranchs sighted, but ROVs outperformed UVS in terms of occurrence. This was mostly due to the ability of ROVs to survey at night and to record a 360-degree video of the transect. Additionally, although UVS is one of the most used and simple survey method, the logistical organisation of dive plans, gear, and divers is ultimately harder than the preparation of the ROV gear and of one single pilot. Compared to catches, ROVs had higher abundance (higher CPUE) and detected very different

species compared to the ones caught with drumlines. The logistics of ROV surveys may be easier than catches, mostly due to the ethics application process and the amount of heavy gear involved in drumline setting. However, ROV surveys require more effort as the pilot must be engaged for the whole duration of the survey, while the soak time of drumlines is mostly “passive” waiting. Finally, BRUVs outperformed ROVs in both abundance and occurrence, except for certain reefs. Similarly to drumline sets, BRUVs do not require active surveying once they have been deployed. In terms of post processing, the analysis of ROV videos requires similar amounts of time compared to videos from UVS (DOVs) and BRUVs. Post-processing is not necessary for drumline catches.

Table 4.7. Summary of main results. Equal sign (=) indicates that the ROV compared to another method was equal or comparable. The minus (-) sign indicates that the ROV was inferior to another method. The plus (+) sign indicates that the ROV was superior to another.

Comparison	ROV vs UVS	ROV vs Drumline catches	ROV vs BRUVs
Relative abundance	=	+	- (+)
Species occurrence	+	= (but different)	-
Logistical demands	-	-	=
Survey effort	=	+	+
Night survey capability	+	+	-
360-degree view capability	+	+	+
Post processing	=	+	=

Experiment 1: Comparing ROVs Against UVS

The comparison of ROV and UVS performance for studies on fish diversity and abundance produced mixed results in previous studies, with some indicating lower performance for ROVs while others show similar effectiveness (Andaloro et al., 2013; Maslin et al., 2021; Pita et al., 2014). Although ROVs in this study sighted more elasmobranchs compared to UVS, the low number of counts likely prevents robust estimates of model effectiveness. Nonetheless, as a similarly performing method, the pilot study suggests that ROV surveys can be used as an alternative to divers UVS (Bryan et al., 2013; Söffker et al., 2011). This is especially true for crepuscular and nocturnal surveys, which are often avoided by divers. In this study Most of the sightings occurred during crepuscular hours, and one nocturnal species (*H. ocellatum*), which often goes unseen by scuba divers during the day, was observed by the ROV at night. Considering that most non-extractive nocturnal fish surveys have been performed with BRUVS (Hart et al.,

2010; Harvey et al., 2012), ROV surveys can offer a complementary mobile-transect method to enhance the reliability of BRUV estimates for elasmobranchs at night (Bassett & Montgomery, 2011). By fitting 4 Go Pros on the top of the ROV, a 360 degree video of the transect can be recorded to determine species ID during post-processing instead of relying on experts ID in situ (Schramm et al., 2020). However, post-processing time is a common challenge with remote video methods. The application of artificial intelligence could significantly expedite post-processing by identifying the presence of elasmobranchs in the video, allowing researchers to review only relevant segments (Jenrette et al., 2022; Sheaves et al., 2020).

Both red light and white light were used for nocturnal and crepuscular surveys. Studies that have investigated the effect of light on the behaviour of fish and elasmobranchs indicate that red light is a safer option for recording occurrences without causing disturbance (Bedore et al., 2013; McFarland, 1990). Although some batoids and sharks are capable of distinguishing colours, many shark species are colour blind (Hart, 2020). Lemon sharks (*Negaprion brevirostris*), are capable of detecting red light to some extent (Cohen et al., 1977). In this study, counts recorded during red and white-light survey did not differ significantly, even though low counts affected model performance. Further trials with both lights are necessary to collect higher counts test the significance of this variable. However, the lights did cause differences in piloting the ROV. Firstly, piloting with red light proved more challenging due to its limited range compared to white light. Conversely, white light resulted in significant backscatter, particularly in the presence of high plankton density, making detections arduous (Figure 4.17). Furthermore, observations of many elasmobranchs during dusk and dawn surveys indicated that they tended to maintain a distance from the ROV. This behaviour suggests that during nighttime operations, they might remain undetected at such distances. With both lights nocturnal navigation along pre-set transects presented considerable challenges. Locating the start of the transect and maintaining the course proved especially difficult in conditions of strong currents and reduced visibility. These findings represent a practical and applicable result to perform surveys with ROVs at night and in limited visibility environments.

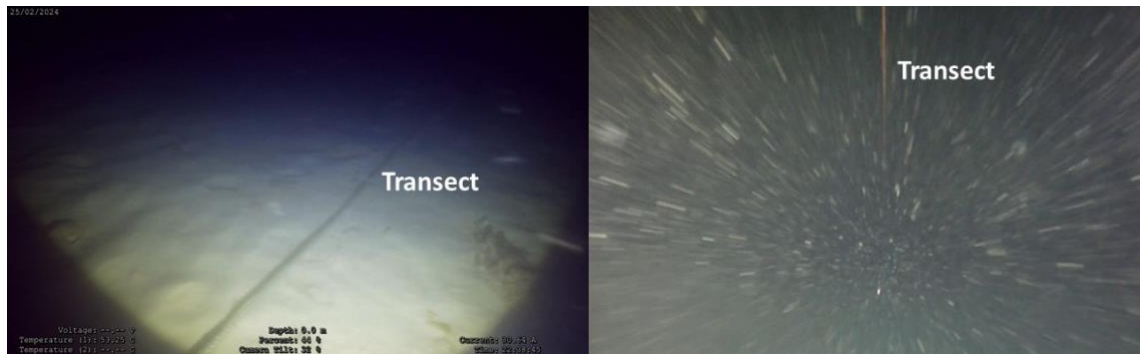


Figure 4.17. Nocturnal visibility with white lights. Different underwater conditions can impact the benefits of using white light, as backscatter can affect visibility.

Experiment 3: Comparing ROVs and Catch Data.

One of the most interesting and significant aspects of the ROV-drumline comparison is the difference in species caught/detected. The ROV primarily detected ray species and smaller reef-associated species, while drumlines predominantly caught large, roaming shark species. Previous studies comparing ROV surveys with catches vary based on the species and the fishing gear used but overall suggest promising applications of ROVs as a non-invasive method to survey fish assemblages and possibly estimating biomass (Mapula et al., 2016b; Norcross & Mueter, 1999; Sward et al., 2019). At Northwest Island, ROV surveys observed nearly the same number of elasmobranchs as drumlines. However, when considering effort (hook soak time for drumlines and survey time for ROVs), ROVs seem more effective. Although these results are significant, the diversity of species detected suggests that combining these two methods could provide the most accurate data on occurrence and abundance.

Given the time and resource constraints of this pilot study, it was not feasible to assess how baited drumlines influenced the relative occurrence of species and their detection during ROV surveys. In a more extended study, it would be advantageous to conduct ROV surveys prior to setting lines to account for the possibility that bait may attract elasmobranch species, thereby increasing sightings during the surveys. Another aspect of this study that could not be systematically tested is the difference in performance between baited (B-ROVs) and non-baited ROVs. B-ROVs were tested on only two out of the five survey days, preventing definitive conclusions regarding its effectiveness. For instance, environmental effects or contextual variables could have affected the occurrence of elasmobranchs in the survey area on the day where B-ROVs were used. Nevertheless, both baited and non-baited ROVs recorded more elasmobranchs per hour compared to catch surveys and detected different species, predominantly rays.

Experiment 4: Comparing ROVs and BRUVs for Deep Water Surveys

The comparative study between ROVs and BRUVS shows notable differences in species detection and number of elasmobranchs sighted. First, BRUVs observed all species detected at this study site while ROVs only detected the most observed species across all depths and reefs. These results align with the differences in abundance of elasmobranchs detected by divers and BRUVS in other comparative studies, likely due to the effectiveness of the bait for attracting elasmobranch and predatory species (Cheal et al., 2021; Munoz & Burton, 2019). In terms of overall abundance, when standardised by time underwater, ROVs detected more observations per hour compared to BRUVS at Ashmore Reef, Flinders Reef, and Willis Reef. These findings highlight the importance of standardising by effort when using ROVs alongside other methods (Sward et al., 2019). For instance, higher accuracy and possibly species diversity could be detected below 30 meters by a combination of ROVs, as a mobile transect survey, and BRUVS, as stationary remote video systems (such as BRUVS). Previous studies using ROVs have been able to ground truth the occurrence of species in deep waters, such as the deepest observation of scalloped hammerhead (*S. lewini*) at 1042 meters (Moore & Gates, 2015), and an observation of sleeper shark at more than 2600 meters deep.

The Challenges of Piloting New Technologies

The Blue ROV2, used in this study, is the most affordable high-performance observation-class ROV on the market and showed promising results for elasmobranch surveys. However, sightings of elasmobranchs at Moore Reef and Northwest Island were sparse, limiting the confidence in determining whether ROVs truly perform better than underwater surveys and drumline catches. Due to the extensive survey effort in the Coral Sea, the comparison between ROVs and BRUV has more robust model estimates. Limited sample sizes are a known challenge in elasmobranch research, mainly due to the highly mobile and/or cryptic nature of many species, as well as their naturally lower abundance (Braccini & Taylor, 2016; Hyde et al., 2022; McCauley et al., 2012). Additionally, as this study aimed to pilot observation-class ROVs for elasmobranch surveys, more effort and attention were devoted to testing different setups and methods rather than gathering numerous replicates. Based on the experiences and trial-and-error of this pilot study, several suggestions can be made for using ROVs, a relatively new technology in this field.

The Effect of the Current

The effect of strong currents on observation-class ROVs are known to the research community using this technology (Makwela et al., 2016). At Moore Reef, the currents exerted significant pressure on the thrusters and battery, making it difficult to locate and reach the transect, particularly at night. To facilitate the approach to the start of the transect, flashing lights were affixed to a buoy marking its beginning. This allowed the ROV to be guided towards the buoy at the surface before following the line down to the bottom. However, high swells often obscured visibility at the surface, hindering the use of the front ROV camera for following the flashing light. Additionally, the currents frequently pushed the ROV sideways while tracing the buoy line downward, leading to instances where the line was lost in limited visibility. Piloting the ROV along the transect was also challenging in strong current as ROV stabilization and depth hold functions contributed to considerable battery strain. Commercially available ROVs may underperform compared to working-class ROVs, particularly in maintaining stabilization in high currents while conserving battery life (McLean et al., 2017). However, the purpose of this study was to test the capabilities of readily available and affordable ROVs, aiming to provide insight into this technology for any research projects, even if limited by funding and gear.

Taking into consideration the impact of the current at Moore Reef, transects at Northwest Island were designed as drift transects. This strategy proved more efficient, enabling longer transects while conserving battery power. Although ROV surveys often prioritize the transect type (Sward et al., 2019), this study shows that drift surveys appeared to be the most effective way to use the Blue ROV2 in high-currents environments. However, when the ROV drifts with the current the pilot needs to stay vigilant for upcoming reefs or structures that could cause the tether to tangle.

Effects on Behaviour and ROV Avoidance

Quantifying the effects of ROVs on the behaviour of elasmobranchs was beyond the scope of this project, but these effects need to be considered in interpreting data from ROV surveys. ROVs have been recorded to have different effects on the behaviour of different species of fish (Lorance & Trenkel, 2006; Trenkel et al., 2004). Other than the shape and size of ROVs, there are two main factors influencing behaviour that could be tested and addressed: noise (often dependent on the mechanics of the thrusters and the speed of the ROV), and lights. Although differences in lighting were considered in this study, white light surveys were always conducted after red light surveys to maximize encounter rates while minimizing disturbance. On the other hand, it became apparent during post-processing that red-light allowed for a

significantly reduced field of view compared to white light, as previously mentioned in other studies (Bassett & Montgomery, 2011). The behavioural effects of lighting could be more accurately tested using a randomized survey design that alternates light colours. The noise of the ROV could possibly triggering a stronger avoidant reaction in elasmobranchs. Behavioural experiments in captivity could systematically record different species' reactions to ROV noise, by using recordings of underwater noise from various ROV models at different speeds, while simultaneously controlling for confounding environmental parameters.

The Value of Multiple Camera Positions

Previous evaluations of ROV performance have indicated that their significantly reduced field of view makes them less suitable for detecting cryptic species (Wetz et al., 2020). To overcome this limitation, the Blue ROV2 used at Moore Reef and Northwest Island were equipped with 4 Go Pros mounted on the top frame, which recorded an approximately 360-degree video around the ROV. At Moore Reef, although overall sightings number remained low, the ROV camera had the highest number of sightings. This was likely due to the large number of white tip reef sharks resting on the bottom. As the ROV followed the transect, the camera was tilted downwards, effectively capturing these individuals on the bottom. Meanwhile, the other cameras recorded higher species diversity compared to the ROV camera alone, but no camera seemed to be more effective than other. This may suggest that no camera position is most likely to record more sightings, but a 360 degrees view guarantees the most species diversity and reduces the likelihood of missing individuals due to limited field of view. Studies using multiple cameras on BRUVs have reported that cryptic species hiding in the background may be missed by the front camera as other species crowd to the front to feed on the bait. Also, smaller individuals may also avoid the front camera due to competitive effects and avoidance of larger predatory species (Whitmarsh et al., 2018).

The Baited ROV

At Northwest Island the ROV was baited during timed swims, leaving behind a trace of bait plume. Although most elasmobranchs were sighted when the ROV was baited, the effect of using attractants with an ROV needs further testing given the low sample effort and lack of comparable data to analyse trends (e.g. surveying with un-baited ROV on the same day and will all other circumstances being equal). Additionally, many of the species observed by the baited ROV were small benthic rays or eagle rays which seem unlikely to follow and track a bait plume in mid water, so the higher occurrence could be co-incidental to a higher abundance of elasmobranchs during that particular day. This approach also requires caution, as larger sharks

may severely damage the ROV or become entangled in the tether as they further investigate the source of the plume. In this study, although sharks appeared 'curious' of the baited ROV, no biting attempts were recorded.

Conclusion

This pilot study tested the feasibility of using ROVs to survey elasmobranchs. Specifically, the BlueROV2 was deployed for elasmobranch nocturnal and deep-water surveys, and its performance was compared to traditional survey methods. Performance was assessed based on species detection, overall abundance, and effort (in time). When ROV performance was evaluated against UVS, BRUVS, and catch surveys, the results were mixed. At Moore Reef, ROVs and UVS yielded similar counts and species richness. When compared to BRUVS across several sites in the Coral Sea, results varied depending on the survey location, with BRUVS detecting higher abundance and most species diversity for most of the reefs visited. At Northwest Island, sightings standardised by effort showed that ROVs surveys were more effective but detected very different species compared to drumlines. Challenges encountered with the method were addressed through improved survey techniques and ROV customization. After strong currents proved problematic at Moore Reef, drift surveys were tested at North West and showed promising results. To enhance the field of view, four cameras were mounted on top, providing a 360-degree view of the transects. The use of different light treatments for nocturnal surveys was also tested, showing no significant difference in species occurrence between treatments, but better navigation overall with white lights. While these findings provide valuable insights into the use of ROVs for elasmobranch surveys, more data are needed to validate the preliminary data presented here. Future research should also examine the effects of lighting, noise, and avoidance behavior. More standardized and comparable efforts could also be useful for deep water ROVs and BRUVS surveys. Overall, ROVs offer advantages over traditional survey methods and can be a useful addition to the elasmobranch research toolbox.

Chapter 5: General Discussion

The research presented in this thesis advances current methods by integrating new technologies, demonstrating how these innovations can expand the spatial and temporal capabilities of traditional approaches. This is achieved through two case studies that combine traditional survey methods with novel technological ideas to improve their efficacy.

In **Chapter 3**, the use of artificial intelligence (AI) supports a flexible and customizable protocol for enhancing photographic identification. The model was able to confirm the stability of unique patterns in adult, a fundamental requirement for re-sightings during long-term studies. On the other hand, when trying to ID immature individuals, the model's low accuracy and precision suggested that, for epaulette sharks (*H. ocellatum*), neonates and juveniles cannot be reliably identified. These findings highlight that AI models can be used beyond simple image sorting to test the reliability and stability of patterns, and that identification may be challenging for immature life stages of other species. **Chapter 3** also suggests advancements in the field of AI for animal re-identification by training and testing a model that can work for small and large datasets, even with limited computational capabilities. These results broaden the application of photo ID to projects with extensive databases and those with limited resources aiming to trial photo ID.

The pilot study in **Chapter 4** aimed to test the efficacy of Remotely operated vehicles (ROVs) to conduct underwater visual surveys for elasmobranchs. The results showed promise in expanding and/or improving survey capabilities in time and space, such as nocturnal and deep-water surveys. At the same time, several environmental and context-dependent variables influenced the efficacy of ROVs, promoting careful considerations when using this technology alongside other methods. Initially designed to test the performance of ROVs against established methods, **Chapter 4** demonstrated the practical benefits of incorporating ROVs into multi-method approaches.

Using ROVs as a Complementary Method to UVS, BRUVS, and Catch Surveys.

The new developments described above build on a wide toolbox of methods used in elasmobranch research, each with its own benefits and limitations. For instance, some of the traditional methods may benefit from incorporating new technologies and novel solutions. Historically, the most used scientific methods for estimating elasmobranch abundance and

occurrence are underwater visual surveys (UVS), capture methods, and baited remote underwater video systems (BRUVS).

The pilot study (**Chapter 4**) aligns with existing literature confirming the applicability of ROVs for fish surveys (Andaloro et al., 2013; Maslin et al., 2021; Wetz et al., 2020), and expands current knowledge by demonstrating that ROVs perform similarly to diver surveys in terms of abundance, and can detect more elasmobranchs species during crepuscular and nocturnal hours. While diver-conducted underwater visual surveys (UVS) are well-trialled and standardized, they are restricted to shallow, clear waters and daytime hours, potentially excluding a significant portion of elasmobranch species. The pilot study indicates that ROV surveys can effectively collect abundance and occurrence data, surpassing the depth and safety limitations of scuba diving (Benz et al., 2007; Lowe et al., 2018; Maslin et al., 2021; Moore & Gates, 2015; Packard et al., 2013).

When ROVs were used alongside BRUVS in the Coral Sea (**Chapter 4**), they detected similar or higher abundance at most reefs, while BRUVS detected more species overall. These findings suggest that ROVs can serve as a complementary, mobile transect method for BRUVS. For instance, using UVS and BRUVS together has shown different results in species abundance and occurrence compared to using them individually (Cheal et al., 2021). With the use of ROVs, deep water and nocturnal mobile surveys could complement BRUVS data.

Additionally, when ROV (and baited ROV, B-ROV) timed swims were performed alongside catch surveys, B-ROVs had the highest CPUE, followed by ROVs, and detected different species compared to drumlines. Drumlines mostly caught large roaming species such as tiger sharks (*Galeodon cuvier*), great hammerhead sharks (*Sphyrna mokarran*), and pigeye sharks (*Carcharhinus amboinensis*), while the ROV observed mostly ray species (e.g., *Aetobatus narinari*, *Neotrygon kuhlii*) and white tip reef sharks (*Triaenodon obesus*). As the ROVs were able to survey in the proximity of baited drumlines, this approach complemented the overall species composition detected in the area. For instance, species composition and size classes are highly influenced by gear type and deployment area (Zhou, 2008). Ultimately, ROVs can be a valuable complementary method to provide an insight on the differences in species catchability (Braccini et al., 2021; Norcross & Mueter, 1999; Talwar et al., 2020). Capture-mark-recapture (CMR) methods can also be implemented with catches to address biases in detectability and abundance (Rago, 2005), but to be effective require substantial investments in resources and long-term

efforts (Cameron et al., 2019), which may not be feasible depending on the context of the study and the research question.

How Complementary Multi-Methods Approaches Improve Detection of Species Occurrence and Estimates of Abundance.

The use of ROVs as a complementary tool for traditional methods aligns with the hypothesis that using multiple methods for detecting occurrence and estimating abundance can help mitigate the intrinsic biases of single methods (Cappo et al., 2004; Colton & Swearer, 2010). The capture-mark-recapture approach is often used with catch surveys to gather demographic data (e.g., growth and survival) and estimates of abundance, but the low recapture probability of elasmobranchs can complicate population models. However, tracking methods can support models with data on movement and survival. For example, a study on broadnose sevengill sharks (*Notorhynchus cepedianus*) along the coast of South Australia found that acoustic recapture rates of tagged individuals were much higher compared to re-captures of longlines in the same area. The study concluded that the inclusion of acoustic data increased the precision of abundance estimates from longlines (Dudgeon et al., 2015). Similarly, a study investigating aggregation patterns of whale sharks (*Rhincodon typus*) used photo ID as a mark-recapture method and telemetry to track individual movements in and out of the aggregation areas (Araujo et al., 2019). Another method often used with CMR studies is the analysis of genetic samples to evaluate population parameters. For example, microsatellite markers, have been used with photo ID-based CMR to compare population estimates from recaptures to the effective population size of white sharks obtained from genetic samples (*Carcharodon carcharias*) (Andreotti et al., 2016). Similarly, census size (N_c) obtained from mark recapture for a population of Indo Pacific leopard shark (*Stegostoma fasciatum*) was compared to microsatellite-derived effective population size (N_e), concluding that estimates from CMR studies resembled effective population sizes (Dudgeon & Ovenden, 2015). BRUVS and UVS, both with divers and ROVs, have been used together collecting data on the distribution and abundance of fish species (Colton & Swearer, 2010). For example, a study on rocky reefs fish assemblages showed that BRUVS were more effective for predatory fish, while UVS detected cryptic species better (Rolim et al., 2022). Similarly, a study found that the combination of BRUVS and transect methods increased the species diversity for reef fish (Schramm et al., 2020). The combination of BRUVs, catches, and eco-sounders can reconstruct reliable representation of species occurrence and abundance in an area, including movement, habitat use, and presence of interconnected prey and predator species (Barnett et al., 2022). While considerable variability is introduced by each type of underwater visual survey (MacNeil et al., 2008; Pais & Cabral,

2018), such as observer bias, visibility, and fish speed (Ward-Paige et al., 2010), using multiple methods can help researchers understand how many individuals may be missed and why (MacNeil et al., 2008; Ward-Paige et al., 2010). In this instance, using ROVs alongside visual transects can not only collect data from inaccessible locations, but can also provide supplementary data to ground-truth and refine data derived from visual surveys. Overall, when it comes to complementary methods, the benefit of adding multi-methods to survey designs may be context dependent, but it often yields the most accurate estimates of abundance. However, even with the data from multiple methods, estimates of abundance are challenging to calculate due to the difficulties in obtaining population models parameters such as survival, recruitment, immigration, and emigration (Dudgeon & Ovenden, 2015; Sollmann et al., 2013). Complementary methods may be more effective to obtain data to support estimates from one main method, such as acoustic tagging can provide data that feeds into the population modelling generated by CMR data (Dudgeon et al., 2015). Also, complementary methods can be used to uncover potential biases or highlight knowledge gaps. For instance, during underwater surveys divers may observe species that are rarely captured in the area, indicating that the fishing gear may not be effective for those species.

From Relative Abundance to Estimates of Abundance

Estimates of relative abundance refer to the number of individuals present in an area at a specific time. These estimates are time and space dependent, but can be used for comparisons with other areas, or for detecting abundance fluctuations in time. On the other hand, absolute estimates of abundance (or density) are more challenging as they require to count all individuals in a population (Seber, 1973). Because of the nature of elasmobranchs, open population models need to be used when estimating absolute abundance for these species, but the data necessary for these models, such as survival and immigration/emigration, are often not available or challenging to collect (Sollmann et al., 2013). However, long-term CMR studies can be used to gather robust estimates of numbers, recruitment, and mortality, resulting in more reliable estimates (Braccini et al., 2019). For example, many fisheries studies rely on long-term CMR data to create stock assessments models (SAMs), which aim to estimate the total number of individuals in a population (Hilborn & Walters, 2013). Although traditional fisheries models are based on single data sources (Beverton & Holt, 2012), SAMs can integrate multiple data sources, such as age composition and catch data (or CPUE), to calculate the closest possible estimate of absolute abundance of fish in a stock (Fournier & Archibald, 1982). Similarly, integrated population models (IPMs) can use count data, demographic data, telemetry data, and CMR data to estimate relative and absolute abundance (Schaub et al., 2024). SAMs are the most common

integrated model used for species of fisheries interest (Maunder & Deriso, 2003), and examples of IPMs have been developed for conservation purposes, such as habitat suitability models (González-Andrés et al., 2021).

Since multi-methods approaches often result in more accurate abundance estimates for elasmobranchs, integrated models are likely to be most appropriate when estimating absolute abundance of elasmobranchs species (Zipkin & Saunders, 2018). With the support of these models, relative abundance from CMR, movement data from tracking studies (Braccini et al., 2016; Dudgeon et al., 2015), and kinship data from genetic material (Andreotti et al., 2016; Delaval et al., 2023) can be combined to derive absolute estimates of abundance. Similarly, species occurrence data from BRUVs could be modelled together with CPUE data from catch survey and relative abundance from ROV transect surveys (McLean et al., 2015; Pita et al., 2014; Santana-Garcon et al., 2014). As shown by the results of the pilot study in **Chapter 4**, data collected from ROVs provided a valuable insight in the relative detectability and abundance estimates collected by BRUVS, UVS, and catch surveys.

As multi-method approaches emerge in elasmobranch science, it is important to standardize data by time or other metrics of effort before interpreting results comprehensively. Previous studies comparing ROVs with other methods for fish surveys have been using different measures of effort such as time of survey and catch per unit of effort (Sward et al., 2019). The ROV pilot study (**Chapter 4**) highlighted the need for standardization for the interpretation of data from multiple sources. When ROVs were employed with BRUVS, initial observations based on MaxN values suggested higher elasmobranch counts with BRUVS. However, upon standardizing by survey hour, ROVs proved equally effective across many surveyed reefs. Notably, the impact of effort standardization was most pronounced when comparing B-ROVs and ROVs sightings with drumline catches standardized by CPUE. By using a measure of CPUE (or sightings per unit of effort) ROVs and B-ROVs accounted for significantly higher elasmobranch detections per unit of survey time compared to catches, despite both methods yielding similar overall counts by the study's conclusion.

New Technologies and Future Directions

Arguably the most prominent technological advancements in the field of elasmobranch science are high-definition underwater cameras, eDNA and genomics, and drones (Butcher et al., 2021; Casas et al., 2022; Conservation X Labs, 2024; Macreadie et al., 2018; Terrasa, 2022). For example, multiple high-resolution cameras, spherical cameras, and acoustic cameras have

been added to BRUVs to address some of the viewpoints and visibility challenges of this method. Drone technology has enhanced the detection of certain elasmobranch species, providing a useful combination between mobile surveys and remote videos (Butcher et al., 2021). Recently, aerial drones have been able to collect whale blow samples for population genetics (O'Mahony et al., 2024), and amphibious drones can complete both aerial and aquatic missions and can be equipped with wireless data relay systems for transmission (Suetsugu et al., 2024).

Artificial intelligence (AI) has the potential to expand the spatial and temporal scales of elasmobranch research by expediting the sorting and analysis of media data (Christin et al., 2019). AI has been applied for annotating fish and shark species from remote underwater videos (Jenrette et al., 2022; Sheaves et al., 2020) and is extensively used in photographic identification (photo ID) (Pierce, 2019). However, in the context of photo ID for elasmobranchs, most models remain simple and require substantial pre- and post-processing effort (Marshall & Holmberg, 2011; Norman et al., 2017). The model proposed in **Chapter 3** not only used DNNs to minimise pre-processing but also incorporated cutting-edge AI innovations such as the similarity network approach (Schneider et al., 2022), and the XGBoost ensemble model (Jahanbakht et al., 2023). These novel solutions resulted in a customizable and versatile model to ID epaulette sharks, and future testing with other species and larger databases is need. When applied to long-term databases, the model can test the reliability of patterns over time, a fundamental requirement for long-term re-sightings and reliable demographic estimates (Armstrong et al., 2020; Hoschke et al., 2023). However, for re-sighting to be effective, individuals must maintain the same pattern over time.

The model developed in **Chapter 3** tested the reliability and persistence of patterns, showing innovative application of AI models beyond image sorting. The model indicated that the patterns of immature individuals could not be reliably used for ID, providing insights into the effectiveness of photo ID for population studies involving species with morphological changes through ontogeny. The model could be tested across several species in the wild, looking at the notable changes exhibited by epaulette sharks, Indo-Pacific leopard sharks (*Stegostoma fasciatum*), and the slight fading of patterns of white-spotted eagle rays (*Aetobatus narinari*), and tiger sharks (*Galeodon cuvier*) (Ebert et al., 2021; Last et al., 2016). If photo ID is applied to these species and photographs of immature stages are present in the database, the model from **Chapter 3** can test the reliability of these patterns for ID. Furthermore, considering the fast and effective processing abilities of AI, a model could be trained to align relative changes in colour patterns with growth rates. Although a closely monitored, captive study would be necessary,

neonates of any species could be photographed at close intervals until patterns stabilize, allowing the model to interpret pattern changes as a proxy for growth. This tool could enable the reliable and non-invasive collection of growth and age parameters from wild populations of species that exhibit pattern changes.

Finally, the protocol proposed in **Chapter 3** can be adopted by photo ID projects worldwide, even those with limited resources. This aspect of the protocol was intentionally designed to ensure that even citizen science projects in remote locations could trial photo ID for any observed species. While the reliability of citizen science data remains debatable in certain contexts (Chin & Pecl, 2018), successful examples of citizen science highlight the potential for expanding the spatial scales of photo ID and ultimately increasing the availability of long-term re-sightings data (Araujo et al., 2017; Armstrong et al., 2019; Hoschke et al., 2023). This type data is crucial for delineating occurrence ranges and possibly estimating the absolute abundance of populations.

Although the use of ROVs as a survey tool requires further testing on the effects of lights and noise on elasmobranch behaviour, this method has proven highly appropriate to complement traditional methods. The AI-integrated photo ID protocol tested in this study offers a chance to use AI beyond image sorting, supporting photo ID projects with varying resource levels. Furthermore, this study advocates for future research to adopt a multi-method approach to species detection and abundance estimation, highlighting the potential of ROVs as a valuable tool in enhancing such strategies. While integrating multiple methods is crucial for enhancing abundance estimates, data analysis must develop integrated models that can incorporate diverse sources and types of data. Meanwhile, AI capabilities promise to enhance the applicability and scope of photo ID, manage large datasets of media, and potentially leverage citizen science for global-scale studies. This emphasizes the collective responsibility of research communities, including funding organizations, proposal writers, and journal reviewers, to ensure the selection of the best combination of methods and appropriate multi-methods analysis for each specific research objective.

Conclusion

In an era of exponential technological growth, unprecedented opportunities exist for traditional methods to incorporate technological advancements, potentially overcoming current spatial and temporal limitations. This thesis focuses primarily on methods used to estimate the abundance and occurrence of elasmobranchs, with particular emphasis on evaluating the efficacy of new technologies. While new technologies are appealing, traditional methods, despite their intrinsic limitations, are standardized, well-known, and reliable. Therefore, the actual benefits, practicality, and performance of new technologies must be rigorously tested before widespread implementation. Additionally, given the constraints on conservation efforts—often limited by time, funding, and resources—governments, funding bodies, and foundations may be hesitant to support new technologies without assurance of their efficacy. This work, therefore, serves as a trial of innovative technologies, aimed at improving existing methods, and providing accessible resources on the trial-and-error process. By sharing the results of these trials, this thesis offers valuable insights for enterprises, research centres, NGOs, and contractors considering the adoption of new technologies. The threatened state of elasmobranch species calls for more representative data on abundance and occurrence, which represents the foundation of species conservation and marine resource management. Adopting and customizing new technologies for research could significantly contribute to addressing major knowledge gaps and consequently develop more effective conservation strategies for elasmobranchs worldwide.

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Appendix A

Table A1. The capturing dates of the pictures taken from each shark in our Baseline dataset (B0) and Time dataset (T0, T1, T2, etc.).

	B0	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
HO_10	22/12/202	27/02/202													
0	1	0	1/04/2021	4/11/2021	14/12/202										
HO_10	22/12/202	11/03/202													
2	1	0	1/04/2021	4/11/2021	14/12/202										
HO_10	22/12/202	11/03/202													
3	1	0	1/04/2021	4/11/2021	14/12/202										
HO_10	22/12/202	11/03/202													
5	1	0	1/04/2021	4/11/2021	14/12/202										
HO_10	22/12/202	21/09/202	20/03/202												
6	1	0	1	4/11/2021	14/12/202										
HO_10	22/12/202	21/09/202	20/03/202												
7	1	0	1	4/11/2021	14/12/202										
HO_10	22/12/202	21/09/202	20/03/202												
9	1	0	1	4/11/2021	14/12/202										
HO_11	22/12/202	21/09/202	20/03/202												
0	1	0	1	4/11/2021	14/12/202										
HO_11	22/12/202	22/06/202	19/08/202	13/10/202											
1	1	1	1	1	4/11/2021	13/12/202									
HO_11	22/12/202	22/06/202	19/08/202	13/10/202											
2	1	1	1	1	4/11/2021	13/12/202									
HO_11	22/12/202	22/06/202	19/08/202	13/10/202											
3	1	1	1	1	4/11/2021	13/12/202									
HO_11	22/12/202	22/06/202	19/08/202	13/10/202											
4	1	1	1	1	4/11/2021	13/12/202									
HO_11	22/12/202	22/06/202	19/08/202	13/10/202											
5	1	1	1	1	4/11/2021	13/12/202									
HO_11	24/03/202	22/08/202													
6	3	2	5/09/2022	12/09/202	19/09/202	10/10/202	17/10/202	5/12/2022	23/01/202	6/02/2023	15/02/202	20/02/202	27/02/202		
				2	2	2	2	2	3		3	3	3		
HO_11	24/03/202	12/08/202	22/08/202	29/08/202	12/09/202										
7	3	2	2	2	2	3/10/2022	19/09/202	10/10/202	17/10/202	5/12/2022	23/01/202	6/02/2023	20/02/202	27/02/202	22/03/202
							2	2	2	2	3	3	3	3	3
HO_11	24/03/202	12/08/202	29/08/202												
8	3	2	2	5/09/2022	3/10/2022	17/10/202	5/12/2022	6/02/2023	15/02/202	20/02/202	27/02/202	22/03/202			
						2			3	3	3	3			
HO_11	24/03/202	12/08/202													
9	3	2	5/09/2022	12/09/202	3/10/2022	10/10/202	17/10/202	5/12/2022	23/01/202	6/02/2023	15/02/202	20/02/202	27/02/202		
				2		2	2	2	3	3	3	3	3		

Table A2. Species observed by each camera and total observation per camera for each camera. Across all transects, light treatments, and time of the day, the ROV camera detected most species, mainly white tip reef shark.

Camera	Species	Number	Total observations
	<i>C. amblyrhynchos</i>	1	
Front	<i>Carcharhinus spp.</i>	1	4
	<i>T. obesus</i>	2	
	<i>Carcharhinus spp.</i>	1	
Right	<i>T. obesus</i>	1	3
	<i>H. ocellatum</i>	1	
	<i>C. amblyrhynchos</i>	2	
Left	<i>N. acutidens</i>	1	4
	<i>Carcharhinus spp.</i>	1	
Back	<i>N. acutidens</i>	1	2
	<i>R. ancylostoma</i>	1	
	<i>C. amblyrhynchos</i>	1	
ROV			6
	<i>T. obesus</i>	5	

Table A3. Species observations divided by red and white light treatment for nocturnal and crepuscular transects.

Species	Dusk ROV		Nocturnal ROV		Dawn ROV	
	RED	WHITE	RED	WHITE	RED	WHITE
<i>Triaenodon obesus</i>	2	1	1	-	2	1
<i>Carcharhinus amblyrhynchos</i>	1	-	-	1	1	-
<i>Negaprion acutidens</i>	1	1	-	-	-	-
<i>Hemiscyllium ocellatum</i>	-	-	-	1	-	-
<i>Rhina ancylostoma</i>	1	-	-	-	-	-
<i>Carcharhinus spp.</i>	1	-	-	-	-	-
Total obs.	6	2	1	2	3	1