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From nets to barcodes: Selecting suitable methods for assessing fish and prawn assemblages in seagrass meadows

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ABSTRACT

Seagrass meadows are vital coastal ecosystems that support fish and prawn assemblages, providing essential resources such as food and refuge. They are especially important as nursery habitats for ecologically and economically important juvenile fish and prawns. However, seagrass ecosystems are declining globally due to their vulnerability to both natural disturbances and anthropogenic impacts. Effective monitoring and management strategies are therefore essential to ensure their conservation and ecological functionality. This review synthesises literature on methods for sampling fish and prawns in seagrass habitats, grouping them into three categories: capture, sensory, and molecular approaches. Capture methods, including beam trawls and seine nets, provide valuable biological data, but are extractive and can be destructive to the surrounding habitat. Sensory methods such as baited remote underwater video systems (BRUVs) and hydroacoustic techniques, offer a non-destructive alternative, but can be negatively influenced by environmental conditions such as turbidity and habitat complexity that are common in seagrass meadows. Molecular approaches, particularly environmental DNA (eDNA) metabarcoding, present a highly sensitive and non-invasive alternative approach, but challenges remain in quantifying species abundance and demographics. To guide method selection, we propose a structured framework of questions and visualisations to assist researchers in selecting the most appropriate sampling methods based on their specific research objectives. Given the biases and limitation of these methods individually, we suggest integrating multiple methods to enhance assessments of marine communities in seagrass habitats. Future research should focus on refining these methodologies to improve the accuracy of biodiversity monitoring in seagrass meadows, whilst minimising environmental impacts.

1. Introduction

Seagrass meadows are amongst the most productive coastal ecosystems, supporting vital ecosystem services valued at billions of dollars globally (Costanza et al., 2014; Grech et al., 2012). These soft-sediment, benthic habitats, consist of marine-adapted plants that can form patchy or continuous vegetation across thousands of square kilometres (den Hartog, 1970; Hemminga and Duarte, 2000; Unsworth and Cullen-Unsworth, 2014). Seagrass meadows support highly diverse faunal assemblages and enhance fisheries by providing essential habitats and food for economically important juvenile fish and prawns, whilst contributing to coastal protection and carbon sequestration (Grech et al., 2012; Orth et al., 2006; York et al., 2018).

Seagrasses can provide dense cover and high canopies that support permanent, seasonal, or transient marine fauna (French et al., 2021; Hyndes et al., 2018). Some species will inhabit seagrass meadows throughout their entire life cycle, and others may use seagrass habitats that are connected to adjacent ecosystems, such as mangroves and coral reefs, to fulfil different life stages (French et al., 2021; Henderson et al., 2017; Honda et al., 2013; Unsworth et al., 2008; Whitfield, 2017). Seagrass meadows function as important nursery habitats among coastal systems, promoting higher densities, growth, and survival of juvenile fish, including economically important marine fish species (Jackson et al., 2015; Unsworth et al., 2019; Whitfield, 2017) and penaeid prawns

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(Coles et al., 1993).

Seagrass habitats can be impacted by indirect and direct pressures, including industrial and urban runoff, coastal development, dredging, and natural events such as floods or storms (Cullen-Unsworth and Unsworth, 2018; Grech et al., 2012; Griffiths et al., 2020; Turschwell et al., 2021). These stressors have contributed to a widespread decline in seagrass habitats across temperate and tropical regions, threatening the fish and prawn communities that rely on these habitats for food, shelter, and nursery functions (Dunic et al., 2021; Orth et al., 2020; Rasheed and Unsworth, 2011; Unsworth and Cullen, 2010; Waycott et al., 2009). Given the anticipated changes in degradation and recovery dynamics associated with increased disturbance in seagrass habitats, there is an urgent need for improved monitoring and management strategies aimed at restoration and conservation of these habitats (Cullen-Unsworth and Unsworth, 2016; Greening et al., 2023; Munsch et al., 2023; Murphy et al., 2021; Nowicki et al., 2017; O'Brien et al., 2018).

Effective monitoring of fish and prawn assemblages in seagrass meadows provide critical insights into ecosystem condition and function, particularly through the use of ecological indicators such as species richness, trophic composition or the presence or absence of specific trophic groups (French et al., 2021; Henseler and Oesterwind, 2023; O'Brien et al., 2018). However, the success of these assessments depends largely on the suitability of the sampling methodology to the environmental context. Seagrass habitats are influenced by a range of environmental conditions including turbidity, upwelling events, nutrient levels, salinity, light availability and seasonal rainfall events that can impact sampling effectiveness (Bridges et al., 1982; Carruthers et al., 2002; De Boer, 2007). High turbidity, common in the tropics due to high seasonal rainfall, poses challenges for visual sampling methods, favouring alternatives methods such as environmental DNA (eDNA), capture methods, or acoustics (Franco et al., 2012; Kumar et al., 2022; Souza Jr et al., 2023). Depth, bathymetry, and currents further influence the spatial distribution and logistics of sampling seagrass meadows (Gullström et al., 2008), with some species recorded as deep as 60 m (Unsworth et al., 2019). Assessing deeper meadows often requires the use of remote sampling techniques, including deep trawls, baited remote underwater videos (BRUVs), drop cameras, and autonomous underwater vehicles (AUVs) (Martin et al., 2023). The selection of appropriate sampling methods requires careful consideration of both the research objectives and the physical and ecological conditions of the seagrass system (Baker et al., 2016; French et al., 2021; Kiggins et al., 2018)

Seagrass meadows form complex three-dimensional habitats, that vary both spatially and structurally, posing significant challenges for selecting the appropriate sampling methodology (Dorenbosch et al., 2007; Heck and Orth, 1980; Henderson et al., 2017; Horinouchi, 2007; Skilleter et al., 2017; Unsworth et al., 2008). This structural complexity creates distinct ecological niches that influence the distribution and behaviour of fish communities present, and their detectability (Henderson et al., 2017). Habitat structure varies at multiple spatial scales – from canopy height and the presence of epiphytes at a small scale, patchiness and seagrass species composition at a medium scale, to broader seascape connectivity at a larger scale (Gullström et al., 2008; Henderson et al., 2017; Hyndes et al., 2003; Jackson et al., 2006; Unsworth et al., 2008). Understanding the life history traits, behavioural patterns, and morphological characteristics of marine fauna within seagrass meadows is therefore crucial for determining suitable sampling methodologies (Henseler and Oesterwind, 2023). Feeding ecology and diel movements influence vertical positioning in the water column, while morphological traits, such as body size, shape, and caudal fin structure, affect the movement of the fish and therefore gear selectivity. A clear understanding of these community dynamics is important for mitigating methodological biases in these diverse habitats.

This review evaluates the common sampling approaches used to assess juvenile and small fish and prawn assemblages in seagrass habitats. We examine the strengths and limitations of capture, sensory and molecular approaches, and propose how integrating multiple methods can enhance biodiversity monitoring in seagrass habitats. Fig. 1 illustrates sampling methods suited to high and low turbidity conditions, and different fish community dynamics, offering guidance for method selection based on environmental condition in tropical seagrass habitats.

2. Sampling techniques for diverse seagrass habitats and faunal assemblages

Assessing fish and invertebrates in seagrass habitats requires a diverse range of monitoring techniques tailored to site-specific conditions and the research objective. These methods can be broadly categorised into three groups: capture methods, sensory methods, and molecular methods. Capture methods involve the physical collection of fauna using gear such as trawl nets, seine nets, pop nets, and traps (Guest et al., 2003; McNeill and Bell, 1992). Sensory methods require an observation of the fauna, either directly through diver led underwater visual censuses (UVCs) or remotely such as baited remote underwater video systems (BRUVs) and remote underwater video systems (RUVs), or sonar (French et al., 2021; Olson et al., 2023). Molecular methods analyse environmental samples, such as water or sediment, that contain shed or excreted genetic material, with eDNA metabarcoding a widely used approach (Taberlet et al., 2012; Thomsen et al., 2012).

These methods can be further classified based on their operational mode into either active or passive methodologies. Passive methods, such as traps, baited and unbaited remote underwater videos (BRUVs and RUVs), and sonar, are stationary, although re-deployable at other locations, and measure the abundance according to activity levels over a generally unspecified area (Dorn et al., 2005; Henseler and Oesterwind, 2023; Portt et al., 2006). In contrast, active methods, such as trawling, seine nets, and camera tows, can define specific sampling areas, enabling the measurement of density per unit area (Morrison and Carbines, 2006). The collection of eDNA can be both active, with immediate on-site filtration (Thomsen et al., 2012; Yamanaka et al., 2016), or passive, with deployments of devices to capture DNA over longer periods of time (Bessey et al., 2021; Chen et al., 2022; Maiello et al., 2022; Zhang et al., 2024).

The assessment of marine biodiversity depends on selecting the most appropriate sampling methodologies, each with distinct advantages and limitations. Each method can be influenced by site-specific conditions, which can be problematic for effectively detecting temporal and spatial changes of marine fauna (DiBattista et al., 2022). Capture-based methods can be influenced by variables such as mesh size, towing speed and/or the bait used, as well as environmental factors such as the weather, turbidity, and habitat complexity (Henseler and Oesterwind, 2023; Kubečka et al., 2012; Morrison and Carbines, 2006; Portt et al., 2006; Rozas and Minello, 1997). Sensory methods, such as BRUVs, are subject to biases from species-specific attraction to bait which can influence biodiversity estimates (Schramm et al., 2020; Stat et al., 2019). Molecular approaches, including eDNA metabarcoding methods, are affected by DNA shedding and degradation rates, as well as water movement, and flushing times, making accurate estimates of species' abundance challenging (Huerlimann et al., 2020; Wang et al., 2021). Additionally, accessibility, visibility, and safety challenges in the marine environment can make it difficult to quantify biodiversity at some sites (Donaldson et al., 2019). Integrating multiple sampling methods can therefore enable a more robust assessment of marine fauna by capturing different species and life stages, mitigating against possible biases associated with a single approach (Henseler and Oesterwind, 2023).

2.1. Capture methods

Capture methods used to assess fish and prawn assemblages include a wide variety of equipment and techniques, often relying on traditional fishing methods that physically capture target animals (Guest et al., 2003; McNeill and Bell, 1992; Pierce et al., 1990). Commonly used capture devices for sampling in seagrass habitats include: beam trawls,

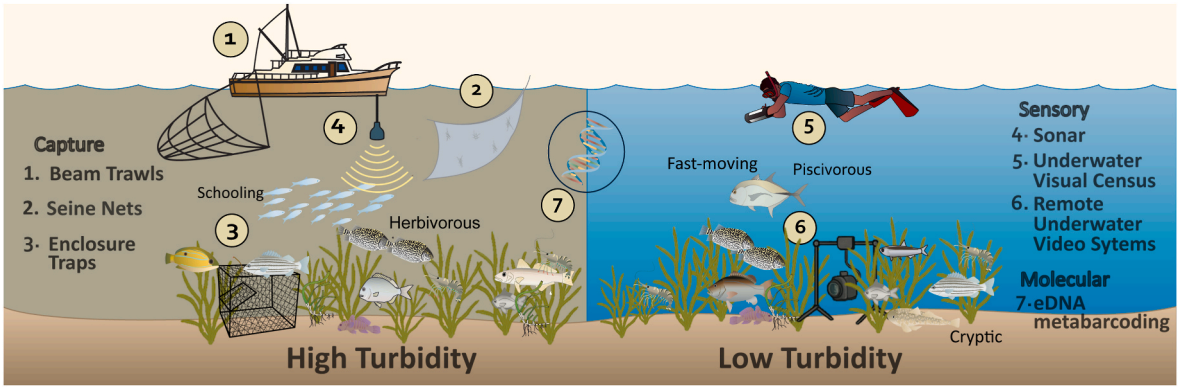

Fig. 1. – Schematic diagram illustrating seven common survey methods (1–7) used to assess fish and prawn communities in seagrass habitats under high and low turbidity conditions. Methods are grouped into three categories: capture-based methods (1–3: trawling, seine nets, and traps), effective in turbid environments but also effective in low turbidity, allowing use across all conditions; sensory-based methods (4–6: sonar, remote underwater video systems (RUVs), and underwater visual census (UVC)), with RUVs and UVCs dependent on low turbidity; and molecular methods (7: environmental DNA (eDNA) metabarcoding), which can detect marine organisms in high and low turbidity. High turbidity environments (left) are represented with brown-toned waters and low turbidity environments (right) with clear blue waters, reflecting their influence on method suitability and fish community visibility. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

seine nets, cast nets, fyke nets, gill nets, minnow traps, enclosure (drop, pop and throw) nets, electrofishing, longline, spearfishing, and hook and line (Coles et al., 1993; Connolly, 1994; Guest et al., 2003; Henseler and Oesterwind, 2023; McNeill and Bell, 1992; Petrik and Levin, 2000; Portt et al., 2006). Capture methods are widely used because these methods provide a measure of catch per unit effort (CPUE) as an indicator of relative fish abundance. Their cost-effectiveness and simple design make them repeatable sampling tools for monitoring habitats (Cappo and Brown, 1996). As sampling techniques evolve and technology advances, integrating emerging approaches with traditional capture methods will enhance marine biodiversity assessments (see Table 1).

2.1.1. *Active capture methods (Beam Trawls, Seine Nets and Enclosure Traps)*

Beam trawls and seine nets are two widely used active methodologies for sampling fish and prawns in seagrass habitats (Coles et al., 1993; Guest et al., 2003; Harmelin-Vivien and Francour, 1992; Jenkins et al., 1996; McNeill and Bell, 1992; Meyer et al., 1999; Watson et al., 1993). These methods can provide detailed biological and morphological data on individual fish including: weight, length, sex, maturity, and physiological condition (Hammerl et al., 2024). Additionally, caught specimens can be further analysed through extraction of otoliths for age and growth studies, genetic samples for population and phylogenetic analyses, and gut content for dietary assessments. Beam trawls are a simple and effective methodology used to identify species within a defined area, but are extractive and can cause destruction to the benthos (Hammerl et al., 2024; Meyer et al., 1999). In seagrass meadows, trawls are a commonly used and reliable method, often capturing a greater species richness and diversity compared to visual methods, such as UVCs or stereo video (French et al., 2021). Trawls are particularly effective at monitoring spatial and temporal variations in small juvenile fish and prawn populations that typically inhabit seagrass meadows, providing standardised, quantitative data on marine fauna whilst sampling across different habitat patches and environmental conditions (French et al., 2021; Hyndes et al., 2018).

Seine nets have been widely used to assess juvenile fish populations in nearshore seagrass habitats, providing a simple and cost-effective method for sampling fauna within a defined area (Blaber and Blaber, 1980; Connolly, 1994; Guest et al., 2003; He et al., 2022; Jenkins et al., 1996; Pierce et al., 1990). These nets are particularly effective in highly turbid environments and have proven successful at sampling rare and cryptic species within seagrass habitats (Baker et al., 2016; Blaber and Blaber, 1980). Comparative studies have shown that seine nets can be more effective than beam trawls at capturing the density and proportion of marine fauna in seagrass habitats (Guest et al., 2003). However, their slower retrieval makes them prohibitive in capturing larger, more mobile fish that seek refuge within the seagrass canopy (French et al., 2021).

Enclosure (pop, drop and throw) traps actively capture fish and prawns within a small area at a single point in time (Portt et al., 2006). These methods are highly suited to vegetated habitats with little water movement (Portt et al., 2006). Connolly (1994) found that pop nets outperformed seine nets in capturing fish, particularly in providing a more accurate estimation of bottom-dwelling species. However, whilst effective for more targeted studies, these enclosure traps prove less suitable for biodiversity assessments due to their limited spatial coverage and labour-intensive deployment (Guest et al., 2003; Rozas and Minello, 1997).

2.1.2. *Passive capture methods (Traps, enclosure traps, and gillnets)*

Passive capture sampling techniques involve leaving fishing gear in place for a period of time before retrieval, relying on fish movement and interaction for capture to occur (Mehdi et al., 2021; Portt et al., 2006). As a result, their effectiveness is influenced by fish behaviour, gear placement, and selectivity, as different gear types can be biased, and introduce selectivity for certain species, sexes, sizes and habitats (Coté and Perrow, 2006; Hammerl et al., 2024; Murphy and Willis, 1996). Passive capture sampling methods are effective in seagrass habitats as they can often be deployed in dense vegetation and high turbidity. These techniques can be also deployed for extended sampling periods, including both day and night deployments, increasing the likelihood of detecting a more representative range of species (Lapointe et al., 2006). This makes passive methods suitable for long-term monitoring, particularly in dynamic seagrass habitats. Common passive sampling gears include minnow traps, gillnets, and enclosure devices for assessing fish abundance (Petrik and Levin, 2000).

Gillnets are a passive sampling method, relying on the fish to actively swim into, and entangle, in the gear. Their effectiveness and selectivity are influenced by several factors, including mesh size, panel length, material used, and the diel activity of the animals, often resulting in biases towards certain trophic groups (Henseler and Oesterwind, 2023). Gillnets can be size-selective, catching larger individuals, whilst underestimating smaller fish (Henseler and Oesterwind, 2023; Portt et al., 2006). They are also less effective at catching species with eel-like or elongated body shapes, emphasising their size selectivity and potential biases in community assessments (Henseler and Oesterwind,

Table 1
The advantages and disadvantages of capture methods to assess biodiversity of marine fauna, applicable to sampling seagrass habitats.

	Methods	Captured Information	Advantages	Disadvantages
Active	Trawling	• Species identification • Species abundance • Species richness • Biomass • Physiological data	• Simple, cost-effective method to collect data from a defined area (Pierce et al., 1990) • Live collection of fish species (Pierce et al., 1990) • More reliable at detecting smaller species (French et al., 2021) • They can be used to measure diel shifts in marine organisms (Cappo et al., 2004) • Trawls can be used in different sea states and levels of turbidity (Cappo et al., 2004)	• Difficulty in capturing fast moving fish (French et al., 2021) • Dependent on environmental conditions, making continuous monitoring efforts more difficult (Oka et al., 2021) • Can cause damage to seagrass (Meyer et al., 1999) • Cause mortality of captured fish species – particularly vulnerable juvenile fish (Baker et al., 2016) • It may be difficult to obtain authorisation and permits for sampling of rare species or in areas off-limits to trawling (Hightower et al., 2013) • Trawling nets are selective of the size of fish caught (Hightower et al., 2013) • Difficult to use in areas with high currents or with bottom obstructions (Hightower et al., 2013)
Active	Seine Netting	• Species identification • Species abundance • Species richness • Biomass • Physiological data	• Considered the best method for determining the relative proportion of species in a seagrass habitat (Guest et al., 2003) • Seined fish can undergo significant stress, but are usually not injured (Portt et al., 2006)	• Benthic fishes are less likely to be captured than mid-water species (Gross et al., 2018) • They are restricted to shallow areas (Henseler and Oesterwind, 2023) • Easily snag on physical obstructions (Pierce et al., 1990)
Active	Enclosure Traps (pop, drop and throw)	• Species identification • Species abundance • Species richness • Biomass • Physiological data	• Provides an accurate estimation of bottom dwelling species in seagrass habitats (Connolly, 1994)	• Not efficient for broadscale surveys (Guest et al., 2003) • Dense vegetation can impede the nets descent through the water column (Portt et al., 2006) • Fast-swimming species are able to avoid falling traps (Kushlan, 1974)
Passive	Gill Nets	• Species identification • Species abundance • Species richness • Biomass • Physiological data	• One of the most efficient passive methods for capturing overall diversity in seagrass habitats (Henseler and Oesterwind, 2023)	• Light, turbidity and net colour can affect catch (Portt et al., 2006) • Catch efficiency can be reduced with time if the nets become fouled with debris (Hamley, 1975). • They are not effective for catching sedentary fish (Portt et al., 2006) • They can injure fish severely, resulting in high mortality (Portt et al., 2006)
Passive	Traps (Minnow)	• Species identification • Species abundance • Species richness • Biomass • Physiological data	• Can be used in structurally complex habitats (Hammerl et al., 2024) • Fish can often be released alive (Miller, 1990) • Easily transportable and can be deployed by one person (Portt et al., 2006)	• Cannot determine the sample area (Hammerl et al., 2024) • Influence of environmental conditions difficult to measure – including currents and bait plume (Stoner, 2004). • Size-selective (Hubert, 1996)

2023).

Minnow traps are small, lightweight, passive sampling devices composed of a mesh frame, designed to trap fish and prawns over an extended period of time (Petrik and Levin, 2000). They are easily deployed by one person, and well suited for use in low currents, particularly within structurally complex habitats such as seagrass meadows (Portt et al., 2006). However, inconsistent estimates of fish diversity and lower catch efficiency have been identified in minnow traps compared to alternative methods such as gillnets (Henseler and Oesterwind, 2023). Minnow traps exhibit selectivity biases, favouring certain mobile species such as seabream, while under-sampling small or sedentary taxa, including pipefish, seahorses, and gobies, despite their documented presence in seagrass habitats (Gray and Bell, 1986; Hyndes et al., 2003; Rudershausen et al., 2003).

Both active and passive capture methods are widely used to assess marine biodiversity and have been used extensively in temperate and tropical seagrass habitats (Coles et al., 1993; Guest et al., 2003; McNeill and Bell, 1992; Watson et al., 1993). Active capture methods, such as beam trawls and seine nets, provide detailed, real-time data on fish and invertebrate populations, which is invaluable for monitoring marine communities (Hammerl et al., 2024). However, these methods are associated with greater habitat disturbance, higher bycatch of threatened species, and increased labour and operational costs (Hammerl et al., 2024; Henseler and Oesterwind, 2023; Meyer et al., 1999). Many research studies using active capture methodologies now require permits before they can be undertaken, which can be difficult to obtain (French et al., 2021).

In contrast, passive methods, such as minnow traps, can be effective in dense, turbid, seagrass habitats providing a low-disturbance and cost-effective alternative (Petrik and Levin, 2000). Passive methods generally require less labour and typically have lower operational costs, but it can be challenging to standardise the sampling effort and area, making direct comparisons with active methods difficult (Portt et al., 2006).

The respective strengths and limitations of passive and active capture methods are summarised in Table 1. Although capture methods can provide detailed biological data on fish and prawns, their extractive nature and disturbance to habitats has led to a decline in their use for biodiversity assessments, especially within marine protected areas (Hightower et al., 2013). Nevertheless, future advancements in capture methodologies, such as refining gear selectivity and mesh size, may enhance their efficiency and ethical application, particularly when biological data is essential for the specific research objectives (Christiansen et al., 2022). However, fewer technological innovations for capture-based methods are being pursued due to the shift in focus towards non-extractive alternatives, including sensory and molecular methods.

2.2. Sensory methods

Visual, acoustic, and sonar methods, under the collective term “sensory methods”, are a non-destructive alternative for assessing fish and prawn assemblages. These approaches vary in their level of interaction with the environment, ranging from direct in situ surveys, such as underwater visual censuses, to remotely operated systems, such as

Table 2
The advantages and disadvantages of sensory methodologies used to assess biodiversity of marine fauna, applicable to sampling seagrass habitats.

	Methods	Captured Information	Advantages	Disadvantages
Active	Underwater Visual Census (UVC)	• Species identification • Species abundance • Species richness	• Methodology is easy to undertake (Holmes et al., 2013) • Non-extractive, therefore reducing impacts on environment (Franco et al., 2012) • Cost-effective, with minimal equipment and personnel needed (Franco et al., 2012) • Data is counted in-situ (Holmes et al., 2013)	• Some species of fish may be attracted or repelled from divers or snorkellers (Watson and Harvey, 2007) • It is difficult to identify cryptic and small species in dense or turbid seagrass areas (Franco et al., 2012; Holmes et al., 2013) • Potential observer bias where there is an overestimation of fish and/or transect boundary (French et al., 2021) • Observer expertise needed for taxonomic identification, which cannot be validated post-fieldwork (Holmes et al., 2013) • Accessibility and safety can make it unsuitable to undertake at the site (Donaldson et al., 2019).
Active	Diver Operated Video	• Species identification • Species abundance • Species richness • Biomass	• Enables identification and size estimation of fish post-fieldwork, with videos stored as a permanent record (Goetze et al., 2019) • Ability to quantify the habitat type and habitat complexity associated with fish assemblages (Goetze et al., 2019)	• Difficulty in surveying cryptic species in seagrass blades due to poor resolution of video image (Holmes et al., 2013) • Increase in the time spent analysing videos post-fieldwork (Jessop et al., 2022).
Passive	Baited and unbaited remote underwater videos (BRUVs and RUVs)	• Species identification • Species abundance • Species richness • Biomass	• They can be deployed in different habitats and depth ranges (Schramm et al., 2020) • Reduced diver error in abundance and size estimations of fish (Baker et al., 2016) • Less reliance on expertise required for taxonomic identification – users can capture image and repeat video playback, with access to identification guides and experts post-fieldwork (Holmes et al., 2013) • Less destructive and commonly used in marine protected areas (Wraith et al., 2013)	• The large amount of footage collected requires longer time periods for data analysis (Langlois et al., 2010) • Small and cryptic fish can be difficult to observe (Smith et al., 2012). • Obstructions to the camera field of view in seagrass habitats can lead to misrepresentation of species abundance (Kiggins et al., 2018) • Baited/Non-baited camera systems may be discriminatory towards certain fish assemblages (Harvey et al., 2007)
Passive	Hydroacoustics	• Species abundance	• Enable rapid survey assessments (Papastamatiou et al., 2020) • Can assess the habitat use and biological interactions between marine fauna (Papastamatiou et al., 2020) • Is non-invasive (Hightower et al., 2013) • Can be used at night and in low visibility (Helminen and Linnansaari, 2021)	• Unlikely to detect smaller animals, invertebrates, and bottom associated mobile marine fauna (Bradley et al., 2023) • Difficult to detect fish identity, size or sex (Bradley et al., 2023; Papastamatiou et al., 2020) • Affected by environmental conditions such as wind, waves or entrapped air (Boswell et al., 2007)

sonar. Method selection can influence species detection and biodiversity estimates, depending on habitat characteristics and species behaviour. A comparison of the strengths and limitations of each sensory method is provided in Table 2.

2.2.1. Active sensory methods

Underwater visual census (UVC), is a commonly used method undertaken by snorkellers or SCUBA divers to assess fish diversity and assemblages. It is considered an active method, in which a diver counts and identifies fish along a transect in a predefined area (Brock, 1954; Edgar and Stuart-Smith, 2014; Goetze et al., 2019). UVCs are cost-effective, with limited impact on the area surveyed, and repeatable, allowing measurements of fish abundance, density, and size (Harvey et al., 2004). However, they rely on the diver's ability to accurately estimate species size and abundance, and correctly identify species, which can introduce observer bias (French et al., 2021). Detecting cryptic and smaller species is particularly challenging in dense or turbid seagrass habitats (Franco et al., 2012; Holmes et al., 2013). UVCs also carry logistical and safety challenges in environments with marine stingers, crocodiles, sharks or strong currents (Donaldson et al., 2019).

Diver-operated videos (DOV) are often incorporated with UVCs, to enhance data quality through post-fieldwork analysis of footage (Goetze et al., 2019). DOV can improve the accuracy of fish measurements and counts, but it may also influence fish behaviour, with some species actively avoiding cameras and seeking refuge in the seagrass canopy (French et al., 2021). Identifying fish from the captured footage has also been shown to be more difficult than in-situ observations, resulting in a lower observed species richness compared to UVCs (French et al., 2021; Holmes et al., 2013). These factors highlight the importance of aligning survey methods with specific environmental conditions and research objectives when monitoring marine fauna in seagrass habitats.

2.2.2. Passive sensory methods

Passive sensory methods are non-invasive techniques that enable the observation of fauna without direct human interference, making them particularly valuable in remote or sensitive marine ecosystems (Cappo et al., 2003; Schramm et al., 2020; Wraith et al., 2013). Among these, remote underwater video systems are widely used to monitor marine biodiversity and are typically deployed as either baited (BRUVs), or unbaited (RUVs) units to assess species abundance across a range of habitats and depths (Rhodes et al., 2020; Schramm et al., 2020). These systems utilise single (mono), or dual (stereo) video setups, with stereo cameras enabling the measurement of individual body lengths and estimation of biomass (Cappo et al., 2003; Langlois et al., 2020). These systems have proven to be effective as they are non-destructive, have low equipment costs, and have the ability to observe rare species, making them a safe and accessible tool for assessing fish and prawn populations (Cappo et al., 2003; Goetze et al., 2019; Langlois et al., 2020; Schramm et al., 2020; Wraith et al., 2013).

The choice between baited or unbaited systems introduces inherent observational biases that can influence the taxonomic composition of observed marine assemblages (Harvey et al., 2007). Baited systems can attract marine fauna over an unknown area due to the dispersal of bait plume, potentially impacting detectability across different trophic groups (Dorman et al., 2012; Harvey et al., 2007; Rhodes et al., 2020; Schramm et al., 2020). Additionally, both BRUVs and RUVs are constrained by uncertainty in the effective detection range at which fish are attracted to the camera system (Cappo et al., 2004). To overcome this challenge, the MaxN metric, defined as the maximum number of individuals of a species observed in one frame, is used to generate standardised estimates of relative abundance (Cappo et al., 2003). This metric reduces the likelihood of overestimating marine species abundance but could underestimate the true abundance of fish populations (Schramm et al., 2020; Sherman et al., 2018). Although BRUVs have been successfully used in seagrass habitats to capture a diverse range of fish communities, their effectiveness may be reduced by obstruction from seagrass blades and reduced visibility in turbid environments (Espadero et al., 2020; Gladstone et al., 2012; Kiggins et al., 2018; Whitmarsh et al., 2014, 2017).

Hydroacoustic methods operate by the transmission and reflection of sound waves through water to infer information about the size, abundance, and spatial distribution of fish populations (Becker and Suthers, 2014; Coté and Perrow, 2006; Giorli et al., 2018; Hammerl et al., 2024; Jones et al., 2021; Sibley et al., 2023). Depending on the research objective, methods can include split-beam echosounders, side-scan sonar and multibeam sonar, acoustic cameras, acoustic telemetry, and passive acoustic monitoring (Boswell et al., 2007; Hammerl et al., 2024). These techniques are particularly advantageous for sampling the marine environment in low visibility, across different tidal ranges, and throughout the diel cycle, enabling insights into fish habitat use during both diurnal and nocturnal periods (Becker and Suthers, 2014; McSpadden et al., 2024; Sibley et al., 2023).

Acoustic methods remain limited in their ability to identify fish to species level, however advances incorporating species-specific acoustic signatures are improving taxonomic resolution (Sibley et al., 2023). Hydroacoustic techniques are frequently used in combination with traditional sampling methods to enhance assessments of fish biomass and size distribution. For example Boswell et al. (2007) demonstrated the value of integrating hydroacoustics alongside gill nets and trawls. Sonar imaging, a specific hydroacoustic technique, involves transmitting a sonar pulse and analysing its reflection to produce two-dimensional representations of fish densities and spatial distributions (Bradley et al., 2023; Daniel et al., 1998). This method has been used effectively to estimate fish abundance in structurally complex habitats such as seagrass meadows (Boswell et al., 2007; Olson et al., 2023; Wilson et al., 2013) and for mapping the spatial distribution of schools of fish (Bradley et al., 2023; Leonori et al., 2021; Rourke et al., 2025). While sonar provides high-resolution data on fish biomass and movement, there has been limited success at distinguishing species or genus (Sibley et al., 2023).

2.2.3. Advances in sensory methodologies: enhancing accuracy through technical innovations

Emerging technologies, such as passive acoustic monitoring (PAM), utilise sound recording devices to analyse soundscapes to provide an indicator of ecosystem health (Lamont et al., 2022). PAM has shown considerable potential for detecting the frequency and occurrence of individual fish species, with applications for understanding spatiotemporal patterns and habitat use by marine fauna (McWilliam et al., 2017). These technological developments represent a promising, non-invasive approach for long-term monitoring of seagrass ecosystems.

The integration of technological advancements in sensory methods further promotes their wider use for ecological assessments. A major limitation of stereo video systems is the time required to process the video footage, and the challenges of accurately identifying fish species (Jessop et al., 2022; Rauf et al., 2019). Advances in machine learning and automated fish recognition technologies are addressing these limitations by enabling the accurate identification of fish from underwater video imagery, significantly reducing manual effort in post-survey video processing (Shafait et al., 2016). Siddiqui et al. (2017) reported a classification accuracy of 94.3 % for fish species in Western Australia using automated recognition models, a performance comparable to that of the identification rate of human observers. Similarly, Connolly et al. (2023) applied machine learning to count and detect fish in sonar datasets, further highlighting the potential of automated systems.

However, these systems are currently limited to predefined species list, with building and training models remaining a time-intensive process, requiring large datasets to ensure accurate classification across a diverse range of habitats (Connolly et al., 2023). Despite these challenges, these advances in automated classification systems offer a cost-effective and feasible alternative to manual identification, with the potential to enhance the consistency, efficiency and taxonomic

resolution of biodiversity surveys (Siddiqui et al., 2017). As these systems continue to evolve, they could become widely adopted for reliable and standardised monitoring of marine fauna in seagrass habitats.

Sensory methods provide a non-invasive alternative for assessing marine fauna assemblages, particularly in sensitive or remote seagrass habitats. Active methods such as UVC enable in-situ sampling, but they can be difficult for identifying cryptic and small species within dense seagrass habitats, introducing observer bias and even repelling fish species (French et al., 2021; Holmes et al., 2013; Watson and Harvey, 2007). In contrast, passive methods, such as BRUVs, minimise disturbance to seagrass habitats, but can be biased towards certain taxonomic groups, especially towards species attracted to the bait (Harvey et al., 2007; Wraith et al., 2013). Additional factors, such as turbidity, habitat complexity, and diurnal changes in fish behaviour, can further influence species detectability and species richness estimates using these approaches (Bacheler et al., 2017; Edgar et al., 2001; Figueroa-Pico et al., 2020). However, advancing technologies, including passive acoustic monitoring and machine learning, are overcoming some of these limitations by streamlining analyses and improving detection accuracy (Connolly et al., 2023; Helminen and Linnansaari, 2021; Shafait et al., 2016; Siddiqui et al., 2017). Therefore, integrating multiple sensory methods has been shown to enhance biodiversity assessments by capturing a broader range of species across temporal and spatial scales in seagrass habitats (Cappo et al., 2004; French et al., 2021; Harmelin-Vivien and Francour, 1992; Morrison and Carbines, 2006).

2.3. Molecular techniques

Molecular techniques, particularly environmental DNA (eDNA), have transformed our ability to assess communities in dynamic marine habitats. Environmental DNA is a non-invasive, molecular technique that detects genetic material shed by organisms in the environment in the form of mucus, decaying matter, blood, faeces, and other biological matter (Kelly et al., 2014; Oka et al., 2021; Taberlet et al., 2012). By extracting the DNA from environmental samples, including water, sediment, and air, eDNA methods enable a highly sensitive tool for detecting species, including those that are rare, cryptic, or difficult to detect using traditional methods (Ficetola et al., 2008; Ruppert et al., 2019; Villacorta-Rath et al., 2022). These approaches enhance detection sensitivity, expand spatial and temporal coverage, and reduce habitat disturbance – key advantages summarised in Table 3. Environmental DNA metabarcoding has been successfully applied across diverse marine ecosystems, from deep-sea environments (Thomsen et al., 2016) to coastal seagrass meadows (Waters et al., 2023).

Environmental DNA methods generally fall into two categories: targeted single-species detection and metabarcoding (McColl-Gausden et al., 2023). Single-species detection uses targeted primers in combination with quantitative PCR (qPCR) or droplet digital PCR (ddPCR) to detect specific taxa (Hunter et al., 2017; Jerde et al., 2011). These methods, in many cases, have shown strong correlations between eDNA copy number and species biomass or abundance, offering a more targeted approach for species-specific monitoring (Guri et al., 2024; Pont et al., 2023; Rourke et al., 2022).

Conversely, eDNA metabarcoding uses general primers targeting conserved genomic regions across taxa, coupled with high-throughput next-generation sequencing (NGS), to detect multiple species simultaneously within a single environmental sample (Taberlet et al., 2012). Environmental DNA metabarcoding has proven to be a valuable and complementary tool for biodiversity assessments, however, it is limited in reliably estimating biomass and absolute abundance of individual species (Lacoursière-Roussel et al., 2016b; Waters et al., 2023). These limitations can be attributed to methodological differences including DNA extraction protocols, marker and primer choice, sequencing depth, completeness of taxonomic reference libraries and differences in bioinformatic pipelines (Bessey et al., 2021; Keck et al., 2022; McElroy et al., 2020). Integrating eDNA with traditional survey methods is often

Table 3
The advantages and disadvantages of eDNA metabarcoding to assess biodiversity of marine fauna, applicable to sampling seagrass habitats.

	Methods	Captured Information	Advantages	Disadvantages
Active	Water Samples Sediment Samples Plankton Tows	•Species identification •Species richness	•Provides species identification without needing visual sight of the animal (Jerde et al., 2011) •Cost-effective and non-destructive (Bessey et al., 2020) •Captures a wide diversity of marine taxa (Thomsen et al., 2012) •Species can be identified from their DNA sequences, which can be more accurate for taxonomic identification at early life stages and for small and cryptic species (Pegg et al., 2006) •Highly sensitive tool for detecting species, including those that are rare, cryptic, or difficult to detect using traditional methods (Ficetola et al., 2008; Ruppert et al., 2019; Villacorta-Rath et al., 2022). •Greater understanding of temporal and spatial distribution of species (Huerlimann et al., 2020; Simpfendorfer et al., 2016) •Samples can be preserved and archived, enabling potential future re-analysation with advancement in metabarcoding methodologies (Deiner et al., 2017) •eDNA can be preserved in the sediment for extended periods of time (Huston et al., 2023; Zhu et al., 2023) •Lower decay rate of eDNA in sediment samples	•Different sized individuals and taxa shed eDNA at different rates, affecting levels of detection (Rourke et al., 2022) •Difference in decay rate of eDNA in the environment leading to false-negative detection (Huerlimann et al., 2020; Rourke et al., 2022) •Abiotic factors such as amount of rainfall, temperature, UV, and pH, can affect persistence of eDNA in marine system, particularly in the tropics (Huerlimann et al., 2020) •Incomplete reference database for taxonomic groups (Keck et al., 2022; Weigand et al., 2019) •Currently cannot capture data on size, sex and absolute abundance of fish (Waters et al., 2023). •Currently no standardised method of collection or metabarcoding workflow (Bessey et al., 2021; Keck et al., 2022; McElroy et al., 2020) •Legacy eDNA can be detected even when a species is no longer present

(*continued on next page*)

Table 3 (*continued*)

	Methods	Captured Information	Advantages	Disadvantages
			compared to water samples (Huston et al., 2023; Sakata et al., 2020)	
Passive	Submerged Sampling Device	•Species identification •Species richness	•Samples can be collected over an extended period of time (Bessey et al., 2021) •Greater detection of rare species by maximising exposure time (Zhang et al., 2024) •Higher sampling rate across broader spatial scales (Bessey et al., 2021) • Potential for automated sample capture and preservation, which provides the potential for remote sample collection (Yamahara et al., 2019)	•Difficulty in quantifying the volume of water (Morris et al., 2024)

recommended to achieve a more comprehensive understanding of fish and prawn assemblages in the marine environment (Afzali et al., 2021; Maiello et al., 2022; Thomsen et al., 2016).

Environmental DNA samples can be collected using a variety of methods, typically categorised as either active or passive (Buxton et al., 2018; Deiner et al., 2017; Huston et al., 2023; Koziol et al., 2019; Sakata et al., 2020; Turner et al., 2015). Active sampling involves the direct collection of environmental material, such as filtering water, or manually collecting sediment, at a specific time or across a defined transect (Bessey et al., 2022; Cananzi et al., 2025). Passive sampling involves deploying materials such as membranes, loose sorbent, or gauze, that remain submerged to capture organic material over time (Bessey et al., 2022; Chen et al., 2022; Maiello et al., 2022). Each approach has distinct advantages and their combined use can improve species detection both spatially and temporally at a site (Zhang et al., 2024).

The choice between water or sediment sampling depends on the target species, system dynamics and monitoring objectives (Sakata et al., 2020). Water samples typically reflect a broader spatial scale and may be more suitable for detecting biological communities (Shaw et al., 2016). The persistence of eDNA in sediment is influenced by environmental conditions and sediment geochemistry, where eDNA can persist for an extended period of time and can be more concentrated than in water samples leading to enhanced detection rates and better assessments of site occupancy (Buxton et al., 2018; Huston et al., 2023; Sakata et al., 2020; Turner et al., 2015).

While eDNA metabarcoding has emerged as a powerful tool for biodiversity assessments in marine systems, its detection can be influenced by environmental and methodological factors (Afzali et al., 2021; He et al., 2022; Pont et al., 2023; Sato et al., 2021; Stat et al., 2019; Thomsen et al., 2016). DNA degradation rates, variability in DNA shedding rates amongst species, and the horizontal and vertical transport and settlement properties of eDNA can influence detection outcomes (Koziol et al., 2019; Plough et al., 2018; Rice et al., 2018). Additionally, inaccurate species detections can also be caused by the transport of eDNA from connected areas, in cases where the organism was otherwise not present in the sampled environment (Buxton et al., 2018; Huston et al., 2023). Conversely, false-negative detections, often due to organism rarity, can result in an underrepresentation of species richness (Collins et al., 2018).

A key limitation for eDNA metabarcoding's broader application for biodiversity assessments is the incompleteness of reference libraries across different taxonomic groups and geographical regions (Keck et al., 2022; Mathieu et al., 2020; Weigand et al., 2019). Taxonomic classification relies on the matching of DNA sequences to a reference library and gaps in this library can result in misidentifications or failure to assign taxonomy to detected sequences (Claver et al., 2023; Cristescu and Hebert, 2018; Hebert et al., 2016; Keck et al., 2022). Many species remain underrepresented in these databases, and addressing these gaps will require targeted sequencing efforts to improve taxonomic coverage (Marques et al., 2021). Further advancements in sequencing technology, such as long-read sequencing and whole genome approaches, may further refine these reference databases and improve species resolution for eDNA metabarcoding approaches (Adams et al., 2019; Claver et al., 2023).

2.3.1. *Active eDNA sampling*

Active eDNA sampling involves the immediate collection of water or sediment from the marine environment to detect species within a specific time frame and location (Bessey et al., 2020). Optimising the conditions for active eDNA sampling requires careful consideration of the volume and number of replicates of water samples, the pore size of the filters, and preservation methods (Kawakami et al., 2023; Rourke et al., 2022). Water sample volumes can vary significantly between research studies, with sample volumes typically ranging from 400 to 6000 mL for detection of fish DNA (Bessey et al., 2020; Turner et al., 2014). Studies show distinct variations in species detections from the volume of water collected, where small-pore-size filters can be easily clogged and precipitation methods are limited by centrifuge capacity (Cooper et al., 2022; Schabacker et al., 2020). In dynamic tropical regions, such as tropical seagrass meadows, environmental variables such as high seasonal rainfall, elevated temperatures, pH variations, and UV irradiation can significantly affect the detection and dispersion of eDNA within the habitat (Barnes and Turner, 2016; Collins et al., 2018; Huerlimann et al., 2020). High turbidity, for example, can quickly clog filter membranes, reducing the quantity of eDNA captured or increasing the concentration of inhibitors that are co-captured (Huerlimann et al., 2020).

Sediment samples, also actively collected, offer a unique advantage by preserving eDNA in the sediment for longer periods of time compared to water samples, enabling a more comprehensive assessment of community assemblages (Buxton et al., 2018; Huston et al., 2023; Picard et al., 2023; Turner et al., 2015; Zhu et al., 2023). The slower decay rates of eDNA in sediment compared to water samples, enable long-term monitoring of species presence (Buxton et al., 2018). However, less is understood about how sediment type affects the accumulation and preservation of eDNA, particularly fish eDNA (Buxton et al., 2018; Huston et al., 2023; Sakata et al., 2020).

Recent studies have explored the use of tow nets for eDNA collection, which allows the processing of larger water volumes and the targeting of larger particles of eDNA (Bowen et al., 2024; Pochon et al., 2024; Schabacker et al., 2020). These studies have highlighted that the use of large pore size tow nets can increase the detection sensitivity for certain taxa when compared to low volume, small pore size samplers (Cooper et al., 2021; Schabacker et al., 2020; Sepulveda et al., 2019; Turner et al., 2014). With logistical challenges associated with large-scale monitoring, high-volume eDNA sampling could provide a more efficient sampling technique for assessing species diversity, especially in seagrass habitats where biodiversity is particularly challenging to assess in highly turbid waters.

2.3.2. Passive eDNA sampling

Passive eDNA sampling involves submerging a sampling device, such as a filter or artificial substrate, for an extended period to accumulate genetic material from the environment over time (Bessey et al., 2021; Morris et al., 2024; Stevens et al., 2024; Zhang et al., 2024). Unlike active sampling, which captures eDNA at a single time point, passive sampling integrates eDNA collection over many hours or days, increasing the likelihood of detecting marine organisms (Bessey et al., 2021; Zhang et al., 2024). Passive sampling is advantageous for long-term monitoring and detecting rare species by maximising exposure time, although different substrates will saturate at different points in time (Zhang et al., 2024). This method can also introduce biases due to challenges in quantifying the volume of water interacting with the device and there is a limited understanding of how environmental factors, such as turbidity and pH, influence sampling efficiency (Morris et al., 2024; Zhang et al., 2024).

While both active and passive eDNA sampling methods have distinct advantages, their suitability depends on the research objectives and environmental conditions that they are deployed in. Active sampling is preferential for targeted, rapid assessments, whereas passive sampling can be effective for covering a higher spatial and temporal resolution (Zhang et al., 2024). Combining both approaches could enhance the robustness of eDNA studies, providing a more comprehensive understanding of marine biodiversity (Zhu et al., 2023). By continuing to optimise and standardise eDNA metabarcoding methodologies, researchers can improve the reliability of eDNA metabarcoding as a tool for assessing marine communities.

2.3.3. Future directions in eDNA monitoring: towards quantifying species abundance and genetic diversity

A key limitation of environmental DNA (eDNA) metabarcoding is its ability to reliably estimate species abundance, limiting its wider use for assessing marine communities. While eDNA metabarcoding has been widely used to detect species presence, emerging evidence suggests that eDNA could also provide an estimate of the abundance and biomass of species within an ecosystem (Lacoursière-Roussel et al., 2016a; Sassoubre et al., 2016; Spear et al., 2021). However, numerous abiotic and biotic factors influence eDNA concentration, making direct abundance estimates challenging (Rourke et al., 2022; Stewart, 2019). Despite these limitations, continued refinement of sampling and analysis techniques suggests that eDNA metabarcoding could provide more quantitative insights into species abundance and biomass in the near future (Ficetola et al., 2008; Lacoursière-Roussel et al., 2016b; Tillotson et al., 2018).

In contrast to eDNA metabarcoding, targeted molecular approaches using qPCR and ddPCR offer greater precision for estimating species abundance and biomass (Doi et al., 2015; Pilliod et al., 2013; Takahara et al., 2012). These techniques quantify the number of DNA copies in a sample, enabling the detection of target species at low concentrations (Guri et al., 2024). Although they require species-specific primer development, qPCR and ddPCR provide highly sensitive, species-specific data, and multiplexing these different primers allows simultaneous quantification of multiple species (Brys et al., 2023).

In freshwater systems, researchers have explored using sequence read counts and site occupancy models as proxies for abundance (Hänfling et al., 2016), as well as combining eDNA metabarcoding with qPCR to improve quantitative accuracy (Pont et al., 2023). However, replicating this level of resolution in marine systems remains challenging given the dynamics of water movement and transport (Stoeckle et al., 2021; Thomsen et al., 2012). To improve the reliability of eDNA-based abundance estimates further methodological advancements are required in sampling protocols, bioinformatic processing, and our understanding of the environmental factors influencing DNA persistence and detection (Rourke et al., 2022).

The emerging use of environmental RNA (eRNA) offers the potential to improve the temporal and spatial resolution of organism detection (Yates et al., 2023). Due to its greater lability, eRNA is believed to degrade more rapidly in the environment than eDNA, potentially reflecting more recent biological activity (Kagzi et al., 2024). Environmental RNA might therefore increase the resolution of organism detection and provide insights into life-history characteristics such as age class, developmental stage, and sex (Yates et al., 2021). However, the widespread application of eRNA in marine systems remains limited, due to significant methodological and analytical challenges (Cristescu, 2019; Yates et al., 2023).

In addition to species detection, high-throughput sequencing of eDNA has shown promise for assessing genetic diversity and population structure through the analysis of haplotypic variation (Adams et al., 2019; Andres et al., 2023; Stat et al., 2017). Sigsgaard et al. (2016) demonstrated that high-throughput sequencing of eDNA could provide genetic diversity estimates of whale shark (*Rhincodon typus*) populations, highlighting its potential to inform ecosystem-level genetic monitoring. In seagrass habitats, population genetic data derived from eDNA could provide valuable insights into biodiversity and population connectivity, which could inform conservation management strategies (Eble et al., 2020). Using genetic markers may enable population-level assessments and improve estimates of species abundance. However, applying eDNA population genetics to biodiversity assessments in seagrass systems would require rigorous validation and significant technological refinement before it can be used to estimate abundance and genetic diversity of fish and prawn populations at scale (Adams et al., 2019; Andres et al., 2023).

Although the use of eDNA for population genetics and absolute abundance estimates remains in its early stages, future developments of models to determine taxonomic, physiological, and environmental variation of eDNA in the environment, could change how this method is integrated within biodiversity assessments (Eble et al., 2020; Tillotson et al., 2018). At present, eDNA metabarcoding serves as a complementary tool to traditional marine survey methods, but future developments suggest that both eDNA and eRNA could both detect species and infer ecological and demographic patterns of a marine community. Prioritising research that refines these techniques will be critical to realising their broader application in marine biodiversity assessments.

3. Capture, sensory, and molecular methods: A summary

Effective monitoring and management of biodiversity in seagrass habitats requires the careful selection of sampling methodologies based on the research objective, the local environmental conditions, and target species of interest. Capture methods remain valuable for collecting detailed morphological and physiological data, but may be unsuitable in sensitive habitats, leading to ethics and permitting issues and can be biased by gear selectivity and species behaviour (Baker et al., 2016; Hightower et al., 2013; Meyer et al., 1999; Morrison and Carbines, 2006; Portt et al., 2006). Sensory methods, such as BRUVs and hydroacoustic techniques, are less invasive and suitable in clear water, though turbidity and habitat complexity within seagrass meadows can reduce detection, particularly of cryptic species (Franco et al., 2012; Holmes et al., 2013; Kiggins et al., 2018; Smith et al., 2012). Molecular methods, such as eDNA metabarcoding, can capture a wide diversity of species, including cryptic, rare, and morphometrically similar marine fauna, and are highly adaptable to various environmental conditions (French et al., 2021; Huerlimann et al., 2020; Thomsen et al., 2012; Waters et al., 2023). However, the accuracy of eDNA metabarcoding depends on robust primer design and the completeness of taxonomic reference libraries, which can be under-represented in most seagrass habitats (Burian et al., 2023; Keck et al., 2022; Miya et al., 2015; Qiu et al., 2023; Zainal Abidin et al., 2022).

To effectively capture the biodiversity within seagrass habitats, an integrated approach that incorporates a combination of sampling methodologies is therefore desirable (French et al., 2021; Harmelin-Vivien and Francour, 1992; Qiu et al., 2023). Currently, eDNA metabarcoding is a powerful complementary tool to conventional

biodiversity methodologies, improving species detection by capturing cryptic and rare species that might be missed by sensory or capture-based methods. Integrating eDNA metabarcoding with BRUVs or UVCs can therefore enhance biodiversity assessments by capturing both rare and cryptic species as well as larger, more mobile taxa (Pegg et al., 2006). This combined approach provides a more comprehensive assessment of biodiversity and minimises the impact of destructive sampling methods (Deiner et al., 2017). However, given its current limitations on providing data on abundance, size and life history, eDNA metabarcoding is best used as a complementary technique that enhances other sampling methodologies. Continued refinement of eDNA methods, alongside integrative sampling approaches and development of advanced technologies, will be critical to maximising the potential for eDNA metabarcoding to be used in long-term monitoring of fish and prawns in seagrass habitats.

4. Selecting optimal sampling methods for fish and invertebrates in seagrass habitats

Successfully sampling marine biodiversity in seagrass habitats presents challenges due to a range of biotic and abiotic factors including turbidity, currents, habitat complexity, and danger of being in the water (Donaldson et al., 2019; Franco et al., 2012; Rozas and Minello, 1997). Given these constraints, selecting appropriate, minimally destructive methods are crucial. To ensure the selection of the most suitable method/s for sampling fish and prawns in seagrass habitats, we propose a systematic set of questions that can be integrated with Fig. 2 and Tables 1–3 to guide the decision-making process.

4.1. What are the objectives of the study?

Designing an effective study relies on selecting sampling methodologies that align with the ecological and biological variables to achieve the study's objectives. The three sampling methods – capture, sensory, and molecular – obtain different metrics on the marine fauna sampled.

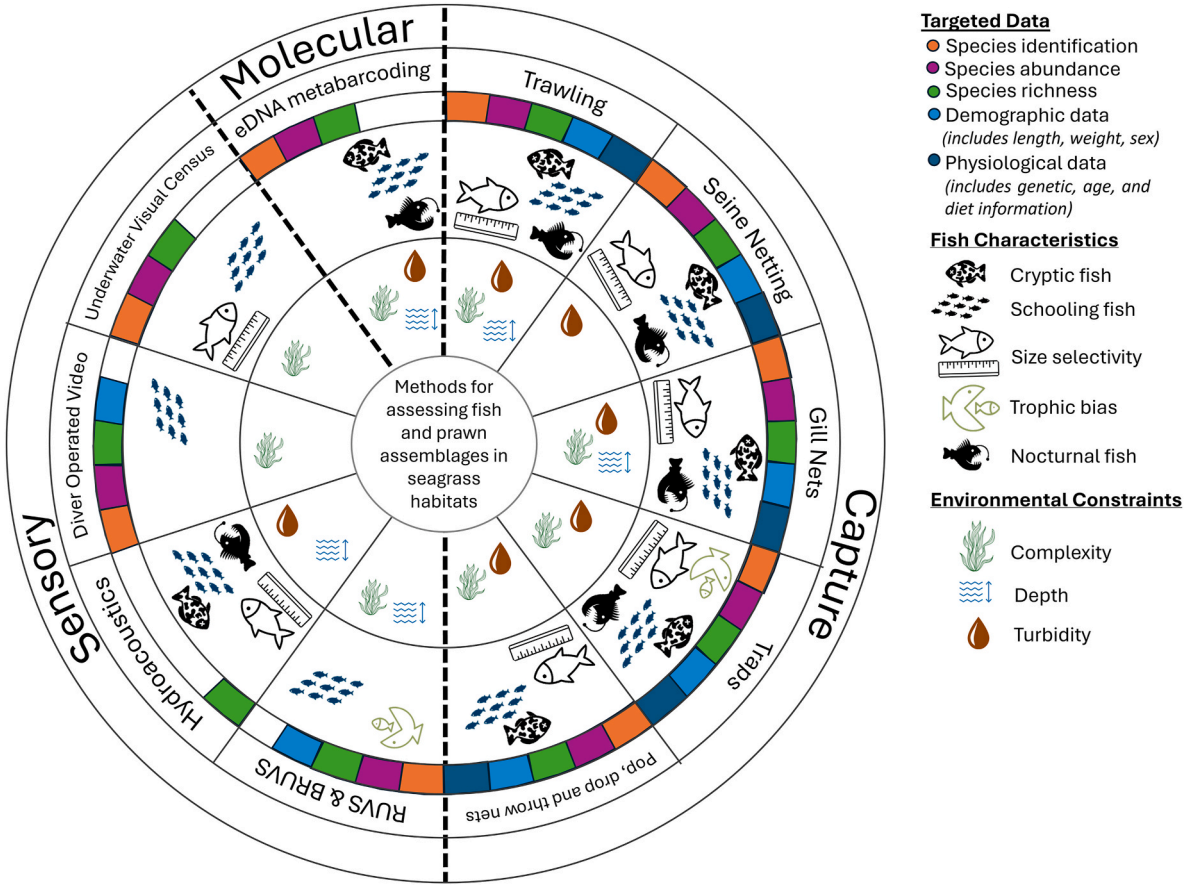

Fig. 2. A decision wheel for assessing fish and prawn assemblages in seagrass habitats categorised by **capture**, **sensory** and **molecular** approaches in the outer ring. The second ring identifies **key methods** applied in seagrass ecosystems from each category. The third ring is colour-coded to indicate the type of biological data collected by each method: **species identification**, **species abundance**, **species richness**, **demographic data** gathered from observation or capture (e.g., length, weight, and sex) and **physiological data** (from physical capture of the specimen, e.g., genetic samples from tissues, age estimations from otoliths, reproductive or disease data through histology or diet from gut analysis or stable isotopes). Some methods, such as BRUVs, are limited in their ability to collect demographics (capturing size estimates but not necessarily sex) but are included in this category due to partial capability. Similarly, eDNA has the potential to provide abundance estimates for fish (Rourke et al., 2022), however inferring abundance from the number of sequences per taxon/per sample can be challenging based on variables that can influence the relative level of DNA amplification and therefore there are some difficulties in determining its absolute value. The fourth ring describes the effectiveness of each method in sampling specific fish characteristics including **cryptic**, **schooling,** and/or **nocturnal** fish (method can be used both in the day and at night). It also highlights whether **size selectivity** needs to be considered with this method e.g. different mesh sizes on nets or traps can capture different size classes of fish and/or prawns; and **trophic biases** e.g. the use of bait in RUVS or traps can bias towards higher trophic levels such as carnivores, scavengers, and piscivores. The fifth ring outlines the environmental constraints that influence the efficacy of the methods including high habitat complexity (high seagrass density and canopy cover), depth (methods that can be applied in shallow to deep environments) and turbidity (those methods that can be applied in highly turbid waters). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

For example, active capture methods, such as trawling, allow the fish to be retained, providing data on age, sex, length, weight, and physical condition (Hammerl et al., 2024). Additionally, these methods enable the estimation of density per unit area, as the area sampled can be approximated by factors such as towing time, speed, and the dimensions of the fishing gear (Hammerl et al., 2024). In contrast, passive visual methods, such as BRUVs, provide abundance estimates using the metric MaxN, which is a conservative estimate of the minimum number of individuals known to be present in a sampling area (Schramm et al., 2020). Passive capture methods, including traps, can be used for estimating fish densities and catch per unit effort (CPUE) (Hammerl et al., 2024). Molecular methods such as eDNA metabarcoding provide an estimate of species richness and are still being refined for providing accurate abundance estimates in marine systems (Hänfling et al., 2016; Pont et al., 2023; Rourke et al., 2022). Ultimately, the choice of sampling method must be chosen depending on the specific metrics required to address the research objective.

4.2. What factors influence sampling suitability at your location?

Selecting appropriate sampling methods require careful consideration of the conditions at the sampling site, which can be determined by both environmental and logistical factors. Physical and biological conditions at the study site, such as turbidity, tides, currents, and diel cycles, can significantly influence the suitability and effectiveness of different sampling methods. For example, active capture methods, such as trawling, may be unsuitable in sensitive environments, or environments with obstacles such as rocky outcrops, due to the risk of damaging habitats or entanglement (Baker et al., 2016). Similarly, sensory methods such as UVCs, are much less suited for sampling in strong currents or turbid waters, as reduced visibility and challenging conditions can impact the accuracy of observations (Franco et al., 2012). Molecular methods, such as eDNA metabarcoding, can be compromised in highly turbid environments, where filtration methods can become quickly clogged by high quantities of suspended organic matter (Huerlimann et al., 2020). Additionally, habitat characteristics such as variations in seagrass species and meadow structures, can influence the marine fauna present and their catchability (Hyndes et al., 2003). For thick, dense, seagrass meadows, sensory methods such as BRUVs are less suitable as they can miss cryptic fish species present or have an obstructed camera view, compromising the accuracy of biodiversity measurements (Holmes et al., 2013; Kiggins et al., 2018; Smith et al., 2012).

Additionally, practical considerations such as site accessibility, regulatory requirements, and anthropogenic activity, must be accounted for in the sample design. Intertidal and subtidal locations will require different methodological approaches, with access to deeper subtidal habitats requiring a vessel. Regulatory restrictions and ethics approval may limit the use of capture-based methods, requiring alternative, non-invasive techniques. Additionally, high levels of boat traffic or ongoing fishing activity may interfere with passive sampling techniques, such as BRUVs, that could bias species behaviour and detection rates.

4.3. What species, trophic groups, and size classes are being targeted in the study?

Finally, it is essential to determine the target species, trophic stage, and size class at the study site to design an effective sampling regime. The life history, morphology, and behaviour of fish and prawns can influence the effectiveness of different sampling methods. For example, behavioural traits vary with different species, with cryptic species harder to detect and fast-moving schooling species difficult to accurately count (Pais and Cabral, 2018). Certain species may exhibit avoidance behaviours in response to the presence of observers, such as elusive species avoiding divers during UVC surveys (Watson and Harvey, 2007).

For capture methodologies, gear selection including mesh size, towing speed, and bait used, are important to consider for targeting different species, trophic groups, and size classes (Henseler and Oesterwind, 2023). For sampling a particular type of fish or trophic group, a single well-chosen sampling method may be more suitable. However, for sampling a broad range of fish and prawn assemblages in dynamic environments, a combination of different methods are more likely to overcome individual biases and limitations (French et al., 2021; Harmelin-Vivien and Francour, 1992; Qiu et al., 2023). By aligning the choice of sampling methods with the life history and ecological characteristics of the target species, researchers can optimise data collection and ensure the study objectives are met.

5. Case scenarios for integrated monitoring in seagrass habitats

To guide method selection in seagrass habitats, we present two practical case scenarios: one for high-turbidity and one for low-turbidity seagrass habitats. These examples provide realistic context for allocating effort across capture, sensory, and molecular methods depending on environmental conditions and study objectives.

5.1. Scenario one: high-turbidity seagrass habitat

In highly turbid seagrass meadows, reduced visibility can present logistical and methodological challenges for monitoring fish and invertebrate communities. Suppose the study objectives are to estimate fish and prawn diversity and measure size structure and condition (e.g., length, weight, histology) of fish and prawns in a recovering seagrass habitat. In this context, a combination of capture-based and molecular methods provides the most robust approach for collecting the data required to address the study objective.

Under poor visibility, capture methods, including both active and passive gear types, are well suited to these environmental conditions. Beams trawls are effective for sampling small, benthic, and cryptic species, enabling the collection of biological metrics such as length, weight, age, and condition, whilst also enabling estimates of density per unit area (French et al., 2021; Hammerl et al., 2024; Henseler and Oesterwind, 2023; Morrison and Carbines, 2006). Minnow traps are an effective complementary passive method for sampling nocturnal or small fish, especially in areas where active sampling may be restricted (Petrik and Levin, 2000). They can be left over an extended period of time and are easily transportable which makes deployment easy to set up for one person (Portt et al., 2006). Capture methods also allow for more detailed metrics to determine condition or demographic information from a subset of retained animals.

Sensory-based approaches such as baited remote underwater videos (BRUVs) or underwater visual censuses (UVCs) may still be applicable in high turbidity during specific windows, such as during clearer tidal windows, but they typically underperform in poor visibility (Franco et al., 2012). An alternative method are acoustic technologies, particularly sonar-based systems, which can be used to detect fish presence, movement and approximate body size even in limited visibility (Helminen and Linnansaari, 2021; Papastamatiou et al., 2020). Sonar can also be used to estimate size structure and abundance of species across diel or tidal cycles (Becker and Suthers, 2014; Bradley et al., 2023; McSpadden et al., 2024). The main limitation of acoustic systems are their cost and difficulty in identifying fish to species level (Sibley et al., 2023), which would make sensory methods more suited as a complementary method for this scenario.

Molecular approaches, such as eDNA metabarcoding, can complement capture methods by expanding species richness estimates, especially for rare or elusive taxa not sampled by capture methods (Ficetola et al., 2008). However, eDNA workflows can be challenging in turbid systems, due to the presence of suspended organic matter, which can clog filters and introduce PCR inhibitors (Holmes et al., 2024; Huerlimann et al., 2020; Kumar et al., 2022; Stoeckle et al., 2021). Methodological adaptations such as pre-filtration, increasing the volume of

water, or collecting multiple replications per site may be required to reduce filter clogging and improve detections under turbid conditions (Capo et al., 2020; Kumar et al., 2022; Takasaki et al., 2021). In addition, qPCR or ddPCR assays targeting key indicator species can be used to provide semi-quantitative abundance estimates (Hunter et al., 2017; Jerde et al., 2011).

For this reason, it is recommended that monitoring efforts in a high-turbidity seagrass meadow, allocate a greater proportion of effort to capture (e.g., approximately 70 %) and molecular (e.g., around 20 %) methods, with limited use of sensory approaches (e.g., less than 10 %).

5.2. Scenario two: A structurally complex seagrass habitat in low turbidity

Low turbidity seagrass habitats are characterised by low particulate loads and enhanced visibility, offering optimal conditions for deploying a wide range of sampling techniques. The study objectives for this scenario are to sample fish and prawn diversity and estimate abundance in a low turbidity, structurally complex seagrass habitat. These conditions enable the use of less-destructive methodologies, including sensory and molecular methods, which can minimise habitat disturbance and produce robust data for monitoring community dynamics.

Sensory methods, such as BRUVs, perform well in low turbidity, enabling species identification, behavioural observations, and abundance estimates, using MaxN, of marine communities (Harvey et al., 2007; Schramm et al., 2020). However, in dense or structurally complex seagrasses, BRUVs may be limited by visual obstructions from seagrass blades, reducing their effectiveness for estimating species abundance (Holmes et al., 2013). Other sensory methods such as DOVs and UVCs, may have difficulty in detecting cryptic species hidden in the seagrass blades, and observer expertise may be required for accurate taxonomic identification (Holmes et al., 2013). A combined approach using UVCs and BRUVs is recommended to maximise species detection and avoid the biases inherent to each method alone (Franco et al., 2012; Goetze et al., 2019). Where time, expertise, or budget is constrained, UVCs may be preferred as a lower-cost alternative, especially when conducted by experienced observers.

Molecular approaches, such as eDNA metabarcoding, can be highly effective in low turbidity, structurally complex environments. Filtration can be more efficient and the likelihood of detecting eDNA signals are increased, although detection rates are still dependent on other environmental factors (Barnes et al., 2021; Kumar et al., 2022). Environmental DNA metabarcoding provides a complementary approach to sensory methodologies in this scenario, providing non-invasive estimates of species richness, including cryptic or nocturnal species, that may evade detection by sensory methods (Ficetola et al., 2008).

Capture methods remain valuable in this scenario, particularly for collecting biological samples, such as tissue for genetics and age/growth/condition studies, and for validating species presence (French et al., 2021; Hammerl et al., 2024). Given the emphasis of non-destructive alternative, for this study objective minnow traps are a suitable passive capture method in dense, structurally complex, seagrass meadows. This method has low impact and can be easily deployed by one person, allowing for the live release after species identification (Hammerl et al., 2024; Miller, 1990; Portt et al., 2006)

In these low-turbidity systems, a shift in focus towards sensory and molecular sampling effort is more feasible, limiting disturbance to these habitats. A monitoring programme might allocate approximately 50 % to sensory methodologies, 35 % to molecular techniques and 15 % of the effort to capture methodologies to retain specimens for species identification. These proportions are dependent on the specific study objectives, target taxa, environmental conditions and logistical considerations.

These two contrasting case scenarios demonstrate the importance of tailoring monitoring strategies to the specific objectives, local environmental conditions, and species and trophic groups targeted for maximising collection of data. Integrating multiple complementary methods can help overcome the inherent limitations of individual techniques, improve biodiversity assessments, and support more robust project planning for seagrass monitoring across different environmental conditions.

6. Conclusion

Monitoring fauna associated with seagrass habitats is challenging due to the dynamic environmental conditions, complex habitat structures, and diverse array of marine fauna that they support. This review highlights the importance of employing a combination of methodologies broadly categorised into capture, sensory, and molecular techniques, each with distinct advantages and limitations depending on study objectives, local environmental factors, and target species. The integration of these methods provides a more holistic approach to biodiversity assessments, where the strengths of one method often compensate for the weaknesses of another method.

Increasingly, coastal management practices are transitioning away from traditional capture-based methodologies towards innovative, less invasive methods that utilise more recent technological advancements. These include developments in artificial intelligence, machine learning, automated data processing and high-throughput sequencing, all of which are improving the accuracy and efficiency of sampling and monitoring seagrass ecosystems (Shafait et al., 2016; Siddiqui et al., 2017).

The future of biodiversity monitoring in seagrass habitats requires an approach that integrates traditional and emerging methodologies. By combining capture, sensory, and molecular methods researchers and managers can generate comprehensive, high-resolution data on fish and prawn assemblages to inform evidence-based conservation and management strategies. This review offers practical guidance on the selection and integration of these methods, summarised in Fig. 2 and detailed in Tables 1–3, to support robust monitoring frameworks in seagrass habitats.

CRediT authorship contribution statement

Darcy E. Philpott: Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation. **Cecilia Villacorta-Rath:** Writing – review & editing, Supervision. **Joseph D. DiBattista:** Writing – review & editing, Visualization, Supervision. **Michael A. Rasheed:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization. **Nathan J. Waltham:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Timothy M. Smith:** Writing – review & editing, Visualization, Supervision. **Paul H. York:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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