



# Current methods and best practice recommendations for skate and ray (Batoidea) research: capture, handling, anaesthesia, euthanasia, and tag attachment

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**Abstract** Skates and rays (Batoidea) play a significant ecological role, contributing to ecosystem services through bioturbation and acting as vital intermediate components of the trophic chain in various aquatic environments. Despite their wide global distribution and ecological importance, batoids receive less attention than their shark relatives, resulting in substantial knowledge gaps that might impede a comprehensive understanding of their conservation status.

This review addresses critical aspects of their capture, handling, tagging, and release to provide readers with crucial information needed to perform research on batoids. Protocols for analgesia, anaesthesia, and euthanasia are also discussed, taking into account the ethical and logistical considerations necessary for research involving this group of species. This information can give researchers and ethics committees the knowledge to conduct and approve studies involving batoids, thereby promoting more effective and ethical research practices.

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## Introduction

Skates and rays, or batoids (class: Chondrichthyes, 26 families and approximately 633 species), are a highly diverse group of elasmobranchs in terms of morphology, distribution, habitat use, and behaviour (Ebert and Compagno 2007; Last et al. 2016). This group is unified by characteristics such as ventral gill slits and a dorso-ventrally flattened body with the full or partial fusion of the pectoral fins to the head and trunk. Adults vary significantly in size, from 25 cm to 6.5 m disc-width or 8 m in total length, and in body shape, with discs forming circular, oval, triangular, heart-shaped or rhombic configurations (Aschliman et al. 2012; Last et al. 2016). Skates and rays are widely distributed from the tropics to polar latitudes and occupy diverse habitats, including freshwater, estuarine, coastal, deepwater, and pelagic environments (Aschliman et al. 2012). While their dorsoventrally-flattened bodies make them highly adapted to life on the seafloor, species can either be benthic, benthopelagic or fully pelagic, with each group exhibiting unique behaviours and ecology (Last et al. 2016).

Currently, 36% of skate and ray species are listed as Threatened (including Vulnerable, Endangered,

or Critically Endangered) on the International Union for Conservation of Nature Red List of Threatened Species (hereafter referred to as the IUCN Red List) (Dulvy et al. 2021), with 9% considered Critically Endangered (as of March 2024; <https://iucnredlist.org>). This is mainly due to overfishing (Dulvy et al. 2021), with skates and rays being taken in targeted commercial fisheries across the globe (e.g., US and European Atlantic skate fishery, Jubenville et al. 2021; and in the world's largest shark and ray fisheries in Southeast Asia, Clark-Shen et al. 2023). Meanwhile, they are also often a substantial component of bycatch in many other coastal fisheries (e.g. South African, Australian and Central American commercial trawl fisheries; Stobutzki et al. 2001; da Silva et al. 2015; Clarke et al. 2016; Morales-Saldaña et al. 2022). Unfortunately, most skates and rays remain an understudied taxonomic group relative to sharks and teleosts and should therefore be considered a priority for further research and conservation action (Dulvy et al. 2017; Berrecil-García et al. 2022; Morales-Saldaña et al. 2022). Understanding their biological characteristics and population parameters (e.g., age and growth), movement and behaviour (e.g., habitat preference, important reproductive areas), and physiology (e.g., energy expenditure, thermal tolerance) in environments with different levels of degradation is critical

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to inform effective management and conservation measures (Stevens et al. 2000), particularly in the face of rapid human development and changing ocean conditions (Flowers et al. 2021; Moreno et al. 2020).

However, studying fully aquatic animals is inherently difficult, and skates and rays' unique ecology and morphology add to these challenges. For instance, many skate and ray species display cryptic behaviours such as burial in sediment and prolonged periods of immobilisation (Gibson 2014). Additionally, their atypical morphology complicates using particular research tools, such as animal-borne tags (Ward et al. 2019). Such tools could provide insights into a range of ecological, behavioural, and physiological aspects of skates and rays in a wide range of environments, for example, in freshwater (Campbell et al. 2012), intertidal areas (Martins et al. 2020a) and deep-sea habitats (Peklova et al. 2014).

In this context, it is imperative to encourage increased research efforts focused on these diverse ecological aspects of skates and rays. However, future studies must rely on well-defined research questions to fill critical knowledge gaps and evidence-based and ethical catching and handling practices to ensure post-release survival, particularly of threatened species. Here, we aim to discuss when and why it is appropriate to capture and handle skates and rays in research settings and further summarise the best capture, handling, and release practices based on the literature and experts' experience. For sharks, there is a body of work on these topics (e.g. Kohler and Turner 2001; Renshaw et al. 2023); however, a research-specific guide for skates and rays is absent. This manuscript aims to guide early career and established researchers and provide a starting point for identifying appropriate methods and techniques to develop well-evidenced protocols for the lesser-studied skates and rays and bolster knowledge on best practice approaches for more studied species.

## Key topics and recommendations

### Ethical and regulatory considerations

While sampling and tagging skates and rays can provide data that can directly inform and improve the population-level welfare of a species, it can also

have a negative impact on the animals. Therefore, before conducting a study, researchers must evaluate the compromise between possible animal suffering against the certainty of benefit to a species/population (following Bateson's cube; Bateson 1986). Additionally, securing licences and relevant permits is often necessary to conduct catching, handling and sampling for research. This may require researchers to provide evidence of how they have followed best practice guidelines and developed in-depth protocols for their focal species. While not all countries or host institutions may require permits or ethical approval protocols, as a researcher there is an ethical obligation to exercise due diligence to minimise the impact(s) of the study on the animal. These changes in ethical consideration while working with animals are also reflected in the increasing number of journals that now require evidence of regulatory approval in an ethical statement within a manuscript as a prerequisite for publication.

The three R's (replacement, reduction, and refinement, hereafter 3 R's) are principles that guide the ethical evaluation of animal use and include and can be used to help support robust, ethical sampling procedures before embarking on sampling work. First adopted in 1959 by Russell and Birch, the central premise is that animals should only be used in research when non-animal alternatives are not possible or feasible.

Replacement refers to methods that replace or avoid the use of animals. Alternate methods could include computer simulations, animal models (e.g. using species of lower conservation concern), or existing datasets to acquire the desired information. For example, Sharkipedia is an open-access database of shark and ray life history traits and abundance time series (Mull et al. 2022a) and has been used to estimate skate and ray sensitivity to exploitation using a modified Euler-Lotka model (Barrowclift et al. 2023). Alternate non-invasive technologies, such as aerial plane and drone surveys and underwater cameras, can provide information on species distribution, habitat use, abundance, size estimates, individual dispersal (e.g., from distinctive markings and photographic identification approaches), and population structure (size and possible sex structure) (Dudgeon et al. 2008; Bansemer and Bennett 2011; Bassos-Hull et al. 2014; Couturier et al. 2014; Armstrong et al. 2020; Setyawan et al. 2022a). For example, Setyawan

et al. (2022a) used aerial drones to study the size distribution, sex and maturity of surface-feeding reef manta rays (*Mobula alfredi*) in Raja Ampat, Indonesia. However, battery life and environmental conditions (e.g., water clarity for drones, accessibility of location, or underwater visibility for underwater cameras) can limit the use of these tools. Likewise, while stock discrimination and movement studies can involve animal tagging, researchers may be able to source samples from fisheries to apply other methods to determine population and movement parameters that are essential for management, such as meristic and morphometrics, parasite analysis (Mackenzie and Hemmingsen 2015), eye lens isotope analysis (e.g. Quaeck-Davies et al. 2018), stable isotope analysis (Navarro et al. 2023), as well as genetic techniques (e.g. cytogenetics, protein electrophoresis, immunogenetics, mitochondrial- and nuclear DNA; Dudgeon et al. 2012). For example, Ferrette et al. (2019) collected muscle tissue samples for genetic analysis from artisanal fisheries bycatch, securing 228 individuals spanning 17 distinct batoid species. However, using fisheries-dependent samples alone comes with a suite of inherent biases related to capture susceptibility, which must also be considered (see Rago 2005 for further details).

Reduction describes the critical evaluation of the minimum number of animals that must be tagged in a study to answer the objective(s), e.g. if appropriate, using a power analysis. However, given the inherently small sample sizes associated with biotelemetry due to high equipment costs, this is often best based on expert judgement and literature review. Additionally, it is vital to consider maximising the number of samples from a single animal, e.g. taking blood, mucosal swabs or tissue sampling, to reduce the required total number of animals. In addition, existing data streams already available through data sharing networks (e.g., the European Tracking Network for telemetry data; Özgül et al. 2024) can complement the proposed sample sizes.

Refinement refers to methods that minimise pain, suffering, distress, and harm to the study animal. Examples of refinement could include the use of analgesia or anaesthesia (see Sect. "Methods and recommendations for anaesthesia and analgesia"), selecting appropriate tags for the animal's size, if appropriate, using suitably sized and shaped holding and recovery tanks, and or the use of lifting mats to maintain the

animal's antimicrobial mucosal layer. The dorsoventrally flattened nature of skates and rays and their unique undulating (Rajiformes) or oscillating (Mobuliformes) swimming mode using their expanded pectoral fins (Di Santo et al. 2017) highlights the need for careful considerations on how to attach electronic tags not to hinder movement behaviour. Particularly given that minimum fish body-weight recommendations and observed effects to date are focused on fusiform teleost fishes (Matley et al. 2024). In addition, researchers should regularly assess the available literature on sampling, handling and tagging techniques to identify methodological developments that minimise adverse effects on the focal species.

### Capture methods and recommendations

Skates and rays are caught in various fishing gears, both as targeted and bycatch species. The gear type can directly impact the condition and survival of the animals caught. Unlike most sharks and teleosts, skates and rays use buccal pumping to ventilate, not ram ventilation (which describes forward motion to force water over the gills; Dapp et al. 2016; Milson and Taylor 2015). Therefore, there is flexibility in the choice of capture technique. Identifying an appropriate capture method depends on several factors, including (i) the primary aim of the study (e.g. whether the effort is targeted or conducted alongside recreational angling or commercial fishing operators), (ii) the desired animal condition (varies with fishing technique from excellent to poor), (iii) the targeted life stage (e.g. juveniles versus adults), and (iv) the focal species' habitat and ecology (i.e. is the species demersal or pelagic). Here, we outline these techniques, indicate when they may be appropriate, and provide examples of their application for various species, life stages, and habitat types. Note that determining the appropriate soak time (i.e. length of time that the fishing gear remains in the water) for sampling is not exclusive to a particular capture method but spans passive and encircling gears. Depending on the study's objective, soak times may need to be adjusted to optimise animal health. For example, in a study that aims to study typical animal movements and not capture-induced effects, nets should be continuously supervised or periodically checked to minimise the time the animal spends ensnared. Extended soak periods may otherwise increase the likelihood of

stress and harm to both the study species and other bycatch species (e.g. sharks, teleosts, sea turtles), e.g. due to physical trauma (Adams et al. 2018) or changeable thermal conditions (Braccini et al. 2012). For example, Ellis et al. (2018) found that the at-vessel morality of skates (Rajidae) caught in tangle nets increased from 1.47 to 6.16% as soak times increased from 13–28 h to 42–53 h, respectively.

### Passive fishing

Passive fishing includes gill nets (e.g. tangle, trammel, wreck nets), longlines, and trapping. Gill nets are generally passive and have multiple floats attached to the surface (also called the “head rope”), a weighted ground line to keep the net stretched to the benthos (“foot rope”), and a large mesh size. For teleosts caught by their gills, if nets are left unsupervised for long periods, this can lead to injury and even death (Marshall 2017). However, if supervised and regularly checked, the incidence of lethal entanglement is vastly reduced as batoids typically become entangled in the net or trapped by their rostrum (White et al. 2017) or serrated barb and not their gills. Gill nets may not be suitable in areas with high current speeds or significant boat traffic due to the increased risk of gear loss, with the number of sets also limited by sample processing times (Roskar et al. 2020). Gill net efficiency for batoid capture is linked to mesh diameter (Walker et al. 2005; White et al. 2017), material (Roskar et al. 2020), soak time (Hubert et al. 2012), net depth versus water height (e.g. loose and bowing nets may more easily capture large-bodied species like *Rhynchobatus* spp.; White et al. 2017), and the hanging ratio of the net (a measure of how tightly the net is stretched along the head and foot rope; Samaranyaka et al. 1997). For example, Roskar et al. (2020) compared gill netting and longline techniques in a shallow water estuary and found that gillnets caught more batoids ( $n$  individuals=444,  $n$  fishing sets=220) versus longline ( $n$  individuals=11,  $n$  fishing sets=294). However, the longlined individuals were in better condition. If information is available, the post-release survival and sub-lethal effects of net capture on a particular target species should inform whether this method is selected.

Longlines consist of a long line with several short lines fitted with baited hooks, either set in the water column (pelagic or drifting longlines) or on

the seafloor (bottom longlines). Longlining can be conducted by researchers or alongside a commercial operator. This technique is only suitable for species that are attracted to bait (Haetrakul et al. 2023). Consequently, planktivorous species (e.g. reef manta ray *M. alfredi*) are unlikely to be caught using this method. Species favouring pelagic or epipelagic habitats in both inshore and deep offshore waters (e.g. *Pteroplatytrygon violacea*) are more likely to be captured in pelagic or drifting longlines when hooks are suspended in the mid-water column (Mas et al. 2015; Piovano and Gilman 2017). However, for species with a predominantly benthic habitat preference (e.g. Lutz’s stingray *Hypanus berthallutzae*, shovel-nose guitarfish *Rhinobatos productus*, and Neotropical freshwater stingrays in general), the highest catch rates are recorded using bottom longlines with hooks closer to the substrate (Almeida et al. 2010; Afonso et al. 2011; Farrugia et al. 2011; Branco-Nunes et al. 2021). Fishing efficacy also depends on gear selection (e.g. hook size and shape) due to variability in species gape sizes (Kerstetter and Graves 2006; Piovano et al. 2010). The selected fishing method can influence the desired size or maturity of captured batoids. For example, White et al. (2017) found that longline-caught giant shovelnose rays (*Glaucostegus typus*) were significantly larger than those caught in gill nets. It was suggested that the escapement of larger animals from nets may have been possible due to the small mesh size combined with the ray’s blunt head shape (as stipulated for tope shark, *Galeorhinus galeus*; Stevens et al. 2000).

Trapping describes when animals are attracted into a permanent or semi-permanent enclosure by bait or because the gear placement seems to provide refuge or safe passage (Storai et al. 2011; Freitas et al. 2021). Animals enter voluntarily through one or more openings and are restricted to a small area they cannot escape. Trapping includes fish traps, corals, tunnel nets, and trap nets. While these methods have been used to capture skates and rays in relatively shallow waters (<50 m deep) of the Mediterranean (Storai et al. 2011) to deepwater seamounts (>600 m) in the Northeast Atlantic (Freitas et al. 2021), they have not been a common approach to capture or study skates and rays but can be very effective. For example, tunnel net fishing involves setting a large mesh net (measuring hundreds of metres) that uses the natural tidal drop to herd fish into one end of the net



called the tunnel, and is often set on shallow (<20 m) sandy substrate (Jacobsen et al. 2021). Pierce and Bennett (2011) secured opportunistic samples of estuary stingray (*Dasyatis fluviorum*) from commercial tunnel-net operators in south-east Queensland. The extensive coverage of tunnel nets (e.g. this operator used a 784 m net with a 75 mm mesh) exceeds that typically used by other methods in shallow estuaries, e.g. seine nets and therefore may be more efficient than other methods.

### Encircling gear

Encircling gear uses seine, cast, and hand nets to encircle and capture skates and rays. These methods aim to target one or more species that tend to aggregate or are found in relatively shallow waters close to shore (Mull et al. 2010; Jirik and Lowe 2012; Farrugia et al. 2014).

Seine nets are similar to gill nets but have a smaller mesh size. The net is typically deployed from a boat or by a small crew when in shallow waters near the shore or a bank around an animal that has been sighted (Farrugia et al. 2014; Bassos-Hull et al. 2014). The net is then pulled in from one or both sides until the animal surfaces. Like other netting techniques (e.g. gill and tangle nets), barbed rays can get tangled in the net and cause stress to the animal. Large commercial seine nets can catch high numbers of skates and rays as bycatch, which may prove harmful and lethal due to the long processing time of releasing tangled animals. For example, in April 2024, a large number of Critically Endangered common eagle rays (*Myliobatis aquila*) (> 1000 individuals) were caught in a beach seine in False Bay, South Africa, and many became entangled in the mesh by their tail barbs, with most dying on landing and after release (SJ Lamberth, South Africa's Department of Forestry, Fisheries and the Environment, pers. comm.). However, if animals are handled quickly and with care, this method can prove one of the least harmful and most effective for skate and ray research (Mull et al. 2010; Jirik and Lowe 2012).

Cast nets are circular nets with small weights around the edge that are thrown out and immediately reeled in. While it requires some skill and training, it has been used to successfully target skate and ray species such as the Atlantic stingrays (*D. sabina*) and whitespotted eagle rays (*Aetobatus*

*narinari*) in specific environments (Choe et al. 2004; Boggio-Pasqua et al. 2022). Cast nets are most successful when targeting pelagic species swimming near the surface of the water column or in shallow waters near the shore. This method does not usually harm captured individuals due to its targeted and rapid nature.

Hand nets and hoop nets can be used to catch benthic species in shallow, coastal habitats (e.g., bays and estuaries, coral and rocky reefs, seagrasses, mangroves, etc.) (Davy et al. 2015; Martins et al. 2020b). Hand nets describe a mesh net attached to a hand-held pole, while similarly, hoop nets have the mesh attached to a hoop and then a pole. This method allows researchers to capture skates and rays resting on the substrate or moving slowly by swimming, snorkelling, kayaking, or even walking in shallow areas with a hand net (Rosa et al. 2010; Davy et al. 2015). This technique also represents a relatively non-harmful method to target specific species of skates and rays across coastal and freshwater habitats.

### Towed gear

Trawling is an active fishing method that uses a single or pair of boats to tow a net along the surface, mid-water, or the benthos. While it is a highly effective method of fishing (Steadman et al. 2021), this method is highly unselective and non-target; bycatch elasmobranch species often make up a significant component of the catch (Clarke et al. 2016, 2017; Last et al. 2016; White et al. 2019). Trawling can be used to collect large quantities of individuals in a relatively short time frame, e.g. Knotek et al. (2020) sampled 612 thorny skates (*Amblyraja radiata*) caught from nine single-day trawl fishing trips ( $n$  tows=27). While beam trawl-caught specimens can prove invaluable in biological studies and survivability, the high volume of fish caught (catch weight) can lead to severe bruising, poor condition, and lower survival rates ( $\geq 45\%$ ) compared to demersal seining (also known as flyshoot fisheries) ( $\geq 77\%$ ) (Enever et al. 2010; Schram et al. 2023). Therefore, when designing studies using trawling methods, it is essential to consider the study aims, the effects of the fishing gear, ethical justifications for capturing fish by trawl, and the release condition of individuals captured.

## Angling

Angling involves the use of hook and line typically in the form of a handline or rod, either from the shore or boat, to target species that are attracted to the bait and lures. While researchers can lead angling efforts, this technique can provide an opportunity to engage with tour operators, anglers and the general public to secure animals for sampling. Mark-recapture techniques (see Sect. "External tagging") often use community engagement and volunteer anglers to leverage the deployment of large numbers of tags across a focal area (Dunlop et al. 2013). However, angling can lead to hook-related injuries (superficial or fatal) and biochemical disruption-related effects (Cooke et al. 2013; Cameron et al. 2023). To reduce animal stress, care should be taken by anglers in the preparation (use of suitable bait, use of appropriately rated tackle, use barbless hooks of the appropriate size and shape), technique (watch the bait to minimise chance of deep hooking, practice efficient fish recovery to avoid exhaustion, employ experienced anglers), and landing (padded mats, use of unhooking tools, cutting deep hooks) of animals. Corrodible hooks, e.g. bronze hooks, may be advisable in the case the hook cannot be safely removed so that it can degrade quicker naturally (see Bègue et al. 2020). Hook type (size, shape) is species-specific and requires careful consideration. Generally, barbless circle hooks are the preferred option when angling for rays, as they can significantly reduce hooking injury, foul-hooking, swallowing of the hook and mortality compared to J hooks (Godin et al. 2012). However, hook preference may differ depending on the fishing technique and the target species' gape size. For example, J hooks have been identified as having a faster self-shedding rate (6 days) than circle hooks ( $44.5 \pm 54.4$  days, mean  $\pm$  SD), which would allow a quicker resumption feeding (pelagic stingray, *P. violacea*; François et al. 2019). The correct choice of tackle will also limit the 'fight time'; for example, heavier tackle should be used as it is capable of landing an animal significantly faster than lighter tackle.

## Methods and best practice approaches to animal handling

For studies not investigating fisheries effects, ensuring captured animals' overall health and survival

will depend highly on well-designed procedures that minimise handling times and stress (Lambert et al. 2018). Handling times and resultant stress vary between species, and studies have found that the associated stress response was more pronounced in more mobile pelagic rays than in more sedentary species (Rangel et al. 2021). Recommended handling techniques vary depending on the gear choice, associated environmental conditions (e.g. if landed from shore, boat or in-water) and the captured species' biological, behavioural, and morphological characteristics. Handling complexity typically increases with the size of the animal (Poisson et al. 2014) and the presence of structures that can hamper the animal's removal from the fishing gear or potentially harm the handler (e.g., stingray stingers, sawfish rostra, whipray tails). Once an animal is captured, to reduce potential harm to the animal or injury to researchers, it is important to restrict its movements in the water or transfer it to a suitable holding space as quickly as possible. In both cases, researchers must provide a non-harmful environment for the skate or ray to be temporarily restrained, restricting rostra, pectoral fins and tail movements to avoid animal and staff injury during procedures. See Table 1 for key handling recommendations.

Before initiating fieldwork, it is essential to carefully evaluate the size and behaviour of the species of interest and potential bycatch using the capture method to help select an appropriate handling strategy, while also accepting an adaptive approach may be needed given an animal's behaviour is unpredictable. This step involves determining whether the animal should or must be removed from the water or freed from the fishing gear before conducting the sampling procedures such as biopsies and tagging. For example, Mobulidae (manta rays and devil fishes) are typically tagged in-situ to avoid handling given their typically large size and difficulty of targeted capture (owing to their zooplanktivorous diet) (Thorrold et al. 2014; Harris and Stevens 2021; Andrzejaczek et al. 2021). This is an essential consideration as skates and rays have loosely connected cartilaginous skeletons that provide limited protection to internal organs once the animal is removed from the water (Poisson et al. 2014), increasing the chance of internal damage if animal handling is not well executed. Throughout all handling procedures, it is vital not to lift an animal by its tail to avoid

**Table 1** Recommendations to guide the proper handling of skates and rays

Recommendation	Further guidance
Identify appropriate techniques for post-capture manoeuvring of animals	<ul style="list-style-type: none"> <li>• Use of rubber nets and large slings made of appropriate material to provide support for the animal's weight</li> <li>• When boarding large skates and rays, if possible, avoid hauling the animal over the gunwale; if necessary, ensure one or two staff members are available to coordinate animal recovery</li> <li>• Use of vessels fitted with water-level gateways or lifting platforms (e.g., dive lifts) to aid boarding and release</li> </ul>
Follow best practice guidelines to reduce animal pain and suffering	<ul style="list-style-type: none"> <li>• Ensure proper handling and support, e.g., avoid holding the entire animal weight from the tail, gills, spiracles, mouth, or head, and ensure the organ cavity is supported</li> <li>• Minimise handling whenever possible and keep hands and surfaces wet to preserve the mucous layer and limit damage to the skin</li> <li>• Cover the animal's eyes with a damp, dark towel to help reduce stress. Note that the type of towel needs to be considered in light of the roughness of the target animal's skin and the potential mucus production. If conducting batch tagging, consider whether a single towel is needed per individual and, if not, the maximum number of reuses across animals. The presence of blood will warrant a towel change; therefore, ensure adequate materials are available</li> <li>• Avoid using a gaff unless it is necessary for the animal's or personnel's safety</li> </ul>
Ensure adequate preparation to maximise researcher safety during animal handling	<ul style="list-style-type: none"> <li>• Use personal protective equipment (PPE), such as single-use surgical gloves, to protect from zoonosis</li> <li>• Use thick gloves and a towel/cloth when handling barbed and spined species</li> <li>• Consider the use of clamps or cable ties to stabilise barbed species</li> <li>• Holding troughs are not appropriate owing to skate and ray morphology. Instead, opt for soft-celled mats or support pillows</li> </ul>
Assess whether holding tanks are appropriate, e.g. on capture, dosing, and recovery post-anaesthesia	<ul style="list-style-type: none"> <li>• Conduct routine temperature and water chemistry checks to ensure the water content is within suitable limits</li> <li>• Constant flow through systems using ambient water is preferable. Where not available, identify how often tanks need a full or partial water change and conditions that affect this cycle, e.g. blood presence should lead to complete water change</li> </ul>
Identify on-deck checks to maintain animal health and condition, particularly if animal transport is necessary	<ul style="list-style-type: none"> <li>• Conduct routine temperature checks and identify appropriate mitigation, e.g., using a sunshade to reduce on-deck temperatures and direct sun exposure</li> <li>• Identify techniques to ensure ventilation, which may depend on the animal's size, e.g. for small animals using a wide-neck wash bottle or for larger animals using a deck hose to aerate the gills</li> </ul>

spinal damage and not to place fingers inside the spiracles to assist with restraining and moving, as this can damage internal structures (Poisson et al. 2014). Planning a post-capture handling strategy also requires understanding the role each researcher will play during the handling process and the preparation of all necessary tools and equipment for rapid handling, sampling and release or euthanasia.

Animals should be kept submerged in water as much as possible to avoid an increased acidosis response when handled out of the water (Weber et al. 2021). For example, Bassos-Hull et al. (2014) placed whitespotted eagle ray (*A. narinari*) in a 2.5 m floating net pen off the side of a boat to hold rays temporarily if more than one ray was caught in a seine net set. If conditions permit, larger animals are better



restrained in the water, usually on a type of net that is buoyed or kept alongside the vessel. If larger animals are removed from the water, transfer slings can be used to manoeuvre the animal to a work area prepared with padded soft-celled mats (or a similar surface) for support. For example, Carlson et al. (2018) advocate using a canvas sling to help remove Mobulid species accidentally caught in purse seine net to avoid direct handling. Ventilation should be supported if possible, such as using a boat hose. The researcher should confirm that the supplied water is free of fuel residues and that the temperature does not fluctuate beyond safe limits, e.g., due to proximity to the boat's engine bay. Animals should not be exposed to direct sunlight whenever possible to reduce skin dehydration. Measures to reduce solar radiation can include a sunshade (Raoult et al. 2019; Wheeler et al. 2023) and damp pieces of cloth (i.e. wet towels) applied to the skin (Poisson et al. 2014).

Meanwhile, smaller animals can be either kept in water or removed from their environment. To facilitate respiration, use handheld bottles, a through-flow system, or place the animal in a holding pen (Mickle et al. 2020) filled with water from its original habitat. Examples of holding tanks range from large tanks onboard a boat (Bassos-Hull et al. 2014) to less complicated holes dug out in beach sand and lined with plastic sheeting (Elston et al. 2023). While holding tanks may prove logistically challenging (e.g. due to limited space onboard a vessel), they can facilitate batch tagging, prevent predation, and enable recovery before release. It is crucial to monitor water quality continuously to maintain optimal conditions. When an open continuous water circulation system is unavailable or unfeasible, portable aerators can maintain dissolved oxygen concentrations in holding tanks. Additionally, partial water changes may be necessary due to the stress of capture and handling, which can quickly degrade water quality and lead to high levels of nitrogenous compounds. Monitoring water temperature to minimise the risk of thermal shock when returning animals to their natural environment is also crucial. In the event of plastic-lined troughs, one researcher should pour water in as the animal is being landed, and the water should then be changed after the animal has been held, sampled, and released.

Researchers should wear appropriate personal protective equipment (PPE) when handling species that have prominent thorns or thorn patches along the

midline, the sides of the tail or the wings (as seen in many Rajidae species; Last et al. 2016), and or stings (e.g. Dasyatidae, Urotrygonidae, Myliobatidae, Aetobatidae and Potamotrygoninae rays, which have one or more venomous-cell-layered barbed caudal stings positioned on the dorsal surface of the tail; Last et al. 2016; Shea-Vantine et al. 2021). It is important to balance sensibility and dexterity to ensure no increase in the risk of damage to both the fish and the personnel during handling. Note that gloves (neoprene and leather) and cloths (Poisson et al. 2024) have been proven ineffective at preventing stinging accidents when restraining and handling stingrays. Although amply used, this technique requires experience and can still result in punctures and injuries if the animal tail is not tightly secured or the cloth is misplaced. Other techniques to avoid wounds and stabilise the tail include the use of chainmail boning or Kevlar® gloves (Marshall et al. 2017) and tail covers made of rigid polyvinyl chloride pipe (PVC) or Styrofoam (Reynolds et al. 2017). The processes mentioned above must be carried out by experienced researchers and with the assistance, if possible, of professionals with experience in handling stingrays.

Nevertheless, extreme caution is still needed when handling some species like freshwater stingrays (Potamotrygoninae) since there are species that have powerful caudal muscles and the ability to direct the sting precisely to the point of stimulation with enough strength to puncture heavy-duty rubber boots, and wooden objects (Castex and Loza 1964; Charvet P. unpublished data). Other methods that need careful consideration and potential licensing include using nylon clamps or cable ties to stabilise an animal's sting, which are cut before the animal's release. Caudal stings are naturally lost and replaced throughout a species' life cycle. For example, the round stingray (*Urobatis halleri*) seems to have an annual barb replacement, while some stingray species within the subfamily Potamotrygoninae lose their stings approximately every six months (Thorson et al. 1988; Lowe et al. 2007a, b). Removing caudal stings is not recommended since the sting in use (i.e. not the one about to be shed) is embedded on the caudal tegument deep enough to cause bleeding (and possibly pain) if removed.

Another group of rays that requires extreme caution when capturing and handling are sawfishes (Pristidae). Sawfish are regarded as the world's most

threatened marine fishes, with all five species classified as highly threatened with extinction (Dulvy et al. 2016). Sawfish species are highly susceptible to entanglement in nets and lines due to their toothed rostrum (i.e. saw). Removal from capture gear can be challenging (Harrison and Dulvy 2014), particularly for large, heavily ensnared individuals. Even juveniles have been reported as capable of causing severe injury to handlers if not adequately secured or controlled (White et al. 2017). Animals, especially large-bodied specimens, are suggested to be kept in the water. Larger animals held alongside a boat should be restrained using a noose at the base of the rostra to avoid the animal thrashing about and injuring itself and handlers (Kyne and Pillans 2014). For release, handlers should carefully untangle any line or net from around the animal's body and rostra, if safe to do so. If unangling is deemed unsafe, cutting the capture gear as close to the animal as possible so that most of the net or line is free from the animal is most likely the best approach (Peverell 2010). Guitarfish and wedgefish, like sawfish, can also suffer from entanglement and poor handling due to their elongated snouts (Pytka et al. 2024). Therefore, the same care suggested for sawfish is recommended for other rhino rays.

Tonic immobility has been identified as an important aid in handling several Rajiformes and Myliobatiformes species (see review by Paez et al. 2023). This reversible behavioural state is characterised by a cessation of voluntary movements (Paez et al. 2023), except vision (Reese et al. 1984) and breathing (Miranda et al. 2014). Inducing tonic immobility involves turning the animal on its back (i.e. inverting its ventral side up) using external pressure combined with physical restraint (Paez et al. 2023). Tonic immobility is thought to reduce visceral pain (Miranda et al. 2006) and prolonged noxious stimulation (Carli et al. 1976). For animals restrained in the water, this is a relatively simple process of inverting the skate or ray, usually by tucking in the wings and rolling the body. For animals restrained in a smaller holding space, lifting the animal out of the water might be necessary when inverting it. In this instance, ensuring that the central body cavity is adequately supported is essential. The benefits of this technique include rapid induction and recovery, limited handling times, and improved post-release welfare (Kessel and Hussey 2015). However, the physiological

effects of tonic immobility on elasmobranchs and the comprehensive list of species where this behaviour is present require further investigation.

#### Assessing animal health, treatment effects, and stress

Throughout a project's conception, a researcher should identify how best to conduct health and vitality assessments of their target species. Stress-induced changes encompass morphological, behavioural and physiological aspects. Research on stress in rays is less extensive than their shark relatives, resulting in a substantial knowledge gap. This gap hinders our understanding of how stress affects physiological pathways and hampers the development of handling protocols to mitigate the effects of allostatic overload. Given the close relationship between rays and sharks, similar stress response mechanisms are expected. Both groups exhibit three phases of stress response: primary, secondary, and tertiary. The primary phase involves neuroendocrine changes, and the secondary phase includes the mobilisation of osmolytes and metabolites, and the tertiary phase may result in medium- and long-term effects such as reduced immune capacity, impaired growth, reproductive issues (Skomal and Bernal 2010; Adams et al. 2018; Jerome et al. 2018; Rangel et al. 2020; Prado et al. 2022; Wosnick et al. 2023a, b; Pytka et al. 2024) and resultant effects on offspring vitality (Guida et al. 2017; Finotto et al. 2021; Finotto et al. 2023).

#### Rapid health and vitality assessments

Evaluation of behavioural proxies during the animal monitoring period can offer a comprehensive view of the recovery process (Sims et al. 2000 and Wosnick et al. 2023a). Health assessments are typically a simple version of an animal health scorecard found in captive aquatic animal husbandry, with its simplicity dictated by project logistics and field conditions. Health assessments should be ideally conducted post-capture and pre-release to monitor any changes in animal health. Assessments should be flexible and modified for specific species and the desired capture method, such as a measure of hook damage for individuals caught on longlines (Silva et al. 2020).

Common forms of health assessments include a semi-quantitative assessment (SQA, see Table S1 in the supplement) (Benoît et al. 2010) and reflex action

mortality (RAMP, Table S2) assessment methods (Davis 2010). Scoring animal health indicators can aid in the selection of appropriate measures, e.g. humane euthanasia. An example of how to apply these techniques and score animal health is provided for thornback ray (*Raja clavata*) in S1 of the supplement. An SQA assessment typically involves classifying body and spiracle movement and the extent of animal injuries on a graded system from excellent (vigorous movement, nil or minor external injuries, e.g. a score of 1), good (weak body movements, nil or minor external injuries; 2), poor (no body movements but with spiracle movements, minor or major external injuries; 3) to deceased (no body or spiracle movements; 4). Thereafter, a RAMP assessment can be used to quantify reflex action scores, either as present (unimpaired with a strong reflex or easily detected; score of 0) or absent (impaired; 2). Assessed reflexes often include ocular tap, whereby the animal is gently tapped on the head behind the eyes and spiracles, and the presence of eye retraction and closure is observed. As well as spiracle closure (do spiracles open and close, e.g. during a five-second observation window) and potential response to wing stimulus, i.e. stroking on the ventral wing surface leads to undulation and flex (see S1 of supplement). Additional information on animal health and condition are typically recorded during the RAMP assessment, which could include but is not limited to, abrasion and bruising (haemorrhaging indicated by pinking of body or fins), bleeding, net marks, scratches, hypo- or hyperventilation, coughing or gasping, oesophageal or rectal prolapse, and exophthalmia (bulging eyes) (list adapted from Catchpole et al. 2015, described in Silva et al. 2020). If conditions permit, assessments should also be conducted after tagging to assess whether the fish can be promptly released or ideally, held in a recovery tank. Given potential objectivity in assessment scoring, overlap between assessing staff should be planned to ensure standardisation between evaluators (Silva et al. 2020).

#### *Other tools to evaluate animal health and estimate survival*

Secondary stress indicators include elevated lactate levels in muscle tissue and depletion of energy stores (Wosnick et al. 2019, 2023b; Rangel et al. 2021; Prado et al. 2022) and a loss of osmo-ionic/urotelic

balance and significant changes in the energy profile, such as glucose, triglycerides, and ketone bodies (Wosnick et al. 2023b). The relationship between blood parameters such as partial pressure of oxygen, base excess, bicarbonate, lactate and glucose and additional information such as fight time, total handling time, body size and water/air temperature can provide insight into the response of the species to these parameters as well as real-time quantitative measurement of stress (Cole et al. 2024; Wosnick et al. 2023b). Handheld blood chemistry analysers can provide insight into the physiological state of the animal and range from simple measuring devices such as blood pH analysers (approx. €500, reusable; Talwar et al. 2017) to devices capable of recording more biochemical parameters (e.g. an i-STAT handheld analyser with a unit cost of approx. €2000, and single-use cartridges around €15 each). However, studies have found considerable intraspecific variation in lactate concentrations related to capture and handling time (Heard et al. 2014; Rangel et al. 2021), with moribund and dead elasmobranchs with lactate ranges of  $> 180 \text{ mg dL}^{-1}$  (Moyes et al. 2006; Wosnick et al. 2023a, b) and information on “ideal” thresholds limited to a few species.

In contrast to direct biochemical measurements, high-resolution tagging approaches can be used to identify recovery behaviours post-capture (Lavender et al. 2022) such as post-release mortality (Knotek et al. 2020). For example, Lavender et al. (2022) studied the vertical activity rates of flapper skate (*Dipturus intermedius*) to catch-and-release angling using archival tags and identified that overall average vertical activity was 38% higher in the 12 h following release compared to undisturbed activity. Though these tags are yet to be widely deployed on skates and rays, tag miniaturisation and resultant developments in attachment techniques will likely increase future deployment rates (e.g. Hussey et al. 2015).

#### *Methods and recommendations for anaesthesia and analgesia*

While pain detection in elasmobranchs is still hotly debated (Snow et al. 1993; Snow et al. 1996; Huntingford et al. 2006; Browman and Skiftesvik 2011; Weber 2011; Rose et al. 2014; Sneddon 2018), these animals react physiologically to stressful situations (Lambert et al. 2018; Bouyoucos et al. 2019).

Therefore anaesthetic and analgesics can minimise handling trauma and resultant associated stress (Neiffer 2021) and should be considered when using invasive sampling techniques. Anaesthesia describes a loss of sensation through the depression of the central nervous system (Martins et al. 2019), while analgesia refers to pain relief through the loss of physical sensation with or without loss of consciousness. Despite the lack of quantitative evidence on skates and rays on the efficacy of anaesthetics and analgesics, or even in fish (see review by Chatigny et al. 2018), legislative requirements in some countries often mandate their use in procedures involving surgical intervention. Given the lack of research on elasmobranch anaesthetics and analgesics, its application should be determined on a case-by-case basis after a cost–benefit analysis of its application. For a thorough, detailed review of this topic, see Neiffer and Stamper (2009) and Mylniczzenko et al. (2014).

Local and general anaesthesia are the two main types of anaesthesia used in surgical tagging. Assessing the applicability of anaesthetics requires careful evaluation of the field project logistics, experimental protocols, and factors relating to the species in question; there is no “one size fits all” solution. For example, while general anaesthesia helps to immobilise a fish, which in turn can improve ease of handling and reduce stress (Sneddon 2012) and enable more precise and controlled surgical procedures, it also requires animals to be removed from their natural environment, which can cause damage to internal organs, skin, and cause significant stress (Brønstad et al. 2016).

Local anaesthesia involves the application of anaesthetics to a specific area to numb the surgical site. It offers an alternative for procedures that do not require full immobilisation and can minimise handling time and complications associated with general anaesthesia. Examples of local anaesthetics used in skate and ray tagging include lidocaine (DeGroot et al. 2020; Cole et al. 2024; Haetrakul et al. 2023) and benzocaine (Stamper 2004). The use of local anaesthetic significantly reduces the amount of time an animal is removed from its natural environment versus general anaesthetics, as no immersion time is required, and the individual can be released immediately post-surgery. However, the time it takes for the anaesthetic to take effect may equate to additional time on deck and may likely offer more relief

post-surgical period. Intramuscular application can lead to variable response times depending on the injection site, e.g., injected into red muscle or white muscle (Mylniczzenko et al. 2014; Williams et al. 2004). The effects of localised anaesthesia have not been thoroughly investigated in elasmobranchs, and careful cost–benefit analysis is required before use.

General anaesthesia renders a fish unconscious and immobile. General anaesthetics include MS-222 (tricaine methanesulfonate), clove oil (active ingredient eugenol ranges from 70 to 90%), and isoeugenol. General anaesthesia is useful for more invasive or longer procedures or species that struggle excessively on capture or handling, ensuring that the fish remain still. While it requires careful monitoring during induction and recovery, general anaesthesia can improve the precision of surgical tagging and reduce stress associated with handling. However, the use of general anaesthesia itself can result in hyperexcitability, specifically in the induction stage (see Mylniczzenko et al. 2014). Following surgical tagging, animals subject to general anaesthesia require post-procedural monitoring. Depending on the chosen anaesthetic, there may also be a legally required holding period prior to release (e.g. MS-222, see Popovic et al. 2012) due to risks to the food chain. Reconsider using any anaesthetic agent on commercially valuable species in areas where commercial fishers might recapture and sell them for human consumption, as this may result in exceeding the maximum residue limits of anaesthetics that can lead to toxic effects (EU 2009). As most elasmobranchs are poikilothermic or “cold-blooded” animals, the time it takes for a drug to be below a safe level for consumption depends on temperature and time (Brønstad et al. 2022). Additionally, intra- and inter-species differences likely affect the time required to metabolise anaesthetics (see Stamper 2007). Aqui-S (isoeugenol is the active ingredient) is currently under extensive investigation as an alternative general anaesthetic that would enable immediate release (Durhack et al. 2020; Trotter et al. 2024). The most reliable indicators to determine if an individual is ready for release are the resumption of spiracle movement (which stops under anaesthesia) and responsiveness to tail grabbing (Neiffer and Stamper 2009). Water quality needs to be closely monitored for faster recovery, particularly dissolved oxygen levels and temperature, to ensure the effective clearance of the administered drug (Neiffer and

Stamper 2009). Moreover, avoid using recirculating water systems during recovery from anaesthesia, as drugs and metabolites excreted into the water can be reabsorbed by the gills and skin, and most mechanical and biological filters cannot effectively remove the administered drugs (Neiffer and Stamper 2009).

Analgesia is also relatively underexplored in fish medicine (Sneddon 2012; Chatigny et al. 2018), and particularly how to appropriately use analgesics under field conditions. The main agents that have been studied to date in relation to fish are opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and local anaesthetics (see review by Chatigny et al. 2018). Published uses of NSAIDs on batoids are limited to aquaria or controlled environments e.g. ketoprofen and meloxicam on bluespotted ribbontail ray (*Taeniura lymma*) (Stamper et al. 2004; Kane et al. 2022; Anderson et al. 2023). Research is needed in this area, particularly on applicable analgesics that are relevant within a field context where the time the animal is out of the water must be finite, and the post-operative evaluation period is often limited.

#### Methods for euthanasia

As many species in this group are at high risk of extinction, the use of non-lethal methods or alternatives to animal sacrifice should be a priority in scientific research. However, in some cases, euthanasia may be necessary and may even be an ethically driven decision (e.g. ending the suffering of critically injured animals encountered on a commercial fishing vessel). Therefore, it is crucial to provide guidelines that aim to minimise suffering and ensure euthanasia is conducted as ethically as possible (Soulsbury et al. 2020). Euthanasia protocols and guidelines can vary by country and even between educational and research institutions (Herrera 2023) or researchers' backgrounds. Researchers must know the standards and recommendations specific to their region and institution. In the absence of local ethics or equivalent committees (e.g., in Small Island Developing States), researchers should adhere to internationally recognised protocols and standards (Sloman et al. 2019).

Euthanasia should be considered as the last option, with the tagging procedure (often regulated) not to be undertaken, or if the condition after the tagging procedure shows that the animal's condition has deteriorated to a level where post-release survival is deemed

unlikely. This decision can be informed using a semi-quantitative health assessment (SQA) and a reflex action mortality predictor (RAMP) assessment (see Sect. "Rapid health and vitality assessments"). Euthanasia should only be carried out by appropriately trained personnel to ensure efficiency and minimise suffering and pain, so it is vital to prepare students, technicians, and assistants for emergencies requiring euthanasia.

The most ethical and humane method for euthanising fish involves immersion in lethal doses of anaesthetic (Neiffer and Stamper 2009), followed by complete destruction of the brain tissue and/or spinal cord. Since dosages vary by species, extensive bibliographical research is necessary. The most commonly used drug for euthanasia is MS-222, which is typically applied at concentrations five to ten times higher than those for anaesthesia (Neiffer and Stamper 2009). Where obtaining anaesthetics is difficult, especially without a veterinarian or in countries with strict pharmaceutical regulations, clove oil or eugenol is recommended. Even in challenging conditions such as field operations, the use of anaesthetics in case of euthanasia is encouraged, as a variety of plastic containers can be transported to create immersion baths. An anaesthetic should be applied directly to the gills or spiracles to expedite the absorption of an anaesthetic in larger fish (that cannot be immersed in a general anaesthetic). Generally, exposure for five to ten minutes post-cessation of opercular movements ensures death (Neiffer and Stamper 2009). If anaesthesia is not possible, animals should be stunned using blunt force brain concussion with a single, forceful strike to the cranium using a blunt instrument such as a mallet. In all cases, death should be ensured via brain tissue and spinal cord destruction using a sharp spiked instrument (e.g. Ikejime or Ikijime Spike) dorso-cranially. If euthanasia is necessary, consider the collection of as many biological samples as possible (e.g. vertebrae, stomachs, blood, tissue samples) and use the cadaver to assist with future training (e.g. handling and tag attachment).

#### Tagging

Selecting a tag attachment method involves identifying ways to minimise tag-related effects (e.g., altered animal behaviour and swimming ability, condition, energetics, or survival) that may otherwise confound

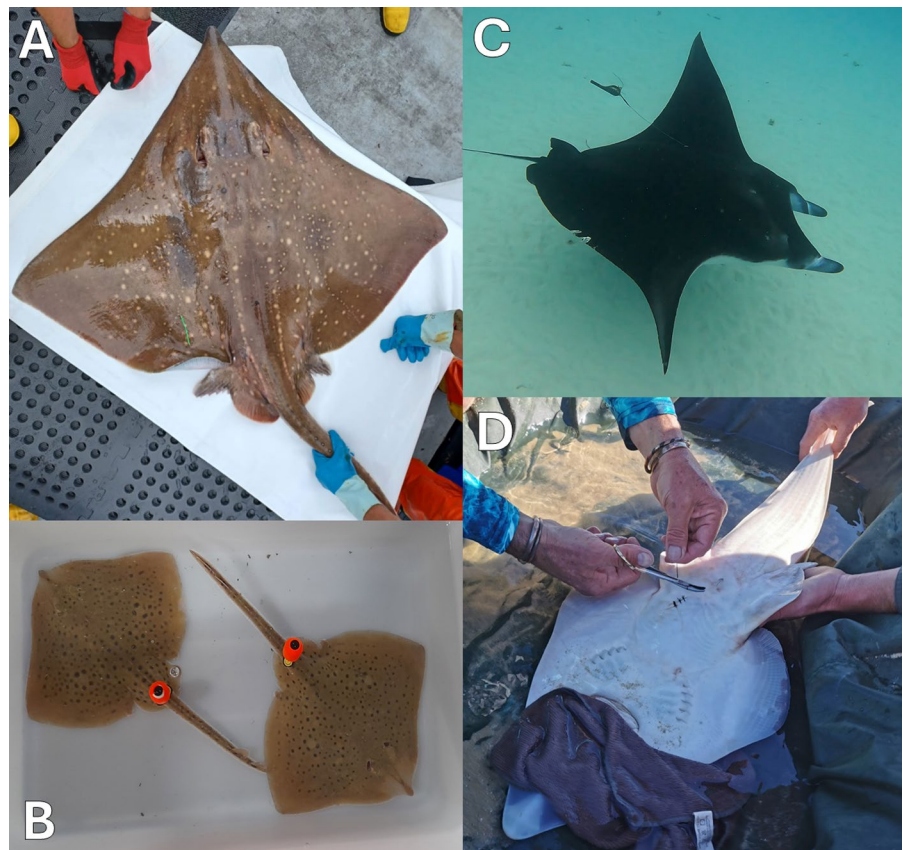


the results (Cooke et al. 2013). Tagging procedures must be appropriate for the animal's size, future growth, body shape, and behaviour and consider the study aim and duration, the endurance of the tag, and the environmental conditions the animal may experience (Holmes et al. 2022). Consideration should also be given to tag burden (the tag weight relative to the animal's weight) and follow appropriate guidelines (see Jepsen et al. 2004). However, note that these guidelines are based on teleost fish, not batoids, where body morphology and intracoelomic dimensions (e.g., internal tag implantation site) differ. For example, research on tag drag and lift for pop-up satellite archival tags (PSATs), has been studied in a flume tunnel system using weights (to simulate an animal) (Grusha and Patterson 2005). This experimental work suggested species-specific weight limits for tag burden, e.g., only applying tags to cownose rays (*Rhinoptera bonasus*) with a weight of > 14.8 kg to avoid tag presence causing a significant energetic burden. Equally, long-term tagging impacts have been

observed for teleosts more so than skate and ray species (see review by Matley et al. 2024).

Researchers have attached a variety of tags to skates and rays (Fig. 1). Tag choice typically depends on the animal's life history characteristics (e.g., body size, target demographic, position in the water column, anticipated scale of movements), tag returns and reporting (e.g., whether the tag requires physical recovery for data download, or whether it must be externally visible for community reporting or satellite uplink) and the study question (i.e. desired resolution of data and the period of deployment) while minimising impact on the animal. See Mull et al. (2022b) for a detailed description of tag types and a decision tree to determine the appropriate technology. Prior to tagging, consideration should be given to the tag configuration to determine data type and quality (resolution), tracking duration (longevity) and the most appropriate method of attachment (see Fig. 1 for an illustrative overview of tag types). While some tag types are strictly external, such as mark-identification

**Fig. 1** Skates and rays tagged with various electronic tags. **A** A dart mark-recapture tag positioned on the wing for large-scale mark-recapture studies of flapper skate, *Dipturus intermedius* (photo credit: The CETUS Project). **B** Pop-off data storage tags attached using Petersen discs to spotted rays, *Raja montagui* (photo credit: Eleanor Greenway). **C** A SPLASH satellite tag on a reef manta ray, *Mobula alfredi* (photo credit: Asia Armstrong). **D** An internally implanted acoustic tag used to study lesser guitarfish, *Acroteriobatus annulatus* (photo credit: Paul Cowley)



(hereafter mark-recapture) tags (Fig. 1A), archival (Fig. 1B) and satellite tags (Fig. 1C), other technologies may be either externally attached or internally implanted (see acoustic tag implantation in Fig. 1D).

### External tagging

External tagging includes conventional or mark-recapture (Fig. 1A) and electronic tags (Fig. 1B–1C) attached to the animal's fins, tail or muscle.

Mark-recapture tagging is a powerful, low-cost tool to identify repeat site use, track longitudinal movements across large spatial scales and model population dynamics (Kohler and Turner 2019). This method involves attaching a conventional tag either through or just beneath the skin of an animal. These tags are typically made of nylon or stainless-steel heads and resistant plastic and have a unique marked alphanumeric identifier to aid later identification upon recapture. Several different types of mark-recapture tags have been used on skates and rays, including jumbo, rototags, self-locking tags, livestock identification tags, Petersen discs, dart tags, and T-bar tags (Grubbs and Musick 2007; Bird et al. 2020).

The appropriate tag type will vary with the size and shape of the studied skate or ray, the life stage (e.g., juveniles vs adults), animal activity levels, ease of application, cost, and the size of the tag versus the size of the animal. Jumbo and roto tags are typically applied using an applicator through a hole in the first or second dorsal fin. Due to their large surface area and associated biofouling (as seen in sharks, Brevé et al. 2016; Dicken and Smale 2006), jumbo and rototags are not recommended. These tag types are also susceptible to vertical movements (Davies and Joubert 1967) and, therefore, shedding. Petersen disc tags (Petersen 1896) involve loading a disc onto a stainless-steel wire, which is then inserted into the ventral side of the wing away from the edge and organ cavity. The dorsal side of the disc displays a unique identifier number, and the sharp end of the wire is snipped and coiled to secure the tag in place. Identifying a minimum animal size that can safely accommodate discs is vital owing to the potential for tag placement to affect animal growth. For example, Ellis et al. (2018) opted only to tag skates (five Rajidae species) > 35 cm in total length. Smaller individuals would require a looser tag fitting to accommodate growth, which would require more

of the wire of the Petersen disc to be exposed, which could otherwise lead to snagging, given the benthic and burying nature of the studied batoids. This technique can also be used to secure electronic tags using specialised tag housings attached to the wing via pins (Silva et al. 2020; Thorburn et al. 2021). In addition, dart and self-locking tags are also suitable for flat-bodied skates and rays. In these cases, a dart tag can be inserted dorsally into a muscle-dense area of the pectoral fin (away from the internal organs), while self-locking tags are typically pierced through the cartilage of the spiracle (Martins et al. 2020a, b, c). Dart tags can be retained in certain ray species for > 12 years (e.g. lesser guitarfish *Acroteriobatus annulatus*, Murray et al. 2023); however, retention time is generally significantly reduced relative to their shark or teleost counterparts. For example, it has been observed that recreationally targeted stingrays and skates in South Africa have retained dart tags from less than two months (e.g. greyspot guitarfish *Acroteriobatus leucospilus*) to almost seven years (e.g. honeycomb stingray *Himantura uarnak*) (Jordaan 2023). The impact of these three small, lightweight tag types on skates and rays is typically negligible when carefully attached. However, infections at the attachment point or extensive muscle damage can occur, and algae accumulation is likely (Brevé et al. 2016).

Satellite tagging is a widely used tool for exploring the broad scale movements, home range, habitat use, and connectivity of skates and rays (e.g. Ajemian and Powers 2014; Omori and Fisher 2017; Kneebone et al. 2020; Brewster et al. 2021). Tag types are categorised as either location-transmitting tags (e.g., smart position and temperature (SPOT) transmitting tags) or pop-up archival transmitting (PAT) tags (Mull et al. 2022b). Although researchers widely use SPOT tags to track various shark species, these tags have limited effectiveness with skates and rays. The limitation arises because SPOT tags require the tagged animals to surface to transmit their geographic positions, a behaviour not typical of skates and rays, though towed-float tags or tethers may aid in this regard (e.g., Ajemian & Powers 2014). However, the use of PATs is on the rise for some surface-oriented species (e.g., Stewart et al. 2018). For example, 22 reef manta rays (*M. alfredi*) were tagged with miniPAT tags in Ningaloo Reef, Western Australia, revealing previously undocumented movements between two World Heritage Areas (Armstrong et al.

2020). Additionally, SPLASH satellite tags deployed on juvenile *M. alfredi* in Raja Ampat, eastern Indonesia, identified a small home range and restricted movements within a discrete nursery area (Setyawan et al. 2022b). In recent years, the growth in satellite tagging data has also resulted in valuable meta-analyses for chondrichthyans, for example assessing fisheries threats (Queiroz et al. 2019), identifying collision risk hotspots from shipping (Womersley et al. 2022), exploring vertical habitat use (Andrzejczek et al. 2022), and providing management recommendations (Womersley et al. 2024).

Researchers have also widely used electronic data storage tags, with or without a pop-off mechanism, to study skates and rays (e.g. Hunter et al. 2005; Pinto et al. 2016; Catchpole et al. 2017; Silva et al. 2020; Thorburn et al. 2021). High-resolution biologging tags record high volumes of data that cannot be readily transmitted, necessitating the physical retrieval of the tag for accessing the stored information. Animal-borne video cameras are an example of such tags that require retrieval to access the recorded data. For example, animal-borne cameras deployed on manta rays (reef manta ray *M. alfredi* and oceanic manta ray *M. birostris*) using suction cups provided insights into their foraging ecology, social dynamics and swimming behaviours (Stewart et al. 2019; Pelletier et al. 2023). However, these observations were constrained by short deployment durations, with a mean of approximately 88 min. Although these tags are gaining popularity among marine megafauna research and show promise in not creating energetically costly drag impacts in mobulas (Fontes et al. 2022), their use in tracking skate and ray movement, habitat use and foraging behaviour remains limited and short in track duration, likely due to cost and challenges in attachment and camera retrieval.

### *In situ external tagging*

In certain situations where environmental conditions and animal behaviour allow, deploying tags and collecting samples without directly capturing or handling the animals using in situ appropriates may be feasible. To date, this has predominantly occurred for larger, pelagic species such as manta rays (i.e., *M. birostris*, Andrzejczek et al. 2021; *M. alfredi*, Harris et al. 2021), although there are also examples of such deployments occurring with larger benthic rays

(broad cowtail ray *Pastinachus atrus*, Speed et al. 2013; Lutz's stingray *H. berthalutzae*; Branco-Nunes et al. 2021). A comprehensive cost–benefit analysis before a study can help guide whether in-situ or direct capture methods are preferable. Before implementing this tagging method, it is vital to consider two factors: (1) how to deploy the tag (i.e., using a tagging pole or by hand), and (2) how to approach the animal in a way that does not alarm it and does not put the safety of the person deploying the tag at risk.

The deployment method depends on the desired tag type and typically involves using a tagging pole, e.g., using a Hawaiian sling to insert a dart carrying the tag into the dorsal musculature (Armstrong et al. 2021). However, in this context, the accuracy of tag placement becomes challenging due to the mobility of the target individual. Despite this, the relatively large size of manta rays reduces the risk of harmful tag placement, explaining why they are preferred candidates for this tagging technique. Additionally, the large size of this taxa generally prohibits direct capture and handling due to ethical and logistical concerns (however, see Kessel et al. 2017). In situ tagging may be conducted using SCUBA diving (e.g. *M. alfredi*, Armstrong et al. 2021), via a free-diver (e.g., *M. birostris*, Graham et al. 2012) or from a boat (e.g. spinetail devil ray *M. mobular*, Canese et al. 2011), with the choice of deployment method dependent on the speed, depth use and behaviour of the target individual. In the case of manta rays, they have a blind spot, making it best to approach from above and behind to deploy the tag. Tagging can be more accessible when the animal is engaged in feeding or cleaning behaviour rather than cruising. For example, in the Seychelles, SCUBA divers deployed satellite tags when *M. alfredi* was aggregating around cleaning stations, while free divers were used for deployment on surface swimming animals (Peel et al. 2020).

### *Internal tagging*

Internal tagging is increasingly favoured in skate and ray research and management because it can provide detailed, long-term data on movements, behaviour, habitat use and reproduction (Ramsen et al. 2017; Frisk et al. 2019). Internal implantation does not create hydrodynamic drag like external tags, has high retention rates, and is thought to have minimal effect on natural animal behaviour (Rub et al. 2014).

Acoustic transmitters and data storage tags (DSTs) are the most common form of internal tag surgically implanted in skates and rays. However, while not yet widely used in batoid research, radio tags, oviduct tags, and gastric implants can glean novel information on reproductive ecology (Sulikowski and Hammerschlag 2023), feeding behaviour, and digestion patterns (Brunnschweiler 2009). Techniques for surgical tagging vary based on species-specific anatomical considerations to ensure minimal impact on fish health and behaviour while maximising tag retention and data reliability. Short-term studies should aim to swiftly attach tags to minimise stress and not promote unusual behaviours by the study animal during the tracking period (Speed et al. 2013). External tagging may be preferable, particularly if there is a need or a chance of recapturing the animal and removing the tag after the study window. When considering surgical tagging, the following questions should be raised: Is surgical tagging required? Does the research team have suitable training and expertise in surgical tagging? Is the size of the tag suitable for the body size? And can surgery be completed in a sterile environment?

Where appropriate, logistically feasible, or legally required, tags should be surgically inserted in conjunction with anaesthesia (see Sect. "Methods and recommendations for anaesthesia and analgesia"). Maintaining an aseptic environment during surgical procedures that involve breaking or cutting the animal's skin is essential to reduce the risk of post-operative infection, maximise survival, and promote animal care (Wagner and Cooke 2005). Although robust guidelines have been developed for aseptic surgery (LASA 2017), implementing aseptic technique during field research can be particularly challenging and requires careful consideration in light of the species, platform, and conditions. While the merits of aseptic technique are still debated in fish field ecology (Jepsen et al. 2013; Mulcahy 2013; Mulcahy and Harms 2014; Fiorello et al. 2016), given the potential benefits of reducing sub-lethal and lethal effects, we would advocate its application. For example, using single-use scalpel blades, hypodermic needles and single-use surgical gloves is strongly recommended. Ideally, instruments should be autoclaved (high-temperature steam at pressure) in advance and transported in a sealed autoclaved container. Instruments must be disinfected between animals where it is not feasible or

practicable to use one set of instruments per animal, for example, by using 70% ethanol followed by a sterile saline solution or sterilised water rinse. Researchers should disinfect instruments (including tag applicators) between individuals; this can be achieved by taking multiple sets of instruments into the field and rotating through sets while previously used ones are re-disinfected. At the bare minimum, sanitising ungloved hands between animals can reduce the chance of contamination and spread of disease. Some countries' federal guidelines state that aseptic techniques, i.e., sterile single-use surgical gloves and sterile tools, must be used (e.g., Canadian Council on Animal Care 1993).

Given the aquatic environment's high microbial load, the suture method must prioritise limiting infection pathways from the external to internal cavities to reduce the risk of tissue inflammation and expedite wound healing (Wagner and Cooke 2005). Best practice includes dissolvable monofilament (rather than braided) suture materials, as these reduce the potential entry pathways for pathogens. Techniques such as interrupted cruciate sutures and double-layer suturing (e.g., initial suturing of the coelomic membrane and muscle, then a second layer of suturing to close the muscle and skin) can create more secure closures, reducing gaps where bacteria could infiltrate. For example, Buckley et al. (2020) used interrupted cruciate sutures to close the incision made for the insertion of an acoustic transmitter (36 mm length) in large-tooth sawfish (*Pristis pristis*) released from a public aquarium. Shorter handling and surgery times are preferable for animal recovery, and the chosen suturing technique should also suit the tagger's capabilities (i.e. skill level) to ensure proper wound closure. Proper wound edge apposition is crucial to creating a watertight seal, particularly for aquatic animals like batoids that often bury themselves. Recognizing species-specific variations, such as skin thickness and healing rates, can also guide the choice of suture technique and materials to enhance overall outcomes. There are limited studies and a lack of guidelines detailing best practice surgical tag implantation in fish, let alone batoids. Wagner and Cooke (2005) considered the range of methods employed by researchers in this field and concluded that improvements in the standards of training and reporting of such procedures would benefit the community. At the bare minimum, sanitising un-gloved hands between animals



can reduce the chance of contamination and spread of disease. Some countries' federal guidelines state that aseptic techniques, i.e., sterile single-use surgical gloves and sterile tools, must be used (e.g., Canadian Council on Animal Care 1993).

### *Animal release*

The same precautions for handling skates and rays apply to their safe release. Understanding the external and internal anatomy of the target species is essential to avoid or minimise potential sublethal and lethal injuries from inappropriate practices (Smith et al. 2004). Due to the dorsal–ventral flattening characteristic of this group, it is essential to support the lower part of the body, either with hands or adapted devices such as a circular stretcher, to prevent organ damage (Smith et al. 2004). Releasing an animal by the tail or cephalic lobes is never recommended (Hutchinson et al. 2017; Reynolds et al. 2017) as it can lead to damage and skeletal injuries. Similarly, releasing the animal by the gill slits and spiracles, especially in larger stingrays, can cause severe injuries such as tearing of the slits or spiracles and dropping the animal due to instability. Holding the animal solely by the pectoral fins is also not advisable, as it provides insufficient support for the internal organs if the lower part of the body is not supported. Whenever possible, consider the use of release devices to reduce the incidence of post-release predation.

Methods for the safe release of tagged skates and rays can vary considerably depending on the platform used for tagging and the size of the animals. Depending on the capture platform (e.g., land or vessel), different approaches and equipment may be required. When releasing skates and rays, consider cumulative capture and handling stress (Rangel et al. 2021), as this can impair swimming and increase predation risk. Before releasing animals, a Reflect Action Mortality Predictor (RAMP) can be used to evaluate animal condition and relies on a positive response to basic reflex indicators, e.g. ocular tap, which results in close and retraction of the eyes (see Sect. "[Rapid health and vitality assessments](#)"). For smaller animals, a sea pen or release box can provide a safe place for animal recovery prior to release. If onboard or land monitoring is not possible, in-water monitoring can be used to identify abnormal post-release behaviour. Safe release using anti-predator cages or

a simple basket can enable a gentle and controlled release of the animals without direct handling. In contrast, larger animals require more robust support mechanisms like slings to ensure their safe release and minimise stress. These slings can support the animal's body during handling and release, reducing the risk of injury. Additionally, it is advisable to avoid releasing animals into areas of high predator abundance or in regions with intense fishing activity, as this may lead to increased predation risks, recapture by fishers and subsequent death due to the accumulation of stress.

### Concluding remarks and future directions

This review has covered a wide range of topics and issues related to practical field research on skates and rays, ranging from ethical and regulatory issues to practical advice on capture, handling, tagging, sampling, and safe release. We aim to provide an informative synthesis of the various issues and guidance for field biologists, members of ethics review committees, and licensing authorities to consider and apply as appropriate to the specific context. Context is central to designing a practical, effective, and logistically feasible protocol before conducting fieldwork, and it relies on two key lines of questioning. Firstly, in line with a cost–benefit approach, how do the research objectives, potential outcomes, and benefits of achieving those outcomes compare to the potential impacts of capturing, handling, and even euthanizing the animal? Secondly, how does the research relate to the broader context of biology and species conservation? For instance, in many areas, recreational and commercial fishers can catch, handle, release, or even kill elasmobranchs without restriction, having far greater impacts than researchers working on the conservation of those same species. In general, elasmobranchs are considered robust to injury and can heal relatively quickly from small to large and severe wounds (Kajjira et al. 2000; Hoyos et al. 2013; Chin et al. 2015; Riley et al. 2009). Consequently, the methods and practices chosen by a researcher and the requirements specified by managing authorities should be reasonable and proportional given these contexts.

Field researchers, ethics committee boards, and managers should practice reflexivity and identify their positionality when considering skate and ray research methods. Reflexivity helps individuals recognize how



their values, beliefs, and experiences (e.g. cultural and institutional settings) shape their viewpoints and interactions, which can otherwise significantly influence an individual's engagement in the research process (Merriam et al. 2001). While common in other sciences, reflexivity's role in conservation is now gaining recognition (Boyce et al. 2022; Pienkowski et al. 2023). Given ongoing debates on research impact versus conservation benefits, individuals should identify and transparently communicate their evidence-based values and beliefs, remaining open to differing views to foster collaborative solutions.

Considering these complexities, we do not advocate any one method or approach. Instead, we invite researchers, managers, and practitioners to carefully consider the options presented here and contextualise them to make evidence-based and transparent judgements regarding the appropriate methods and approaches and why. Regardless of the methods or approach, proper training for all personnel involved in capturing, handling, sampling and releasing skates and rays is essential. Training programs should cover species-specific anatomy and handling techniques, the use of appropriate equipment, emergency procedures, and safe release guidelines. Ensuring everyone understands the importance of minimising animal stress and injury is crucial. Regularly updating training materials based on the latest research and best practices can help maintain high standards of care and post-release survival. Furthermore, research groups running projects with specific species should create their own best practices manuals based on their expertise and learning experiences and share these with the broader research community to help develop well-evidenced research protocols on a species-specific basis. Disclosing methodological failures and improvements will help contribute to a broader understanding of best practice approaches for researchers, which is critical considering the scarcity of species-specific release recommendations for skates and rays.

While this review provides recommendations, several clear data gaps were identified in the process that must be addressed in order to promote animal welfare and develop skate and ray protocols:

1. Species-specific best practice manuals for researchers to sample and release species, considering their morphological, physiological, and behavioural characteristics. These manuals should include photos and or illustrations of best practices and common mistakes to facilitate understanding by a broad audience.
2. Empirical studies are needed to gather evidence on the effectiveness of anaesthesia, particularly local anaesthetics, analgesics and antiseptics for all skate and ray species. This information is essential to ensure the safety and well-being of the animals during and after surgical procedures.
3. Species-specific research is needed to determine how long anaesthetics remain within fish tissues. This will help identify appropriate safe release times for commercial species at risk of being consumed by humans and other animals.
4. Well-developed euthanasia protocols are needed for a range of species, particularly in cases of irreversible damage caused by capture or improper handling that will lead to mortality.
5. Species-specific research into the physiological effects of capture stress, animal management, and tagging is needed. These studies should consider differences between sexes and life stages, as well as the impacts of various fishing methods, management protocols (e.g., duration out of water), and tag types.
6. Targeted research into species-specific physiological markers and behavioural proxies for animal stress, condition and health. These studies are crucial for improving both fishing management practices and scientific research methodologies.
7. Targeted research into tag burden for batoid species, including minimum recommended animal sizes for implanted and externally attached tags, to minimise tag impact on natural behaviour and animal energy budgets.
8. Future work and field protocol guidelines are particularly needed for deep water skates and rays, including best practice capture, handling, tagging (both internal and external), and safe release. These guidelines should be tailored to the unique challenges and conditions associated with deep water environments.

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## Declarations

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