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"Use of pathology to better inform the welfare impact assessment of bycatch and entanglements"

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Introduction

‘Consider the likely difference in response if the results of long standing right whale entanglements (i.e. emaciation, starvation, chronic pain, and infection) were observed on a daily basis by humans as they went about their lives in urban, suburban or rural communities rather than remaining out of sight at sea. If the equivalent of fixed fishery traps and nets was to be set on land, with a comparably slow and painful death for wildlife, the responsible industry could be subject to consumer revolt, irrespective of whether there was an actual law concerning such an interaction.’

Moore and van der Hoop (2012)

There is a growing public, scientific and policy recognition that assessing the impacts of human activities on marine animal welfare is as important as more traditional assessments based on health, causes of mortality and population dynamics. This is particularly relevant for bycatch, the unintended capture of non-target species including whales, dolphins, and porpoises in fishing gear. Bycatch poses a significant threat to cetacean populations, but it also raises significant welfare concerns which have only been partly explored (Soulsbury *et al.* 2008, Dolman and Brakes 2018). Recently there has been greater interest in the welfare implications associated with anthropogenic impacts on cetaceans, based upon their extraordinary cognitive and communication abilities, and the longevity and strength of their social bonds, jointly suggesting that cetaceans possess a strong and refined sentience and a capacity for suffering and enjoyment (IWC 2017, Nicol *et al.* 2020, Rae *et al.* 2022).

To fully evaluate the impact of bycatch events on cetaceans there is a need to understand the pathophysiological processes that occur. Without this knowledge it is not possible to inform those responsible for protecting these species and the marine environment as a whole. Research in this area is challenging. Understanding the processes surrounding terminal events and the diagnosis of peracute underwater entrapment, in particular, continues to present problems despite ongoing research. Likewise, the effects of chronic entanglement are only now becoming apparent and further research is needed.

In light of this the UK government working through its stranding networks embarked upon a scoping study to review current knowledge and advise on future research in the area of bycatch pathophysiology. The workshop held at the ECS conference, Catania in 2024 provided a platform to bring together researchers in the fields of pathology and welfare to discuss current knowledge, areas of future research and develop collaborative approaches to the evaluation of welfare in cetacean species. These proceedings provide a record of the presentations given during the workshop and highlight areas of future research.

I would like to thank all those who were involved in the workshop. These include the presenters – Rebecca Boys, Lonneke IJsseldijk, Eva Sierra, Sandro Mazzariol, Ellie MacLennan and Helen Chadwick. In addition, those that helped organise the workshop include Rob Deaville, Andrew Brownlow, James Barnett and the ECS organising committee in Catania. I would also like to acknowledge the Department for Environment, Food and Rural Affairs, UK for their financial support during the project and in supporting the workshop. Finally, I would like to thank all those who attended and I hope that the interest shown is carried forward to improve cetacean welfare.

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Introducing animal welfare science into pathological assessments of cetacean bycatch and entanglement

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While conservation biology is generally well understood and integrated in policies to assess threats to the survival of wild populations, animal welfare science has received limited attention, often being dismissed as a discipline guided by emotive environmental crusaders (Clegg et al., 2021; Papastavrou et al., 2017). Animal welfare science has, until now, mostly focussed on captive, particularly domesticated, animals and examines these at the individual level. This discipline applies scientific methods to understand the state of an animal as it attempts to cope with its environment at a given time, allowing for an understanding of threats to an individual animal's quality of life (Broom, 2008; Broom & Fraser, 2007).

These disciplines have generally been considered disparate, yet in many real-life problems there are implications for both conservation (in terms of species survival) and animal welfare (Beausoleil et al., 2018; Papastavrou et al., 2017). In fact, in some cases the impacts on individual welfare may become apparent much earlier than population level effects, so integrating welfare science could improve species conservation (Bejder et al., 2022; King et al., 2015; Lusseau & Bejder, 2007; New et al., 2014). Yet, a previous study found that less than 1% of all articles pertaining to marine mammals that were published between 1950 and 2020 featured any reference to welfare (Clegg et al., 2021). Thus, it is important that we develop assessment frameworks to integrate conservation biology and animal welfare science. This will provide the most scientifically-informed, holistic evaluation of animals to achieve objectives in conservation management (Beausoleil et al., 2018).

What is animal welfare science?

An important step in the development of a framework that considers animal welfare is to understand how welfare is being conceptualized, as the approach to understanding welfare will influence how it is assessed and how the outcomes of such assessments are evaluated in terms of decision-making (Beausoleil et al., 2018). There is no single accepted definition of animal welfare. There are three views on what is important to enable an understanding of animal welfare: biological function, natural state, and affective state. Those emphasizing the biological function view believe that good welfare is the minimisation of physiological stress (Barnett & Hemsworth, 2003; Broom, 1991; Hurnik & Lehman, 1988). The natural state view reflects the idea that the environment should enable animals to perform their natural behaviours (Alrøe et al., 2001; Kiley-Worthington, 1989). This is often the focus in captive facilities where the aim is to provide a natural environment for the animals. Lastly, the affective state view, focuses on what the animal is feeling and how it is experiencing its life (Duncan, 1996; Duncan, 2004; Fraser, 2003; Preece & Chamberlain, 1993). This was developed with animal preference and motivation followed by affective neuroscience, and considers how mental states result from physical conditions. Nowadays, there is a general consensus that all three of these aspects are interrelated (Appleby, 1999), and so contemporary animal welfare science encompasses physical (basic health and functioning) and behavioural states, and the cumulative effects that these have on animal mental (affective) state (Mellor, 2016; Mellor et al., 2020).

Concepts of animal welfare evolved in relation to production animals and aimed to protect against deliberate cruelty that was of public concern (Mellor et al., 2009; Ohl & van der Staay, 2012). In recent years humans have acknowledged their increasing effects on free-ranging wildlife and the need for welfare to be considered in conservation (Butterworth, 2017; Freire et al., 2021; Hampton & Hyndman, 2019; Paquet & Darimon, 2010; Scholtz, 2017). However, there is some controversy about what our responsibilities are towards wild free-ranging animals, but generally there is consensus that we should minimise any negative impacts or threats that we cause (Kuba, 2018; Miller et al., 2018).

Understanding an animal's welfare state

Welfare assessments provide insight into the state of an animal, relating to the outcome of sensory information from internal and external inputs processed by the animal's brain (Mellor & Reid, 1994). This incorporates both biological functioning and affective state approaches, since biological functioning underlies affective experience and affective experience influences biological functioning (Mellor & Beausoleil, 2015). Therefore, to perceive their welfare state, animals must be both sentient (able to perceive and feel) and conscious (Mellor & Reid, 1994).

To assess an animal's welfare state we need to understand the potential affective states they may experience. These welfare states are subjective, that is they belong to the animal and are not directly observable. These subjective affective states are valenced, they are positive or negative. Negative states are welfare compromising, such as pain and fear (Beausoleil & Mellor, 2017). In contrast, positive states are welfare enhancing (Mellor & Beausoleil, 2015) such as pleasure from socializing (Spinka & Wemelsfelder, 2011), or behaviours that reduce negative experiences (e.g., relief of thirst). These experiences can also vary in their intensity and duration. Although, the reduction of negative experiences may improve welfare state, this alone cannot represent good welfare. Therefore, when we are considering the management of human impacts, the priority should be to relieve negative experiences (Fraser & Duncan, 1998; Mellor, 2016; Yeates & Main, 2008).

The affective states that an animal can experience will be an outcome of its evolution. Therefore, different animals will have different sets of experiences and be able to experience things to differing degrees. For example, cetaceans have the ability to echolocate, to understand what affective experiences cetaceans may have related to this ability, we can think about the functional importance and evolutionary needs of the animals that led to this ability and infer potential affective states that may be experienced if this capacity was thwarted.

Undertaking welfare assessments

Since affective states are subjective, they cannot be measured directly. Therefore, to undertake welfare assessments we rely on inference from available data. Specifically, we use data from multiple measurable and observable indicators of physical, behavioural and physiological states, many of which have validated links to mental experiences (Beausoleil & Mellor, 2015, 2017). For example, an animal observed to be in emaciated body condition, has likely not been feeding for some time and is probably experiencing feelings of hunger.

In the first stage of a welfare assessment a comprehensive list of indicators should be developed. It is important that these indicators are practically measurable, so that they can be applied. They also need to be valid, that is we need to be sure that they reflect what we understand them to be measuring, so that data is appropriately used (Beausoleil & Mellor, 2017; Boys et al., 2022b; Harvey et al., 2020). We can do this by correlating across multiple measures, for example examining behaviour antemortem with the pathophysiological findings postmortem (Boys et al., 2023; Fernandez et al., 2017; Fraser, 2008). By collecting data from multiple measurable indicators, we can have better evidence-based inferences about welfare state.

Indicators can be animal-based (e.g., body condition or behaviours) or may be resource- or management-based (e.g., environmental conditions, human interventions). Animal-based indicators provide more direct evidence of the animal's welfare state than resource-/management-based indicators. Welfare indicators can be further categorised into 'welfare status' or 'welfare alerting' (Harvey et al., 2020). Welfare status indicators provide explicit evidence of an animal's physical state or external situation and therefore more directly reflect its welfare status (i.e., subjective mental experience). They include some animal-based indicators (e.g., external injuries, specific behaviours). In contrast, welfare alerting indicators do not provide information directly related to an animal's welfare state, but rather represent factors that might compromise that state in some animals exposed to those conditions (i.e., they represent a welfare risk) (Harvey et al., 2020). These include some animal-based indicators (e.g., age class, reproductive status), but also include all resource-/management-based indicators. The main value of welfare alerting indicators is in directing observers to more closely evaluate and monitor animal-based parameters to corroborate or refute the effects of the alerting parameters on the animal's current and future welfare state (Boys, Beausoleil, Pawley, Betty, et al., 2022; Harvey et al., 2021; Wemelsfelder et al., 2000).

Aside from indicator types, we should also consider the duration and intensity of any impacts, as this will affect how compromised welfare becomes and alter outcomes of welfare assessments. To understand how these measures relate to the animal's positive or negative welfare state, we need species-specific knowledge in terms of their anatomy, physiology and behaviour as well as understanding of what is 'normal' under various conditions (Boys, Beausoleil, Pawley, Littlewood, et al., 2022a; Harvey et al., 2020). Unfortunately, for many wild species there are limited data on various indicators to enable validation (Hill & Broom, 2009). This is the case for much behavioural and physiological data in free-ranging marine mammals due to limited habitat accessibility, human avoidance and unobservability for prolonged periods. In these cases, expert elicitation can also be used to inform evaluations of subjective experiences from observed indicators and has been used to inform welfare assessments of cetaceans previously (Boys, Beausoleil, Pawley, Littlewood, et al., 2022b, 2022a; Nicol et al., 2020; Serres et al., in review). For example, experts can consider available evidence from other species with similar neurological systems to infer the likelihood of affective states being experienced in various situations.

Once we have feasible and validated indicators we can use welfare assessment frameworks to guide our evaluation of an animal's welfare state at a given time. Welfare assessment frameworks use validated links between the measurable indicators of physical functional states and mental experiences that they likely reflect (Beausoleil et al., 2018; Beausoleil & Mellor, 2015, 2017). A welfare assessment framework which has been extensively used as part of the scientific method to assess animal welfare and applied to cetaceans, is the Five Domains Model (Mellor et al., 2020; Mellor & Reid, 1994).

The Five Domains Model structurally represents the understanding that physical and mental states are linked and facilitates assessment of welfare based on this understanding. However, it explicitly separates physical/functional impacts from affective experiences that impact animal welfare (Mellor et al., 2020). It includes three physical/functional domains (nutrition, physical environment, health) and one situation-related domain (behavioural interactions). Compromise in any of the four domains are used to infer the potential cumulative impacts on the fifth domain (mental state) which determines the animal's overall welfare state (Mellor and Reid 1994, Mellor and Stafford 2001).

Understanding welfare from pathology of bycatch and entangled cetaceans

From an animal welfare science perspective, the data that can be gathered from postmortem examinations is critical to understand what the animal is likely to have experienced whilst alive and conscious (Boys et al., 2023; Câmara et al., 2020; Fernandez et al., 2017). For bycaught and entangled cetaceans, we know that there are a range of related external injuries and internal lesions that can be observed at postmortem (Bernaldo de Quiros et al., 2018; Cassoff et al., 2011; Dolman & Brakes, 2018; Dolman & Moore, 2017; Knowlton et al., 2022; Moore & van der Hoop, 2012). Based on these known pathological consequences, we understand that the affective states being experienced by animals are likely to be negative and we can begin to consider the quality of potential mental experiences that these cetaceans may have. Here, I provide some general thoughts about potential affective experiences that could be considered when assessing welfare in bycatch and entanglement cases. This is by no means exhaustive and should be used only to help facilitate discussions.

It is possible that being bycaught or entangled reduces the ability to forage, which overtime could result in experiences of weakness and hunger. The environment of being caught or trapped and in a situation that is unpredictable, limiting the animal's control of its own movement, could lead to experiences of fear, anxiety and a feeling of lack of agency or control. It is also possible that whilst trying to escape from a net an animal will experience exhaustion and then weakness. The external injuries such as wounds in the skin and blubber or to joints are likely to cause pain.

For bycaught animals that may be trapped underwater for prolonged periods especially whilst struggling to escape a net, the inability to replenish oxygen stores could lead to the experience of some form of breathlessness, which could further lead to dizziness. For entangled animals, we need to consider the impacts of continued negative experiences caused by pain which have the potential to last for days or weeks, leading to prolonged welfare compromise (Moore, 2014).

Although, possibly considered to be of lesser importance, we should also contemplate the welfare impacts of social disruptions that may be caused by bycatch and entanglement. Cetaceans are generally social species and the inability to interact with conspecifics and removal of or serious compromise to an individual can result in social disruption to both the impacted individual and to the wider pod. For animals that are entangled such social disruption might lead to experiences of loneliness and isolation. We can also consider the welfare and survival impacts on indirectly affected individuals such as unweaned calves if the mother is bycaught or entangled. For animals that are released from nets, either in bycatch or entanglements, there is also the consideration of response to human intervention/handling which is likely to be experienced as threatening and could lead to experiences of fear and panic.

Conclusions

A key aspect of welfare assessments is the duration of negative experiences prior to loss of consciousness. This is because welfare is a feature of the individual animal and how it is experiencing its own life (Mellor & Reid, 1994). Pathology in bycatch and entanglement cases may be able to inform welfare assessments by : estimating the time between an injury and unconsciousness or death occurring; differentiating animals that died rapidly in nets versus those that succumbed to injuries some time after being caught; identification of injuries that may represent particular behaviours being performed that could be suggestive of escape/avoidance and therefore provide additional understanding of the state of the animal prior to unconsciousness; are particular age classes or sexes becoming entangled or bycaught more often and what implications this may have on the wider population.

The systematic collection of postmortem data could help to identify indicators that are feasible to assess and that are valid to understand the welfare experiences of bycaught or entangled animals. The CBIIS framework could be implemented to collect and analyse pathology data from a welfare perspective which will allow us to understand, from an evidence base, the relative importance/influence of different aspects of bycatch and entanglement. This should provide a robust, transparent method that highlights true welfare compromise and will provide evidence-based data to identify key issues and help to facilitate policy discussions on how these impacts could be addressed and how potential future risks could be mitigated to improve management.

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Peracute Underwater Entrapment (PUE) – the pathway to death

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Fisheries interaction and PUE

Peracute underwater entrapment (PUE) occurs following interaction with fishery gear. Both the behaviour of the animal and the nature of the fishery will impact on the welfare of the cetacean from the onset of PUE until the death of the animal following entrapment.

Broadly, net fishery types fall into the 2 categories in UK waters; static and non-static. Static nets are 'set' by different methods and in different locations to catch relevant target species and these include gill nets and drift nets. Gill nets are fixed' at a particular site, whereas drift nets are mobile, drifting with the prevailing currents. Non-static nets include trawling, purse seine and ring netting, ring nets being similar to but smaller than purse seine nets. Detailed information on fisheries methodology falls outside the scope of this document but useful references include Tregenza *et al.,* (1997a), Monizur *et al.,* (1997), Luque *et al.,* (2006).

Bycatch rates of marine mammals vary between different fisheries with the highest rates reported in certain types of trawl fishery and gill nets, including trammel nets (https://www.cornwallgoodseafoodguide.org.uk/cornish-fishing-methods.php).

In static net fisheries, gillnet and tangle net fisheries are most often implicated in the bycatch of harbour porpoises, one of the two species most frequently bycaught in UK waters, with other gear types taking harbour porpoises less frequently (Northridge 1991, Dolman *et al.,* 2016). Similarly, in Swedish waters, 70% of bycaught porpoises were from a variety of static nets with the remaining 30% reported from trawls (Lunneryd *et al.,* 2004). The exact reasons why porpoises are particularly prone to entrapment in static nets is unknown, but it is likely partly due to their behaviour. Nielsen *et al.,* (2012) found that porpoises do not usually actively approach gill nets, concluding that bycatch seems to be the result of individual animals accidentally being caught, likely due to attention shifts or to auditory masking (compromising capacity to 'hear') reducing their ability to detect the nets using echolocation. Sleep may also influence odontocete entanglement in static nets, as echolocation is reduced (Goley 1999). Other factors may include scavenging, inexperience, curiosity, carelessness and distractions, for example predator escape or play behaviour.

The other species most frequently bycaught in UK waters, common dolphins, may also be caught in static nets. In contrast to harbour porpoises, this species may be attracted to fishing boats and subsequently entrapped when static nets are being shot or hauled, rather than while they are set/static. This was illustrated by Tregenza *et al.,* (1997b), who found that common dolphins were around a boat in two of the three bycatch events they witnessed, either during or within 15 minutes of the shooting of the net. They also reported that one common dolphin was hauled up alive, indicating that it was entrapped during or shortly before hauling.

In non-static net fisheries, midwater trawls pose a greater risk to marine mammals than bottom trawls as they are usually large in size and towed at higher speeds; moreover, they target small, schooling species such as squid and herring, which are common prey species for marine mammals (Northridge 1988). Species foraging at mid-water depths are therefore highly vulnerable to mid-water trawls (Fertl and Leatherwood 1997).

Behaviour may also be a significant factor in why dolphin species such as the common dolphin are frequently bycaught in trawls. Trawl interactions generally occur when dolphins exploit them as a feeding opportunity and accidentally become entrapped as they are actively feeding in nets. Dolphins may recognise characteristic engine noises produced by trawl vessels during different stages of fishing leading them to feed during certain periods such as gear deployment or haul back (Leatherwood 1975, Fertl and Leatherwood 1997, Pace *et al.,* 2003). Individuals may get bycaught as the net forms into a U-shape, due to friction between the headline of the net and the surrounding water, creating spaces that may lead to entrapment (Northridge 1988). Entrapment may also occur when the trawl changes speed or direction, altering the shape of the headline and the size and shape of the net and its mouth, or when the boat slows or stops to haul in the catch (Fertl and Leatherwood 1997, Zollett and Rosenberg 2007, FAO Technical Guidelines for responsible fisheries 2021, SGFEN 200).

It is clear therefore that the behaviour of different species influences the risk of entrapment in different fisheries. Foraging activity around trawl nets by common dolphins could change diving behaviour and physiology which would then impact on more proximate factors associated with the PUE event and subsequent death. This would contrast with the more passive/accidental entrapment seen in harbour porpoises. Factoring in the potential effect of these differences on the welfare and time to death of an animal after PUE is challenging and at this stage it is unlikely that the physiological differences immediately prior to entrapment can be separated from those thereafter. In addition, it is unknown if physical factors once entrapped (e.g. the crushing by target species in the net) would also affect behaviour and/or physiology up to the point of death or release.

Death following Peracute Underwater Entrapment

Once an animal has become entrapped 2 scenarios are possible; death following hypoxia/asphyxiation, or release/escape with return to the surface to breath. Both of these have significant welfare impacts on the individual and although death may be regarded as the greatest impact, injuries and other pathologies (i.e. hypoxic brain injury) may lead to long-term welfare issues should the animal survive. Little or no research has been done on cetaceans in either scenario and as such comparative physiology/pathology taken from terrestrial species has been used here to outline the path to death in PUE. Direct observation of PUE has not been documented in UK waters. Therefore certain assumptions need to be made based upon other similar scenarios and those described in terrestrial species. One recorded case in the UK indicated significant activity following entrapment of a harbour porpoise resulting in fixed net displacement suggesting marked behavioural and physiological change associated with entrapment detected during a passive acoustic monitoring experiment(Jamie MacAulay, WMMC Barcelona 2019 short presentation and paper in manuscript).

There are significant differences between death following immersion in water between terrestrial and aquatic mammals. Drowning in terrestrial mammals has been studied both following natural causes and experimentally, and there is a large volume of knowledge on this matter, however diagnosis can be challenging in some cases. The different physiology (and anatomy) of marine mammals means the cause of death in prolonged immersion is more akin to asphyxia/suffocation as opposed to drowning.

Drowning and asphyxia

McEwen and Gerdin (2016) and McEwen (2016) provide a review both of drowning and non-drowning asphyxiation predominantly in terrestrial animals and the following synopsis outlines current knowledge. Drowning is the process of experiencing respiratory impairment from submersion/immersion in liquid and outcomes are death, morbidity or no morbidity (WHO 2005 cited in McEwen and Gerdin (2016)). Other terms such as wet or dry drowning should ideally no longer be used. Drowning results in rapid and persistent hypoxia following the introduction of liquid at the entrance of the airway. Arterial oxygen tension reduces immediately and is followed quickly by acidosis and hypercapnia. A terrestrial animal will initially show breath holding or a few deep respiratory movements plus vagal mediated laryngospasm resulting in decreased arterial oxygen and increased CO_2. Bradycardia occurs with an initial increase in blood pressure that subsequently decreases. Dogs will struggle violently for approximately 1.5 minutes during which liquid is swallowed and enters the stomach. Once unconscious the larynx relaxes with water subsequently aspirated into the lungs. After 3 minutes there is spasmodic convulsion and seizure activity and the electroencephalogram (EEG) becomes isoelectric between 3 and 3.5 minutes with cardiac asystole or observed death usually in 5 minutes although this can take up to 10 minutes.

From a pathophysiological point of view the following features are seen-pulmonary oedema, excess catecholamine release, vasoconstriction, cardiac arrhythmia, pulmonary hypertension and right to left shunting. Death due to saltwater aspiration is more lethal than freshwater as hypertonic seawater increases the formation of alveolar oedema with surfactant washout and decreased lung compliance.

In comparison, asphyxia refers to death by rapid cerebral anoxia and hypoxia due to accidental or non-accidental causes. Non-anaesthetised animals subjected to strangulation by any method will struggle due to the severe physiological ‘air hunger’ that occurs before there is a loss of consciousness. EEG becomes isoelectric at 40 seconds to 2 minutes, with apnoea at 4 minutes and cardiac arrest between 9-10 minutes after the onset of strangulation.

Suffocation occurs through choking, smothering, inadequate environmental oxygen and chemical asphyxiants. Obstructive asphyxia results in immediate dyspnoea, convulsions, bradycardia and apnoea, isoelectric EEG, agonal respiratory movements and cardiac arrest in 4-6 minutes. During asphyxia animals may traumatise themselves as they struggle and therefore may have abrasions, contusions or lacerations depending upon the environment and cause.

Considerations for cetaceans (and other marine mammals)

When considering death by drowning/asphyxiation in marine animals differences in their physiology need to be taken into account. Marine mammals show a very well described diving response of apnoea and bradycardia which is initiated by activation of the cold receptors of the face by the ophthalmic and maxillary divisions of the trigeminal nerve. The bradycardia results in decreased cardiac output and tissue perfusion with blood shunting to the heart, lung and brain with decreased peripheral blood flow as a result of increased peripheral resistance. This effectively results in decreased peripheral metabolism. Interestingly seals are able to modulate heart rate with respect to dive time duration at the start of the dive, however entrapment/entanglement would create a dilemma for the animal (Costa 2007).

Soulsbury *et al.,* (2008) provide a comprehensive review of the animal welfare implications of cetacean deaths associated with fisheries (particularly for by-catch). They recognise the effects of injury and death with regards to welfare, and highlight other aspects including the social implications for species as a result of interaction with fisheries. Specifically, bycatch of cetaceans encompasses a range of welfare issues including; a) asphyxiation, b) physical injuries, c) physiological and psychological stress, d) social disruption. They suggest the primary welfare concern of by-catch is the stress associated with asphyxiation.

In light of this one of the key areas discussed is the time for asphyxia to occur. Soulsbury *et al.,* (2008) refer to Leaper *et al.,* (2006) indicating that the theoretical aerobic dive limit (TADL) may be useful in assessing asphyxiation times. The following table is adapted from Soulsbury *et al.,* (2008) and provides some indication of the potential asphyxiation times in some of the species seen in UK waters (collated information can also be found in Noren and Williams (2000), Ponganis (2011), Teilmann *et al.,* (2007));

Species	**TADL (mins)**	**Maximum Dive Time (mins)**
Harbour porpoise	2-5.5	5.5
Bottlenose dolphin	3-5	8
Minke whale	10-18	13
Fin whale	29	15
Sperm whale	43-54	73

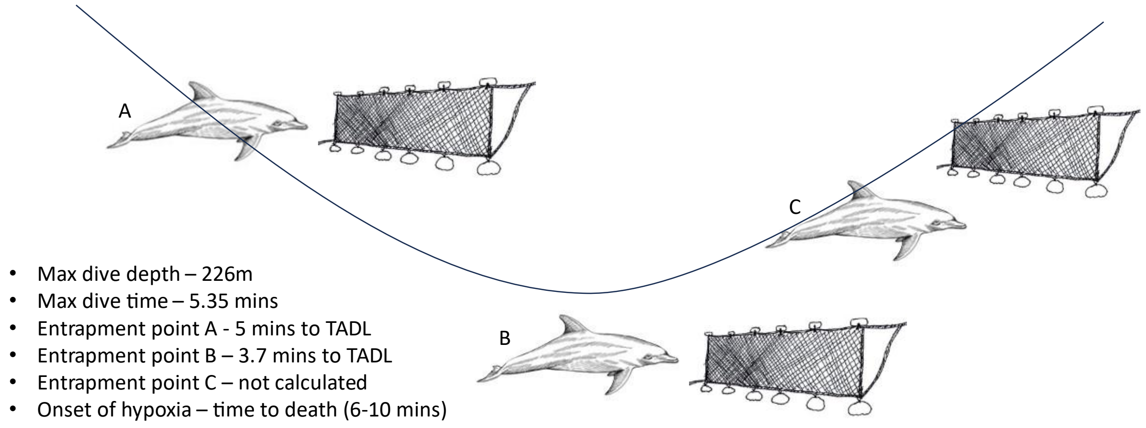
They do indicate that data is very limited and the overall time to asphyxiate is difficult to determine. However they do suggest it is likely to occur somewhere between TADL and the maximum dive duration.

Leaper *et al.,* (2006) discuss the factors affecting the time of death for whales following entanglement in fishing gear. A cetacean (or pinniped) that is entangled underwater is potentially in a terminal forced dive situation and can adopt 2 strategies - induce a rapid and profound dive response, or start to struggle. Based upon the pathological findings noted in assumed acute bycatch situations the latter would appear to be a common scenario. The metabolic rate following entrapment is heavily influenced by the amount of physical activity of the animal following this event. Entrapment and struggling however provides a significant physiological problem-by struggling there will be re-distribution of blood flow to muscles with increased oxygen consumption. In some cases of entrapment dolphins will enter a catatonic state which is thought to be associated with accentuated bradycardia.

The theoretical aerobic dive limit (TADL) gives an approximate indication of the time likely to elapse before the animal experiences extensive anaerobic respiration. This can be useful indicator for the approximate time to death following entrapment as many species frequently have dive durations close to or just beyond their TADL. Leaper *et al.,* (2006) suggest that although there are some uncertainties inherent in predicting time to death once TADL has been exceeded it seems possible that death could occur within minutes of reaching this point. Average dive times were not felt to be useful for assessing time from entanglement to unconsciousness/death. It is however suggested animals may survive without oxygen for periods of some multiple of the TADL.

The position of the fishing gear in the water which the animal becomes entangled within may influence the time to death as animals entrapped at their maximum dive depth or during the ascent would have less available oxygen than those entrapped early in the dive. In the former scenario struggling would rapidly metabolise available oxygen and hasten the onset of unconsciousness/death. Westgate *et al.,* (1995) studied the diving behaviour of harbour porpoises in the Bay of Fundy and found the maximum recorded dive depth and duration was 226 m and 5.35 mins. If an animal became entrapped on the dive descent (A) it may have up to 5 mins before the TADL is exceeded and hypoxic injury may start to occur, at maximal dive depth (B) (226 m obtained at 98 s based on a maximum descent speed of 2.3 m/s), the animal would then have 3.7 minutes before reaching the same state and obviously on the ascent the time would be less still (C). These figures are only approximate and relate to harbour porpoises in the Bay of Fundy. This may vary at other locations and a range of calculations would need to be undertaken for other species.

The dive cycle and entrapment after Westgate and others 1995



- Max dive depth – 226m
- Max dive time – 5.35 mins
- Entrapment point A - 5 mins to TADL
- Entrapment point B – 3.7 mins to TADL
- Entrapment point C – not calculated
- Onset of hypoxia – time to death (6-10 mins)

What this does infer is that death does not occur immediately upon entrapment and the effects of hypoxia in cetaceans (and other marine mammals) are delayed due to their physiological differences. If we assume that once hypoxia starts a similar timeline to death as for terrestrial species occurs then there is an additional 6-10 minutes before death. On this basis for the above example the animal could experience a period of 11- 15 minutes of potential suffering prior to death (on descent entrapment), however the point of unconsciousness is unknown in this period.

As events are not observed we can only make approximate calculations for the time to deaths providing a range of times. Assessment of other metabolic parameters to provide a more defined time on bycatch specimens would in effect be the ‘holy grail’ to provide a welfare assessment for PUE.

Hypoxic injury in peracute underwater entrapment (PUE)

As previously mentioned hypoxia constitutes the most important process in PUE resulting in unconsciousness and death of cetaceans (Soulsbury *et al.,* 2008). Their unique physiology and metabolism mean the assessment and evaluation of any hypoxic insult is challenging. Evidence of asphyxial death is mainly provided through circumstantial evidence and necropsy findings in cetaceans (Moore *et al.,* 2013). To date no detailed studies have been undertaken to gain an understanding of the process in cetaceans. In light of this studies in terrestrial species are important as these provide some information on the changes that occur during asphyxiation. Recent developments in immunohistochemistry and metabolomics mean it may be possible to progress knowledge in the species of interest in relation to hypoxic injury.

Reduced oxygen availability (hypoxia) impacts most significantly upon cardiac and central nervous system function. Cetacean physiology has adapted to periods of hypoxia through changes in metabolism of peripheral tissues (switching to anaerobic respiration) and alterations in blood flow to ensure critical organs receive adequate oxygen concentrations for aerobic respiration (Ponganis and Williams 2016).

Much work has been undertaken in humans in relation to perinatal hypoxic/ischaemic brain injury at parturition however this is not directly applicable to the scenario of peracute underwater entrapment. It is unknown whether cetaceans surviving a period of hypoxia as a result of PUE show long term brain injury/pathology.

Evidence of acute brain ischaemia or hypoxia and differentiation from agonal hypoxia is of particular interest in humans but is highly challenging (Barranco *et al.,* 2021). Hypoxic/ischaemic injury to the brain results in functional arrest but can be the result of a number of different causes including trauma, chemical events (i.e. toxicosis), respiratory and cardiac arrest, asphyxiation or obstruction of cerebral or cervical blood vessels. Differences exist between ischaemia and hypoxia with the former characterised by a reduction or absence of cerebral blood flow leading to irreversible neuronal injury and death whereas hypoxia is associated with a lack of oxygen in the blood leading to increased cerebral blood flow with reversible alteration of brain function. In asphyxiation cerebral circulation continues but an increase in plasma carbon dioxide occurs with an associated reduction in partial pressure of oxygen.

The brain is not homogenous in its response to ischaemia/hypoxia with areas varying in susceptibility. These have been well defined in humans but similar work has not been undertaken for cetaceans although it is likely similar areas/cell types would be involved (frontal lobe, hippocampus, Purkinje cells and globus pallidus).

Very acute ischaemic/hypoxic injury is nearly impossible to detect histologically with changes only becoming apparent after at least 4-6 hours. Therefore if death occurs after a few minutes or within just a few hours it is not possible to identify the areas of brain damage through conventional macroscopic and histologic examination. Despite this problem alterations both in structural and functional proteins within the brain occur over a short period and detection of these changes through either immunohistochemistry or metabolomic study may help to facilitate the diagnosis of equivocal cases of peracute underwater entrapment and in future be able to provide information on the duration of hypoxia/hypoxic interval which would be useful in the assessment of welfare in these circumstances.

Barranco *et al.,* (2021) reviewed the use of immunohistochemistry (IHC) in the post-mortem diagnosis of cerebral hypoxia and ischaemia in man. Immunohistochemistry was used in an attempt to visualise pathological processes before the appearance of routine histological morphologic lesions. They identified a number of useful markers including Calbindin-D28K, MAP2, S-100, Tau protein and SMI 32. A number of other markers including HIF-1, VEGF, Cox-2, C-fos, HSP70, albumin and GFAP and vimentin were not deemed sensitive enough to be of use. It is important however to understand the limitations as sample type, time after death and autolysis were found to be important factors, all of which equally apply to the material normally available from cetacean species. Research on the validation and assessment of immunohistochemistry markers on the central nervous system of cetaceans was undertaken by Orekhova *et al.,* (2022) and within this paper they evaluated a number of markers. One dolphin, that was presumed to have asphyxiated in a net, displayed increased cytoplasmic neuronal Apaf-1-IR, a higher ratio of cytoplasmic : nucleolar DGK- ζ -IR, and no nuclear neuronal Aβ and no evidence of Bcl-2-IR. Immunohistochemistry may be able to provide a tentative constellation of staining ('fingerprint') that could be helpful in establishing evidence of hypoxic injury. However the use of metabolomic studies assessing transcription factors and epigenetic parameters may be more useful than immunohistochemistry alone as this could provide information on real-time biological modifications occurring during an hypoxic insult.

Experimental work undertaken by Locci *et al.,* (2021) using pigs as a model evaluated metabolomics and histopathology in the diagnosis of mechanical asphyxia death. Heart and brain were assessed using routine histopathology, immunohistochemistry and metabolomic analysis of plasma taken during asphyxia. 2 groups of pigs were utilised - one where asphyxia was induced mechanically (through airway obstruction under anaesthesia) and the other having induced ventricular fibrillation. Although the combination of routine histology and IHC (desmin and Troponin-C on heart, and GFAP and S100B on brain) was found to be quite sensitive in the identification of tissue injury (cardiac and cerebral micro-infarction) secondary to cardiac arrest differentiation between the 2 experimental insults was not possible. Metabolomics however did provide useful differentiation. Key metabolites were lactate, succinate, malate, fumarate, glutamate, hypoxanthine, uridine and cytidine which were significantly increased in asphyxial associated cardiac arrest versus that associated with ventricular fibrillation. This was influenced by the asphyxial period with differences becoming apparent after 5 minutes. Of the metabolites hypoxanthine shows time-related behaviour with increases associated with increased asphyxial period – this latter observation supports previous findings and may be helpful in establishing a hypoxic index/duration to help assess welfare. Further work in relation to cetaceans is needed in this area to establish key metabolomic parameters that would be of value, particularly as these animals show significant differences in their metabolism and response to hypoxia under normal circumstances. Camara *et al.,* (2020) investigated cardiac injury following stranding events in dolphin species using a combination of biochemical analysis (cardiac troponin I and creatinine kinase), histology and immunohistochemistry (myoglobin, fibrinogen, and cardiac troponin I and C). This proved valuable in detecting cardiac injury but application to PUE may be compromised due to the short period of time between entrapment and death. It may, however, be feasible to use metabolomics to identify pre-morphologic change in cardiomyocytes.

One important factor to consider in progressing knowledge in this area in cetaceans is the availability and nature of the material available for assessment. It is known that following death changes in human tissue transcriptomes occurs (Ferreira *et al.,* 2018) and this is highly likely in other species. A systematic evaluation of the effects of post mortem interval on transcriptomes is needed in cetaceans to assist in assessing any transcriptional changes and their effect on the metabolome.

Further research

1. Identify 'watershed' areas in the brain critical in hypoxic injury;
2. Studies to identify immunohistochemical markers associated with hypoxia in cetaceans;
3. Studies utilising metabolomics to identify hypoxic markers in body fluids and tissues taken at necropsy;
4. Assessment of the effects of delayed post mortem time and autolysis on IHC and metabolomics markers;
5. Undertake wider studies on comparisons between peracute underwater entrapment and other causes of death in an attempt to validate the test specificity for the former;
6. Develop a 'fingerprint' of change that allows confirmation of asphyxia associated with PUE and an hypoxic index to help assess welfare.

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Peracute Underwater Entrapment in the UK - Cetacean Bycatch Injury Impact Score Delphi Assessment

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Introduction

Bycatch, defined as the accidental entanglement of non-target species in fishing gear (Reeves *et al.,* 2013), affects various sensitive and protected species in the UK including seabirds, elasmobranchs, and marine mammals (Northridge *et al*., 2017; Silva and Ellis 2019; Luck *et al*., 2020; Cleasby *et al*., 2022). Bycatch is widely recognised as being the leading manmade cause of small cetacean mortality both globally, and in the UK (Read *et al*., 2006; Deaville *et al.,* 2021, Moore *et al*., 2021). Despite efforts by organizations like Sea Mammal Research Unit (SMRU) and the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) to implement observer schemes and catch sampling programmes, challenges persist in accurately determining the number of sensitive marine species caught in UK fisheries due to low observer coverage and limited space for monitoring equipment onboard fishing vessels (Wildlife and Countryside Link 2023). The Cetacean Strandings Investigation Programme (CSIP) gathers data on the bycatch-induced mortality of stranded marine mammals, which further contributes to the understanding of spatial and temporal occurrence of bycatch in the UK (Deaville *et al.,* 2021). These monitoring efforts collectively aim to provide a comprehensive understanding of the impact of bycatch on marine mammal populations in UK waters.

What we know about bycatch from strandings data

Data spanning over 30 years from the CSIP indicates that the primary species affected by bycatch in the UK, as diagnosed through necropsies, are predominantly the harbour porpoise (*Phocoena phocoena*) and the short-beaked common dolphin (*Delphinus delphis*). Bycaught harbour porpoises have been recorded UK-wide, although the highest numbers occur in the southwest of England and to a lesser extent in Wales. Records of short-beaked common dolphin bycatch occur almost entirely in the southwest of England. (Deaville et al., 2021). In contrast to bycatch, the highest rates of mortality associated with chronic entanglement occur in Scotland and are specific to large whales.

Southwest England as a bycatch hotspot

The coastal waters of the Celtic Sea are ecologically significant for a diversity of sensitive and protected species, but also for species of economic importance to the commercial fishing industry (Bendall and Hetherington 2021). The southwest of England represents a large proportion of the UK's fishing fleet, providing 10% of the economic output of all UK fisheries in 2022 (Uberoi et al., 2022). Vessels in the southwest UK operate a wide variety of fishing methods, however nearly half of landings into southwest ports between 2018-2019 were from trawl nets and gill nets (Bendall and Hetherington 2021), which are widely recognised as being 'high-risk' gear types for cetaceans (Reeves *et al*., 2013; Northridge *et al*., 2017). The spatial overlap with high-risk fishing gear and species demonstrating high levels of use of coastal waters such as harbour porpoise and common dolphin likely contributes to increased levels of fisheries interaction in the southwest UK. Furthermore, the southwest UK is characterised by its extensive coastline and is subject to prevailing wind and wave patterns in the North Atlantic which can transport marine debris and organisms from offshore towards the UK coast. Bycatch occurring offshore in the North Atlantic could therefore be easily transported to the southwest UK coastline (Peltier *et al.,* 2012; Moore *et al*., 2020), increasing the number of bycaught individuals being recovered in this region.

Cetacean Bycatch Injury Impact Scoring System (CBIIS) and application for peracute underwater entrapment (PUE)

A recent scoping study funded by the UK government aided the development of the Cetacean Bycatch Injury Impact Scoring (CBIIS) tool to assess the impacts on welfare of individuals in Peracute Underwater Entrapment (PUE) cases. CBIIS aims to provide a simple, reproducible, and robust tool to assess the impact of injuries from bycatch events, drawing on assessment criteria from current available guidance for terrestrial animals eg. Five Domains Model for Animal Welfare Assessment and Monitoring (Mellor and Beausoleil 2015), NOAA 'serious injury criteria' (Anderson et al., 2008) and internationally standardised assessment tools for terrestrial animal trapping welfare (ISO 1999(a); ISO 1999(b)). CBIIS uses a scoring system based on necropsy as a first stage. Information gathered at necropsy will be used to provide information on the anatomic site and severity of ante-mortem lesions. A holistic assessment of injuries will include consideration of any pain, loss of function, sensory function or systemic effects caused by the injury as described below.

- Mild (yellow) – minimal to mild pain/discomfort, no loss of function/physical impairment (i.e. can swim normally), no sensory loss, no systemic effects.
- Moderate (orange) – moderate pain, mild to moderate loss of function/physical impairment, variable sensory loss if system involved, systemic effects seen.
- Severe (red) - severe pain, marked loss of normal function/physical impairment, sensory loss if system involved, pronounced systemic effects (e.g. shock, hypovolaemia, effects on 'energetics' etc), death.

If this method is validated, CBIIS could be applied to investigate differences in impacts of PUE across different species and fishery types. It may be possible to use CBIIS to map different lesions to different fishery types or geographic areas and potentially highlight vulnerable species, age groups, or regions of concern.

Delphi validation of CBIIS

The aim of the practical session was to operate a 'trial run' of the CBIIS scoring sheet alongside a PUE case study to receive feedback on the process and application of the CBIIS system. Following the workshop, a full Delphi assessment will be undertaken to derive a validation of the Cetacean Bycatch Injury Impact Scoring tool as a simple, robust, and reproducible tool to assess the impact of injuries from Peracute Underwater Entrapment cases. The Delphi method (Dalkey and Helmer 1963) will be used to obtain consensus on the opinions of experts, termed panel members, through a series of structured questionnaires. As part of the process, the responses from each round are fed back in summarised form to the participants who are then given an opportunity to respond again to the emerging data. Delphi is therefore an iterative multi-stage process designed to combine opinion into group consensus (Hasson et al., 2000). Participants will be provided with post mortem reports with images and case backgrounds for two cases of PUE. Individuals will then be asked to complete a brief questionnaire rating each ante-mortem lesion according to the CBIIS scoring system, along with a rating of how confident they are in their response. Once survey responses have been collected anonymously, responses will be fed back to participants and individuals will have the opportunity to respond again. This process will then be repeated for two further PUE cases.

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Postmortem findings in harbour porpoises retrieved from gillnets

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Bycatch is one of the most significant threats affecting harbour porpoises (*Phocoena phocoena*) in the North Sea. In Dutch waters, predominantly bottom-set gillnets are used. From June 2013-June 2017, a Remote Electronic Monitoring project was conducted by Wageningen Marine Research (WMR), funded through the Dutch Government. 14 Dutch commercial bottom-set gillnet fishing vessels were equipped with closed-circuit television cameras, with the aim of assessing bycatch rates. More details are reported in Scheidat et al. (2018). During this project, 8 bycaught harbour porpoises were brought ashore for postmortem examination. An additional 4 harbour porpoises were opportunistically retrieved from gillnets between 2008-2019 and reported to Utrecht University for postmortem research. These 12 individuals of 'certain bycatches' formed the basis of a targeted study on postmortem findings in harbour porpoises related to bycatch in gillnets (IJsseldijk et al. 2021).

Firstly, a literature review was conducted to establish criteria that aid in the assessment of bycatch in small cetaceans, divided into 4 topics: 1) Findings related to the drowning process, 2) Findings related to contact with or hauling of the net, 3) Findings related to disentanglement of bycaught animals, and 4) Findings related to the health status of the bycaught individuals. A total of 25 criteria were established. Secondly, it was tested which of these criteria applied to the 12 bycaught harbour porpoises. These 12 individuals were necropsied, following the best practice ACCOBAMS/ASCOBANS guidelines (IJsseldijk, Brownlow & Mazzariol, 2019), and diet analysis were conducted at WMR. From two very fresh animals (DCC1), the inner ears were analysed by Dr Maria Morell to assess potential hearing damage. There were 10 juveniles, size range: 93.5 to 117 cm, of which 7 were males and 3 females. In addition, there were 2 adults: a 10-year-old pregnant female (141 cm) and an 8-year-old resting female (171 cm). Eleven cases were fresh (DCC1-2) at the time of retrieval from the net. The adult resting female was decomposed (DCC4) and later believed to have been caught post-mortem.

Of the 25 criteria, "superficial incisions," "encircling imprints," and "recent ingestion of prey" were observed in the vast majority of the confirmed bycatch cases. Criteria like "pulmonary oedema," "pulmonary emphysema," and "organ congestion" were also frequently observed, although considered non-specific as an indicator of bycatch. Notably, previously mentioned criteria as "favourable health status," "absence of disease," or "good nutritional condition" did not apply to the majority of our bycaught porpoises. The animals from which the inner ears were assessed both had haemorrhage in the cochlea, 1 due to parasite migration (Morell et al. 2017). These findings combined may indicate that there is an overall reduced fitness of harbour porpoises inhabiting the southern North Sea or a higher chance of a debilitated porpoise being bycaught, and could result in an underestimation of bycatch rates when assessing stranded animals. It should be noted that different species, different fisheries or other geographical location will likely result in other findings and conclusions.

Next, bycatch was assessed among the stranded harbour porpoises. A total of 612 individuals were included in this study (IJsseldijk et al. 2022), with selection criteria being: stranded between 2008-2019, very fresh to moderate decomposition (DCC1-3) and availability of gross- and histopathological reports, including photographs, for retrospective evaluation. In total, 17% of stranded porpoises most likely died following bycatch. These were mainly juveniles (73%), but also adult (21%) and neonates (6%). The most were in good nutritional condition at the time of death (NCC: 55% good, 35% moderate, 10% poor). In total, 86% had clear and distinct lesions consistent with net entanglement. Scavenging hampered the assessment of the other individuals, and their diagnosis was thus based on other findings pointing towards an acute or traumatic cause of death, with bycatch deemed most likely. Most bycaught porpoises had pulmonary oedema (89%), the majority had prey remains in stomachs (82%, excl. neonates) and half presented subcutaneous bruising, haemorrhage in the central nervous system, or acute skull or mandible fractures (51%). Finally, multiple organ congestion was seen in 29%. Less than 1/3 of all porpoises in the bycatch category were considered “healthy” at the time of bycatch (30%).

The most important conclusions drawn from these studies were that the bycatch diagnosis remains challenging to assign, with netmarks as the best indicator. Scavenging hampers the assessment of netmarks, as well as decomposition. Notably, also dead animals can become bycaught, highlighted by the DCC4 cases landed by fishermen in the REM project. Finally, the overall health status of harbour porpoises in the southern North Sea can be considered concerning. This poses the question whether diseased animal are at higher bycatch risk?

Welfare was not considered in these studies, but it is clear that some findings, like subcutaneous haemorrhage and fractures, would have been extremely painful for those individuals involved. Bycatch was always an acute cause of death in these animals, with generally little indication that bycatch can be considered prolonged (e.g., no signs of tissue response other than haemorrhage, or no tissue response at all, like in some netmarks). However, there have been three cases of chronic entanglement in porpoises from Dutch waters (<1% of all investigated animals). These cases are a significant welfare concern, but raises the questions whether this should be considered bycatch or marine debris entanglement.

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Histopathology and immunohistochemistry approaches for a presumptive diagnosis of peracute underwater entrapment (PUE) in cetaceans

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Bycatch is a major cause of cetacean mortality worldwide, responsible for thousands of deaths each year. Diagnosing bycatch, particularly in cases involving peracute underwater entrapment (PUE) syndrome, remains challenging. Diagnosis relies on an increasingly recognized set of gross and microscopic diagnostic criteria, though none are pathognomonic. In this study, we present a systematic approach to the postmortem investigation of PUE in cetaceans, focusing on confirmed (directly observed) and highly suspected cases. Our primary emphasis is on histopathological findings, with special attention to cardiac alterations.

All stranded dolphins in the study underwent necropsy, and comprehensive laboratory analyses were performed to rule out infectious diseases that could potentially confound the interpretation of observed lesions. The most frequent histopathological findings included mild acute degenerative changes in skeletal muscle, fragments of striated muscle within the alveolar spaces, alveolar oedema and emphysema in the lungs, haemorrhages and acute degenerative changes in the myocardium, the presence of intracytoplasmic hyaline globules in the liver, and leucocytosis along with intravascular clear spaces in blood vessels. These findings provide valuable insights into the pathophysiology of PUE syndrome and its effects on vital organs, particularly the heart.

Additionally, we performed a Masson's trichrome stain, which confirmed the presence of degenerative lesions within the myocardium. These lesions were associated with the empty round spaces observed histologically, which were interpreted as gas bubble emboli, indicative of possible gas embolism. The trichrome staining clearly highlighted the areas of tissue damage, further supporting the degenerative nature of the observed changes. Furthermore, immunohistochemical analysis was conducted in heart samples using an antibody against heat shock protein 70 (HSP 70). This analysis revealed localized immunostaining, particularly in the blood vessels. Such findings are consistent with previous studies reporting the expression of HSPs in response to acute ischemia in cardiac tissue. The presence of HSP immunostaining in this case may indicate a stress response due to ischemic injury, correlating with the vascular pathology observed during the analysis.

Post-mortem evidence used to target mitigation measures: experience in the Mediterranean Sea within the LIFE DELFI project

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Introduction

Every year, thousands of cetaceans are victim of the interactions with fishing activities and many others are not included in the statistics due to the absence of standardized diagnostic frameworks and the difficulty to evaluate decomposed carcasses as well as the unrecorded cetacean strandings in inaccessible locations. In light of this a multi-tiered structure based on the “Best practice on cetacean post-mortem investigation and tissue sampling” joint ACCOBAMS and ASCOBANS document (IJsseldijk, L.L., Brownlow, A.C., Mazzariol, S., 2019) has been adopted for the examination and evaluation of stranded cetaceans on the Italian coastline.

The Approach

According to the aforementioned post-mortem investigation protocol (IJsseldijk, L.L., Brownlow, A.C., Mazzariol, S., 2019), a framework has been developed with a 3 tier approach to assess the findings following investigation, taking into consideration expertise, human resources and logistics, as described below;

TIER 1 - External examination and stranding data collection: determination of life history and fishery interaction occurrence

Tier 1 is intended for a wide range of operators with basic training in cetacean biology. External examination data allows for the collection of information on the life history of the stranded animal(s), including external signs and findings of interaction with fishing. The cause of death, including interaction with fisheries, cannot be determined.

Interaction with fishing activities can only be hypothesized with positive evidence, and the absence of external findings does not support the absence of interaction.

The Tier 1 examiner can report the following interactions with fisheries: entanglement (presence of active/passive fishing gear).

TIER 2 - Post-mortem investigations and tissue sampling: assessment of fishery interaction category

Tier 2 is aimed at responders (veterinarians or trained biologists, depending on country legislation) with basic experience in cetacean post-mortem investigations and tissue sampling. This tier allows for gross evaluation and description of the general aspect of the carcass and major findings, but not the cause of death. Using this information, examiners may be able to categorize the type of the fishery interaction. Tissue sampling allows for subsequent, targeted investigation.

The Tier 2 examiner can report the following fishery interactions;

Findings confirming the interaction with the fishery - fishing interaction in the animal history, net marks/linear signs (acute or chronic), presence of fishing gears (differentiate passive and active fishing gear), presence of fishing gear around larynx (differentiate passive and active fishing gear), presence of fishing gear (entanglement) or fragments in the gastro- intestinal tracts (ingestion)

Findings suggesting the interaction with the fishery - presence of recent feeding

TIER 3 - Post-mortem examination with diagnostic aims: determination of cause of death

Tier 3 is for trained veterinary pathologists who can provide a comprehensive assessment of post-mortem findings by performing ancillary analyses designed to evaluate all possible cause of death, the presence of any ongoing infection and interpreting all data collected at post-mortem. Tier 3 may allow determination of the role of fishery interaction in the death of the animal, assessing mechanism and manner of death and thus the cause.

The Tier 3 examiner can identify the specific fishing gear and fishing interaction.

Evaluation at Tier 3 requires appropriate skills and expertise as well as logistical and laboratory equipment. In addition to a complete necropsy, the following must be confirmed/stated:

- the carcass decomposition condition code (DCC)
- Confirmation of fishery interaction
- Presence or absence of other ongoing diseases
- Assessment of mechanism of death

For post-mortem investigation evidence is categorised as “certain/pathognomonic’’, “consistent” and “suggestive” with respect to the type of interaction with the fishery (i.e. by-catch with active fishing gear, by-catch with passive fishing gear, chronic entanglement, laryngeal entanglement, ingestion).

Information is collected by investigators and interpreted by reference to tabular definitions as described in the document ‘LIFE DELFI Dolphin Experience: Lowering Fishing Interactions LIFE18 NAT/IT/000942 Action A3 Framework for fishery interaction’.

Evidence and data collected in Tier 1 and 2 are useful in assessing any interaction between the stranded individual and fishing activities. At these levels, information suggesting an interaction with fishing activities is useful to stakeholders involved in fisheries and environmental policy and management.

The cause of death and the possible relationship to fishing can only be reported during Tier 3 evaluation, which allows for a deeper investigation of the interaction, requiring a complete necropsy and specialized expertise in forensic pathology. This tier supports the interpretation of interaction with fishing activities during post-mortem examinations, evaluation of gross and microscopic evidence and all other related exams, regardless of whether this interaction may have caused or contributed to the stranding or death of the animal. Suggestions and procedures included in Tier 3 should be used throughout necropsy performed by a trained veterinary pathologist, as they are a supporting tool for evaluating and interpreting key findings.

The Method

To facilitate the 3 tiered approach to investigations a number of definitions related to interaction with fishing activities have been established including those used for the forensic and anatomo-pathological evaluation (IJsseldijk, L.L., Brownlow, A.C., Mazzariol, S., 2019) and are used by each investigator to record data/information on the fishery interaction.

The following definitions related to interaction with fishing activities are currently being used;

Fishery interaction: any behaviour that leads a marine animal to have contact with a fishing gear or operation.

Active fishing gear: gear that is moved to catch fish by trapping or encirclement (e. g., trawlers).

Passive fishing gear: gear that is left in place for a period before being retrieved (e. g. set nets, gillnets, longlines).

Ghost net: fishing nets or part of them that have been abandoned or lost. Sometimes these nets may aggregate together.

Entanglement: is defined as the entrapment of an animal in marine debris (fishery related or otherwise) or active fishing gear. The impact of entanglement in fishing gear is a global issue impacting more than 260 species including marine mammals, sea turtles and seabirds (Derraik, 2002). Immediate effects of entanglement include acute mortality, serious injury, minor injury, or no injury. Long-term effects include health deterioration, decreased reproductive capacity, chronic injury, impairment and energy burden, long-term sub-lethal effects or no impact. The deleterious effects of entanglement occur most frequently at the level of the individual (Asmutis, 2004; Wells *et al.*, 1998). For smaller cetaceans, entanglement can result in death by drowning due to the difficulty of these animals have in breaking free from the net (McCulloch and Goldstein, 2011). While a special emphasis of the effects of marine debris and interaction with fishing gear by marine mammal management agencies has been on commercial fisheries, not the same pressing interest is directed at the impact of recreational fisheries. Among anthropogenic threats to marine wildlife, entanglement is considered a high priority for the welfare and conservation of these species. The entrapment of cetaceans or part of them in fishing-related debris (ghost nets) is defined as passive entanglement (Macfadyen *et al.*, 2009). Entanglement due to direct interaction of cetaceans with operating fishing gear is considered active entanglement (i.e. bycatch or PUE). Competition for the same resource or opportunistic feeding is considered the primary cause of small cetacean by-catch in fishing gear (FAO, 2018).

Peracute Underwater Entrapment (PUE) – acute entanglement: acute mortality of marine mammal caused by entanglement and forced submersion and can entail complex determinations of ultimate cause of death (Moore *et al.*, 2013).

Chronic entanglement: persistence of fishing gear in a region of the body over a long period causing chronic pathological signs (i.e. entanglement in ghost nets or part of it; secondary by-catch event in which the animal survived by ripping the net).

Tier 3 post-mortem investigations

Trained veterinary pathologists are required for tier 3 investigations as they provide a comprehensive assessment of post-mortem findings by performing ancillary analyses designed to evaluate all possible causes of death, the presence of any ongoing infection and interpreting all data collected at post-mortem. Full necropsy should be undertaken whenever possible to establish the cause of death and whether fishery interaction has been implicated in the cause of death or contributed to it.

Interpretation of the necropsy findings relies on identifying lesions/changes that vary from conclusive to circumstantial evidence of fishery interaction. These include;

1. Evidence of direct signs of fishery interaction (specific to each category)

Presence of fishing gear: fishery gear or part of them still on the body (rostrum/mandible, head, pectoral flippers, dorsal fin, peduncle, fluke) including rope around the tail stock that was added to enable removal from a net (Cox *et al.*, 1998; Moore *et al.*, 2013).

Marks/linear signs: acute: fresh fine or deep skin linear lesions with alteration of skin, colour, furrows and impressions encircling or present at the level of the whole body, rostrum/ mandible, head, pectoral flippers, dorsal fin, peduncle, fluke, prescapular; lacerations at the gape of the mouth; chronic (constriction lesions): linear necrotic and fibrotic lesions (de Quirós *et al.*, 2018; Moore *et al.*, 2013).

Penetrating wounds: lesions caused by sharp tools (Cox *et al.*, 1998; Moore *et al.*, 2013).

Mutilation: acute: partial or complete missing of fin or flippers; the lesion appears without chronic inflammatory reaction; chronic: partial or complete missing of the dorsal fin or pectoral flippers due to trauma or chronic entanglement; microscopically, the lesion shows chronic inflammatory reaction and granulation tissue as well as diffuse fibrosis and signs of tissue remodelling; in this case, the animal survives but can present signs of the past interaction with fishing gears.

Fractures: in the mandible (fractured beaks), other parts of the cranium, and ribs, broken/lost teeth (Kuiken, 1994; Cox *et al.*, 1998; Jepson *et al.*, 2013; Moore *et al.*, 2013).

2. Other fishery interaction - associated lesions

Capture myopathy: to be confirmed through histopathological examination (multifocal acute degenerative changes in cardiac and skeletal muscles) and IHC with anti-fibrinogen and anti-myoglobin antibodies. (Note-this condition can be also be found in other stress-related conditions as in live strandings).

Separation of the rectus abdominis muscles: rupture of the linea alba with concomitant separation of the left and right muscles from each other (Epple *et al.*, 2020).

Decompression gas bubbles: presence of gas bubbles disseminated in the cardio-vascular system and organs (both sub-capsular and in the parenchyma) (De Quiros *et al.*, 2012).

Linea alba herniation: entrapment of the peritoneum, often in addition to mesentery (including the omentum, medial umbilical ligaments, median umbilical ligament, and/or falciform ligament) through the internal lamina of the rectus sheath or linea alba that showed evidence of an acute response (Epple *et al.*, 2020).

3. Non-specific findings

Airway and pulmonary changes: macroscopic lesions: stable froth/ blood-tinged watery fluid in the airways; heavy oedema and congestion, multifocal emphysema and atelectasis, diffuse hyperinflated lungs, incomplete collapse of the lungs, pulmonary subserosal petechiae; microscopic lesions: perivascular oedema and haemorrhage, (Duignan *et al.*, 2003; Epple *et al.*, 2020; Jepson *et al.*, 2013; Puig-Lozano *et al.*, 2020). Pulmonary perivascular oedema is frequently associated with PUE cases (Epple *et al.*, 2020).

Absence of other pathologies: absence of other severe pathological processes that could compromise health status and, possibly, lead to death. It is important to differentiate between infection and infection with evidence of disease. The mere presence of a pathogen with no manifestations of disease cannot be considered significant.

During post-mortem examination it can be difficult to determine the origin of materials removed from entangled cetaceans and to assess whether the origin of the entanglement signs represents a by- catch event, in which the animal manages to rip the net, or a passive entanglement event in fishing-related debris. Therefore, from a pathological point of view, these cases fall into the same category of fishery interaction (i.e. chronic entanglement). It is critical to stress the importance of making this determination because incorrect assumptions about the source and origin of entanglements could funnel time, resources and subsequent policy in the wrong direction.

A number of necropsies have identified lesions and changes of note within the catchment area of the stranding investigations project. The following brief descriptions outline findings, interpretation and implications of 3 important events for cetaceans.

Larynx entanglement or laryngeal strangulation: the condition in which the larynx ('goose-beak') gets wrapped and/or twisted in ingested fishery gear. This is observed particularly in dolphins depredating fishing gear (gillnets) that, upon swallowing a portion of the net, with or without the prey, instead of reaching the forestomach becomes entrapped in the larynx. The fishing gear can be of different types and mesh sizes and can encircle the larynx at different depths and levels from top to the base. Trapped gear involving the larynx can cause displacement, dislocation, compression, obstruction or chronic lesions with serious and fatal consequences for feeding, breathing (asphyxia) or health deterioration. When visible and present, fishing net can be seen hanging from the mouth, sometimes entangling flippers or other appendages, and is often the first indication during external examination. Otherwise, the net may be present only at the level of the larynx extending caudally to the oesophagus. According to Gomerčić *et al.* (2009), the most frequent pathological changes affecting the larynx are oedema, mucosal injury, and exuberant granulation. The severity of the lesion reflects the time interval from strangulation/entanglement to death. The main issue for odontocetes is the position of the larynx which makes it vulnerable to foreign body (for example parts of fishing nets) entanglement during deglutition. In fact, as described by Gomerčić *et al.* (2009): "the larynx is elongated into a tubular extension, the laryngeal spout, that transverses the digestive tract into the nasal cavity, where remains in the erect position during deglutition". This structural adaptation allows inspired air to flow directly from the blowhole and nasal cavity to the larynx and trachea, while ingested food passes through large alimentary canals lateral to the laryngeal cartilages via paired pyriform sinuses (Reidenberg and Laitman 1987; McLeod *et al.*, 2007), contrasting to that seen in terrestrials mammals. It does, however, predispose to laryngeal entanglement. Of note is the regional occurrence of this finding with the majority of cases occurring in the Adriatic Sea.

Ingestion: the active consumption/feeding of marine debris causing physical blockage/obstruction at various levels of the digestive system, leading to injury, pain and death. This occurs particularly in species with non-selective feeding behaviour (raptorial feeders and suction feeders) that may confuse and consequently ingest marine debris in the same foraging areas or in close proximity to actual food items (Werner *et al.*, 2016). In order to study the impact of marine debris ingestion on marine mammals during post-mortem examinations, it is recommended to adopt the "Evidence Based Diagnostic Assessment framework for cetacean necropsies on marine debris ingestion and common data collection"

(Annex 5 IWC/SC/68B/REP03 and ASCOBANS/MOP9/ Inf.6.2.3a). The framework represents an effective tool for assessing and categorising the presence of fishery-related debris in the digestive system of marine mammals.

Intentional injury: the situation where a fisherman intentionally injures/hurts the cetacean (i.e. shooting, amputating the fin in animals still alive, while disentangling the animal from the net). Globally, pelagic and coastal fisheries consider cetaceans as undesirable competitors, or responsible for gear damage or damage and reduction of catch. In the Mediterranean context, due to frequent daily direct contact with the fishing industry stakeholders, dolphins were a target to eradicate perceived competitors to the fishing industry (Bearzi *et al.*, 2010; 2008) or their meat was regularly consumed as a traditional food (Curci and Brescia, 2015). Today, the implementation of legislation, protection measures and public awareness have reduced the impact of this threat, despite the persistence of practice irrespective of national and international regulations such as the black market in dolphin meat (Curci and Brescia, 2015), the use of dolphins as bait (Mintzer *et al.*, 2018), and the direct injury by fishermen who blame dolphins for poor fishing yields (McLaughlin, 2017; Squires, 2017). Any injury deliberately inflicted on a dolphin could occur due to a number of different reasons using many different weapons. The injury could present many different characteristics depending on the weapon and the position of the aggressor. Injury may occur pre or post-mortem, on board or directly at sea or while the dolphin is entangled/by-caught in the net; in any case, it is common to observe injury inflicted on the dorsolateral aspect of the animal that is consistent with the fishermen's position just above (Puig-Lozano *et al.*, 2020) or following mutilation or amputation of appendages (flippers, fluke, dorsal fin) if the animal is entangled in the net (Moore *et al.*, 2013). Depending on this, the injury can be single or multifocal, superficial or penetrating, from a firearm, a blunt instrument or a sharp tool.

Conclusion

The stranding investigations, evaluation and interpretation contribute evidence to the larger EU funded project 'Dolphin Experience: Lowering Fishing Interactions LIFE18 NAT/IT/000942 (LIFE DELFI)'. The main aim of LIFE DELFI is the reduction of dolphin mortality caused by fishing activities. The project also contributes to the Regulation on the conservation of fishery resources and the protection of marine ecosystems (2016/0074) and its robust monitoring and mitigation measures reduce interactions between cetaceans and fishing gear in line with the Marine Strategy Framework Directive (2008/56/EC) and the EU Biodiversity Strategy to 2020.

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Chronic entanglement – Impacts on the individual

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Introduction

Bycatch and entanglement describe the incidental capture of non-target species in fishing gear and marine debris. This is a growing problem globally (IWC 2017) which can have devastating long-term conservation impacts. For example entanglement is a major causal factor in the endangered and critically endangered status of the Arabian Sea humpback whale (*Megaptera novaeangliae*), vaquita (*Phocoena sinus)*, and North Atlantic right whale (*Eubalaena glacialis*) (Minton 2008; Taylor *et al.,* 2017; Knowlton *et al.,* 2012). While the focus of national and international bycatch and entanglement research and legislation has historically centred on such conservation and population level impacts, concerns regarding the welfare implications of entanglement to individual animals, and calls for the inclusion of these within future fisheries policy and management decisions have been increasing in recent years (Clegg *et al.,* 2021, Nicol *et al.,* 2020, IWC 2017, Dolman and Moore 2017).

Moore and van der Hoop (2012) provide a concise review of the impacts on welfare of large whales following chronic entanglement, which can be bioenergetically costly events (van der Hoop et al. 2016), impairing an animal's ability to breathe, feed, swim and reproduce. Any welfare impact on wildlife species needs to include the nature of the harm caused, its duration, the number of animals affected and their capacity for suffering. These authors along with Dolman and Brakes (2018) recognise that although the peracute underwater entrapment and asphyxia of small cetaceans is undesirable, the duration of suffering is relatively short (although longer than most terrestrial animals) compared to the effects of entanglement in larger cetaceans, where often a prolonged time course is involved. They identify 5 key impacts in large whales; 'drowning', increased drag, emaciation, infection and severe tissue damage, and the timeframe. Lethally entangled right whales tend to die over periods of about six months, however some cases can persist for multiple years (Moore and van der Hoop 2012). For example a North Atlantic right whale known as Snow Cone (NOAA 2024) was entangled for at least 18 months prior to the last recorded sighting of her in September 2022. Snow Cone was towing ropes from at least two separate entanglement events and was in extremely poor health, evidenced by a heavy external parasite burden, thin body condition, slow movement, and visible chronic injuries, and is presumed dead (NOAA 2024). In addition non-lethal entanglement can result in behavioural and physiological stress responses (as indicated by increased cortisol levels - see 'The stress response and bycatch') which will impact on the course of the entanglement.

The five key impacts are discussed further below. It is important to recognise that many of these impacts are interconnected, creating a high degree of complexity when interpreting the pathophysiological events experienced by these individuals. Over the last 20-30 years there is an increasing body of knowledge in our understanding of chronic entanglement in large whales and the information discussed here is drawn from relevant literature. Much of the work has been done in North America especially on the North Atlantic right whale (NARW) in light of the critical endangered status of this species and the need to have a greater understanding of the anthropogenic impacts on individuals and the species as a whole. Although differences occur in other large whale species general principles are still applicable, but further research is needed in many areas to obtain a better understanding of how chronic entanglement affects individual welfare.

In Scotland, the Scottish Marine Animal Stranding Scheme (SMASS) investigates cetacean, seal, shark and turtle entanglements, and the Scottish Entanglement Alliance (SEA) works closely with the fishing industry to better understand the scale, impacts and potential solutions to reduce this threat. Through these programmes at least 12 species of cetacean, shark and turtle have been reported entangled in Scottish waters, and although the total number of cases reported to SMASS remains relatively low (n=130, 1992 - present) both the incidence and severity of injuries sustained by animals as a result has been steadily increasing over the past decade (Fig. 1). Entanglement is now the largest identified cause of non-natural mortality in baleen whales in Scottish waters, with around half of deaths investigated by the scheme attributed to this. Of these around 30% were chronic, whereby the animals were entangled for a period of weeks or months, resulting in debilitating injuries and representing a significant welfare concern. For example, recent entanglements investigated by SMASS have included cases where animals have experienced fin amputations, fractures, deep tissue lacerations and infections, and scarring indicative of long-term suffering (Fig. 2) (MacLennan *et al.,* 2021). Leaper *et al.,* (2022) conservatively estimate that six humpback whales, 30 minke whales, and 29 basking sharks become entangled in creel fishing gear annually in Scottish waters. However large data gaps in our understanding of the true incidence and nature of interactions between fishing gear and large marine animals remain, largely due to underreporting of entanglements by fishers and the low likelihood of retrieving carcasses for examination. For example less than 5% of entanglements encountered by creel fishers, a sector that is not monitored by on-board observers or remote electronic monitoring (REM), are thought to be formally recorded (MacLennan *et al.*, 2021).

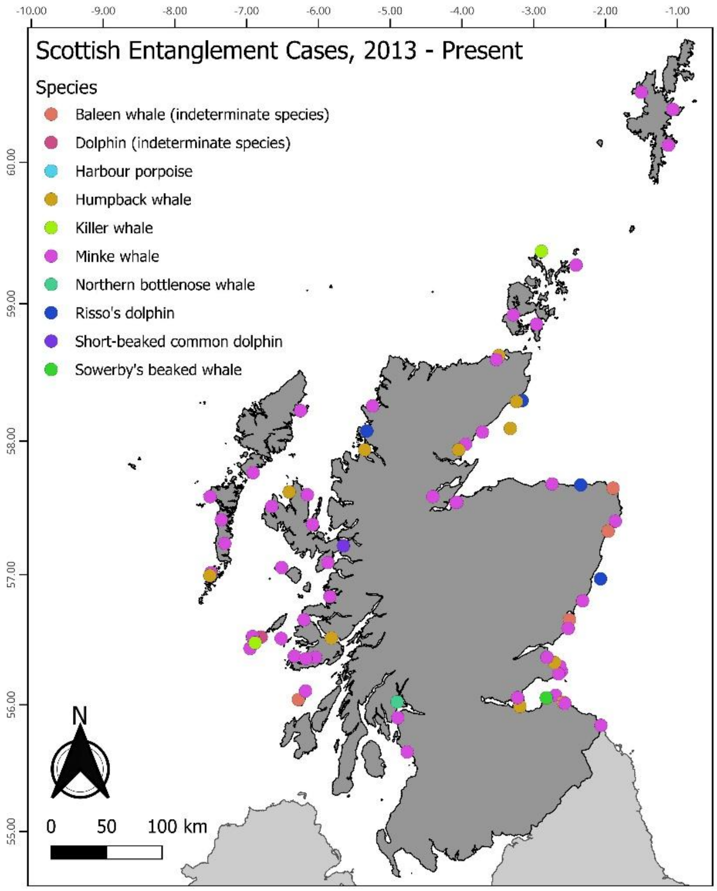

Figure 1: Entanglement cases involving cetaceans reported to the Scottish Marine Animal Stranding Scheme, 2013 – present.

Fig 2: Examples of injuries sustained as a result of chronic entanglement. A.) A Sowerby's beaked whale with a severed pectoral fin; B.) A minke whale with deep lesions extending into the bone and a fractured mandible; C.) A humpback whale with deep infected cuts caused by entangling ropes around the pectoral fin.

‘Drowning’

Following interaction with fishery gear entanglement can lead to peracute underwater entrapment (PUE) with hypoxia and death, or chronic entanglement. With the latter, 2 outcomes are possible – disentanglement either naturally or with human intervention, or persistent retention of gear with subsequent detrimental effects on the animal invariably leading to death.

The likelihood of peracute underwater entrapment is related to the age class and size of the whale, the type of fishery gear involved and the extent of entanglement. In studies undertaken by Lien (1994) and Cassoff *et al.,* (2011) they found the younger age classes (calves and juveniles) were more at risk due to their smaller size, with juveniles less than 11 metres being the most susceptible. This is the result of insufficient body weight and strength to break lines and move to the surface to breathe. Another important factor was found to be the number of body parts affected by the entanglement. Fishery gear fixed to 5 or more body parts resulted in increased likelihood of PUE as a result of decreased mobility and likelihood of breakage of fishery gear. The pathophysiology associated with PUE in these larger species would be similar to that described for small odontocetes (see ‘PUE-the pathway to death’). It is also important to recognise that larger animals that remain entangled become progressively debilitated, less buoyant and have greater energetic demands in rising to the surface leading to eventual exhaustion, inability to surface and subsequent hypoxia/death. This is sometimes exacerbated by direct effects on the respiratory capacity of the affected animals through constriction on thoracic and abdominal structures and impaired function of the nares (blowhole) which is documented in large baleen whales (Cassoff *et al.,* 2011). Many of the agonal pathological processes seen in these animals are the same as those seen with PUE.

Example: In May 2023 a juvenile female humpback whale examined by the SMASS team was determined to have died as a result of drowning due to entanglement. No entangling materiel remained on the animal however there were chronic active entanglement lesions around the tail stock. Abrasions and bruising consistent with a 15mm rope and approximately 100 litres of aspirated seawater suggested the animal had struggled to free itself for several hours before drowning.

Increased drag

Any towed body results in drag with more thrust required for forward movement leading to increased energy output by the animal. A large number of factors affect drag. Different sizes, shapes and types of fishery gear are major factors in the degree of drag. The position of entangled gear (the latter associated with the centre of mass), body shape/condition and inherent buoyancy of the animal are also important with the latter 2 being variable depending on the duration and impact of the entanglement on the animal (van der Hoop *et al.,* 2016). The effects of drag on whales has been studied in-depth by van der Hoop *et al.,* (2016 and 2017b). Both swimming and diving behaviour in NARW were variably impacted and on average there was a 1.5 to 3.1fold increase in drag. Irrespective of the factors there was a direct effect upon energy requirement leading to a negative energy balance.

In baleen whales oral entanglement will have a direct effect on feeding ability, however it can also change the hydrodynamics of swimming and energetics. Baleen whales form a hydrostatic oral seal during normal swimming and any impairment of the seal results in decreased swimming performance (Lambertsen *et al.,* 2005, Cassoff *et al.,* 2011). Oral entanglement is common in these species and as such represents a significant risk to the welfare of these individuals.

The amount of drag in itself is not a predictor of fate of an entangled individual. The duration is the key factor as this reflects the additional energetic cost to the animal which impacts on its health. The more long-standing an entanglement event is the greater the negative health impacts resulting in decreased survival (van der Hoop *et al.,* 2016).

Example: In October 2019 an adult female Sowerby's beaked whale live stranded and died in East Lothian, Scotland. This animal had an entanglement lesion in the form of a circumferential loop of thin cord embedded in the cervical/ thoracic region behind the head. The cord had worked its way through the skin and blubber and was lying on the top of the underlying muscle fascia, with granulation and epidermal remodelling over the top of the rope. Goose barnacles were attached to the remaining cord and based on the depth of the tissue trauma and the damage to the flank, it is likely this cord around the head had at some point been attached to longer and heavier material/s, creating drag and compromising the whale's swimming and foraging capabilities (the animal was in thin body condition and there was no evidence of recent ingesta).

Emaciation

The literature describing chronic entanglement cases consistently highlights the issue of loss of body condition (Cassoff *et al.,* 2011, Pettis *et al.,* 2017, Moore *et al.,* 2013). This is multifactorial in nature with key elements including foraging ability, ability to ingest food, and energetic requirements. Body condition is the metric used to estimate relative energetic reserves. It is correlated with survival and reproductive success and is used to assess and monitor overall health of animal populations. Lipid/energetic reserves are stored and mobilised from a number of tissues including the blubber, visceral tissues and muscles in large whale species. The characteristics of these tissues including thickness and chemical composition represent the primary indicators of body condition. A variety of methods are available to assess body condition including postmortem measurements (i.e. blubber thickness), photogrammetric measurements and visual assessments of body contours. Lipid catabolism results in significant reductions in body girth and blubber thickness and can be the result of changes in environmental conditions or particular life history events (van der Hoop *et al.,* 2016). Entanglement of NARW resulted in a visible reduction in body condition between 16 and 356 days (median 259 days) (of note is the median time frame for improved body condition following disentanglement of 342 days) (Pettis *et al.,* 2017). When assessing body condition a number of factors need to be considered. These include the need for good species specific baseline data (e.g. yearly cycle, sex, breeding activity), and awareness of species differences in utilisation of different fat stores and the impact this has on measurements (van der Hoop *et al.,* 2016, Pettis *et al.,* 2017, Tessa Plint, personnel communication). Further research in this area is needed.

Those species undergoing long migration with associated fasting and reproductively active individuals have evolved physiologically to deal with these energetically demanding periods. However the unpredictable nature of chronic entanglement, that can occur at any point in the animals annual cycle or lifetime, mean affected animals have no mechanism to deal with such an energetically demanding insult resulting in the negative impacts seen in these events. Research suggests that the energetic demands of entanglement are similar to other predictable life history events (van der Hoop 2016).

With loss of body condition there are significant impacts on the reproductive activity of individuals. NARW research has found that there is significant reduction in recruitment to the breeding population of chronically entangled individuals (even those that subsequently become free of gear) which has a large conservation impact in this species and is likely present in other species (Reed *et al.,* 2024).

In baleen whales it is common for fishing gear to become entangled in oral structures including the baleen plates, tongue, jaw and other soft tissue structures, and this alone is thought to constitute the greatest risk of starvation in affected individuals (Cassoff *et al.,* 2011, Moore and van der Hoop 2012, Moore *et al.,* 2013). Involvement of oral structures directly impacts on foraging and the ability to ingest food. Changes in body condition itself further exacerbate the foraging ability due to changes in buoyancy which directly affect diving (van der Hoop *et al.,* 2017a).

Example: A juvenile male humpback whale stranded in Dunbar, east Scotland in Spring 2019. The whale had been observed to have been entangled in creel fishing gear eight weeks prior to stranding and had several chronic lesions, including deep full skin thickness rope abrasions with associated remodelling of the tissue margins and evidence for chronic bacterial infection. Rope remained attached to the pectoral region and had cut deep into the blubber layer, and a rope encircling the head and lower jaw had likely restricted the animal's ability to fully open its mouth. The animal was emaciated and in very poor nutritional condition, with a high internal parasite burden and limited intestinal contents, and no indication of recent successful feeding.

Injuries, infection and severe tissue damage

Injuries associated with chronic entanglement can result in trauma. Fishing gear (or marine debris) that becomes attached to the animal without resulting in peracute underwater entrapment may be either lost quickly (however traumatic injuries may persist) or gear/debris may remain attached. The fishing gear type, quantity, site of entanglement and duration are important factors in the injuries sustained. A wide range of lesions have been recorded and documented and for more detail the reader is referred to Cassoff *et al.,* (2011), Moore *et al.,* (2013), and Sharp *et al.,* (2019) who illustrate the range of lesions seen in chronic entanglement and the effects on the animals.

Briefly, pathological findings in these cases include; (a) the presence of gear/debris; (b) gear impressions and/or unhealed injuries including abrasions, lacerations, amputation and contusions (plus damaged baleen or teeth); (c) absence or presence of infected and/or healing wounds associated with typical entanglement lesions; (d) loss of body condition through to emaciation; (e) abnormal skin condition and higher than average cyamid loads; (f) atrophy of appendages; (g) fracture with or without healing reaction; (h) disuse osteopenia/atrophy. Histopathology changes mirror those seen grossly and include-acute and chronic inflammation, healing (fibrosis/bony reaction), haemorrhage associated with bruising, vascular thrombosis, evidence of infection, myodegeneration/necrosis, atrophy of fat, muscle and bone, and adrenal cortical hyperplasia/hypertrophy with lipid degeneration.

Injuries may be acute, subacute or chronic in nature. They may also change in their severity over time – some healing with no loss of function and others progressively debilitating the individual (i.e. deep flipper lacerations associated with persistence of gear leading to osteomyelitis and arthritis of underlying skeletal structures). This complicates the interpretation of lesions seen at necropsy with respect to assessing the impact on the animal especially when no observational data is available over time. It is important to recognise that the effects of injuries sustained or physical impact of fishery gear can, and often do, have impacts on other body systems. The effects of body condition loss and reproduction have already been discussed. No/little information is available on the systemic action of cytokines, the metabolic changes as a result of injuries, protein loss from large wounds or even the effects of pain (an important factor to consider in welfare). Information on endocrine changes is more available although the paucity of baseline data on a daily, annual and lifetime scale is a problem when interpreting data (Atkinson *et al.,* 2015). It is unknown whether increased levels of parasitism often seen in chronic entangled individuals are the result of chronic stress and/or reduced body condition. Externally cyamid infection is used as an indication of chronic debility in large whales (Cassoff *et al.,* 2011). Infection is frequently related to the extent and chronicity of the sustained injuries and overall body condition is used as a metric of health in NARW individuals (Pettis *et al.,* 2004). High internal parasite burdens have been documented in a number of chronically entangled cases examined by SMASS. However no research has been undertaken into the levels of endoparasitism seen in chronically entangled whales and further work in this area is recommended to try and establish the presence of a possible causal link.

Necropsy data forms an important part of assessing the health status of chronically entangled individuals. For example, dissemination of infection to distant organs such as the lung and liver will impact upon the health of the individual which can only be directly assessed at necropsy. External observations are limited in their scope and use although observation of live affected individuals where the progression of lesions can be studied is of great value in understanding the impact on the animal.

Example: In 2010 a juvenile male minke whale stranded in Argyll on the west coast of Scotland. Both lower mandibles showed evidence of a chronic entanglement, with deep, healing lesions extending into the bone, likely caused by either a rope or some sort of strapping material. The right mandible had re-granulated however in the left osteomyelitis was present in the bone, which had led to a pathological fracture which would have impaired feeding.

Timeframe

One of the most important factors in assessing the welfare impact of chronic entanglement is the duration of the event. This primarily affects the energetic demands on the individual, however progression of injuries with their sequalae are also important factors in survival. On the assumption the animal survives the initial interaction with the fishery gear the duration of the entanglement can vary significantly from days to years (Moore and van der Hoop 2012). As such the suggestion by Nicol *et al.,* (2020) that welfare risk is the product of intensity x duration is of particular importance in chronic entanglement. They also indicate that duration should be expressed as a proportion of the animal's expected natural life span. Having said this even short-term entanglement will have negative impacts with long lasting effects (i.e. reduced reproductive output) (van der Hoop *et al.,* 2016, Reed *et al.,* 2024). Therefore, to evaluate the welfare of an affected individual it is important to take a holistic approach to assessing the different parameters, factors, and findings in each case in relation to duration to provide a more robust welfare assessment.

Assessing duration is problematic however, due to the cryptic nature of entanglement. Ideally detailed observational data could provide the necessary information however this is frequently lacking, for example of 66 cetacean entanglements recorded by SMASS since 2013, there has been only one entanglement case where it could be said with certainty that the animal had been entangled for a prolonged period (at least eight weeks) prior to death, as a result of a photo-ID match. Therefore there is a need to use other methods to assess duration. Pathological assessment of injuries is a tool that can be used albeit we still have poor information on wound healing in cetaceans and the timelines involved (see – 'Wound healing and assessment of duration in cetaceans'). One promising method in baleen whales is to utilise glucocorticoid analysis in baleen. Baleen has been used for the retrospective re-creation of multiple years of glucocorticoid hormone concentrations at approximately monthly intervals in humpback, bowhead and North Atlantic right whales providing information on the likely time of onset of entanglement which is associated with increasing glucocorticoid levels (Lysiak *et al.,* 2018, Rolland *et al.,* 2019, Lowe *et al.,* 2021). As such baleen analysis may therefore prove extremely useful in providing a time frame based upon the stress response in chronic entanglement.

The preferred tool for assessing cetacean welfare is the adapted 5 domains model (Welfare Assessment Tool for Wild Cetaceans) developed by Nicol *et al.,* (2020). Although Moore and van der Hoop (2012) identify 5 key impacts there is a need to translate the component parts of these impacts into the 5 domains tool. Pathological assessment alone of stranded individuals cannot provide the full welfare 'score' and there is a need to undertake more research in areas such as behaviour, endocrinology, physiology and metabolomics. As part of this process however the development of an injury impact tool is valuable in assisting welfare experts in working towards providing more robust and reliable information on the effects of chronic entanglement on whale species.

Cetacean Bycatch Injury Impact Scoring System (CBIIS) and application for chronic entanglement

As a first step towards more holistic welfare assessments, a recent scoping study funded by the UK government has aided the development of the Cetacean Bycatch Injury Impact Scoring (CBIIS) tool to assess the impacts of welfare of individuals in both Peracute Underwater Entrapment (PUE) and chronic entanglement cases. CBIIS aims to provide a simple, reproducible, and robust tool to assess the impact of injuries from bycatch and entanglement events, drawing on assessment criteria from current available guidance for terrestrial animals including the 5 Domains Model for Animal Welfare Assessment and Monitoring (Mellor and Beausoleil 2015), NOAA's 'serious injury criteria' (Anderson *et al.,* 2008) and internationally standardised assessment tools for terrestrial animal trapping welfare (ISO 1999(a); ISO 1999(b)). CBIIS uses a scoring system based on necropsy as first stage. Information gathered at necropsy is used to provide information on the anatomic site and severity of ante-mortem lesions and includes consideration of any pain, loss of function, sensory function or systemic effects caused by the injury as described below, and for chronic cases, duration of suffering.

Severity:

- Mild (yellow) – minimal to mild pain/discomfort, no loss of function/physical impairment (i.e. can swim normally), no sensory loss, no systemic effects.
- Moderate (orange) – moderate pain, mild to moderate loss of function/physical impairment, variable sensory loss if system involved, systemic effects seen.
- Severe (red) - severe pain, marked loss of normal function/physical impairment, sensory loss if system involved, pronounced systemic effects (e.g. shock, hypovolaemia, effects on 'energetics' etc), death.

Duration:

- Acute – Fresh, uninfected injuries which may show no or early inflammation +/- early healing.
- Sub-acute – Wounds show early healing reaction including early granulation tissue where second intention healing occurring.
- Chronic – Advanced healing reaction with fibrosis and scarring.

To validate the CBIIS method as a tool to assess the welfare impacts of PUE and chronic entanglement across different species and fishery types, it was tested at a European Cetacean Society (ECS) conference workshop in April 2024 by approximately 40 veterinary pathologists, cetacean specialists and animal welfare experts. Participants were asked to use the CBIIS scoring sheet using one PUE and one chronic entanglement case. The results of this 'trial run' were then used to develop a Delphi assessment (Dalkey and Helmer 1963), which is an iterative multi-stage process designed to combine opinion into group consensus (Hasson *et al.,* 2000). The first Delphi round has since been completed and the second and final round will be launched in July 2024.

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Welfare assessment of bycatch

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Introduction

Bycatch represents a significant issue for marine mammals (and other marine species) from both conservation and welfare aspects. It can be defined as the unintentional capture of non-target species in fishing gear including entanglement in nets and ropes. Much has been written on the diagnosis of individual cases and impact at the population level of bycatch, particularly in areas of conservation biology. However, the potential welfare impacts on affected individuals have only been partly explored (Dolman and Brakes 2018, Soulsbury *et al.*, 2008). More recently there has been greater interest in the welfare implications associated with anthropogenic impacts on cetaceans, based upon the extraordinary cognitive and communication abilities of cetaceans and the longevity and strength of their social bonds, jointly suggesting that cetaceans possess a strong and refined sentience and a capacity for suffering and enjoyment (IWC 2017, Nicol *et al.,* 2020). Broadly speaking bycatch falls into two categories - peracute underwater entrapment (PUE) leading to rapid death, and the more chronic effects of entanglement/capture which have implications for health and welfare of the affected individuals and their conspecifics.

Soulsbury *et al.,* (2008) provide a comprehensive review of the animal welfare implications of cetacean deaths associated with fisheries (particularly for PUE bycatch). They recognise the effects of injury and death with regards to welfare and highlight other aspects including the social implications for species as a result of interaction with fisheries. Specifically, bycatch of cetaceans encompasses a range of welfare issues including; a) asphyxiation, b) physical injuries, c) physiological and psychological stress, and d) social disruption.

They suggest the primary welfare concern of PUE bycatch is the stress associated with asphyxiation. This along with other aspects of PUE are discussed in greater depth in other sections of this report – 'PUE-The pathway to death' and 'The stress response and bycatch'. However a number of other injuries/physiological changes may occur immediately prior to entrapment and during the short phase between entrapment and death. Therefore to fully assess the welfare of an individual during a PUE bycatch event it is important to capture all of the changes.

Dolman and Brakes (2018) discuss the welfare impacts associated with bycatch and entanglement. They suggest that the suffering of an odontocete captured in fishing gear would occur over a period of minutes and possibly hours prior to death. Larger whales would be affected for a more prolonged period due to their ability to carry the entangling gear to the surface. Asphyxiation/drowning and the sustained injuries associated with interaction with the gear are important considerations for acute bycatch. Non-lethal entanglement can result in behavioural and physiological stress responses (as indicated by increased cortisol levels) which would impact on the outcome of the entanglement.

IWC/66/WKM&WI Rep01 (Report of the workshop to support the IWC's consideration of non-hunting related aspects of cetacean welfare) (2017) reports on the workshop at which welfare aspects associated with bycatch (amongst others) were discussed and debated. During this meeting a number of points were agreed upon, including the use of an adapted version of the 5 Domains model (Mellor 2016) for analysing the welfare costs of various situations. With regards entanglement, impacts include - acute impacts (e.g. underwater entrapment); chronic death (e.g. bleeding out, infection, starvation, killed while mobility is impaired); physical wounds (pain) and deformity; energetic costs and other possible non-lethal impacts of disturbance (fleeing contact); and possible displacement. Entanglement was felt easier to assess with regards the welfare threats due to trauma and injury being visible compared to some other activities (e.g. whale watching).

During the IWC workshop a proposed scoring system was suggested for assessing the impacts of entanglement utilising the 5 Domains model. As part of this some definitions were applied. An 'acute' impact was defined according to the time taken for the animal to drown, anything over a longer time span (hours to weeks or months) was defined as 'chronic'. One of the issues recognised was the effects of chronic impacts of even minor welfare burdens which may have a cumulative effect impacting on the overall resilience of the animal taking it beyond their coping capacity. A method to assess cumulative impacts was felt important to establish in future. One area of particular interest that the group made recommendations upon was the monitoring of wound healing, wound progression, and time to death in wild cetaceans that incurred vessel-strike or entanglement injuries in order to provide greater understanding of the welfare implications on individuals (in this current report the section 'Wound healing and assessment of duration in cetaceans' provides background and recommendations to progress this issue).

Moore and van der Hoop (2012) provide a concise review of the impacts on welfare of large whales following chronic entanglement. Any welfare impact on wildlife species needs to include the nature of the harm caused, its duration, the number of animals affected, and their capacity for suffering. Although drowning of small cetaceans and pinnipeds is undesirable, the duration of suffering is relatively short (although longer than most terrestrial animals – see 'PUE-the pathway to death') compared to the effects of entanglement in larger cetaceans, where often a prolonged time course is involved. They identify 5 key impacts in large whales; drowning, emaciation, increased drag, infection and severe tissue damage, and the timeframe (lethally entangled right whales tend to die over periods of about 6 months, some cases can persist for multiple years). This is further explored in the section 'Chronic entanglement – Impacts on the individual' in this report.

Assessment of injuries – marine mammals

To date a quantitative scoring system has not been developed for injuries and physiological changes associated with marine mammal bycatch events. In the USA a standardised system for the assessment of serious and non-serious injury to cetaceans is currently in use by the National Oceanic and Atmospheric Administration (NOAA)/National Marine Fisheries Service (NMFS). This was developed as a requirement of the Marine Mammal Protection Act (MMPA) which requires the NMFS to prepare stock assessment records for marine mammals under the jurisdiction of the United States which summarise human-caused mortalities and serious injuries to marine mammals.

The Act defines an injury as the following;

‘A wound or other physical harm. Signs of the injury include, but are not limited to, visible blood flow, loss of or damage to an appendage or jaw, inability to use one or more appendages, asymmetry in the shape of the body or body position, noticeable swelling or haemorrhage, laceration, puncture, or rupture of eyeball, listless appearance or inability to defend itself, inability to swim or dive upon release from fishing gear, or signs of equilibrium imbalance. Any animal that ingests fishing gear, or any animal that is released with fishing gear entangling, trailing or perforating any part of the body will be considered injured regardless of the absence of any wound or other evidence of an injury’

Serious injury is defined as; ‘any injury that is likely to result in mortality.’

Key *causes of* injury include: hooking (longline, troll, recreational), entanglement (trap/pot, gillnet, monofilament, longline), entrapment (trawl, seine), and collisions (vessel hull, propeller).

Key variables contributing to whether an injury should be considered serious include:

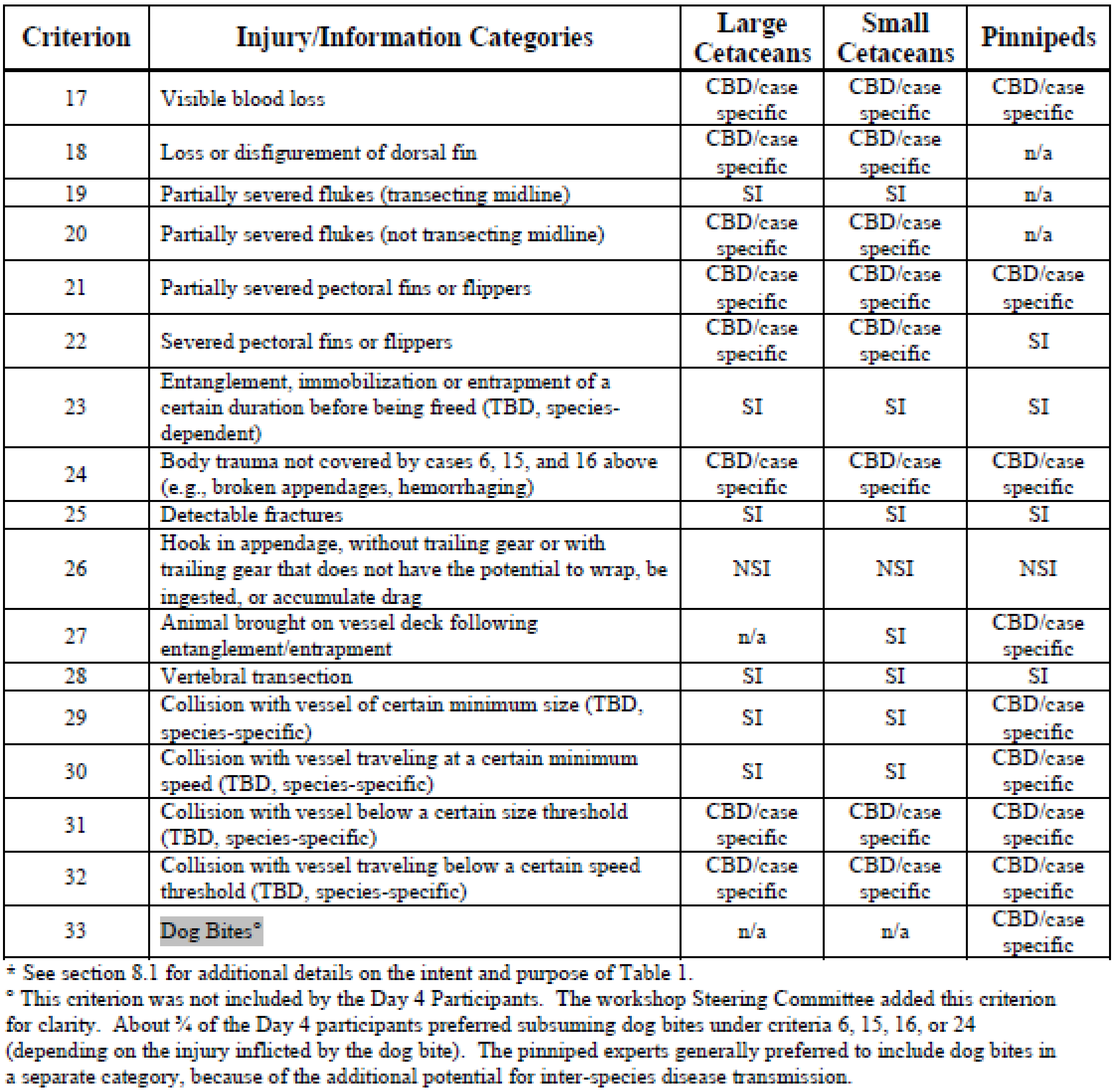
- Animal age.
- Animal health.
- Animal behaviour.
- Injury type (e.g., puncture, laceration, blunt trauma, compression).
- Injury location (e.g., mouth, head, body, flipper, tail, internal).
- Injury size.
- Injury duration (e.g., short, repeated or chronic).
- Entanglement type (e.g., hooked, constricting line, loose line, anchored, entrapment).
- Entanglement size (e.g., size, length and number of branches of line; number of buoys, traps or anchors; volume of netting).
- Entanglement constriction (e.g., tight, loose, multiple wraps).
- Entanglement duration.

Workshops held in 1997 (Angliss and DeMaster 1998) and 2007 (Anderson *et al.,* 2007) discussed and developed a consensual agreement on the serious injury criteria. The following table (Table 1) has been taken from Anderson *et al.,* (2007) and shows the ‘working’ criteria used. As can be seen there are still areas where more information is required before a specific criteria can be ascribed to a case. Uncertainty was a factor that was highlighted in this latter workshop report and reflects the difficulties in injury assessment associated with human-marine mammal interaction.

Table 1: Recommended Serious Injury Criteria for Different Taxonomic Groups *

SI = Serious Injury; **NSI** = Not Serious Injury; **CBD**/**case specific** = Potential SI, but either 1) insufficient information about the impact of a particular injury, or 2) additional factors must be considered on a case-by-case basis to determine the severity; **n/a** = not applicable; **TBD**= To Be Determined; ▨ = areas lacking near-complete agreement among Day 4 participants.

Criterion	Injury/Information Categories	Large Cetaceans	Small Cetaceans	Pinnipeds
Pre-Existing Guidance (included in Angliss and DeMaster (1998) and/or NEFSC publications, retained with no changes)				
1	Ingestion of gear or hook	SI	SI	SI
Modified Criteria (some aspects retained from guidance provided in Angliss and DeMaster (1998) and/or NEFSC publications, with some changes or additions)				
2	A free-swimming animal observed at a date later than its human interaction, exhibiting a marked change in skin discoloration, lesions near the nares, fat loss, or increased cyamid loads, etc.	SI	SI	SI
3	Gear constricted on any body part, or likely to become constricting as the animal grows	SI	SI	SI
4	Uncertain whether gear is constricting, but appendages near the entanglement's point of attachment are discolored	SI	SI	SI
5	Anchored/immobilized (not freed)	SI	SI	SI
6	Head trauma (including eye injuries)	SI	SI	SI
7	Hook in mouth (excluding case 9 below), no trailing gear	CBD/case specific	SI	SI
8	Hook confirmed in head (excluding mouth), no trailing gear	NSI	SI	CBD/case specific
9	Hook confirmed in lip only, no trailing gear	n/a	CBD/case specific	CBD/case specific
10	Gear attached to free-swimming animal with potential to 1) wrap around pectoral fins/flippers, peduncle, or head; 2) be ingested; or 3) accumulate drag	CBD/case specific	SI	SI
11	Animal freed from gear and released without gear	CBD/case specific	CBD/case specific	CBD/case specific
12	Social animal separated from group or released alone	CBD/case specific	CBD/case specific	CBD/case specific
13	Dependent animal (e.g., calf, pup) alone post-interaction	SI	SI	SI
14	Wrap(s) of gear around pectoral fin/flippers, peduncle, head, abdomen, or chest	CBD/case specific	SI	SI
New Criteria				
15	Deep, external cut or laceration to body	CBD/case specific	CBD/case specific	CBD/case specific
16	Body cavity penetration by foreign object or body cavity exposure	SI	SI	SI



Criterion	Injury/Information Categories	Large Cetaceans	Small Cetaceans	Pinnipeds
17	Visible blood loss	CBD/case specific	CBD/case specific	CBD/case specific
18	Loss or disfigurement of dorsal fin	CBD/case specific	CBD/case specific	n/a
19	Partially severed flukes (transecting midline)	SI	SI	n/a
20	Partially severed flukes (not transecting midline)	CBD/case specific	CBD/case specific	n/a
21	Partially severed pectoral fins or flippers	CBD/case specific	CBD/case specific	CBD/case specific
22	Severed pectoral fins or flippers	CBD/case specific	CBD/case specific	SI
23	Entanglement, immobilization or entrapment of a certain duration before being freed (TBD, species-dependent)	SI	SI	SI
24	Body trauma not covered by cases 6, 15, and 16 above (e.g., broken appendages, hemorrhaging)	CBD/case specific	CBD/case specific	CBD/case specific
25	Detectable fractures	SI	SI	SI
26	Hook in appendage, without trailing gear or with trailing gear that does not have the potential to wrap, be ingested, or accumulate drag	NSI	NSI	NSI
27	Animal brought on vessel deck following entanglement/entrapment	n/a	SI	CBD/case specific
28	Vertebral transection	SI	SI	SI
29	Collision with vessel of certain minimum size (TBD, species-specific)	SI	SI	CBD/case specific
30	Collision with vessel traveling at a certain minimum speed (TBD, species-specific)	SI	SI	CBD/case specific
31	Collision with vessel below a certain size threshold (TBD, species-specific)	CBD/case specific	CBD/case specific	CBD/case specific
32	Collision with vessel traveling below a certain speed threshold (TBD, species-specific)	CBD/case specific	CBD/case specific	CBD/case specific
33	Dog Bites°	n/a	n/a	CBD/case specific

* See section 8.1 for additional details on the intent and purpose of Table 1.

° This criterion was not included by the Day 4 Participants. The workshop Steering Committee added this criterion for clarity. About ¾ of the Day 4 participants preferred subsuming dog bites under criteria 6, 15, 16, or 24 (depending on the injury inflicted by the dog bite). The pinniped experts generally preferred to include dog bites in a separate category, because of the additional potential for inter-species disease transmission.

Table provided courtesy: NOAA Fisheries

This system only broadly categorises injuries into three criteria – 'serious injury', 'not serious injury' and 'cannot be determined/case specific'. No guidance or indication is given to suggest that the scoring system can be used cumulatively on any one animal (although this would seem logical and is briefly alluded to in the text) and it does not apply a numerical score to each injury. Definitions as to impact of each injury are not provided. Participants also highlighted the lack of consistency and clarity on definitions to distinguish serious from non-serious injury and it was felt there was need to move to a quantitative rather than a qualitative approach.

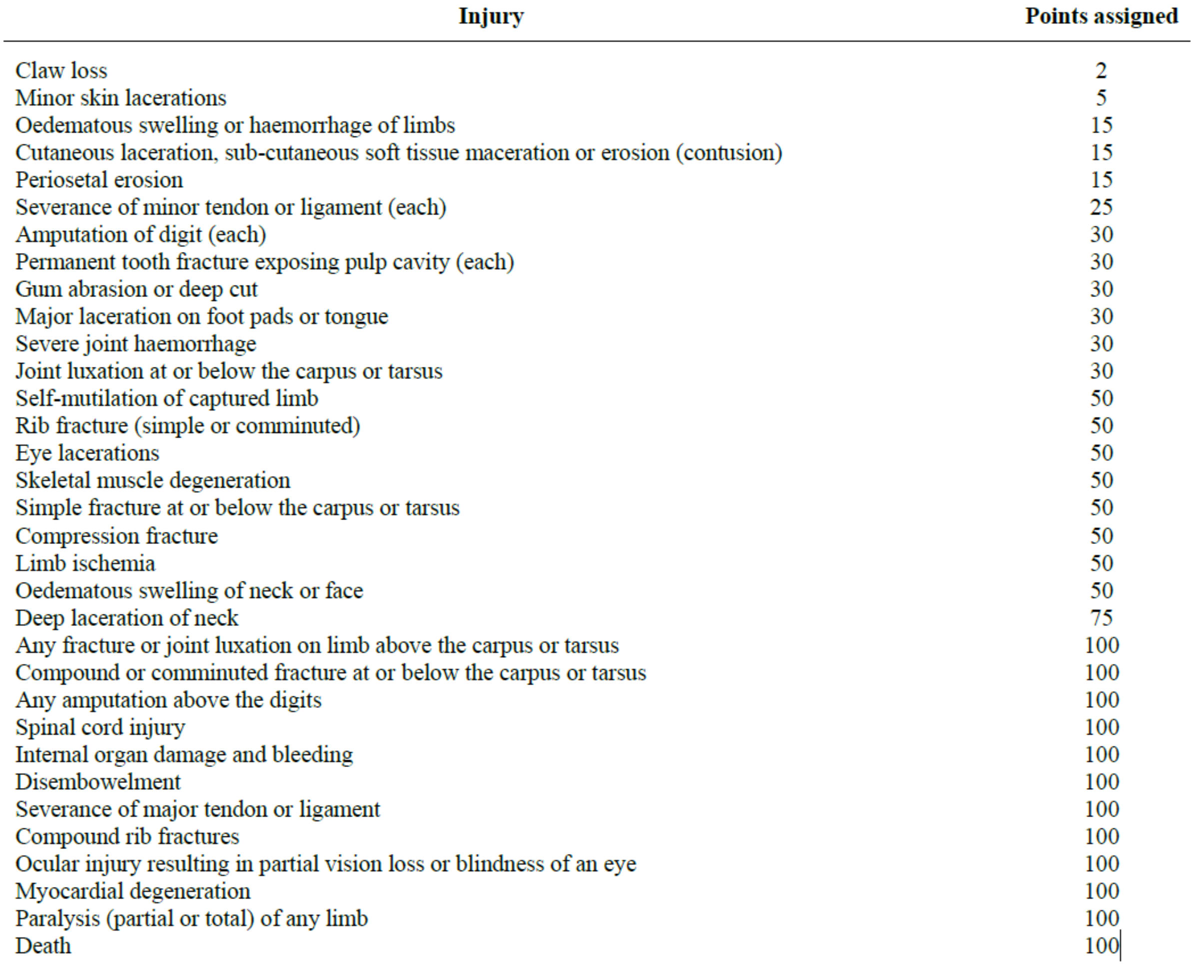
Section 6 of the Anderson *et al.,* (2007) report discusses areas of the pathobiology of injuries in human-marine mammal interactions. Within this Moore describes the use of a scoring matrix to characterise and evaluate propeller wounds. Mention is then made of a system used to subjectively score significant parameters associated with entanglement trauma. At that time the model was being refined with the aim of ranking cases in terms of severity, and comparing the ultimate outcome. No further information has been found on the latter. Rotstein discusses the consequences of injury. His definition of serious injury is where death occurs instantaneously (peracute), in a short period (acute) and over a more prolonged time period (chronic), or results in significant debility that affects feeding, mobility and reproduction. He emphasises the need for full carcass assessment as internal injuries form part of any evaluation. The pathological consequences of any injury comprise the anatomic and physiologic changes. The former are easier to document and assess, and the latter require an understanding of the pathological processes as a consequence of injury (i.e. the hormonal stress response, effects of blood loss etc). Using animal factors and the sources/nature of the trauma he suggests categorisation of the injury and response to the injury could be developed for marine mammals similar to that used for human trauma scoring.

Assessment of injuries – terrestrial mammals

In light of the suggestion that a more quantitative approach to assessing injuries sustained by cetaceans in human-animal interactions is needed, exploration of other comparable methodologies is helpful. In humans a number of trauma scales have been developed which assess anatomic and physiological changes relating to prognosis/outcome and are used to guide treatment (Lecky *et al.,* 2014). However there are few/no objective scales available for interpreting the impact of injury on animals. One area where this has been implemented and partly standardised is in terrestrial mammal trapping. This has been driven by the need to ensure equipment and procedures to trap wild animals are improved to reduce the welfare impact on individuals following concerns raised by the scientific community, animal welfare organisations and the public (Iossa *et al.,* 2007, Proulx *et al.,* 2022). In 1999 the International Organisation for Standardisation (ISO) published an agreed process for testing and assessing mammalian traps. The ISO standards did not offer definitions of acceptable standards of animal welfare, but were seen as an initial step towards ensuring and improving the welfare of trapped wild mammals.

Iossa *et al.,* (2007) suggested a different approach was needed to assess welfare trapping standards based on; a) the individual animal and context, b) location of the wounds, c) the nature and pain associated with the injuries, and d) the long-term survival and fecundity of the individual and impacts of removal of the animal from the population if released. The reliance on assessment of physical injury alone (as per the ISO standard) is clearly insufficient and a more comprehensive approach to welfare is needed. A combination of behavioural (including anxiety and psychological responses to capture), physiological responses and physical injuries associated with trapping is needed to fully assess the welfare of any given trapping technology (Proulx *et al.,* 2022). Injury-based trauma scales are currently the best method available to relate injuries to welfare for terrestrial mammals. Physical injury evaluation between trap types used for terrestrial mammals utilises a point scoring system based upon the severity of the lesion. Proulx *et al.,* (2022) provide an updated/refined scoring system following ISO standards (Table 2 reproduced below) which assigns points to each injury type seen. An overall injury score for an animal is the summation of the individual lesions. Injuries considered serious (≥ 50) are given when they are likely to impact on the welfare and survival of any released animal, or when a series of minor injuries (< 50 points for each injury) amount to ≥ 50 points and have a compounded effect.

Table 2. Injury-scoring system for the assessment of restraining trap systems (Proulx *et al.,* 2022). Table reproduced by kind permission of Dr. Gilbert Proulx, Alpha Wildlife Research & Management Ltd.



Injury	**Points assigned**
Claw loss	2
Minor skin lacerations	5
Oedematous swelling or haemorrhage of limbs	15
Cutaneous laceration, sub-cutaneous soft tissue maceration or erosion (contusion)	15
Periosetal erosion	15
Severance of minor tendon or ligament (each)	25
Amputation of digit (each)	30
Permanent tooth fracture exposing pulp cavity (each)	30
Gum abrasion or deep cut	30
Major laceration on foot pads or tongue	30
Severe joint haemorrhage	30
Joint luxation at or below the carpus or tarsus	30
Self-mutilation of captured limb	50
Rib fracture (simple or comminuted)	50
Eye lacerations	50
Skeletal muscle degeneration	50
Simple fracture at or below the carpus or tarsus	50
Compression fracture	50
Limb ischemia	50
Oedematous swelling of neck or face	50
Deep laceration of neck	75
Any fracture or joint luxation on limb above the carpus or tarsus	100
Compound or comminuted fracture at or below the carpus or tarsus	100
Any amputation above the digits	100
Spinal cord injury	100
Internal organ damage and bleeding	100
Disembowelment	100
Severance of major tendon or ligament	100
Compound rib fractures	100
Ocular injury resulting in partial vision loss or blindness of an eye	100
Myocardial degeneration	100
Paralysis (partial or total) of any limb	100
Death	100

Translating this into bycatch events is challenging. Although a trauma scoring system is helpful in assessing welfare of cetacean bycatch, the animal experiences a number of physiological and behavioural changes during any episode which need to be factored in. This has been highlighted over a number of years by different authors (Soulsbury *et al.,* 2008, IWC 2016, Dolman and Brakes 2018, Nicol *et al.,* 2020). The development of a trauma scale for bycaught cetaceans may however go some way to assessing welfare impacts. It must be recognised that the physiological and behavioural aspects are important and further research is required before they can be successfully incorporated into any welfare scoring system. For cetaceans this is problematic as it is recognised that even under experimental situations evaluating the physiological changes seen in trapped terrestrial mammals has significant challenges (Proulx *et al.,* 2022). As discussed in other areas of this report (see ‘The stress response and bycatch’) assessment may be feasible for some parameters in cetaceans although further research is needed.

The 5 domains model/welfare assessment tool for wild cetaceans (WATWC)

Originally conceived to assess welfare in experimental animals, the 5 domains model has been subsequently applied to a wider range of species and scenarios (Mellor and Beausoliel 2015, Mellor 2016, and Mellor *et al.,* 2020). It is currently considered the most comprehensive method to assess cetacean welfare (IWC 2017) and has been applied to cetacean stranding welfare (Boys *et al.,* 2022), whale watching (Nicol *et al.,* 2020), and ship strike events (Rae *et al.,* 2022). It is also used as a standardised welfare assessment for the North Atlantic Right Whale, to provide a holistic way to understand the cumulative effects of anthropogenic activities at both the individual and population levels (King *et al.,* 2021). It should be recognised that assessment of wild cetaceans in relation to bycatch welfare is challenging due to limitations on observations in the marine environment and our current state of knowledge in areas of pathophysiology and behaviour. This does not, however, preclude applying the 5 domains model to provide an assessment of bycatch and entanglement welfare.

Nicol *et al.,* (2020) provide an adapted version of the 5 domains model for human-cetacean interactions, having developed the Welfare Assessment Tool for Wild Cetaceans (WATWC). As with the original 5 domains model, it is divided into four physical/functional domains and one affective experience domain. In all of the applied welfare scoring systems that have been published for anthropogenic insults, it is recognised that there is a lack of empirical data for assessing cetacean welfare, and Rae *et al.,* (2022) state that 'expert opinion is the best (or only) method available to develop a picture of the overall welfare impact of a scenario'. Opinion from experts and stakeholders in the relevant field has therefore been sought when applying the WATWC model using the Delphi technique, as group opinion is more representative than that of the individual.

To assist in assessing welfare Nicol *et al.,* (2020) developed a numerical scoresheet to score the maximum intensity of each factor/domain (1=least, 10=most) in domains 1-4, and an overall judgement of the harm for domain 5. It is interesting to note that in the 5 domains model Mellor and Beausoliel (2015) opted not to use a numerical grading scheme 'in order to avoid facile, non-reflecting averaging of scores as a substitute for considered judgement, and to avoid implying a degree of precision that is not achievable'. They instead use a 5 tier impact scale (A-E corresponding to mild, moderate, marked, severe and very severe) for each of the domains, which represent increasingly negative impacts on the animal concerned. Three factors were used for grading;

1. Severity of the physical/functional impacts in domains 1-4
2. The related intensity and duration of the inferred affective impacts and their reversibility (the latter probably of little value in PUE as death is the outcome but in cases of chronic entanglement these represent important factors)
3. Whether or not the impacts may need mitigation and/or be ended (via relocation to a benign condition, intervention of animal care/veterinary treatment, and/or by euthanasia (again of little value in PUE))

Based upon this they provide 'levels' of welfare score as shown below;

Grade A and B – no/low-level but tolerable negative affects

Grade C and D – intermediate

Grade E – exceptionally unpleasant negative effects of high intensity

For domains 1-4 grading uses well-validated indices in animal management and veterinary assessments. It is important to note that many of these indices have not, or have only partly, been developed for cetaceans.

Peracute Underwater Entrapment (PUE) WATWC assessment

If the WATWC model is applied to PUE the following areas of welfare concern can be identified. For each domain deemed relevant brief discussion is given highlighting the problems in assessing the welfare impact and potential methods that can be used to improve the accuracy of any assessment.

Survival-related factors (potentially observable/measurable)

Domain 1 – Nutrition – N/A

Domain 2 – Physical environment

a) Constriction, confinement, trapping, entangling – this can be measured through direct observation of the event and/or via the evidence of injuries obtained at necropsy.

b) Unpredictable events – it goes without saying that PUE is an unpredictable event associated with novel fishery gear interaction. It is important to recognise some individuals may seek out fishery activities for foraging purposes so the impact of this is difficult to assess. Experience of the individual and overall ‘fitness’ are important but difficult to assess and quantify.

Domain 3 – Health

a) Internal injury – measure by objective assessment by proposed trauma scoring system

b) External injury – measure by objective assessment by proposed trauma scoring system

c) Compromised respiration – further research required to establish method for assessing hypoxia duration - see ‘PUE-the pathway to death’.

d) Loss of sensory function – this would involve impairment of auditory and visual senses primarily. Although necropsy findings would be helpful they may not represent the full effects on the individual.

Domain 4 – Behavioural interactions (see also Domain 2)

a) Separation from conspecifics - this is dependent on the social structure of the species and the individual themselves.

b) Limitations on communication or interaction with conspecifics – this is dependent on the time to death and other events the animal is experiencing. It’s significance is unknown.

Affective experience (non-observable, inferred from Domains 1-4)

Domain 5

a) Pain from internal or external cause – unlike domestic species where research has been undertaken to assess/evaluate pain little is known about this in wild cetaceans. There is a need to infer this factor from the necropsy finding and proposed trauma scale (see below).

b) Anxiety, fear, panic – this is alluded to previously in the discussion regarding the stress response and further research is required in ‘measuring’ the acute stress response in cetaceans and PUE.

c) Discomfort – this is primarily a behavioural/emotional response requiring observational data however a greater understanding of the acute stress response and neurophysiology may assist in measuring this more objectively. Physical assessment of the trauma using the trauma scale may be of partial value.

d) Fatigue, exhaustion, lethargy – from observed data cetacean behaviour in PUE events can result in the former 2 components in this area. It may be possible to assess this at necropsy but further development of markers to indicate increased muscular activity/metabolic change are needed.

e) Confusion – this is difficult to assess and would require observational information to assist in judgement.

f) Breathlessness, dizziness – with the onset of hypoxia the animal would have a desire to breath. As mentioned under Domain 3 assessment of markers for hypoxia would assist in evaluation.

g) Other cetacean-specific mental state – unknown factors but maybe relevant.

Reviewing the separate factors of the domains clearly identifies large gaps in our current knowledge and presents difficulties in applying an accurate WATWC score to a PUE event. At present the development and refinement of a trauma score (see below) would go some way to quantify the impact of PUE on an individual and it can be used by welfare experts to provide more robust answers to welfare associated issues in bycatch. Further research in other areas (i.e. behaviour and physiology) is needed to provide a more robust and reliable assessment of welfare in these events.

Chronic Entanglement WATWC assessment

Chronic entanglement has a different set of components/factors to consider to those seen in PUE. Limited direct observation of living affected individuals has meant research is already being undertaken in many of the areas of interest (see ‘Chronic entanglement – Impacts on the individual’). Ongoing development of a series of analytical tools to assess areas such as the stress response, energetics, etc. will provide welfare experts with tools to establish a clearer understanding and evaluation of the welfare experiences of these animals. Duration is an important factor in the welfare assessment of chronic entanglement and is discussed further in ‘Chronic entanglement – Impacts on the individual’ and later in this document. The following discusses the different factors in the WATWC assessment for chronically entangled cetaceans.

Survival-related factors (potentially observable/measurable)

Domain 1 – Nutrition

a) Foraging ability- direct and indirect observational data is needed to establish foraging ability which could include restrictions to the animal’s ability to dive, and/or to open its mouth. The impact of reduced foraging is on body condition, blubber/lipid volumes and consistency, and protein status – these can be evaluated both before death and at necropsy.

b) Ability to ingest food– measure by objective assessment using proposed trauma scoring system.

c) Energetic requirements-although research has been undertaken to calculate the energetic demands of chronic entanglement, without knowledge of the effects of drag, points a) and b) above and other factors such as the impact of infection, a definitive figure is difficult to achieve. Reliance of changes in body condition and lipid/protein status are currently the most useful method of assessment.

Domain 2 – Physical environment

a) Constriction, confinement, trapping, entangling – this can be measured through direct observation and/or via the evidence of injuries obtained at necropsy.

b) Unpredictable events – chronic entanglement is an unpredictable event associated with novel fishery gear interaction. As entanglement can occur at any time point in the annual cycle or lifetime the animal cannot ‘budget’ for the event. Evaluation of this factor requires in-depth knowledge of the ecology and physiology of these animals.

Domain 3 – Health

a) Internal injury – measure by objective assessment using proposed trauma scoring system.

b) External injury – measure by objective assessment using proposed trauma scoring system.

c) Compromised respiration – factors involved include direct effects of constriction, physical obstruction of the nares (blowhole), difficulty in rising to surface, and terminal events when exhausted and unable to surface resulting in hypoxia. Some assessment can be made based on necropsy findings although measurement of blood oxygen is more definitive.

d) Loss of sensory function – this could involve impairment of auditory and visual senses primarily. Although necropsy findings would be helpful they may not represent the full effects on the individual.

e) Functional impairment – the direct effects of gear can be judged at necropsy although assessing this in the live animal would give a greater understanding of the impact. Functional impairment is not merely the direct result of the entanglement. The effects of chronic stress on immune system function, effects on reproduction, loss of muscle mass (and locomotor ability) etc are factors that cross other domains but need consideration.

f) Parasitism- the significance on welfare of parasitism is unknown in large cetaceans. External parasitism due to cyamid infection is directly related to external injuries (both their extent and chronicity) and generalised debility. It is used as a metric of body condition in NARW and is therefore useful in providing information on the overall impact of entanglement. Immunosuppression secondary to chronic stress may predispose to infection whether that involves ecto- or endo-parasites.

g) Dehydration – cetaceans derive water from two sources - ingested food and metabolic water ‘released’ following lipid catabolism. Chronic entanglement can result in decreased food intake, however the utilisation of fat stores to maintain energy needs partly negates the effects of reduced water intake. Only once lipid stores are exhausted will dehydration become critical. Therefore assessment of ability to feed, and lipid and protein metabolism, need to be considered in this factor.

h) Loss of body condition – this is related to reduced food intake, increased energetic demands, and effects of injury/infection. Direct measurement of weight, blubber thickness and muscle mass are valuable in this assessment. Other semi-quantitative measures including body profile analysis are well established although better suited to the live animal.

Domain 4 – Behavioural interactions (see also Domain 2)

a) Separation from conspecifics - this is dependent on the social structure of the species and the individual themselves. Of particular note are cow/calf interactions which may result in maternal separation and/or reduced survival of younger animals.

b) Limitations on communication or interaction with conspecifics – this is dependent on the time to death and other events the animal is experiencing. Its significance is unknown.

c) Disturbed or inadequate sleep or rest. It may be possible to evaluate this through direct observation of the live animal, the use of remote tracking devices and data loggers.

d) Altered time budgets – see above. Direct observation and remote data loggers would provide invaluable information on this. Reported observations indicate changes in foraging behaviour and swimming/diving activity. The result of changes in time budgets may be judged from body condition however this is very difficult to relate directly to changes in behaviour alone.

Affective experience (non-observable, inferred from Domains 1-4)

Domain 5

a) Pain from internal or external cause – unlike domestic species where research has been undertaken to assess/evaluate pain, little is known about this in wild cetaceans. There is a need to infer this factor from the necropsy finding and proposed trauma scale (see below).

b) Anxiety, fear, panic – this is alluded to previously in the discussion regarding the stress response, and further research is required in ‘measuring’ the chronic stress response in cetaceans experiencing chronic entanglement. Measurement of glucocorticoids in body fluids, tissues and baleen is available to assist in providing a ‘metric’ of severity.

c) Discomfort – this is primarily a behavioural/emotional response requiring observational data, however a greater understanding of the stress response and neurophysiology may assist in measuring this more objectively. Physical assessment of trauma using the proposed trauma scale may be of partial value.

d) Fatigue, exhaustion, lethargy – from observed data these parameters can be assessed. It may be possible to assess this at necropsy but further development of markers to indicate increased muscular activity/metabolic change are needed.

e) Confusion – this is difficult to assess and would require observational information to assist in judgement.

f) Breathlessness, dizziness – factors associated with compromised respiration (see Domain 3) contribute to these experiences. They are best assessed through in vivo observation and ante mortem blood gas analysis.

g) Malaise - malaise is defined as a general feeling of discomfort, illness, or lack of well-being. Quantifying this is challenging but physical changes observed in the live animal and at necropsy, plus ancillary testing (e.g. white blood cell counts, cortisol levels etc) are useful in establishing an inferred score.

h) Anger, rage, irritation – direct observational information would be of value however greater understanding of cetacean behaviour is required.

i) Hunger - this could be inferred from the body condition and components thereof.

j) Other cetacean-specific mental state – unknown factors but maybe relevant.

Cetacean Bycatch Injury Impact Score (CBIIS)

Each of the welfare assessment systems discussed have their attributes when it comes to trying to establish a similar system for bycaught cetaceans. To successfully develop a robust and repeatable system for these species that can be easily applied, there is a need to provide definitions for the severity/impact of any injury before a nominal 'score' can be attributed to an individual or suite of lesions. This project set out to utilise necropsy data, but it is recognised that behavioural aspects and the physiological responses are important in welfare assessment and as such a definitive 'welfare score' cannot be established without further research in these areas. This does not preclude the usefulness of a necropsy-based assessment but judgement of other factors such as pain and function (which are best assessed in the live animal) need to be considered. Inevitably there is a degree of subjectivity in any assessment, but the definitions allow judgement of a small set of important criteria which can be used to provide an overall impact assessment on the animal. The CBIIS system has been designed to use empirical necropsy data with supporting evidence from a limited number of ancillary tests. As further research is done in areas of physiology it may be possible to refine or provide additional information, for example cortisol level measurement, that could provide a more quantitative approach.

Four parameters are assessed as they represent important features associated with the impact of any injury sustained by an animal. These parameters are pain, loss of function/physical impairment, loss of sensory function, and systemic effects. The reason for utilising each of these is discussed below.

1. Pain – it is universally accepted that pain is an important factor in assessing welfare. Pain is defined as 'an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage' (Raja *et al.,* 2020). Pain is an important evolutionary adaption warning about dangers in the environment, wounds and disease. There are three categories of pain recognised;
    a. Nociceptive pain - caused by noxious stimuli which is a physiological protective system to detect and minimise contact with dangerous and noxious stimuli resulting in an immediate reaction via withdrawal reflex, intrinsic

unpleasantness of sensation and emotional anguish, and overrules most other neural functions.

b. Inflammatory pain - associated with tissue damage and immune cell infiltration. This assists in healing by discouraging physical contact and movement.
c. Maladaptive pain (pathological pain) - which is the result of abnormal functioning of the nervous system and maybe secondary to damage of the nervous system (neuropathic pain) or dysfunctional pain when no inflammation is present (Woolf 2010).

Pain initiates behavioural, physiological and psychological responses with adaptive learning leading to improved organism survival (Ding *et al.,* 2022). Bycatch events will result in variable degrees of pain whether they are PUE or chronic, in which the duration of the injuries add to the welfare implications (Moore and van der Hoop 2012).

Assessment of pain in any species including humans is very challenging with high degrees of subjectivity. To overcome this the use of objective markers such as blood pressure, heart rate variability, respiratory rate, electroencephalogram and nerve conduction velocity have been suggested, but nothing has been found satisfactory (Gigliuto *et al.,* 2014, Shekhar *et al.,* 2023). In animals, evaluating behavioural aspects such as vocalisation, withdrawal, lameness, posture etc. have been utilised but species differences as to how pain is manifest is problematic (Gigliuto *et al.,* 2014). No specific assessment criteria have been established for cetaceans to date, meaning evaluation of pain is difficult and non-standardised. Various adaptations to pain are seen in the animal kingdom, and specifically with cetaceans salinity is thought to stimulate increased pain sensation, with sodium chloride leading to water absorption at the site of wounds with increased swelling and compression on nociceptors. Ding *et al.,* (2022) suggest cetaceans may have undergone molecular evolution in pain and analgesic traits, making them more sensitive to pain in certain situations and conversely more effective in analgesia helping to relieve suffering.

Without further research it is not possible to provide definitive scores for pain assessment and it is important to be conscious not to assess pain based on an anthropomorphic approach. However because pain is fundamentally recognised as an important factor in suffering and welfare, it should be assessed and judged for each injury sustained in bycatch events. This can be undertaken at a relatively superficial level i.e. a net impression mark would only be considered as inducing minimal/mild pain whereas extensive, deep laceration of a flipper with involvement of muscle, tendon and bone would be considered severe. Pain is also not a constant as, for example, wound healing will change the level of sensory perception and therefore pain response. Currently no method to adjust a ‘pain’ score is proposed to take this into consideration.

2. Loss of function/physical impairment – injuries sustained in bycatch events, whether PUE or chronic entanglement, can result in loss of function/physical impairment. This depends upon the site, severity and duration of the insult. It should be possible to assess any injury at the time of necropsy relatively accurately and consider its impact on the animal. It is important to recognise that these injuries will also be involved in other areas of assessment including induction of pain, sensory loss and systemic effects. Examples of physical injuries include; severance or partial severance of a flipper or fluke which would directly affect the swimming ability and therefore feeding and social interaction with conspecifics; fracture of ribs directly compromising

respiratory function; and fracture of the mandible or maxilla in cases of PUE, which would lead to loss of feeding ability (despite the short period between entrapment and death it would be still regarded as a very significant injury as should the animal escape it would impact upon it survivability – this approach to assessment is similar to that used by NOAA and in terrestrial animal trapping evaluation). It may also be the result of direct physical impairment associated with fishery gear e.g. retention of fishery gear leading to increased drag or impairment of feeding if oral structures are involved.

3. Loss of sensory function – cetaceans evaluate their environment through a number of different sensory modalities, responding to both biotic and abiotic factors. As the sensory system is pivotal in initiating both behavioural and physiological change any impact on sensory function would compromise the animals' ability to adapt and survive, hence the importance of this parameter in assessing the impact of any injury. The major sensory modalities in cetaceans include; hearing, equilibrioception (balance), vision, somatosensory (touch, pain, temperature and body position), electroreception, magnetoreception and chemoreception (Kremers *et al.,* 2016). The importance of these varies and identifiable physical changes at necropsy is limited to hearing, equilibrioception and vision. Without behavioural observations and direct measurement of the sensory apparatus and subsequent physiological change, a comprehensive assessment of the loss of any sensory function is challenging in cetaceans. However injury to the aforementioned systems such as bruising of auditory mandibular fat or physical injuries to the eye can be discerned and judgement made as to the impact on the animal during bycatch events. It is important to recognise that different species and even individuals within species perceive their environment in different ways and as such it is not possible to be entirely accurate as to how other animals 'understand' their environment. This should not, however, preclude an assessment being made.

4. Systemic effects – injuries can have a more systemic impact affecting other body systems either through direct effects or via mediators (e.g. cytokines, hormones etc). An example of an immediate and serious systemic effect is severance of a major artery to a flipper resulting in hypovolaemic shock and death. Effects may be overt, for example loss of body condition due to oral injury and inability to feed, or more subtle secondary to chronic stress with increased cortisol and aldosterone levels (see 'The stress response and bycatch'). Long-term effects include decreased reproductive activity due to cachexia and debility. Infection at the site of wounds is common and may have a direct impact through loss of function but there may be evidence of dissemination of infection to other organ systems (affecting their function) with or without endotoxaemia. The release of inflammatory mediators from areas of inflammation/infection such as TNF can result in pyrexia and inappetence in other species and may be relevant to cetaceans. It is therefore important to consider all necropsy findings in a holistic manner when assessing the systemic effects of any injury.

As already mentioned it is important to recognise that these 4 criteria overlap but by using these it is possible to 'build a picture' of the overall impact of the injuries identified at necropsy to facilitate a better understanding of the welfare impact associated with bycatch. A method to present bycatch injury data is proposed that allows easy visualisation and data evaluation - the Cetacean Bycatch Injury Impact Score (CBIIS).

Cetacean Bycatch Injury Impact Score (CBIIS) evaluation and recording

Having established a suggested method for assessing bycatch injury impact the recording and ‘visualisation’ of the data is important to consider. The main requirements are ease of use, repeatability, applicability for data analysis, and that these are understandable to a wide audience. The following describes the suggested approach:

During or after the necropsy each lesion or lesion type is judged based on the four criteria previously discussed;

-Pain

-Impact on function

-Sensory disruption

-Effects on the whole body (systemic effects)

Only ante/pre-mortem lesions relevant to the bycatch event should be assessed and recorded as it is these insults/injuries sustained during the event that are important. Where multiple lesions of the same type are present these should be individually scored.

A 3 level ‘scoring’ system was opted for as it was felt to be easier to differentiate between the levels of impact, and as such was more repeatable between observers. A numerical system has not been used in support of the 5 domains model (Mellor and Beausoliel 2015) and to overcome an overly prescriptive approach allowing the assessor to make a better judgement of the lesions present.

The following ‘scores’ are suggested with relevant levels of assessed criteria and the corresponding grades used by Mellor and Beausoleil (2015) (in parentheses);

Mild – minimal to mild pain/discomfort, no loss of function/physical impairment (i.e. can swim normally), no sensory loss, no systemic effects (Grade A and B – no/low-level but tolerable negative affects).

Moderate – moderate pain, mild to moderate loss of function/physical impairment, variable sensory loss if system involved, systemic effects seen (Grade C and D – intermediate).

Severe - severe pain, marked loss of normal function/physical impairment, sensory loss if system involved, pronounced systemic effects (e.g. shock, hypovolaemia, effects on ‘energetics’ etc), death (Grade E – exceptionally unpleasant negative effects of high intensity).

To facilitate the recording of injury scores a list of the reported ante/pre-mortem lesions associated with bycatch have been compiled to make recording relatively straight forward (see Table 3 and 4 for PUE and chronic entanglement respectively). This also allows for future additions should novel lesions be identified - these can be assessed on the same criteria and given a ‘score’. This allows flexibility and capture of injuries that may arise due to future changes in fishery methods.

In cases other than PUE, evaluation of duration should be undertaken. Three broad categories are used- acute, subacute and chronic- corresponding to those used by Moore *et al.,* (2013). These are based upon standard pathological time periods but have been modified to aid those less familiar with the presentation of lesions to make a better judgement. These are based on macroscopic changes which should correlate to microscopic findings, however the latter can provide more accurate information. It should be borne in mind that if there is any doubt about the duration of an injury following macroscopic evaluation, the collection of appropriate samples for histological examination is advised to clarify the situation and improve confidence in duration scoring.

- Acute (A): Fresh, uninfected injuries which show no or early inflammation (minutes to hours).
- Subacute (SA): Wounds show early healing reaction including early granulation tissue where second intention healing is occurring (hours to days).
- Chronic (C): Advanced healing reaction with repair and remodelling, fibrosis/scar formation, secondary bacterial involvement or loss of body condition (weeks to years).

It is inevitable that some cases or lesions will present difficulties in assigning a score, whether that be the result of autolysis, unfamiliarity with the lesion nature/type, or the assessor's experience. Where this is the case the degree of uncertainty/confidence should be rated on a scale of 0 (Not at all confident) to 4 (Very confident) for each injury. Capturing assessor confidence helps identify injury types that are difficult to assess and highlights areas needing further research to improve the assessment tool.

Where novel lesions not previously reported are seen but thought to be relevant to the bycatch event, these can be added using the same principles as described above and recorded in the same way.

Having undertaken an assessment of the injuries and applied a score, the outcome is readily visualised using a colour coding system. The severity of each lesion type, i.e. mild, moderate and severe, is colour coded yellow, orange and red respectively. For each animal this provides a 'pattern' of lesion severity which is easy to relate to those unfamiliar with the impact of the pathology seen. Collation of a larger number of cases into a spreadsheet format subsequently allows for the identification of lesion patterns which may relate to fishery types and/or be species specific.

Illustrations

To illustrate how the CBIIS grading scheme would be presented, a worked-up assessment of a PUE and chronic entanglement case is provided in Appendices 1 and 2 respectively. In addition, a sample of 15 PUE bycatch cases were assessed and collated into a spreadsheet format to provide an illustration of how larger data sets could be compiled for analysis (Table 5).

Table 3. Reported PUE associated injuries recorded at necropsy

Organ system	PUE injury type/pathology	Reference(s)
Integument	Net impression marks	Ijsseldijk *et al.,* 2020, Puig-Lozano *et al.,* 2020, Bernaldo de Quiros *et al.,* 2018, Jepson *et al.,* 2013, Moore and Barco 2013, Read and Murry 2000, Kirkwood *et al.,* 1997, Kuiken 1994
	Net cuts/lacerations	Ijsseldijk *et al.,* 2020, Puig-Lozano *et al.,* 2020, Bernaldo de Quiros *et al.,* 2018, Jepson *et al.,* 2013, Moore and Barco 2013, Read and Murry 2000, Kuiken 1994
	Subcutaneous haemorrhage	Ijsseldijk *et al.,* 2020, Jepson *et al.,* 2013, Moore and Barco 2013, Read and Murry 2000, Kirkwood *et al.,* 1997, Kuiken 1994
Muscular system	Muscular haemorrhage	Ijsseldijk *et al.,* 2020, Puig-Lozano *et al.,* 2020, Jepson *et al.,* 2013, Kirkwood *et al.,* 1997
	Separation of rectus abdominus	Epple *et al.,* 2020
	Acute peritoneal hernia	Epple *et al.,* 2020
Skeletal system	Mandible/maxilla fracture with haemorrhage	Ijsseldijk *et al.,* 2020, Puig-Lozano *et al.,* 2020, Jepson *et al.,* 2013, Read and Murry 2000
	Rib fracture and haemorrhage	Jepson *et al.,* 2013, Read and Murry 2000
	Skull fracture with haemorrhage	Jepson *et al.,* 2013, Kirkwood *et al.,* 1997, Kuiken 1994
	Flipper bone fracture with haemorrhage	Read and Murry 2000
	Vertebrae fracture with haemorrhage	Read and Murry 2000
Alimentary system	Tooth fracture/loss	Puig-Lozano *et al.,* 2020, Moore and Barco 2013, Read and Murry 2000, Kuiken 1994
	Haemabdomen	Puig-Lozano *et al.,* 2020
Special senses	Ocular hyphema	Ijsseldijk *et al.,* 2020
Systemic	Hypoxia	Soulsbury *et al.,* 2008

Table 4. Reported chronic entanglement associated injuries recorded at necropsy

Organ system	**Chronic entanglement injury type/pathology**	**Reference(s)**
	Body condition (good/moderate/poor)	Puig-Lozano *et al.,* 2020, Moore *et al.,* 2013a, Moore *et al.,* 2013b, Cassoff *et al.,* 2011
Integument	Gear retention	Sharp *et al.,* 2019, Rolland and Graham 2019, Moore *et al.,* 2013a, Moore and Barco 2013,Cassoff *et al.,* 2011
	Rope/net impression marks	Moore *et al.,* 2013a, Moore and Barco 2013, Cassoff *et al.,* 2011, Moore *et al.,* 2006
	Abrasions	Sharp *et al.,* 2019, Moore *et al.,* 2013a, Moore and Barco 2013, Cassoff *et al.,* 2011, Moore *et al.,* 2004
	Lacerations/incisions (superficial, blubber, deep tissues)	Puig-Lozano *et al.,* 2020, Rolland and Graham 2019, Sharp *et al.,* 2019, Moore *et al.,* 2013a, Moore *et al.,* 2013b, Moore and Barco 2013, Cassoff *et al.,* 2011, Moore *et al.,* 2006, Moore *et al.,* 2004
	Contusions/subcutaneous haemorrhage/haematoma	Puig-Lozano *et al.,* 2020, Sharp *et al.,* 2019, Moore *et al.,* 2013a, Moore and Barco 2013, Cassoff *et al.,* 2011, Moore *et al.,* 2004
	Parasitism - external (cyamid)	Moore *et al.,* 2013a, Cassoff *et al.,* 2011
	Focal - local infection/inflammation, abscess	Moore *et al.,* 2013a, Cassoff *et al.,* 2011
Muscular system	Muscle injury - Degeneration/necrosis	Rolland and Graham 2019, Sharp *et al.,* 2019, Moore *et al.,* 2013a, Cassoff *et al.,* 2011
	Tendon/ligament injury - transection	Moore *et al.,* 2013a
	Focal - local infection/inflammation, abscess	Moore *et al.,* 2013a
Skeletal system	Fracture - site important	Moore *et al.,* 2013a
	Periostitis	Sharp *et al.,* 2019, Moore *et al.,* 2013a, Cassoff *et al.,* 2011, Moore *et al.,* 2004
	Focal - local infection/inflammation - osteomyelitis, septic arthritis	Sharp *et al.,* 2019, Moore *et al.,* 2013a, Cassoff *et al.,* 2011, Moore and others 2006 *et al.,*
	Arthritis - non-septic	
	Osteopenia	Sharp *et al.,* 2019, Moore *et al.,* 2013a, Cassoff *et al.,* 2011, Moore *et al.,* 2006
	Pseudoarthrosis	Moore *et al.,* 2013a

	Healing - including sequelae i.e. callus, mal-union, scoliosis, sequestrum etc	Sharp *et al.,* 2019, Moore *et al.,* 2013a, Moore *et al.,* 2004
	Flipper/fluke/fin atrophy, ischaemia and infection	Puig-Lozano *et al.,* 2020
	Amputation	Sharp *et al.,* 2019
Alimentary system	Baleen loss/mal-alignment	Sharp *et al.,* 2019, Moore *et al.,* 2013a, Moore *et al.,* 2004
	Ulcerative stomatitis - tooth/baleen fracture with soft tissue +/- bone involvement	Puig-Lozano *et al.,* 2020, Sharp *et al.,* 2019, Moore *et al.,* 2004
Systemic	Systemic inflammation/infection	Cassoff *et al.,* 2011
	Systemic agonal events	

Appendix 1

Example of CBIIS application for PUE

Juvenile male common dolphin reference EX-C01-17

Known bycatch - hauled aboard a fishing vessel in a bass net.

Key Necropsy findings;

Right mandible: 3mm wide fresh encircling notch in lip with associated haemorrhage; two near 1mm wide curvilinear faint marks running on to lip further caudally, the first 12mm caudal to notch, the second 6mm caudal to cranial mark; two further faint curvilinear marks of similar width on caudal right mandible running on to lip.

Left mandible: near 1mm wide linear fresh wound encircling lip near tip

Just under 1mm wide linear mark running over caudal maxilla, just cranial to cranial point of melon; on right side of maxilla this separated into 2-3 linear marks; on left side this formed a distinct fresh wound encircling lip. One 7cm long, 1mm wide black linear mark dorsal to right eye. One irregular, near 1mm wide mark running caudal and dorsal to left eye. One faint linear near 1mm wide mark running over dorsal mid thorax to a knot impression to right of dorsal midline; at knot, a second linear mark ran at an angle of approximately 160° cranioventrally towards right pectoral; a third, very short linear mark ran out from knot at an angle of approx. 45° caudal to second mark; 4.5 cm lateral to first knot, a second knot was just visible on second mark described above. Pattern of diamond shaped marks over right thorax and flanks (caused by cargo net). One 5mm wide suspect rope mark running dorsoventrally over mid left tail stock. One approx. 1.5 cm diameter fresh shallow wound with associated haemorrhage on left caudal tail stock just cranial to insertion of fluke.

Rostral maxilla: deep, irregular split in tip, running 2.5 cm caudally with significant associated haemorrhage. Comminuted fracture of maxillary bone, with two fragments on either side of midline exposing central cartilage.

Necropsy photographs illustrating some of the relevant lesions

Fig. 1. Net impression marks over thorax (image credit CMPT-CSIP)

Fig. 2. Net impression mark over maxilla/beak (image credit CMPT-CSIP)

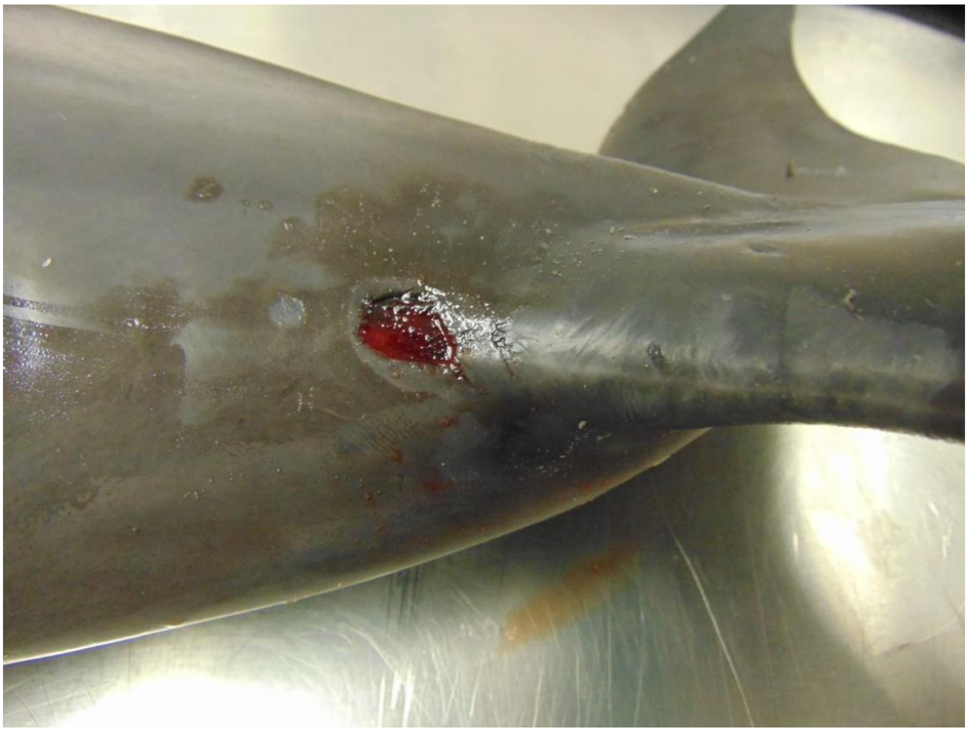


Fig. 3. Fresh shallow wound with associated haemorrhage on left caudal tail stock just cranial to insertion of fluke (image credit CMPT-CSIP)

Fig. 4. Rostral maxilla with deep, irregular split in tip, running 2.5 cm caudally with significant associated haemorrhage (image credit CMPT-CSIP)

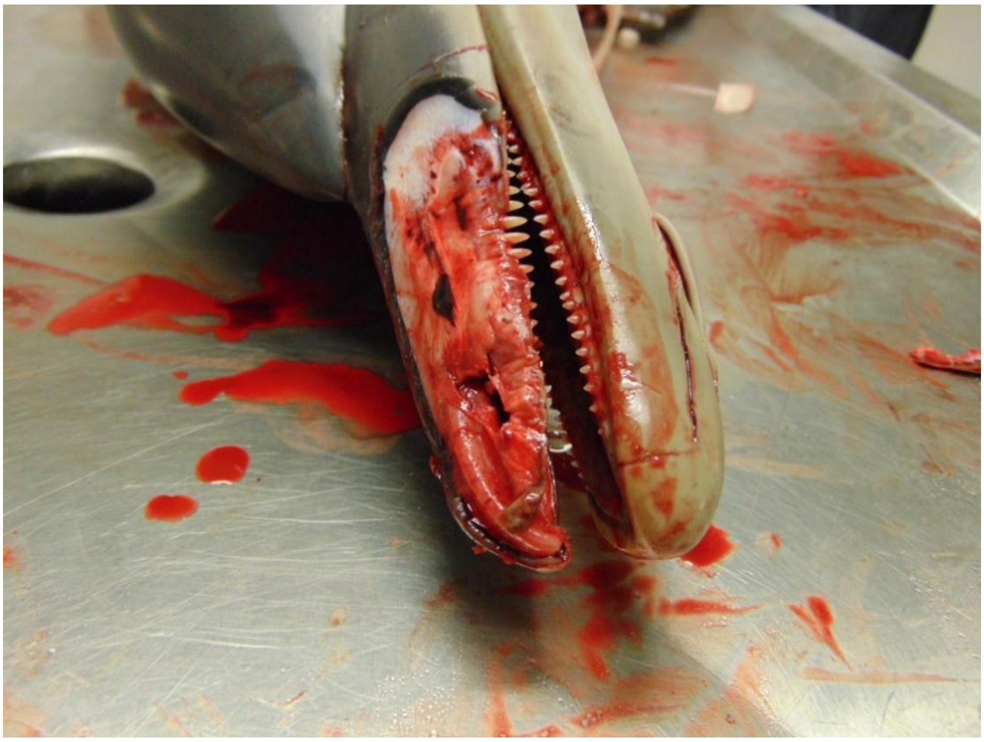


Fig. 5. Comminuted fracture of maxillary bone, with two fragments on either side of midline exposing central cartilage (image credit CMPT-CSIP)

Organ system	Documented ante mortem lesions in PUE	Case No. EX-C01-17
Integument	Net impression marks	🟨
	Subcutaneous haemorrhage	
	Net cuts/laceration	🟧
Muscular system	Muscular haemorrhage	
	Separation of rectus abdominus	
Skeletal system	Rib fracture and haemorrhage	
	Mandible/maxilla fracture with haemorrhage	🟥
	Flipper bone fracture with haemorrhage	
	Vertebrae fracture with haemorrhage	
	Skull fracture with haemorrhage	
Body cavities	Acute peritoneal hernia	
	Haemabdomen	
Cardiovascular system	Thoracic rete mirabile haemorrhage	
Respiratory system		
Alimentary system	Tooth fracture/loss with haemorrhage	
Urinary system		
Lymphatic system incl. spleen etc		
Endocrine		
Genital system		
Nervous system		
Special senses	Ocular hyphema	
Systemic	Hypoxia	🟥

Mild = yellow, Moderate = orange, Severe = red

Synopsis:

EX-C01-17 sustained numerous mild net impression marks over the carcass, moderate net cuts/lacerations involving the rostral maxilla and left caudal tail stock, and a severe injury with a comminuted fracture of the maxilla. Death was due to hypoxia.

Appendix 2

Example of CBIIS application for chronic entanglement

Juvenile male humpback whale (*Megaptera novaeangliae*), reference M245/19

Observed to have been chronically entangled for at least four weeks prior to having been found stranded.

Key Necropsy findings;

Poor body condition with little free lipid in the blubber layer. No evidence of recent feeding.

Focally extensive, encircling, deep ulceration around base of left pectoral fin with associated remodelling of the tissue margins and evidence for chronic bacterial infection in the skin, subcuticular tissues and draining lymph nodes. Rope remaining attached to the pectoral region was 9-12mm in diameter and had cut deep into the blubber layer, notably around the left scapulo-humoral joint. Mild cyamid burden associated with wound.

Rope of a different colour encircled the head and lower jaw restricting the animal’s ability to fully open its mouth. Accompanying deep erosion/ulceration of skin to deep dermis.

High parasite burden noted specifically a large burden of intestinal *Bolbosoma sp.* worms.

Large volumes of fluid in the lungs and airways consistent with sea water aspiration and drowning as the proximal cause of death.

A systemic, heavy, pure growth of *Streptococcus agalactiae* isolated from all organs subjected to culture.

Necropsy photographs illustrating some of the relevant lesions

Fig. 1. Encircling rope impression mark and erosion/ulceration of head and lower jaw (image credit SMASS)

Fig. 2. Encircling rope impression mark demonstrating erosion/ulceration of head and lower jaw (image credit SMASS)

Fig. 3. Focally extensive, encircling, deep ulceration around base of left pectoral fin with associated remodelling of the tissue margins (image credit SMASS)

Organ system	Documented chronic entanglement injury type/pathology	Case reference	Chronicity
		M245/19	A/SA/C
	Body condition (good/moderate/poor)	🟥	C
Integument	Gear retention	🟥	C
	Rope/net impression marks	🟧	C
	Abrasions		
	Lacerations/incisions (superficial, blubber, deep tissues)	🟥	C
	Contusions/subcutaneous haemorrhage/haematoma		
	Parasitism - external (cyamid)	🟨	C
	Focal - local infection/inflammation, abscess	🟧	C
Muscular system	Muscle injury - Degeneration/necrosis		
	Tendon/ligament injury - transection		
	Focal - local infection/inflammation, abscess		
Skeletal system	Fracture - site important		
	Periostitis		
	Focal - local infection/inflammation - osteomyelitis, septic arthritis		
	Arthritis - non-septic		
	Osteopenia		
	Pseudoarthrosis		
	Healing - including sequelae i.e. callus, mal-union, scoliosis, sequestrum etc		
	Flipper/fluke/fin atrophy, ischaemia and infection		
	Amputation		
Alimentary system	Baleen loss/mal-alignment		
	Ulcerative stomatitis - tooth/baleen fracture with soft tissue +/- bone involvement		
	Endoparasitism	🟨	C
Systemic	Systemic inflammation/infection	🟥	A
	Systemic agonal events	🟥	A

Mild = yellow, Moderate = orange, Severe = red

Chronicity; A = acute, SA = subacute, C = chronic

Synopsis:

M245/19 Entanglement resulted in restriction in left pectoral movement, chronic deep ulceration and secondary infection - these would result in pain, loss of function and impact on systemic factors such as swimming, feeding ability etc. Gear retention impacted notably on feeding ability contributing to poor body condition. Rope impression marks reflect gear retention and would result in mild/moderate pain. Ecto- and endo-parasitism were mild and likely associated with debility and presence of an ulcerated wound. Death was due to debility, aspiration of seawater and terminal septicaemia.

Example CBIIS Grading of PUE cases from Cornwall																
Organ system	**Documented ante mortem lesions in PUE**	**Cetacean reference**														
		CW-C16-22	**EX-C01-17**	**EX-C28-17**	**CW-C24-23**	**EX-C10-17**	**EX-C12-17**	**EX-C13-18**	**EX-C17-16**	**EX-C27-18**	**EX-C28-18**	**EX-C14-15**	**EX-C19-15**	**EX-C19-16**	**CW-C11-23**	**CW-C18-23**
		CD	**CD**	**CD**	**CD**	**CD**	**CD**	**CD**	**CD**	**CD**	**CD**	**HP**	**HP**	**HP**	**CD**	**CD**
Integument	Net impression marks	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
	Subcutaneous haemorrhage			🟨	🟨				🟨	🟨			🟨		🟨	
	Net cuts/laceration		🟧			🟨	🟨	🟨	🟧	🟨	🟨	🟨	🟧	🟨	🟧	🟨
Muscular system	Muscular haemorrhage	🟨				🟧			🟨				🟧			
	Separation of rectus abdominus															
Skeletal system	Rib fracture and haemorrhage															
	Mandible/maxilla fracture with haemorrhage		🟥						🟥							
	Flipper bone fracture with haemorrhage															
	Vertebrae fracture with haemorrhage															
	Skull fracture with haemorrhage															
Body cavities	Acute peritoneal hernia															
	Haemabdomen															
Cardiovascular system																
Respiratory system																
Alimentary system	Tooth fracture/loss with haemorrhage				🟧											
Urinary system																
Lymphatic system incl. spleen etc																
Endocrine																
Genital system																
Nervous system																
Special senses	Ocular hyphema	🟧		🟧	🟧	🟧		🟧		🟧		🟧	🟧	🟧		🟧
Systemic	Hypoxia	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	CD=common dolphin HP = harbour porpoise															

Scoring system re impacts of PUE bycatch injuries		
🟨 Mild – minimal to mild pain/discomfort, no loss of function/physical impairment (i.e. can swim normally), no sensory loss, no systemic effects	🟧 Moderate – moderate pain, mild/moderate loss of function/physical impairment, variable sensory loss if system involved, systemic effects seen	🟥 Severe - severe pain, marked loss of normal function/physical impairment, sensory loss if system involved, pronounced systemic effects (e.g. shock, hypovolaemia, effects on 'energetics' etc), death

Table 5. CBIIS collated data of 15 PUE bycatch cases

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Wound healing and assessment of duration in cetaceans

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Introduction

Wound healing in all mammalian species follows the same sequence of events irrespective of the species involved. However the timing of these events is highly variable both within and between species, and a number of extrinsic and intrinsic factors can impact upon the chronology.

A wound can be defined as damage to any part of the body due to the application of mechanical force (Ressel *et al.,* 2016). The energy of any impact on the body needs to exceed the resistance and compliance of the affected tissue to produce visible damage. Although sharp trauma (i.e. stab injury) is seen in cetacean species, blunt trauma resulting in abrasion, contusion and laceration is probably more commonly encountered.

Abrasion results in skin injury where the epidermis is removed/lost by friction or compression with minimal dermal involvement. With contusion there is no epidermal disruption but haemorrhage is seen beneath the skin. Laceration secondary to crush injury or stretching forces on the skin is characterised by tearing of the skin itself, but it is common to also find components of abrasion and contusion in such lesions.

Traumatic injuries in cetaceans can be the result of a number of different causes; abrasions on solid objects, conspecific/inter-species aggression, anthropogenic trauma (i.e. fishery gear entanglement or propeller strikes) and thermal burns secondary to exposure. In the context of bycatch those of importance are anthropogenic trauma (leading to abrasion, contusion and laceration) and less frequently thermal injury. A range of injuries have been encountered associated with bycatch, from impression marks with or without contusion to incised skin lesions through to extensive lacerations with variable involvement of deeper structures including muscle and bone (Moore and Barco 2013, Moore *et al.,* 2013). Injuries sustained have both a local and systemic impact on the welfare of the animal. The nature and duration of the insult, and site are key factors in the severity of the lesion and impact on the individual.

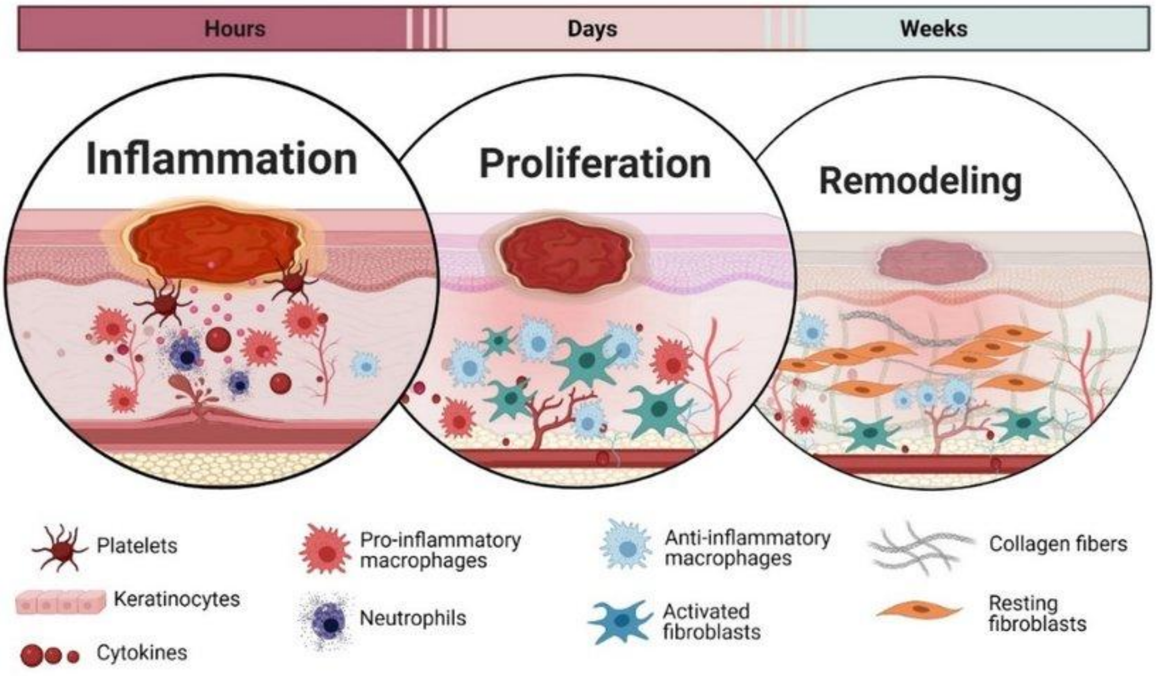
Assessment of duration of any injury is important to give some idea as to the chronicity as this is an important factor in evaluating the welfare associated with the lesion. However it is important to understand the sequence of events in wound healing before discussing the assessment of duration/age of lesion. The following information is derived from a series of review papers and experimental models and provide information on findings in terrestrial species. More specific findings relating to cetaceans are discussed later.

The healing process

In the absence of any surgical/medical intervention wounds will heal by second intention. This is a dynamic and complex process with 4 phases which often overlap (Gupta and Kumar 2015, Petkovic *et al.,* 2021[illustration]);

1. Coagulation and haemostasis-initial stages of wound healing are associated with the formation of a blood/fibrin clot which produces matrix for the next steps. Cytokines and growth factors released from the clot and damaged tissue stimulate the next phase of healing by inducing an inflammatory cell infiltrate.

2. Inflammation-within minutes to hours inflammatory cells (initially neutrophils followed by macrophages) infiltrate tissues adjacent to the wound to fight local infection and remove necrotic debris. Cytokine secretion by leucocytes controls and directs further healing processes. Of note however is that uncontrolled inflammation will destroy early migration of reparative cells and arrest healing.
3. Proliferation-there can be considerable overlap with the above phase. Proliferation of epidermal, dermal and deeper tissues is the predominant feature with re-epithelialisation, the production of a primary extracellular matrix and angiogenesis.
4. Wound remodelling leading to scar formation-during this stage tissue remodelling and differentiation are the principal processes with reorganisation of the collagen matrix and transformation of fibroblasts to myofibroblasts with subsequent wound contracture.



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Species differences

As previously mentioned there is inter-and intra-species variation in the healing process particularly in relation to the timing of the different phases. Braiman-Wiksman *et al.,* (2007) reviewed the wound healing process in mice experimentally. Chronologically the following phases were seen;

1. Day 1-3-blood clot, activation of the epidermal edges and early inflammation (neutrophils)
2. Day 4-7-scab formation, and migration of epidermal edges, early granulation tissue formation and inflammation (predominantly macrophages and lymphocytes)
3. Day 8-12-scab detachment, new epidermis (differentiated by day 12), dermal closure with granulation tissue and attenuation of inflammation

4. Day 12-30-advanced healing with matrix remodelling, terminal differentiation of epidermis, increased elastic fibre content and increased wound strength

Important features included;

1. Epidermal closure preceded all other parameters with keratinocyte migration as an early step across the defect with fully differentiated epidermis preceding dermal closure. Migration of keratinocytes promotes the other stages and is therefore important in initiation of healing. Any impact upon this would alter wound healing itself.
2. As previously mentioned inflammation is a response to fight infection and activates both dermal and epidermal processes through cytokines. If excessive it impairs healing with decreased migration of reparative cells and can arrest healing progression. Where chronic inflammation is present there is collapse of the extracellular matrix and the formation of necrotic foci within the wound.
3. Collagen formation is an important initiating step in wound healing with the deposition of premature non-structured collagen which shows poor histological staining. Fibroblast proliferation is an important early phase and occurs as inflammation is decreasing. The presence of mature granulation tissue indicates advanced healing.
4. Remodelling involves dermal reorganisation to increase strength and elasticity that occurs early after full re-epithelialisation.

Barington *et al.,* (2018) undertook experimental work to assess second intention healing in pigs and evaluated both gross and histological changes. This work was undertaken to assist in the forensic evaluation of skin wounds. They provided a decision tree for assessing age of wounds based on key features identified during the healing process. They determined a method to assess the thickness of granulation tissue macroscopically and used this to estimate the age of the wound. However a number of factors are known to interfere with granulation tissue formation (e.g. individual animal variation, age of animal, local infection and degree of tissue loss) which complicated interpretation. As such only broad ranges of healing duration could be made. Further research specifically to assess different components of granulation tissue has subsequently been published (Pankoke *et al.,* 2023). Utilising immunohistochemistry to evaluate neovascularisation and myofibroblast numbers, using CD31 and α-smooth muscle actin (α-SMA) respectively, assessment of blood vessel density in superficial and deep regions of the wound, and α-SMA staining provided some degree of temporal accuracy in their experimental animals, however this was not always replicated in the forensic cases they tested.

Barington *et al.,* (2018) made a number of observations of note including the application of special stains in wound assessment, key features of porcine wound healing and differences to other species including humans;

1. Perivascular neutrophilic infiltration is observed from 1 hour to day 18. This is not present in porcine wounds inflicted post-mortem or seconds before death.
2. Between 18-35 days haemosiderophages are present within the wound. In man these are first seen at day 3 and increase in numbers to day 8.
3. Although the ratio of neutrophils to macrophages can persist when ageing the lesion any wound contamination, infection or ongoing trauma influences this ratio.
4. Histologically endothelial cell proliferation, angiogenesis and fibroblast proliferation occur at 12 hours, 2 days and 3 days respectively.
5. Granulation tissue is present at 4 days histologically and grossly evident at 5 days. The depth of granulation tissue is influenced by infection, ongoing trauma and

sequestration of necrotic tissue. Granulation tissue was a maximum thickness at 10 days before regressing.

6. Using special stains (Masson's trichrome and Picrosirius red) new collagen was identified at day 4 with increasing collagen content over the 35 day experimental period.
7. Epidermal basal cell hyperplasia and migration started at 12 hours and complete epithelialisation was present between 18-35 days.

Ressel *et al.,* (2016) reviewed information from the literature as well as utilising their own experience in estimating repair of skin injuries/wounds in veterinary mammalian species. As previously indicated they state that there are great differences in wound healing times between different species. The following table is adapted from their review with additional personnel observations (Mark Wessels) and provides a working guideline for the histological assessment of open wounds in terrestrial mammalian species;

Time interval	Histologic changes
<30mins	Extravasated erythrocytes (haemorrhage)
30min-4hrs	Small number perivascular neutrophils present
8-12hrs	Increasing neutrophils with small number macrophages at wound periphery. Central necrosis
16-48hrs	Increasing macrophages at wound periphery. Fibrin - MSB yellow (<16hrs), red (>16hrs)
	Maximum number neutrophils at wound periphery at 24hrs
	Epidermal migration starts at periphery 24-48hrs
	Overt necrosis in central wound >32hrs
	Maximum number macrophages at periphery 48hrs
2-4 days	Fibroblast migration starts Haemosiderin identifiable with Perls Prussian Blue Neovascularisation starts 3-4ds
4-8 days Start of granulation tissue formation	New collagen deposition 4 days
	Pronounced neovascularisation 4-8 days. If bruising significant in injury abundant haemosiderin
	Maximum number lymphocytes at wound periphery 6 days
8-12 days	Decreasing leucocytes, fibroblasts and neovascularisation Increasing collagen size and quantity
>12 days	Regression of cellular activity in epidermis and dermis
	Diminished dermal vascularity
	Epidermal basement membrane identifiable with PAS
	Maximal fibroplasia day 14 followed by progressive maturation and contraction
	Early collagen birefringence day 14
	Overt collagen birefringence day 21

Table 1: Terrestrial mammalian wound healing chronology

No published information is available on injury to deeper structures including muscle and bone but Ressel *et al.,* (2016) have provided a general guide for the assessment of healing for each tissue;

Muscle injury (the normal course of healing is haemorrhage, regeneration and repair);

1. Macrophages appear within 12 hours.
2. Myotube formation and contact with adjacent viable segments occurs at 10-14 days.
3. Centralised nuclei are present in myofibres at 7-21 days.

Bone injury with fracture;

1. Unstable fracture with haematoma-0-24 hours
2. Unstable fracture with undifferentiated mesenchymal cells and neovascularisation-24-48 hours
3. Unstable fracture with early woven bone-36 hours
4. Stable fracture with primary callus cartilage-4-6 weeks
5. Stable fracture with woven bone progressing to lamellar bone-months to year

Cetacean wound healing assessment

One of the confounding factors associated with assessment of wound healing in cetaceans is the ability to closely observe affected individuals over time. Only a small number of papers have been written on experimentally induced wounds in cetaceans. From naturally occurring events it is reasonable to say that a similar process to that described for terrestrial mammals is seen in cetaceans (and other marine mammals). There are however a number of differences that impact upon wound healing in the species involved;

1. The aquatic/marine environment in which wounds are continually bathed in salt water is in contrast to that experienced by terrestrial mammals.
2. One key difference, associated with the environment, is the absence of a solid fibrin clot or scab. The wound is covered in degenerate cells/debris which acts as a protective barrier to further injury, and is the result of effects of seawater osmolarity.
3. Cetaceans have increased proliferative capacity of the epidermis as a result of the micro-anatomy whereby interdigitation between epidermis and dermis leads to significantly increased numbers of stratum basale/germinativum cells.
4. Deep wounds involving the blubber heal well. This is thought to be the result of the presence of antimicrobial compounds (organo-halogens and isovaleric acid), and stem cell activity within adipose tissue. Adipocytes are thought to dedifferentiate to pre-adipocytes and mesenchymal stem cells and also undergo trans-differentiation to myofibroblasts during the healing process (Zasloff 2011, Griffeth *et al.,* 2014).

Su *et al.,* (2022) utilised stranded Fraser's dolphins with cookie-cutter shark lesions to provide a framework assessing healing progression in cetacean skin. This, however, does not provide defined time lines for the different stages seen but it does allow classification of wounds. The authors quote broad time lines of granulation tissue being present within 'days' and the wound is closed within 2 months. 5 stages were seen;

Stage 1-new wound

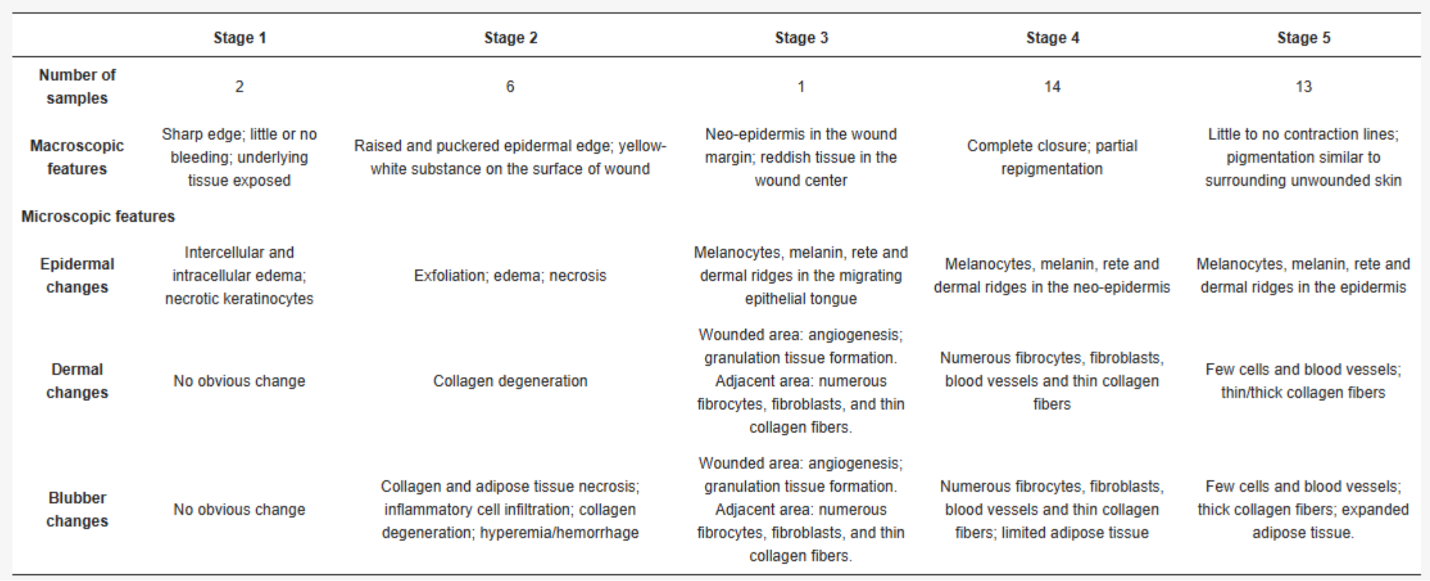
Stage 2-initial healing without granulation tissue

Stage 3-healing with granulation

Stage 4-healed with cellular and vascular blubber

Stage 5-healed without cellular and vascular blubber

The following table is reproduced from their paper and highlights the relevant macroscopic and microscopic features of each stage;



	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Number of samples	2	6	1	14	13
Macroscopic features	Sharp edge; little or no bleeding; underlying tissue exposed	Raised and puckered epidermal edge; yellow-white substance on the surface of wound	Neo-epidermis in the wound margin; reddish tissue in the wound center	Complete closure; partial repigmentation	Little to no contraction lines; pigmentation similar to surrounding unwounded skin
Microscopic features					
Epidermal changes	Intercellular and intracellular edema; necrotic keratinocytes	Exfoliation; edema; necrosis	Melanocytes, melanin, rete and dermal ridges in the migrating epithelial tongue	Melanocytes, melanin, rete and dermal ridges in the neo-epidermis	Melanocytes, melanin, rete and dermal ridges in the epidermis
Dermal changes	No obvious change	Collagen degeneration	Wounded area: angiogenesis; granulation tissue formation. Adjacent area: numerous fibrocytes, fibroblasts, and thin collagen fibers.	Numerous fibrocytes, fibroblasts, blood vessels and thin collagen fibers	Few cells and blood vessels; thin/thick collagen fibers
Blubber changes	No obvious change	Collagen and adipose tissue necrosis; inflammatory cell infiltration; collagen degeneration; hyperemia/hemorrhage	Wounded area: angiogenesis; granulation tissue formation. Adjacent area: numerous fibrocytes, fibroblasts, and thin collagen fibers.	Numerous fibrocytes, fibroblasts, blood vessels and thin collagen fibers; limited adipose tissue	Few cells and blood vessels; thick collagen fibers; expanded adipose tissue.

Table 2: Outline of the macroscopic and microscopic features of wounds of different healing stages in Fraser’s dolphins (Su *et al.,* 2022).

An additional feature of note to help stage assessment was the configuration of collagen fibres within the dermis. In stage 4 these fibres are parallel with numerous fibroblasts, fibrocytes and blood vessels. In stage 5 collagen bundles thickness increases and cell numbers decrease.

Few papers have been produced detailing the chronology of healing events in both the natural and experimental setting, and although there are many reports discussing wounds observed in cetaceans, they give little information on chronology (i.e. Lockyer and Morris 1987). Experimentally bottlenose dolphins show a similar sequence and timing of events to terrestrial mammals (Bruce-Allen and Geraci 1985) but beluga whales take five times longer, a difference attributed to the markedly thickened epidermis (Geraci and Bruce-Allen 1987). Experimental incisions were however relatively superficial unlike the more extensive lesions often seen in wild animals. One of the major challenges in assessing healing chronology in the natural setting is the need to have repeated and relatively frequent observations of individuals at suitably close proximity to allow accurate gross assessment of the wound to identify key stages in wound healing. The following table collates data from a number of sources where this has been done (plus data from the experimental work highlighted in blue). Fruet *et al.,* (2016) studied the healing process of dart biopsy lesions where tissue from both the epidermis and blubber layer were collected. These small wounds subsequently healed by granulation and do provide some information on the chronology. Zasloff (2011) provides detailed chronological data on 2 bottlenose dolphins following shark attacks, one of which received veterinary care. The different stages of wound healing correspond to those of Su *et al.,* (2022) and the observational data and interpretation regarding timing of wound healing has been judged from the descriptions of wounds in the different papers (11 naturally occurring events, the 2 experimental series are excluded) and correlated with the 5 stages. It should be stressed that this exercise is very approximate and open to interpretation but is currently the only information available.

Stage		**Fruet *et al*., 2016**	**Olaya-Ponzone *et al.*, 2020**				**Bloom and Jager 1994**		**Dwyer *et al*., 2014**	**Zasloff 2011**		**Elwen and Leeney 2010**
	Species	**BND**	**CD**	**CD**	**CD**	**CD**	**BND Infected**	**Non-infected**	**BND**	**BND**	**BND**	**HD**
Stage 1		Day 1	Day 1		Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
Stage 2							Day 3	Day 3		Day 2		
Stage 3				Day 1 +25		Day 9+35	Day 12	Day 18	Day 23	Day 5	Day 7	Day 19-23
Stage 4		Day 18-35	Day 60	Day 42	Day 53	Day 63	Day 65+	Day 65		Day 30	Day 28	Day 39
Stage 5			Day 69				Day 128	Day 128		Day 49		

BND = bottlenose dolphin   CD = common dolphin   HD = Heaviside's dolphin

Day 1 represents the first observation of the wound which may relate to different stages depending on when in the healing process the wound/animal was first observed.

The animal described by Bloom and Jager (1994) had both infected and non-infected wounds which have been separately recorded here.

From the above it is possible to give a range of times related to the different stages of the healing process;

Suggested duration in relation to stage based upon above data (excluding experimental data)

Phase	**Day**
Stage 1-new wound	Day 1
Stage 2-initial healing without granulation tissue	Days 2-3
Stage 3-healing with granulation	Days 5-35
Stage 4-healed with cellular and vascular blubber	Days 18-65
Stage 5-healed without cellular and vascular blubber	Days 49-128

Factors affecting wound healing

A number of factors influence the speed and ‘success’ of wound healing. Ideally the repair process should return the affected tissue to its previous state with no impairment of function. Any reduction or loss of function can compromise the long-term welfare of the individual. In man delayed wound healing and non-healing can result in chronic wounds which represent an intractable clinical problem and are frequent causes of morbidity and mortality (Gupta and Kumar 2015).

The primary structural factor in impaired healing is disorderly collagen formation. Both local and systemic factors can affect wound healing with some of these working synergistically. A number predisposing factors are unknown and the following have been adapted to reflect what may be seen in cetaceans;

Local factors

1. Oxygenation of the wound
2. Blood supply
3. Persistence of any foreign body
4. Infection-wounds continually exposed to water increase the chances of infection becoming established. Abscess formation and chronic wound infection is predisposed to in areas of high levels of waste water/sewerage discharge (Bloom and Jager 1994)
5. Ectoparasites - cyamid loads are often higher at wound sites which may influence wound healing (Moore *et al.,* 2013)
6. Environmental factors-water temperature is known to effect the speed of wound healing. Decreased blood supply to the skin associated with cold water is known to limit cell regeneration rates of the epidermis in seals (Feltz and Fay 1966)-this may also be true of cetaceans (Elwen and Leeney 2010).
7. Warmer water results in faster wound healing (Olaya-Ponzone 2020). Constant irrigation with salt water has been cited as an aid to wound healing (Corkeron *et al.,* 1987). Salinity itself is an important factor with higher salinity associated with more rapid healing (Hurst and Orbach 2022). It is also been suggested that in some wounds the constant movement of water over the wound site may slow healing by keeping the wound open through the physical removal of epidermis from the exposed, forward facing edge of the wound (Dwyer *et al.,* 2014).
8. Initial wound size
9. Movement of tissue at the site of the injury i.e. wounds overlying movable joints would be expected to heal slower
10. Site of the injury-Fruet *et al.,* (2016) found biopsy lesions on the dorsal fin slower to heal than those from the trunk of the animal

Systemic factors

1. Age-younger animals have a higher rate of tissue regeneration
2. Systemic disease processes (i.e. cardiovascular disease, hepatic and renal disease)
3. Nutrition - protein and vitamin/minerals are important in wound healing. If any animal is nutritionally compromised this could have an adverse effect on healing
4. Chronic stress-increased levels of corticosteroids will affect tissue regeneration and repair
5. Immunosuppression-any factors affecting the inflammatory response will influence the speed of healing and predispose to infection

Assessing duration/chronicity of wounds in cetaceans

To help quantify the significance of any wound a standardised assessment of the affected tissue is necessary. A number of factors should be considered when assessing the impact of any injury on an individual including;

1. The site of the wound
2. Tissues involved
3. Duration of the lesion
4. Functional impact of the injury
5. Systemic effects of the wound
6. Effects on conspecifics

This section deals with the assessment of wound duration and provides a gross and histological standard methodology for wound evaluation.

Gross examination

The following information should be recorded at the time of necropsy with photographs taken whenever possible;

1. Site of the wound
2. Length, greatest width and depth of the wound
3. Tissue types involved e.g. skin only, skin and blubber, muscle etc
4. Presence or absence of fresh blood/haemorrhage
5. Presence or absence of fresh exudate
6. Presence or absence of epidermal regeneration at the wound edges
7. Presence or absence of granulation tissue
8. Evidence of complete closure
9. Any loss of pigment
10. Evidence of wound contracture
11. Presence of any foreign body material
12. Presence of any infection

Following gross evaluation tentative staging of the wound based upon Su *et al.,* (2022) can be made.

Collection of wound material for histological evaluation

The extent of any wound will dictate sampling for histology. It is important that all relevant aspects of the wound are sampled appropriately. It is also important to recognise that the healing process is dynamic. To provide some degree of accurate assessment of duration it is the most chronic portion of the wound that needs to be collected and evaluated (i.e. areas of the wound that have been healing for longest).

Assessing wounds in cases of PUE to determine whether lesions occurred before death, at the time of death or after death can be challenging. The short period between entrapment and death means little tissue reaction will be present. Tissue disruption with epidermal loss, fissure formation and injury to the underlying dermis/subcutis may be present in ante- and post-mortem lesions. Haemorrhage is the most likely insult seen but Ressel *et al.,* (2016) suggest it is not always a reliable indicator of ante-mortem injury. The majority of lesions examined histologically show no evidence of haemorrhage or other confirmatory ante-mortem change (personnel observation, MW). Blubber has the ability to deform and absorb external pressure readily, and as such cutaneous injury overlying blubber is less likely to show significant haemorrhage. Areas of skin that show little or no ability to deform such as that overlying bone or dense connective tissue (fins and flukes) are more likely to show haemorrhage similar to that seen in terrestrial animals which frequently show variable haemorrhage (dependant on the nature of the insult) overlying the skull, bony protuberances and firmer subcutaneous tissues. It would therefore be sensible to take samples in suspected PUE from lesions overlying similar areas to improve the chances of detecting haemorrhage.

For narrow wounds it should be possible to collect a transverse section of the affected tissues consisting of both cut margins and the deep portion of the wound extending to subjacent normal tissue (see diagram below).

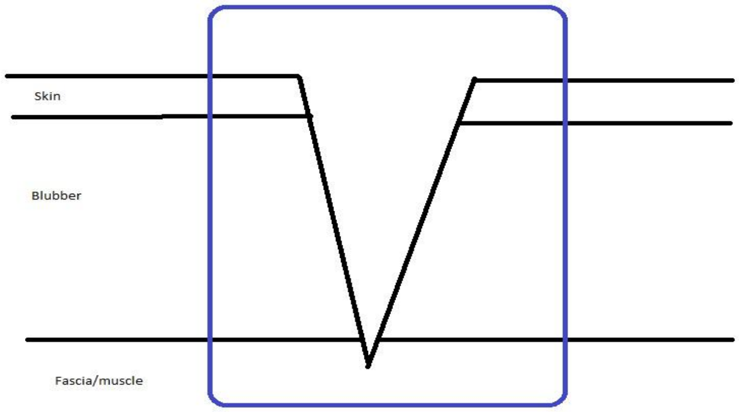


Fig 1. Sampling of narrow wounds

For wounds that are deep and wide it is unlikely the above areas could be captured in their entirety. As such the lateral edges of the wound including the epidermis and dermis, the blubber layer, and the deep portion of the wound including normal underlying tissue can be sampled separately (see diagram below).

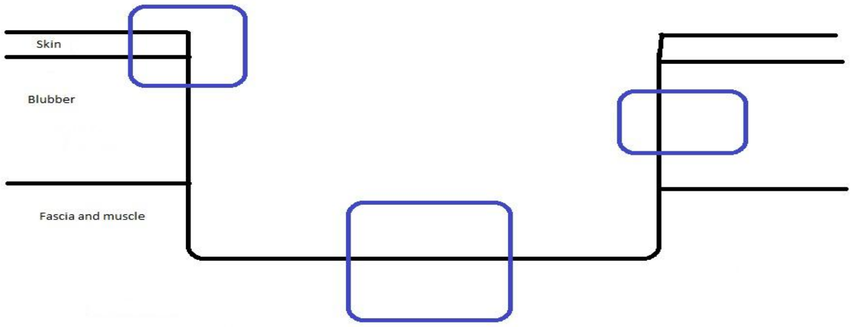


Fig 2. Sampling sites of a large wound

All tissues should be fixed in 10% neutral buffered formalin and clearly labelled with site of origin before submission to the processing laboratory. Tissue trimming should ensure that all relevant areas of the wound and adjacent unaffected tissue are taken for evaluation. Tissues can be processed routinely and the following stains used - haematoxylin and eosin, Masson's trichrome, Perl's Prussian blue, elastin stains and, if available, picrosirius red (the latter used in conjunction with polarised light).

The pathologist should assess each part of the wound including the epidermis, dermis, blubber, fascia and any deeper tissue paying particular attention to relevant features of healing such as re-epithelialisation and differentiation, inflammation, angiogenesis, fibroplasia, the extracellular matrix components (collagen and elastin), and any evidence of remodelling.

Areas identified of greatest chronicity should be evaluated to provide an approximate age of the lesion. These areas are likely to be at the interface between normal and abnormal (wound) tissue. Careful assessment particularly of the extracellular matrix collagen and any remodelling will help to establish chronicity. Using Masson's trichrome and picrosirius red and judging the overall elastin content are key in this process (Barington *et al.,* 2018). The wound should then be histologically staged as per Su *et al.,* (2022) and, where deeper tissues are involved, using suggested timelines from Ressel *et al.,* (2016). Once staging has been established wound duration can then be judged based upon collated data in the previous table ('Suggested wound duration in relation to stage').

It is important to recognise the limitations of any evaluation and judgement as a number of factors affecting wound healing will influence assessment of duration. As previously stated species differences in wound healing can be marked so only broad assessment of duration can be made until further information is made available on healing times and staging for each species.

Further areas of research

1. Obtain better, accurate information of wound healing duration and staging in different cetacean species through direct observation and pathological assessment;
2. Utilise specialised techniques to further assess different stages of wound healing e.g. antiloricin for epidermal differentiation, CD31 angiogenesis and PCNA for proliferation of epidermal and mesenchymal wound healing components. Utilising artificially intelligence to assess extracellular matrix components and cellularity;
3. Obtain a greater understanding of blubber adipose tissue with regards to healing including the antibacterial effects and stem cell activity/derivation;
4. Investigate growth factor and cytokine levels in different areas of the wounds on how these coordinate wound healing.

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ECS 2024 workshop program

"Use of pathology to better inform the welfare impact assessment of bycatch and entanglements"

8.30 am Start

8.30 Introduction – reasons behind the workshop and what we hope to achieve – Mark Wessels, MMPath Hunton, Bedale, North Yorkshire, UK

8.45 Applying welfare science to marine mammals- Rebecca Boys, Research Associate, Cetacean Ecology Research Group, School of Natural Sciences, Massey University, Auckland, NZ

9.30 PUE- The pathway to death – Mark Wessels, MMPath Hunton, Bedale, North Yorkshire, UK

10.30-11.00 Coffee/tea

11.00 Postmortem findings in harbour porpoises retrieved from gillnets - Lonneke Ijsseldijk, Division of Pathology, Faculty of Veterinary Medicine, Department of Biomolecular Health Sciences, Utrecht University, NL

11.30 Histopathology and immunohistochemistry approaches for a presumptive diagnostic of peracute underwater entrapment (PUE) in cetaceans - Eva Sierra, Departamento de Morfología, Anatomía y Anatomía Patológica Comparadas, Universidad de Las Palmas de Gran Canaria, Spain

12.00 Post-mortem evidence used to target mitigation measures: experience in the Mediterranean Sea within the LIFE DELFI project - Sandro Mazzariol and Guido Pietroluongo, Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Italy

Lunch 12.30-2 pm

2 pm Afternoon session

2.00 pm Welfare assessment in bycatch – Mark Wessels, MMPath Hunton, Bedale, North Yorkshire, UK

Introduction to PUE in the UK and PUE CBIIS Delphi assessment' - Helen Chadwick, Centre for Ecology and Conservation (CEC) University of Exeter, UK

Chronic entanglement of cetaceans in Scottish waters – recent examples and next steps – Ellie MacLennan College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

Interactive workshop session for participants

4-4.30 pm Coffee/tea

Wound healing and duration assessment in cetaceans – Mark Wessels, MMPath Hunton, Bedale, North Yorkshire, UK

Final discussion session – can the CBIIS tool be applied by welfare experts as part of their assessment?

List of participants

Anna Maria Roos

Anna Uzonyi

Cinzia Centelleghe

Sarah Wund

Linnea Cervin

Mariana Macieira

Luca Spadotto

Gail Leeming

Juliette Drevelle

Andréa Lobao

Eleonora Barbaccia

Agathe Serres

Etienne Levy

Andrea Fariñas-Bermejo

Beatriz Reis

Marie Petitguyot

David Jacinto

Shirin N Rahman

Vicky Ward

Mark Peter Simmonds

Matthew Perkins

Hélene Mattys

Miguel Grilo

Natalie Arrow

Rachel Lennon

Evangelia Layla Mpinou

Rob Deaville

James Barnett

Andrew Brownlow

Ellie MacLennen

Nick Davison

Mariel ten Doeschate

Helen Chadwick

Rebecca Boys

Eva Sierra

Sarah Dolman

Sandro Mazzariol

Lonneke Ijsseldijk

LaetitiaNunny

Emma Naeve-Webb

Mark Wessels

Alan Knight

Alicia Quirin

Karina Vishnyakova

Evridiki Kontemeniotou

Alba Haro

Paula Alonso

Graham Pierce

Marco Vecchiato