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Evaluating the potential of photo-identification as a monitoring tool for flapper skate (*Dipturus intermedius*)

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Abstract

- 1. Flapper skates (*Dipturus intermedius*) were once widespread in European shelf waters but are currently classified as critically endangered by the International Union for Conservation of Nature due to historical overexploitation. Novel monitoring approaches are needed to assess the efficacy of management measures, such as dedicated marine protected areas, for the conservation of relict skate populations.
- 2. Flapper skates possess distinctive dorsal spot patterns, which could potentially be used for individual recognition using photo-identification (photo-ID) approaches. This study assessed the potential of photo-ID as a method for individual recognition of a relict population of skates within a dedicated marine protected area in western Scotland (UK), which has long been targeted by directed recreational angling. A collection of 486 photographs of 373 separate skate capture events from 2011 to 2016, taken with standard mobile phones and compact cameras, was studied using visual pairwise comparison methods to determine number of individuals and recapture rates.
- 3. Results indicated that adult flapper skates were individually recognizable with a high degree of certainty through comparison of spot patterns, assuming appropriate lighting conditions. A total of 226 individuals were identified, of which 77 (34%) were recaptured at least once. The average recapture interval was 308 days (SE: 29.4 days), with the longest recapture interval to date being 4.4 years. Spot patterns among recaptured tagged or otherwise uniquely identifiable skates were found to remain stable over timescales of months to >1 year.
- 4. Results indicate that photo-ID, based on photographs sourced through citizen science approaches, can provide a low-cost alternative means of monitoring flapper skate presence and distribution for the purposes of underpinning management decisions.

KEYWORDS

elasmobranchs, fish, fishing, marine protected area, monitoring, ocean, protected species, recreation, sublittoral

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1 | INTRODUCTION

Understanding of animal ecology has been transformed through the development of methods allowing reliable identification of individuals over extended periods. The ability to identify individual animals also provides significant benefits to conservation research and practice. Individual identifications aid in answering important questions on population sizes, distributions, and habitat requirements and are particularly beneficial at low population sizes (Parra, Corkeron, & Marsh, 2006). The largest individual-identification studies are based on bird ringing, with hundreds of thousands of birds being individually tagged each year in the UK and Ireland alone (Walker et al., 2016). Individual identification is particularly important when studying animals in small populations as it allows individual behavioural variability to be taken into account (Austin, Bowen, & McMillan, 2004) and also allows an assessment of growth and mortality rates in situations where large statistically valid cohorts are unavailable (Clutton-Brock & Sheldon, 2010). In addition, certain individuals may have particular ecological or societal significance, and regular recording of recognizable individuals may facilitate outreach efforts (e.g. through sponsorship/adoption).

One way in which individual identification can be achieved is by using artificial tags or markers (Silvy, Lopez, & Peterson, 2012). Many different tag designs are available, ranging from physical tags such as bird rings or seal flipper tags, which must be read after recapture, to passive integrated transponder (PIT) tags and active acoustic tags whose presence is recorded when they are near a receiver, to telemetry tags transmitting data via satellite over great distances (e.g. Ehrenberg & Steig, 2003; Gibbons & Andrews, 2004; Matthiopoulos, McConnell, Duck, & Fedak, 2004; Pomeroy, Smout, Moss, Twiss, & King, 2010). Such methods can be controversial, particularly in studies involving rare species, as the tagging process, tag presence, or subsequent injury may induce stress, impact long-term welfare, and/or affect the very behaviours under study (Calvo & Furness, 1992; Dann et al., 2014; Kohler & Turner, 2001). An alternative, less invasive identification method involves recording presence of "natural tags," which can include vocalizations (e.g. Terry, Peake, & McGregor, 2005) or DNA (e.g. Taberlet & Luikart, 1999; Woods et al., 1999). However, such studies most commonly rely on visible external features, such as skin markings (e.g. Arzoumanian, Holmberg, & Norman, 2005; Gilkinson, Pearson, Weltz, & Davis, 2007; Würsig & Jefferson, 1990), or variations in the shapes of structures, such as ears or fins (Towner, Wcisel, Reisinger, Edwards, & Jewell, 2013; Würsig & Jefferson, 1990).

Photo-identification (hereafter referred to as photo-ID) methods are based on the ability to identify individual animals based on photographs of distinctive natural marks (e.g. skin pigmentation patterns, fin shapes, scars), which can then be used to reliably identify each individual over time and across space. The method works best in cases where recapture rates are relatively high (i.e. populations are not enormously large and contain individually recognizable animals) and individuals can be reliably photographed without excessive effort. If animals have insufficiently discrete external marks to be visually distinguishable, conventional methods such as tagging may be more appropriate. Photo-ID studies also benefit from a relatively high proportion of identifiable individuals in a population (>50%; Castro & Rosa, 2005; Marshall, Dudgeon, & Bennett, 2011; Meekan et al., 2006), although lower proportions can still provide useful information (Würsig & Jefferson, 1990).

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The method has been applied to a wide range of marine species (often, though not exclusively, large, mobile, and long-lived vertebrates) to assess population abundance, residency, migration pathways, life history parameters, and social structures (e.g. Graham & Roberts, 2007; Karczmarski, Würsig, Gailey, Larson, & Vanderlip, 2005; Smith et al., 1999; Würsig & Jefferson, 1990). Photo-ID methods can also allow application of mark-recapture techniques to estimate population size (Hammond, 1986). In this case, more stringent assumptions apply, including the requirement that natural marks remain recognizable over time and have an approximately equal probability of being (re)sighted. If marks change appreciably over extended periods such that the animal is no longer recognizable, or if animals are unlikely to be recognizable in all but the best observational conditions, using such data as the basis for mark-recapture analyses would produce biased estimates of demographic parameters.

To date, most photo-ID studies in the marine environment have focused on marine mammals, which are regularly available at the surface to be recorded by visual observers (e.g. Baird et al., 2009; Langtimm et al., 2004; Würsig & Jefferson, 1990). There is, however, significant potential for applying this approach to other marine megafauna, including elasmobranchs (see Marshall & Pierce, 2012). Historically, most identification projects on elasmobranchs followed the protocols set out by early marine mammal work and used the dorsal fin of some species to identify individuals. This was successful in great white sharks (Carcharodon carcharias; Gubili et al., 2009) and basking sharks (Cetorhinus maximus; Gore, Frey, Ormond, Allan, & Gilkes, 2016). However, this restricts photo-ID to species that exhibit behaviour allowing regular sightings of the dorsal fin from the water surface. Following on from studies on marine mammals showing that markings on the skin or pelage can reliably be used to identify individuals of certain species (e.g. Paterson et al., 2013), the skin markings of clearly marked elasmobranch species such as whale sharks (Rhincodon typus; Arzoumanian et al., 2005; Graham & Roberts, 2007) have shown to reliably allow for the identification of individuals. This approach is now being used to study numerous other shark and ray species bearing visually identifiable markings (e.g. Bansemer & Bennett, 2008; Castro & Rosa, 2005; Dudgeon, Noad, & Lanyon, 2008; Klimley & Anderson, 1996; Marshall et al., 2011; Van Tienhoven, Den Hartog, Reijns, & Peddemors, 2007).

The ability to reliably identify elasmobranchs over time is a potentially significant tool to underpin or validate crucial assumptions about management approaches for these long-lived species, such as the establishment of marine protected areas (MPAs; e.g. Gormley et al., 2012; Schofield, Katselidis, Dimopoulos, & Pantis, 2008; Wilson, Reid, Grellier, Thompson, & Hammond, 2004). As such, understanding mark stability becomes very important. While markings in many species of marine mammals (e.g. seals) have been shown to be stable from pup stages through to adulthood, allowing them to be used for identification throughout the animal's life (Paterson et al., 2013), the stability of markings on elasmobranch skin is still poorly understood. While there are numerous studies demonstrating that skin markings in some species are highly stable, allowing individual identification over periods of years to decades (Anderson, Chapple, Jorgensen, Klimley, & Block, 2011; Holmberg, Norman, & Arzoumanian, 2009; Meekan et al., <u>1362 |</u>WILEY

2006), there are also examples of markings changing over time (Robbins & Fox, 2012).

Photo-ID, on its own, is non-invasive (Pauli, Whiteman, Riley, & Middleton, 2010) and thus avoids injury to animals during and post tagging through tag loss, fouling, and so on (Kohler & Turner, 2001). The method has also become progressively more practical in marine environments as digital cameras, underwater housings, and computers have become cheaper, more capable, and more widely available. This also means that photographs can be collected by members of the general public through directed citizen science projects, potentially resulting in significant expansion of sampling effort and increased engagement with project outcomes by local stakeholders (Dickinson et al., 2012; Dickinson, Zuckerberg, & Bonter, 2010; Marshall & Pierce, 2012). However, the basic requirement for a clear view of the subject remains, and so the majority of studies use photographs of parts of the animal visible above the surface or taken in clear water. For animals in deep, turbid or fast-moving water, one potential solution is to attract animals to bait, so that they can be photographed in situ or even captured and briefly brought to the surface for photography (Dala-Corte, Moschetta, & Becker, 2016).

The flapper or common skate (*Dipturus intermedius*), previously considered part of the *Dipturus batis* species complex, is the largest member of the family Rajidae found in European shelf and slope waters (Dulvy et al., 2006; Griffiths et al., 2010; Iglésias, Toulhoat, & Sellos, 2010; Last, Weigmann & Yang, 2016). Originally widespread, the species is now classified as Critically Endangered by the International Union for Conservation of Nature, largely due to its low intrinsic population growth rate and high sensitivity to overfishing (Dulvy et al., 2006). Although effectively extirpated across much of its historical

range (Brander, 1981; Jennings, Greenstreet, & Reynolds, 1999; Walker & Hislop, 1998), small populations persist in some areas, including along the western and northern coasts of Scotland (Dulvy & Reynolds, 2002). In recent years, increasing efforts have been put into conservation of these populations, including a 2009 landings ban for EU fishing vessels (although the species remains at risk from bycatch in multispecies trawl fisheries; Simpson & Sims, 2016).

One relict population of flapper skates occurs in inshore waters of western Scotland, centred on the Firth of Lorn (Figure 1). This area contains a number of deep basins (>100 m), where skates have long been, and continue to be, caught by recreational sea anglers. By 1975, concern about declining skate numbers led to the development of a tag-recapture programme aimed at the sea angler community (1975-2008), which generated considerable amount of data on skate movements and site fidelity (Little, 1995, 1997, 1998; Neat et al., 2015). These data were pivotal in clarifying the significance of the area to flapper skate, resulting in the designation in 2016 of the Loch Sunart to the Sound of Jura Nature Conservation MPA (Figure 1) by the Scottish Government (2016). The MPA conservation order has as its main objective the continued conservation of flapper skates within the Loch Sunart to the Sound of Jura MPA. Importantly, this does not preclude recreational angling or even commercial fishing using mobile gears within the MPA boundaries, although the latter activity is now restricted to the periphery of the MPA. Recreational angling for this species in Scotland is only permitted under a catch-and-release policy.

Monitoring skates within this MPA is a challenge for Marine Scotland and Scottish Natural Heritage (SNH), the competent government authorities. Traditionally, spatial data on skates in this area have been collected through close collaboration with the sea angling



FIGURE 1 Overview of the Loch Sunart to the Sound of Jura MPA (western Argyll, Scotland, UK). Main water bodies within the MPA, site boundaries and approximate angling locations where skates were photographed are indicated. The 100 m isobath indicates deep basins within the Firth of Lorn where catches of flapper skates have historically been high

community, using external identification tags applied by volunteer anglers and charter skippers (as described by Neat et al., 2015). Initially, skates were tagged using cattle ear tags on the trailing edge of the wing; later, anglers switched to Floy[™] dart tags that were inserted in the skates' dorsal wing muscle. This tagging approach proved effective in generating distribution, behavioural, and movement data for flapper skates. Nonetheless, concerns have increasingly been raised over risks to tagged animals' health and/or behaviour through improper tag application, as well as the potential for tag damage or loss (Jepsen, Thorstad, Havn, & Lucas, 2015; Marshall & Pierce, 2012; Thorstad, Økland, & Heggberget, 2001). More recently, implanted PIT tag technology has been applied to address these concerns (Kohler & Turner, 2001). Presently, only a small number of trained local charter skippers undertake PIT tagging in Argyll, limiting numbers of skates thus tagged. Moreover, as a dedicated PIT scanner is required to record recapture events, recaptures of PIT-tagged skates by individual anglers may go unrecorded. For these reasons, there was a desire to develop alternative methods to monitor distribution, recurrence, and movement of individual skates within, and across the boundaries of, the MPA in conjunction with existing tagging programmes.

Flapper skates represent a potential suitable candidate for photo-ID studies for several reasons. Animals typically possess a dark brown upper (dorsal) side with lighter spots arranged in a variety of patterns (Neal & Pizzolla, 2006; Stehmann & Bürkel, 1984). Spots are generally distributed in a broadly bilaterally symmetrical pattern but vary widely in terms of placement, size, clarity, and overall density, suggesting that they could enable photo-ID of individual skates. In addition, the species is long-lived (~50 years; Du Buit, 1977, although this work describes "Raja batis" [D. batis] caught off France, which may in fact refer to D. flossada) and occurs predictably in particular areas; as a large predator, it is expected to occur at comparatively low densities, potentially allowing for reasonable recapture rates. Flapper skates typically inhabit deep waters (>50 m), where they cannot be readily observed visually by divers (Neat et al., 2015), which would ordinarily limit the utility of photo-ID approaches. During sea angling, however, hooked skates are often briefly brought aboard to allow safe hook removal and collection of size measurements before being released. While aboard, skates are also often photographed by anglers and/or charter boat skippers. A database of such photographs, assuming sufficient quality, could thus provide a novel means of recording the presence of a greater number of individually identifiable skates across a wider area than can presently be achieved through tagging. Initial studies suggested that photo-ID methods could provide a viable additional monitoring strategy for this species (Bradley, 2012; Cooper, 2012).

The aims of the present study were to: (i) confirm whether photographs of captured flapper skates collected by anglers/skippers would be sufficiently clear to allow identification of individuals using spot patterns and other features visible on their dorsal sides; (ii) determine whether such individuals could be reliably re-identified over timescales of months to years by means of such photographs; and (iii) assess the utility of photo-ID methods to monitor flapper skates in the Loch Sunart to the Sound of Jura MPA as a complementary approach to tagging studies.

2 | METHODS

2.1 | Data collection

Skates were captured during the course of regular angling trips by recreational sea anglers aboard RC's charter vessel Laura Dawn II, using rod-and-line techniques. All captures occurred in the central Firth of Lorn at two locations within the MPA boundaries (Figure 1); for reasons of commercial confidentiality, more detailed capture locations were unavailable for this study. Lines were baited using squid (Loligo vulgaris), octopus (Eledone cirrhosa), saithe (Pollachius virens), or mackerel (Scomber scombrus). Captures typically occurred in water between 120 and 170 m depth, with up to seven skates recorded captured per trip. Although large skates are particularly sought after by anglers, small (<50 cm wingspan) juveniles were captured occasionally as well. Following capture, skates were briefly landed on deck for hook removal, recording of measurements and tag numbers (when available), and collection of one or more photographs before being released. Photographs were taken if convenient and/or upon request from the customer; not all captured skates were therefore photographed. For the same reason, pictures nearly always included anglers posing with their catch, photographed under a wide range of lighting conditions and diverse camera angles.

For the purposes of this study, a 'trip' was defined as a single day on which angling occurred and at least one photograph of one or more captured skates was taken. No data were available on the total number of trips when no skates were caught or where no photographs were taken; use of photograph date metadata as indicators of angling activity was assumed to provide a reasonable approximation of overall effort distribution. Based on logbook data, approximately 80, 50, and 100 dedicated skate angling trips were undertaken for the years 2014, 2015, and 2016 respectively. A total of 486 photographs were available for this study, all taken by the same person (RC). A small number (37) of photographs were taken during 2011-2013 using a digital Olympus[™] S1030SW camera. From 2014 onward, the remaining 449 photographs were all collected using a Samsung[™] mobile phone camera. All photographs were taken from the deck of the vessel at a distance of ≤ 2 m from the skate, but the height of the camera above the deck and the camera angle relative to the skates' anteroposterior body axis varied widely, although the skates were facing the camera in almost all cases. Photograph dimensions varied between 850 × 720 pixels and 2592 × 4608 pixels. Date and time were logged as metadata for each photograph.

2.2 | Validation

An ongoing SNH/Marine Scotland tagging programme provided an opportunity to test the validity of the photo-ID approach. During March 2016, 39 skates were caught, equipped with PIT tags, and photographed by SNH as part of an ongoing study on skate movements. These photographs and PIT tag records were subsequently matched to tag records and photographs collected later in 2016 during routine angling trips. Matching PIT tag codes would provide independent confirmation of recaptured skates' individual identities and allow an assessment of long-term spot pattern stability.

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The 486 photographs were sorted into 373 unique capture events based on associated date and time metadata. Although the available photographs spanned a timeframe from August 2011 until October 2016, 92% of photos were taken from 2014 onward. Sixteen photos contained multiple (two or three) skates, each of which was considered to represent a separate capture event.

Photographs were taken using two cameras (one digital, one mobile phone camera) and under varying conditions, causing considerable variability in photograph quality due to different light conditions, parts of the skate being blocked by people, camera height above deck, angle of skate towards camera, and so on. Prior to matching, the best photograph of each capture event was selected and graded according to several basic parameters describing both picture and mark quality (Urian et al., 2015; Wilson, Hammond, & Thompson, 1999). Apart from blocking out peoples' faces for confidentiality purposes, no post-processing of photographs was undertaken. Photograph quality was assessed based on the following binary scale:

Poor quality

- i. Photograph is not in focus.
- ii. Photograph has insufficient resolution to reliably detect spot patterns.
- iii. The skate is being held up or otherwise not lying flat on deck.
- iv. Less than 50% of main body surface (excluding tail) is visible due to poor lighting conditions (shade and/or glare), attached sediment, obstruction by people or objects, and/or being photographed from a very low angle.
- v. The skate is photographed from the back, preventing a clear view of spots around the head and leading edges of the fins.
- vi. The skate is very small (<50 cm width), as it is not known at what age skates' spot patterns stabilize.

Good quality

- i. Photograph is in focus.
- ii. Photograph has sufficient resolution to reliably detect spot patterns.
- iii. The skate is approximately flat on deck and photographed from the front or side (\leq 90° of anteroposterior body axis).
- iv. Most (>50%) of main body surface (excluding tail) is visible, allowing spots to be observed clearly. Poor lighting conditions (shade and/or glare), attached sediment, and/or obstruction by people or objects may locally affect spot visibility.

Photographs were graded as "poor quality" on this scale if one or more of the listed criteria were noted. Poor-quality photographs were not used in the present analysis to minimize the risk of incorrect reidentification (Urian et al., 2015). Good-quality photographs were only identified as such if all the listed characteristics were observed. Given that most photographs were taken with the skate facing the camera, matching efforts focused on spot patterns on the front of the body. Spot patterns around the head (inclucing the rostrum), the proximal part of the vertebral column, and the leading edge of the

pectoral fins proved particularly useful for photo-ID. The presence and location of individual spots, linear aggregations, and spot clusters relative to skates' eyes, spiracles, tip of rostrum, anteroposterior body axis, and/or pectoral fin edge were used to confirm individual identities. Occasionally, spots on other parts of the body, notably the pelvic fins and the tail, could also be used, but these were considered of secondary importance. Examples of locations of spot patterns used for photo-ID in this study are provided in Figure 2a-d.

The vast majority of skates possessed clearly visible spot patterns spread across their entire dorsal surface. This meant that mark quality was typically sufficiently good to allow individual identification even if only a portion of a skate's dorsal surfaces was visible. Nevertheless, some skates displayed only very few spots, which might affect identification probability of such poorly marked animals. For the present study, a skate's mark quality was therefore also classified using the following binary scale:

Poorly marked	The skate possesses no or very few, small spots; the dorsal surface appears almost monochrome.
Clearly marked	The skate possesses numerous spots (i.e. tens to hundreds), either clustered in discrete locations or distributed more evenly across the dorsal surface

Photographs were compared by eye on two adjacent computer screens in an iterative pairwise comparison process, such that each photograph was compared with each other photograph on two separate occasions. Two observers (SB and VAM) undertook comparisons independently as an additional check. Pictures were considered a match only if spot patterns (as opposed to scars, etc. on the skin) were identical. To ensure this was the case, multiple areas across the body surface were compared, and spots of increasingly small size were used to confirm or reject matches as appropriate. In practice, most skates' spots were sufficiently distinctive to make a clear judgement on whether or not two photographs were of the same individual. Spots used for photo-ID purposes were generally ≥ 1 cm in diameter, to avoid undue reliance on features that might not be clearly distinguishable in all photographs. Matching was further aided by recording the skate's gender (based on larger female size and presence/absence of male secondary characteristics, such as claspers and alar thorns; McEachran & Konstantinou, 1996) and any permanent injuries (fin nicks, scars, etc., including those resulting from historical tagging efforts). Confirmed individuals were given an individual alphanumeric code (e.g. Di000001) for inclusion in a master database. Inter-sex differences in (re-) capture rates were assessed through χ^2 tests (Zar, 1999). Graphs underpinning results were created using the R package ggplot2 (Wickham, 2009).

3 | RESULTS

3.1 | Effort summary

Photographs were collected during 165 chartered sea angling trips between August 18, 2011, and October 23, 2016. The greatest number of trips, as well as highest rates of recorded capture events per trip, occurred during 2014-2016 (Table 1). Trips took place

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FIGURE 2 An example of the types of spot patterns (circled for added emphasis) used in the matching process. (a) Overview of the entire individual (Di000184, captured May 18, 2016); (b) Close-up of head, with distinctively spotted areas on the rostrum and cranium; (c, d) Aggregations of large and small spots on right and left pectoral fin respectively. Matching often relied on using spot patterns across the skates' dorsal surface. Original image copyright R. Campbell

throughout the year during the 2014–2016 period, excluding January. However, 57% of trips in these years occurred during the March–May period, which is the peak season for dedicated skate angling (Figure 3a). Average catch rates (number of skates per trip) remained broadly consistent between years; there was, however, considerable variability within years (Figure 3b). Gender could be determined for 96% of skates photographed during 2014–2016; on average, 81% of these skates were females. A small subset of individuals was designated "gender unknown," pending better photographs of these individuals becoming available.

3.2 | Identification

Twenty-four of the 373 original capture events (approximately 6%) were excluded from further analysis based on poor-quality photographs, mainly due to poor lighting conditions, skates being lifted off the deck, and the photograph having been taken from behind the animal (Table 1). It is worth noting that several pictures of skates photographed from behind were of sufficient quality to allow retrospective successful matching with other good-quality photographs, but this could not be achieved consistently in all cases. Six capture events involved very small (<50 cm wingspan) skates, likely juveniles. These were also excluded from further analysis because they were typically being lifted completely off the deck by anglers while being photographed, preventing a clear view of the dorsal surface. This left 349 unique capture events with at least one good-quality photograph available for potential matching, resulting in the identification of 226 individual flapper skates.

3.3 | Spot pattern variability

Although most skates possessed many spots, the observed degree of variability between individual skate's patterns was considerable (Figure 4). The size and clarity of individual spots also varied greatly within and between individuals. Although spot patterns were broadly bilaterally symmetrical in terms of locations of distinctive aggregations of spots, considerable variation between left and right sides was observed. Some skates' spots were clearly delineated with a sharp outer edge, while other spots were less distinct or presented a "doughnut" appearance with a dark centre surrounded by a lighter ring. Some skates also possessed highly distinctive dark spots. The often large number of unique spot patterns observable across skates'

 TABLE 1
 Summary of recorded angling trips and number of identified/non-identified skates, 2011-2016

	2011	2012	2013	2014	2015	2016	Total
Total number of trips	1	8	3	45	40	68	165
Total number of capture events:	1	12	5	92	86	177	373
involving only poor-quality photographs (including juveniles)	0	0	1	10	4	9	24
involving at least one good-quality photograph	1	12	4	82	82	168	349



FIGURE 3 (a) Average number of recorded angling trips by calendar month (±SE; 2014–2016). (b) Average number of skates captured and photographed per trip (±SE; 2014–2016). Note that in (b), November and December data are based on two trips and one trip respectively

dorsal surfaces facilitated individual identification even when only part of the skate was visible. This enabled reliable identification of skates even where only part of the dorsal surface was visible, if enough unique spot patterns could be observed. A small number of skates (n = 9 individuals) possessed only a small number of clear markings (see Figure 4, top right), and these were accordingly separated into a distinct "poorly marked" category. However, all available pictures of these individuals were sufficiently clear to allow reliable identification both amongst themselves and in comparison with other skates.

3.4 | Recapture rates

Recaptures of PIT-tagged skates of known identity during 2016 allowed changes in individual spot patterns to be investigated across intermediate timescales (months). Of 39 photographed skates equipped with PIT-tags in March 2016, 11 individuals were subsequently recaptured, sometimes multiple times (n = 16 events in total). Both PIT tag identities and photographs were collected for 11 recapture events, involving eight individuals. Recapture intervals in these eight cases ranged from 45 to 122 days. In all cases, spot patterns were found to have remained essentially static during these periods (Figure 5a,b). Spot pattern stability was also verified through recaptures of malformed or extensively injured individuals, which were likely to possess unique injury patterns. The present database contained a single such individual (Di000063), a female with an extremely recognizable, large longitudinal scar and damaged rostrum likely resulting from a historical injury (Figure 5c,d). This individual was originally caught and photographed three times in 2014 and 2015, before being PIT-tagged and photographed during the March 2016 campaign. Maximum recapture interval length was 665 days, during which this skate's spot pattern was found to have remained stable. If, as seems likely, the degree of spot pattern stability observed in these PIT-tagged or otherwise uniquely identifiable skates is representative of the entire local population, then skates may remain individually recognizable by their spot patterns over multiple years, supporting the utility of photo-ID assessment methods.

Skate recapture events, identified through visual matching of photographs, occurred regularly throughout the study period. A total of 123 recapture events were recorded across years, albeit concentrated in 2016, when more skates were caught (20, 31, and 72 recapture events in 2014, 2015, and 2016 respectively). Of 226 identified individuals, 77 (34%) were recaptured at least once, of which 24 (10% of total) more than once, during the six years covered by the photographic database (Figure 6a). There were no significant differences in recapture rates between males and females (χ^2 test: p = 0.4199; v = 5 df). Recapture intervals varied widely, from <1 day (i.e. the same individual being recaptured within the same angling trip) to 1613 days (equivalent to 4.4 years). Excluding same-day recaptures, the average time between successive recaptures was 308 days (SE: 29.4 days), or just under 1 year (Figure 6b). Similarly, average time between first and last (most recent) recapture was 495 days (SE: 42.9 days). Three of the nine "poorly marked" skates were reported recaptured (each only once), with an average recapture interval of 346 days.

Skates were observed to gain and lose a variety of non-permanent marks over time. Such marks were superimposed over their permanent pigmentation patterns and were sometimes visually prominent. Some skates had extensive longitudinal scars across one or both pectoral fins, potentially derived from predation attempts, intraspecific aggression or interactions with fisheries (Figure 7a,b). A few animals possessed extensive white or grey patches of unknown origin, which appeared to change appearance over time (Figure 7c,d). Many photographed skates suffered from ectoparasites, particularly skate



FIGURE 4 Illustration of spot pattern variability among individual skates (from top left to bottom right: Di000047, Di000061, Di000178, Di000186, Di000206, Di000212). Also note variation in spot size, shape, and intensity within each individual skate. Original images copyright R. Campbell

leeches (*Pontobdella muricata*). After the leeches let go (or were removed by anglers), their bite wounds eventually healed, leaving behind small, bright scars that slowly faded (Figure 7e,f). These scars tended to be a paler colour than the spots themselves, but were of comparable dimensions to smaller spots. While the presence of such scars could theoretically confound accurate matching if only their immediate surrounding area were observed, their presence would be unlikely to obscure overall spot patterns, and so the potential for incorrect matches is minimal.

A discovery curve was plotted to explore the relationship between the detection rate of "new" individuals against an everincreasing number of capture events (Figure 8; Williams, Dawson, & Slooten, 1993). As shown in Figure 8, the detection rate of new individuals had slowed down over time but had not yet stabilized. Since little is known about skate recruitment rates and movement patterns, these preliminary results should be treated with some caution, but they do suggest that the real population of flapper skates in the Firth of Lorn section of the Loch Sunart to the Sound of Jura MPA might well exceed several hundred individuals. This is in line with earlier observations by Neat et al. (2015) regarding the adjacent Sound of Jura (Figure 1).

4 | DISCUSSION

This study indicates that analysis of photographs of caught and released flapper skates, taken by sea anglers and charter skippers, can provide a simple, non-invasive means of monitoring presence of individual animals. Adult skates possess distinctive spot patterns on their dorsal surfaces that remain recognizable over periods of several years. Visual comparison of spot patterns on recaptured PIT-tagged or otherwise distinctive individuals suggested that changes to spot patterns were insignificant over these timescales. Most spot patterns in this study were sufficiently clear to be recognizable in photographs taken for nonscientific (i.e. advertising, recreational) purposes using commonly available (mobile phone) camera equipment. This suggests that photo-ID based on photographs submitted by members of the public (notably anglers) can provide a low-cost alternative source of information on flapper skates' residency and movement patterns in the Loch Sunart to the Sound of Jura MPA and further afield, as well as allowing eventual estimation of abundance through capturemark-recapture (CMR) modelling (Hammond, 1986).

The photographic database underpinning the present analysis was biased in several respects. Not all captured skates were photographed

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FIGURE 5 Examples of apparent spot pattern stability in flapper skates. White ellipses denote notable white and dark spot patterns used for matching. (a) Skate Di000097 on March 13, 2016, having just been PIT-tagged; an external acoustic tag was also applied in this individual for a different study. (b) The same skate recaptured on July 11, 2016. (c) Permanently scarred skate Di000063 photographed on May 22, 2014 (note extensive partially healed gash along left side of rostrum). (d) The same skate photographed following recapture and PIT-tagging on March 17, 2016. Note the white raised feature, likely a leech scar, behind Di000063's right eye in 2014, but which was no longer prominent in 2016 (c, d; white square). In contrast, dermal scarring along the leading right pectoral fin edge remained visible (c, d, white dashed ellipse). Original images copyright R. Campbell

before release due to various factors (e.g. skipper busy, poor weather conditions, skate too small to command interest). Sampling of skates occurred in only two discrete locations within the Firth of Lorn (Figure 1). CMR modelling based on 280 skates tagged in the adjacent Sound of Jura indicated significant heterogeneity in recapture rates (Neat et al., 2015), suggesting that the region contained a mixture of resident and transient individuals. This implies that recapture rates among "local" skates might be relatively high. Most skates photographed in this study were not recaptured, suggesting that the population in the Firth of Lorn might be relatively large and/or that animals might be more mobile than previously thought. The present photo-ID dataset also lends itself to CMR analysis to estimate absolute abundance of flapper skates in the Firth of Lorn. Determining appropriate abundance estimation scenarios based on this photo-ID dataset, and evaluating CMR modelling assumptions, will represent an important next step in studying this population.

Most skates could be reliably assigned to gender, with the exception of a small number of cases involving young skates or skates where rear portions of the body were not visible. Although young flapper skates do possess clearly visible spots, it is not known at what

age they develop the full adult spot pattern and thus become eligible for inclusion in the database. Skin patterns of animals of many species, including elasmobranchs, change during the first few years of life (e.g. Compagno, 1984; Wilson & Martin, 2003). Limited data from captive-bred barndoor skate (Dipturus laevis, a closely related species) suggest that hatchling skates might already possess some form of the adult spot pattern at birth (Parent, Pépin, Genet, Misserey, & Rojas, 2008), but further work is required to confirm the age at which flapper skates' spot patterns become fixed. Once established, the stability of these spot patterns over the life of the individual also needs to be assessed. The permanence of markings on elasmobranch skin is still poorly understood, and there are conflicting studies in the literature both supporting long-term stability (Anderson et al., 2011; Holmberg et al., 2009; Meekan et al., 2006) and short-term variation (Robbins & Fox, 2012). Our study suggested that spot patterns in flapper skates were stable over periods of at least 4 years; nonetheless, this apparent long-term stability of markings needs to be better understood before photo-ID can be fully relied upon as a long-term (i.e. decadal) monitoring tool for this species. The continuing combination of photo-ID work and identification tagging



FIGURE 6 (a) Distribution of number of recapture events (brown) per individual skate, 2011–2016. To date, most skates have only been captured once. (b) Distribution of all observed recapture intervals in years. To date, most recaptures have occurred within 1 year of previous capture

will allow this to be investigated further, as individuals can be identified by PIT tag number, allowing comparison of photographs for changes. A succession of tag recapture records across years supported by good-quality photographs will allow in-depth assessment of spot pattern stability as well as retention rates of non-permanent marks, such as scars and other injuries.

Capture and release through recreational angling can cause substantial stress in fish and may result in injury or even death under certain conditions (e.g. Campbell, Patino, Tolan, Strauss, & Diamond, 2009; Gallagher, Serafy, Cooke, & Hammerschlag, 2014). Effects of stress associated with capture and release on long-term health of flapper skates are presently unknown. The present study took advantage of ongoing recreational angling activities to gather information on flapper skates using a non-invasive tool (photo-ID), which eliminated additional stresses associated with physical tagging. In this manner, photo-ID can help reduce stresses experienced by skates during the capture and release process. Care should always be taken to return skates to the water as quickly as possible.

Photographs used in this study were taken for the benefit of customers and as circumstances allowed. As a result, they varied widely in terms of camera angle relative to the deck, ambient light levels, extent of light reflection or shadow across the skate, and how much of skates' dorsal surfaces was visible. This complicated attempts to match poor-quality photographs, which were therefore excluded from the present analysis. To improve the probability of a reliable match, multiple spot patterns were used across the dorsal surface area, such that a match could still be made even if part of the skate was obscured or poorly lit. However, further work is required to understand which areas are crucial for successful photo-ID.

While flapper skates possess many markings that can be used for identification, this is facilitated if pictures are taken in a particular manner:

- Photographs should be in focus and taken at as high a resolution as possible.
- The skate should be photographed while lying flat on deck (i.e. not lifted or held up, to prevent spot pattern distortion through bending of fins or body).
- The photograph should be taken from as close to vertical (i.e. looking straight down onto the skate) as possible, to ensure that all spots are clearly visible and foreshortening of distant spots is avoided.
- The entire skate's dorsal surface should be clearly visible to maximize the number of spots that can be used for identification.
- Care should be taken that spot patterns are not obscured by people, obstacles, shadows, and reflections, where possible and practical.
- Efforts should be made to include the pelvic region in the photograph, to allow determination of gender. Adult males' claspers, if present, should be included.
- A scale reference should be included in the photograph.

This guidance is presently under development for wider distribution among charter skippers. Improved consistency in photography will, in future, hopefully allow greater automation of detection processes through use of dedicated software (e.g. I³S, Wildbook™; Arzoumanian et al., 2005; Holmberg et al., 2009; Van Tienhoven et al., 2007), although the aforementioned large variability between photographs made such tools less practical here.

The near-ubiquitous presence of high-quality cameras and camera-equipped mobile phones among anglers and the general public means that the probability of obtaining a photographic record of each individual skate capture event is far greater than when relying on tagging technology. In addition, only minimal training is required to

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FIGURE 7 Examples of changes to skates' dorsal surfaces over time. (a) Skate Di000003 captured on March 28, 2012. (b) The same individual captured on August 27, 2016, now with extensive pale scarring across the right pectoral fin (white arrows). (c) Skate Di000054 captured on May 9, 2014, with atypical irregular white markings on the left pectoral fin. (d) The same individual recaptured on July 16, 2016; changes to the white markings are notable. (e) Skate Di000191 captured on May 27, 2016, with attached skate leech (*Pontobdella muricata*; white box) on left pectoral fin. (f) The same individual recaptured on June 29, 2016. Several new, bright white marks, likely leech scars, are now visible (white boxes). Examples of matching spot patterns are indicated by white ovals for purposes of comparison. Original images copyright R. Campbell



FIGURE 8 Discovery curve showing the increase in numbers of new identified individual skates against the cumulative number of capture events during the study (N = 349 capture events during 2011–2016). A hypothetical scenario in which each capture event involved a new individual was included for comparative purposes. The divergence between both lines is driven by increasing numbers of recaptures

collect good-quality photographs. There is, therefore, the potential to engage the wider Scottish sea-angling community in a dedicated collaborative citizen science project to photographically record captures of skates across and outside the Loch Sunart to the Sound of Jura MPA and compile these observations in a central catalogue. The present photographic database is intended to form the basis of such a photo-ID catalogue of flapper skates in Argyll waters, which will also seek to incorporate current and historical images from other sources (e.g. scientific surveys). Such a catalogue will increase understanding of common skate abundance, distribution, and movement patterns in and around the MPA. It will also improve understanding of factors affecting individual skates' health, including prevalence and healing rates of dermal infections, injuries and scars. In future, collection of photographs for the photo-ID catalogue could also be accompanied by sampling of genetic tissue to increase understanding of population substructure (e.g. Barker, Nosal, Lewallen, & Burton, 2015; Griffiths et al., 2010; Wright & Bentzen, 1995). In this manner, photo-ID data will generate novel insights into flapper skate biology and abundance. Such data will complement existing monitoring approaches to assist in the conservation of this critically endangered species in Scottish waters and beyond. More broadly, this approach should also be considered for studying other elasmobranch and teleost species of conservation concern that are targeted by catch-and-release recreational angling programmes.

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CONFLICT OF INTEREST

The authors are unaware of any existing conflicts of interest that would preclude or bias publication of the results contained in this manuscript.

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