Hunting the hunter: using genetic profiling for improved management of shark attacks on humans

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Short communication

Title (max 15 words – n=14)

Hunting the hunter: using genetic profiling for improved management of shark attacks on humans

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Summary (max. 150 - n=140)

DNA evidence is routinely used to identify individual predators responsible for attacks on people and livestock in terrestrial settings. However, the use of transfer DNA techniques in aquatic environments for similar purposes is a recent development. To date, DNA barcoding has been used successfully to identify shark species depredating fish catches and biting surfboards and neoprene surfaces. In this study we demonstrate the successful DNA barcoding and fingerprinting of individual sharks from transfer DNA collected directly from the wounds of two shark bite victims. The successful use of DNA techniques to identify both species and specific individuals responsible for shark bites opens the door to selective removal of these individuals as an innovative shark bite risk management strategy. This selective approach would be a more effective, eco-responsible, cost-effective and ethical solution for vulnerable taxa than ongoing non-selective culling campaigns.

Recent decades have seen the adoption of DNA forensic techniques for solving wildlife poaching cases and identifying culprits in terrestrial predator attacks on humans[1,2]. Analysis of mitochondrial DNA (mtDNA 'barcoding') is used to identify species[3], whereas analysis of nuclear DNA (nDNA 'fingerprinting') can distinguish between individuals of the same species[4]. Microsatellites (short tandem repeats, STRs) are the preferred nuclear markers for DNA fingerprinting because of their polymorphic and co-dominant nature, strict adherence to Mendelian inheritance and high reproducibility[5]. STRs have been used to solving poaching cases involving wild boar[6] and Sardinian Mouflons[7,8], and to identify individual brown bears (*Ursus arctos*) and tigers (*Panthera tigris*) responsible for fatal attacks on humans[9,10]. The source of DNA in these cases was either hair or blood, but successful DNA fingerprinting has also been achieved using saliva left in dog bite wounds on a child in Japan[11] and livestock in Italy[12]. In these cases it was important to identify the specific "problem individual"[13,14] responsible for the attack in order to eliminate the threat.

Shark bites on humans, commonly referred to as "shark attacks", cause about ten fatalities each year[15] but receive extensive sensationalized media coverage leading to distorted public perceptions of risk[16]. This biased perception is reinforced by the existence of chronic clusters of bites in certain areas such as northeastern Brazil (Western Central Atlantic Ocean), Reunion Island (Indian Ocean) or the coasts of Australia, New Caledonia or Hawaii (Eastern Central Pacific)[17]. Common shark bite mitigation measures include the use of nets or repellent barriers to limit contact between ocean users and sharks[18], early detection of the animals by human spotters[19], drones[20] or telemetry[21], and personal shark repellent devices[22,23]. However, non-selective mass shark culling campaigns are also still used despite the lack of evidence for their effectiveness. Previous studies concluding that culling campaigns improve human safety[24,25] lack appropriate controls and show apparent trends that may simply reflect the natural rarity and stochasticity of fatal bites on humans. Other studies have shown culling campaigns do not reduce shark bites[26]. Indiscriminate culling of 174 tiger sharks and 125 bull sharks between January 2014 and November 2020 did not prevent the occurrence of six fatal bites around La Reunion Island (2,512 sq.km) during this period (an average of 1 fatality per year), compared to five fatal bites between 2010 and 2013 (an average of 1.2 fatalities per year). Recreational ocean use

around La Reunion Island has decreased significantly since 2014[27], suggesting shark bite risk may actually have increased during the period of culling. The effectiveness and ethical appropriateness of shark culling is increasingly criticized by scientists and the general public alike[28,29,30].

The rationale for mass shark culling is two-fold; (1) the culprit responsible for a bite incident may be among the sharks removed, and (2) reducing the density of sharks reduces shark bite risk. Reducing animal densities to mitigate problematic behaviors can be effective in cases where the majority of individuals are engaged in these behaviors, such as great cormorants (*Phalacrocorax carbo sinensis*) depredating fish catches[31], sika deer (*Cervus nippon*) damaging trees[32], or wild boar damaging crops[33]. However, shark bites on humans are atypical behavior for sharks – they are very rare events despite high levels of human ocean recreation activities around the world. It is possible that problem individuals exist in shark populations just as they do in populations of terrestrial predators[15] and shark bites result from the atypical behavior of a few such individuals. Sharks that bite people may be at the extreme end of the behavioral spectrum for traits such as boldness (vs. shyness) and risk taking (vs. avoidance)[34]. If problem individuals exist in shark populations then they could be selectively removed following bite incidents to prevent future incidents, as is the case in terrestrial environments with large predators[14]. Genetic profiling could be used to unequivocally identify both the species and the individual responsible for bite incidents. However, this would be contingent on DNA persisting in bite wounds despite the potential for seawater to wash it away. Recent studies have successfully used transfer DNA recovered from depredated fish catches[35], neoprene and surfboards[36] to identify the culprit shark species. However, to date no study has identified both the shark species (via barcoding) and the individual within that species (via fingerprinting) using transfer DNA recovered directly from shark bite wounds in human flesh.

Here we demonstrate the successful use of transfer DNA recovered from the wounds of two recent shark bite victims to identify the culprit species and demonstrate that a different individual was responsible for each bite. We discuss the implications of DNA profiling for shark bite mitigation.

Results

Two swimmers were recently bitten by sharks in waters off St. Martin (French West-Indies) and the neighboring island of Nevis (West-Indies). Both incidents were witnessed by observers that visually identified the culprit as a tiger shark (*Galeocerdo cuvier*) in both cases. Medical personnel swabbed the bite wounds within a few hours of each incident. We successfully recovered shark mitochondrial DNA from these swabs and performed a barcoding analysis confirming the visual identification of a tiger shark in each case. All six swabs collected from the St Kitts-and-Nevis incident matched reference tiger shark mtDNA (4>98% match, 2>94% match). Five swab samples from St Martin yielded a >98% match to *Galeocerdo cuvier* and one failed. The transfer DNA recovered from both victims also allowed the fingerprinting of both shark individuals based on 25 composite short tandem repeats (STR) (see S1). A comparison of the two individual genetic profiles showed that the two bites were perpetrated by two different individuals (see S2). As these were first-time analyses, it took several weeks to obtain results. With preparation, the waiting time for barcoding and fingerprinting results could be reduced to an average of 36-40 hours and 5-7 days, respectively. Such delays would perfectly fit with the inclusion of DNA profiling in a short and long terms innovative strategy of shark risk management (Fig. 1).

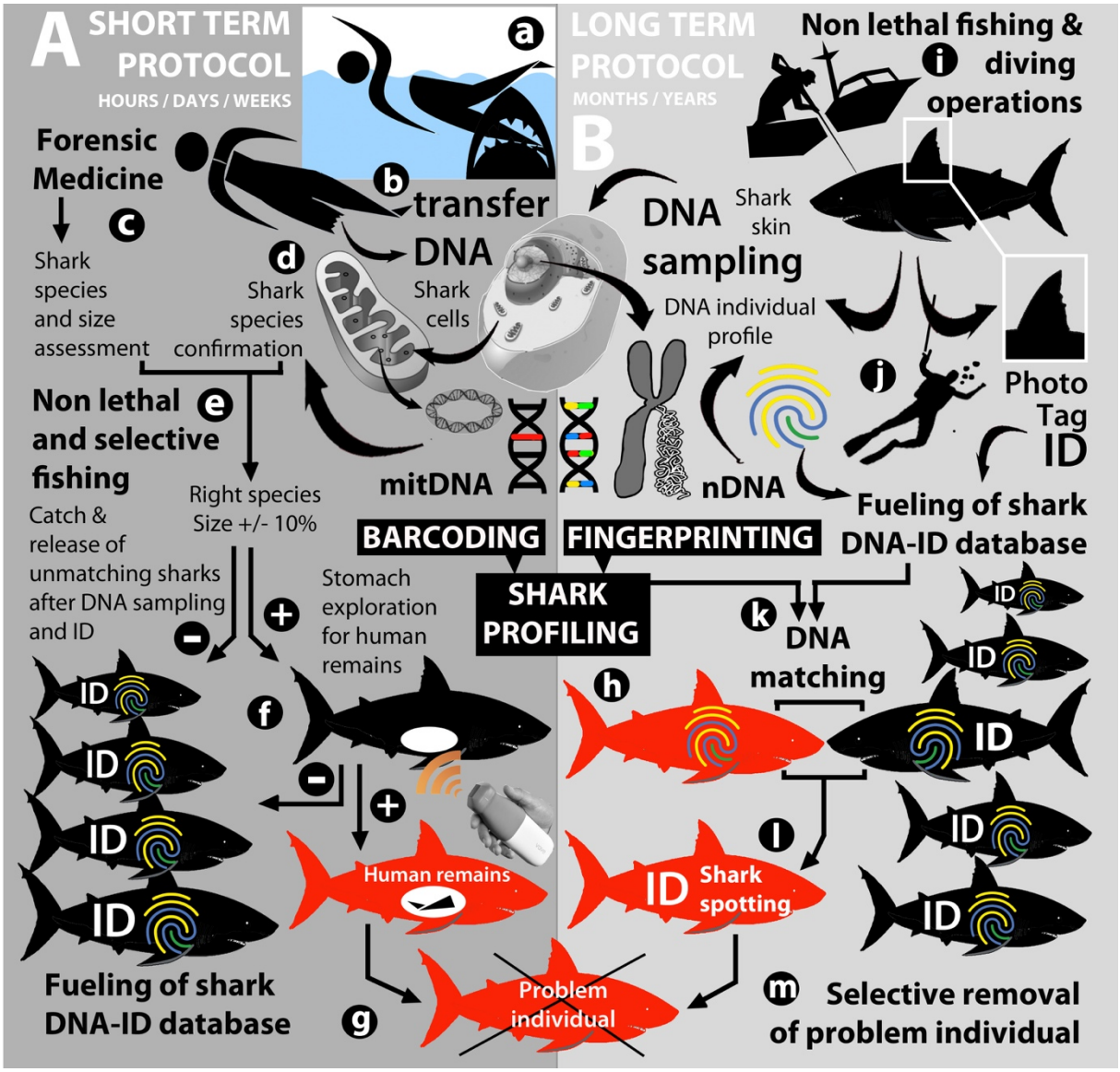

Fig. 1 – Suggested genetic profiling strategy for selective removal of shark problem individuals. A: Short-term response (hours/days) would use barcoding analysis to identify the culprit species. B: Long-term response (months/years) would use genetic fingerprinting to profile the culprit individual. (a) Wound-swabbing would be conducted within a few hours of a shark bite incident. (b) Forensic analysis of the bite would provide an assessment of the size of the shark and the potential species. (c) The culprit species would be confirmed through barcoding (delay: 36-40 h) and the synthesis of information would allow (d) non-lethal and selective fishing within the days after the attack. DNA samples would be taken from all captured sharks and those outside the culprit size estimate (error margin of +/- 20 cm) would be ID tagged, photographed and released. This information would be stored in a central database with the individual genetic shark profile and the means to re-identify it

(photo-ID or ID tag) (e). Stomach contents of sharks matching the culprit species and size would be examined via ultrasonography or physical eversion in order to identify potential human remains. In absence of remains, the animal would be released; in presence of human remains, the animal would be culled (f). The transfer DNA from the human wounds would also allow (g) the fingerprinting of the animal responsible for the bite. (g) Long-term measures would include routine collection of shark DNA profiles and ID tagging or photography via non-lethal fishing or diver surveys at specific monitoring sites to build a shark identification database. (h) If a culprit shark DNA profile matches a known individual in the database, that specific individual would be targeted for selective removal based on unique external characteristics (ID tag or photo).

Discussion

We successfully barcoded and fingerprinted shark transfer DNA samples collected by swabbing bite wounds in two separate incidents. The swabbing was performed by different individuals in each case, the samples were stored in 90° alcohol and a regular freezer prior to analysis and standard protocols for DNA extraction, amplification and sequencing were applied to obtain the identification results. These results demonstrate that transfer DNA analyses could be a standard component of the forensic analysis of shark bite incidents, and would complement existing wound analyses used to estimate the size of the shark involved.

The ability to identify individual culprits provides a potential alternative to mass-culling. Non-lethal fishing could be used to try and find the individual responsible. The stomach contents of captured sharks meeting the culprit species (determined via barcoding) and size criteria (within a +/-10% error) could be non-lethally examined using a portable ultrasound unit to scan for human remains (especially bones that are more resistant to digestive juices). Our successful fingerprinting of transfer DNA from bite wounds could allow the culprit to be definitively identified and selectively removed in a similar manner to terrestrial predators that kill humans. However, the concealing nature of the ocean and wide-ranging behavior of some large shark species makes this approach more challenging than in

138 terrestrial settings where large predators typically occupy well-defined home ranges, exhibit
139 territoriality or defend carcasses and can be relatively easily found. Possible solutions to this
140 considerable challenge include pre-emptively creating genetic reference databases of large sharks
141 using non-lethal sampling methods (e.g. biopsy sampling of free-swimming or captured individuals).
142 Following a severe shark bite incident, the fingerprint obtained from transfer DNA could be compared
143 to the database to identify a specific culprit that would be externally identifiable from an identification
144 tag or photo-ID. Once identified as responsible for a bite on humans and found again thanks to a
145 monitoring sites, the problem individuals could be removed (Fig. 1).
146
147 Existing examples of animal genetic profile databases include brown bears (*Ursus arctos*) (n=479)[31]
148 and rhinoceros (species)(n>3,900). In Africa, the RhODIS® (Rhinoceros DNA Index System)
149 database facilitated the criminal prosecution of several poachers via DNA matches between the
150 database and confiscated material[37]. Estimating shark population sizes is challenging and prone to
151 large error margins but existing population size estimates (e.g. 1200 bull sharks around La Reunion
152 island[38], 2,500–6,750 white sharks off Eastern Australia and New Zealand[39]) illustrate the scale of
153 sampling required to create genetic profile databases. Although shark species primarily responsible for
154 biting humans may range over thousands of kilometers of ocean, they also return to specific locations
155 within their home ranges. For example, satellite-tagged bull sharks and tiger sharks have moved over
156 hundreds of kilometers in the Pacific[40] and thousands of kms in the Western Atlantic[41], respectively,
157 before returning to artificial provisioning sites.
158
159 Selective approaches have the potential to be less costly than existing culling campaigns. La Reunion
160 island had a 2018 annual budget of 2.2 million USD for shark bite risk management, including
161 660.000 USD for shark culling. Eighty (65 tiger and 15 bull) sharks were culled at a cost of US$8,250
162 per shark, but two fatal bites still occurred in January and May 2019[30]. Creating a La Reunion shark
163 DNA fingerprint database (using 20-loci as for the present study) would cost of 660.000 USD based
164 on the current shark population size estimate and analysis costs of US$40-50 per shark. The field

sampling (i.e. fishing) costs would be comparable to those for the lethal culling campaign and would be spread over several years.

The substantially lower ecological impact of selective shark removal compared to mass culling should also be weighed in the cost-benefit analysis. Shark populations are already severely overfished in many locations around the world[42]. The removal of large numbers of top level predators reduces population genetic diversity and may result in undesirable cascade effects through marine food webs. Similar negative impacts may result from large scale shark culling. Selective removal of a small number of sharks known to have bitten humans would bring shark bite mitigation in line with the management of terrestrial predators responsible for targeting humans.

Material and methods

DNA extraction and sequencing

DNA was extracted from the swab tips from St Martin (n=6 swabs) and St Kitts-and-Nevis (n=6 swabs) using the Gentra Puregene DNA Purification Kit (Qiagen) following manufacturer instructions. DNA extraction quality was visualized on a 2% agarose gel.

Taxonomic identification was performed using the 260 bp CO1shark25F – CO1shark315R fragment (CO1shark25F -5' AGCAGGTATAGTTGGAACAGCCC 3' and CO1shark 315R -5' GCTCCAGCTTCTACTCCAGC 3') (Fotedar et al., 2019). Mitochondrial sequences were amplified using Quiagen reagents kit (preciser), with 2.5µl Tampon TAQ 10X, 2µl MgCl2 25 mM, 2.5µl dNTPs 2 mM, 0.6µl of each primer (10µM), 0.1µl Taq polymerase (5u/µl), Ultra Pure H2O, 5µl QSolution, for a final volume of 25µl. PCR programs consisted of an initial denaturing step at 95 °C for 3 min., followed by 35 cycles of 95 °C for 30 sec, 62 °C for 45 sec, and 72 °C for 30 sec, finished by 10 min at 72 °C and then held at 4°C (Thermocycler Eppendorf nexus gradient). PCR products were all ran on a 2 % agarose gel. PCR products were sequenced by GenoScreen (Lille – France) using an Applied Biosystem's 3730xl DNA Analyzer.

Sequence data were analyzed with BioEdit 7.2.5 and exported to the BLAST function (fasta format) from GenBank. CO1shark25F – CO1shark315R sequences were assigned allowing a best match score > 98%.

Genomic DNA Microsatellite markers were amplified using Type-it Microsatellite PCR kit (Qiagen, Hilden, Germany) in a final volume of 10 µL including 5 µL Type-it Multiplex PCR Master Mix (2X), 0.04 µL of each primer (25 µM forward and reverse primers diluted in TE pH 8 buffer) and 1 µL of DNA. PCR programs consisted of an initial denaturing step of 15 min at 95 °C, followed by 40 cycles of 1 min at 95 °C, 1 min at specified annealing temperature (53°C, 56° or 58°C – see table 1), 72 °C for 1 min, and a final elongation step at 72°C for 20 min. Due to the very low genomic DNA quantity, all loci were amplified in Monoplex, PCR products were sequenced by GenoScreen (Lille – France) using an Applied Biosystems 3730 Sequencer. GeneScan 500 LIZ (Applied Biosystems) was used for accurate sizing. Allele sizes were scored and checked manually using GENEMAPPER 3.7 (Applied Biosystems).

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Author contributions
E.C. was the Coordinator of the project, and conceived the project together with S.P., C.M., J.L. and T.V. M.V. was involved in supervising the DNA sampling, storage and transportation. S.B. conducted the barcoding and fingerprinting analysis and forensic validation under S.P. supervision. The genetic data analyses were carried out by S.P.T.V. provided overall input to the paper as external experts. E.C. drafted the paper that was improved and accepted by all co-authors.

Supplementary material
S1: Table S1 Genetic characterization of twenty-two microsatellite loci in tiger shark (*Galeocerdo cuvier*)

S2: Table S2 Fingerpriniting unmatching based on comparison of STRs from both sharks.

Additional information
Supplementary Information accompanies this paper at http://www.

Competing financial interests: The authors declare no competing financial interests. Reprints and permission information is available online at http://

Figures

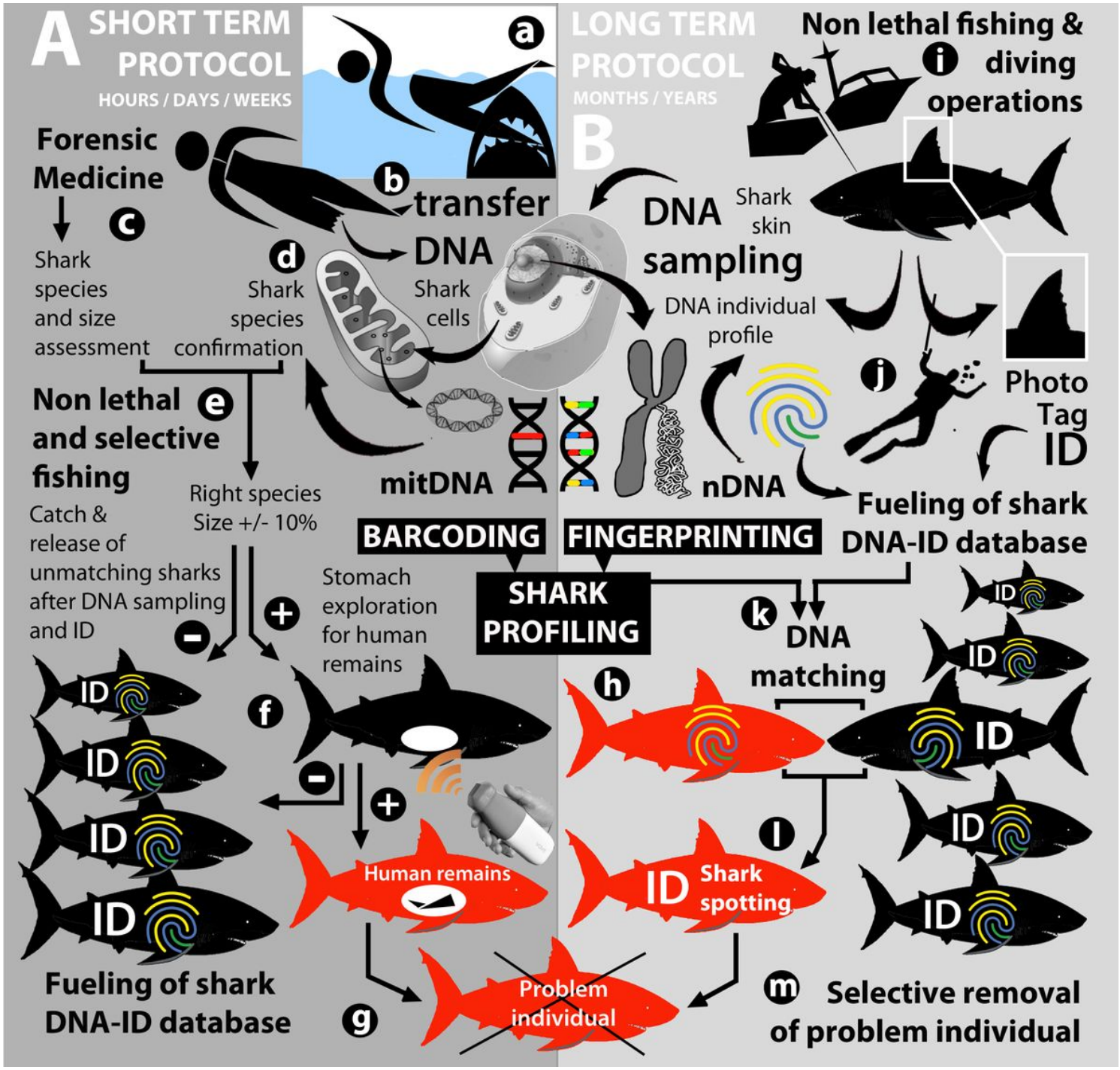

Figure 1

Suggested genetic profiling strategy for selective removal of shark problem individuals. A: Short-term response (hours/days) would use barcoding analysis to identify the culprit species. B: Long-term response (months/years) would use genetic fingerprinting to profile the culprit individual. (a) Wound-swabbing would be conducted within a few hours of a shark bite incident. (b) Forensic analysis of the bite would provide an assessment of the size of the shark and the potential species. (c) The culprit

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Supplementary Files

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- NCOMMS2117878S1TableS1STR.pdf
- NCOMMS2117878S2Table2fingerprintingunmatching.pdf