# Juvenile hawksbill turtles (*Eretmochelys imbricata*) fare well with good welfare: assessing welfare of captive-raised turtles to infer post-release survivability

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*- Alfred North Whitehead (mathematician and philosopher)*

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# ABSTRACT

Hawksbill turtles (*Eretmochelys imbricata*), like other sea turtle species, hold ecological, cultural, and financial value, but are threatened with extinction. Of all living sea turtle species, hawksbill turtles are one of the most at risk as they are targeted for their unique shell patterning. Conservation programs that aim to increase the number of hawksbill turtles in the population could help prevent their extinction. Some intervention strategies involve removal of early life-stage individuals from the wild for a period of rearing under human care. These individuals have the potential to contribute to hawksbill turtle populations by increasing the number of turtles surviving to later life-stages in the population, but only if their rearing is well managed. The success of these programs, whereby success is based on the turtles' potential for post-release survivability, hinges on the hawksbill turtles maintaining positive welfare and retaining key physiological responses and behavioural skills. To achieve this, husbandry and housing should be tailored to meet the species and size-class requirements whilst turtles are under human care and release protocols should be optimised. Currently, there is a paucity of data surrounding the effects of captive rearing on sea turtles and comparative data from free-living conspecifics.

Hawksbill turtles were collected as hatchlings from the North Queensland management unit (northern Great Barrier Reef) in March 2019. Eleven hatchlings were kept at the James Cook University Turtle Health Research facility until May 2022 and studied in this thesis. Using the Five Domains Model of welfare as a framework, the holistic fitness of the turtles was assessed relative to their readiness for release and post-release survivability. Firstly, a scoping review was conducted to determine the best measures of welfare in the conservation setting of rehabilitation centres. Assessment metrics and expected outcomes should be specific to the species and life-stage in question and also feasible to implement in a conservation setting. Furthermore, use of environmental enrichment devices should be encouraged to promote positive welfare of turtles whilst under human care; specifically, feeding, tactile, and structural devices. Secondly, a safe and effective method of attaching satellite trackers to small, juvenile hawksbill turtles was successfully optimised and trialled. The methodology resulted in good adhesion of the trackers for >3 months, without impeding turtle growth or causing deformation and damage to the scutes. Thirdly, the turtles were tested for their physiological response to stress, measured via concentration of corticosterone and lactate in the blood. Stressors were: 1) handling and sample collection; 2) an unfamiliar, acute (5 minutes) stressor; 3) an extended stressor, during which trial satellite trackers were attached; and 4) transportation to the release site. The upper basal limits (median 0.10 ng/ml

corticosterone and 0.30 mmol/L lactate) of captive-raised turtles were lower than reported in free-living turtles but turtles did show a significant response to, and subsequent recovery from, all stressors. The magnitude of the response, as well as time to peak and time to recovery, varied amongst stressors and between biomarkers, with corticosterone response and recovery generally quicker than lactate.

Fourthly, after more than 2 years under human care, the hawksbill turtles were released and observed in-water for up to 25 minutes at the point of release to record their behaviours. Turtles were released when new-recruit sized (357 – 444 mm curved carapace length), near to the holding facility (central Great Barrier Reef). The turtles were observed engaging in naturalistic behaviours that are commonly recorded in free-living turtles, including swimming, resting, surfacing to breathe, grooming, and investigating. The sixth key survival behaviour commonly recorded in free-living turtles that was not recorded during the release event was feeding. Although this behaviour was not observed in the captive-raised turtles during the release event, it was observed whilst under human care (grazing on algae on the tanks) and was inferred later from the satellite tracker transmissions (up to 15 months). The trackers were attached to the turtles using the protocol developed earlier in the thesis and transmitted data for a maximum of 422 days (median 182 days), a longer transmission time than previously recorded for tracked head-started hawksbill turtles. The turtles were tracked to investigate if and how captive-raised turtles that had never before entered the ocean would disperse from the release site. All of the turtles remained within the natural range of free-living hawksbill turtles. However, the turtles did not behave uniformly with some staying near to the release site, and some travelling north, or travelling south. Furthermore, there was a divide between those who migrated from reef to reef and those who remained close to the coast, with the longest transmissions coming from turtles whose final location was coastal.

Overall, following a Five Domains model framework, the hawksbill turtles were assessed and concluded to have good welfare and be ready for release into the wild. In comparison to free-living hawksbills, the study turtles were assessed as having naturalistic physiological responses and displaying behavioural skills key to survival indicating a good chance of post-release survival. Data collected following the release did not conclusively speak to the long-term survival of the turtles, but they did provide a snapshot indication that turtles were able to adapt to living in the wild at least in the short-term. Additionally, these data provided valuable new insight to inform future conservation efforts and improvements for future turtles under human care practices. The key improvement identified was integration of more environmental enrichment devices to encourage natural foraging. This thesis found that positive conservation and welfare outcomes can be achieved for captive-raised hawksbill turtles and a degree of post-release survivability maintained.

# PUBLICATIONS AND PRESENTATIONS

## Peer reviewed publications

### Published

**Diggins** RL, Grimm J, Mendez D, Jones K, Hamann M, Bell I, and Ariel E (2023). Confirmed feasibility of a satellite tracker attachment method on small juvenile hawksbill turtles *Eretmochelys imbricata*. Marine Ecology Progress Series, 704, 119-130.

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### Under review or in preparation

**Diggins** RL, Mendez D, Jones K, Erickson K, Bell I, and Ariel E (*under review*) Captive-raised juvenile turtles display naturalistic behaviours when first released into the ocean.

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## Conference presentations

### Oral presentations

**Diggins** RL, Grimm J, Mendez D, Jones K, Hamann M, Bell I, and Ariel E (2021, December 9). *One size does not fit all: Satellite tracker attachment methods for small juvenile hawksbills* [Oral Presentation]. College of Public Health, Medical and Veterinary Sciences Higher Degree by Research Student Conference 2021, Townsville, Australia.

**Diggins** RL, Rudd D, Munns S, Kophamel S, Jones K, Mendez D, and Ariel E (2021, September 6 – 8). *Measuring stress response of juvenile hawksbill turtles to satellite tracker attachment via blood*

*sampling* [Oral Presentation]. Cohort Doctoral Studies Program 10 Year Anniversary Conference "Health Research: Making Connections", Townsville, Australia.

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### Poster presentations

**Diggins** RL, Mendez D, Jones K, Erickson K, and Ariel E (2023, March 21-25). *Behaviour of juvenile hawksbill turtles in captivity and upon release into the ocean* [Poster presentation]. International Sea Turtle Symposium 41, Session: In-water Biology, Cartagena, Colombia.

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## "Three Minute Thesis" (3MT) entries

**Diggins** RL (2021, August 13). *Tracking turtle toddlers: Attaching satellite trackers to juvenile hawksbills* [Oral presentation]. College of Public Health, Medical and Veterinary Sciences Three Minute Thesis 2021, Townsville Australia. (https://vimeo.com/583412810).

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| BCI | Body condition index |
| CCL | Curved carapace length |
| CITES | Convention of International Trade in Endangered Species of Wild Fauna and Flora |
| CMS | Convention on Migratory Species |
| DES | Department of Environment and Science |
| EE | Environmental enrichment |
| EED | Environmental enrichment device |
| GBRMPA | Great Barrier Reef Marine Park Authority |
| GLMM | Generalised linear mixed model |
| IQR | Interquartile range |
| IOSEA | Indian Ocean and South-East Asia |
| IUCN | International Union for Conservation of Nature |
| JBR | John Brewer Reef |
| JCU | James Cook University |
| MoU | Memorandum of Understanding |
| MU | Management unit |
| nQld | North Queensland |
| PCV | Packed cell volume |
| RMU | Regional management unit |
| SCL | Straight carapace length |
| SPREP | Secretariat of the Pacific Regional Environment Programme |
| SSC | Species Survival Commission |
| TED | Turtle exclusion device |
| THR | Turtle Health Research |
| WWF | World Wide Fund for Nature |

# CHAPTER 1: GENERAL INTRODUCTION

## Sea turtle conservation

### Importance of sea turtles

Sea turtles are marine dwelling reptiles that are threatened with extinction despite having inhabited Earth's oceans for 120 million years (Bjorndal & Jackson, 2002). There are seven extant species of sea turtles from six genera, within two families. Hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*), green (*Chelonia mydas*), flatback (*Natator depressus*), olive ridley (*Lepidochelys olivacea*), and Kemp's ridley (*Lepidochelys kempii*) turtles belong to Cheloniidae, and leatherback turtles (*Dermochelys coriacea*) are the only species in Dermochelyidae. Owing to the length of their collective existence on Earth, sea turtles have become integral to the healthy functioning of their ecosystems, with each stage of their complex life history intrinsically linked and adding value to their environment and all organisms within it (Bjorndal & Jackson, 2002).

All sea turtle species follow similar life cycles, wherein turtle hatchlings emerge from nests on the beach before entering the ocean. There are some species-specific variations in the developmental phase: flatback turtles remain in the neritic zone for all their developmental stages; loggerhead, green, hawksbill and Kemp's ridley turtles have an oceanic stage for several years after which they return to the neritic zone (shallow coastal) as new recruits to feed and grow; and leatherback and olive ridley turtles are believed to complete all developmental stages entirely in the oceanic zone (Bolten, 2003). Once they reach sexual maturity (which can take decades), sea turtles periodically migrate back to their natal region for reproduction and females come ashore to lay their eggs (Zbinden et al., 2007). Although their distribution varies by species, sea turtles collectively inhabit much of the world's oceans, linking ecosystems and peoples across the globe.

### Ecological importance

One ecologically important aspect of sea turtle life history is the transfer of nutrients to nutrient-poor coastal regions when adult females undertake nesting migrations (Diane et al., 2017; Lovich et al., 2018). After a clutch of hatchlings has emerged, any unhatched eggs and remnant shell fragments in the nest add nutrients into the beach and help prevent coastal erosion by sustaining native vegetation that stabilises the sand dunes (Bouchard & Bjorndal, 2000; Vander Zanden et al., 2012). Turtle eggs and emerged hatchlings are an important food source for many species of birds,

fishes, reptiles, mammals, and crustaceans. Larger turtles are prey to apex predators such as crocodiles and sharks (Lovich et al., 2018). Some sea turtle species graze on algae and seagrass, assisting with algal control and maintenance of seagrass beds (Scott et al., 2020) which are vital to the healthy functioning of coral reef ecosystems (Lovich et al., 2018). Except for their reproductive migrations, turtles spend most of their adult life foraging in one location. This high site fidelity, coupled with their long lifespan, makes sea turtles great environmental health indicators for their habitat (Aguirre & Lutz, 2004).

## Societal significance and economic implications

By the time *Homo sapiens* emerged, sea turtles were already well established (Pereira et al., 2022). Therefore, it is not surprising that turtles are central to so many folktales, mythologies, and cultures, often representing the creation of the Earth (Godley et al., 2020). Turtles have been deeply embedded in the culture and traditions of many groups and societies worldwide since their origin, with traditional use of turtles including consumption for ceremonial and ritualistic occasions such as weddings (Kitolelei et al., 2022). These traditions have been maintained in many indigenous cultures, including among First Nations peoples of Australia and numerous island communities throughout Oceania (Rudrud, 2010). Turtles are economically important to some of these cultural groups who rely on turtle meat and eggs not only as a source of protein but also as a high-value trade item (Mejías-Balsalobre et al., 2021). For example, in remote parts of Papua New Guinea, there are communities that trade turtles for rice and other items that they are unable to produce themselves (RD personal observation).

Sea turtles continue to hold their value in modern day society. As iconic and charismatic megafauna, turtles are universally known and admired, and have even been characterised in several forms of popular culture. This popularity makes turtles an excellent marketing tool for tourism hotspots that overlap with sea turtle nesting and foraging grounds and even as the basis of entirely turtle-based tourism ventures (Frazier, 2005; Wilson & Tisdell, 2003). The International Ecotourism Society (2015) defined eco-tourism as: "responsible travel to natural areas that conserves the environment, sustains the well-being of the local people, and involves interpretation and education". This definition highlights the interrelatedness of the cultural and financial value of nature, in this case sea turtles (Senko et al., 2011). Yet, despite the great importance of sea turtles, they are subjected to numerous threats, including anthropogenic, which put them at risk of extinction.

## Conservation status and threats

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species classifies the global extinction risk status of animal, plant, and fungus species and also provides information pertinent to the implementation of conservation actions for those species. Species classified as "Vulnerable", "Endangered" or "Critically Endangered" are deemed "threatened", meaning that they are considered to be at high risk of extinction in the wild without intervention (IUCN, 2023). Six of the seven species of sea turtles are currently considered threatened globally (IUCN, 2023), and the seventh species, flatback turtles, are classified Data Deficient and thus their extinction risk is undetermined (Red List Standards & Petitions Subcommittee, 1996). Loggerhead, olive ridley, and leatherback turtles are classified Vulnerable (Abreu-Grobois & Plotkin, 2008; Casale & Tucker, 2017; Wallace et al., 2013); green turtles are Endangered (Seminoff, 2004); and hawksbill and Kemp's ridley turtles are Critically Endangered (Mortimer & Donnelly, 2008; Wibbels & Bevan, 2019). Given the ecological, economic, and social importance of sea turtles outlined above, it is imperative to conserve their populations.

In general, the aim of conservation is to support and stabilise turtle populations by optimising the number of individuals that reach maturity and reproduce, which can be achieved by supplementing recruitment and/or improving survivorship (Rees et al., 2016). However, the global population of each species comprises multiple, evolutionarily distinct, regional populations (Wallace et al., 2010). Each population's specific biology and ecology influence its ability to recover from individual losses, and therefore also determine the risk of extinction of that specific population (Bolten et al., 2011; Rees et al., 2016). As such, extinction risk can be either species specific or population specific. For example, although hawksbill turtles are classified Critically Endangered globally, in Australia they are considered Vulnerable, and based on state legislation, the North Queensland population is Endangered (Department of Environment and Science, 2021). Therefore, it is necessary to define the target population before considering management strategies. Populations can be determined at nesting level (management units; MU) or regionally (regional management units; RMU) to include the wider geographic dispersal of the turtles (Moritz, 1994; Wallace et al., 2010). The regional approach is useful for threat analyses since the likelihood and impact of each threat can vary spatially (Wallace et al., 2011). The IUCN Species Survival Commission (SSC) Marine Turtle Specialist Group identified the five major threats to global sea turtle populations as direct take, fisheries bycatch, coastal development, pathogens and pollution, and climate change (Fuentes et al., 2023; State of the World's Sea Turtles). However, when assessing threats, it is necessary to understand the disparity between species and within species (regional populations), and how

turtle susceptibility to each threat differs between life-stages (Klein et al., 2017). These specificities are important when considering actions to mitigate threat impacts.

## Direct take

Turtles and turtle eggs are harvested for consumption and traded both legally and illegally in parts of Asia, Africa, and the Americas (Humber et al., 2014; Lagueux & Campbell, 2005). Some uses of the turtles include food, medicine, part of celebratory or religious ceremony, leather, oil, and jewellery (Rice & Moore, 2008). In Australian waters, the *Native Title Act, 1994*, and *Torres Strait Treaty, 1985*, permit self-regulated harvesting by Australian Aboriginal and Torres Strait Islanders and Papua New Guineans from villages specified in the Torres Strait Protected Zone (*Torres Strait Fisheries Act, 1984*). Whilst there remains a lack of reporting on harvesting, many Australian First Nations peoples are actively working to conserve sea turtle species (Department of Environment and Science, 2021). Sea turtles of all species and size-class are caught and traded; however, turtles are most susceptible to collection during nesting events, when laying eggs or resting in nearby coastal waters for breeding purposes or between nest laying (Marco et al., 2012). Turtle-centred tourism has been introduced and tested as an alternative income in several communities with traditional (extractive) turtle use (Pegas et al., 2013; Sardeshpande & MacMillan, 2019). Such eco-tourism practices have resulted in reduced harvest of sea turtles and their eggs and have sustained financial revenue for local communities but only in cases where local stakeholder groups have been centrally involved with the tourism and management plan development (Abd Mutalib et al., 2013; Campbell et al., 2007; Marcovaldi & Dei Marcovaldi, 1999; Mendes et al., 2019; Pegas et al., 2013; Sardeshpande & MacMillan, 2019; Stewart et al., 2016).

Although some legal consumption and trade of sea turtles occurs in various regions, it happens on a relatively small scale compared with the illegal international trade. Trade of all sea turtle species, in entirety or part, is prohibited throughout most of the world under the *Convention of International Trade in Endangered Species of Wild Fauna and Flora* (CITES). However, illegal trafficking is lucrative and persists despite legislation and conservation efforts to stop it (Easter et al., 2023; Mancini et al., 2011). Each year, tens of thousands of sea turtles are removed from the global population for illegal trade, with green and hawksbill turtles particularly targeted (Senko et al., 2022). An assessment of exploitation impact on RMUs has highlighted the seriousness of direct take on hawksbill populations in the Western Pacific, and it has been calculated that millions of hawksbill turtles have been killed over the past century for their shells (Senko et al., 2022). Several studies have also highlighted the significance of the North Queensland hawksbill stock and of Australian waters for supporting multiple populations of hawksbill turtles in the Western Pacific

region (Barr et al., 2021; Bell et al., 2020; Hamilton et al., 2021; Jim et al., 2022; Madden Hof et al., 2023b).

## Fisheries bycatch

Fisheries bycatch encompasses any non-target species that is caught from any type of fishing and is a major driver in the loss of marine biodiversity, globally (Lewison et al., 2014). Sea turtles are particularly susceptible to being caught in the fishing gear (e.g., long-lines, gill nets, and trawl nets) and often die from perforation or drowning (Work & Balazs, 2010). Mass mortality events, associated with net entanglement, are documented but not well represented in scientific literature (Duncan et al., 2017; Guimaraes et al., 2018). Pelagic, demersal, and coastal fisheries, from small to large scale, all contribute to the decline of sea turtle populations, catching juveniles and adults (Casale & Heppell, 2016). Madden Hof et al. (2023a) recently determined that gillnet and ringnet fisheries are highly impacting North Queensland hawksbill turtles and that underreporting of bycatch is obscuring the full effect of fisheries in the Western Pacific. Ghost nets in the Arafura Sea have been found to greatly impact post-hatchling hawksbill turtles (Hamann et al., 2021). Strategies to mitigate bycatch include modification of gear and the use of bycatch reduction devices known as turtle exclusion devices (TEDs). These devices have been implemented in several fisheries regionally, but on a global scale, there is still more to be done to mitigate this driving factor of sea turtle decline (Casale & Heppell, 2016; Poirier et al., 2018; Segniagbeto et al., 2017). Additionally, implementation of spatial or temporal closures of fisheries based on areas/periods of significance to sea turtles can help to reduce sea turtle bycatch (Madden Hof et al., 2023a). Recently, the Australian Government made $20 million available for commercial operators in the South East Trawl Fishery to surrender their fishing permits to relieve pressure on the targeted fin fish (Department of Agriculture Fisheries and Forestry, 2023), which is likely to also help reduce bycatch of marine creatures, including sea turtles.

## Coastal development

Increasing global human populations, and the associated expansion of urban areas and coastal industries such as tourism and port operations, apply enormous pressure on coastal environments (Biddiscombe et al., 2020). Furthermore, associated land use changes often negatively impact the availability and quality of habitat required for healthy turtle populations (Fuentes et al., 2020). This degradation can vary in severity (Pike, 2008; Venizelos, 1991) and have long-term effects on sea turtle populations. Disruption to sea turtle populations is particularly problematic where developments occur in prime nesting beach locations or foraging grounds for juvenile turtles (Hill et al., 2019; Taylor & Cozens, 2010). Beachfront developments at known nesting sites have been

shown to alter nesting behaviours of female sea turtles, reduce the number of successful nests, and disorientate hatchlings thereby delaying or preventing them from reaching the shoreline (Taylor & Cozens, 2010), resulting in fewer hatchlings entering the population. These disturbances are primarily caused by encroachment of available nesting areas, compaction of sand by construction vehicles, light and noise pollution from restaurants and hotels, damage to nests by beach furniture, and disturbance to nesting females by human presence (Hernández et al., 2007; Kaska et al., 2010; Maldonado, 2014; Oliver de la Esperanza et al., 2017; Roe et al., 2013; Taylor & Cozens, 2010). Donlan et al. (2010) used expert opinion to rank threats to turtles by species and geographical region and identified coastal development as being one of the most impactful threats to hawksbill turtles. Some mitigation efforts have been made with the development of 'turtle friendly lighting' and social campaigns to reduce light pollution and encourage beach users to modify their behaviours in key nesting areas/periods with varying degrees of success (Hettiger, 2021; Long et al., 2022; Mascovich et al., 2023). In contrast, there are some tourism operations that have modified their facilities and regulate the activities of their guests to minimise disturbance to the turtles (e.g. Mon Repos and Heron Island in Queensland, Australia). These turtle and wildlife-based tourism centres can have additional positive output for turtle conservation through raising funding and awareness as well as providing opportunities for valuable research to be collected (Read et al., 2019).

## Pollution and pathogens

Increased population and development of infrastructure can result in nutrient loading, for example from excess sewage (Camacho-Cruz et al., 2019; Sánchez et al., 2013). Changes to the nutrient composition in the marine environment can inhibit the growth of seagrass, thereby reducing food availability for some sea turtle species. Conversely, nutrient changes can promote growth, resulting in seagrass outcompeting corals, creating an imbalance in the ecosystem (Burkholder et al., 2007). Pathogens and heavy metals can also enter the marine environment from a number of sources including hospital effluent, excess sewage, and industrial and agricultural run-off (Ahammad et al., 2021). Reduced water quality and presence of chemical pollutants can cause sickness in the turtles and increase their susceptibility to diseases, including fibropapillomatosis (Jones et al., 2016; Jones et al., 2022). In addition, poorly managed rubbish, including discarded fishing gear, that is inadequately disposed of can enter the ocean as marine debris (Retama et al., 2016; Wilson & Verlis, 2017). Marine debris has the potential to entangle sea turtles, impeding their ability to swim and, in some cases, may cause drowning (Gall & Thompson, 2015). Furthermore, ingestion of marine debris by turtles may lead to gut impactions and/or leeching of chemicals into the bloodstream. This may result in buoyancy disorders, starvation and/or

poisoning (Clukey et al., 2018; Nicolau et al., 2016). Entanglement and ingestion can affect sea turtles of any size (Schuyler et al., 2016) and marine debris can also act as a physical barrier for hatchlings on the beach preventing them from reaching the ocean and entering the population (Katsanevakis, 2011).

### Climate change

Climate change impacts the nesting beaches in various ways, resulting in altered suitability of the beach and the sediment from which it is formed, and subsequently reduced output with fewer hatchlings entering the population (Fuentes et al., 2019; Fuentes et al., 2010; São Miguel et al., 2022). Firstly, there is growing concern over rising temperatures causing feminisation of nests, which occurs due to the temperature-dependent sex determination of sea turtles (Jensen et al., 2018; Tanner et al., 2019). This imbalance in the female-male ratio then compromises the reproductive capability of the population as these hatchlings sexually mature (Patrício et al., 2021; Wibbels et al., 2003). Furthermore, if nest temperatures exceed approximately 34°C, there is likely to be high or complete mortality of the nest (Bladow & Milton, 2019). Secondly, increased storm intensity can result in large areas of beach, as well as the nests themselves, being washed away, resulting in fewer nests being successfully laid and fewer sea turtles successfully hatching (Mishra et al., 2023). It is also possible that increased sea level rise and storm energy could wash away low-lying cays that are important turtle nesting grounds (Fellowes et al., 2024). Such storms can also result in loss of seagrass and coral coverage from sedimentation and wave action, reducing food availability for foraging turtles (Correia & Smee, 2022; Edmunds & Gray, 2014). Thirdly, nest success is also reduced by increased precipitation and rising sea levels (up to 21 cm by 2030), which can inundate the nests, drowning the eggs (Martins et al., 2022; Von Holle et al., 2019). Whilst the unhatched sea turtles can survive some level of inundation, mortality is dependent on time submerged and developmental stage of the turtle embryos (Limpus et al., 2020). Coastal systems are most resilient to climate change when they have high biodiversity and minimal modification and development involving hard structures that hinder supply of sediment (Kennedy, 2024).

## Barriers to effective conservation

Unfortunately, there are many barriers to conservation of long-lived, migratory, marine megafauna such as sea turtles (Lascelles et al., 2014). These barriers can be ecological, economic, and logistical or social in nature and can hinder the feasibility, or likelihood of success, of conservation initiatives (Klein et al., 2017; Rees et al., 2016).

## Ecological

Sea turtles are long-lived and consequently have a delayed time (>10 years) to maturity (Department of Environment and Science, 2021). Their population replacement and stability rely on the laying of many eggs (50 – 120 per clutch) as most will not make it to adulthood (Bell et al., 2020). The combination of large clutch sizes and delayed time to maturity means any decrease in population at this stage is unlikely to be detected for years or even decades. Delayed detection of the population trend then hinders evaluation of conservation effectiveness in relation to how well the outcome fulfills the overall aim of increasing and stabilising the population. There are also differences in sea turtle biology and ecology between species and between populations across ontogenetic stages. Some examples include: size at maturity; time to maturity; somatic growth rate; number of eggs per clutch; number of clutches per season; inter-nesting period; pivotal temperatures for sex-determination; size at each life-stage; location of foraging grounds; diet; and migration behaviours (Avens et al., 2021; Crouse et al., 1987; Hamann et al., 2021). When planning conservation actions for sea turtles, it is important to understand the biology and ecology of the target species, population (RMU), and life-stage that will be affected. However, much of this information is unknown at the level required to make informed decisions on conservation actions (Wildermann et al., 2018).

## Economic and logistic

Any long-term management plan needs to consider the scale at which it can operate and availability of resources (funds, infrastructure, and skilled staff) (Klein et al., 2017). Effective management of specific, identified key areas (e.g., nesting beaches, inter-nesting habitats, and foraging grounds) can help conserve individual populations, but the size and often remoteness of these important habitats make them difficult to manage (Kot et al., 2022; Ricardo et al., 2018). Furthermore, some turtle species travel great distances (transoceanic) during their early developmental stage and may recruit to foraging grounds distant from their natal region (Luschi et al., 2003). Once mature, those turtles will migrate back to their natal region for reproduction, and this breeding migration can span thousands of kilometres between foraging and nesting grounds. However, it is rarely possible to also protect these migration routes, which may align with or cross shipping lanes (Iverson et al., 2020). Additionally, sea turtles frequently migrate across jurisdictional boundaries (Blumenthal et al., 2006; Tanabe et al., 2023). Where the nesting and foraging habitats of a population are situated in different countries, protecting only one of these important habitats may not be sufficient to effectively conserve the population (Tanabe et al., 2023). Therefore, a multi-jurisdictional management plan is needed. Such plans are difficult to implement because of

differences in conservation commitments, legal frameworks, compliance monitoring ability, and financial means (Dutton & Squires, 2011; Lascelles et al., 2014). There are, however, groups comprising multiple member states that are working to develop joint regional plans for sea turtle management. For example, the Secretariat of the Pacific Regional Environment Programme (SPREP) has developed a *Regional Marine Turtle Action Plan, 2021 – 2025.* Additionally, the *Memorandum of Understanding on the Conservation and Management of Marine Turtles and their Habitats of the Indian Ocean and South-East Asia (IOSEA Marine Turtle MOU)* is a framework under the *Convention on Migratory Species (CMS)* in which 35 Signatory States agreed to conserve and support region turtle populations. From these collaborations, and under the CMS, species-specific assessments have been produced to aid in the development of a Single Species Action Plan (Hamann et al., 2021). In practice, the need for multi-jurisdictional agreements for management, combined with the lack of physical borders or fences in the marine environment, continue to make sea turtle conservation challenging.

## Social

Conservation initiatives can be community-led, private sector or government based, and are most effective when based on solid social and scientific research (De la Cruz-González et al., 2018; Fernández-Llamazares et al., 2021). Governmental policy implementation is often driven by social demand for change, and at a local level, compliance often depends on community engagement (Barrios-Garrido et al., 2019). Therefore, a full understanding of the social and cultural context as well as the community expectations and support for sea turtle conservation is pivotal to the success of any conservation action (Barrios-Garrido et al., 2019). Involvement of stakeholders in the development, implementation, and operation of a sea turtle management strategy can also vastly improve the likelihood of a successful intervention (Lewison et al., 2015; Risien & Tilt, 2008; Senko et al., 2011). For example, involvement of fishers and other stakeholders in Brazil (Da Silva et al., 2010) and America (McClellan et al., 2011) were noted as key to reducing turtle bycatch in fisheries. As such, a greater social effort, including relationship building, respect, and decolonisation of conservation and research, is needed to improve wildlife conservation outcomes generally, and for sea turtles specifically (Barrios-Garrido et al., 2019; Vierros et al., 2020). Social considerations in sea turtle conservation and management need not necessarily conflict with ecological conservation goals. Use of an integrated socio-ecological systems framework approach to conservation planning could help with identifying management strategies with the best potential outcome for the ecosystem with ecological and social benefits (Ban et al., 2013).

## Conservation actions and controversies

There is some evidence to indicate that increasing the survivorship of large juveniles, sub-adults, and adults is a key driver for sustaining populations (Donlan et al., 2010), and therefore conservation actions should be focused on these life-stages (Crouse et al., 1987). However, the ecological, economic/logistical, and social difficulties associated with addressing the threats that most target these life-stages, make such strategies less feasible. Many of the previously mentioned threats have their greatest impact on the nesting to post-hatchling stages of sea turtle development; therefore, it would seem logical to prioritise conservation efforts on nesting turtles and emerging hatchlings. Frazer (1986) calculated that for every thousand sea turtles that hatch, just one is likely to reach adulthood. This number is now likely much smaller given that the anthropogenic pressures driving most of the abovementioned key threats have increased since Frazer's estimation. Low-cost interventions that target early life-stages on a large scale can have a beneficial conservation impact (Donlan et al., 2010). Furthermore, the knowledge gap on early life-stages of turtles is still vast and targeting conservation efforts on early life-stage would create opportunities to bridge this gap and further inform other conservation strategies (Wildermann et al., 2018). It is, therefore, imperative to implement conservation actions to increase recruitment into the population now whilst longer-term management strategies for protecting older life-stages are further developed.

In-situ monitoring of wild sea turtle populations (nesting and foraging) aids conservation efforts by determining long-term population trends in size and structure over time and identifying key turtle habitats (López-Castro et al., 2022). Monitoring and protection of nesting beaches can also help to evaluate conservation efforts for the target population; however, population decline cannot be prevented without direct actions. Conservation actions can vary in their levels of human intervention. In-situ (leaving nests on the beach) and ex-situ (moving nests to artificial hatcheries) nest management can aid predator evasion, prevent overheating and water inundation of the nests, and manipulate the sex ratio of hatchlings (Tomillo et al., 2021). More controversial conservation actions involve removal of sea turtles from the wild population to be held under human care, either temporarily or permanently. Controversy surrounding this type of strategy derives from the increase in potential risks as the level of human interference increases because rearing turtles in captivity may have unknown effects on their survivability post-intervention (Mullin et al., 2023). However, due to the extinction risk and declining populations of many sea turtles, it is important to use research to answer the unknowns rather that to discount these potential management options altogether (Fuentes et al., 2015).

Examples of conservation strategies involving the temporary removal of sea turtles from the population include head-start and rehabilitation facilities. Rehabilitation centres take sick and diseased individuals from the population with the aim of releasing them once healthy so that they have a chance to reach adulthood (if juvenile) or continue to breed (if adult already), thus helping to sustain the population (Flint et al., 2017; Kaska et al., 2011; Melvin et al., 2021). Head-start programs aim to fulfill both conservation objectives by: 1) reducing the high mortality rate in sea turtle early life-stages (eggs and hatchlings), increasing recruitment to the population; and 2) releasing sea turtles into the ocean at a later stage of development, when they are larger and will hypothetically have increased survivorship (probability of annual survival) and therefore greater chance of reaching maturity and reproducing (Blumenthal et al., 2021; Nasiri et al., 2023). Rehabilitation centres and head-start facilities are often operated by small charities and community groups with varying levels of the ecological understanding, technical skills, infrastructure, and access to funding required to successfully fulfill the conservation aim. Conservation initiatives can also indirectly aid by generating advocacy and funding for turtle conservation (Shum et al., 2023). Furthermore, some research facilities also temporarily house turtle hatchlings so they can be studied ex-situ under experimental and near-natural conditions prior to their release. The James Cook University Turtle Health Research Centre, also known as "The Caraplace", is one example of this.

Head-starts are the subject of considerable debate in the realm of sea turtle conservation. Effectiveness of the hatchery component of the head-start process (objective 1: recruitment) can be determined by calculating the percentage of eggs that hatch and hatchlings that enter the ocean, compared with wild counterparts if data are available. Additionally, the percentage of head-started turtles still alive by the end of the rearing process can be compared with known estimates of percentage of wild counterparts that survive to the same size-class, which is usually neonate or post-hatchling. However, increasing survival to release size does not necessarily mean they will survive to adulthood and reproduce once released (objective 2: survivorship). Interference of the natural ecology of turtles by rearing them under human care could jeopardise their fitness in terms of their survivability post-release (Phillott, 2023). Key captivity-related behavioural issues that have been identified as reducing survivability fitness include an inability to forage, desensitisation to humans, lack of a predator stress response mechanism, and display of stereotypic behaviours (indicative of chronic stress) (Arena et al., 2014). Stress response is key to the survival of captive-held turtles once they have been released into the wild. Maintenance of short-term physiological and behavioural stress responses to intermittent stimuli is necessary for avoiding predation and may be indicative of good fitness and survivability (Preston et al., 2020).

However, chronic stress from long-term exposure to poor husbandry practices or subpar housing conditions can cause negative physiological and behavioural issues, reducing fitness and survivability (Johnstone et al., 2012; Mills et al., 2014; Usategui Martín, 2020). As such, appropriate housing and husbandry of sea turtles is important for their wellbeing whilst under human care and for their post-release survivability. This is one of the key criticisms of head-start practices in cases where subpar facilities and husbandry practices render sea turtles in poor health, either resulting in mortality pre-release or release of unfit individuals into the wild population (Orós et al., 2020). Unfit individuals are unlikely to survive and are also a possible disease risk to the wild population if they have been released without health checks (Mullin et al., 2023). In such cases, head-starting would be at best an ineffective conservation action and at worst damaging to the wild population.

If turtles are unfit and therefore likely to have decreased survivability when released, this raises two questions.

**1) How do we determine whether a turtle is fit to be released?**

There is currently a lack of general consensus or clearly stated identifiers in the literature for pre-release fitness determination of head-started turtles. Some publications note that measures of fitness for head-started turtles to be released included visual inspection, body condition scores, and growth (Stacy et al., 2023). Although behaviour is suggested as an indicator for inferring fitness (Deem & Harris, 2017), it is not commonly mentioned in published studies of head-started turtles. Other publications state that turtles were released after a set time-period and give little to no indication whether any health or fitness parameters were tested. More broadly, fitness or health of an animal under human care is most holistically assessed using the current gold standard "Five Domains Model" (Mellor, 2017) approach, which encompasses physical health, nutrition, environment, behaviour, and mental state. This is the standard which has for a long time been applied in the discipline of Animal Welfare Science and which is being slowly integrated into the newer discipline of Conservation Welfare. Therefore, it should be possible to use the Five Domains Model of welfare as a surrogate of turtle fitness and identify metrics that can be used to determine if a turtle under human care is fit (ready) to be released.

**2) How do we infer survivability post-release?**

True assessment of the fitness surrogate validity would be detection of head-started turtles laying viable eggs because true biological fitness of an animal is its ability to contribute to population growth (Grafen, 2015). However, due to the extended time to sexual maturity and difficulty in detecting head-started turtles on nesting beaches (long-term and expansive monitoring), using

biological fitness as an indicator is often not feasible. Post-release survivability can be inferred from immediate and short-term monitoring of the turtles, and survivorship can be modelled using data collected via satellite telemetry (Abalo-Morla et al., 2018; Robinson et al., 2021). However, there are logistical issues of attaching trackers to small turtles, and the cost and skill required for data collection and analysis is high. Furthermore, having trackers attached may reduce survivability if attachment causes excessive stress to the turtle or negatively affects their behaviour and growth. Therefore, it is necessary to develop practical and repeatable methods of assessing turtle survival post-release.

## Summary

Despite their ecological, economic, and socio-cultural importance, sea turtles are globally threatened with extinction. There are many threats to their populations and mitigation efforts are being made. However, mitigation is complicated by the spatial scale of the threats, long distance migrations of sea turtles, and complicated political and management requirements. Sea turtle conservation efforts aim to increase recruitment to and survivorship of the population so that they can increase and stabilise. Conservation aims can be addressed using varied conservation actions and levels of intervention. Most contentious are actions that involve removal of wild turtles from the population for a temporary period under human care, such as head-start programs. These programs are contentious due to the potential harm to the turtles whilst in captivity and the lack of evidence that these programs meet their conservation aim. Development of husbandry practices, release protocols including indicators of pre-release fitness (readiness), and methods for assessing survivability and likely survivorship post-release would improve release outcomes and provide evidence of the effectiveness of this conservation action. Measures of pre-release fitness or release readiness, release protocol, and post-release assessment of survivability would all need to be adapted and made specific to the species, region, and life-stage of the turtles being released. In doing this, it would be possible to determine the success of the conservation action in meeting its goal.

## Situating this thesis

Given the species and size-class variation in biology and ecology of sea turtles, welfare assessments should be species and size-class specific (Diggins et al., 2022). Extinction of hawksbill turtles would have ecological, cultural, and economic implications and is currently considered a likely possibility due, in part, to their unique shell patterning, which makes them a specifically targeted species for direct commercial take. The North Queensland hawksbill population was once considered the largest in the world but is in decline and estimated to reach nesting extirpation as early as 2036 (Bell et al., 2020), with low survivorship of turtles that leave Australian waters (Madden Hof et al., 2023b; Madden Hof et al., 2023a). This stock has been identified as a High Priority stock for conservation actions (Department of Environment and Science, 2021). Conservation programs that aim to increase turtle recruitment to the population are particularly important for preventing hawksbill turtle extinction.

Head-start facilities that target the earliest and most highly predated life-stages of sea turtles have the potential to make a large contribution to hawksbill turtle populations but only if they are well managed (Blumenthal et al., 2021; Evans et al., 2022; Maggeni & Feeney, 2020). Currently, there is a paucity of data surrounding the effect of captive rearing on survivability of sea turtles post-release and markers of fitness for release. Specifically in question are loss of survival-dependent behaviours, including changes to their stress response systems. Therefore, to raise hawkbill turtles temporarily under human care and ensure a successful release, it is necessary to determine effective measures of welfare and to document the effects of captive rearing on juvenile hawksbill turtles prior to and upon release into the wild. Furthermore, satellite telemetry can be used as a tool to infer short-term survivability, provided that optimal tracker attachment techniques are used and their effects on the small juvenile hawksbill turtles are documented (Diggins et al., 2023).

Having identified these knowledge gaps, this thesis aimed to assess readiness for release of captive-raised hawksbill turtles following a framework based on the Five Domains Model and to use in-water observations at the release event and satellite tracking data to infer post-release survivability. The outcome of this research will inform the development of better husbandry practices and release protocols and add data-driven evidence to the ongoing debate surrounding the viability of head-start facilities as a conservation tool for hawksbill turtles specifically and sea turtle species in general.

# Thesis aims

## Aim 1

Determine the best evidence-based measure of welfare for sea turtles whilst under temporary human care.

- Review the suitability of welfare assessments for turtles under human care, accounting for:
    - interpretation of what constitutes positive welfare when sea turtles are intended for release to the wild; and
    - feasibility of the assessment methods.
- Explore the use of and design considerations for environmental enrichment devices to promote positive welfare for turtles in a rehabilitation setting.
- Identify welfare metrics that can be used to assess the readiness of sea turtles under human care to be released into the wild.

## Aim 2

Optimise method of attaching satellite trackers to small juvenile hawksbill turtles for post-release monitoring without jeopardising turtle welfare.

- Develop, test, and confirm a protocol for the successful attachment of satellite trackers to small juvenile hawksbill turtles where success was defined by:
    - trackers remaining firmly attached for more than 3 months; and
    - attachment method leaving minimal scute damage or disfigurement whilst allowing turtles to continue growing.

## Aim 3

Characterise and compare physiological indicators of stress response in captive-raised juvenile hawksbill turtles after 2 years under human care.

- Determine basal range of corticosterone and lactate concentrations in captive-raised juvenile hawksbill turtles.
- Assess how captive-raised juvenile hawksbill turtles respond to the following stressors:
    - handling and blood sampling protocol;
    - short-term stressor (5-minute stimulation);
    - tracker attachment (1 hour) and dry-docking (12 hours); and

- transportation from turtle housing facility to release site.

### Aim 4

Assess whether juvenile hawksbill turtles maintain naturalistic behaviour after 2 years under human care.

- Document the behaviours of captive-raised hawksbill turtles on first entry into the ocean via in-water observation.
- Document the dispersal of captive-raised juvenile hawksbill turtles on release using satellite telemetry.
- Compare in-water behaviours of recently released juvenile hawksbill turtles with free-living hawksbill turtles reported in published studies.

### Aim 5

Make recommendations to improve husbandry and release protocols for greater welfare and post-release survivability of captive-raised juvenile hawksbill turtles.

## Thesis structure

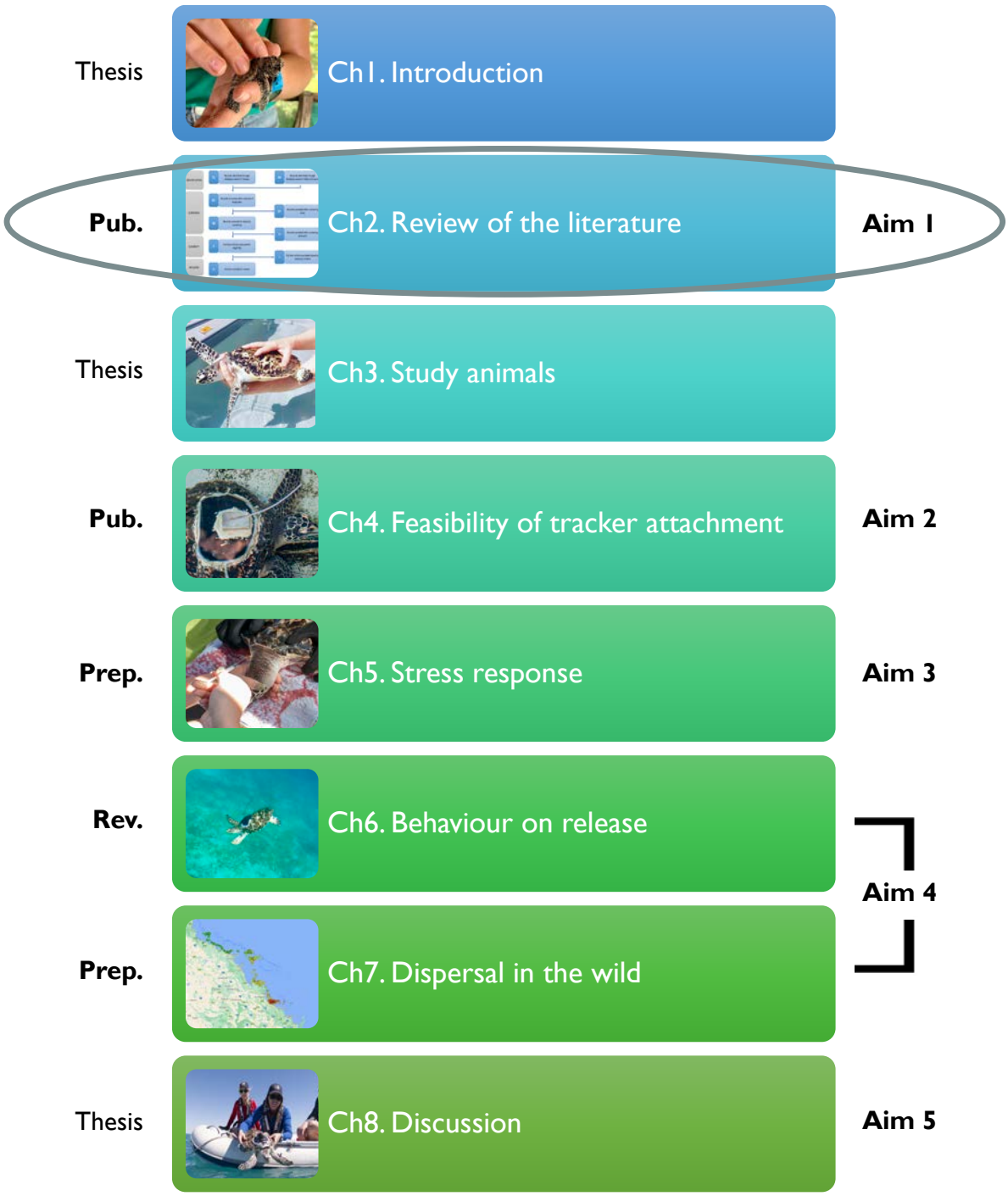
The thesis structure is laid out in the following diagram, which will be used to map progress throughout the thesis at the start of each chapter. The current chapter (Ch) is circled in grey. The thesis aims addressed in each chapter are indicated to the right-hand side of the chapter title. The publication status of each chapter is indicated to the left-hand side of the chapter title. Status labels are as follows:

- Thesis: the chapter is for the thesis only and will not be published
- Pub.: the chapter is already published
- Prep.: the chapter is being prepared as a manuscript
- Rev.: the chapter has been prepared as a manuscript and submitted to a journal for peer-review

| | | |
|---|---|---|
| Thesis |  Ch1. Introduction | |
| **Pub.** |  Ch2. Review of the literature | **Aim 1** |
| Thesis |  Ch3. Study animals | |
| **Pub.** |  Ch4. Feasibility of tracker attachment | **Aim 2** |
| **Prep.** |  Ch5. Stress response | **Aim 3** |
| **Rev.** |  Ch6. Behaviour on release | **Aim 4** |
| **Prep.** |  Ch7. Dispersal in the wild | |
| Thesis |  Ch8. Discussion | **Aim 5** |

# CHAPTER 2: REVIEW OF THE LITERATURE

## Thesis structure



# Background and aims of this chapter

## Rationale

Conservation of sea turtles requires current populations to be increased and stabilised through actions to promote recruitment into and survivorship (annual likelihood of survival at a given life-stage) of the population (Godley et al., 2020). Some organisations aim to increase the survivorship of individuals within a population by providing temporary refuge for the turtles in a captive environment before releasing them back into the wild. Rehabilitation centres house turtles of any life stage that are likely to die from disease and injury without treatment intervention. Head-start organisations house hatchling turtles in a nursery until they have grown and developed into a later life-stage with fewer natural predators. Additionally, there are some research facilities that temporarily house hatchling turtles under human care primarily to study components of their development before releasing them into the wild at a later life-stage, consequently simulating a head-start. In each case, it is possible to calculate the proportion of turtles that survive to the point of release; however, it is more difficult to determine turtle survivorship post-release (Abalo-Morla et al., 2018). Furthermore, survival to the point of release is not in itself indicative of survivability (ability to survive) once released into the wild (Mathews et al., 2005). If a turtle with low survivability is released from human care into the wild and fails to thrive, its survivorship has not been improved and, therefore, the conservation action has not fulfilled its objective.

Reduced survivorship to the point of release and post-release is one of the key criticisms of head-start facilities (Orós et al., 2020; Rodríguez et al., 2023). Likewise, low survival rates have been inferred for rehabilitated turtles post-release (Flint et al., 2017). In conservation science, physical fitness indicators and metrics are most commonly used to determine whether an animal is ready for release (Harrington et al., 2013). However, the observed low rate of sea turtle survival post-release indicates that it may be beneficial to further investigate potential indicators of survivability to guide metrics to evaluate turtle readiness for release (Mathews et al., 2005). Welfare is a holistic measure of health (considering the relationship between physical and mental health) used to assess animals under any type of human care (Mellor, 2017). Furthermore, negative welfare is known to reduce animal survivability post-release (Beausoleil et al., 2018; Harrington et al., 2013; Harvey et al., 2020; Swaisgood, 2010; Walker et al., 2012). Therefore, concepts considered in animal welfare science, and the emerging field of conservation welfare (Beausoleil et al., 2018; Swaisgood, 2010), could provide a good framework for identifying readiness metrics for indicators of sea turtle survivability post-release. Additionally, housing and husbandry standards as well as

transportation and release protocol should be considered in terms of optimised welfare to increase turtle survivability post-release (Harrington et al., 2013).

Welfare assessments should be tailored to suit the needs of the specific animal (species and life-stage) and the context of its captivity (reason for being held under human care) (Mellor, 2017). Holistic welfare assessments have been developed for many farmed animals and those in zoos (Harvey et al., 2020) but are lacking for sea turtle species. Furthermore, the long-term requirements of sea turtles kept permanently under human care will differ from those intended for release into the wild and these differences should be taken into consideration when determining which metrics and values represent 'positive welfare'. For example, positive welfare for turtles held permanently under human care might be achieved by habituation to human presence to reduce or prevent chronic stress (Hutchins, 2006). In contrast, positive welfare for turtles intended for release might be achieved by fostering retention of a fearfulness of humans and a physiological and behavioural response to short stress events, which would be required for survival post-release (Guy et al., 2013).

Welfare and health of sea turtles are inconsistently reported from head-starting facilities and rehabilitation centres; however, they are documented in rehabilitation literature (Manire et al., 2017b). Therefore, this literature review identified sea turtle welfare metrics and possible methods of assessment, focusing on welfare of turtles in a rehabilitation setting. Additionally, the feasibility of each turtle welfare assessment method was considered in terms of its implementation in a conservation setting and implications to the turtle. Potential for welfare improvement in sea turtles by means of environmental enrichment devices was also explored. The findings from this review can extend beyond rehabilitation centres and were used in the context of this thesis to guide husbandry, housing, and release protocol for the hawksbill turtles in the study, including use of identified metrics for assessment of release readiness.

## Thesis aim 1

Determine the best evidence-based assessments of welfare for sea turtles under temporary human care.

### Chapter aims

- Review the suitability of welfare assessments for turtles under human care, accounting for:
    - interpretation of what constitutes positive welfare when sea turtles are intended for release to the wild; and
    - feasibility of the assessment methods.

- Explore the use of and design considerations for environmental enrichment devices to promote positive welfare for turtles in a rehabilitation setting.
- Identify welfare metrics that can be used to assess the readiness of sea turtles under human care to be released into the wild.

## Research Outputs

**Diggins** R, Burrie R, Ariel E, Ridley J, Olsen J, Schultz S, Pettett-Willmett A, Hemming G, and Lloyd J (2022). A review of welfare indicators for sea turtles undergoing rehabilitation, with emphasis on environmental enrichment. *Animal Welfare*, 31(2), 219-230 https://doi.org/10.7120/09627286.31.2.006 (Fig. 2.1).

**Diggins** R, Burrie R, Ariel E, Ridley J, Olsen J, Schultz S, Pettett-Willmett A, Hemming G, and Lloyd J (2019, September 16 – 18). *Use of environmental enrichment devices for improved welfare of hospitalised turtles* [Poster abstract]. Sea Turtle Foundation Health and Rehabilitation Symposium 2019, Gold Coast, Australia.

**Diggins** R, Burrie R, Ariel E, Ridley J, Olsen J, Schultz S, Pettett-Willmett A, Hemming G, and Lloyd J (2021, December 9). *Review of welfare and environmental enrichment for sea turtles undergoing rehabilitation* [Poster presentation]. College of Public Health, Medical and Veterinary Sciences Higher Degree by Research Student Conference 2021, Townsville, Australia.


*Figure 2.1. Front cover from the Animal Welfare journal issue in which the manuscript is published, featuring a photograph of a juvenile green turtle by Rebecca Diggins.*

# A review of welfare indicators for sea turtles undergoing rehabilitation, with emphasis on environmental enrichment

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## Introduction

Welfare for animals under human care is an evolving concept and one that is implemented by individual organisations (Flint et al., 2017), resulting in varied welfare outcomes for the animals. Accredited institutions of the World Association of Zoos and Aquaria or the Zoo and Aquarium Association Australasia, for example, are bound by regulated welfare standards. For animals undergoing rehabilitation, however, welfare standards are set by specific national or state legislation, which is not always so clear or well-regulated (Englefield et al., 2019) and often aimed at terrestrial animals and too general to be of direct relevance to sea turtles.

There are multiple ways to consider welfare. Dawkins (2008) proposed that animal welfare be determined and defined by two questions: 1) are the animals healthy; and 2) do the animals have what they want? Ideally, the desire is for animals to experience 'good' welfare. Identifiable in the Five Freedoms of animal welfare (Farm Animal Welfare Council, 1993), and recognised by (Barnett & Hemsworth, 2009), are three primary facets of welfare: basic health and functioning, psychological or affective states, and natural living. The current industry standard for welfare assessment is the Five Domains Model (Mellor, 2017), which assesses animals holistically based on four physical domains (nutrition, environment, physical health, behaviour) and a fifth, mental domain. Originally this model was developed as an assessment of welfare compromise for animals held in research, teaching, and testing environments (Mellor & Reid, 1994). Subsequently, it has been updated to include additional categories of animals under human care, such as domestic, livestock and zoo, and to incorporate and emphasise positive states of welfare (Mellor & Beausoleil, 2015).

There is no single, fully inclusive method in the determination of welfare specifically for sea turtles; however, a species-specific welfare assessment based on the Five Domains model could benefit them. A similar assessment was developed by Clegg et al. (2015) for captive cetaceans. A species-specific assessment metric for sea turtles would have to consider individual requirements of species due to the variation between the seven species in diet and behaviours observed naturally in the wild. Whitham and Wielebnowski (2009) developed a three-step process for the maintenance of welfare for the individual animal. These involve: 1) the development of a welfare

scoresheet (based on extensive knowledge of normal parameters for the particular species); 2) the validation of the scoresheet through a six-month behavioural and physiological assessment; finally resulting in 3) a welfare scoresheet personalised to each species. Such an assessment tool would be useful in a rehabilitation setting for sea turtles to ensure positive welfare, therefore promoting speedy recovery.

The rehabilitation setting is a specific environment that would require the assessment to have different considerations than if it were for sea turtles housed in zoos or aquaria without intention of release to the wild. Common causes of hospitalisation for sea turtles include boat strike, ingestion or entanglement in fishing gear or marine debris, limb damage or loss, fibropapillomatosis or other disease, and floating syndrome (Flint et al., 2017). Each cause of hospitalisation requires consideration when housing and treating the turtles during rehabilitation. The average time spent by sea turtles in rehabilitation centres has decreased over the last couple of decades but can range from one day to more than a year, with the average time to release after rehabilitation being approximately four months (Flint et al., 2017). Furthermore, since the aim of a rehabilitated turtle is to release it back into the wild, it is important to limit turtle-human interactions, which might be more common in an aquarium setting. Therefore, for an assessment of turtles undergoing rehabilitation, it is most important to determine the desirable state a turtle must reach before it can be released and how quickly this can be measured (Deem & Harris, 2017).

Following Cyclone Yasi in January 2011, in Australia's Far North Queensland, the region experienced a significant increase in sick, injured, and stranded sea turtles (Meager & Limpus, 2012). Several turtle rehabilitation centres opened in response to this increase, and the College of Public Health, Medical and Veterinary Sciences, James Cook University (JCU) was transiently part of this response. Close observation of these wild animals spurred research into environmental enrichment (EE) for sea turtles in rehabilitation (Lloyd et al., 2012), many of which have to spend months in plain plastic tanks whilst undergoing treatment. Newberry (1995) defined EE as an "improvement in the biological functioning of captive animals resulting from modifications to their environment." Hoy et al. (2010) later organised enrichment strategies under eight classifications: feeding, tactile, structural, auditory, olfactory (i.e. exposing the animal to the smell of its prey), visual, social, and human-animal interaction. Maple and Perdue (2013) suggested that 'cognitive' also be included in this list. Ideally, one environmental enrichment device (EED) will be able to satisfy multiple different enrichment styles.

With an anticipated increase in hospitalised turtles following future cyclones and anthropogenically induced environmental damage, a thorough review to assess measures of

welfare is critical, particularly in regard to how EE can increase speed of recovery and optimise chance of survival upon release back into the wild. This review covers suitable welfare assessment methods and how they can be adapted for turtles in rehabilitation, examples of past EE studies, and a discussion on the design of appropriate EEDs for sea turtles in rehabilitation. Detailed explanations of auditory and olfactory EEDs are not included in this review, as there is little information on the uses of these in sea turtles.

## Materials and methods

A scoping review was conducted to explore the literature pertaining to use of EEDs in turtles as a measure of welfare. Two databases were used for the search: Scopus and Web of Science. Ovid Medline was tested but yielded no relevant results so was excluded. Search terms were (environment*) AND (enrich* OR welfare OR entertain*) AND (turtle* OR cheloni* OR testudine* OR reptile* OR loggerhead* OR leatherback* OR hawksbill* OR Ridley OR terrapin*) AND (rehab* OR hospital* OR clinic* OR recover* OR captiv* OR recuperat*). Searches included the full date range of each database (Scopus: 1970–present); Web of Science: 1965–present) for articles related to environmental enrichment and welfare of non-pet testudines. The reference lists of the most relevant papers were used to look for additional papers that had been missed in the database search.

From the literature search, excluding duplicates, 87 articles were identified. Titles and abstracts were reviewed against the selection criteria, which narrowed the results to 15 articles. Any literature not directly pertaining to turtles interacting with environmental enrichment was excluded. All types of environmental enrichment were included and both marine and freshwater turtle studies were included; however, tortoises were excluded. Assessment of full texts reduced the total to 11 articles (Fig. 2.2), of which only one was specifically relating to environmental enrichment for rehabilitation of hospitalised sea turtles. Due to the lack of specific literature, this paper reviews wider literature in the context of the Five Domains as they relate to sea turtles.

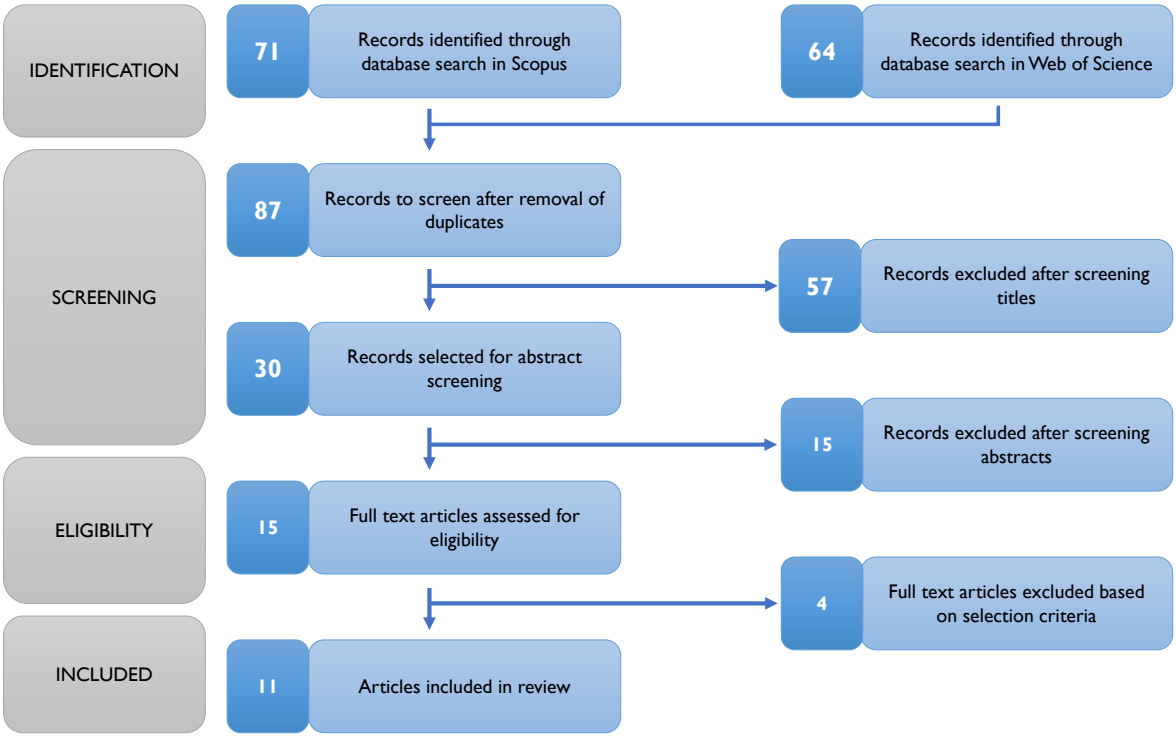



*Figure 2.2. PRISMA flow diagram of scoping review search. Papers were excluded if they did not directly discuss enrichment of freshwater or sea turtles. Papers were included even if they were not in the context of rehabilitation. Only one paper directly discussed implications of environmental enrichment of turtles in a rehabilitation setting. Review papers were excluded.*

## Assessing sea turtle welfare in a rehabilitation setting

### Physical health evaluation

Assessing physical health in sea turtles is met with many challenges, mostly due to the absence of reliable physical and biochemical reference values (March et al., 2018). However, there are several general parameters that are relevant across all animal species, and these can be considered in a modified version for sea turtles undergoing rehabilitation.

Presence of disease and injury in a captive setting are normally considered indicators of poor welfare (Barber et al., 2013); however, in the rehabilitation setting, this assessment of welfare may be less useful as turtles enter the establishment already diseased/injured. Therefore, it is more logical to assess recovery rate and absence of husbandry mutilations. These can be routinely evaluated by sea turtle carers and veterinarians in rehabilitation centres based on visual inspection, behaviour, and activity levels. An unpublished example of a green turtle physical exam score card (Fig. 2.3) is provided from an Australian rehabilitation centre (courtesy of Dr Duane March). The level of epibionts and external parasites on admission can be visually assessed and easily treated

with a freshwater bath on entry. Internal parasite infections are assumed and treated as a standard rule; however, these parasites may be resistant to treatment and therefore cause ongoing problems during rehabilitation.

| **Animal ID** | | | | | **Location** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Comment** | | | | | **Date:** | | **M** | **Tu** | **W** | **Th** | **F** | **Sa** | **Su** |
| **Demeanour** | Bright, alert, responsive | 0 | Quiet, alert, responsive | 1 | Non-responsive | 2 | | | | | | | |
| **Swim ability** | Strong upright | 0 | Weak upright | 1 | Strong/Weak circling | 2 | | | | | | | |
| **Skin Appearance** | Healthy | 0 | Minor lesions | 1 | Generalised sloughing | 2 | | | | | | | |
| **Skin Epibiotic load** | <10% | 0 | 10 - 50% | 1 | >50% | 2 | | | | | | | |
| **Fibropapill-omatosis** | Nil | 0 | <5 lesions | 1 | 5+ lesions | 2 | | | | | | | |
| **Carapace Epibiotic load** | <10% | 0 | 10 - 50% | 1 | >50% | 2 | | | | | | | |
| **Carapace integrity** | Firm | 0 | Soft at margins | 1 | Generalised weakness | 2 | | | | | | | |
| **Plastron** | Convex | 0 | 0 - 3 cm Concave | 1 | >3 cm Concave | 2 | | | | | | | |
| **Plastron integrity** | Clean | 0 | Moderate damage | 1 | Marked damage | 2 | | | | | | | |
| **Muscle tone** | Strong | 0 | Poor | 1 | Absent | 2 | | | | | | | |
| **Buoyancy** | Neutral | 0 | Abnormal buoyancy, able to dive | 1 | Abnormal buoyancy, unable to dive | 2 | | | | | | | |
| **Neurological exam** | Jaw tone present | 0 | Jaw tone reduced | 1 | Jaw tone absent | 2 | | | | | | | |
| | Palpebral present | 0 | Palpebral reduced | 1 | Palpebral absent | 2 | | | | | | | |
| | Menace present | 0 | Menace reduced | 1 | Menace absent | 2 | | | | | | | |
| **Total** | | | | | | | | | | | | | |

*Figure 2.3. Green turtle (Chelonia mydas) physical exam score card. Developed in consultation with participants in a workshop at the Turtle Health and Rehabilitation Symposium 2017, Townsville, Australia, facilitated by Duane March and implemented at Dolphin Marine Magic, Coffs Harbour, Australia.*

Reproductive fitness may not be a reliable indicator of good welfare as captive animals have been known to reproduce well despite poor environments, and the opposite is also true (Wickins-Dražilová, 2006). Specifically, for sea turtles undergoing rehabilitation, it is a poor indication of

welfare as it would not be feasible to replicate the environmental conditions appropriate for successful reproduction in sea turtles. Furthermore, many of the individuals undergoing rehabilitation are sexually immature.

Stress has been linked to negative welfare (Broom et al., 1993) and therefore assessment of stress could be an indicator of welfare in sea turtles undergoing rehabilitation. Activation of the hypothalamic-pituitary-adrenal axis, and the subsequent release of glucocorticoids are commonly used to determine levels of stress (Hunt et al., 2016; Stabenau & Vietti, 2003). Glucocorticoid measurements may provide an indication of acute or chronic stress, depending on the chosen method of collection (blood, saliva and faecal/urine for acute stress, and samples of integumentary structures for chronic stress); however, there are numerous issues to this evaluation technique (Jessop et al., 2004a). Primarily, stress associated with reptile-capture and blood and saliva collection can interfere with results (Silvestre, 2014). Additionally, glucocorticoids may be released in response to arousal, and not aversive stimuli (Latham, 2010). Furthermore, there are incongruences as to the correlation of glucocorticoid levels to stress levels in sea turtle literature (Gregory et al., 1996; Jessop et al., 2002a; Jessop et al., 2002b). Finally, there seems to be a delay in green turtles' (*Chelonia mydas*) adrenocortical responses to stress (Jessop, 2001). There may also be potential for adrenal fatigue in animals that are chronically debilitated (March et al., 2018). Ironically, many of these parameters are obtained via invasive collection techniques, which may cause undue stress and actually decrease the welfare of the animal (Mason & Veasey, 2010).

A number of blood parameters normally used to assess health in mammals were found to be of limited prognostic value for green turtles undergoing rehabilitation in Australia (March et al., 2018). Although some of the parameters would provide a general indication of health, such as heterophil count and haematocrit level, none were correlated to recovery. This could be because of the particular suite of diseases encountered locally. The heterophil to lymphocyte ratio and blood glucose levels have been used to assess stress response (Davis et al., 2008; Krams et al., 2012), but it is clear that more research is needed to provide reliable prognostic biomarkers for each species of marine turtle in rehabilitation.

With all of these inconsistencies in mind, as well as the expense, specialised skillset, and human-turtle contact required, measurement of glucocorticoid levels and other blood parameters are not ideal adjunctive methods of health assessment for determining welfare status of sea turtles. Of course, they are necessary for determining the health and rehabilitation status of the turtles.

## Nutritional evaluation

Sea turtles entering rehabilitation centres are frequently emaciated and therefore weight gain is a priority. Some literature has shown that adult green turtles appear to do very well on high protein diets in captivity (Amorocho & Reina, 2008; Wood & Wood, 1981). High weight gain is achievable on such diets, which can be either animal matter (Caldwell, 1962) or commercially prepared high protein, readily digestible pellets (Wood & Wood, 1981). However, it is important to consider the optimal diet for sea turtles undergoing rehabilitation. There is a natural variation in the diets of wild sea turtles of different species and life stages (Arthur et al., 2009; Limpus & Limpus, 2000). Therefore, diet needs to be tailored to the specific nutritional requirements of the individual to reflect their natural preferences. A number of rehabilitation centres have been known to feed turtles a high protein diet to encourage rapid weight gain, irrespective of species (EA, personal communication 2021). For a predominantly herbivorous species, such as the green turtle, this does not reflect a natural diet and may lead to uraemia and hypercholesterolaemia (March et al., 2018).

Weight gain by itself is not necessarily an indicator of welfare; however, it can be used in conjunction with body condition scoring (Limpus et al., 2012) to show progress for rehabilitation of emaciated sea turtles. Body condition reflects not only the availability of appropriate and nutritious food items in the captive setting, but also appetite and physiological ability to convert food to build muscle and support activity. This method can be applied for sea turtles, where the body condition index (BCI) is recorded regularly, and release is dependent on having achieved a BCI consistent with wild populations (Bjorndal, 1980). A more accurate method of scoring body condition would be bio-impedance analysis as that would differentiate between weight gain caused by fluid, fat, or muscle (Kophamel et al., 2023). However, this requires specialised equipment and training, as well as additional human-turtle interactions. Melvin et al. (2021) have also found evidence that malnutrition is a key factor in mortality of sea turtles undergoing rehabilitation and suggest monitoring metabolomic profiles for earlier diagnosis and treatment of metabolic failure.

Whilst poor body condition/weight loss is often precipitated by stress, it is also influenced by diet, activity levels (Mason & Mendi, 1993), and disease. Cachexia is a common finding in sea turtles presenting to rehabilitation clinics (March et al., 2021). Ideally, in a rehabilitation setting, each turtle's diet would be formulated to cater for maintenance, whilst taking activity levels and disease status into consideration. Overall, measuring weight in conjunction with body scoring is a useful method to assess welfare. It is minimally invasive and can be obtained on a weekly basis by rehabilitation staff and carers.

## Environmental evaluation

The environmental domain for a captive turtle can be evaluated in two stages: 1) the initial set-up of the tank; and 2) the ongoing maintenance of tank conditions. Considerations when designing an enclosure for sea turtles should include substrate, structure/shape, size, depth, material, and colour (New South Wales Government, 2020; Stamper et al., 2017). Substrate, structure, and material for a sea turtle tank should consider that turtles are likely to ingest anything small enough (Hoopes et al., 2017). Particularly in a rehabilitation setting, it would be disadvantageous to put turtles in an environment where they may do more harm to themselves through ingestion or scraping against rough surfaces. Juvenile green turtles showed a preference toward the colour blue under experimental settings; therefore, implementation of blue tanks may improve their comfort (Hall et al., 2018). Tanks should be deep enough to provide refuge, but weak turtles are at risk of drowning, and so fitness of the turtle needs to be considered (Stamper et al., 2017). These features of the environment are likely to remain constant throughout the entire rehabilitation period and so anticipated length of time in captivity (as well as species) should be considered at set-up. This is particularly relevant to enclosure size as turtles must have sufficient space to manoeuvre and engage in positive natural behaviours (Stamper et al., 2017).

Environmental conditions that can be regularly and simply monitored to ensure comfort for sea turtles include temperature, light, ultraviolet, salinity, and other water quality parameters (Stamper et al., 2017). Sea turtles have a range of tolerability for each of these parameters; if they are not well-monitored and maintained, it is possible that sea turtles already in a weakened state, such as those undergoing rehabilitation, might become further compromised by sub-optimal environmental conditions. For example, as ectotherms, reduced temperatures will reduce the efficiency of the digestive and immune system, which would be detrimental for underweight sick turtles (Hoopes et al., 2017). These are all environmental conditions that are always essential to the physical well-being of sea turtles; however, variety in non-essential environmental stimuli has been shown to positively affect welfare of other animals (Burghardt, 2013) and should, therefore, be considered for use with sea turtles. EEDs can be introduced to do this and the change in behaviour of the turtles can be used to assess the impact on welfare.

## Behavioural evaluation

It has commonly been perceived that stereotypic behaviour is indicative of either past and/or present poor welfare (Mason et al., 2007; Mason, 1991; Mason & Latham, 2004). Indeed, the presence or absence of stereotypic behaviour remains one of the best validated measures of animal welfare (Maple & Perdue, 2013). Mason et al. (2007) proposed that stereotypic behaviour,

as a broad term, should refer to "repetitive behaviour induced by frustration, repeated attempts to cope and/or central nervous system (brain) dysfunction." In the rehabilitation setting, changes in behaviour could be due to brain damage caused by parasites such as spirorchiid flukes (Glazebrook et al., 1989) or coccidia (Gordon et al., 1993) or, alternatively, it could be environmentally induced as a result of boredom or reduced welfare. This is particularly likely if the turtles are kept in sterile, empty hospital tanks, devoid of environmental enrichment.

Abnormal behaviours indicating stress in turtles include grafting of jaw (rasping of ramphotheca), pseudo-vocalisation (squeaks or whines), pattern swimming, poor posture when resting at the bottom of the tank (flopped and lifeless rather than propped up on front flippers), and boundary exploration (related to exploratory and escape activity) (Arena et al., 2014; Tynes, 2010). Leatherback turtles (*Dermochelys coriacea*) are particularly difficult to keep in captivity due to their inability to register boundaries. They are continuous swimmers and can cause additional damage to themselves if allowed to swim into the sides of a rehabilitation tank (Jones et al., 2000; Levy et al., 2005). Turtles recently hospitalised, or handled in and out of the water, may display behavioural floating for a period. This could be as a response to stress or a preference to be at the surface due to weakened physical condition (Manire et al., 2017a). Buoyancy disorder due to gas accumulation within the coelomic cavity will be discussed later. Associated with the presence of or contact with humans, other stress-related behaviours include cloacal evacuations upon handling, projection of penis or hemi-pene, voluntary regurgitation of food, and human-directed aggression. Often these signs are related to fear and are common in overly restrictive and inappropriate environments (Warwick et al., 2013).

Stereotypic behaviour tends to be associated with negative welfare in healthy animals (i.e. in zoos/aquaria), but in the case of sick turtles, it can actually illustrate improved health via increased energy levels. However, if they are to be kept longer for full rehabilitation, stereotypic behaviours should be discouraged. EEDs are a useful tool, commonly used in captive settings to discourage stereotypic behaviours and encourage positive behaviours (Mason et al., 2007). Consequentially, observing animals for the presence or absence of negative behaviours could be used as a proficient welfare evaluation measure, and potentially as a means of determining the effectiveness of EEDs, particularly in turtles that have spent several months in rehabilitation. Additionally, comparing captive animal behaviour with wild animal behaviour (Burghardt et al., 1996; Phillips et al., 2011; Smith & Litchfield, 2010) is another measure of welfare. The more a captive-held animal engages in behaviour exhibited in the wild, the better its welfare is deemed. Similarly, the effectiveness of EE can be deduced by comparing the proportion of time an animal is engaged in a type of behaviour before and after introduction of an EED (Lloyd et al., 2012; Therrien et al., 2007).

## Mental evaluation

The physical domains (health, nutrition, environment, and behaviour) all contribute to the mental state of the turtles (Mellor, 2017). The affective state of an animal can be assessed via study of its behaviour (Bracke & Hopster, 2006). Stress fever and tachycardia, both physiological responses associated with emotion in other vertebrates, have been observed in iguanas (*Iguana iguana*) (Cabanac, 1999) and wood turtles (*Clemmys insculpta*) (Cabanac & Bernieri, 2000). Cabanac (1999) also discovered that rather than venture into a cold environment to obtain food, iguanas preferred to remain in a warm environment, suggesting that their motivation was influenced by sensory pleasure. Therefore, it appears that basic affective states exist in reptiles, turtles included. In the assessment of affective states, there is a potential issue of over-anthropomorphosis and evaluator bias.

## Using EEDs to monitor welfare

Modification of the environment to provide more opportunities and promote positive behaviours can be used to infer the affective state of the turtles and assess their welfare. EEDs should be designed to increase positive affective state of turtles but must also be suitable for the rehabilitation setting. EEDs are all designed to enhance environmental opportunity and choice, but depending on the specific device, could also promote positive behavioural expression, increase fitness, and aid nutrition. Thus, contributing to a positive affective state for the turtles and improved welfare. It is on this premise that EEDs may be able to contribute to a speedier recovery and shorter rehabilitation time of hospitalised turtles.

The psychological and physical benefits of EEDs are well documented in captive mammals (Mellen & Sevenich MacPhee, 2001; Newberry, 1995; Young, 2013), but less so in the case of marine and terrestrial reptiles (de Azevedo et al., 2007; Eagan, 2019; Maple & Perdue, 2013). Reptiles have previously been considered too sedentary to interact with, and thus benefit from, EE (Bennett, 1982; Burghardt, 2013). Turtles housed at JCU proved this to be a misconception by actively interacting with EEDs (Lloyd et al., 2012). Furthermore, a literature review by Lambert et al. (2019) found multiple studies that showed sentience in reptiles, including multiple turtle species. We therefore found it timely to conduct a thorough review of past reptile-specific EED studies as well as to draw from existing knowledge of wild sea turtle ecology to explore the potential for EEDs in assisting with rehabilitation of hospitalised turtles.

## EEDs for turtles undergoing rehabilitation

At this point, it is necessary to make a distinction between EE for hospitalised turtles and those that are permanently captive (such as in public aquaria). For all captive turtles, it is desirable for their captive conditions to be as similar to their wild conditions as practically possible (Newberry, 1995). Hospital settings, however, are often not conducive to this as they must remain sterile to reduce likelihood of infection, for example. As such, EEDs should aim to stimulate natural behaviours safely without jeopardising the necessary sanitation standards of a hospital setting or the safety of the turtle. Therefore, EEDs should encourage 'preferred' naturalistic living. The term 'preferred' is used to omit negative aspects of naturalistic living, such as famine and predation (Hutchins, 2006). Predatory avoidance behaviours correlated with stress could reduce longevity of animals in long-term captivity, which would be associated with negative welfare. However, anti-predator responses are necessary for temporarily captive turtles to ensure a good chance of survival on release. Turtles intended for release after rehabilitation, therefore, need to maintain a level of fearfulness, which could be promoted through subjection to occasional and temporary unpleasant stimuli (Guy et al., 2013). With respect to this, it is difficult to prepare sea turtles for natural life in an artificial environment, especially in a rehabilitation setting where emphasis is on improving health and fitness. An ideal welfare evaluation plan for sea turtles in the rehabilitation setting would adhere to the following considerations:

- Be **safe** for the turtle;
- Be **feasible** in the rehabilitation setting;
- Be **cost-effective**;
- Be **easily implemented** by carers without the requirement for specialised skills or training;
- Be **minimally invasive to induce little or no stress** on the turtles, which is especially important as these turtles are diseased and/or injured and added stress is likely to exacerbate immunosuppression, subsequently lengthening recovery time;
- Accurately measure **stress in conjunction with behavioural assessment**;
- Require **minimal human-turtle contact**; and
- Require a **short-term** evaluation of welfare variables to provide a reliable indication of welfare.

## Feeding enrichment

Turtles in the wild appear to feed in bouts – early to mid-morning and mid to late afternoon (Ogden et al., 1983) – and therefore reproducing this pattern in the captive setting to maintain the natural rhythm may be beneficial for release. Food-oriented devices appear to be a very effective form of EE (Maple & Perdue, 2013). As a reflection of their natural foraging behaviour, hunting of live jellyfish, ctenophores, and squid would be a valuable EED for turtles in captivity or those undergoing rehabilitation. However, the ethical dilemma associated with live feeding, biosecurity, and the availability of such prey may exclude this EED. The lettuce feeders on the tank floor reported by Therrien et al. (2007) may prove an interesting activity for turtles as this mimics grazing behaviour (Hart & Fujisaki, 2010; van de Merwe et al., 2009) and serves a dual purpose, as a hiding place.

Injuries and ailments of each individual turtle need to be considered when designing the EED. 'Floating syndrome', which affects the turtle's buoyancy, can be caused by air trapped in the lungs, coelomic cavity, or intestine of the turtle. The air upsets diving proficiency, which prevents the turtle from reaching the tank floor, resulting in major feeding constraints (Norton, 2005). However, occasional bottom feeding for floating turtles would encourage them to try to dive down when they have enough energy. A possible alternative could consist of a frozen ice block containing squid and vegetable matter, such as cos lettuce and nori, to encourage foraging and provide the turtles with a focused interactive activity for an extended period of time. Entanglement is another common cause of turtle hospitalisation. Entanglement may result in amputation of a flipper, causing restricted movement, which also needs consideration when designing EEDs. In general, natural foraging on the tank floor should be encouraged as well as a disassociation between humans and food.

## Tactile enrichment

Hoy et al. (2010) described tactile EE as "the provision of objects that are physically stimulating to the animal." To reflect their natural environment, turtles may benefit from the inclusion of muddy or sandy floor bottoms, perhaps contained within a tray to maintain ease of cleaning and water drainage; however, this is unlikely to be feasible in a sterile rehabilitation setting. Employment of stones too large to ingest, however, could provide excellent enrichment, for green turtles in particular (EA, JL personal observation), as they are attracted to rocky rubble to perform self-cleaning behaviours (Heithaus et al., 2002). Whilst captive turtles have been observed to swim under brooms in order to groom themselves (Brill et al., 1995; Lloyd et al., 2012), turtles have also been known to eat the broom bristles. Consequentially, this EED comes with risks and, if

utilised, should only be provided under supervision. Provision of a 'waterfall', as well as toys such as hoops and balls, would provide valuable tactile enrichment (Burghardt, 2005).

## Structural enrichment

In promoting naturalistic living, turtles should have access to shallow water for resting (Brill et al., 1995). This can be achieved in the form of a platform suspended from the wall of the tank or positioned in the centre of the tank. Alternatively, water levels could be lowered for floating turtles, to enable them to reach the tank floor and right themselves with their flippers. Turtles should also have deeper parts in their tanks, ideally with 3D structures that could mimic caves (Brill et al., 1995). A pipe on the tank floor, large enough for hiding their head, allows turtles to hide and/or exclude external stimuli during resting periods (Lloyd et al., 2012; Therrien et al., 2007). Hatchlings and young post-hatchlings can be buoyant and so EEDs on the tank floor may not be appropriate. Therefore, mounting pipes to the side of the tank or in shallow water for young or floating turtles would provide a suitable refuge.

## Social and visual enrichment

Sea turtles in restricted environments should be housed individually due to their typically solitary tendencies (Heithaus et al., 2002) and documented aggression in overcrowded facilities (Arena et al., 2014) and during mating (Schofield et al., 2007). However, cohabitation with other species, such as a green turtle and brown tang (*Acanthurus nigrofuscus*) or yellow tang (*Zebrasoma flavescens*) (Losey et al., 1994) could potentially act as a form of social EE. Inter-species cohabitation would also provide visual enrichment (something to look at), whilst additionally satisfying the natural behaviour of the green turtle to be clean. However, Zamzow (1998) showed that whilst this cohabitation may be beneficial for control of ectoparasites, reef fish may serve as vectors in the spread of fibropapillomatosis or create an opportunity for infection if the turtle is wounded during cleaning. This would also require additional husbandry for the fish, which would be costly to the rehabilitation facility in terms of time and money.

## Cognitive and human-animal enrichment

Maple and Perdue (2013, p. 108) described cognitive enrichment as: "challenging and stimulating an organism's memory, decision-making, judgment, perception, attention, problem-solving, executive functioning, learning and species-specific abilities." A training routine using associative learning (Lopez et al., 2001; Wilkinson et al., 2007; Wilkinson et al., 2009) would provide this type of enrichment and has been proven possible in marine turtles (Bartol et al., 2003; Mellgren &

Mann, 1998). However, since rehabilitation turtles only remain in facilities temporarily, training may not be a worthwhile form of EE due to the potential time investment required for it to be successful. Additionally, although human-turtle interactions may be encouraged in aquaria to increase familiarity and reduce stress (Claxton, 2011), they should be limited in temporary captive settings. Turtles may have extensive long-term memory (Bartol et al., 2003; Davis, 2009; Davis & Burghardt, 2012); therefore, human-turtle interactions could cause potential overdependence and 'trust' towards humans. Lack of caution towards humans would be disadvantageous to the turtles after release as it could lead to injury (Addison & Nelson, 2000).

## Past examples of EE in captive turtles

A case study from a Spanish rehabilitation centre, based on the work of Therrien et al. (2007), showed that EE aided in the successful rehabilitation and release of a sea turtle that was previously considered unfit for release due to a flipper amputation (Monreal-Pawlowsky et al., 2017). Recognising the limitations of implementing EE in a rehabilitation environment, enrichment was based on feeding, tactile and structural stimuli. Enrichment primarily involved eating live food and aimed to prepare the turtle to avoid unnatural objects in the water, such as buoys. Despite being in captivity for 10 years, including a 2-year rehabilitation period, two-months of EE was sufficient to prepare the turtle for release into the wild. This successful release was confirmed by ten-month transmission from a satellite tag that showed the loggerhead turtle (*Caretta caretta*) crossed an expansive body of water. It is unknown how quickly a turtle might be released with a timelier introduction to EE as no specific studies for this were found in the literature. However, it is important to note that EE in this case study was administered over a short time-period, easy to implement, cost-effective and required minimal human interaction as a webcam was used for monitoring.

Research was undertaken on the effects of EE on four captive display sea turtles (three loggerhead turtles and one blind green turtle) in Florida (Therrien et al., 2007). The behaviour of the turtles was assessed both with and without enrichment present. The EEDs were designed to stimulate their tactile sense, increase exploratory swimming, and satisfy their need to forage. The study showed that there was a significant increase in amount of time engaged in naturalistic behaviours with the use of EEDs. The devices for the blind turtle were modified to suit its special needs and successfully decreased the stereotypical behaviour and increased the exploratory behaviour of the animal. In an enrichment study of captive-raised, collectively housed green turtles intended for release, Kanghae et al. (2021) found that enrichment devices decreased negative behaviour. Specifically, the turtles exposed to enrichment had fewer bite wounds than turtles without

enrichment and without other health parameters affected. EE appears to be just as effective for marine reptiles as it is for mammalian species, and should be encouraged for captive sea turtles, including disabled ones, and particularly when housed collectively.

A preliminary study on hospitalised sea turtles, conducted by Lloyd et al. (2012) arrived at similar conclusions. Lloyd et al. (2012) demonstrated that there was an overall decrease in pattern swimming and resting behaviours observed amongst the turtles in the presence of EE. Additionally, it was found that each turtle responded to different EEDs in their own specific ways, highlighting the apparent variances in natural behaviours and preferences between individuals. It is also important to consider the possibility that turtles will habituate to an EED if given unrestricted access to it. Consequentially, EEDs should be rotated and their use potentially supervised (Lloyd et al., 2012). Furthermore, the placement of structural elements of the captive environment should be altered two to three times a year to maintain their novelty factor (Hawkins & Willemsen, 2004).

Relatively few studies on EE in sea turtles are published. For this reason, we have included studies on freshwater turtles. Case et al. (2005) assessed the preference as well as the physiological and behavioural effects of enriched versus barren environments on 38 box turtles (*Terrapene carolina*). Preference for the habitat-enriched environment was apparent. Following the preference tests, turtles were housed for a 1-month period in one of the two environments. Behaviourally, turtles with habitat enrichment spent less time engaged in negative behaviours, and physiologically they had significantly lower heterophil to lymphocyte ratios than turtles in the barren environment. This illustrates that turtles prefer EE, that enrichment improves their welfare, and importantly, that this improvement can be observed in their behaviour. Similarly, studies by Tetzlaff et al. (Tetzlaff et al., 2018; Tetzlaff et al., 2019c; Tetzlaff et al., 2019a) found that even captive-born *T. carolina* intrinsically preferred enriched habitats, and that enriched environments, along with time for growth in captivity, might aid survival post-release.

Food-centred enrichment for freshwater turtles has also been studied. (Bryant & Kother, 2014) used puzzle-based feeding enrichment devices to successfully increase time spent feeding and promote foraging behaviour of Fly River turtles (*Carettochelys insculpta)* on display at ZSL London Zoo, UK. Bannister et al. (2021) introduced scented and unscented enrichment devices pre-feeding to reduce negative behaviour in a group of freshwater (*Pseudemys sp* and *Trachemys scripta ssp*) display turtles at Tynemouth Aquarium, UK. Presence of enrichment devices pre-feeding successfully reduced escape behaviour and turtles showed greater interest in scented devices than unscented, indicating that olfactory enrichment is appropriate for captive turtles.

Burghardt (2005) observed 'play' behaviour in a captive Nile soft-shell turtle (*Trionyx triunguis*) that was introduced to five EEDs: two basketballs of different colours, a hoop, a rubber fill hose, and live fish for feeding. Burghardt (2005, p. 82) defined play as "repeated, incompletely functional behaviour differing from more serious versions structurally, contextually, or ontogenetically, and initiated voluntarily when the animal is in a relaxed or low stress setting." These EEDs were introduced in an effort to reduce boredom-induced self-mutilation (Burghardt et al., 1996). It was observed that this soft-shelled turtle played with the EEDs for 21% of observed time. This play is longer than juvenile captive mammals, including primates, which play between 1 and 10% of the time (Fagen, 1981). Burghardt (2005) also mentioned object play behaviour in another two Nile soft-shelled turtles at Toronto Zoo, as relayed by reptile curator Robert Johnson. Indeed, there are other examples of play in turtles, including object play in a loggerhead turtle (Burghardt, 2005), locomotor play in a wood turtle and social play in Emydidae turtles (Burghardt, 2005). Therefore, EEDs designed to encourage play should be considered for hospitalised turtles in order to increase welfare and reduce rehabilitation time.

## Animal welfare implications

Maintaining positive welfare of animals under human care is of utmost importance. When considering appropriate methods to assess welfare status and promote positive welfare some distinctions need to be made specifically for sea turtles undergoing rehabilitation. Species and life-stage specific considerations need to be made but also limitations due to the hospital environment should be considered. The Five Domains model of welfare can be applied to assess welfare of sea turtles, and reviewed for appropriateness, effectiveness, and feasibility for application in the rehabilitation setting. Physical health evaluation methods are highly specialised, invasive, and expensive and not easily implemented by rehabilitation staff. Nutritional evaluation should always be carefully considered with rehabilitation turtles and more research is needed to assess effects of poor diet on the physical health of sea turtles in captivity. The environmental implications on welfare of turtles undergoing rehabilitation can be difficult to manage due to the need for the environment to be sterile and easily cleaned, which makes this domain difficult to assess. The behavioural domain is easily assessed by rehabilitation staff and can be used to infer mental state of the sea turtles. For this reason, behavioural assessment of turtles and mental affective states whilst undergoing rehabilitation should be routinely undertaken to promote positive welfare.

The limited literature shows that sea turtles respond to EEDs and can benefit from enrichment to improve their welfare whilst in captivity. They have been observed to have basic affective states, engage in play behaviours, and to respond positively to the introduction of EEDs. Through the

use of EEDs (including devices to encourage foraging, complex multi-dimensional environments, and hides), designed according to the requirements of the rehabilitation centre and the needs of the individual turtle, it is possible to cover the three main facets of welfare, and thereby assist in the recovery and preparation of rehabilitated turtles for release back into the wild. The authors hope that this literature review will contribute to the recognition of the advantages and significance of EE in hospitalised sea turtles, and to encourage turtle rehabilitators to effectuate and employ EEDs. Future research projects may also assess the impact of various EEDs to determine the most beneficial of these on the welfare of hospitalised and other captive sea turtles, through welfare measures such as a reduction in stereotypic behaviour and faster recovery times, the ultimate goal being to improve the welfare of sea turtles held in confinement.

## Addendum to publication

The published literature search was last conducted on 28 June 2021. An updated literature search was conducted on 9 October 2023 using the same methodology outlined in the Materials and methods section above with an amended date range of 2021 – present. The search yielded an additional 40 papers after removal of duplicates. This number was reduced to two experimental papers after screening for relevance as per inclusion and exclusion criteria, and these papers are summarised below.

Thomson et al. (2021) published a continuation of the study by Bannister et al. (2021), already included in this published review. Thomson et al. (2021) focused more specifically on the colour of the enrichment devices and found that individual turtles had different responses to enrichment but generally preferred the colour yellow to red, white, or green. Overall, there was little interest in the enrichment, which was hypothesized to be caused by habituation to the devices, although it was found that novel enrichment changed the behavioural budgets of the turtles, specifically in response to time engaged in negative escape behaviour. For turtle characterised as passive, the escape behaviour increased with introduction of novel enrichment, whereas for turtles characterised as active, time engaged in escape behaviour decreased in presence of novel enrichment. This highlights the need to assess behaviours and response to enrichment at an individual level, or at least by groupings related to classical reptile behaviours: shyness–boldness, exploration–avoidance, activity–passivity, and sociability–aggression (Waters et al., 2017).

Escobedo-Bonilla et al. (2022) also published a review of EED use with sea turtles, including the same studies covered in my review. However, they also discussed a fifth case study of an EE program designed for a single olive ridley turtle held at their rehabilitation facility for two years

prior to introduction of EE. The turtle had both front flippers amputated due to damage from net entanglement and the EEDs were introduced as a means to reduce stress and boredom, inferred from time spent in active behaviours. EE was delivered by tactile, structural, or feeding devices and indeed appeared to increase activity level of the turtle by reducing time spent resting and increasing time engaged in random swimming. This case study shows the value of EEDs for improving welfare of sea turtles held under human care for extended periods of time.

# Summary of thesis and chapter aims

## Thesis aim 1

Determine the best evidence-based assessments of welfare for sea turtles under temporary human care.

### Chapter aims

### Chapter aim 1

- Review the suitability of welfare assessments for turtles under human care, accounting for:
    - interpretation of what constitutes positive welfare when sea turtles are intended for release to the wild; and
    - feasibility of the assessment methods.

Any animal undergoing a period of human care needs to have its welfare assessed and monitored to ensure the holistic health of the animal is maintained. Sea turtles are no exception to this. The Five Domains Model of welfare is considered the current gold standard by which to assess animals under human care. The first four domains are physical indicators (nutrition, environment, physical health, and behaviour) and the fifth domain is the mental state. Common assessments include growth and body condition scoring (nutrition); observation of naturalistic behaviours and response to enrichment (environmental); measurement of various haematological and biochemical markers (physical); and presence/absence of stereotypic behaviours and time engaged in naturalistic (wild) behaviours (behaviour). Despite previous misconceptions about reptilian need for such stimulation, turtles have been shown to have an affective state and to respond positively to improved welfare by means of environmental enrichment. The mental health of turtles can also be indicated by behavioural response of turtles to various stimuli (or lack thereof) and by presence or absence of stereotypies.

Positive welfare is generally considered highest when captive conditions encourage naturalistic living, stimulate natural behaviours, physical and nutritional metrics are within natural range of healthy wild counterparts, and there is an absence of stereotypic behaviours displayed. However, for turtles that are intended to be released into the wild, such as those undergoing rehabilitation, in a head-start centre, or in some cases in research facilities, there are additional welfare considerations needed to prepare the turtles for survival post-release. For example, turtles under temporary human care should not be conditioned to human presence as this would be detrimental to their survival in the wild. Furthermore, some short sporadic stress events would help to maintain natural predator avoidance responses.

For animals undergoing rehabilitation it is vital to monitor welfare in a way that is feasible, practical, and limits chronic stress to the animal. Feasibility and effectiveness of these domains for assessing welfare of sea turtles undergoing rehabilitation were reviewed and it was determined that the mental state can be best assessed through behavioural changes. Results also showed that certain welfare assessment methods may be less appropriate for short-term captivity experienced during rehabilitation (e.g. reproduction). Additionally, the hospital environment limits the ability to address some of the domains (e.g. biosecurity, feasibility, and safety of turtle might be compromised). Some methods of assessing welfare (e.g. blood collection to measure levels of stress biomarkers) might be difficult to implement in certain centres due to the expense, skill, and equipment required and also cause undue stress to the animals during assessment.

## Chapter aim 2

- Explore the use of and design considerations for environmental enrichment devices to promote positive welfare for turtles in a rehabilitation setting.

A scoping review of the literature was conducted using Scopus and Web of Science to investigate use of environmental enrichment devices (EEDs) as a measure of welfare in sea turtles. Behavioural assessments using EEDs were found to be well-documented; however, most EED studies pertained largely to livestock or zoo animals. Furthermore, studies rarely concentrated on reptiles, and specifically sea turtles. This review found that only three of the nine environmental enrichment strategies described in the literature suit the specific requirements of sea turtles in rehabilitation: feeding, tactile, and structural. Feeding EEDs encourage foraging, tactile EEDs can be used for grooming and investigation, and structural EEDs promote natural resting behaviours. Furthermore, EEDs both promote positive behaviours as well as limit negative behaviours in sea turtles and can, therefore, help to promote positive welfare in sea turtles under human care.

## Chapter aim 3

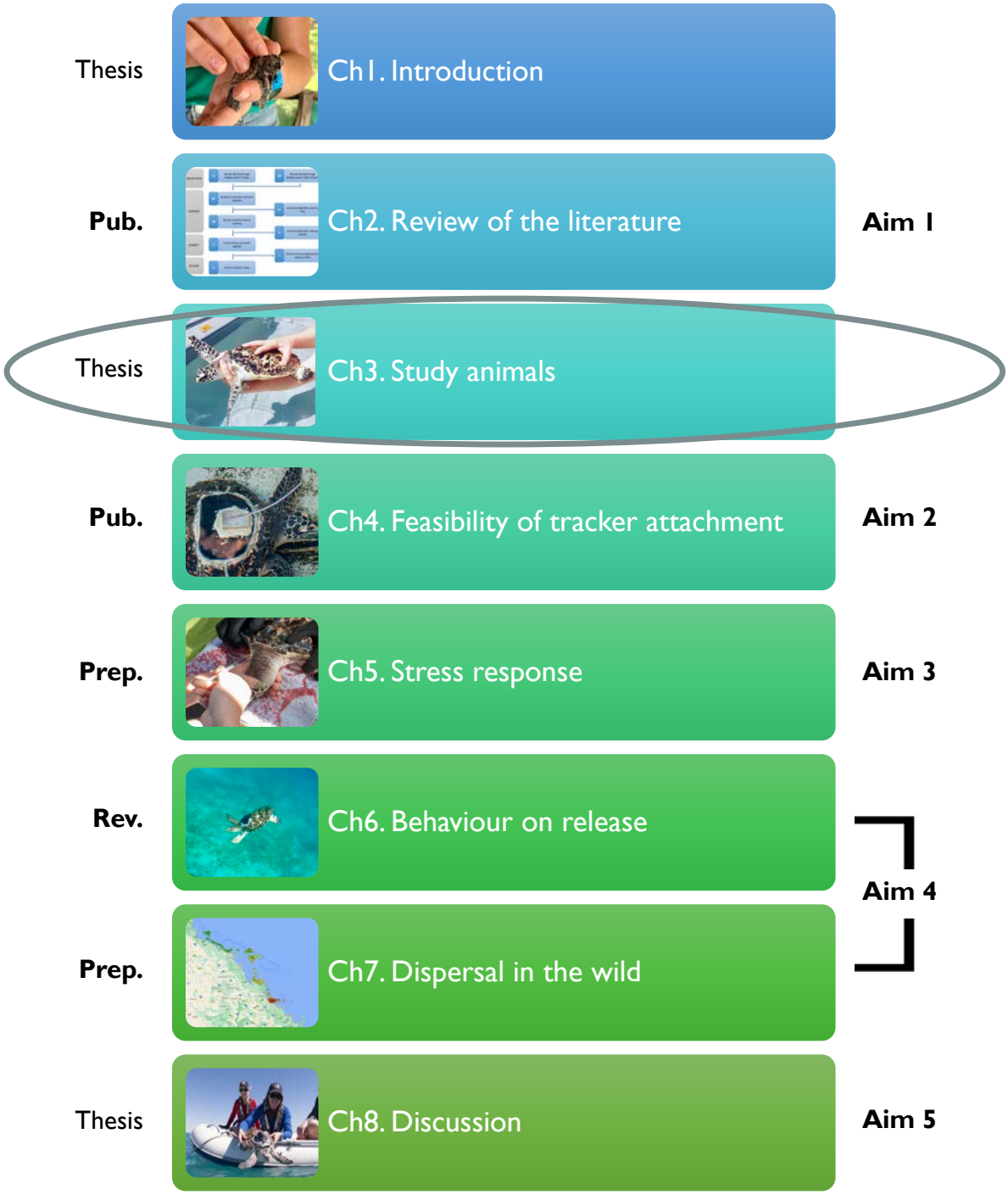
- Identify welfare metrics that can be used to assess the readiness of sea turtles under human care to be released into the wild.

Physical health can be inferred via evaluation and monitoring of behaviours because presence of injury or disease can affect activity levels and stress response and some neurological diseases result in distinct behavioural changes. Nutritional health can be inferred from appetite and daily defecation rates as well as the body position of the turtle (i.e. propped up on flippers and alert or flat on tank floor and weak). Environmental health with regards to poor salinity, temperature or water quality can be indicated behaviourally by a change in activity level of the turtles. Furthermore, turtles should be housed in an environment that stimulates natural behaviours such as resting under a shelter. Behavioural health itself can be assessed by time engaged in positive versus negative behaviours, where positive behaviours are represented by time engaged in naturalistic behaviours as observed in sea turtles living in the wild. Mental affective state can be inferred by absence or reduction of stereotypic behaviours.

Overall, behavioural assessments may be the most feasible measure of welfare across community-based conservation settings. Furthermore, there are additional welfare metrics that can be easily considered in all settings. Physical health checks should include visual inspection for disease or injury and nutritional health assessment should consider growth rate and body condition of the turtles. In research settings there is also capacity to test biochemical stress markers (physical health) which, in combination with the abovementioned metrics, can provide a more holistic understanding of sea turtle readiness for release. These metrics were applied to the hawksbill turtles studied in this thesis to assess their readiness for release into the wild, documented in the following chapter.

# CHAPTER 3: STUDY ANIMALS

## Thesis structure



# Background and aim of this chapter

## Rationale

The same group of 11 hawksbill sea turtles (*Eretmochelys imbricata*) were studied in each subsequent experimental chapter of this thesis (Chapters 4 – 7). This chapter presents the details of their collection, care, and release, including the assessment of their readiness for release into the wild. Methods that are specific to the individual investigations reported in this thesis are presented in detail in the relevant chapter.

### Chapter aims

- Describe, in detail, the collection, care (husbandry and welfare considerations), and release of the study turtles.
- Outline the assessment used to determine the turtles' readiness for release into the wild.

# Turtle collection, care, and release

## Permits, support, and collaborations

Research supporting this thesis strictly adhered to the James Cook University (JCU) Animal Ethics approval (A2586) and was conducted under the permissions and approvals of the Queensland Government's Department of Environment and Science (DES, approval: WA0012830) and Great Barrier Reef Marine Park Authority (GBRMPA, Marine Parks Permit: G20/44009.1). Collection of the turtles was facilitated by DES, trackers were attached in collaboration with rangers from the Gudjuda and Girringun Aboriginal Corporations, and turtles were released with support of the Manbarra Elders of Palm Island, Queensland, Australia.

## Turtle collection

The 11 hawksbill turtles studied in this PhD were collected as hatchlings from the North Queensland (nQld) hawksbill stock on Milman Islet, in the northern section of the Great Barrier Reef, Queensland, Australia (11.167°S, 143.017°E). They were collected on 17 March 2019 as part of a regular turtle monitoring project conducted by DES with volunteers from the JCU Turtle Health Research (THR) group and other research students. The hatchlings were collected as they neared the water, after emerging from the same nest and undertaking their natural run to the ocean. As the collected hatchlings emerged from their nest in daylight, it is very likely that mortality would have been high due to possible dehydration, thermal stress, and predation

(Pankaew & Milton, 2018; Tomillo et al., 2010). Accordingly, the collection of these hatchlings is likely to have had no impact on reproductive output from the island. The collected hatchlings were kept cool and hydrated in a dark crate with moist towels during transportation to JCU, Townsville, Australia. On arrival at the JCU THR facility, hatchlings were placed in individual tanks and left to acclimatise to their new habitation for 9 days before feeding was slowly initiated (Higgins, 2002) with small pieces of shrimp less than the size of their head. The volume of feed was increased from one piece to ten pieces over 2 weeks. Once faeces were observed in each tank, the turtles were transitioned to a composite gel diet as outlined below and detailed in the facility's husbandry manual (Turtle Health Research, 2021). There was an additional twelfth hawksbill hatchling collected with the 11 documented in this thesis; however, it failed to thrive and was euthanised as a welfare measure to prevent suffering. The necropsy and pathology results indicated an undetermined cause of illness. As such, throughout this thesis, chapters will only refer to the 11 turtles that were collected, studied, and released into the wild.

## Turtle care

### The facility

The purpose of JCU's purpose-built research facility, "the Caraplace", is to enhance our understanding of sea turtles during their cryptic developmental phase, known as "the lost years" (Mansfield et al., 2021). The research facility was designed to house hatchling to post-hatchling turtles individually for up to 2 – 3 years. The number of turtles that can reside at the facility at any one time is dependent on their size and expected growth rate (species-specific) because the turtles are allocated larger tank space as they grow (approximately 50 – 1,000 L).

Seawater within each self-contained, open-air tank system was recirculated, filtered (50-100 µm), and sterilised (40 W ultraviolet bulb). Seawater temperatures in all tank systems were changed seasonally (25 – 30°C) to simulate the natural, seasonal oscillation of shallow water reef temperatures in the Great Barrier Reef (Australian Institute of Marine Science; Lough, 1998). Throughout all investigations undertaken as part of this thesis research, the hawksbill turtles were housed individually across three tank systems. Each tank system comprised two raceways containing either one or two turtles per raceway, separated by a divider (Fig. 3.1). There were fixed cameras permanently situated above two of the tank systems to record the turtles continuously, day and night (Fig. 3.2). Data were accessible via Nx Witness Client (5.0.0.36634).

*Figure 3.1. Hawksbill turtles in their tanks, either two turtles sharing one raceway separated by a divider (left) or only one turtle in the raceway (right). Tank measurements for two turtles sharing one divided raceway were approximately 1.5 m (length) x 0.6 m (width) x 0.4 m (depth).*

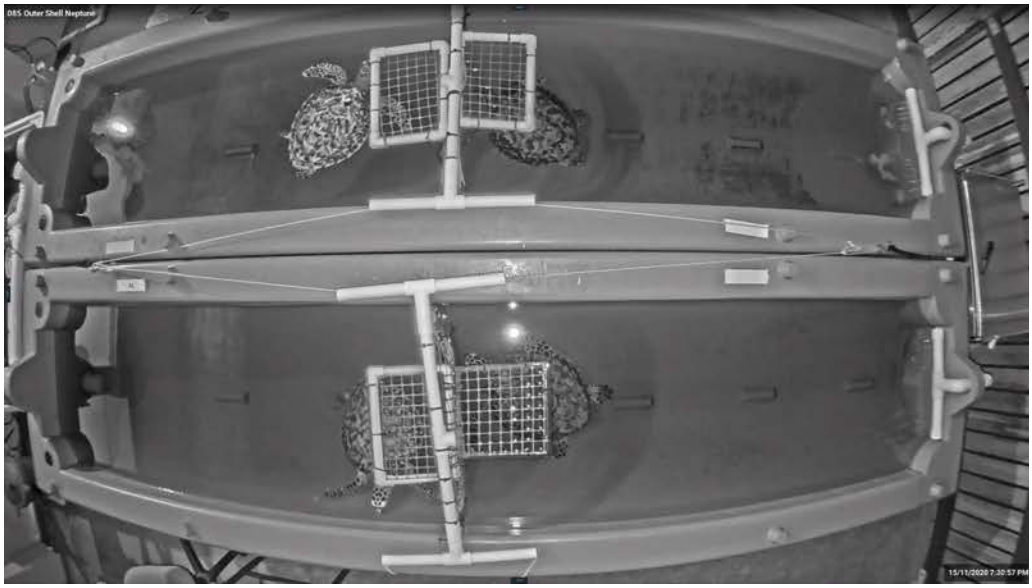



*Figure 3.2. Night view of turtles in one self-contained tank system, "Neptune", captured by fixed camera and viewed in Nx Witness Client.*

## Husbandry and welfare considerations

The hawksbill turtles were cared for under the strict guidelines of the facility's husbandry manual (Turtle Health Research, 2021) and standard operating procedures (WLD-16). A team of 20 – 40 trained volunteers cared for the turtles at the facility. Training was monitored by the facility manager and each day's husbandry team had an experienced Team Leader to organise tasks. I started as a volunteer at the Caraplace in 2016 and was training and coordinating volunteers by

the following year. Prior to commencing my doctoral candidacy, I attended and organised numerous field trips where I gained experience in turtle handling and sample collection from several species of sea and freshwater turtle. Prof. Ellen Ariel and I, along with the facility manager, modified the handling techniques as the hawksbill turtles grew to ensure the safety of the turtles and volunteers.

The hawksbill turtles were fed a mixed diet of blended fish (human-grade fillets and whole fish) and green vegetables, formulated into a cube with a gelatine binder. The food cubes, which also contained supplementary vitamins, were cut into smaller pieces for feeding and total feed weight was determined by the weight of the turtles (daily feed was 3 – 5% of their body weight, updated fortnightly). Turtles were fed Monday to Friday and fasted on Saturday and Sunday (Higgins, 2002), and were not fed directly by hand but rather food was dropped or thrown into the tanks whilst volunteers stood back. This feeding method was implemented to promote a disassociation between humans and food so that the hawksbill turtles would have a greater chance of survival when released into the ocean (Diggins et al., 2022; Smulders et al., 2021). Furthermore, this method aimed to encourage the turtles to forage for food as they would in the wild. Foraging was further encouraged by only partially removing the algae that grew naturally in the tanks.

In addition to preparing the food and feeding the turtles, volunteers cleaned the housing systems, checked the water quality in each system (temperature, salinity, nitrate levels), and monitored the health of the turtles. Typically, husbandry occurred Monday to Friday with only brief checks for equipment function and turtle welfare made on weekends. Turtle health was monitored via daily in-water observations and fortnightly out-of-tank inspections and indicated by changes in their behaviour or physical health (visual signs of disease or injury). This combination of daily and fortnightly monitoring was used to ensure that any changes could be quickly reported, an intervention implemented (if required), and improvement monitored. Throughout their time under human care, several different environmental enrichment devices (EEDs) were introduced to the turtles under the supervision of volunteers and Team Leaders. EEDs were only used periodically to maintain the novelty of each device. Three types of EED were used (Diggins et al., 2022): feeding (to encourage foraging), tactile (to encourage grooming and investigation), and structural (for resting and head hiding).

Growth of the turtles was also recorded as an indicator of welfare in conjunction with monitoring of the turtles for consistency in food intake and defecation. Turtle growth was recorded fortnightly, with measuring and weighing conducted concurrently to minimise handling of the turtles. Straight carapace length (SCL) of the turtles was measured using callipers for the first few

months, after which, curved carapace length (CCL) was measured with a soft measuring tape. Weight and CCL of the turtles throughout the investigations of this thesis are detailed in each relevant chapter. However, by the end of their 25.5 months at the research facility, the hawksbill turtles had grown to 357 – 444 mm (median 389.0 mm; IQR 373.5 – 404.5 mm) CCL and weighed 4,445 – 7,830 g (median 5,760.0 g; interquartile range (IQR) 5,013.8 – 6,926.3 g). Hawksbill turtles typically recruit to neritic foraging grounds in Queensland with a CCL of 35 cm or more (Chaloupka & Limpus, 1997); therefore, the study turtles were deemed to be a suitable size for release in a nearshore reef.

## Release

### Assessment of readiness for release

Readiness for release was based on the assessment outcomes of overall welfare and holistic fitness of the turtles following the Five Domains Model of welfare (Mellor, 2017). A positive overall outcome in welfare assessment was considered indicative of turtles having sufficient potential survivability for living in the wild once released. Under each of the five domains – physical health, nutrition, environment, behaviour, and mental state – surrogates of health were identified (Table 3.1). The physical health and nutrition domains are closely interrelated given that poor nutrition often leads to poor physical health and increased disease susceptibility (Manire et al., 2017b). With respect to this, low activity levels and poor posture may also be measures of a weakened state indicative of poor nutrition (Manire et al., 2017b). However, since it would not be possible to distinguish whether poor nutritional welfare was solely responsible for the activity level and posture without other underlying physical health issues, these parameters were included in the assessment only once, under the physical health domain.

*Table 3.1. Parameters, methods, and summary of outcomes for surrogates of health under the Five Domains Model (Mellor, 2017) of welfare for a group of juvenile hawksbill turtles raised under human care for 2 years. Assessment outcomes indicate that turtles were good candidates for release into the wild.*

| Welfare Domain | Surrogate | Parameter | Method | Assessment Outcome |
|---|---|---|---|---|
| **Physical Health** | Injury/disease status | Presence/absence of lesions, fractures, abrasions, etc. | Physical examination by a veterinarian | No indication of injury or disease |
| | | Activity level | Visual observation | Turtles had good buoyancy and were not lethargic |

| | | | | |
|---|---|---|---|---|
| | | Body posture | Visual observation | Propped up on flippers and alert |
| | | Neurological disease behaviour (swimming in circles) | Visual observation | No sign of neurological disease |
| | Systemic stress response | Corticosterone and lactate concentration (also packed cell volume) | Blood sampling | Turtles showed stress response to and recovery from acute and extended stressors |
| **Nutrition** | Steady growth | Mass and length over time | Morphometrics | Growth rate faster than published estimates of wild hawksbill turtles* |
| | Good appetite | Daily feeding /defecation | Visual observation | Turtles consumed all their food and regularly defecated |
| | | Body condition index (BCI) | Morphometrics | Turtle BCIs ranged from 1.03-1.37, indicative of good body condition in small juvenile hawksbill turtles** |
| **Environment** | Naturalistic interaction with structural environment | Time engaged in naturalistic resting behaviour | Activity budget | Most resting time spent under the provided structures |
| | Appropriate environmental conditions | Salinity, temperature & water quality readings | Water testing | Environmental conditions remained stable except temperature which followed natural seasonal variation |
| **Behaviour** | Naturalistic behaviours | Ability to feed, surface to breathe, rest, and swim with good buoyancy | Ethogram | Turtles displayed all behaviours necessary for survival post-release |
| | | Fear response to human presence via change in activity level | Visual observation | Turtles had higher activity levels with increased human presence |
| | | Behavioural response to acute stress via increased movement | Visual observation | Turtles engaged in more frenzied movement after stressor and later |

| | | | | |
|---|---|---|---|---|
| | | | | returned to calm movement |
| **Mental State** | Positive affective state | Presence/absence of stereotypic behaviours | Ethogram | No behavioural indication of chronic stress |

* Diggins et al. (2023)

** Nishizawa and Joseph (2022)

## Physical health outcomes

From their physical health assessments, the turtles were found to be in good physical health. They also showed a systemic response to and recovery from stressors (detailed in Chapter 5). There were no physical or behavioural signs of disease or injury; the turtles had no fractures, lesions, abrasions, and had a good general level of activity as well as alertness when resting. Furthermore, there were no signs of neurological disease such as swimming in circles, which is thought to be caused by parasites in the brain (Manire et al., 2017b).

## Nutritional welfare outcomes

The nutritional assessments of appetite and growth showed that the study turtles grew faster than predictive growth models for wild hawksbill turtles (Diggins et al., 2023) and that they had good body condition indices for hawksbill turtles of their size-class (Nishizawa & Joseph, 2022). The higher growth rate of the captive turtles may be partially explained by limited data for wild hawksbill turtles in this specific size-class and is also likely due to provision of high-quality food, lack of predators, suitable environmental conditions, and reduced energy expenditure. The growth rate was not considered to be problematic for release because the outcomes of appetite assessment were positive. The regularity of feeding and defecation indicated no gut impaction and the good body condition indices show that the turtles were not underweight nor overweight for their size and species (Nishizawa & Joseph, 2022).

## Environmental welfare outcomes

Environmental assessments included assessment of the water quality in which the turtles lived as well as their interactions with structures in their tanks. Water temperature, salinity, and quality were tested regularly (see Husbandry and welfare considerations) and found to be consistent, except for water temperature which was adjusted to follow seasonal temperatures. For the environmental interaction, sea turtles in the wild often rest under coral ledges or rocks (Matley et al., 2021); therefore, environmental welfare of turtles under human care was considered positive when the turtles used a structure in their tank to rest under. The study turtles were

observed spending much of their time under human care resting under the waterfalls and platforms, comparable to their expected natural behaviours, and so this was assessed as positive.

## Behavioural welfare outcomes

Behaviours essential to survival in the wild include the ability to forage and feed, surface to breathe, rest, and swim with good buoyancy (Jeantet et al., 2018). The turtles were observed regularly displaying each of these behaviours whilst under human care. Although the turtles were fed regularly, they were also observed grazing on algae that was growing in the tanks (Bell, 2013). Also essential to post-release survivability is the retention of a behavioural response to unknown stressors, representative of predator avoidance behaviour (Preston et al., 2020), and fearfulness of humans (Hayes et al., 2017). Observed changes in the displayed behaviours and activity levels of the turtles in response to increased human presence and unknown stressors were considered positive indicators that these naturalistic behaviours had been retained.

## Assessment of mental state and overall readiness for release

Stereotypic behaviours identified in Diggins et al. (2022) as being indicative of chronic stress were not observed in the study turtles by the husbandry volunteers, facility manager, or researchers whilst the turtles were under human care. Chronic stress can also be inferred via an allostatic load index, which show the physiological response of humans and non-human animals to cumulative strain over time (Edes et al., 2018; Seeley et al., 2022). Whilst two biomarkers of stress have been evaluated in this thesis (Chapter 5), an allostatic load index was not determined.

Overall, the assessment outcomes under each welfare domain discussed above were considered generally positive. The final part of the pre-release assessment was a clinical health check by an experienced veterinarian (wild animals including turtles) who declared the turtles fit for release.

## Release protocol

Prior to release, a satellite tracker (Wildlife Computers) and a titanium flipper tag (Stockbrands Company, Pty.Ltd., Perth, Western Australia; day of) were attached to each turtle. Satellite trackers were attached 5 days prior to release per the protocol detailed in Chapter 4 (Diggins et al., 2023) to monitor turtle dispersal post-release (Chapter 7). The titanium tags were supplied by DES and attached to the trailing edge of the flipper on the day of release. During transportation from the research facility to the release site, the hawksbills were kept separate, each contained in a 54 L tub with a modified lid (small cut-out window to allow air circulation and visual checks of the turtles) and cocooned in warm, damp towels. Mode of transportation was via car from the

research facility to the Townsville marina and then from the marina to the release site via the "Reef Sentinel" (Queensland Parks and Wildlife Services' vessel). Turtles were visually monitored for signs of distress or discomfort (excess movement: flipper flapping or attempts to climb out the tub) throughout the transportation process and calmed by gently covering their eyes.

The release site was John Brewer Reef (Fig. 3.3), Queensland, Australia (18.633°S, 147.067°E). Selection of this reef was based on: 1) proximity to the Caraplace; 2) distance from the main, populated coastline (approximately 74 km offshore); and 3) consultation with GBRMPA and DES regarding logistical, ecological, and social considerations. Turtles were released within the lagoon at John Brewer Reef, which was approximately 5 m deep and comprised a mixture of sand, coral rubble and coral bommies. The Reef Sentinel was anchored in the sand and turtles were released from the tender, which trailed 20 m off its stern attached by a rope. For the first two turtles released the tender was in neutral and the main vessel had its engine switched off; for all releases following, both motors were off. This set up was designed for safety and to maintain stability and continuity whilst minimising environmental damage to the corals and disturbance to the turtles.


*Figure 3.3. Map showing turtle collection, housing, and release sites for the 11 hawksbill turtles studied in this thesis. Map modified from Google Maps (Imagery ©2023 TerraMetrics, Map data ©2023 Google).*

The turtles were released over 2 days, 6 – 7 May 2021, with six turtles released the first day and five the second. The release was organised this way to maintain positive welfare of each turtle while collecting high-quality data. Turtles were released and observed between 10:00 and 14:00 each day. Three in-water observers filmed each individually released turtle using a combination of GoPro cameras (Hero 3, Hero 4, and Hero 8); one snorkeler filmed from the surface whilst two divers followed the depth of each turtle (Chapter 6). The release strategy implemented for the hawksbill turtles was based on a proof of concept involving a clutch of green turtles (*Chelonia mydas*) that had been previously collected and housed at the research facility. When the green turtles were released at John Brewer Reef (1 year prior to the hawksbill turtle release), they were observed displaying naturalistic behaviours (Department of Environment and Science et al., 2019).

## Summary of chapter aims

### Chapter aims

#### Chapter aim 1

- Describe, in detail, the collection, care (husbandry and welfare considerations), and release of the study turtles.

Newly hatched and emerged hawksbill turtles were collected from Milman Islet and transported to the James Cook University Turtle Health Research facility. Husbandry protocols, ethics considerations, and appropriate permits have been documented in detail. The turtles were released offshore at John Brewer Reef after approximately two years under human care.

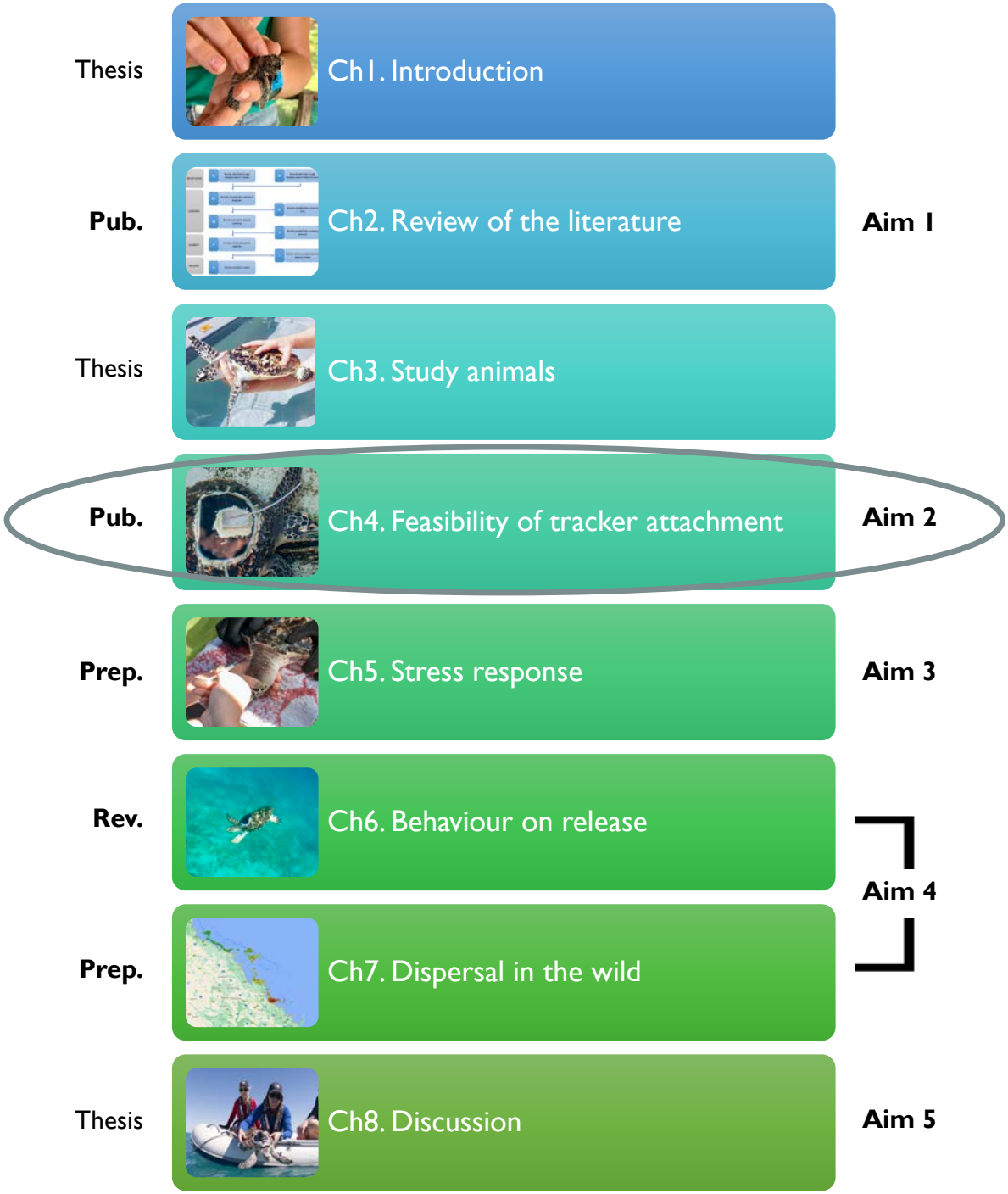
#### Chapter aim 2

- Outline the assessment used to determine the turtles' readiness for release into the wild.

Assessment of readiness for release into the wild was conducted following the Five Domains Model of welfare, in which each aspect of the turtles' welfare (physical health, nutrition, environment. Behaviour, and mental state) was considered. Overall, the turtles were deemed to have good welfare and potential survivability for living in the wild and were therefore considered to be ready for release.

# CHAPTER 4: TRACKER ATTACHMENT FEASIBILITY

## Thesis structure



# Background and aims of this chapter

## Rationale

To fulfil the aims of this thesis, satellite trackers had to be attached to the carapace of each study turtle prior to release. However, no method of satellite tracker attachment specifically adapted for small juvenile hawksbill turtles had been previously published. As such, it was necessary to determine a suitable method of attachment to ensure longevity of the trackers whilst maintaining positive welfare for the turtles.

## Thesis aim 2

Optimise method of attaching satellite trackers to small juvenile hawksbill turtles for post-release monitoring without jeopardising turtle welfare.

### Chapter aims

- Develop, test, and confirm a protocol for the successful attachment of satellite trackers to small juvenile hawksbill turtles where success was defined by:
    - trackers remaining firmly attached for more than 3 months; and
    - attachment method leaving minimal scute damage or disfigurement whilst allowing turtles to continue growing.

## Research outputs

**Diggins** RL, Grimm J, Mendez D, Jones K, Hamann M, Bell I, and Ariel E (2023). Confirmed feasibility of a satellite tracker attachment method on small juvenile hawksbill turtles *Eretmochelys imbricata*. *Marine Ecology Progress Series*, 704, 119-130. https://doi.org/10.3354/meps14216.

**Diggins** RL (2021, August 13). *Tracking turtle toddlers: Attaching satellite trackers to juvenile hawksbills* [Oral presentation]. College of Public Health, Medical and Veterinary Sciences Three Minute Thesis 2021, Townsville Australia. (https://vimeo.com/583412810).

**Diggins** RL, Grimm J, Mendez D, Jones K, Hamann M, Bell I, and Ariel E (2021, December 9). *One size does not fit all: Satellite tracker attachment methods for small juvenile hawksbills* [Oral Presentation]. College of Public Health, Medical and Veterinary Sciences Higher Degree by Research Student Conference 2021, Townsville, Australia.

# Confirmed feasibility of a satellite tracker attachment method on small juvenile hawksbill turtles (*Eretmochelys imbricata*)

**DOI: https://doi.org/10.3354/meps14216**

## Introduction

Conservation of sea turtles requires an understanding of their ecology to develop effective management measures (Levy et al., 2017; Rees et al., 2016; Yeh et al., 2021). Sea turtle behaviour is specific to each life-stage (Bolten, 2003; Mansfield et al., 2021; Mansfield et al., 2014) and species (Plotkin et al., 2002), necessitating the study of every species, at each life-stage, and often in different regions. Yet, because the non-uniformity of threats in space and time across the range of the species is not evenly represented in the literature, there have been several studies advocating for expanded research in understudied species and age-classes (Godley et al., 2008; Hazen et al., 2012). Sea turtle habitat usage and behaviour are often inferred via platform-based observations or telemetry data (Hart et al., 2012; Hays & Hawkes, 2018; Horrocks et al., 2001; Robinson et al., 2020). Satellite tracking is one of the most commonly used techniques; however, published sea turtle satellite tracking studies have predominantly focused on adult-sized turtles, primarily loggerheads (*Caretta caretta*) and green turtles (*Chelonia mydas*) (Godley et al., 2008). While the methods for attaching satellite tags to adult-sized turtles are well established (Balazs et al., 1996), published studies often lack detail on the tracker attachment method and do not specify whether any methodological amendments were made to account for species or life-stage differences. Recently, there has been an increase in studies tracking smaller size-classes of marine turtles and investigating more appropriate attachment methods and tracker types based on turtle life-stage (Mansfield et al., 2017; Mansfield et al., 2021; Mansfield et al., 2014; Pabón-Aldana et al., 2012; Putman & Mansfield, 2015). This expansion of research is important because optimising a safe and durable satellite tracker attachment method for all sea turtles, specific to species and size-class, would help improve tracking outcomes and help clarify ontogenic movement patterns and foraging ground usage.

Sea turtles have different diets, habitats, growth rates, and morphology throughout their development (Bolten, 2003). These distinctive characteristics define the life-stage of the turtle, and for immature turtles, they generally correlate with turtle size (Van Buskirk & Crowder, 1994). There is some consensus on the approaches used to determine the minimum size of maturity, with variation between and within species (Bjorndal et al., 2014; Phillips et al., 2021). However, the term 'juvenile turtles' is more subjective and has been used to refer to many different stages

of immature turtles (Morafka et al., 2000), including dispersal stage turtles (neonates, i.e. those in their first year of life; or post-hatchlings, i.e. offshore oceanic stage) and post-dispersal stage turtles recently transitioned to neritic foraging habitats (new recruits), and sub-adults (Bolten, 2003; Crouse et al., 1987).

One key difference influencing the choice of satellite tracker attachment method in juvenile hard-shelled turtles compared to adult-sized turtles is variation in carapace morphology (Wyneken, 2001). The standard method of attachment for hard-shelled turtles, based on Balazs et al. (1996) is a direct attachment of the satellite tracker to the keratinised carapace scute using an epoxy-based adhesive, with or without strips of fibreglass for extra stability. However, this method is difficult to use in juvenile turtles because: 1) the base area of the tracker can be larger than the scute size; 2) the carapace morphology of smaller-sized juvenile turtles, such as post-hatchlings, is such that they often have a raised ridge of vertebral scutes (Mansfield et al., 2012); and 3) juvenile turtles are still growing, and growth can affect tracker retention. Furthermore, this epoxy-based method is unsuitable for use on other species such as leatherback turtles (*Dermochelys coriacea*), or flatback turtles (*Natator depressus*) (Fossette et al., 2008; Sperling & Guinea, 2004).

Attaching satellite trackers to small-sized juvenile turtles such as dispersal stage or those recent recruits to neritic habitats presents a further issue. Although smaller and lighter satellite trackers have been developed, attachment methods need to account for the accelerated growth rate in these earlier life-stages compared with later ones (Bellini et al., 2019). Attachment techniques developed for adult turtles use hard epoxies, which if used on juvenile turtles could inhibit growth rates, hence a requirement for some level of flexibility in the attachment method has been identified (Mansfield et al., 2012; Seney et al., 2010). Furthermore, if an attached tracker spans multiple scutes, traditional direct attachment methods could jeopardise scute growth and shape since scutes grow marginally (Wyneken, 2001). Additional challenges relate to carapace morphology. 1) Oceanic dispersal stage loggerhead and Kemp's ridley (*Lepidochelys kempii*) turtles and new recruit (post-dispersal) hawksbill (*Eretmochelys imbricata*) turtles have a distinct vertebral ridge running the length of the carapace (Fig. 4.1a); 2) in juvenile hawksbill turtles the first vertebral scute may be convex (RD, EA personal observation; Fig. 4.1b); and 3) the carapace is uniquely formed of overlapping scutes (Salmon et al., 2018), which are slightly raised at the trailing edge (Fig. 4.1c). Consequently, the uneven surface of their carapace reduces the available flat surface area for best contact with the satellite tracker. To counter this, a high volume of epoxy would be required to build a flat area, adding weight, height, and surface area to the attachment site, as well as producing extra heat during epoxy curing. Thus, tracker attachment techniques for smaller-sized juvenile turtles require testing to develop techniques that minimize potential shell damage

and do not impede growth or cause negative behavioural changes (Mansfield et al., 2017; Mansfield et al., 2021; Mansfield et al., 2014; Putman & Mansfield, 2015).

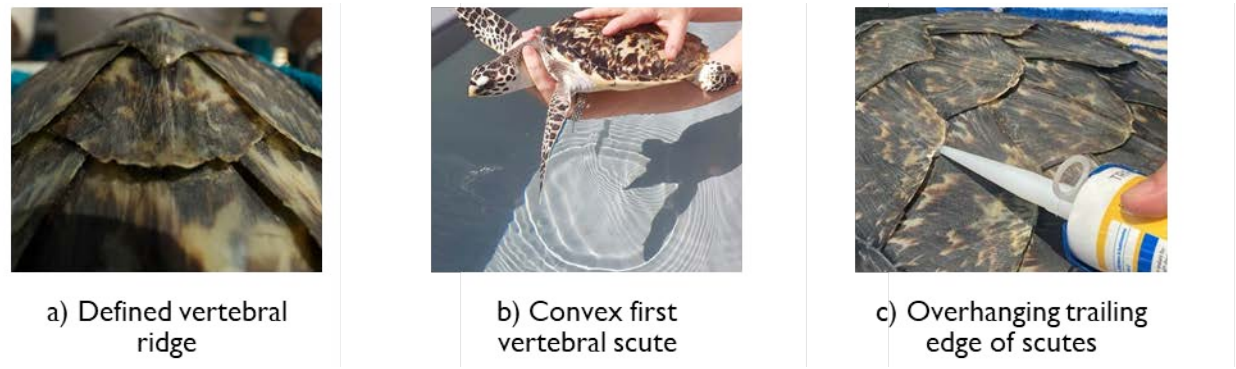



*Figure 4.1. Three morphological variations of a juvenile hawksbill turtle carapace that make satellite tracker attachment challenging: a) defined vertebral ridge; b) convex first vertebral scute; and c) overhanging trailing edge of scutes.*

The challenge of attaching satellite trackers to smaller-sized juvenile hawksbills is similar to those when designing techniques to track juvenile loggerhead and green turtles as well as oceanic-stage Kemp's ridley turtles (Seney & Landry Jr, 2011). The published methods of satellite tracker attachment for these species found that the optimal techniques differed between green and loggerhead turtles (Mansfield et al., 2021), and both techniques (Mansfield et al., 2012; Seney et al., 2010) could be used as a starting point to explore options for attachment to smaller-sized juvenile hawksbills. For example, Mansfield et al. (2012) tested four direct attachment methods and two indirect attachment methods for solar satellite trackers on neonate loggerhead turtles (12 – 25 cm straight carapace length (SCL)). They concluded that both indirect attachment methods used on the neonate loggerhead turtles were deemed unsuitable as they restricted normal growth (Mansfield et al., 2012) and it would be reasonable to assume the same problem would occur if these methods were used to attach tags to similarly sized juvenile hawksbill turtles. From Mansfield et al. (2012) study, the method that yielded the best adhesion over time for loggerheads used a neoprene-silicone attachment on an acrylic base coat. This method of attachment was also thought to mitigate the challenge of carapace unevenness in juvenile hawksbill turtles and enable longer attachments.

A technique by Seney et al. (2010) used a combination of epoxy-based adhesive and a neoprene base to allow for the growth of new recruit-sized juvenile loggerhead turtles. This method could be transferable to comparably sized juvenile hawksbill turtles; however, the unique scute morphology of juvenile hawksbill turtles necessitates validation of the method as some

modifications may be required to reduce the development of perimeter gaps between the neoprene and carapace (Seney et al., 2010) and enable longer attachment duration. Hence the aim of this study was to optimise and test the Seney et al. (2010) protocol for the attachment of satellite trackers to small-sized juvenile hawksbill turtles (similar in size to new recruits from the same population) by confirming that trackers remained firmly attached for more than 3 months and caused minimal scute damage or disfigurement after tracker removal.

## Materials and methods

### Study animals

Eleven hawksbill turtle hatchlings were collected from Milman Islet, Queensland, Australia (11.167°S, 143.017°E) on 17 March 2019 with authorisation from the Department of Environment and Science (Permit reference: WA0012830). Hatchlings emerged naturally from one nest and were allowed to run the course of the beach but were collected before entering the water. The hatchlings were transported to James Cook University (JCU), Townsville, Australia, and raised in a purpose-built facility following the facility's husbandry manual and standard operating procedures (WLD-16) under JCU Animal Ethics permit A2586. Turtles were housed individually in open air, recirculating seawater systems. The water was filtered under a 40 W ultraviolet bulb and maintained at seasonal temperatures ranging from 25 – 30 °C to reflect natural temperature variation on the Great Barrier Reef (Lough, 1998). Prior to the commencement of the trials, the turtles in this study weighed between 1,990 and 3,890 g (median 2,940 g; IQR 2,467.5 – 3,265 g) and had a curved carapace length (CCL) of 267 – 345 mm (median 314 mm; IQR 297 – 328 mm). For comparison, in Queensland, the size range of hawksbill turtles classed as new recruits to the foraging habitats is 322 – 418 mm CCL (Limpus, 1992b; Limpus et al., 2008), hence classifying them as small juveniles (Robinson et al., 2021).

### Turtle diet and growth observations

Throughout the study, the turtles were fed a blend of human-grade whole fish, fish fillets, mixed vegetables, and gelatine, combined with vitamins and solidified into cubes for consumption. Turtles were fed between 3 and 5% of their body weight on weekdays, with the aim of all turtles weighing at least 5 kg by release. Growth was measured as an indicator of welfare rather than for a specific growth study so individual feed amount was not considered critical. Turtles were weighed every 1 – 2 weeks throughout the study to ensure continuous growth and adjust feed quantities accordingly. Daily observations of the turtles were noted for welfare monitoring, and weekly checks of the trackers were also recorded throughout the study to look for signs of tracker

dislodgement or formation of perimeter gaps in the neoprene. If the trackers had detached during the 3-month test period (per the study objective), or turtle behaviour indicated reduced welfare, this attachment methodology would have been discontinued and rejected. Turtles were measured periodically from when they arrived at the JCU facility to when the trial commenced. Turtles could not have CCL measured whilst replica trackers were attached, but CCL was measured again at the end of the trial period after replica tracker removal. SCL was not recorded during this experiment. We applied a linear mixed effect model with treatment, and a random intercept of individual turtle ID, to turtle growth data from 17 March 2020 to the date of replica tracker attachment to predict turtle growth rates for the period of tracker attachment (CCL in mm/day). Data were modelled using R 4.1.1 (R Core Team, 2023), with tidyverse (Wickham et al., 2019) and lubridate (Grolemund & Wickham, 2011) packages in RStudio 1.4.1717 (RStudio Team, 2023).

## Replica tracker attachment

Replica trackers modelled after genuine satellite trackers were constructed and used in this proof-of-concept study. Protite Clear Casting and Embedding Resin and a metal wire antenna were used to create replica trackers matching the approximate shape, dimensions, and weight of the SPOT-387 satellite tracker produced by Wildlife Computers (59 x 29 x 23 mm, 39 g) (Fig. 4.2). One turtle, the heaviest at the time, had its replica tracker attached on 2 November 2020. The remainder were attached on 27 November 2020 (n= 6) and 28 November 2020 (n= 4).

a) Replica tracker b) Wildlife Computers SPOT-387 tracker

*Figure 4.2. Comparison of a) replica tracker used in this study and b) SPOT-387 satellite tracker manufactured by Wildlife Computers.*

## Carapace preparation prior to attachment

The turtles were scrubbed with a toothbrush and fresh water 2 days prior to tracker attachment to reduce the time spent cleaning on the day of attachment. The first three vertebral scutes and first two pairs of costal scutes were 'flossed' using a thin damp cloth to remove algae and other debris accumulated under the overlapping scutes (Fig. 4.3a). On tracker attachment day, each turtle was dried and then weighed (g) and measured (±1 mm CCL). After weighing, turtles were placed on top of a clean towel on a table and held in place by one person who kept the turtles' eyes covered (Fig. 4.3b) while a second person prepared the carapace and attached the trial tracker.

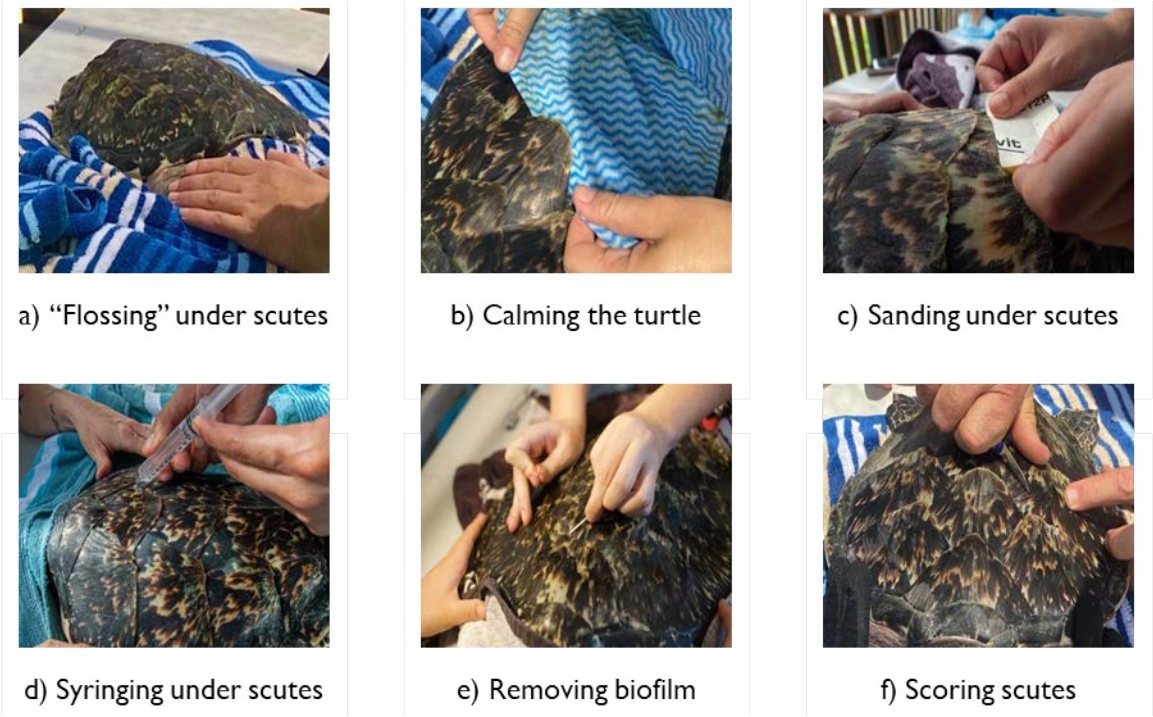



*Figure 4.3. Graphic depiction of preparing carapace for neoprene and satellite tracker attachment: a) using an absorbent cloth to "floss" underneath scutes; b) covering turtle's eyes to keep it calm during carapace prep; c) using 60-grit sandpaper to sand underneath overlapping scutes; d) using a syringe filled with fresh water to clean underneath overlapping scutes; e) removing biofilm from underneath overlapping scutes with a toothpick; and f) using a serrated pocket knife to score scutes in preparation for neoprene attachment.*

Sandpaper (60-grit, as per Seney et al. (2010)) was used on top of the first three vertebral scutes and the first two pairs of lateral scutes of the carapace and underneath overlapping scute edges to remove algal biofilm and to assist with successful adhesion (Fig. 4.3c). Sanding always occurred uni-directionally to limit damage to the brittle scute edges. After sanding, the carapace was wiped

in a craniocaudal direction with fresh water and a cloth. The gaps underneath the scutes were also cleaned by syringing freshwater (Fig. 4.3d), wiping with a thin cloth, and using a toothpick to remove as much biofilm as possible (Fig. 4.3e). This process of sanding and washing with freshwater was repeated three times to ensure the carapace was as clean as possible. Following this cleaning protocol, the first two vertebral scutes and the first two pairs of costal scutes were scored superficially with a serrated knife to create additional surface area for the neoprene and epoxy to grip (Fig. 4.3f). A new cloth was then used to wipe down the carapace with acetone (Seney et al., 2010), and the gaps underneath the scutes were also syringed and flossed with acetone one final time and air dried in preparation for epoxy application.

### Neoprene and tracker attachment

In this study, 3 mm neoprene was selected as it was comparable to other studies (Seney et al., 2010), thick enough to help level the uneven surface of the scutes and vertebral ridge, and presumably flexible enough to allow turtle growth without tracker loss. Prior to carapace preparation, neoprene was prepared by soaking in a double cycle of bleach bath (liquid chlorine as sodium hypochlorite) for 1 hour and then overnight in fresh water to disinfect the neoprene and remove any grit. Neoprene size and shape (approximately 15 x 15 cm, with rounded edges) were adjusted for each turtle individually to ensure complete coverage of the second vertebral scute and partial coverage of the surrounding 6 scutes (Fig. 4.4a). The silicone used was Sikasil® Pool as it is water, ultraviolet, weathering, and fungal resistant and has high elasticity. Silicone was prepared by discarding the volume contained within the neck of the tube and was then applied to the carapace. It was used to fill gaps under the raised scutes, ensuring that there was also a visible line of silicone along all scute edges that would be covered by the neoprene (Fig. 4.4b). The silicone was smoothed and flattened to ensure complete contact with the scutes, especially as some gaps started to form when the silicone was setting. Therefore, more silicone was added to fill any gaps that formed during drying. When the silicone was touch dry, the next phase of neoprene attachment was initiated.

The epoxy used was Sika AnchorFix®-3+ as it is one of the fixatives commonly used by researchers for satellite tracker attachment on hard-shelled turtles (Shimada et al., 2012; Shimada et al., 2016). Epoxy was prepared the same way as the silicone, by discarding a small amount before applying a thin layer to cover the second vertebral scute. This area was subsequently built up to flatten out the raised curve created by the first vertebral scute (Fig. 4.4c). Epoxy was also applied to the surrounding scutes that would be covered by neoprene, starting from the silicone lining the second vertebral scute and working outwards towards the marginal scutes (Fig. 4.4d).

Care was taken not to allow bubbles to form in the epoxy, as this would reduce adhesion. Epoxy was smoothed, and bubbles were removed by hand or by using a toothpick (Fig. 4.4e). Epoxy was applied up to the silicone edges but not atop. The epoxy was allowed to dry until it had a tacky consistency before applying the neoprene. To attach the neoprene, some pressure was applied by hand to the centre and worked concentrically and outwardly to remove air bubbles. The neoprene was held firmly in place for a few minutes to ensure complete contact with the adhesive to the carapace (Fig. 4.4f).


*Figure 4.4. Graphic depiction of lining the scutes with silicone and attaching the neoprene with epoxy: a) round shape of neoprene patch, cut to fit individual turtles; b) silicone barrier being created underneath the scutes to allow for unrestricted growth; c) applying epoxy to the first scute in attachment method; d) applying epoxy to surrounding scutes; e) using a toothpick or gloved finger to remove air bubbles from applied epoxy; and f) neoprene patch being first applied in the centre and then pressed down in an outward direction.*

After the neoprene was secured, a silicone barrier was created, outlining where the tracker would be attached (Fig. 4.5a). Epoxy was applied to the underside of the tracker (Fig. 4.5b), which was then directly applied close to the top edge of the neoprene. Additional epoxy was added around each edge of the trial tracker and up the sides of the tracker to ensure good adhesion to the neoprene. The silicone barrier assisted with the epoxy build-up (Fig. 4.5c). The epoxy was allowed

to cure for at least an hour before a final ring of silicone was added to the edge of the neoprene (Fig. 4.5d) to act as an additional barrier to algal growth under the neoprene. Turtles were kept dry-docked in individual containers for 12 hours to allow the epoxy to cure, each on a towel dampened with saltwater to prevent dehydration. Flippers were tucked at the side of the carapace and covered by a towel to prevent the animals from knocking the trial trackers or getting epoxy on their flippers during the epoxy drying time. Turtles were also monitored throughout the process for signs of distress, such as rapid flipper movement, and calmed by gently covering their eyes. Antifoulant is usually painted onto satellite trackers before deployment to reduce algal growth; however, due to potential toxicity (Amara et al., 2018), it was not applied in the trial setting of recirculated sea water. This is also why the 12 hours curing time was adhered to in this experiment. However, using the same epoxy in the field setting, it would be possible to release the turtles back into the ocean after a shorter time (approximately 6 – 8 hours) if overnight containment was not possible (EA, MH, IB, KJ, JG personal observation).

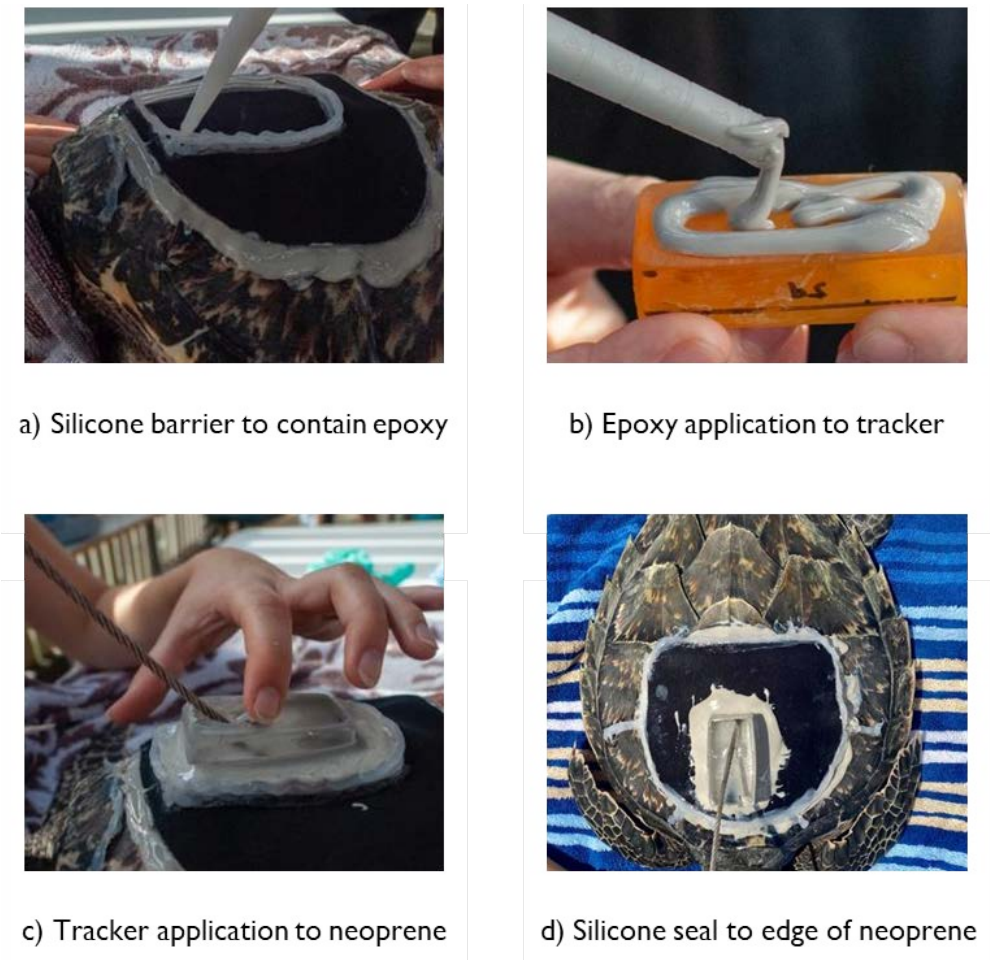



*Figure 4.5. Graphic depiction of tracker attachment to neoprene and final silicone seal added around the edge of the neoprene: a) creating a silicone dam to assist with epoxy build-up around the sides of the*

*tracker; b) epoxy applied directly to the bottom of the trial tracker; c) positioning the tracker inside the silicone dam; and d) final silicone layer added to the edge of the neoprene.*

### Replica tracker removal

As all trackers remained firmly attached at the end of the trial, the first replica tracker was intentionally removed on 1 March 2021, approximately 4 months after the attachment. The remaining replica trackers were removed over 4 days (11, 12, 16, and 17 March 2021), approximately 3.5 months after attachment. Removal of trackers occurred approximately 1.5 months before attaching the genuine Wildlife Computers SPOT 387 satellite trackers for a subsequent dispersal study. Turtles were first dried, weighed and photographed before tracker removal. After tracker removal, they were measured (CCL), photographed, and reweighed.

## Results

Replica satellite trackers were successfully attached to 11 small juvenile hawksbill turtles (267 – 345 mm CCL). The first attachment was undertaken initially to test and confirm the attachment method and the remaining ten trackers were attached within one day of each other. The attachment process took approximately 1 – 1.5 hours per turtle, excluding curing time and pre-cleaning. The total in-air weight of the tracker (39 g to replicate the SPOT-387 trackers), plus the epoxy, silicone, and damp neoprene, was 4.5 – 7.8% of turtle body weight at the start of the trial and 2.5 − 3.9% of turtle body weight at the end of the trial (average ±SE tracker and attachment material weight was 162.0 ±4.42 g).

### Attachment success

All trial trackers remained firmly attached with no evidence of neoprene peeling from the carapace or the tracker from the neoprene for at least 3 months, meeting our objective. Algae was found on the tracker and neoprene (Fig. 4.6a), but this had no noticeable effect on the adhesion. On removal of the replica trackers, the carapace scutes under the replica trackers were undamaged (Fig. 4.6b); however, the neoprene attached to the replica trackers had to be removed in pieces. Therefore, it was not possible to compare our results with the photograph of the neoprene used in the Seney et al. (2010) study.

a) Algal growth on neoprene and tracker

b) Scutes post replica tracker removal

*Figure 4.6. a) Algal build-up on neoprene and replica tracker by the end of the study and b) undamaged scutes photographed after removal of replica tracker.*

## Growth of turtles during trial

Total weight gained throughout the experiment ranged from 1640 g to 2980 g, and CCL growth ranged from 50 mm to 94 mm (Fig. 4.7). Median weight gain and CCL growth across all 11 turtles was 2230 g (IQR 1995− 2595 g) and 56 mm (IQR 52−63 mm), respectively. The weight gain per turtle from start to end of the study ranged from 67% body weight increase to 114% body weight increase (median 76%, IQR 72−84%). The CCL growth per turtle from the start to the end of the study ranged from a 14% increase to a 28% increase (median 18%, IQR 17−22%) (Table 4.1). Repeated measurements indicated a steady CCL increase (Fig. 4.8) and weight gain throughout the trial (Fig. 4.9) in accordance with each individual turtle's growth trajectory prior to the study. There was no statistical difference between the calculated daily growth (CCL in mm/day) of turtles before the replica trackers were attached and after the replica trackers were removed ($t1 = -1.674$, $p = 0.096$).

When comparing the calculated daily growth rate of the turtles during the trial (weight or CCL gained divided by the number of days in the trial), there was a median increase of 20.9 g/day (IQR 19.4 − 24.0 g) and 0.5 mm/day (IQR 0.5 − 0.6 mm) weight and CCL, respectively. Calculated daily turtle growth rates ranged from 15.2 to 25.4 g/day in weight gain and 0.5 to 0.8 mm/day in CCL increase. The calculated annual growth rate of the turtles in this trial from 1 year old to 2 years old averaged 228.4 mm/year in CCL, including the attachment trial period.

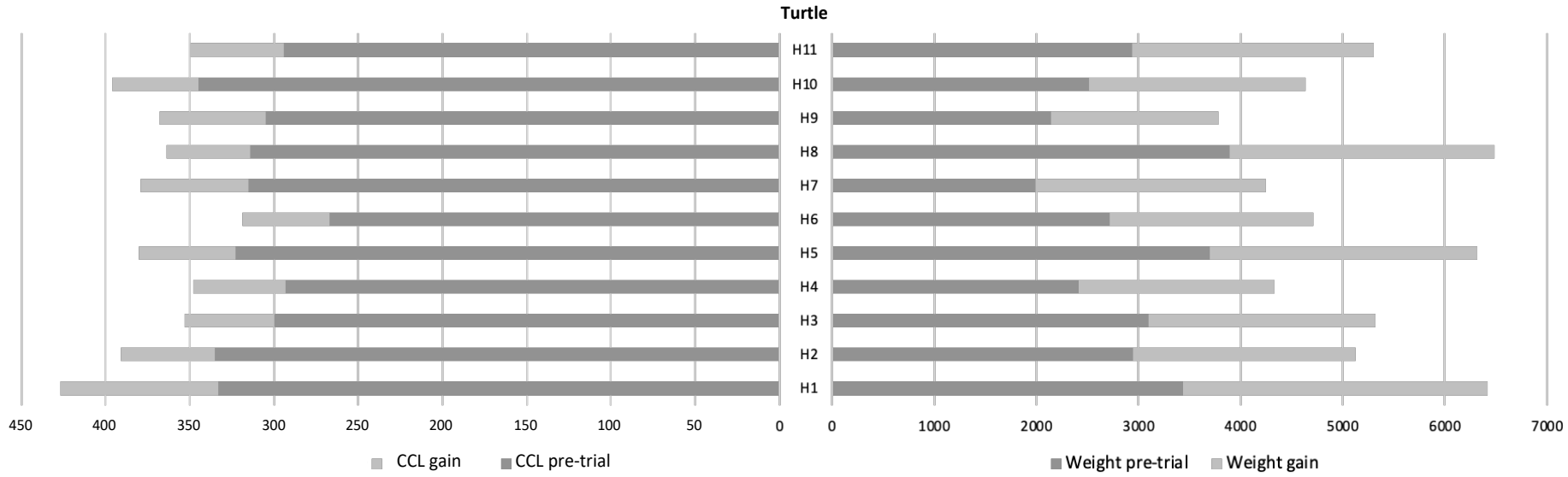



*Figure 4.7. Curved carapace length (CCL, mm) and weight (g) of each turtle pre- and post-trial. Dark grey bars indicate weight/CCL pre-trial, and light grey bars indicate the weight/length gained during the trial. Dark and light grey bars collectively indicate each turtle's total weight/CCL at the end of the trial.*

*Table 4.1. Number of days each turtle had trial tracker attached and the increase in weight (g and %) and curved carapace length (CCL; mm and %) gained from start to end of the trial.*

| Turtle ID | Study Use | Days with Tracker | Start Weight (g) | End Weight (g) | % Weight Increase | Start CCL (mm) | End CCL (mm) | % CCL Increase |
|---|---|---|---|---|---|---|---|---|
| H01 | Test | 119 | 3435 | 6415 | 87% | 333 | 427 | 28% |
| H02 | Trial | 108 | 2950 | 5125 | 74% | 314 | 370 | 18% |
| H03 | Trial | 103 | 3095 | 5325 | 72% | 323 | 376 | 16% |
| H04 | Trial | 108 | 2415 | 4330 | 79% | 300 | 355 | 18% |
| H05 | Trial | 103 | 3695 | 6315 | 71% | 335 | 392 | 17% |
| H06 | Trial | 103 | 2715 | 4710 | 73% | 294 | 346 | 18% |
| H07 | Trial | 108 | 1990 | 4250 | 114% | 267 | 331 | 24% |
| H08 | Trial | 108 | 3890 | 6485 | 67% | 345 | 395 | 14% |
| H09 | Trial | 108 | 2145 | 3785 | 76% | 293 | 356 | 22% |
| H10 | Trial | 103 | 2520 | 4635 | 84% | 305 | 356 | 17% |
| H11 | Trial | 108 | 2940 | 5300 | 80% | 315 | 371 | 18% |

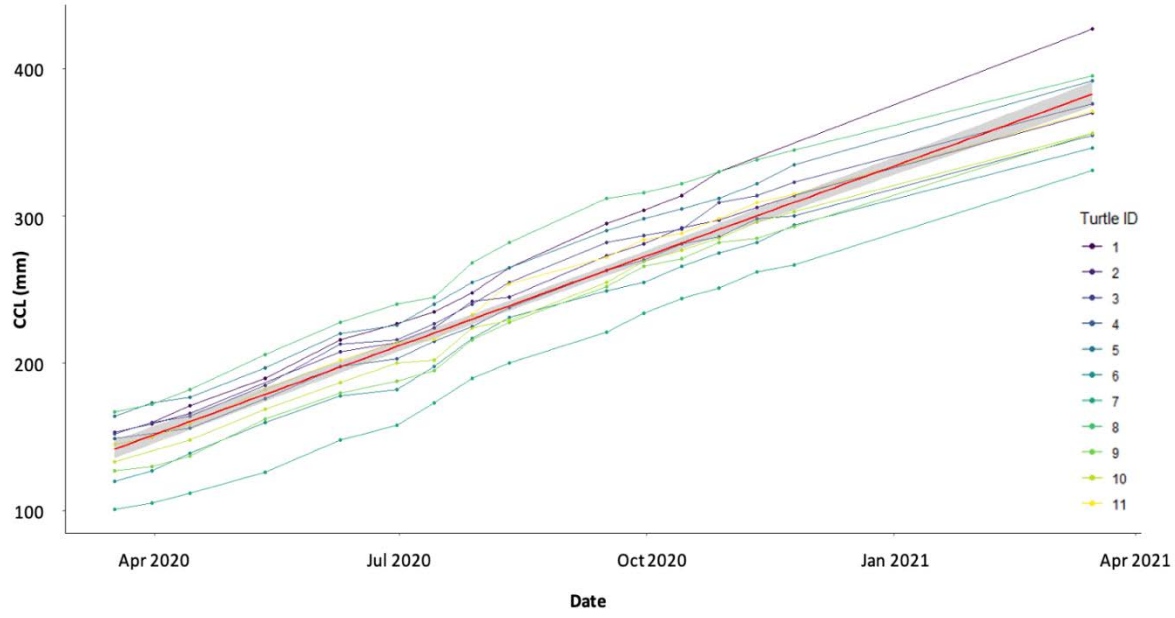



*Figure 4.8. Curved carapace length (CCL) increase of the 11 hawksbill turtles (Turtle ID H01 – H11) from March 2020 (1 year old) to March 2021 (2 years old). No CCL measurements were taken during the trial period with replica trackers attached (November 2020 – March 2021; grey box). The red line is the linear regression of averaged turtle CCL over time, and the grey area is the confidence interval.*

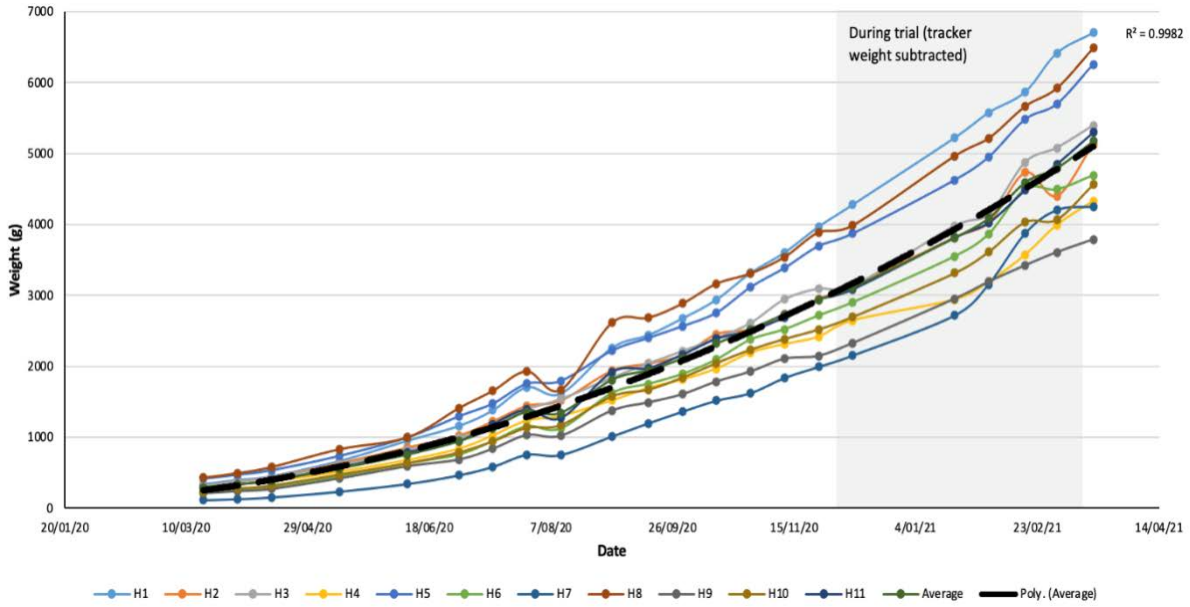



*Figure 4.9. Weight increase of the 11 hawksbill turtles (H01 – H11) from March 2020 (1 year old) to March 2021 (2 years old). Shaded area depicts the replica tracker attachment trial period, with tracker and attachment material weight subtracted (as calculated individually per turtle). Final weights displayed are after replica trackers were removed at the end of the trial.*

## Discussion

This study confirmed the feasibility of a method for satellite tracker attachment to small juvenile hawksbill turtles that were a similar size to new recruit turtles beginning to forage in benthic neritic areas (Velez-Zuazo et al., 2008). Furthermore, this attachment method did not result in tracker detachment, damage to scutes, or short-term reduction of growth within the 3 to 4-month study period. This is the first known study to test a tracker attachment method for any size of juvenile hawksbills, so there are no comparable data in the published literature. Whiting and Koch (2006) reported the outcome of a single juvenile hawksbill turtle that was satellite tracked from the Cocos (Keeling) Islands, but no attachment methodology was described. Similarly, other studies have satellite-tracked juvenile hawksbill turtles, but without detailing whether any adaptations from the techniques commonly used on adult turtles were used to fit trackers to juvenile hawksbill carapaces (Martinez-Estevez et al., 2021; Robinson et al., 2021).

### Attachment success

Although the hawksbills were housed in a clean environment, the exposure to natural light enabled some algal build-up on the neoprene and replica trackers. The turtles were routinely scrubbed throughout the trial, as per in-house husbandry procedures; however, any algae on the neoprene

and trackers were not removed. Algal growth is an issue for satellite tracking as it can inhibit the functioning of the sensors, thus preventing the upload of data from the trackers to the satellite system (Hays et al., 2007), and algal growth under the tracker can cause it to become detached. Without application of antifoulant, there was algal growth on the neoprene and trial trackers; however, there was no evidence of the neoprene peeling, as noted by Seney et al. (2010). In our trial, the additional silicone seal applied around the edge of the neoprene likely helped prevent the peeling of the neoprene and algal growth underneath it.

The use of neoprene for the attachment of satellite trackers to small juvenile turtles has been documented by Mansfield et al. (2012) to accommodate the vertebral ridge and by Seney et al. (2010) to allow for shell growth. Both benefits of using neoprene to attach trackers to the juvenile turtles in this study were noted. Primarily, neoprene provided the flexibility needed to account for accelerated growth in this size-class that could otherwise cause trackers to fall off prematurely or cause malformation of the carapace (Seney et al., 2010). Similar to loggerhead turtles, juvenile hawksbills have reduced attachment points on their carapaces due to the ridged shape and small size of their scutes. Neoprene can provide an anchor point across multiple scutes, whereby the additional thickness helps to create a flatter surface for adhesion, and the flexibility of the layers allows for carapace growth. Seney et al. (2010) compared different thicknesses of neoprene without indicating any preference in the results. In our trial, 3 mm neoprene was found to be thick enough to account for the unevenness of the carapace whilst being flexible enough to accommodate growth. Furthermore, this study used neoprene patches that were larger than previous studies and shaped to cover the most cranial seven scutes of the carapace to maximise surface area for adhesion. The continued development of smaller trackers could eventually make the need for neoprene redundant; however, the likely size of a tag to fit one scute would be difficult to specify as the size and shape of scutes can vary greatly between individuals. Moreover, the 'bumpiness' of the individual scutes (Salmon et al., 2018) prevents a tracker from sitting well on just one scute alone; hence previous tracking studies have often targeted larger juveniles (Hays et al., 2021) to which the tracker could be attached more easily.

Sanding or scoring of the carapace is common during satellite tracker attachment (Balazs et al., 1996), presumably to reduce the biofilm and increase surface area for better adhesion and longevity of tracker attachment (Hoffman, 2020). However, detailed assessments of potential scute damage post-tracker removal are lacking in the literature. This study found that sanding and scoring were not evident after replica tracker removal 3 – 4 months later, indicating no long-term physical damage from this procedure. Additionally, in terms of welfare, the turtles in our

trial had been handled and gently scrubbed as part of weekly husbandry since being at the research facility. This process may have helped reduce their stress, as they did not display signs of discomfort such as hyperactivity or rapid body movement (Arena et al., 2014) during sanding, acetone wash or epoxy application, or over the following few days.

## Growth of turtles during trial

Calculated growth rate estimates of wild hawksbill populations vary between regions but there is consensus in the literature that hawksbill growth rates differ by age and are highest in juvenile turtles (Bell & Pike, 2012b; Bellini et al., 2019; Chaloupka & Limpus, 1997; Snover et al., 2013). In our study, the turtles followed a linear growth pattern and grew throughout the trial without detectable damage to, or misshaping of, the underlying scutes as noted for the hard attachment methods studied in Mansfield et al. (2012). This could be attributed to the silicone that was applied to the edges of the underlying scutes, as Seney et al. (2010) indicated that the silicone could provide the necessary flexibility for scute growth. It should be noted, however, that the turtles observed by Mansfield et al. (2012) were much smaller than those in this study or Seney et al. (2010). In comparison with annual growth estimates of wild juvenile hawksbill populations (33 – 108 mm/year CCL), the captive-raised animals used in our study had a higher growth rate (Avens et al., 2021; Bellini et al., 2019; Bjorndal & Bolten, 2010; Bjorndal et al., 2016; Blumenthal et al., 2009; Boulon, 1994; Cañas-Uribe et al., 2020; Chaloupka & Limpus, 1997; Diez & van Dam, 2002a; Hart et al., 2013; Hawkes et al., 2014; Krueger et al., 2011; León & Diez, 1999; Limpus, 1992b; Llamas et al., 2017; Montero & Pena, 1996; Santos et al., 2019; Van Houtan et al., 2016; Wood et al., 2013), which is a common outcome of captive-reared turtles. Thus, our technique should be applicable to slower-growing wild turtles (Bjorndal & Bolten, 2010; Bjorndal et al., 2016). Since all study turtles were from the same clutch, they may have genetically inherited high growth rates (Heppell et al., 2002). However, there was also individual variation in growth between study turtles.

## Limitations of study

Although this study validated the described tracker attachment method, the attachment duration and lack of a control group are limitations to consider when interpreting our results. The study animals were scheduled to be released into the wild in early May 2021, fitted with genuine Wildlife Computers Spot-387 trackers; therefore, trial trackers were deliberately removed prior to this. Although we could not determine maximum attachment time by allowing natural detachment of the trackers, our replica trackers remained intact for 3.5 to 4 months, with no noticeable adverse

effects to the turtles. Given that Spot-387 trackers have an expected battery life of 5 to 6 months, the 3-month minimum criterion used in our study was considered adequate to assess the attachment method for real-world application. The method deemed most likely to succeed, based on current literature, was modified, and uniformly applied to all 11 hawksbills. No turtles were trialled as controls for growth without tracker attachment because of potential intra-species variations in natural growth rate and carapace shape and the relatively low number of trial turtles. However, all trackers in this study were successfully attached, and all turtles continued to grow according to their individual calculated trajectory, providing a good indication that the genuine trackers would stay intact for the following dispersal study on release into the wild without influencing turtle welfare.

## Considerations for future studies

The vertical distance between overlapping scutes was not measured in this study. Doing so could have provided further insight into the roles of silicone and neoprene and should therefore be included in future studies. One important difference between the study environment and the natural environment is the lack of complexity in habitat structure in the captive setting of this study. Although turtles did have access to a platform under which they could wedge themselves, this is minimal compared to the possible damage hawksbills can do to trackers when foraging and resting in natural reef or rock-based systems (Hays & Hawkes, 2018; Storch, 2004). One further consideration regarding the tracker attachment method that was not addressed in this study was the potential drag that could be experienced by turtles tracked in the wild given the size of the finished attached tracker (tracker plus neoprene and adhesives) compared to the size of the turtles. Drag negatively affects turtles by reducing their swimming speed to cope with increased energetic demands (Jones et al., 2011) and, therefore, future studies should test for this. Finally, future studies should consider obtaining an in-water weight of the tracker. Although the epoxy and tracker are heavy, the positively buoyant neoprene may reduce the weight of the attachment in water.

## Conclusion

Publishing replicable tracking data for sea turtles is important and requires optimised protocols. For best data collection and animal welfare results, tracker attachment methods should be tested and adapted for each species and life-stage. The methods developed for neonate green and loggerhead turtles (Mansfield et al., 2021; Mansfield et al., 2012; Seney et al., 2010), adapted for oceanic-stage Kemp's ridley turtles (Putman & Mansfield, 2015; Seney & Landry Jr, 2011), and

described here for new recruit-sized juvenile hawksbill turtles demonstrate attachment longevity (>3 months) in a little studied size-class of turtles without notable negative welfare implications (no notable scute damage or disfigurement). As such, this study serves as a valuable tool for researchers and conservation groups aiming to study juvenile hawksbill turtles' dispersal patterns and foraging ground usage. Moreover, the method developed here may be adapted to other aquatic reptiles in future studies.

# Summary of thesis and chapter aims

## Thesis aim 2

Optimise method of attaching satellite trackers to juvenile hawksbill turtles for post-release monitoring.

### Chapter aims

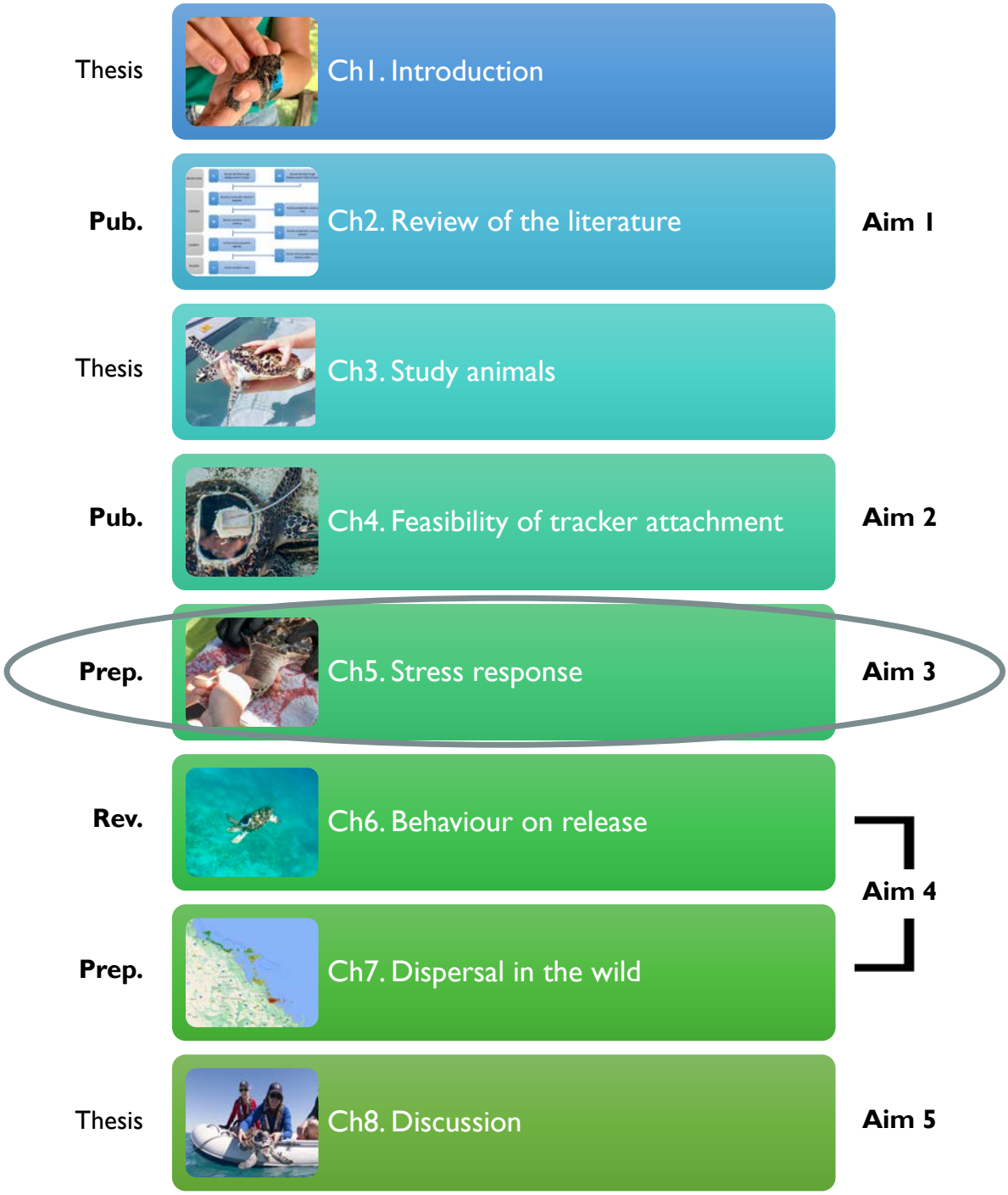
### Chapter aim 1

- Develop, test, and confirm a protocol for the successful attachment of satellite trackers to small juvenile hawksbill turtles where success was defined by:
    - trackers remaining firmly attached for more than 3 months; and
    - attachment method leaving minimal scute damage or disfigurement whilst allowing turtles to continue growing.

This study's objective was to confirm the feasibility of an attachment method that would allow small-sized juvenile hawksbill turtles (~267 – 345 mm curved carapace length) to continue growing, without tracker loss or damage to underlying scutes. Replica trackers were made of resin (simulating Wildlife Computer Spot-387 trackers), and attached with epoxy, silicone, and neoprene, using a technique modified from those used on neonate loggerheads and Kemp's ridleys. Throughout the study (3.5 months), replica trackers remained attached, the turtles grew up to 11⁴% heavier and 25% longer, and all turtles appeared clinically healthy and active. Furthermore, all scutes were undamaged after tracker removal. Therefore, this method of attachment will ensure the best chance of success when attaching the genuine trackers for the hawksbill release.

# CHAPTER 5: PHYSIOLOGICAL STRESS RESPONSE

## Thesis structure



# Background and aims of this chapter

## Rationale

Stress response is important for survival in free-living sea turtles, particularly for evading predators. Furthermore, metrics of stress can be used to infer physical and behavioural welfare status, which is a holistic indicator of health and fitness. Therefore, understanding the stress response of turtles temporarily under human care for conservation or research purposes is important for assessing welfare and fitness whilst in care and for inferring survivability once released into the wild. Maintaining positive welfare whilst under human care, including retaining the ability to respond to stressors, will therefore, aid in achieving positive outcomes for the turtles whilst under human care and post-release. All sea turtles that are temporarily kept under human care undergo some form of transportation to their release site, which could increase their stress levels at the point of release. Furthermore, in some cases, sea turtles are fitted with satellite trackers to gain further understanding of their behaviour, including migration patterns. For turtles temporarily held under human care, these satellite trackers can also aid in evaluating the success of the release by providing evidence of post-release survivability and informing future release protocols. However, the process of attaching the trackers, as well as the continued presence of the trackers, are likely to cause further stress to the turtles, which may decrease their survivability at the point of release. Stress response in reptiles can be measured numerous ways, including via corticosterone and lactate concentrations in the blood; however, limited measurements of corticosterone or lactate have been reported in captive-raised juvenile hawksbill turtles (Eretmochelys imbricata). Without knowledge of basal stress levels or an understanding of stress response in turtles, it is difficult to improve their welfare whilst under human care and during release into the wild to maximise their post-release survivability.

## Thesis aim 3

Characterise and compare physiological indicators of stress response in captive-raised juvenile hawksbill turtles after 2 years under human care.

### Chapter aims

- Determine basal ranges of corticosterone and lactate concentrations in captive-raised juvenile hawksbill turtles.
- Assess how captive-raised juvenile hawksbill turtles respond to the following stressors:
    - handling and blood collection;

- short-term stressor (5-minute stimulation);
- tracker attachment (1 hour) and dry-docking (12 hours); and
- transportation from turtle housing facility to release site.

## Research outputs

**Diggins** RL, Rudd D, Munns S, Kophamel S, Jones K, Mendez D, and Ariel E (2021, September 6 – 8). *Measuring stress response of juvenile hawksbill turtles to satellite tracker attachment via blood sampling* [Oral Presentation]. Cohort Doctoral Studies Program 10 Year Anniversary Conference "Health Research: Making Connections", Townsville, Australia.

*This chapter is a modified version of a drafted manuscript that is being prepared for later submission:*

**Diggins** RL, Rudd D, Munns S, Bairos-Novak K, Jones K, Kophamel S, Mendez D, and Ariel E (*in preparation*). Measuring biochemical stress response of captive-raised juvenile hawksbill turtles (*Eretmochelys imbricata*) via corticosterone and lactate in the blood.

# Measuring biochemical stress response of captive-raised juvenile hawksbill turtles (*Eretmochelys imbricata*) via corticosterone and lactate in blood

## Introduction

Stress is a response to a given stimulus or stressor (Mills et al., 2014), resulting in physiological, psychological, and behavioural outcomes (Hing et al., 2016). These outcomes are important for survival at both the individual and species levels, for example when evading predators (Karaer et al., 2023). A reduced response to an acute stressor (short-term) might, therefore, result in decreased ability to survive (survivability) in the wild. As such, it is imperative that any animals removed from the wild and held temporarily under human care with the intention of eventual release back into the wild retain their innate ability to respond to acute stressors. However, animals raised under human care without predatory threats may become desensitised to acute stressors (stress acclimation (Romero, 2002)). Desensitisation could then result in a weaker stress response and unfavourable outcomes post release. Thus, demonstration of captive turtles' ability to respond to stressors can be an indicator of post-release survivability potential.

Conversely, long-term exposure to unsuitable husbandry and housing conditions can result in a prolonged and elevated state of chronic stress, sometimes referred to as "distress" (Linklater et al., 2010). Chronic stress is associated with negative welfare wherein the inability to recover from persistent physiological and psychological stressors leads to unfavourable fitness (Fischer & Romero, 2019). Adverse outcomes from a chronic state of stress include a weakened immune system (physiological) and self-mutilation (behavioural) (Marino et al., 2020; Pizzutto et al., 2015). In summary, acute stress induces a useful response in the short term as it allows the individual to react quickly to the stimulus presented (e.g. for predator avoidance). In contrast, chronic stress reduces the long-term health, fitness, and overall welfare of the animal. Hence, the ability to respond to stressors must be retained (short-term response and recovery) but not sustained (chronic state) to promote positive welfare for animals whilst under human care and post-release survivability potential.

Sea turtles are a group of marine animals comprising seven extant species that are threatened with extinction and therefore prioritised for conservation (Klein et al., 2017). Some conservation strategies involve holding turtles under human care, including rehabilitation of sick and injured turtles (Melvin et al., 2021); head-start programs that protect hatchlings from predation for a

period so they enter the ocean at a larger size (Kanghae et al., 2023); zoos and aquaria that engage and educate the public and sometimes also fundraise for turtle conservation (Ballantyne & Packer, 2016); and research into the ecology and biology of each sea turtle species to better inform management and conservation programs for improved outcomes (Usategui-Martín et al., 2021). While turtles are held under human care, their caretakers have an ethical responsibility to ensure positive welfare for the turtles (Brando & Buchanan-Smith, 2018), including maintaining innate acute stress response capability and disassociation from humans whilst avoiding exposure to phenomena that may induce chronic stress.

In some cases, turtles may be exposed to stressors for an extended period of time (several hours) prior to release in the wild. For example, some sea turtles are released with a satellite tracker attached to their carapace for research and monitoring purposes (Hays & Hawkes, 2018). Attaching a satellite tracker requires extended physical contact with the turtle and a period of dry docking for the adhesive agent to cure (Diggins et al., 2023), both of which would likely induce a stress response. Additionally, the tracker itself may cause irritation and increased drag, depending on the attachment method, triggering further stress (Seeley et al., 2022). Therefore, where a turtle will be satellite tracked on release, the stress responses caused by tracker attachment and presence of the tracker itself should be considered. A final consideration for optimising welfare and survivability of all sea turtles being released into the wild is potential stress caused by transportation to the release site (Hunt et al., 2020).

To ensure the best welfare for turtles under human care and their potential post-release survivability, husbandry, housing, and release conditions and protocols should be optimised. Positive welfare can be achieved by understanding how turtles respond to stress and how these responses can best be measured. Physiological stress response can be inferred via biochemical markers measured in the blood, saliva, and faeces (Karaer et al., 2023). Production of corticosterone (stress hormone regulator in reptiles) is often measured as the inability to release corticosterone can be an indication of reduced health and fitness (Gormally & Romero, 2020). One less commonly recorded marker is lactate, which forms from anaerobic metabolism during excessive movement such as predator evasion or hatchling dispersal (Pereira et al., 2012). An increase in lactate is typically indicative of a behaviour requiring intense physical exertion often in response to an acute stressor. However, in sea turtles subjected to extended periods of stress (i.e. satellite tracker attachment and transportation prior to release into the wild), it is unknown how the lactate concentrations would vary with regards to production and recovery. To provide a more complete understanding of the stress response and recovery of the turtles, basal

(Johnstone et al., 2012; Romero, 2002) ranges of corticosterone and lactate must first be determined so that scope (magnitude) of the stress response, time to peak, and time to recovery can be calculated.

Whilst stress has been measured in several species of free-living turtles, fewer studies have investigated stress response in captive-raised turtles (Usategui-Martín et al., 2021). Furthermore, stress response is also specific to species and life-stage (Fischer & Romero, 2019). As a critically endangered species, hawksbill turtles (*Eretmochelys imbricata*) could particularly benefit from conservation programs that promote population growth by releasing healthy turtles into the wild. However, the lack of available data for stress response of hawksbill turtles, both free-living (Jessop et al., 2004a) and captive-raised (Kawazu et al., 2022), hinders the effectiveness of such programs.

Therefore, the aim of this study was to characterise the physiological basal levels and stress response of captive-raised juvenile hawksbill turtles by measuring variation in their blood concentration of corticosterone and lactate in response to 1) general handling and blood collection; 2) an acute (short-term) handling stressor; 3) an extended stressor (satellite tracker attachment and dry docking); and 4) transportation to release site. The main hypotheses were that the captive-raised turtles would have low basal concentrations of corticosterone and lactate compared with free-living turtles, and that concentrations would increase differently in response to each external stimuli presented, with recovery back to basal within 24-hours of stimuli cessation. Additionally, it was hypothesised that transportation to release site would cause corticosterone and lactate concentrations to increase.

## Materials and methods

### Study animals

Captive-raised juvenile hawksbill turtles were collected as hatchlings from the northern Great Barrier Reef (11.167°S, 143.017°E). The 11 study turtles (H01 – H11) were collected on 17 March 2019 (Department of Environment and Science permit WA0012830) and housed at James Cook University (JCU; Animal Ethics permit A2586) until release into the wild on 6 – 7 May 2021. Collected turtles were housed individually at the purpose-built JCU Turtle Health Research facility (detailed description in Chapter 3 and Diggins et al. (2023)). Briefly, turtles were reared in recirculating sea water systems with ultraviolet filter, and temperature controlled between 25 – 30°C to match seasonal Great Barrier Reef water temperatures.

## Study design

The physiological stress responses of captive-raised, juvenile hawksbill turtles were measured across four studies, conducted between November 2020 and May 2021. The first three studies were conducted solely in the research facility at JCU, and the fourth was conducted mostly in the research facility, with the final blood sample collected on the boat at the release site (John Brewer Reef, Australia, 18.633°S, 147.067°E). Of the 11 hatchlings raised at the JCU Turtle Research Facility, 10 were included in the first three studies and all 11 were included in the final study. One turtle was excluded from the first three studies because it was part of another experiment at the time (Diggins et al., 2023), but it was included in the final study to record its levels of corticosterone and lactate just prior to being released. At the start of the first, third, and fourth study, the turtles were weighed, and their curved carapace lengths (CCL) were measured. The study design and timelines are summarised below (Fig. 5.1).

| Stress Study | Blood Sampling (turtle$^{-1}$) | Number of Turtles | Timeline | Turtle Weight (g) | Turtle CCL (mm) |
|---|---|---|---|---|---|
| 1) Handling & Sampling | **4.3 ml**<br>12 samples<br>4 days | 6 turtles<br>4 turtles | 6 – 9 Nov 2020<br>7 – 10 Nov 2020 | *Range*: 1,835 – 3,540<br>***Median*: 2,605**<br>*IQR*: 2,328.75 – 2,892.5 | *Range*: 262 – 338<br>***Median*: 302**<br>*IQR*: 287.75 – 312.75 |
| 2) Acute Stressor | **5.5 ml**<br>13 samples<br>4 days | 6 turtles<br>4 turtles | 14 – 17 Nov 2020<br>15 – 18 Nov 2020 | Not re-weighed | Not re-measured |
| 3) Extended Stressor | **5.5 ml**<br>13 samples<br>4 days | 6 turtles<br>4 turtles | 28 Nov – 1 Dec 2020<br>29 Nov – 2 Dec 2020 | *Range*: 1,990 – 3,890<br>***Median*: 2,827.5**<br>*IQR*: 2,441.3 – 3,058.8 | *Range*: 267 – 345<br>***Median*: 308.5**<br>*IQR*: 295.5 – 321.0 |
| 4) Transportation | **1.5 ml**<br>3 samples<br>2 days | 6 turtles<br>5 turtles | 5 – 6 May 2021<br>6 – 7 May 2021 | *Range*: 4,445 – 7,830<br>***Median*: 5,970**<br>*IQR*: 5,062.5 – 6,682.5 | *Range*: 357 – 444<br>***Median*: 389**<br>*IQR*: 373.5 – 404.5 |

*Figure 5.1. Study design and timeline for four studies measuring stress response of captive-raised hawksbill turtles (Eretmochelys imbricata), including morphometrics: Study 1) handling and sampling; Study 2) acute stressor; Study 3) extended stressor; and Study 4) transportation.*

## Study 1: Response to handling and sampling

Study 1 was designed as a control to examine the turtles' potential physiological stress response to being handled and having multiple subsequent (repeat) samples collected. Each turtle had 12 samples collected, totalling 4.3 ml of blood per turtle. Samples were collected over a 4-day period from 6 to 10 November 2020. Turtles were returned to their tanks between each collection.

## Study 2: Response to acute stressor

Study 2 was designed to measure the turtles' immediate physiological stress response to an unfamiliar acute stressor as well as the following handling and blood collection stressors. For the acute stress event, turtles were positioned into dorsal recumbency and had their ventral axillary regions stimulated for 5 minutes using light touch from the researchers. That is, the turtles were turned over onto their carapaces and the soft tissue region between their flippers and plastrons were tickled/stroked, causing them to wriggle. Each turtle had 13 blood samples collected over a 4-day period, totalling 5.5 ml blood. The turtles were collected from their tanks, had an initial blood sample collected, and then were immediately subjected to the acute stressor. This was directly followed by a second blood collection and first temperature reading before turtles were returned to their tanks. Following this, the turtles underwent 11 further blood sampling collections and were returned to their tanks between each collection.

## Study 3: Response to extended stressor

Study 3 was designed to measure the physiological stress response of the turtles to satellite tracker attachment and prolonged time out of the water (dry-docked). Each turtle had 13 blood samples collected over a 4-day period, totalling 5.5 ml blood. Turtles were removed from their tanks for the first blood sample collection, immediately after which the satellite tracker was attached per Diggins et al. (2023) (a process that took approximately 60 minutes). Turtles remained dry-docked for the following nine blood collections but were placed back into their tanks after the tenth, which was the final blood collection for day 1. Turtles were returned to their tanks between each of the final three blood collections.

## Study 4: Response to transportation stress

Study 4 was designed to measure the physiological stress response of the turtles to being transported from their tanks to the release site. This study also captured their response to being semi-dry docked the night before release, which was necessary to allow drying of the anti-foulant applied as part of the satellite tracker attachment process. Each turtle had 3 blood samples collected over a 2-day period, totalling 1.5 ml blood. Turtles were removed from their tanks for the first blood collection, after which antifoul was applied to their satellite trackers to slow algal growth that would block transmission. Turtles were then placed into a modified holding tank overnight, in which they were semi submerged, to allow antifoul to dry. Although the circulating water temperature was approximately 25°C, to match seasonal temperatures, the air temperature could not be controlled for, resulting in lower-than-expected core temperatures of

the turtles overnight. The following morning, turtles were collected from their holding tanks and had a second blood sample collected before being transported to their release site. The third and final blood sample was collected at the release site after mooring was completed. Turtles released on 6 May 2021 were sampled on 5 – 6 May and turtles released on 7 May 2021 were sampled on 6 – 7 May.

## Blood sampling schedule

Since it was unknown how long it would take for the corticosterone and lactate concentrations to peak and return to basal post exposure to stressors, blood samples were collected at increasing intervals, starting from 30 minutes (the shortest possible interval due to logistic constraints; Fig. 5.2). Studies 1 – 3 followed the same sampling intervals with the exception of an initial stressor (acute in Study 2 and extended in Study 3). Studies 1 – 3 each lasted 4 days per turtle, but with a staggered start; six turtles were sampled starting on the first date of each study and four turtles were sampled starting the second date. Study 4 was conducted several months later, just prior to release, and followed different sampling intervals from Studies 1 – 3.

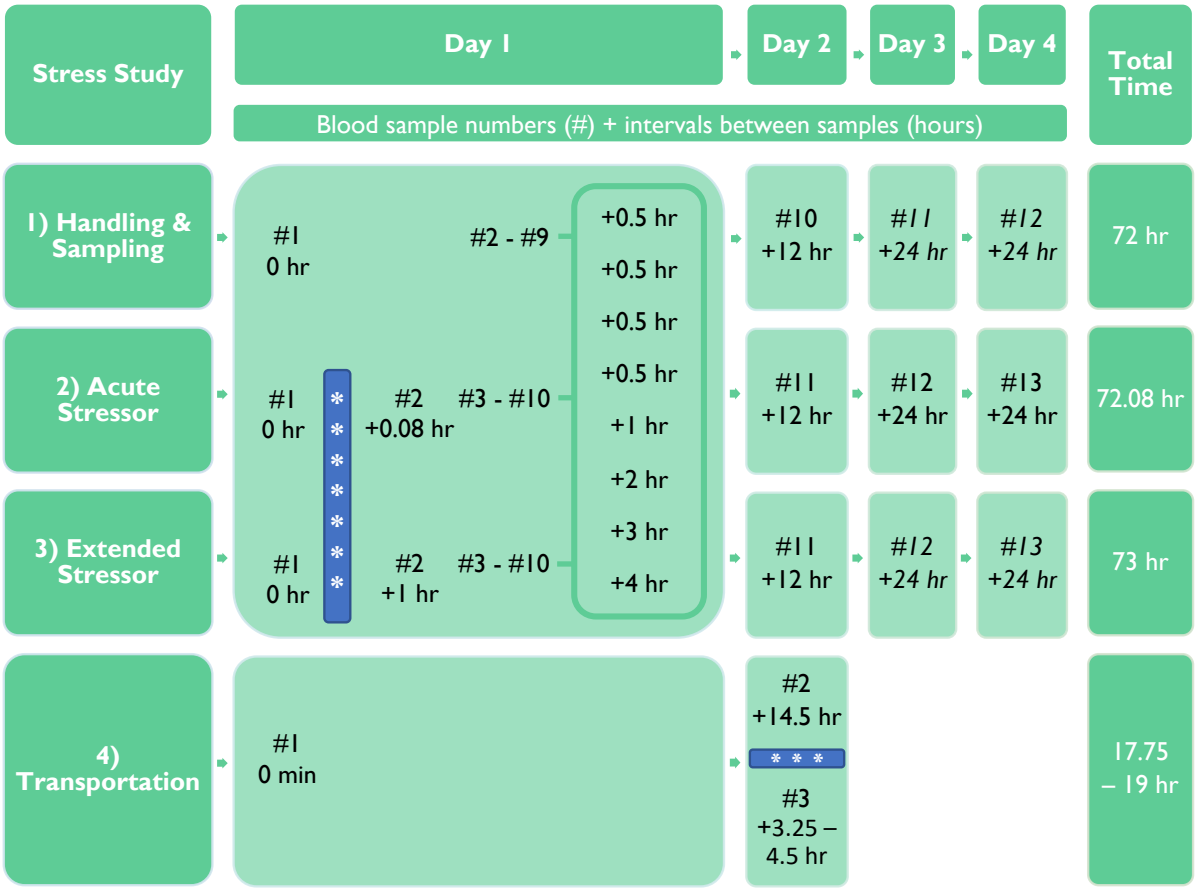


*Figure 5.2. Blood sampling schedule for four studies measuring stress response of captive-raised hawksbill turtles (Eretmochelys imbricata): Study 1) handling and blood collection; Study 2) acute stressor; Study 3) extended stressor; and Study 4) transportation. Introduction of stressors are indicated by blue bar with asterisks between sample numbers (#).*

## Animal welfare considerations

Given the temporal proximity of the first three studies (study design above) and lack of published literature regarding sea turtle blood regeneration time, a conservative approach was taken to calculate the cumulative volume of blood that could be collected. The total blood collected per turtle for each study was kept within safe limits of blood collection based on the weight of the smallest turtle. The smallest turtle weighed 1,835 g at the start of the first study, with approximately 73.4 – 146.8 g of blood (4 – 8% of body weight). Since 10% of total blood can be collected (Mader & Rudloff, 2006), it was deemed safe to collect up to 14.7 ml of blood per turtle across the first two studies (9.8 ml collected per turtle by end of second study). At the start of the third study, turtles were reweighed, and the weight of the smallest turtle had increased to 1,990 g, hence the total blood that could be safely collected per turtle by the end of the third study was 15.9 ml (15.3 ml cumulatively collected from first three studies). The fourth and final study occurred 5 months after the end of the previous study, at which time the smallest turtle weighed 4,445 g, indicating that 35.6 ml could be collected (16.8 ml total blood collected by end of all stress studies). As a further measure, packed cell volume (PCV) was recorded at the start and end of each study to detect potential health deterioration of the turtles (e.g. anaemia, dehydration) (Reséndiz & Lara-Uc, 2018).

## Blood Collection Protocol

Turtles were removed from their enclosures by experienced handlers and placed on a solid surface with the head lower than the body to encourage them to naturally extend their necks. The neck of each turtle was towel dried and then sanitised with an ethanol wipe prior to blood collection. A 25-gauge 1.5-inch needle and 1 ml syringe were used for Studies 1 – 3 to collect either 0.4 ml (for a standard sample) or 0.7 ml (if PCV was also being measured) of blood on each collection. By Study 4, the turtles were larger so 21-gauge 1.5-inch needles and 1 ml syringes were used. Blood was collected from the dorsal cervical sinus, alternating between left and right side of the neck for each subsequent sample. Collected blood was dispensed into a serum separator tube, labelled with the sample number and time of blood collection, and then stored on ice until processed. Cloacal temperature of each turtle was recorded immediately following blood

collection using a Thermistor Thermometer (Model 8402-20) temperature probe. Vaseline® petroleum jelly was used to lubricate the end of the probe, which was inserted no more than 3 cm into the cloaca and held until temperature stabilised. The probe was sanitised with ethanol between each use. Cloacal temperatures ranged from 26.5 – 30.1°C (mean 28.2 ±0.03°C) for Study 1, 2, and 3 and 15.0 – 29.3°C (mean 24.4 ±0.68°C) for Study 4 (Appendix Suppl. Table 5.1).

## Blood Processing Protocol

### Packed cell volume testing

The PCV was recorded as the average of duplicate capillary tubes, for the first blood sample of each study for each turtle. Samples were spun in an Eppendorf centrifuge at 3,500 rpm for 2 minutes and haematocrit was manually determined. PCVs of the captive-raised hawksbill turtles were compared amongst turtles and across studies and also compared with previously reported PCV of wild hawksbill turtles (22 – 48%) (Crooks et al., 2023; Muñoz-Pérez et al., 2017; Stacy et al., 2023; Stewart et al., 2023; Whiting et al., 2014).

### Corticosterone and lactate sample preparation and analysis

Blood samples were set aside in an air-conditioned room (24°C) for approximately 20 minutes to clot. They were then spun in an ELMI or Thermo Scientific centrifuge at 1500 rpm for 10 minutes, following which the serum was aliquoted into pre-labelled polymerase chain reaction tubes. Prepared samples were immediately stored in a freezer (-20°C) and the entire rack was moved to a -80°C freezer at the end of each trial for long-term storage. Corticosterone levels were determined using Enzo Life Sciences corticosterone ELISA kit (category ADI-901-097), 480 (5x96) well, and protocols. A Beckman Coulter AU analyser was used to measure lactate levels.

## Data analysis

Data were analysed using R version 4.3.1 2023-06-16 (R Core Team, 2023) in R Studio 2023.09.0 Build 463 (RStudio Team, 2023). Packages used were tidyverse (Wickham et al., 2019), lubridate (Grolemund & Wickham, 2011), janitor (Firke, 2023), lme4 (Bates et al., 2014), tidyr (Wickham et al., 2023), and ggplot2 (Wickham, 2016). PCV data were not normally distributed (Shapiro-Wilk test; $p<0.05$); therefore, difference in PCV amongst turtles and across studies were tested using Kruskal-Wallis and Wilcoxon Signed Rank tests.

Corticosterone and lactate concentration data distributions were also not normally distributed (Shapiro-Wilk test; $p<0.05$). Corticosterone data were log (base 10) transformed and lactate and

time since first bleed (minutes) data were square root transformed. Effect of bleed duration (the length of time taken to collect the blood sample) on lactate and corticosterone concentrations were tested using Spearman's rank correlation and change point analysis testing (Romero & Reed, 2005). No correlation was found; however, data points where collection exceeded 3 minutes were discarded from analysis as this is standard protocol (Romero, 2002). Basal corticosterone and lactate range upper limits were calculated from the 25th quantile of each turtle's data set (collated data from Studies 1 – 3), based on a quantile regression of lactate and corticosterone per turtle by time, which showed increased variation past the 25$^{th}$ quantile. Differences within and between each turtle's basal dataset for each study were tested using Kruskal-Wallis and Wilcoxon Signed Rank tests.

Lactate and corticosterone responses to each stress study were modelled using a Gamma distribution generalised linear mixed model (GLMM) with log-link, including blood collection time as a polynomial, and using turtle as a random factor. Possible physiological (turtle temperature, turtle weight, turtle CCL, and time of day) and methodological (person collecting the blood sample, person handling the turtle, and time from sample collection to sample freezing) confounding factors were tested in each model. Additionally, peak concentrations, scope (calculated as factorial increase from grouped basal to individual peak), time to peak from initial sample collection (sample 1), and time to recovery (calculated as time from peak to first data point below the grouped basal) were calculated and reported.

## Results

### Packed cell volume

The PCV of the captive-raised, small juvenile hawksbill turtles ranged from 27.5 – 47.5 (median 34.0; IQR 31.0 – 35.0) across Studies 1 – 4, and there was no significant difference in PCV amongst turtles using data from all studies ($p=0.1696$). The PCV range of turtles throughout this study were comparable with reports of healthy wild hawksbill turtles (Crooks et al., 2023; Muñoz-Pérez et al., 2017; Stacy et al., 2023; Stewart et al., 2023; Whiting et al., 2014). PCV range per study was as follows: Study 1) 29.0 – 36.5 (median 34.5); Study 2) 29.0 – 34.5 (median 33.5); Study 3) 28.0 – 35.0 (median 30.75); and Study 4) 27.5 – 47.5 (median 37.5). No significant difference was found amongst Studies 1 – 3 using the combined turtle PCVs per study ($p>0.05$); however, there was a significant increase (Wilcoxon Signed Rank test) in PCV of Study 4 compared with Studies 1 ($p=0.018$), 2 ($p=0.018$), and 3 ($p=0.016$).

## Basal stress

Basal concentrations of corticosterone and lactate in the blood of juvenile hawksbill turtles were calculated for each turtle individually based on the 25$^{th}$ quantile of each turtle's data across Studies 1 – 3 (Table 5.1). Individual basal levels of corticosterone ranged from 0.07 to 0.12 ng/ml (median and mean 0.09 ng/ml), and the range in individual basal lactate concentrations was 0.22 – 0.36 mmol/L (median and mean 0.29 mmol/L). Grouped basal corticosterone and lactate concentrations were calculated as mean ±1 standard deviation. One grouped basal concentration of corticosterone was calculated as 0.10 ng/ml for all turtles. Data below the grouped basal concentration (0.10 ng/ml) were compared among turtles and among studies and no significant difference was found in these comparisons ($p>0.05$). Three grouped basal concentrations of lactate were calculated at 0.25, 0.30, and 0.36 mmol/L (low, median, and high grouped basal concentrations, respectively). Three grouped basal concentrations were considered more appropriate than one due to significant variation amongst turtles in data below the single grouped basal concentration (0.33 mmol/L; $p=0.003075$). Data below each grouped basal lactate concentration showed no significant difference among turtles ($p>0.05$). There was also no significant difference among studies for low and high grouped basal lactate ($p>0.05$). However, data below the grouped basal lactate for turtles in the medium basal group were significantly different between studies ($p=0.02551$) with Study 1 data lower than Study 2 data ($p=0.036$).

*Table 5.1. Individual and grouped upper basal ranges of corticosterone and lactate concentrations in captive-raised juvenile hawksbill turtles (Eretmochelys imbricata), H02 – H11. Med = medium.*

| | CORTICOSTERONE (ng/ml) | | LACTATE (mmol/l) | |
|---|---|---|---|---|
| **TURTLE** | **Individual Basal** | **Grouped Basal** | **Individual Basal** | **Grouped Basal** |
| **H02** | 0.09 | 0.10 | 0.29 | 0.30 Med |
| **H03** | 0.08 | 0.10 | 0.25 | 0.25 Low |
| **H04** | 0.09 | 0.10 | 0.36 | 0.36 High |
| **H05** | 0.07 | 0.10 | 0.24 | 0.25 Low |
| **H06** | 0.07 | 0.10 | 0.34 | 0.36 High |
| **H07** | 0.09 | 0.10 | 0.34 | 0.36 High |
| **H08** | 0.09 | 0.10 | 0.30 | 0.30 Med |
| **H09** | 0.12 | 0.10 | 0.30 | 0.30 Med |
| **H10** | 0.09 | 0.10 | 0.25 | 0.25 Low |
| **H11** | 0.09 | 0.10 | 0.22 | 0.25 Low |

## Study 1: Response to handling and sampling

The turtles showed a physiological response to and recovery from stress caused by handling and blood sampling, indicated by changes in their corticosterone and lactate concentrations throughout Study 1 (Fig. 5.3). Corticosterone peaked at a median concentration of 0.49 ng/ml (IQR 0.47 – 0.71 ng/ml), with a median scope of 4.88-fold above grouped basal (IQR 4.74 – 7.12-fold). Median peak in lactate concentration was 1.09 mmol/L (IQR 0.86 – 1.31 mmol/L), with a lower median scope increase of 3.90-fold (IQR 3.21 – 4.43-fold). When analysed collectively, the turtles reached their peak corticosterone (Fig. 5.3a) and peak lactate (Fig. 5.3b) concentrations approximately 0.5 hours and 2 hours after sample 1, respectively. Corticosterone was no longer significantly higher than grouped basal concentration (recovery time) 4.5 hours post peak ($p=0.497$) and lactate recovery was recorded 3 hours post peak ($p=0.308$). Both corticosterone and lactate concentrations were reported as recovered to basal at sample 7, which was collected 5 hours post sample 1. The best fitted model (GLMM) for corticosterone accounted for 53% of the variation and included turtle temperature ($t=3.29$, $p=0.001$) as a fixed effect (Appendix Suppl. Table 5.2.) The best fitted model (GLMM) for lactate accounted for 50% of the variation and included no additional fixed effects (Appendix Suppl. Table 5.3).

There was individual variation amongst turtles (Fig. 5.4) with respect to their peak concentrations, scope (increase from basal to peak), time to peak, and time to recovery for both corticosterone and lactate. The range in peak corticosterone concentrations recorded was 0.35 – 1.07 ng/ml with a calculated increased scope ranging from 3.48 (H04) to 10.69-fold (H05). Peak lactate concentrations ranged from 0.78 – 3.23 mmol/L, at a factorial scope increase ranging from 2.17 (H07) – 11.88-fold (H05). Most turtles (n=8) only had an increased scope within approximate 2 – 4.5-fold, and only two turtles (H04 and H05) had a scope of approximately 9 – 12-fold. Other than the peak concentrations already reported, some turtles showed additional spikes in their corticosterone and lactate concentrations, most notably 12 hours post sample 1. Time to reported peak corticosterone ranged from 0.5 hours (n=5) to 2 hours (n=2) post sample 1. Two turtles reached their peak corticosterone concentration 1.5 hours post sample 1 and the peak for the final turtle was reported at sample 1, after which its next peak was 1.5 hours post sample 1. For recovery, most turtles (n=8) were not reported as recovering to grouped basal corticosterone levels by the end of the first day of sampling, with one turtle (H05) never recorded below grouped basal throughout Study 1. However, all turtles showed a decline to near basal levels within the first day. For the two turtles with full recovery to basal corticosterone concentrations reported within the first day of sampling, one recovered within 1.5 hours post

peak and the other within 6.5 hours. Lactate recovery time also showed large variation; however, only one turtle (H06) had no reported concentrations within basal by the end of the first day of sampling. For the other nine turtles, recovery from peak lactate concentration was reported within 1.5 (n=1), 2 (n=1), 3 (n=2), 4 (n=1), 6 (n=1), 6.5 (n=1), 9 (n=11), and 11 (n=1) hours.

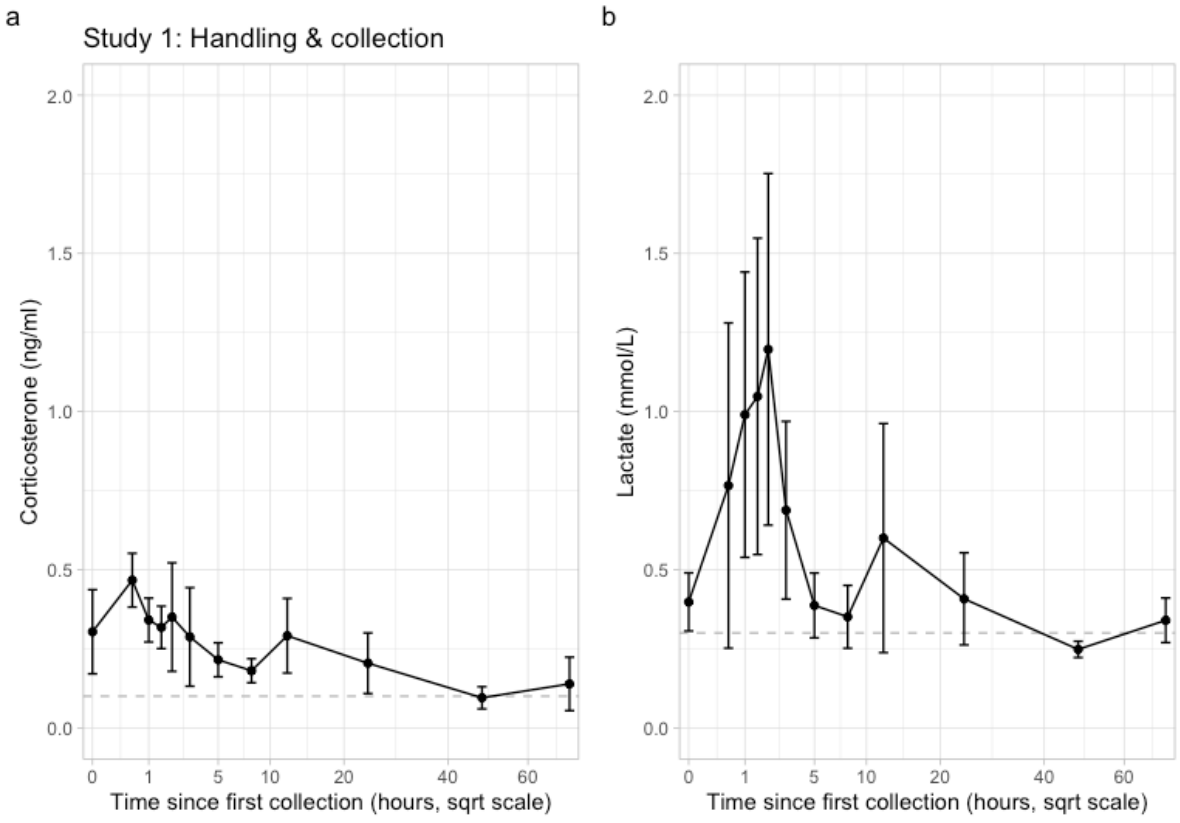



*Figure 5.3. Change in a) corticosterone (ng/ml) and b) lactate (mmol/L) concentrations over time for a group of 10 juvenile hawksbill turtles (Eretmochelys imbricata), resulting from general handling and sampling (Study 1). Figure displays mean values (using log10 transformed data) with 95% confidence interval bars. X axis is square-root scaled and Y axis is capped at a) 2 ng/ml and b) 2 mmol/L. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L).*

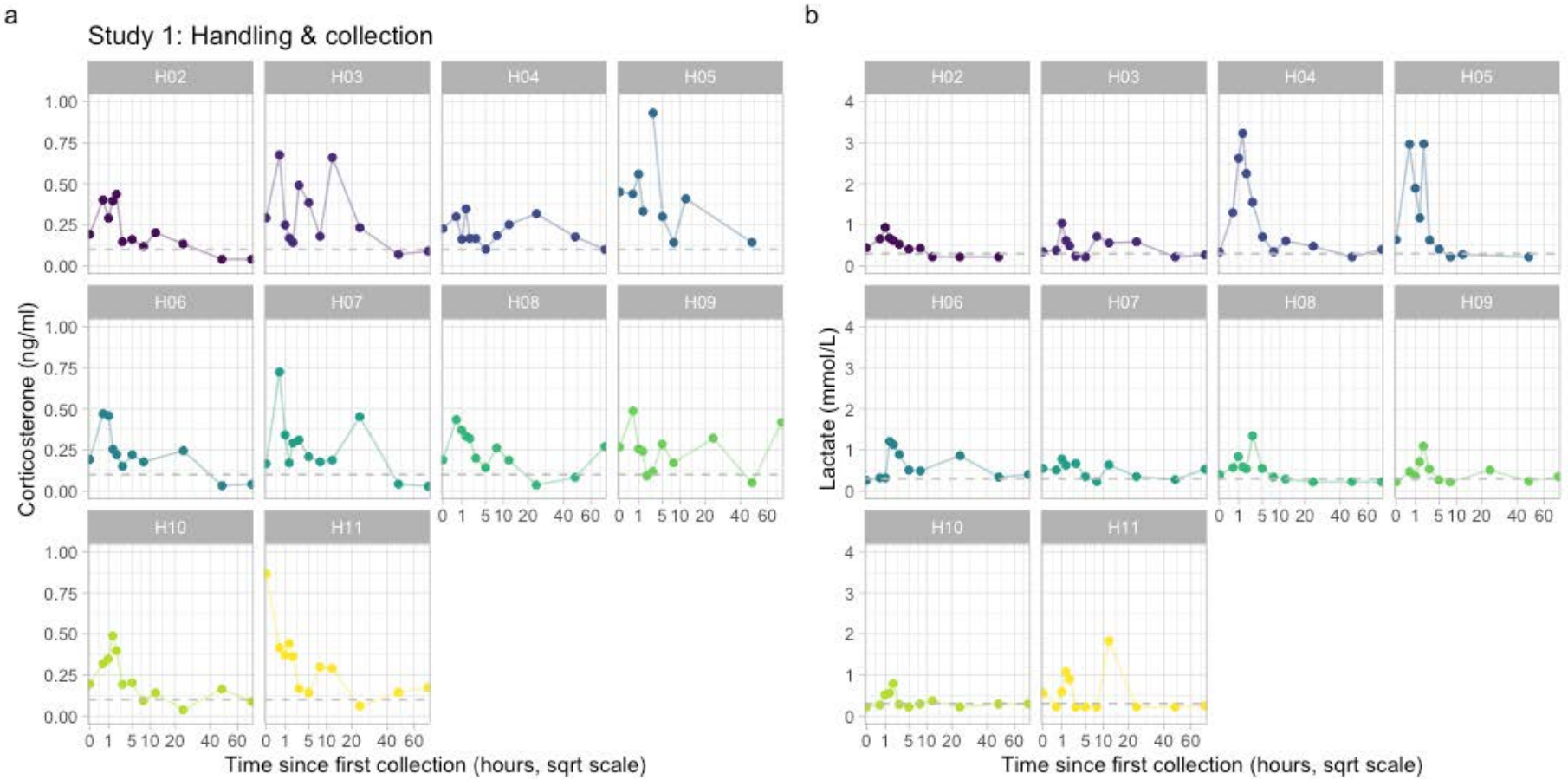



*Figure 5.4. Change in a) corticosterone (ng/ml) and b) lactate (mmol/L) concentration over time for each of 10 juvenile hawksbill turtles (Eretmochelys imbricata) resulting from general handling and sampling throughout Study 1. X axis is square-root scaled and Y axis is capped at a) 1 ng/ml and b) 4 mmol/L. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L).*

## Study 2: Response to acute stressor

In response to the acute stressor, the turtles' corticosterone concentrations showed a small peak and a quick recovery whereas the lactate concentrations showed a large peak followed by a longer recovery time. Corticosterone peaked at a median concentration of 0.23 ng/ml (IQR 0.19 – 0.29 ng/ml), with a median scope of 2.32-fold increase from grouped basal (IQR 1.88 – 2.89-fold). Collectively, time to peak corticosterone concentration was approximately 1 hour (Fig. 5.5a), and recovery to grouped basal was recorded 0.5 hours post peak ($p=0.916$), at the next sample collection. Median peak lactate concentration was 10.51 mmol/L (IQR 8.55 – 11.58 mmol/L), and the median scope increase was 34.12-fold (IQR 25.06 – 42.42-fold). Collectively, time to peak lactate concentration was approximately 0.5 hours post sample 1 (Fig. 5.5b); however, lactate concentrations at samples 2 (≈5 minutes), 3 (≈35 minutes), and 4 (≈65 minutes) were not significantly different ($p<0.05$). Recovery to a lactate concentration not significantly different from grouped basal was recorded in sample 9 ($p=0.065$), approximately 7.5 hours post peak. The best fitted models (GLMM) for corticosterone and lactate accounted for 51% and 94% of the variation, respectively. The corticosterone model (Appendix Suppl. Table 5.4) included turtle temperature ($t=-5.96$, $p<0.001$) and bleeder (only one bleeder significantly different: $t=3.04$, $p=0.003$) as additional fixed effects, whereas the lactate model (Appendix Suppl. Table 5.5) included only bleeder (only one bleeder significantly different: $t=4.15$, $p<0.001$) in addition to sampling time.

Individual variation amongst turtles was lower for corticosterone than for lactate (Fig. 5.6). Peak corticosterone concentrations ranged from 0.17 – 0.39 ng/ml, with a scope increase of 1.71 (H02) – 3.92-fold (H09) higher than grouped basal. Peak lactate concentrations ranged from 5.89 – 13.00 mmol/L, which was a scope increase of 16.36 (H07) – 52.00-fold (H11) higher than grouped basal. Time to recorded peak corticosterone took approximately 1 hr for half of the turtles (n=5), with three turtles peaking sooner (≈5 minutes, n=2; ≈35 minutes, n=1), and two peaking later (≈2 hours, n=1; ≈8 hours, n=1). Two turtles were not recorded as returning to grouped basal corticosterone concentrations within the first day of sampling, although both showed decreased concentrations from the peak. Of the other eight turtles, time from peak to recorded concentration below grouped basal took up to 0.5 (n=2), 1 (n=2), 1.5 (n=1), 2.5 (n=1), 3 (n=1), and 4 (n=1) hours. The corticosterone concentration of Turtle H02 peaked the first day of sampling at sample 9 (≈8 hours post sample 1); however, it showed a spiked increase in corticosterone concentration at sample 3 (≈35 minutes post sample 1), and reduced corticosterone concentration at the following sample (≈30 minutes post spike). Time to recorded peak lactate ranged from approximately 5 minutes (n=4) to 1 hour (n=2), with the remaining four

turtles reaching peak lactate concentration at approximately 0.5 hours. Time to recorded recovery of lactate concentration varied from 4.5 hours (n=2) to 12 hours (n=2), with other turtles recovering to grouped basal lactate within 7 (n=2), 7.5 (n=1), and 8 (n=2) hours. One turtle (H11) did not return to grouped basal lactate levels within the first sampling day; however, did reach near grouped basal at sample 9, approximately 7.5 hours post its peak.

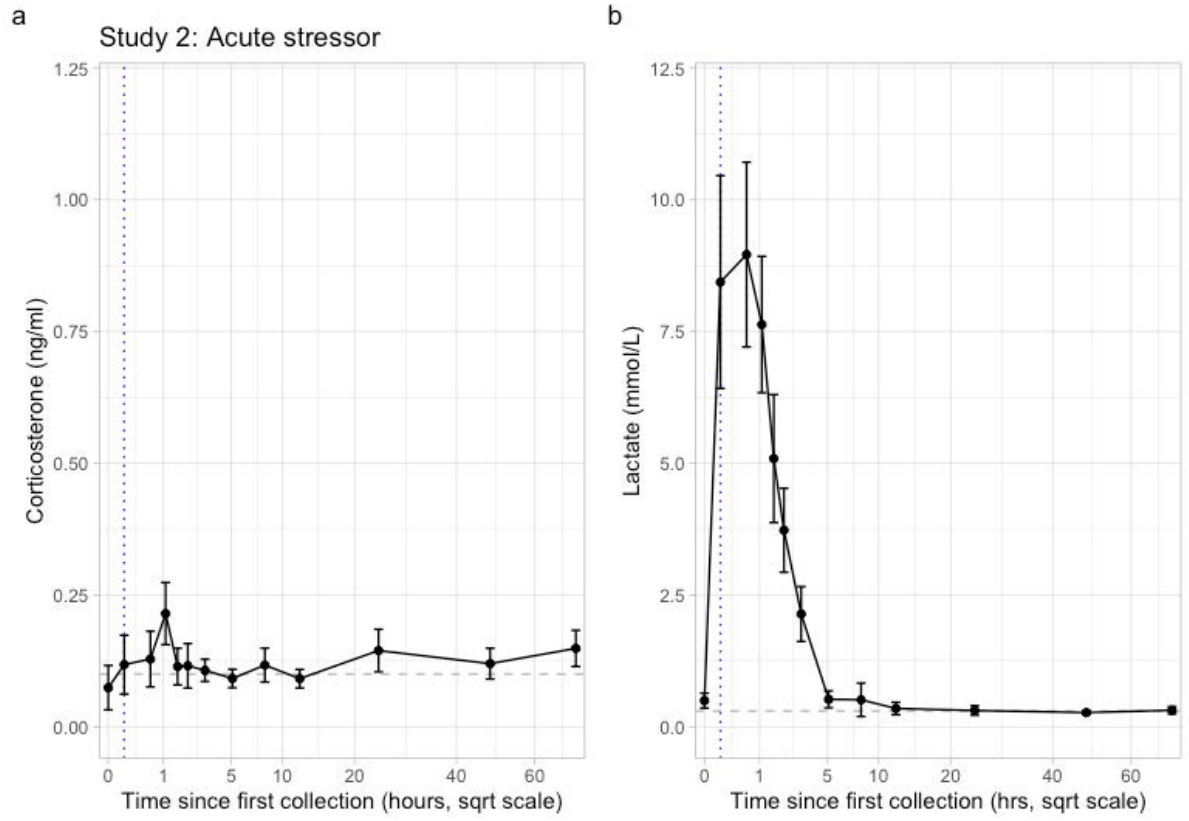



*Figure 5.5. Change in a) corticosterone (ng/ml) and b) lactate (mmol/L) concentrations over time for a group of 10 juvenile hawksbill turtles (Eretmochelys imbricata), resulting from an acute (5 minute) stressor (Study 2). Figure displays mean values (using log10 transformed data) with 95% confidence interval bars. X axis is square-root scaled and Y axis is capped at a) 1.25 ng/ml and b) 12.5 mmol/L. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L). Dotted blue lines indicate blood sample after which turtles were returned to their tanks.*

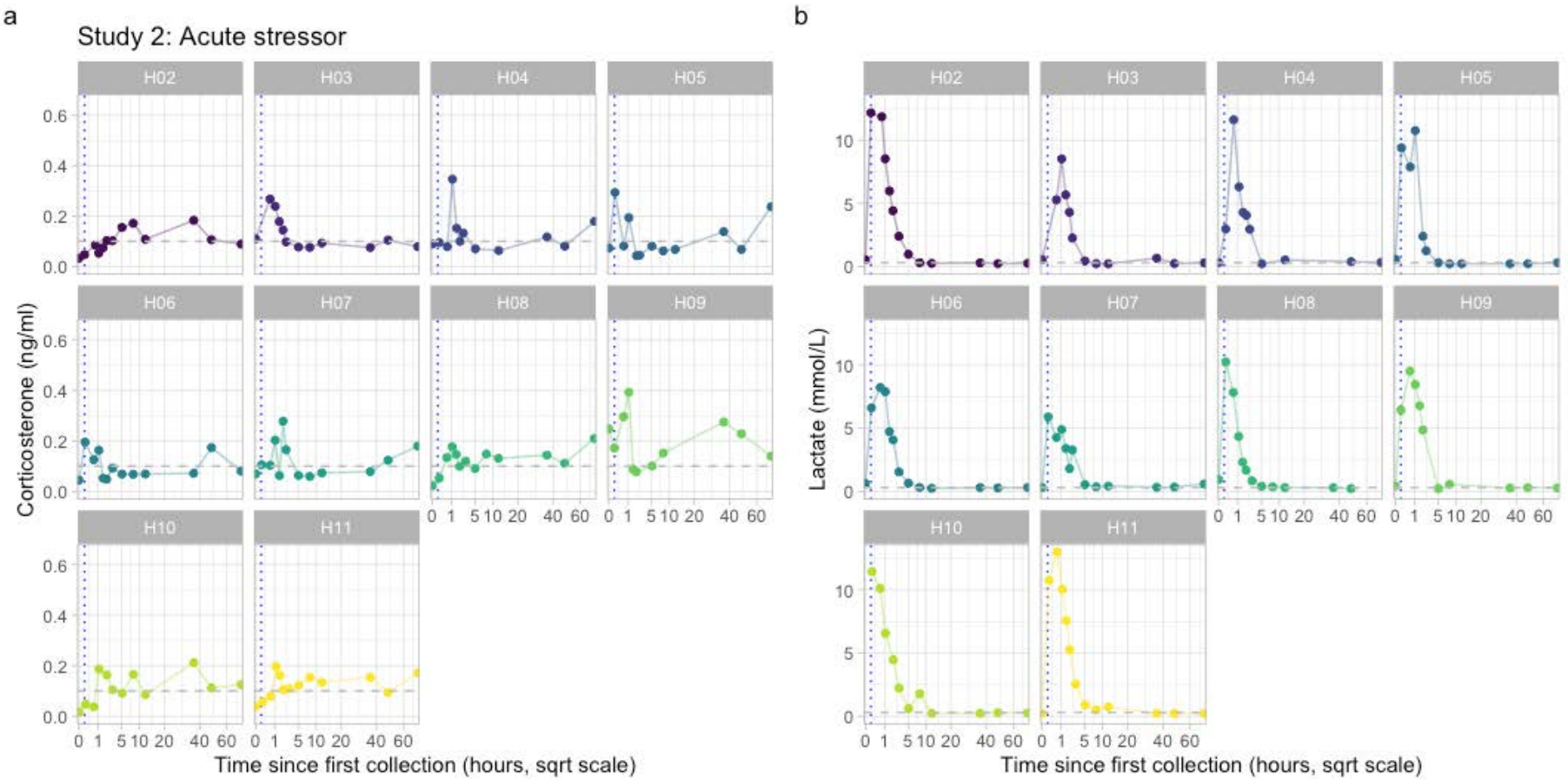



*Figure 5.6. Change in a) corticosterone (ng/ml) and b) lactate (mmol/L) concentration over time for each of 10 juvenile hawksbill turtles (Eretmochelys imbricata) resulting from an acute (5 minute) stressor (Study 2). X axis is square-root scaled and Y axis is capped at a) 0.65 ng/ml and b) 13 mmol/L. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L). Dotted blue lines indicate blood sample after which turtles were returned to their tanks.*

## Study 3: Response to extended stressor

In response to the extended stressor of replica satellite tracker attachment and subsequent dry-docking, the turtles' corticosterone and lactate concentrations stayed above grouped basal for an extended period of time before full recovery back to basal. Concentrations of corticosterone and lactate peaked at a median of 0.37 ng/ml (IQR 0.21 – 0.50 ng/ml) and 3.92 mmol/L (IQR 2.62 – 10.15 mmol/L), respectively. The median scope increase of corticosterone concentration was 3.74-fold (IQR 2.0 – 5.00-fold) compared with 10.88-fold (IQR 9.90 – 37.18-fold) for median lactate concentration scope. Collectively, time from sample 1 to recorded peak corticosterone and lactate concentrations was 1 – 2.5 hours (Fig. 5.7a) and 2 – 2.5 hours (Fig 5.7b), respectively, with samples within these times showing no significant difference from one another ($p>0.05$) due to large variation amongst turtles (Fig 5.8). The turtles were recorded as returning to basal corticosterone and lactate concentrations by samples 7 (1.5 – 3 hours post peak) and 9 (6.5 – 7 hours post peak). Both corticosterone and lactate concentrations recovered to grouped basal whilst the turtles were still dry-docked. The best fitted model (GLMM) for corticosterone only accounted for 39% of variation and included weight of the turtles ($t=-2.94$, $p=0.004$) as an additional fixed effect (Appendix Suppl. Table 5.6). The best fitted model (GLMM) for lactate accounted for 73% of the variation and included no additional fixed effects beyond sample time (Appendix Suppl. Table 5.7).

There was a large amount of individual variation amongst turtles for both corticosterone and lactate response to the extended stressor study (Fig. 5.8). The corticosterone response in particular showed several spikes and decreases in concentration, notably at the final sample of the day, just prior to being returned to their tanks (13 hours post sample 1). The lactate concentrations also showed a grouped spike at this final sample pre return to tank. Peak corticosterone and lactate concentrations ranged from 0.17 – 0.95 ng/ml and 0.97 – 13.00 mmol/L, respectively. The range in scope increase was 1.69 (H10) – 9.53-fold (H09) for corticosterone and 3.88 (H10) – 45.16-fold (H05) for lactate concentrations. Individual peak corticosterone concentrations were recorded at 1 (n=1), 1.5 (n=3), 2 (n=2), 2.5 (n=2), and 3 (n=2) hours post sample 1. Time to individual peak lactate concentrations had a smaller range with peak reported at 1 (n=2), 1.5 (n=3), 2 (n=1), and 2.5 (n=4) hours post sample 1. Time to recovery (grouped basal) from peak was generally achieved quicker for corticosterone than lactate concentrations. Time to recovery in corticosterone was achieved within 0.5 (n=1), 1 (n=4), 1.5 (n=2), 2 (n=1), 3 (n=1), and 6.5 (n=1) hours. Only half (n=5) of the turtles were recorded as recovered to grouped basal by the end of the first day of sampling, with recovery achieved within 4.5 (n=1), 7.5 (n=2), 8

(n=1), and 12 (n=1) hours. The remaining five turtles whose lactate concentrations did not return to below grouped basal within Day 1 did, however, show recovery to near basal.

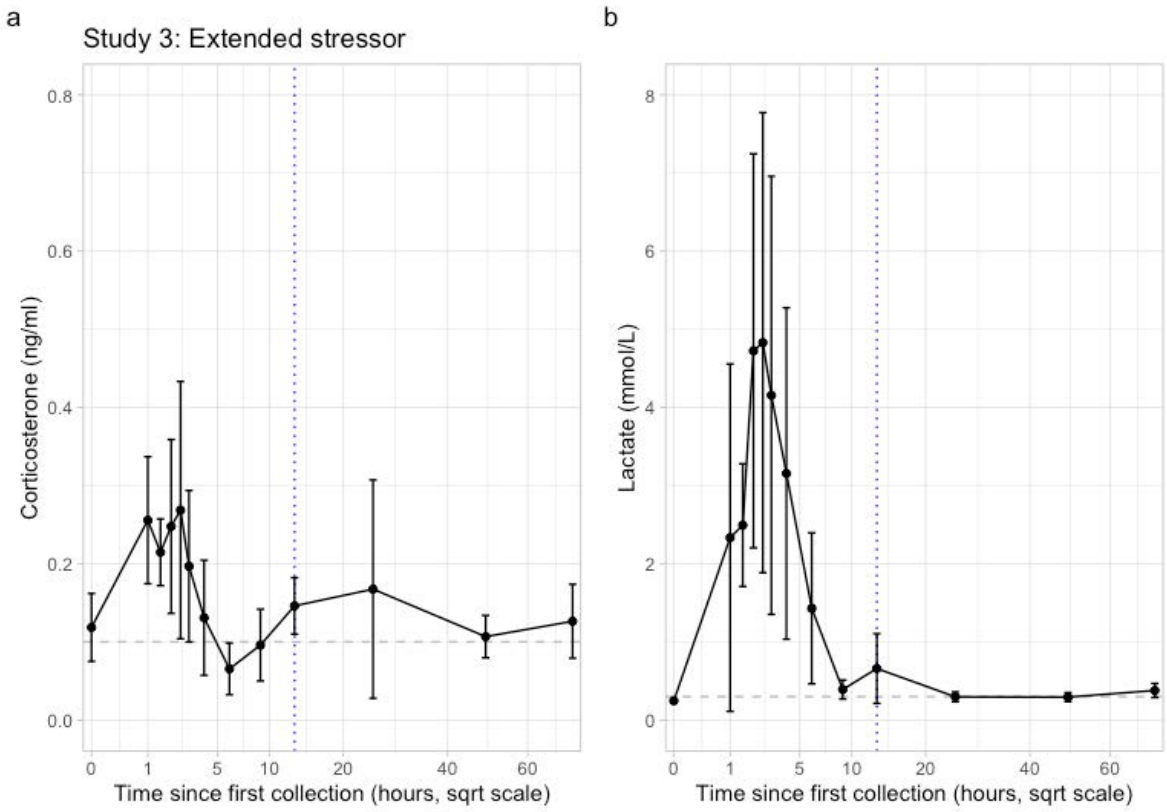



*Figure 5.7. Change in a) corticosterone (ng/ml) and b) lactate (mmol/L) concentrations over time for a group of 10 juvenile hawksbill turtles (Eretmochelys imbricata), resulting from an extended stressor (replica tracker attachment + dry docking, approximately 13 hours total; Study 3). Figure displays mean values (using log10 transformed data) with 95% confidence interval bars. X axis is square-root scaled and Y axis is capped at a) 0.8 ng/ml and b) 8 mmol/L. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L). Dotted blue lines indicate blood sample after which turtles were returned to their tanks.*

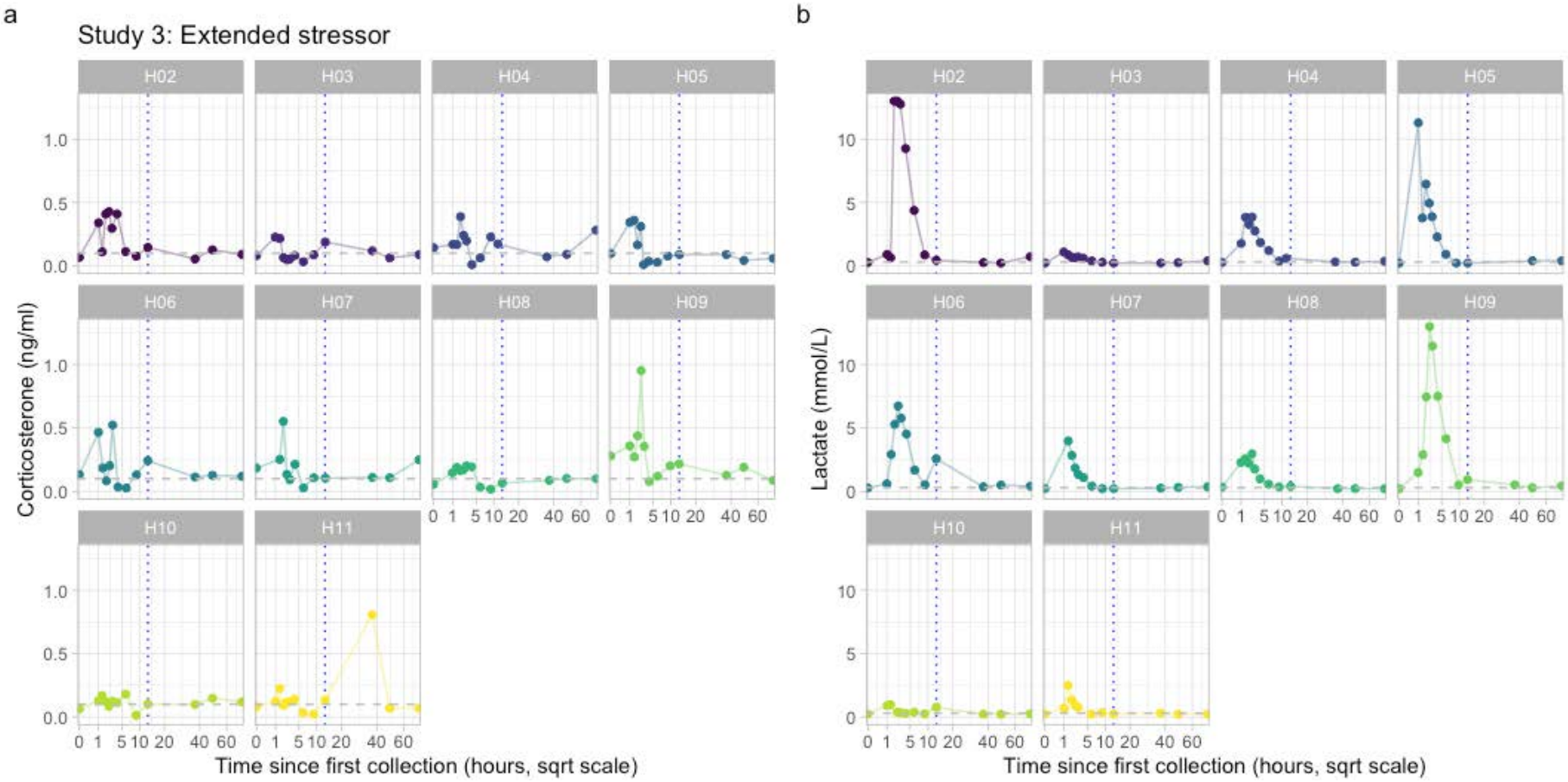



*Figure 5.8. Change in a) corticosterone (ng/ml) and b) lactate (mmol/L) concentration over time for each of 10 juvenile hawksbill turtles (Eretmochelys imbricata) resulting from an extended stressor (replica tracker attachment + dry docking, approximately 13 hours total; Study 3). X axis is square-root scaled and Y axis is capped at a) 1.3 ng/ml and b) 13 mmol/L. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L). Dotted blue lines indicate blood sample after which turtles were returned to their tanks.*

## Inter-study comparison

Overall, the corticosterone response was far smaller than the lactate response by concentration, but the concentration variation trends observed in response to the different stressor events were similar (Fig. 5.9). Corticosterone and lactate concentrations both increased in response to each study stressor and then recovered to grouped basal after a given period. Corticosterone response was greatest in the first study, with sample 1 already well above basal concentration. In response to the acute stressor (Study 2), corticosterone concentrations showed a small peak and quick recovery, whereas in response to the extended stressor (Study 3), corticosterone concentrations showed a marginally higher peak but with a longer recovery time. Lactate response was greatest in Study 2 as a response to the short stressor; however, some turtles also showed equally large increases in lactate concentrations in Study 3 as a response to the extended stressor. The time to peak was much shorter in the acute stressor study (Study 2) than in the extended stressor study (Study 3); however, turtles recovered to basal lactate concentrations by sample 9 in both Study 2 and Study 3. The largest individual variation was observed for lactate concentrations in response to the extended stressor (Study 3). All individual data summaries for peak, scope, time to peak, and time to recovery are documented in Appendix Suppl. Table 5.8.

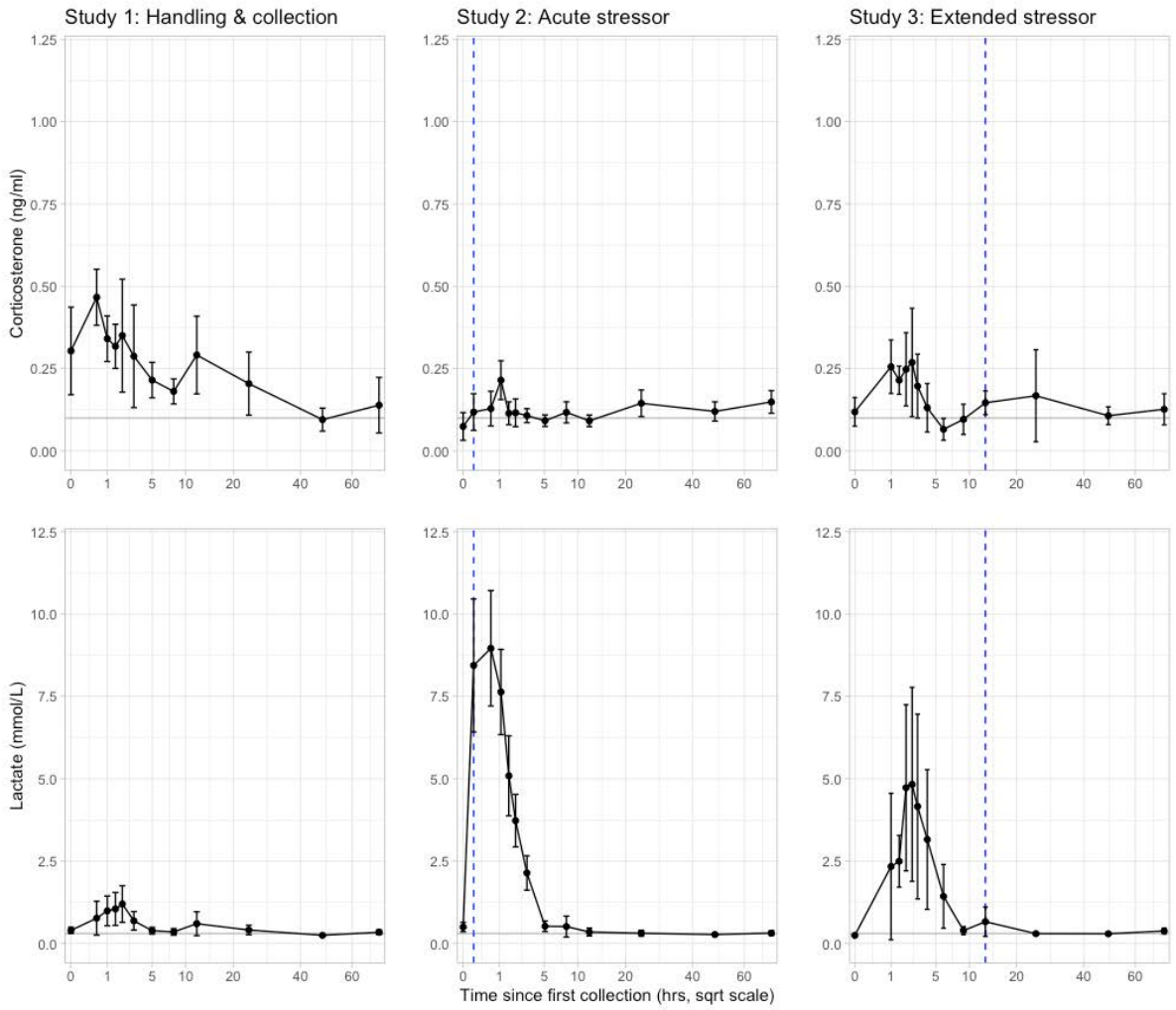



*Figure 5.9. Change in corticosterone (ng/ml) and lactate (mmol/L) concentrations over time for a group of 10 juvenile hawksbill turtles (Eretmochelys imbricata), resulting from handling and blood collection (Study 1), an acute stressor (Study 2), and an extended stressor (Study 3). Figure displays mean values (using log10 transformed data) with 95% confidence interval bars. Axes are scaled; the lactate concentration axis is 10-fold higher than corticosterone. Dashed grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L). Dotted blue lines indicate blood sample after which turtles were returned to their tanks.*

## Study 4: Response to transportation stress

The median blood concentrations of corticosterone (Figure 5.10a) collected from the turtles the afternoon before release (sample 1: day before release) was 0.17 ng/ml (IQR 0.10 – 0.18 ng/ml), which was higher than the previously calculated basal. After being kept semi-dry overnight, their temperatures had decreased by approximately 10 °C and their corticosterone concentrations (sample 2: pre-transport) had increased to a median concentration of 0.27 ng/ml (IQR 0.20 – 0.39

ng/ml), 1.59-fold higher than the previous sample. After transportation to the release site, the turtles were sampled again (sample 3: post-transport), and their corticosterone had increased to a median concentration of 0.41 ng/ml (IQR 0.33 – 0.44 ng/ml), which was 1.52-fold higher than prior to transportation and 2.41-fold higher than the previous day (sample 1). There was individual variation within each sample, particularly notable at sample 3, which was post-transportation and prior to release. According to the best fitted model (GLMM), which included turtle temperature as a fixed effect in addition to sampling number, the corticosterone concentration was above basal at all three sampling points (Appendix Suppl. Table 5.9). The pre-transportation and post-transportation (shortly prior to release) corticosterone concentrations were 2.7-fold and 4.1-fold higher than basal, respectively.

Lactate concentration (Figure 5.10b) response varied less than corticosterone, and concentrations were within basal range at all three sampling points (Appendix Suppl. Table 5.10). At sample 1: day before release, the median concentration was 0.28 mmol/L (IQR 0.19 – 0.33 mmol/L), excluding one anomalous sample reported at 3.93 mmol/L. The following morning, at sample 2: pre-transportation, median lactate concentration had reduced to 0.13 mmol/L (IQR 0.11 – 0.16 mmol/L), which was a 2.15-fold reduction. After transportation to the release site, median lactate concentrations at sample 3 had increased to 0.30 mmol/L (IQR 0.26 – 0.42 mmol/L), which was a 2.31-fold increase from pre-transportation. The best fitted model (GLMM) for lactate concentration in Study 4 included only sampling number as a fixed effect but accounted for only 22% of the variation.

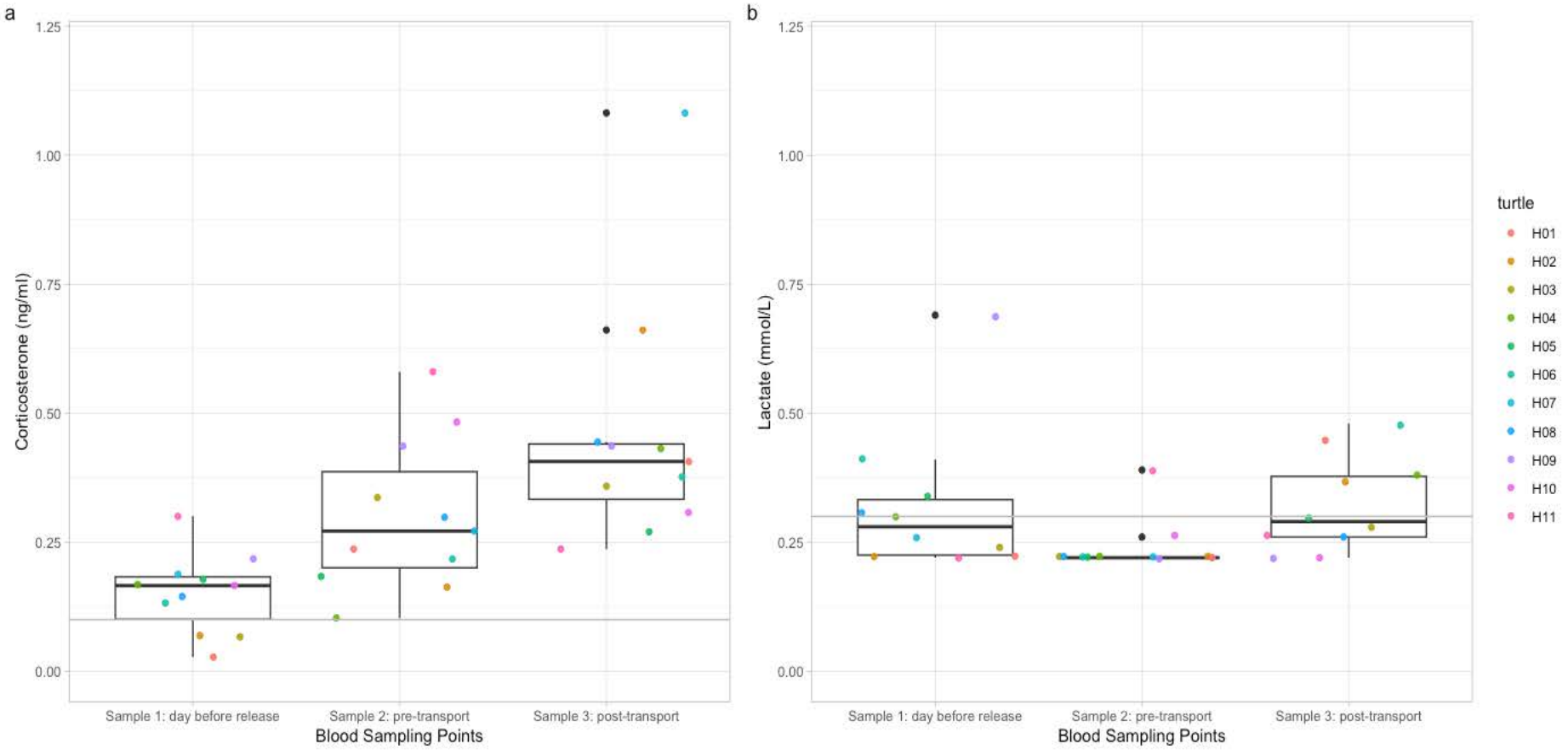



*Figure 5.10. Concentrations of a) corticosterone and b) lactate recorded in 11 juvenile hawksbill turtles (Eretmochelys imbricata) at three time points: Sample 1) day before release; Sample 2) morning of release (pre-transport); and Sample 3) shortly prior to release (post-transport). Grey lines indicate a) the grouped basal concentration of corticosterone (0.10 ng/ml) and b) medium grouped basal concentration of lactate (0.30 mmol/L). Black dots indicate outliers.*

## Discussion

Ability to respond to stressful stimuli physiologically, behaviourally, and mentally is a trait necessary for survival; for example, sea turtles use these mechanisms to escape predation. It is, therefore, crucial to ensure that animals temporarily removed from the wild for conservation or research purposes also retain this essential innate response to maintain survivability following release. Behaviour is often assessed as an indicator of stress, particularly in relation to mental welfare (i.e. presence or absence of stereotypic behaviours); however, understanding the physiological changes that occur in response to stressors can add valuable insight to the health and welfare status of the animal (Korte et al., 2007; Usategui Martín, 2020). Quantifying physiological systemic stress response can be used as a surrogate to assess physical health and welfare of captive turtles. These studies used corticosterone and lactate concentrations in the blood to measure physiological stress response of juvenile hawksbill turtles raised in captivity for approximately 2 years since hatching naturally. Although corticosterone is frequently used to assess animal stress (Gormally & Romero, 2020), limited data are published for juvenile hawksbill turtles of this size and there are very limited data available for biomarkers of stress in any species of sea turtles kept or raised under human care. Lactate is less commonly studied as a biomarker of stress but is important as it elevates in response to capture and restraint in some animals, including reptiles (Abreu da Fonseca et al., 2020; Molinaro et al., 2022). All the turtles recorded in this study showed a response to handling stress, an acute stressor and an extended stressor via increased corticosterone and lactate concentrations in the blood and were able to regulate their systems to return to basal concentrations. Therefore, the hypothesis that captive-raised sea turtles maintain a systemic stress response in which they show a peak and a recovery phase was found to be true. It was also hypothesised that basal range in captive-raised hawksbill turtles would be lower than in wild hawksbill turtles because turtles in captivity are subjected to fewer natural stressors (e.g. not subject to depredation nor food deprivation).

Basal range of any physiological biomarker of stress (corticosterone and lactate in this case) should encompass concentrations required for the turtles to function (Johnstone et al., 2012). This is distinguishable from baseline levels, which are used in field studies to denote samples collected within 3 minutes of capture because it is unknown whether the animal was within basal range pre-capture (Romero & Reed, 2005). The basal range of corticosterone concentration in the blood of the study turtles was low in comparison with the baseline of wild conspecifics (Jessop et al., 2004a), as expected. Although the size of the wild juvenile hawksbill turtles was not stated, the baseline reported by Jessop et al. (2004a) was approximately 6-fold higher than the upper basal

range in this study. Furthermore, Kawazu et al. (2022) reported a concentration of corticosterone for captive-raised hawksbill turtles (median of 0.8 – 0.9 ng/ml) that was similarly low to turtles from this study, despite a disparity in turtle size. Lactate has rarely been collected in hawksbill turtles, although Muñoz-Pérez et al. (2017) recorded it for hawksbill turtles (41 – 82 cm CCL) sampled within an average of 27 minutes post-capture, with blood lactate concentrations ranging from 0.9 – 2.7 mmol/L (mean 1.6 mmol/L). The basal ranges for lactate concentrations of turtles in this study were no higher than 0.35 mmol/L and lactate was found to increase significantly within 5 minutes of an acute stressor and so a direct comparison is not possible. Stress response in vertebrates has been found to vary between populations, within populations by sex, and even within individuals seasonally, diurnally, and ontogenically (Johnstone et al., 2012; Landys et al., 2006). This complicates the interpretation of results within a single study group and hinders comparison between groups of the same species (Flower et al., 2015). However, it is important to ascertain basal ranges for each population or group studied to understand the extent of their physiological stress response to introduced stressors. This knowledge can in turn be used for ongoing health and welfare checks, particularly for animals kept or raised under human care.

The first calculation of basal ranges in this study were made using the concentrations of corticosterone and lactate at the first sample of each day (1 – 4) for the first three studies (responses to handling, acute stressor, and extended stressor). The assumption was that these concentrations would be low because no handling had yet occurred (per day) and it was expected that sufficient time had elapsed since sampling ended the previous day for these samples to be independent. However, this method did not yield the best results because corticosterone concentration increases were highest in the first study and were particularly high at the start of the first study, above the upper basal range. Lactate concentrations at the start of the first study were also slightly above upper basal range. This was most likely a combination of the novelty of the blood sampling procedure stressor at the start of the first study, to which they partially acclimated throughout Studies 1 – 3 (Moszuti et al., 2017), and improvements to the sample collection workflow. The collection of repeated blood samples for multiple turtles in these studies required a large team of research assistants but as the team grew more experienced with the protocol, fewer people were required at each sample. This was particularly true on days 3 and 4 of each study because only one sample was collected per turtle on each of those days. Furthermore, corticosterone fluctuates diurnally, and therefore using data only collected at 06:00 or 07:00 would have likely biased the calculated basal range (Jessop et al., 2002b; Kawazu et al., 2022). Therefore, using the 25$^{th}$ quantile of the dataset gave a better representation of the upper

basal range than if the first bleed of each day had been used, which would have been more akin to a "baseline" measure than a "basal" one.

It is important for turtles kept or held under human care to acclimate and not attenuate their response to the stress from routine husbandry so that health and welfare are maintained (Johnstone et al., 2012). For turtles under human care, stress response varies by frequency of human interactions, with increased handling frequency resulting in reduced corticosterone response (Usategui-Martín et al., 2021). The hawksbill turtles in this study were acclimated to regular handling throughout their time under human care, as indicated by low basal concentrations (upper limit: 10 ng/ml corticosterone; 0.25, 0.30, and 0.36 mmol/L lactate) and lack of physical or behavioural indicators of chronic stress (Diggins et al., 2023). However, they also showed a significant response ($p<0.001$ corticosterone and lactate) to the handling and blood collection procedure in Study 1, as predicted, and indicated by increased concentrations of corticosterone and lactate (peak at 4.88-fold and 3.90-fold above basal, respectively). Corticosterone response to handling stress has been shown in other species of captive and free-living sea turtle (Abreu da Fonseca et al., 2020; Flower et al., 2015; Hunt et al., 2019; Hunt et al., 2016; Hunt et al., 2020; Usategui-Martín et al., 2021) and lactate response has also been noted in free-living turtles (Abreu da Fonseca et al., 2020) and captive crocodiles (Molinaro et al., 2022). Of note, Crooks et al. (2023) found that lactate dehydrogenase also increased for free-ranging hawksbill turtles with time out of the water and handling.

In addition to the peak concentrations, several spikes in corticosterone and some in lactate concentration were observed for individual turtles throughout the studies. There was a notable peak in corticosterone and lactate at approximately 19:00 in Study 1 and in Study 3 to a smaller magnitude. The increase in corticosterone could be partially explained by natural diurnal fluctuation. However, it is more likely that the lactate and corticosterone concentrations increased as a stress response to the lights turning on (Mancera & Phillips, 2023), with some turtles recorded displaying panicked swimming and escape behaviours immediately after. The numerous spikes indicate that the turtles were not only responding to the initial stress of being handled and sampled but also to additional stressors, likely including increased human presence (Carter et al., 2021). Behavioural and physical changes in the hawksbill turtles had been observed on previous occasions, pre-study, whereby the turtles lost weight following an event in which numerous people were near the tanks without any handling of the turtles (RD personal observation). Desensitisation to humans is a concern for captive-raised sea turtles because of the potential negative implications on their survival post-release (Wright et al., 2020). Therefore, the

findings of this study are important because the turtles were observed to have a physiological stress response to handling and human presence despite being captive raised for 2 years.

Another key concern regarding temporarily holding sea turtles under human care for later release (usually for conservation purposes) is how the captive environment, free from predators, might affect their ability to respond to acute stressors such as depredation post-release (Tetzlaff et al., 2019b). The hawksbill turtles in this study showed a response to the acute stressor that was 2.3-fold higher than basal corticosterone and 34.1-fold higher than basal lactate concentrations. Therefore, these captive-raised turtles retained their ability to respond to a novel acute stressor, and to recover back to within their basal ranges within hours post-cessation of the stressor. Lactate is a particularly important biomarker for physiological stress response of reptiles because it is produced by anaerobic glycolysis as a result of muscle exertion, for example when escaping predators (Crooks et al., 2023; Donovan & Gleeson, 2001). The sharp increase in lactate concentration recorded after just 5 minutes in Study 2 (acute stressor) was a good indicator that the turtles would have the ability to physiologically respond to depredation threat. The extreme peak in lactate concentrations documented in the acute stressor response (Study 2) was likely caused by the increased muscle exertion from flipper movement of the turtles during the stressor, which was facilitated by being positioned in dorsal recumbency. This was also the only study in which lactate concentrations peaked before corticosterone. Again, this was due to the increased movement and therefore glycolysis causing quick build-up of lactate to fuel the muscles (Rabinowitz & Enerbäck, 2020).

When comparing the captive-raised hawksbill turtles in this study with the free-living immature hawksbill turtles recorded by Jessop et al. (2004a), the stressor protocol in Jessop was most similar to the extended stressor study. Corticosterone response to the extended stressor in captive-raised hawksbill turtles increased 3.74-fold higher than basal compared with the wild turtles whose response was 11.01 to 19.52-fold higher than their recorded baseline. The difference in scope between captive-raised and free-living hawksbill turtles was up to 5.22-fold, which is comparable to the difference between basal and baseline (approximately 6-fold). A further difference between the free-living and captive-raised hawksbill turtles is that after 5 hours of being dry-docked, free-living turtles maintained high corticosterone concentration, whereas the captive-raised turtles had returned to basal range within the same timeframe. This could be partially explained by the positioning of the free-living turtles in dorsal recumbency (on their carapace) throughout the stress protocol in comparison with the captive-raised turtles in this study that were positioned plastron down. Given the purpose of this study was to determine the stress

response to having a tracker attached, for which turtles would be kept on their plastrons, it is possible that free-living turtles may also show some recovery during the dry-docking time if kept calm. Keeping turtles calm would be preferable to prevent stress attenuation, which could reduce the turtles' health pre-release (Caliani et al., 2019).

Difficulty in interpretation can come from distinguishing between reduced stress response due to acclimation versus attenuation. The first has a positive effect on health and welfare, whereas the second has a negative effect (Johnstone et al., 2012). This point has been argued in a stress study of rhinoceroses during translocation (Linklater et al., 2010). Furthermore, translocated crocodiles are susceptible to adverse outcomes due to overproduction of lactate from stress response (Molinaro et al., 2022; Nevarez, 2019). The same result has not been reported in sea turtles, most likely because they are prey animals with high tolerance to anoxia, compared with crocodiles, which are ambush predators and generally sedentary (Schmitz, 2017; Warren & Jackson, 2008). Additional health and fitness indicators should be measured to determine the cause of the reduced corticosterone production. Allostatic load is a concept that considers cumulative degradation of welfare due to repeated or chronic stress. This concept is widely used in research of chronic stress on humans but has been less commonly applied to non-human animals (Seeley et al., 2022). Development of a species-specific allostatic load index can help monitor and predict health, welfare, and likelihood of mortality at individual and population levels (Edes et al., 2018). In this case, it would also help to determine whether animals were experiencing stress acclimation or attenuation. For the turtles in this study, other welfare assessments determined the turtles as being clinically healthy before release.

During the transportation from research facility to release site, the concentrations of both corticosterone and lactate increased (1.52-fold and 2.31-fold, respectively). In comparison with the extended stressor study outcome where corticosterone returned to basal within 4 hours of stressor commencement, corticosterone concentration after approximately 4 hours of transportation was 1.52-fold higher than the pre-transportation sample. Since there were no samples collected during transportation, it is plausible that corticosterone concentrations peaked after approximately 1 hour (per Study 2 and 3) and were in the recovery phase. A study of transportation stress in Kemp's ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) turtles found a post-transport (<6-hour duration) increase of 1.35-fold and 2.69-fold in corticosterone concentrations, respectively (Hunt et al., 2020). Interestingly, in the same study, lactate decreased 2.00-fold post-transport in Kemp's ridley turtles but increased 4.47-fold in loggerhead turtles, compared with a 2.31-fold increase in lactate from the captive-raised hawksbill turtles in this study.

This disparity in stress response further highlights the need to study species individually to gain the best understanding of their health and welfare status and requirements for release protocol (Hunt et al., 2020).

Lactate concentrations throughout the hawksbill transportation study were generally low and not significantly above basal range at any point measured ($p>0.099$), whereas corticosterone concentrations were above basal at all points ($p<0.022$). The reduction in lactate is most likely attributed to the large decrease in temperature experienced by the turtles during this study (>5°C) (Adamovicz et al., 2018). As ectotherms, reduction in temperature resulted in reduced movement by the turtles and therefore low concentrations of lactate (1.92 – 2.77-fold below grouped basal). Maintaining their summer temperatures in winter, whilst keeping them semi-dry docked to allow anti-fouling paint to dry, was attempted but not achieved. Corticosterone, conversely, has been shown to increase in response to low temperature stress (Dupoué et al., 2013). This could partially explain why the first sample had corticosterone concentrations above basal range. Seasonal changes could also explain some variation between lactate and corticosterone in the final study compared with the first three, although seasonal studies have been found to be specific to species, size-class, and region (Gregory et al., 1996; Miguel et al., 2020; Moore & Jessop, 2003; Romero, 2002). It is possible that the basal range of corticosterone may have increased in the 5 to 6-month period between Study 1 – 3 and Study 4, with considerable growth of the turtles in that time (median weight and CCL increase of 2.11 and 1.26-fold, respectively). However, a study of captive-raised loggerhead turtles found no correlation between turtle size and corticosterone concentrations (Usategui-Martín et al., 2021). It is more likely that in the time between studies the turtles' desensitisation to the protocol regressed and their sensitivity to handling re-established to be similar to the results of Study 1 (handling and blood sampling). A final consideration as a possible reason for the higher than basal corticosterone concentrations in the first sample of Study 4 is that samples were collected shortly after the afternoon husbandry routine, meaning that there was a greater human presence around the tanks despite no interaction with the study turtles.

The hawksbill turtles' corticosterone and lactate responses (scope, time to peak and time to recovery) differed between the acute and extended stressors. Corticosterone concentrations peaked at approximately 1 hour post sample 1 with both stressors but recovery was 1 – 2.5 hours shorter after the acute stressor than the extended one. Lactate concentrations reached their peak 1.5 – 2 hours sooner following the acute stressor than the extended stressor, but interestingly the recovery time from peak to within basal range was 0.5 – 1 hour longer for the acute stressor

than the extended stressor. This could be due to the magnitude of lactate stress response in the acute stressor trial being 3-fold larger than in the extended stressor study. However, the total time that lactate concentrations remained elevated above basal range was longer in response to the extended stressor (9 hours total) than the acute stressor (8 hours). Understanding the responses to different types of stressors is, therefore, important when determining at what time to sample and with which biomarker (Gormally & Romero, 2020). A review interpreting stress indices by Johnstone et al. (2012), proposed a two-axis model for physiological stress with four testable characterisations of stress: transient (short-term, mild), acute (short-term, severe), prolonged (long-term, severe), and moderate (long-term, mild). Following this model, the four experimental studies within this overarching study could arguably be characterised the same way: Study 1) handling and sampling (transient); Study 2) acute stressor (acute); Study 3) extended stressor (prolonged); and Study 4) transportation (moderate). Understanding the different responses to each stressor would help with selecting an appropriate sampling design including choice of biomarkers, interpreting results of future studies or health and welfare checks, and determining best time point for release.

There are several other important considerations when selecting stress metrics, particularly in a conservation setting such as a sea turtle head-start or rehabilitation centre. These include the timescale of the stressor requiring evaluation (Gormally & Romero, 2020), feasibility (time, money, skillset, available resources, ability to control for temperature) of the collection and analysis methods (Diggins et al., 2022), and species and life-stage of the individual (Jessop et al., 2004b; Jessop, 2001; Moore & Jessop, 2003; Pereira et al., 2012). Ideally, several metrics should be considered in parallel, collected within minimal handling time. Specifically, it has been advised that lactate and corticosterone should be analysed concurrently in sea turtles (Abreu da Fonseca et al., 2020). This would provide more reliable interpretation of the data and a better overall picture of the animal's health and welfare (Seeley et al., 2022).

Stress response is highly variable (Flower et al., 2015; Jessop et al., 2004a), which means that large cohorts would be required to make general assumptions. The results of these studies are relevant only to the individuals recorded; however, they do aid in filling the large knowledge gap of juvenile hawksbill and captive-raised turtle stress response. Although the sex of the study turtles remained undetermined, no significant difference between male and female immature hawksbill turtles was found in a study of free-living turtles (Jessop et al., 2004a). Even within this small cohort of turtles, there was large individual variation in both corticosterone and lactate response, for example, the statistically-based determination of three basal range limits of lactate concentration. Although only

one corticosterone basal range was determined in this study, it is possible that more variation would have been detected with a larger number of turtles as only one turtle in this group had a higher basal limit.

This study evaluated the physiological stress response of captive-raised hawksbill turtles using corticosterone and lactate concentrations as biomarkers. Research extending from the current study could involve capture of free-living hawksbill turtles from the same population and of a similar size for temporary holding before conducting the same or similar studies. Study of turtles that have lived in the wild but are temporarily held under human care would help to further answer questions regarding the effects of captivity on sea turtles and how this may help or hinder conservation efforts. It would also be beneficial to measure additional stress biomarkers, particularly those that can be detected with minimal additional sampling effort required. Studying the behavioural response to stressors alongside the physiological metrics would add another dimension and assist with understanding stress response, thus helping conservation programs promote positive welfare for temporarily captive sea turtles.

## Conclusion

It is important to understand species-specific and size-class specific stress responses of sea turtles to maximise positive welfare state of turtles kept or raised under human care. Some level of stress acclimation is desired so that turtles are not overly disturbed by routine husbandry, housing, and care and do not experience stress attenuation in response to their conditions. Furthermore, for turtles intended for release, individuals should retain the ability to respond to and recover from both short and extended stressors so that post-release survivability is not reduced. This can be achieved by handling the turtles for short periods of time as part of routine husbandry procedures and periodically introducing acute stressors during husbandry (and research) of captive turtles. Sea turtles held under human care that maintain optimal stress responses are more likely to reach appropriate fitness for release with good health and welfare outcomes. The hawksbill turtles in this study had a lower basal range than wild conspecifics but similar to adult captive-raised hawksbill turtles from another region. Additionally, these study turtles displayed a corticosterone and lactate response under four different stressors and recovered to basal range within hours of stressor cessation. Therefore, this study showed that small juvenile hawksbill turtles raised under human care can maintain innate stress response mechanisms indicative of good health and fitness and positive welfare, with a positive outlook for post-release survivability.

# Summary of thesis and chapter aim

## Thesis aim 3

Characterise and compare physiological indicators of stress response in captive-raised juvenile hawksbill turtles after 2 years under human care.

## Chapter aims

### Chapter aim 1

- Determine basal ranges of corticosterone and lactate concentrations in captive-raised juvenile hawksbill turtles.

The basal range upper limit of the group of captive-raised juvenile hawksbill turtles was found to be 0.10 ng/ml, with individual upper limits of basal concentrations ranging from 0.07 – 0.12 ng/ml (n=10). The individual upper limits of basal range of lactate concentrations ranged from 0.22 – 0.36 mmol/L (n=10). Turtles were categorised into three distinct groups for basal lactate limits: 1) low (0.25 mmol/L); 2) medium (0.30 mmol/L); and 3) high (0.36 mmol/L), with a statistically significant difference found for data points below respective basal limits amongst the three groups.

### Chapter aim 2

- Assess how captive-raised juvenile hawksbill turtles respond to the following stressors:
    - handling and blood collection;
    - short-term stressor (5-minute stimulation);
    - tracker attachment (1 hour) and dry-docking (12 hours); and
    - transportation from turtle housing facility to release site.

The captive-raised juvenile hawksbill turtles showed a physiological stress response to all four stressors applied, as demonstrated by variations in corticosterone and lactate concentrations in the blood. Turtles showed the largest corticosterone response to the first study, which only sought to measure handling and sampling stress effects. This indicates that the turtles are still sensitive to human presence, despite being raised in captivity with regular human interaction through daily husbandry, including weekly handling for grooming and measurement of morphometrics. Turtles showed the largest lactate response to the acute stressor, which is unsurprising since lactate is formulated during glycolysis to energise the muscles and the acute stressor stimulated flipper movement. The extended stressor caused a more sustained physiological stress response than the acute stressor, with concentrations of both corticosterone

and lactate remaining above upper basal limit for longer. Although it was expected that the stress response may remain above basal range for longer during tracker attachment and dry-docking of the turtles to allow the adhesive agent to dry, it was unknown whether stress biomarkers would fall back within basal range before turtles were returned to the water. Both lactate and corticosterone concentrations returned to within basal range before turtles were returned to the water, showing an indication of acclimation to the stressor. Lactate response was stronger than corticosterone response for the acute and extended stressors in both scope from basal range to peak and time to recovery form peak to basal range. However, lactate was lower in the transportation study due to decreased temperatures resulting in reduced flipper movement. Corticosterone and lactate concentrations both increased significantly from basal range during transportation to the study site, but neither were measured at higher than peak concentrations from the previous three studies, from which the turtles were all able to recover back to basal range within a few hours.

# Appendix

*Suppl. Table 5.1. Median, interquartile range (IQR), and range of hawksbill turtle (Eretmochelys imbricata) cloacal temperatures during four stress studies: 1) Handling and blood sampling; 2) acute stressor; 3) extended stressor; and 4) transportation stress.*

| | 1) Handling | 2) Acute | 3) Extended | 4) Transport |
|---|---|---|---|---|
| **Median** | 28.6 | 28.4 | 27.8 | 25.7 |
| **IQR** | 28.3 - 28.9 | 27.9 - 28.7 | 27.4 - 28.2 | 22.9 - 28.0 |
| **Range** | 27.1 - 30.1 | 27.5 - 29.8 | 26.5 - 28.8 | 15.0 - 29.3 |

*Suppl. Table 5.2. Summary of model best fitted to explain corticosterone response of 10 hawksbill turtles (Eretmochelys imbricata) in Study 1) handling and blood sampling.*

| Parameter | Coefficient | SE | 95% CI | t(110) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | -48.03 | 13.88 | (-75.55, -20.52) | -3.46 | < .001 |
| bleed n (1) | 0.93 | 0.19 | (0.54, 1.31) | 4.78 | < .001 |
| bleed n (2) | 1.30 | 0.19 | (0.91, 1.68) | 6.66 | < .001 |
| bleed n (3) | 0.97 | 0.20 | (0.58, 1.36) | 4.92 | < .001 |
| bleed n (4) | 0.87 | 0.20 | (0.48, 1.27) | 4.35 | < .001 |
| bleed n (5) | 0.82 | 0.21 | (0.41, 1.23) | 3.93 | < .001 |
| bleed n (6) | 0.54 | 0.22 | (0.11, 0.97) | 2.49 | 0.014 |
| bleed n (7) | 0.17 | 0.25 | (-0.32, 0.66) | 0.68 | 0.497 |
| bleed n (8) | 0.08 | 0.24 | (-0.39, 0.55) | 0.35 | 0.726 |
| bleed n (9) | 0.51 | 0.24 | (0.03, 0.98) | 2.12 | 0.036 |
| bleed n (10) | 0.59 | 0.20 | (0.19, 0.98) | 2.94 | 0.004 |
| bleed n (11) | 0.13 | 0.21 | (-0.28, 0.54) | 0.64 | 0.521 |
| bleed n (12) | 0.60 | 0.23 | (0.15, 1.05) | 2.66 | 0.009 |
| turtle temp c (log1p) | 13.54 | 4.11 | (5.39, 21.69) | 3.29 | 0.001 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.09 | | | | |
| SD (Residual) | 0.44 | | | | |
| Model: log1p(cort_ngml) ~ factor(bleed_n) + log1p(turtle_temp_c) (126 Observations) | | | | | |
| Residual standard deviation: 0.437 (df = 110) | | | | | |
| Conditional R2: 0.526; Marginal R2: 0.504 | | | | | |

*Suppl. Table 5.3. Summary of model best fitted to explain lactate response of 10 hawksbill turtles (Eretmochelys imbricata) in Study 1) handling and blood sampling.*

| Parameter | Coefficient | SE | 95% CI | t(109) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | -0.61 | 0.09 | (-0.78, -0.44) | -7.01 | < .001 |
| bleed n (1) | 0.13 | 0.10 | (-0.07, 0.32) | 1.30 | 0.197 |
| bleed n (2) | 0.35 | 0.10 | (0.16, 0.55) | 3.57 | < .001 |
| bleed n (3) | 0.53 | 0.10 | (0.33, 0.72) | 5.34 | < .001 |
| bleed n (4) | 0.56 | 0.10 | (0.37, 0.76) | 5.71 | < .001 |
| bleed n (5) | 0.63 | 0.10 | (0.43, 0.83) | 6.17 | < .001 |
| bleed n (6) | 0.35 | 0.10 | (0.16, 0.55) | 3.59 | < .001 |
| bleed n (7) | 0.10 | 0.10 | (-0.09, 0.30) | 1.02 | 0.308 |
| bleed n (8) | 0.07 | 0.10 | (-0.13, 0.26) | 0.70 | 0.486 |
| bleed n (9) | 0.30 | 0.11 | (0.09, 0.50) | 2.80 | 0.006 |
| bleed n (10) | 0.14 | 0.10 | (-0.06, 0.34) | 1.36 | 0.177 |
| bleed n (11) | -0.09 | 0.10 | (-0.28, 0.11) | -0.88 | 0.381 |
| bleed n (12) | 0.07 | 0.10 | (-0.14, 0.28) | 0.66 | 0.512 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.09 | | | | |
| SD (Residual) | 0.24 | | | | |
| Model: sqrt(lac_min_corr) ~ factor(bleed_n) (124 Observations) | | | | | |
| Residual standard deviation: 0.241 (df = 109) | | | | | |
| Conditional R2: 0.502; Marginal R2: 0.425 | | | | | |

*Suppl. Table 5.4. Summary of model best fitted to explain corticosterone response of 10 hawksbill turtles (Eretmochelys imbricata) in Study 2) acute stressor.*

| Parameter | Coefficient | SE | 95% CI | t(114) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | 66.97 | 11.66 | (43.88, 90.07) | 5.75 | < .001 |
| bleed n (1) | -0.63 | 0.17 | (-0.96, -0.29) | -3.72 | < .001 |
| bleed n (2) | -0.07 | 0.17 | (-0.40, 0.27) | -0.41 | 0.682 |
| bleed n (3) | -0.04 | 0.17 | (-0.38, 0.29) | -0.27 | 0.791 |
| bleed n (4) | 0.61 | 0.16 | (0.29, 0.92) | 3.79 | < .001 |
| bleed n (5) | 0.02 | 0.19 | (-0.35, 0.39) | 0.11 | 0.916 |

| | | | | | |
|---|---|---|---|---|---|
| bleed n (6) | 0.09 | 0.18 | (-0.27, 0.45) | 0.51 | 0.612 |
| bleed n (7) | 0.19 | 0.19 | (-0.18, 0.55) | 1.00 | 0.319 |
| bleed n (8) | 0.24 | 0.19 | (-0.14, 0.62) | 1.25 | 0.214 |
| bleed n (9) | 0.49 | 0.20 | (0.09, 0.89) | 2.42 | 0.017 |
| bleed n (10) | 0.45 | 0.19 | (0.07, 0.82) | 2.37 | 0.020 |
| bleed n (11) | 0.28 | 0.16 | (-0.04, 0.60) | 1.71 | 0.090 |
| bleed n (12) | 5.75e-03 | 0.17 | (-0.34, 0.35) | 0.03 | 0.974 |
| bleed n (13) | 0.06 | 0.18 | (-0.29, 0.42) | 0.36 | 0.719 |
| turtle temp c (log1p) | -20.56 | 3.45 | (-27.39, -13.73) | -5.96 | < .001 |
| bleeder (ellen) | 0.05 | 0.10 | (-0.15, 0.25) | 0.51 | 0.613 |
| bleeder (kezia) | 0.49 | 0.16 | (0.17, 0.82) | 3.04 | 0.003 |
| bleeder (sara) | 0.20 | 0.12 | (-0.04, 0.45) | 1.65 | 0.102 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.14 | | | | |
| SD (Residual) | 0.37 | | | | |
| Model: log1p(cort_ngml) ~ factor(bleed_n) + log1p(turtle_temp_c) + bleeder (134 Observations) | | | | | |
| Residual standard deviation: 0.369 (df = 114) | | | | | |
| Conditional R2: 0.512; Marginal R2: 0.444 | | | | | |

*Suppl. Table 5.5. Summary of model best fitted to explain lactate response of 10 hawksbill turtles (Eretmochelys imbricata) in Study 2) acute stressor.*

| Parameter | Coefficient | SE | 95% CI | t(112) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | -0.68 | 0.08 | (-0.83, -0.53) | -9.06 | < .001 |
| bleed n (1) | 0.26 | 0.08 | (0.09, 0.43) | 3.06 | 0.003 |
| bleed n (2) | 1.68 | 0.08 | (1.51, 1.84) | 19.87 | < .001 |
| bleed n (3) | 1.71 | 0.08 | (1.55, 1.87) | 20.87 | < .001 |
| bleed n (4) | 1.64 | 0.08 | (1.48, 1.80) | 20.29 | < .001 |
| bleed n (5) | 1.42 | 0.09 | (1.23, 1.60) | 15.13 | < .001 |
| bleed n (6) | 1.24 | 0.09 | (1.06, 1.42) | 13.78 | < .001 |
| bleed n (7) | 0.96 | 0.09 | (0.78, 1.14) | 10.44 | < .001 |
| bleed n (8) | 0.24 | 0.09 | (0.06, 0.42) | 2.68 | 0.008 |
| bleed n (9) | 0.17 | 0.09 | (-0.01, 0.36) | 1.86 | 0.065 |
| bleed n (10) | 0.10 | 0.09 | (-0.07, 0.28) | 1.18 | 0.240 |
| bleed n (11) | 0.03 | 0.08 | (-0.14, 0.19) | 0.34 | 0.734 |
| bleed n (12) | -2.86e-03 | 0.09 | (-0.17, 0.17) | -0.03 | 0.973 |

| | | | | | |
|---|---|---|---|---|---|
| bleed n (13) | 0.07 | 0.09 | (-0.11, 0.24) | 0.79 | 0.433 |
| bleeder (ellen) | 0.07 | 0.05 | (-0.03, 0.17) | 1.44 | 0.154 |
| bleeder (kezia) | 0.31 | 0.07 | (0.16, 0.45) | 4.15 | < .001 |
| bleeder (sara) | 0.04 | 0.05 | (-0.06, 0.13) | 0.72 | 0.472 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.02 | | | | |
| SD (Residual) | 0.18 | | | | |
| Model: sqrt(lac_min_corr) ~ factor(bleed_n) + bleeder (131 Observations) | | | | | |
| Residual standard deviation: 0.178 (df = 112) | | | | | |
| Conditional R2: 0.939; Marginal R2: 0.938 | | | | | |

*Suppl. Table 5.6. Summary of model best fitted to explain corticosterone response of 10 hawksbill turtles (Eretmochelys imbricata) in Study 3) extended stressor.*

| Parameter | Coefficient | SE | 95% CI | t(122) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | 5.18 | 2.57 | (0.10, 10.26) | 2.02 | 0.046 |
| bleed n (1) | 0.11 | 0.25 | (-0.38, 0.60) | 0.45 | 0.657 |
| bleed n (2) | 0.90 | 0.26 | (0.39, 1.41) | 3.52 | < .001 |
| bleed n (3) | 0.77 | 0.25 | (0.27, 1.26) | 3.08 | 0.003 |
| bleed n (4) | 0.75 | 0.25 | (0.26, 1.25) | 3.03 | 0.003 |
| bleed n (5) | 0.81 | 0.25 | (0.31, 1.31) | 3.23 | 0.002 |
| bleed n (6) | 0.57 | 0.25 | (0.07, 1.06) | 2.27 | 0.025 |
| bleed n (7) | 0.25 | 0.25 | (-0.24, 0.75) | 1.01 | 0.315 |
| bleed n (8) | -0.44 | 0.25 | (-0.93, 0.06) | -1.76 | 0.082 |
| bleed n (9) | -0.11 | 0.25 | (-0.60, 0.38) | -0.44 | 0.661 |
| bleed n (10) | 0.36 | 0.25 | (-0.13, 0.85) | 1.45 | 0.150 |
| bleed n (11) | 0.46 | 0.25 | (-0.04, 0.96) | 1.82 | 0.071 |
| bleed n (12) | 0.05 | 0.25 | (-0.44, 0.54) | 0.20 | 0.840 |
| bleed n (13) | 0.20 | 0.25 | (-0.29, 0.69) | 0.80 | 0.425 |
| weight g (log1p) | -0.95 | 0.32 | (-1.60, -0.31) | -2.94 | 0.004 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.11 | | | | |
| SD (Residual) | 0.58 | | | | |

Model: log1p(cort_ngml) ~ factor(bleed_n) + log1p(weight_g) (139 Observations)
Residual standard deviation: 0.582 (df = 122)
Conditional R2: 0.358; Marginal R2: 0.335

*Suppl. Table 5.7. Summary of model best fitted to explain lactate response of 10 hawksbill turtles (Eretmochelys imbricata) in Study 3) extended stressor.*

| Parameter | Coefficient | SE | 95% CI | t(120) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | -0.60 | 0.13 | (-0.86, -0.33) | -4.48 | < .001 |
| bleed n (1) | -0.09 | 0.13 | (-0.34, 0.17) | -0.68 | 0.495 |
| bleed n (2) | 0.86 | 0.13 | (0.59, 1.12) | 6.45 | < .001 |
| bleed n (3) | 1.02 | 0.13 | (0.76, 1.27) | 7.90 | < .001 |
| bleed n (4) | 1.20 | 0.13 | (0.93, 1.46) | 9.00 | < .001 |
| bleed n (5) | 1.16 | 0.13 | (0.90, 1.42) | 8.98 | < .001 |
| bleed n (6) | 1.07 | 0.13 | (0.81, 1.32) | 8.23 | < .001 |
| bleed n (7) | 0.92 | 0.13 | (0.66, 1.19) | 6.94 | < .001 |
| bleed n (8) | 0.56 | 0.13 | (0.31, 0.82) | 4.37 | < .001 |
| bleed n (9) | 0.09 | 0.13 | (-0.17, 0.34) | 0.69 | 0.494 |
| bleed n (10) | 0.27 | 0.13 | (0.01, 0.52) | 2.09 | 0.039 |
| bleed n (11) | -4.12e-03 | 0.13 | (-0.27, 0.26) | -0.03 | 0.975 |
| bleed n (12) | -0.02 | 0.13 | (-0.28, 0.23) | -0.17 | 0.868 |
| bleed n (13) | 0.09 | 0.13 | (-0.17, 0.34) | 0.69 | 0.494 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.16 | | | | |
| SD (Residual) | 0.31 | | | | |

Model: sqrt(lac_min_corr) ~ factor(bleed_n) (136 Observations)
Residual standard deviation: 0.311 (df = 120)
Conditional R2: 0.733; Marginal R2: 0.661

*Suppl. Table 5.8. Individual data summaries for peak, scope, time to peak, and time to recovery of corticosterone (CORT; ng/ml) and lactate (mmol/L) concentrations of 11 hawksbill turtles (Eretmochelys imbricata; H02-H11) in response to four stress studies. 1) Handling and blood sampling; 2) acute stressor; 3) extended stressor; and 4) transportation stress. Min: minimum; max: maximum; LQ: lower quartile; UQ: upper quartile.*

| Turtle | Handling | | Acute Stressor | | Extended Stressor | |
|---|---|---|---|---|---|---|
| | CORT | Lactate | CORT | Lactate | CORT | Lactate |
| **Peak (CORT ng/ml; Lactate mmol/L)** | | | | | | |
| **H02** | 0.44 | 0.94 | 0.17 | 12.17 | 0.43 | 13.00 |
| **H03** | 0.68 | 1.04 | 0.27 | 8.52 | 0.23 | 1.09 |
| **H04** | 0.35 | 3.23 | 0.35 | 11.62 | 0.39 | 3.85 |
| **H05** | 1.07 | 2.97 | 0.29 | 10.76 | 0.36 | 11.29 |
| **H06** | 0.47 | 1.21 | 0.19 | 8.22 | 0.52 | 6.74 |
| **H07** | 0.72 | 0.78 | 0.28 | 5.89 | 0.55 | 3.98 |
| **H08** | 0.43 | 1.34 | 0.18 | 10.25 | 0.20 | 2.96 |
| **H09** | 0.49 | 1.09 | 0.39 | 9.52 | 0.95 | 13.00 |
| **H10** | 0.49 | 0.79 | 0.19 | 11.45 | 0.17 | 0.97 |
| **H11** | 0.87 | 1.08 | 0.20 | 13.00 | 0.23 | 2.50 |
| **Median** | 0.49 | 1.09 | 0.23 | 10.51 | 0.37 | 3.92 |
| **Min** | 0.35 | 0.78 | 0.17 | 5.89 | 0.17 | 0.97 |
| **Max** | 1.07 | 3.23 | 0.39 | 13.00 | 0.95 | 13.00 |
| **LQ** | 0.47 | 0.86 | 0.19 | 8.55 | 0.21 | 2.62 |
| **UQ** | 0.71 | 1.31 | 0.29 | 11.58 | 0.50 | 10.15 |
| **Scope increase (factorial) from grouped basal to peak** | | | | | | |
| **H02** | 4.37 | 3.13 | 1.71 | 40.57 | 4.28 | 43.33 |
| **H03** | 6.76 | 4.16 | 2.67 | 34.08 | 2.26 | 4.36 |
| **H04** | 3.48 | 8.97 | 3.46 | 32.28 | 3.88 | 10.69 |
| **H05** | 10.69 | 11.88 | 2.93 | 43.04 | 3.60 | 45.16 |
| **H06** | 4.70 | 3.36 | 1.95 | 22.83 | 5.24 | 18.72 |
| **H07** | 7.25 | 2.17 | 2.78 | 16.36 | 5.52 | 11.06 |
| **H08** | 4.35 | 4.47 | 1.76 | 34.17 | 2.01 | 9.87 |
| **H09** | 4.87 | 3.63 | 3.92 | 31.73 | 9.53 | 43.33 |
| **H10** | 4.88 | 3.16 | 1.86 | 45.80 | 1.69 | 3.88 |
| **H11** | 8.66 | 4.32 | 1.97 | 52.00 | 2.25 | 10.00 |
| **Median** | 4.88 | 3.90 | 2.32 | 34.12 | 3.74 | 10.88 |
| **Min** | 3.48 | 2.17 | 1.71 | 16.36 | 1.69 | 3.88 |
| **Max** | 10.69 | 11.88 | 3.92 | 52.00 | 9.53 | 45.16 |
| **LQ** | 4.74 | 3.21 | 1.88 | 25.06 | 2.07 | 9.90 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **UQ** | 7.12 | 4.43 | 2.89 | 42.42 | 5.00 | 37.18 |
| **Time to peak (hr) from first blood collection** | | | | | | |
| **H02** | 1.98 | 0.98 | 8.07 | 0.08 | 2.42 | 1.92 |
| **H03** | 0.48 | 0.98 | 0.57 | 1.05 | 0.98 | 0.98 |
| **H04** | 1.47 | 1.47 | 1.12 | 0.62 | 2.00 | 2.50 |
| **H05** | 2.07 | 2.07 | 0.10 | 1.08 | 1.50 | 1.02 |
| **H06** | 0.50 | 1.50 | 0.10 | 0.60 | 3.07 | 2.53 |
| **H07** | 0.48 | 0.98 | 2.07 | 0.10 | 2.00 | 1.50 |
| **H08** | 0.50 | 2.98 | 1.08 | 0.13 | 2.97 | 2.48 |
| **H09** | 0.50 | 2.02 | 1.08 | 0.58 | 2.57 | 2.57 |
| **H10** | 1.47 | 1.97 | 1.10 | 0.12 | 1.42 | 1.42 |
| **H11** | 0.00 | 1.45 | 1.13 | 0.62 | 1.48 | 1.48 |
| **Median** | 0.50 | 1.48 | 1.09 | 0.59 | 2.00 | 1.71 |
| **Min** | 0.00 | 0.98 | 0.10 | 0.08 | 0.98 | 0.98 |
| **Max** | 2.07 | 2.98 | 8.07 | 1.08 | 3.07 | 2.57 |
| **LQ** | 0.49 | 1.46 | 0.35 | 0.12 | 1.49 | 1.43 |
| **UQ** | 1.47 | 2.00 | 1.13 | 0.62 | 2.53 | 2.50 |
| **Recovery time (hr) from peak to grouped basal** | | | | | | |
| **H02** | 45.97 | 10.95 | 63.95 | 7.98 | 6.52 | 35.03 |
| **H03** | 47.47 | 1.95 | 1.97 | 6.97 | 1.02 | 12.08 |
| **H04** | 70.58 | 6.55 | 0.98 | 4.47 | 2.05 | 32.92 |
| **H05** | Not basal | 5.98 | 0.50 | 6.98 | 1.50 | 8.00 |
| **H06** | 47.55 | 46.55 | 1.48 | 7.45 | 1.05 | 34.38 |
| **H07** | 47.47 | 3.95 | 2.97 | 7.92 | 1.07 | 7.60 |
| **H08** | 23.43 | 8.93 | 0.97 | 11.85 | 3.02 | 34.52 |
| **H09** | 1.52 | 2.98 | 0.52 | 4.47 | 1.55 | 46.43 |
| **H10** | 6.45 | 2.98 | 4.00 | 11.95 | 1.00 | 7.50 |
| **H11** | 23.93 | 1.50 | 46.88 | 35.42 | 0.50 | 4.50 |
| **Median** | 45.97 | 4.97 | 1.73 | 7.68 | 1.28 | 22.50 |
| **Min** | 1.52 | 1.50 | 0.50 | 4.47 | 0.50 | 4.50 |
| **Max** | 70.58 | 46.55 | 63.95 | 35.42 | 6.52 | 46.43 |
| **LQ** | 6.45 | 2.98 | 0.63 | 7.10 | 1.01 | 7.53 |
| **UQ** | 47.47 | 8.34 | 3.74 | 10.88 | 1.93 | 34.48 |

*Suppl. Table 5.9. Summary of model best fitted to explain corticosterone response of 11 hawksbill turtles (Eretmochelys imbricata) in Study 4) transportation stress.*

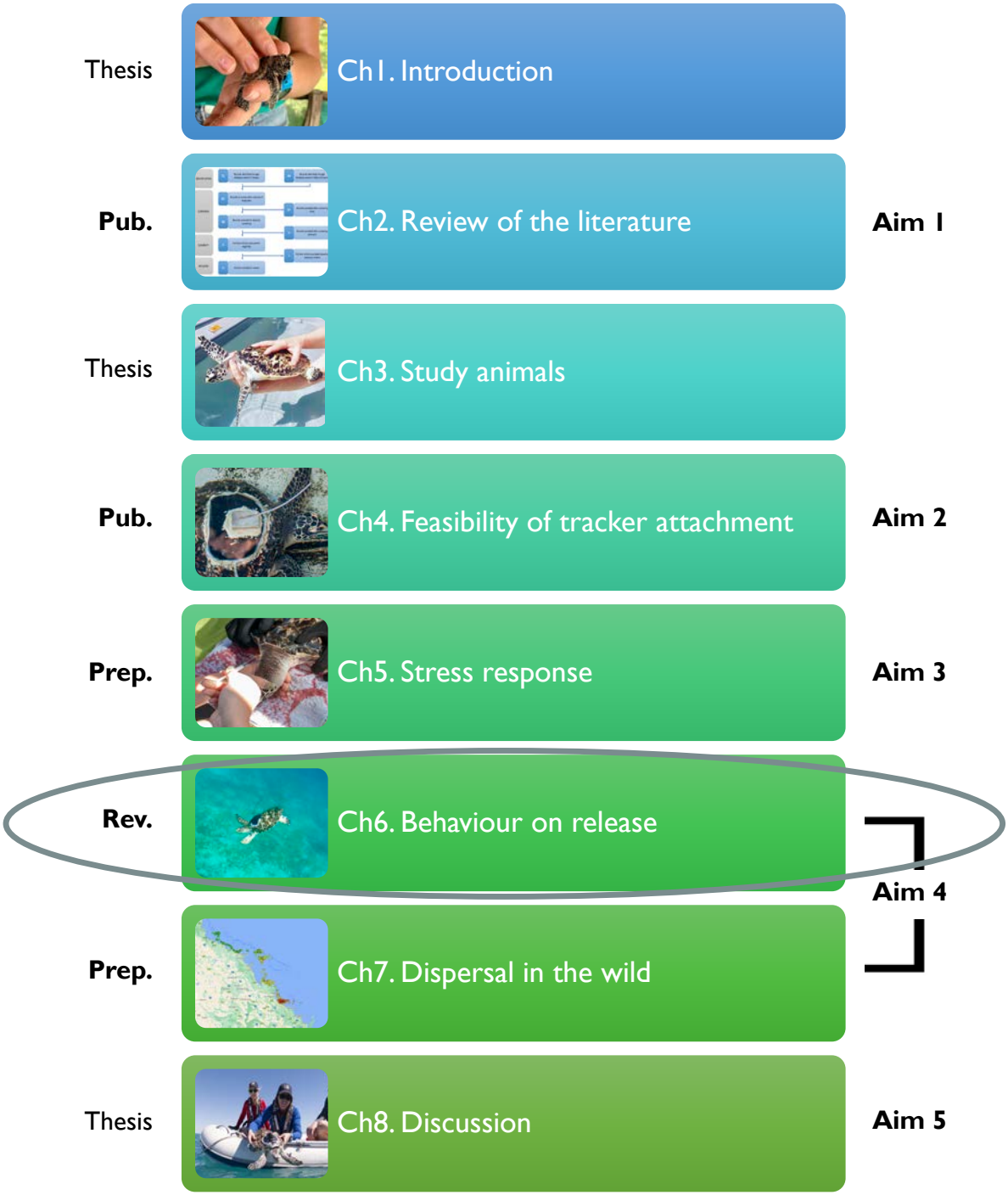
| Parameter | Coefficient | SE | 95% CI | t(37) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | 2.63 | 1.57 | (-0.55, 5.80) | 1.68 | 0.102 |
| bleed n (1) | 0.35 | 0.15 | (0.05, 0.65) | 2.40 | 0.022 |
| bleed n (2) | 0.53 | 0.19 | (0.14, 0.93) | 2.74 | 0.009 |
| bleed n (3) | 1.10 | 0.17 | (0.77, 1.44) | 6.66 | < .001 |
| turtle temp c (log1p) | -1.47 | 0.46 | (-2.41, -0.54) | -3.18 | 0.003 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 9.16e-09 | | | | |
| SD (Residual) | 0.32 | | | | |
| Model: log1p(cort_ngml) ~ factor(bleed_n) + log1p(turtle_temp_c) (44 Observations)<br>Residual standard deviation: 0.321 (df = 37)<br>Conditional R2: ; Marginal R2: 0.746 | | | | | |

*Suppl. Table 5.10. Summary of model best fitted to explain lactate response of 11 hawksbill turtles (Eretmochelys imbricata) in Study 4) transportation stress.*

| Parameter | Coefficient | SE | 95% CI | t(38) | p |
|---|---|---|---|---|---|
| **Fixed Effects** | | | | | |
| (Intercept) | -0.60 | 0.09 | (-0.78, -0.42) | -6.82 | < .001 |
| bleed n (1) | 0.18 | 0.11 | (-0.04, 0.40) | 1.69 | 0.099 |
| bleed n (2) | -0.12 | 0.11 | (-0.33, 0.10) | -1.11 | 0.273 |
| bleed n (3) | 0.12 | 0.11 | (-0.09, 0.34) | 1.16 | 0.252 |
| **Random Effects** | | | | | |
| SD (Intercept: turtle) | 0.12 | | | | |
| SD (Residual) | 0.32 | | | | |
| Model: sqrt(lac_min_corr) ~ factor(bleed_n) (44 Observations)<br>Residual standard deviation: 0.317 (df = 38)<br>Conditional R2: 0.224; Marginal R2: 0.108 | | | | | |

# CHAPTER 6: BEHAVIOUR ON RELEASE

## Thesis structure



# Background and aims of this chapter

## Rationale

Conservation programs that temporarily keep turtles under human care with the aim of releasing them into the wild, thus strengthening the population, include head-start programs and rehabilitation centres. Head-start programs monitor nests and raise hatchlings until they are larger and theoretically more likely to survive once released into the wild. However, some researchers have questioned whether innate behaviours key to survival may be lost during captive-raising, reducing survivability on release. However, no published studies have assessed in-water behaviour of captive-raised turtles for comparison with free-living turtles, which would be particularly useful for the critically endangered hawksbill turtle.

## Thesis aim 4

Determine whether juvenile hawksbill turtles maintain naturalistic behaviours after 2 years under human care.

### Chapter aims

- Document the behaviours of captive-raised hawksbill turtles on first entry into the ocean via in-water observation.
- Compare in-water behaviours of recently released juvenile hawksbill turtles with free-living hawksbill turtles reported in published studies.

## Research Outputs

**Diggins** RL, Mendez D, Jones K, Erickson K, Bell I, and Ariel E (*under review*) Captive-raised juvenile turtles display naturalistic behaviours when first released into the ocean.

**Diggins** RL, Mendez D, Jones K, Erickson K, and Ariel E (2023, March 21-25). *Behaviour of juvenile hawksbill turtles in captivity and upon release into the ocean* [Poster presentation]. International Sea Turtle Symposium 41, Session: In-water Biology, Cartagena, Colombia.

# In-water observations of captive-raised juvenile hawksbill turtles (*Eretmochelys imbricata*) immediately upon release into the ocean

## Introduction

Of the seven extant species of sea turtle, three are categorised by the International Union for Conservation of Nature (IUCN) as globally vulnerable (Abreu-Grobois & Plotkin, 2008; Casale & Tucker, 2017; Wallace et al., 2013), one as endangered (Seminoff, 2004), two as critically endangered (Mortimer & Donnelly, 2008; Wibbels & Bevan, 2019), and one as data deficient (Red List Standards & Petitions Subcommittee, 1996). Vulnerable, endangered, and critically endangered statuses all indicate that a species is at risk of extinction unless their global population increases. Many pressures contribute to the threatened species status of these animals, including numerous anthropogenic and climate-based factors such as deliberate and accidental catch or egg harvesting, and increased flooding or overheating of nests (Fuentes et al., 2011; Hamann et al., 2013; Hamann et al., 2010; Rees et al., 2016). Chelonians lay large clutches (approximately 50 – 120 eggs, species-dependent) but have no parental input for the survival of their offspring (Hirth, 1980). Therefore, the high mortality rate seen at this early life-stage (eggs and hatchlings) is expected due to high levels of predation, with approximately 1 in 1,000 hatchlings reaching maturity (Frazer, 1986). Unfortunately, the addition of numerous anthropogenic stressors, including the above-mentioned examples, further decreases the survival rate of hatchlings (Martins et al., 2021b; Rees et al., 2016). The combination of natural and non-natural stressors can overpower the natural resilience and elasticity of turtle populations, causing a drastic, and oftentimes unsustainable, decrease in the number of turtles reaching maturity (Patrício et al., 2021). Furthermore, the extended lifespan and time to maturity for sea turtles (Bowen et al., 1992; Meylan et al., 2011) can result in decades passing before the detection of negative impacts, such as an overly poor nesting season, or feminisation of a particular population (Chatting et al., 2021).

Turtles experience the highest mortality rates in their earliest life stages (egg incubation, hatching and post-hatchling dispersal), when they are small and highly vulnerable to predation (Gane et al., 2020). Furthermore, research has shown that increased body size of turtles is correlated with survivability (Tetzlaff et al., 2019c). Therefore, targeting intervention efforts at these early years could therefore lead to large conservation gains by increasing the number of individuals entering the population (Evans et al., 2022) and hopefully surviving to adulthood. Following this logic, head-start programs were developed with the intention to increase recruitment (Burke, 2015) by: 1) protecting nests to increase the number of successful hatchlings; and 2) raising the post-hatchlings

in captivity for a relatively short period (usually <2 years) to release them back into the wild at a larger size than newly hatched turtles (Shaver, 2007).

Studies have shown that head-started turtles are able to grow to maturity and reproduce (Bell et al., 2005; Shaver, Rubio, et al., 2016). However, head-start programs are perceived by some as an experimental and controversial management technique (Bennett et al., 2017; Seigel & Dodd Jr, 2000) because turtles are raised in an artificial environment, without full understanding of consequences or probability of survival and reproduction on release (Bennett et al., 2017). Specifically, there have been concerns regarding potential disease introduction from poor husbandry practices and rearing conditions (Flanagan, 2000; Seigel & Dodd Jr, 2000), potential alteration of behaviours critical for post-release survival (East et al., 2013; Meylan & Ehrenfeld, 2000; Okuyama et al., 2010), and lack of post-release analysis of fitness and health (Bennett et al., 2017). The long time to maturity in sea turtles complicates predictions of reproductive success and survival rates; however, short-term success of a head-start program can be inferred from satellite tracked data analysed for behaviour and dispersal of turtles on release over a 1 – 2 year period (Abalo-Morla et al., 2018; Okuyama et al., 2010). To date, there has been little published literature reporting on and analysing turtle behaviours observed both in captivity and on release into the wild, and how these may affect the probability of survival post-release into the wild. Furthermore, comparing the observed behaviours of head-started turtles with free-living turtles of a similar life-stage or size could help to determine whether captivity affects behaviour (Carlson & Tetzlaff, 2020).

Critically endangered turtle species such as the hawksbill (*Eretmochelys imbricata*) could highly benefit from head-start management intervention (Evans et al., 2022; Nasiri et al., 2023). In-water behavioural surveys of juvenile hawksbill turtles have been conducted in Honduras (Baumbach et al., 2022; Dunbar et al., 2008; Hayes et al., 2017; Wright et al., 2020), Panama (Diez et al., 2002b), Seychelles (Houghton et al., 2003; von Brandis et al., 2010), Brazil (Proietti et al., 2012), and Saint Kitts and Nevis (Stimmelmayr et al., 2010). Commonly reported behaviours included swimming, feeding, investigating, resting, and surfacing to breathe. Furthermore, previous studies observed free-living hawksbill turtles, with only one considering the effect of temporary captivity (Dunbar et al., 2008). Therefore, this study aimed to document the behaviours of captive-raised hawksbill turtles on first entry into the ocean and compare these with behaviours reported in published studies on free-living hawksbill turtles.

## Materials and methods

### Permits and approvals

All work was conducted ethically and responsibly under the following authorisations: James Cook University (JCU) Animal Ethics (approval A2586); Department of Environment and Science (DES; approval: WA0012830); Great Barrier Reef Marine Park Authority (Marine Parks Permit: G20/44009.1).

### Study animals and holding facility

Eleven hawksbill turtles were collected from Milman Islet, Queensland, Australia (11.167°S, 143.017°E) after hatching naturally and running a few metres down the beach. Hatchling collection occurred on 17 March 2019 and, shortly thereafter, turtles were transferred to JCU's Turtle Health Research facility in Townsville, Queensland, Australia. The turtles remained in filtered natural sea water in this purpose-built facility (Diggins et al., 2023), with husbandry procedures per the facility's manual (WLD-16), until they were a similar size to the smallest (new recruits) of those naturally found inhabiting the North Queensland coastline from which the hatchlings were originally collected (Limpus, 1992b). Just prior to release, at 2 years of age, the study turtles weighed 4,445 – 7,830 g (median 5,760.0 g; interquartile range (IQR) 5,013.8 – 6,926.3 g) and measured 357 – 444 mm (median 389.0 mm; IQR 373.5 – 404.5 mm) in curved carapace length (CCL). Turtles were fitted with satellite trackers five days prior to release, following methods outlined in Diggins et al. (2023). They were also tagged on the day of release on the trailing edge of their flipper with titanium tags (Stockbrands Company, Pty.Ltd., Perth, Western Australia) provided by DES.

### Study release site

The hawksbill turtles were transported to the site in individual tubs (54 L storage crate with modified lids, (Fig. 6.1), dry-docked and wrapped in damp towels to prevent overheating. Transportation to the release site was via a Queensland Parks and Wildlife marine park vessel. The release site selected was John Brewer Reef, Queensland, Australia (18.633°S, 147.067°E), in the reef lagoon area between coral bommies. The boat was anchored at approximately 5 m depth (surrounding lagoon depth varied) and visibility ranged from 10 – 20 m. This reef was selected due to its proximity to the research facility whilst being distant from populated coastal areas. To ensure the best possible welfare for the turtles during the release and high-quality data collection, six turtles were released on 6 May 2021 and the remaining five turtles were released on 7 May

2021. Turtles were released between 10:00 and 14:00 each day. The release was supported by and in collaboration with Manbarra Elders and rangers from the Gudjuda and Girringun Aboriginal Corporations.

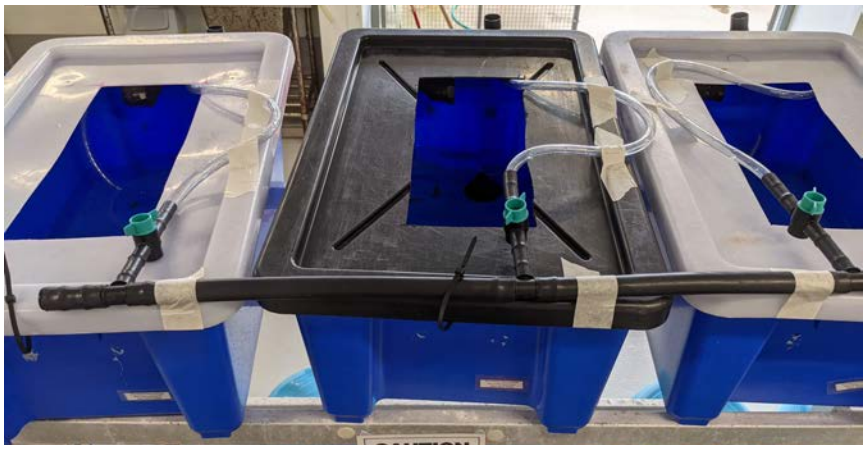


*Figure 6.1. Photograph of transportation containers with modified lids.*

## Turtle release and in-water observation

The main vessel was anchored in a sandy spot in the lagoon to maintain position whilst limiting damage to the environment, and the tender was secured to its stern using approximately 20 m of rope to maximise distance. Both boats had their engines turned off to ensure safety and reduce disturbance to the turtles on release, except the first two turtles released for which the tender motor was in neutral. Turtles were released one at a time from the tender and concurrently filmed (GoPro Hero 3, 4, and 8) by one snorkeler from the surface and two scuba divers matching the turtles' depth. Observers recorded themselves prior to each turtle's release to facilitate later video synchronising for analysis. Observational research methodology was selected to facilitate data gathering and comprehension of behaviour in a non-intrusive approach. Direct observation of the turtles via video recording allowed capture of the turtles' in-water behaviour, immediately on release into the ocean.

Response of turtles to the scuba divers was recorded as an additional observation as it was expected that human presence could influence turtle behaviour. To reduce the possibility of this interference, scuba divers and snorkellers attempted to stay at least 2 m away from the turtles whilst recording them (Hayes et al., 2017; Meadows, 2004). On occasions when turtles swam directly toward the divers or snorkellers, the observers remained still and allowed the turtles to freely choose their direction. If any turtle swam away faster than divers could easily follow, the recording was stopped, and the dive terminated. To minimise stress to the turtles, enable

comparison with other published data, and ensure dive safety requirements were met, the maximum time observing each turtle was capped at 25 minutes. Divers recorded maximum depth of each turtle via depth gauge (Suunto D4 or Mares Smart). Water temperature (°C), visibility (m), current (perceived by divers at depth), wave action (Beaufort scale), and weather were also recorded at the time of each turtle's release.

## Coding classifications

Recordings from the observers were synchronised and overlayed into one video file per turtle and were watched independently by two experienced observers (RD, EA), each of whom recorded the end time for all behaviours in a continuous manner throughout the duration of the recording. All behaviours and relative depths were recorded as narrative to ensure no observational data were missed. Behavioural categories (Table 6.1) were determined based on previously published classifications (Dunbar et al., 2008; Hayes et al., 2017; Houghton et al., 2003; Whilde et al.), combined with preliminary observations of the study turtles whilst in captivity. Further, individual behaviours were grouped into types of behaviour, for example 'locomotion' is a type of behaviour that covers *swimming*, *hawksbill walk* and *relaxed swimming*. Relative depth (surface, water column or benthos) was recorded along with positioning within the environment (benthic type) at the release site. The response of the turtles toward the divers during release was recorded as 'focused on', 'aware of', 'ignoring' and 'moving away from the divers' (definitions provided in Table 6.1).

*Table 6.1. Ethogram of behaviours observed (including descriptions of in-water positioning of turtles whilst performing each behaviour and reaction to the in-water data recorders) during the release of captive-raised juvenile hawksbill turtles into the wild.*

| Type of Behaviour | Behaviour | Code | Description of Behaviour |
|---|---|---|---|
| Locomotion | Orientation | **O** | Random swimming or lack of movement immediately upon release, until next distinctive behaviour |
| | Swimming | **SW** | Deliberate movement at moderate speed (including ascent and descent for taking breaths) |
| | Hawksbill Walk | **HW** | Walking/crawling/gliding motion over benthos, propped up on flippers and pushing off benthos with asymmetric flipper movement |
| | Relaxed Swimming[a,b] | **RS** | Slow swim/glide using synchronised front flippers for propulsion, mostly horizontal, without attempt to escape, including exploring environment |

| | | | |
|---|---|---|---|
| Locomotion/ Predator avoidance | Escape | **ESC** | Fast swimming away from divers, evasive manoeuvring, and carapace flashes (turning sideways and directing the top of the shell towards a potential predator) |
| Focused | Investigating[a, b, c] | **IN** | Examination of and interaction with anything in their immediate vicinity, including potential food items |
| | Grooming[b] | **GR** | Rubbing of carapace with flippers or against solid structure |
| Surfacing | Surfacing to Breathe[a] | **SB** | Recorded from first breath to last breath before next distinctive behaviour |
| Rest | Resting[a, b, c] | **RR** | Stationary, or minimal flipper movement to counter swell/current, on benthos or under coral ledge |
| **Relative Depth** | **Position during Activity** | **Code** | **Description of Relative Depth** |
| | Surface | **SF** | Breaking the surface or less than 0.5 m from the surface |
| | Water Column | **WC** | More than 0.5 m below the surface but not on the benthos |
| | Benthos | **BT** | On or just above the sea floor (ie pushing off benthos) |
| **Habitat** | **Benthic Type** | **Code** | **Description of Habitat** |
| | Sand | **SA** | Completely sandy bottom, with/without algae |
| | Coral Rubble | **CR** | Mixture of sand covered in pieces of dead coral |
| | Coral Bommie/Reef | **CB** | Either an individual coral bommie or a small patch of coral reef |
| **Type of Response** | **Response** | **Code** | **Description of Response to Diver** |
| Reacting[d] | Focused | **F** | Watching diver constantly; moving toward, circling or facing diver while stationary |
| | Away | **A** | Deliberately moving away and sometimes looking back |
| | Aware | **W** | Watching diver intermittently throughout a distinct behaviour |
| Ignoring | Ignoring | **I** | Not looking at diver for the entire period of a distinct behaviour |

[a]Modified from Dunbar et al. (2008)
[b]Modified from Whilde et al.
[c]Modified from Houghton et al. (2003)
[d]Modified from Hayes et al. (2017)

## Data analysis

All video footage was analysed by two independent researchers (RD, EA). Both sets of observations were then compared for consistency. Where researchers recorded different behaviours, results were discussed until a consensus was reached by both researchers. Total time observed and proportion of observed time spent in each activity were calculated for each turtle. Due to the small sample size, a Shapiro-Wilk test for normality was performed and data were

confirmed to be not normally distributed ($W=0.66031$, $p<0.05$). To determine whether behavioural profiles (proportion of time engaged in all behaviours) varied between turtles, the Fisher's exact test was used. To test for differences between time engaged in each behaviour Kruskal-Wallis test, Dunn test with Holm adjustment, Friedman test, and pairwise Wilcoxon signed rank test with Bonferroni adjustment were used. Median and interquartile ranges were calculated to account for the non-normal distribution of data. To allow comparison with published literature, mean, standard error, and range were also calculated. Furthermore, a table was created comparing published hawksbill behavioural data with proportion of time study turtles engaged in each behaviour as well as some key study parameters (Table 6.2).

*Table 6.2. Comparison of proportion of observed time spent engaged in each behaviour across published studies of juvenile hawksbill turtles (CCL: curved carapace length; SCL: straight carapace length).*

| **Behaviour** | **This study** | **Dunbar et al. (2008)** | **Hayes et al. (2017)** | **Wright et al. (2020)** | **Proietti et al. (2012)** | **Blumenthal et al. (2008)** |
|---|---|---|---|---|---|---|
| | *Captive raised* | *Partial Captivity* | *Free-living* | *Free-living* | *Free-living* | *Free-living* |
| Locomotion/ Swimming | 60.1% [3% escape] | *78.9%* | *57.9%* | *33.4%* | *48%* | *35% [5% escape]* |
| Investigating | 1.1% | 15.0% | 16.3% | 33.0% | | |
| Grooming | 11.3% | | 0.1% | | | |
| Breathing | 14.0% | 4.2% | 4.0% | 4.1% | | 5% |
| Resting | 13.5% | 1.1% | 0.4% | 0.0% | 20.3% | 40% |
| Feeding | 0.0% | 0.8% | 16.5% | 29.5% | 28.9% | 13% |
| Reacting | *2.9% | | 3.4% | | | |
| Interacting | | | 1.4% | | 2.8% (fish) | |
| Number of turtles | 11 | 19 | 61 | 37 | 257 | 39 |
| Observation total (mins) | 123.6 11.2±3.6 turtle$^{-1}$ | 368.6 19.4# turtle$^{-1}$ | 823.9 13.3±7.5 turtle$^{-1}$ | 557 15.1# turtle$^{-1}$ | N/A | N/A |
| Observation per turtle (mins) | 0.5 - 25.0 | 3 - 48 | 1.2 - 36.0 | Not stated | Instantaneous observation | Instantaneous observation |
| Time of day | 10:00-14:00 | 9:00-15:30 | 0900-1600 | 0830-1630 | 0600-1900 | "daytime" |
| CCL (cm) | 35.7 – 44.4 | 21.8-46.0 (SCL) | unknown | ~40-65 | 24.5-75 | 26.4-58.4 (SCL) |
| Location | N Queensland, Australia | Roatán, Honduras | Roatán, Honduras | Roatán, Honduras | Brazil | Cayman Islands |

*For this study, "reacting" was considered most similar to "away from diver", per description of "reacting" behaviour in Hayes et al. (2017).
#Calculated as not stated in the publication.

# Results

## In-water behavioural observations

Eleven turtles were observed for a total of 123.6 minutes. The mean duration of in-water observation per turtle was 11.2 ±3.6 minutes (0.5 – 25.0), but there was a clear divide between turtles who quickly disappeared out of sight ("short observed", n=6, median 0.8 minutes, IQR 0.5 – 1.5 minutes) and those that stayed to explore their immediate surroundings ("long observed", n=5, median 24.6 minutes, IQR 22.4 – 25.0; Fig. 6.2). This divide was not likely caused by release date and accompanying conditions as there was an even split of turtles in the short and long observed groups for each of the two release dates. The behaviour profiles (percentage of time turtles were engaged in each behaviour) of the combined short observed turtles (n=6) were significantly different ($p<0.001$) from the combined long observed turtles (n=5).

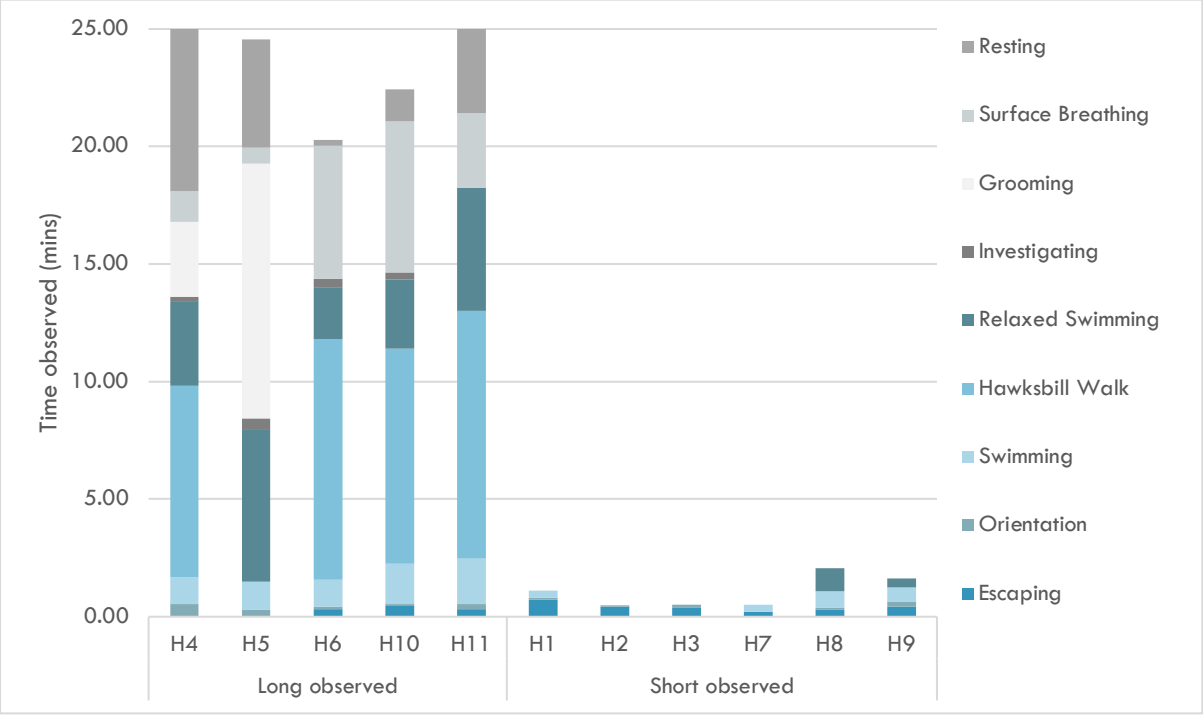



*Figure 6.2. Total time (min) each turtle (H1-H11) was observed engaging in each behaviour.*

Collectively, the long observed turtles (n=5) displayed all listed behaviours (Fig. 6.3). No individual turtle (n=11) displayed every reported behaviour; however, five of the behaviours (*Orientation*, *Swimming*, *Relaxed Swimming*, *Surfacing & Breathing*, *Resting*, and *Escape*; Table 6.1) were observed

in each of the long observed turtles (n=5). Of the short observed turtles collectively (n=6), only four of the listed behaviours were displayed (*orientation*, *swimming*, *relaxed swimming*, and *escape*), with *escape* the only behaviour observed in all six turtles. Only one turtle out of all eleven released had no clear *orientation*. For the remaining ten turtles, *orientation* lasted an average (median) of 0.12 minutes (IQR 0.08 – 0.22). *Investigating* was recorded in four of the five long observed turtles (median 0.28 minutes, IQR 0.22 – 0.38); *escape* in three (median 0.32 minutes, IQR 0.00 – 0.32); and *grooming* in only two (median 0.00 minutes, IQR 0.00 – 3.17).

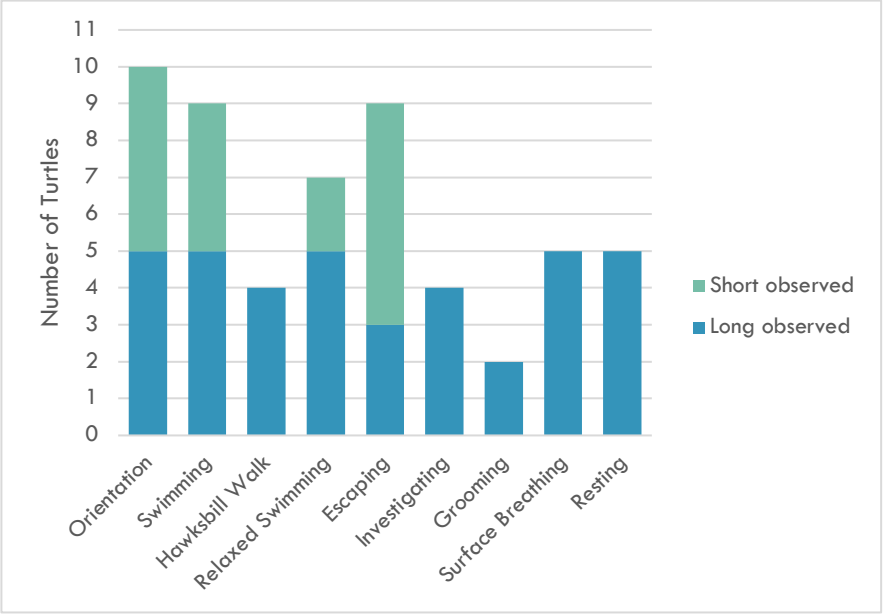



*Figure 6.3. Total number of turtles (n=11) observed engaging in each behaviour, displayed by long observed (n=5) and short observed (n=6) groupings.*

As a proportion of the observed time (Fig. 6.4), the short observed group spent most of their time demonstrating *escape* behaviours (38.9%), followed by *swimming* (30.4%). For the long observed group, *hawksbill walk* (Table 6.1) was the most observed (32.5%), followed by *relaxed swimming* (17.4%). *Surfacing to breathe* and *resting* were the next most frequently observed behaviours for the long observed group, recorded for 14.8% and 14.2% of the observed time, respectively. When all behaviours were compared between all turtles (n=11), there was a significant difference (p=0.02038) between each behaviour as a proportion of the total observation time, but no pairwise differences. However, combined locomotive behaviours (*swimming*, *hawksbill walk*, *relaxed swimming, orientation, escape*; Table 6.1) were observed statistically more of the time (60.1%) than *resting* (13.5%; p=0.00058), *grooming* (11.3%; p=0.00050), *surfacing to breathe* (14.0%; p=0.00058), and *investigating* (1.1%; p=0.00052). The long observed group (n=5) spent 57.9% in *locomotion*, which was significantly more time proportionally than *resting* (14.2%; p=0.079) and

*surfacing to breathe* (14.8%; p=0.079). Locomotion for the short observed group (n=6) was recorded 100% of the observation time (Fig. 6.4).

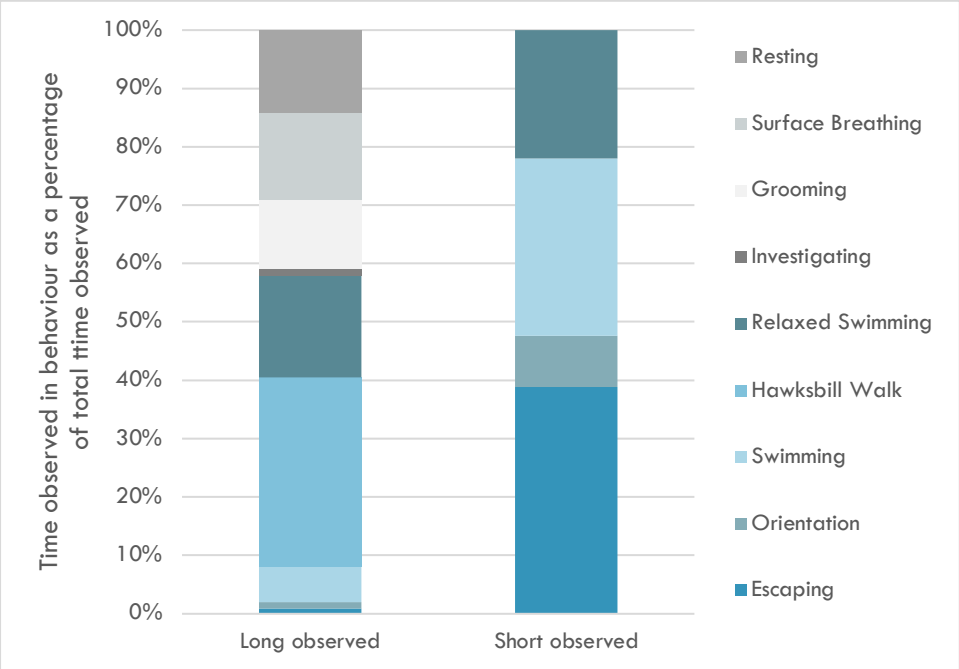



*Figure 6.4. Combined profiles of the behaviours recorded for juvenile hawksbill turtles in the long (n=5) and short (n=6) observed groups, as a percentage of the total observed time for each group.*

Of the 11 turtles released, 9 displayed *escape* behaviours once or twice during observation (mean 1.2; median 1). The median time turtles spent displaying *escape* behaviours was 0.38 minutes (IQR 0.24 – 0.43). Only one turtle escaped at the surface for a total of 0.18 minutes, whereas seven turtles escaped in the water column and six along the benthos for 1.62 and 1.75 minutes, respectively. The median time spent escaping at each depth per turtle was 0.18 minutes (IQR) at the surface, 0.30 minutes (IQR) in the water column, and 0.24 minutes (IQR) along the benthos. Four turtles escaped at two depths (water column and benthos, n=3; surface and benthos, n=1) and the other five turtles escaped at only one depth (water column, n=2; benthos, n=3). Carapace flashing in response to observer presence (turning sideways and directing the top of the shell towards a potential predator), was included as an *escape* behaviour, and was observed in five turtles (long observed turtles, n=3; short observed turtles, n=2) on seven occasions: five times in the water column and once each at the surface and along the benthos. The remaining four short observed turtles all displayed *escape* behaviours but without carapace flashing, and the remaining two long observed turtles displayed no type of *escape* behaviour at all (including carapace flashing).

## Comparison of in-water behaviour across published studies

The captive-raised turtles in this study displayed behaviours that would be expected in free-living hawksbill turtles of a similar size and life-stage, including *swimming*, *resting*, and *investigating* (Table 6.2). Observed proportion of time turtles were engaged in *swimming* behaviours (combined *locomotion*) in this study were comparable to those reported by other published studies of free-living turtle populations (Table 6.2). The *escape* behaviours that were described in this study (3% of observed time) were also similar to that noted in the Blumenthal et al. (2009) study (5% of observed time) on juvenile hawksbill turtles in the Caribbean. The proportion of time these study turtles engaged in *resting* behaviour fell within the range of free-living turtle studies. *Investigating* fell below the published range observed in free-living turtles, although it was omitted from some studies. The main behaviour that was expected but not observed was *feeding*, which was a commonly observed behaviour (13 – 29.5%) in studies of free-living juvenile hawksbill turtles (Blumenthal et al., 2008; Hayes et al., 2017; Proietti et al., 2012; Wright et al., 2020). This lack of feeding was similarly observed; however, in the study of wild-caught but captive held turtles (Dunbar et al., 2008), which engaged in *feeding* only 0.8% of the observed time. Time spent *grooming* was found to be higher in the captive-raised turtles of this study than time spent displaying this behaviour by free-living turtles in one study, although this behaviour was only observed in two of our study turtles. *Surfacing* time was observed proportionally more in this study than that of free-living populations; however, much of the observations in the present study was contributed to by two individual turtles that remained at the surface and were periodically breathing whilst swimming away from divers.

## Response to divers

Between the two groups, only the long observed turtles spent any of the observed time ignoring the divers (Fig. 6.5). Long observed turtles spent most of their time being aware of the divers (55.0% of observation time, median 12.02 minutes, IQR 12.00 – 12.97), and the least time was spent actively moving away from the divers (8.9% of observation time, median 1.98 minutes per turtle, IQR 1.13-2.60). Conversely, short observed turtles showed some form of response to divers throughout the entire study, spending most of their time actively moving away from divers (49.2% of observation time, median 0.43 minutes per turtle, IQR 0.38 – 0.50). Each turtle in the short observed group moved away from the divers. Long observed turtles all spent time focused on or aware of divers, and four of the five spent time moving away from or ignoring divers.

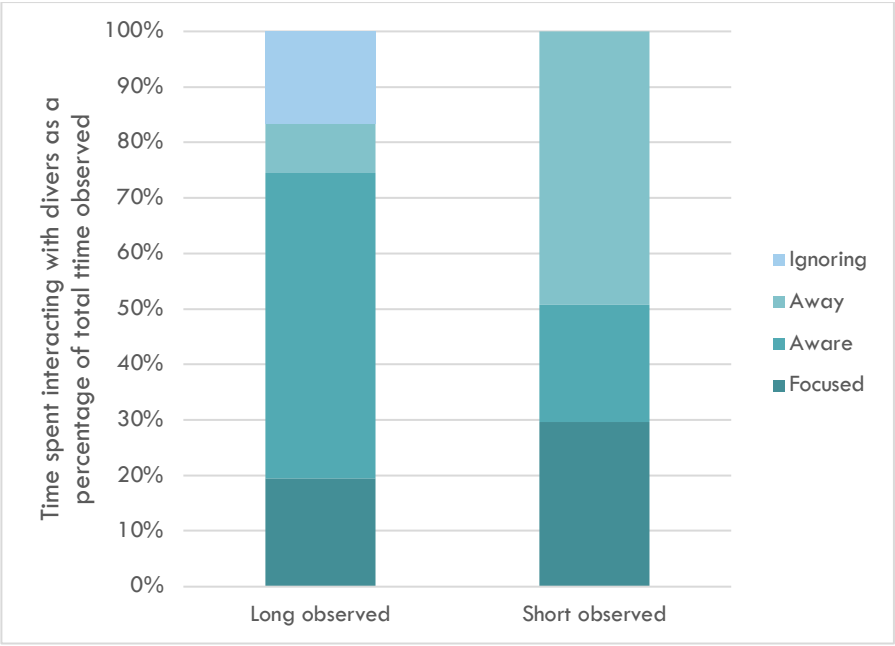



*Figure 6.5. Proportion of time each group (long observed, n=5; short observed, n=6) of newly released hawksbill turtles spent interacting with the divers (ignoring: green; responding: orange).*

## Diving behaviours

### Dive profiles

All eleven turtles were observed diving below the surface, and all except one reached the benthos during observation. Of the five long observed turtles, four turtles had two distinct dive periods during their observation, and the other had three dives (Fig. 6.6). Subsequent dives got progressively longer, and all five long observed turtles spent much longer diving than at the surface, as a proportion of the observation time. Two of these five turtles were still on the benthos at the end of the observation time, indicating that their final dive would have been longer than was recorded. Median dive duration for the five long observed turtles, including two capped dive times (two turtles were at depth at the end of the observation), was 11.08 minutes (IQR 4.57 – 12.36). The maximum depth for the observed dives of all turtles (n=11) was 7.5 m, ranging from 3.3 – 7.5 m (median 4.0 m, IQR 3.7 – 6.1).

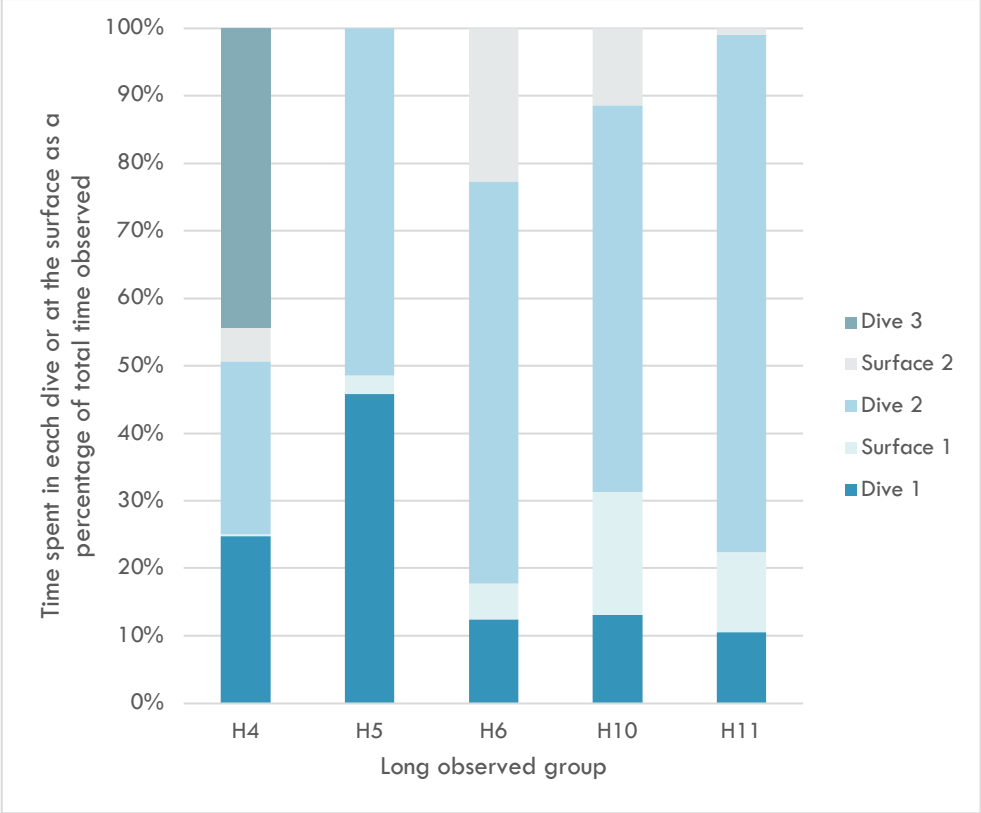



*Figure 6.6 Time spent on each dive and total time spent at the surface as a proportion of the total observed time for each of the five juvenile hawksbill turtles (long observed group) that were recorded for more than one dive.*

## Depth and habitat profiles

Amongst the long observed group, all benthic habitats were explored (sand, coral bommie, coral rubble), whereas only 'sand' was recorded for the short observed group (Fig. 6.7). Proportionally, the long observed group spent most of their observed time on the benthos (74.5%, median time 18.00 minutes, IQR 13.75 – 21.58), whereas the short observed group were mostly occupying the water column (59.0%, median time 0.59 minutes, IQR 0.33 – 0.80).

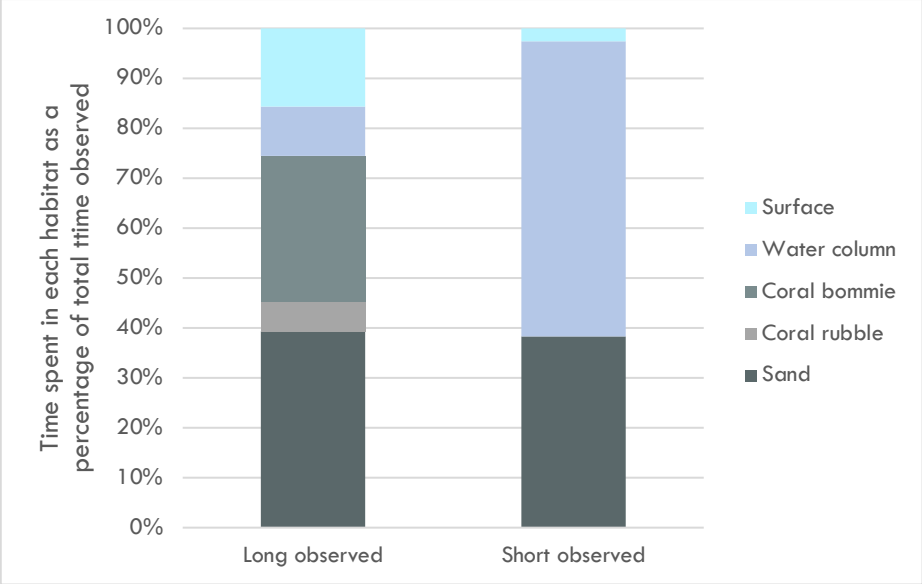



*Figure 6.7. Proportion of time the long observed (n=5) and short observed (n=6) groups of turtles spent at each relative depth (Light blue: surface, dark blue: water column, yellow/brown shades: benthos) and on each benthic substrate (sand, coral reef/bommie, coral rubble).*

## Discussion

All eleven of the captive-raised juvenile hawksbill turtles were able to dive and display good buoyancy control upon first release into the ocean. Nine distinct behaviours were recorded over the total observation period; however, no individual turtle exhibited all nine behaviours. Seven of the turtles reacted to the divers throughout their observation periods, and four turtles spent at least some time completely ignoring the divers. Of the turtles with multiple dives recorded, subsequent dive length increased, with most of the observed time spent on the benthos. Observed behaviours were critical to survival in the wild, and included swimming, surfacing to breathe, resting, investigating, and escape or predatory avoidance. Comparison with published observations of free-living juvenile hawksbill turtles will allow a first insight into whether captive-raised turtles can maintain naturalistic behaviours, regardless of the stay in captivity and the presumed stress of the release process.

### In-water behaviours

Despite availability of food in the release environment, feeding was the only behaviour commonly noted in published studies of free-living turtles that was not observed during the initial release period (maximum 25 minutes) recorded in this study. These study turtles had not been fed in the 2 days prior to release to reduce metabolic demand (Hicks & Bennett, 2004; Wallace & Jones,

2008; Wyneken, 2017) but would likely still have had energy reserves to support them without the need to forage immediately on release. This, and the relatively short observation time, may explain the absence of this behaviour. Similar observations were found by Dunbar et al. (2008) in wild-caught turtles (1.2-10.2 kg, n=19) that had been held in captivity for an extended period (a few weeks to 8 months). When released back into the wild, after an unspecified number of days of fasting ("not fed on the day of release", page 5), feeding was recorded but at a far lower occurrence (0.8%) (Dunbar et al., 2008). Another possible explanation is that the turtles were prioritising other behaviours because they were in an entirely new environment. Although *feeding* was not recorded during the observation time in this study, the turtles were observed grazing on naturally grown algae from the bottom of their tanks during captivity (RD, EA, KE personal observation). Additionally, the satellite trackers attached to these turtles transmitted for several months, indicating the ability of the turtles to feed and sustain themselves in the wild post-release (see Chapter 7). Grazing on algae is a natural part of the hawksbill's diet (Bell, 2013) and thus, ability of the study turtles to continue this foraging behaviour upon release is highly likely. Foraging was reported in another head-started juvenile hawksbill study, inferred via satellite telemetry (Okuyama et al., 2010).

*Feeding,* along with *investigating* and *swimming,* are mostly diurnal behaviours of turtles and *resting* mostly occurs at night (Hayes et al., 2017). In this study, all data were collected during the day and so less time spent *resting* would be expected. However, the observed proportion of *resting* was highly variable across the published literature, and it is possible that *resting* was high in this study because of the lack of observed *feeding*. The lack of time spent *investigating,* even though it was daytime, could also account for some of the extra time ordinarily allocated to *resting* as the turtles did not need to be actively looking for food. High proportion of time *grooming* could also be linked to the extended resting periods since the grooming observed in this study involved rubbing the carapace on coral and rock on the benthos where turtles could also rest.

Combined *swimming* (locomotive) behaviours recorded in this study sit at the top end of the range from published literature. Similarly, a high proportion of time engaged in *swimming* was also recorded for temporarily captive turtles by Dunbar et al. (2008) who supposed this may be explained by lower foraging needs at the time of release than in free-living populations from other studies. Lengthy *surfacing* observations of two specific turtles in this study that were swimming away from divers at the surface and intermittently breathing could have been reclassified as *swimming*, further increasing the proportion of time turtles engaged in *swimming*. Furthermore, other studies did not specify *escape* as its own behaviour; however, distinguishing between *escape*

and *swimming* provides a more detailed insight into the behaviours and response of the turtles to potential predators or disturbances like scuba divers or snorkellers in the water. Therefore, *escape* is a key survival behaviour, and we deemed it an important behaviour to assess in a captive-raised turtle population and that should also be included in future studies of both free-living and captive-raised turtles.

While a few studies have based their behavioural classifications on the work of Houghton et al. (2003), there remains a lack of consistency and standardisation of behavioural definitions, which hinders direct comparison between study populations (Diez et al., 2002b; Meadows, 2004). Specifically, *investigation* was difficult to classify if not foraging-related and occupied a small proportion of observation time in this study compared with other published studies (Table 6.2). An additional difficulty in cross-comparison of studies is that several studies have focused on only a few, pre-targeted behaviours, such as *resting* and *foraging* (Houghton et al., 2003; Proietti et al., 2012; Stimmelmayr et al., 2010), rather than describing the full range of observed behaviours. Liu et al. (2009) identified the importance of such specificity by documenting 75 detailed behaviours observed in captive four-eyed turtles (*Sacalia quadriocellata*), which coded to 8 main categories. Developing similar extensive ethograms of species-specific sea turtle behaviours for use across populations would greatly benefit this area of research.

Behaviours not previously described and/or categorised were *orientation* and *hawksbill walk*. *Orientation* was deemed a necessary addition to account for the release procedure, specifically the period when the captive-raised turtles were first released into the ocean and exposed to sights, sounds, smells, depths, and waves that they had never experienced. For some turtles, it was a frozen state with little movement, or it was a rotation in all directions to get a bearing (or *orientation*) before starting a defined behaviour. The *hawksbill walk* was also not previously described; however, it could possibly be considered locomotion by one study or foraging by another due to lack of consistent and well-defined terms. *Hawksbill walk* is described here specifically because this behaviour was also observed in captivity (EA, RD, KE personal observation) and therefore possibly influenced by the shallower tank depth of 0.6 m. Blumenthal et al. (2008) described the behaviour of the turtle at each stage of its diving profile, noting that there was a stage of "pulling along the bottom with flippers", which is listed in their related figure as "Feeding (crawling along the bottom)", but could have been categorised as *hawksbill walk*, *investigating*, *feeding* or 'locomotion' in other studies.

## Human-turtle interactions

### Response to scuba divers

Reactions to divers were a combination of focusing on, aware of, and escaping from divers, and was observed in all 11 study turtles. This could be due partially to proximity to the turtles, as found in previous studies of free-living turtles, or possibly because of habituation to humans (Hayes et al., 2017). Despite the possible effect of diver or snorkeller presence on turtle behaviour, this method of data collection still provides valuable insight into turtle behaviour that would likely be difficult to collect via other means. Examination of changes in behaviour caused by in-water presence of divers and snorkellers has yielded mixed results. A study of immature (mean CCL 47 cm, 30 – 75 cm) green turtles (*Chelonia mydas*) in Hawaii found that recreational snorkeller presence did not affect the mean time turtles engaged in each behaviour; however, it did increase the number of bouts of each behaviour (Meadows, 2004). This was attributed to turtles switching between behaviours when interrupted by snorkellers. Meadows (2004) also observed an increase in the number of surfacing events in the presence of snorkellers; therefore, it is possible that snorkeller presence in our study also increased *surfacing* time. However, detailed description of swimming speed was lacking from Meadows (2004) study, which could have been used to determine if the increased surfacing was a result of increased metabolic demand from faster, "panicked" swimming away from snorkellers. Observation of juvenile hawksbill (size range unspecified) behavioural response to divers in a marine protected area in Roatán, Honduras, also found no effect on time turtles spent swimming (Hayes et al., 2017). However, hawksbill turtles spent significantly less time investigating, eating, and breathing when divers were within 1 – 2 m. Also, conversely to the findings of Meadows (2004) for green turtles, Hayes et al. (2017) found no significant change in the median number of bouts of activities during diver presence. So, while some turtles in this study were aware of and actively moved away from divers on occasion, their responses to in-water divers appeared to be in-line with those of free-living turtles and did not prevent expression of naturalistic behaviours.

### Maintenance of natural behaviours in captive reared turtles

One key husbandry consideration for sea turtles temporarily kept in captivity with intention of release into the wild is preventing habituation to and dependence on humans (Diggins et al., 2022). Husbandry practices at the JCU Turtle Health Research facility have been developed to maintain positive welfare of the turtles (good nutrition, maintained water quality, and water temperatures set to mimic natural variation (Arena et al., 2014)), whilst limiting human-turtle interactions. Whilst reducing stress to animals during captive rearing is important for maintaining positive

welfare, loss of the "fight or flight" stress response in animals due for release would be detrimental to their survival on release (Diggins et al., 2022)). For example, one concern noted in the literature is the risk of turtles swimming up to boats or fishermen for food if they have been acclimatised to feeding by humans (Smulders et al., 2021). Approaching boats out of lack of fear and potential drive for food could result in injury/death caused by accidental capture in fishing nets (Lamont et al., 2022) or boat strike (Chaloupka et al., 2008; Phu & Palaniappan, 2019). Food was never fed directly to the turtles but rather dropped or thrown into the tank from a short distance, after which feeders promptly stepped away from the tanks. Employing similar practices in any temporary captive-holding environment, including head-starts, research facilities and rehabilitation centres, would be prudent for discouraging human habituation and therefore maximising chance of survival post-release (Diggins et al., 2022).

Noise and movement from boats could be considered a form of human-caused disturbance representing 'predation' that should result in a prey response from the turtles (Frid & Dill, 2002). As such, boat presence should result in predator avoidance behaviours; however, some studies have found a lack of risk-avoidance behaviours in turtles inhabiting areas high in predators (Foley et al., 2007; Hammerschlag et al., 2016; Hammerschlag et al., 2015). Therefore, boat presence may not actually result in increased escape or predatory avoidance behaviour. Indeed, Wright et al. (2020) found that juvenile hawksbill behaviour was not influenced by boat presence; however, a number of turtle sightings was positively correlated with boat presence. One possible explanation for this correlation would be turtle habituation to the tourism activity as this study was conducted within a marine protected area that provides refuge for foraging hawksbill turtles (Hayes et al., 2017). In contrast, turtles from this study were observed changing direction to avoid the boats. For the first two turtles released, the tender motor was on in neutral, causing extra noise stimulus, and both turtles swam away immediately. For all remaining turtle observations, the boat engines were turned off prior to release. Furthermore, all study turtles showed escape behaviours and several displayed carapace flashing, which is an evasive manoeuvre turtles use to avoid predation. Therefore, this study demonstrates the innate behaviours that are key to survival in the wild are still present in captive-raised turtles.

## Dive profiles and behaviours

Despite spending their first 2 years in a captive environment with a depth of only 0.6 m, all turtles in this study dived below this depth and displayed good buoyancy control on release into the ocean. The average dive duration of our turtles was at the lower range of, or shorter than, recorded in published studies of free-living turtles (Blumenthal et al., 2008; Houghton et al., 2003;

van Dam & Diez, 1996; von Brandis et al., 2010). However, dive duration and depth have been shown to be positively correlated with body mass, which could contribute to this difference as our turtles were at the lower end of the size range (Blumenthal et al., 2008). Additionally, some temporarily captive juvenile hawksbill turtles have been observed making an initial short dive when released, followed by dives of longer duration after the second or third breath (Dunbar et al., 2008), which was also observed in this study. An extended observation of these study turtles might, therefore, have resulted in an increased average dive duration.

The average dive depth of the turtles in this study was comparable with free-living turtle observations (von Brandis et al., 2010). The maximum depth, however, was considerably shallower than most because turtles were released in a shallow lagoon and did not have access to deeper water during the observation period. Furthermore, previous studies have found that the dive depth profile of juvenile hawksbill turtles is strongly correlated with time of day, whereby greater depths are accessed at night for resting (Blumenthal et al., 2008). Since this study did not include night-time observations, it is likely that the turtles would have dived deeper later, and therefore, is not an indicator that captive-raised turtles are unable to dive to normal maximum depths.

The proportion of surfacing and breathing time recorded in this study was approximately 3.2-fold more than observed in free-living populations, according to the literature (Table 6.2). However, some of the surface time in this study was derived from two turtles that spent part of their observation time swimming away from the divers within 1 m of the surface, whilst intermittently surfacing to breathe. Whilst not recorded as an escape behaviour, this increased metabolic demand (swimming) could account for the larger than expected surface time as time between breaths decreases when metabolic demand is increased (Wyneken, 2017). If the surfacing and breathing time of those two turtles are discounted from analysis, the proportion of time the other nine hawksbill turtles engaged in this behaviour (4%) falls within the published range.

## Limitations of the study

When working with critically endangered species, small sample sizes can be a limiting factor in the research (Kophamel et al., 2022). Removal of turtles from the wild as hatchlings has ethical implications and permitting restrictions on numbers, etc. Being able to research hawksbill turtles in a controlled and optimised environment, simulating the wild but free from certain stressors, provides excellent opportunity to advance our knowledge of their early development. Having the capacity to rear them under the best welfare conditions and unifying their history, aids further

justification for the temporary holding of the turtles in this study. An additional welfare consideration during release was limiting potential stress to the turtles. This was accounted for by: 1) releasing the turtles over two days, which limited the waiting time for turtles on the main vessel while the others were being released and recorded; and 2) maintaining adequate distance between turtles and divers, not chasing the turtles, and capping the maximum observation time at 25 minutes. As a result of these mitigatory actions, some behaviours may have been underrepresented, with *foraging* not observed at all. It is possible also that releasing the turtles across two days introduced additional confounding factors, with some variability in underwater visibility and Beaufort scale; however, current and water temperature were similar across all releases. Another possible limitation of this specific study was the potential for the attached satellite tracker to influence the turtles' behaviour. This is a potential limitation in all studies of behaviour derived from tracking data. However, this was likely mitigated by attaching replica trackers to the turtles whilst they were in captivity, which were swapped for the genuine trackers shortly prior to release (Diggins et al., 2023).

## Management and conservation implications

Measuring the success of a captive-raised turtle release is difficult because true success is marked by successful reproduction, which could take decades and would be very difficult to record (Bell et al., 2005; Shaver, Rubio, et al., 2016). Satellite tracking of released turtles can provide a quicker indication of success via inference of behaviours (Okuyama et al., 2010) and short-term survival of the turtles (Abalo-Morla et al., 2018). However, satellite trackers are expensive, and analysis of the data requires access to software and specialised skill. Most facilities that release captive-raised or temporarily captive turtles are community run head-start and rehabilitation centres, which are often restricted in their resources (Laurance et al., 2012). Recording and analysing the first behaviours of sea turtles released into the ocean after a period of captivity provides an immediate insight into survival likelihood. It is also easily replicated, which is important for cross-study comparisons and will provide a better overall understanding of the effects of captivity on behaviour of sea turtles. The hawksbill turtles in this study were observed engaging in naturalistic behaviours, key to survival, immediately on release, despite being raised in captivity and having never before entered the ocean. This innate nature of sea turtle behaviour is important because it shows that turtles raised in head-start facilities or kept in rehabilitation centres for extended periods of time can retain the required behavioural instincts to survive in the wild, provided that they are kept under appropriate housing and husbandry to promote positive welfare.

### Conclusions

Eleven hawksbill turtles were raised in captivity for 2 years before being released into the ocean for the first time. Despite having spent their lives up to that point in tanks <1 m deep, all turtles were able to dive below the surface and displayed good buoyancy control. Other behaviours observed during release included *swimming*, *surfacing to breathe*, *resting*, and predator avoidance manoeuvres (including *escape*), which are all naturalistic behaviours exhibited by wild juvenile hawksbill turtles. This suggests that key survival behaviours are innate in turtles and not lost during captivity and, therefore, captive-raised hawksbill turtles can still have a positive outcome when released into the wild.

## Summary of thesis and chapter aims

### Thesis aim 4

Determine whether juvenile hawksbill turtles display naturalistic behaviours after 2 years under human care.

### Chapter aims

#### Chapter aim 1

- Document the behaviours of captive-raised hawksbill turtles on first entry into the ocean via in-water observation.

All turtles were able to dive and swim with good buoyancy control immediately upon release. Nine behaviours key to survival in the wild were recorded, including swimming, escape, resting, and investigating. Locomotive behaviours combined (orientation, swimming, relaxed swimming, "hawksbill walk", and escape) constituted most of the total observation time (55.7%). The turtles in this study also spent time surfacing to breathe, resting, and grooming. Most turtles spent time aware of, focused on, or moving away from the observers, although four turtles also spent some time completely ignoring the observers. Average dive duration for turtles with multiple dives observed (n=5) was 11.08 minutes (median; IQR 4.57 – 12.36) minutes, and in each case the first dive was shorter than subsequent dives. The average dive depth for all turtles (n=11) was 4.0 m (median; IQR 3.7 – 6.1 m), ranging from 3.3 – 7.5 m.

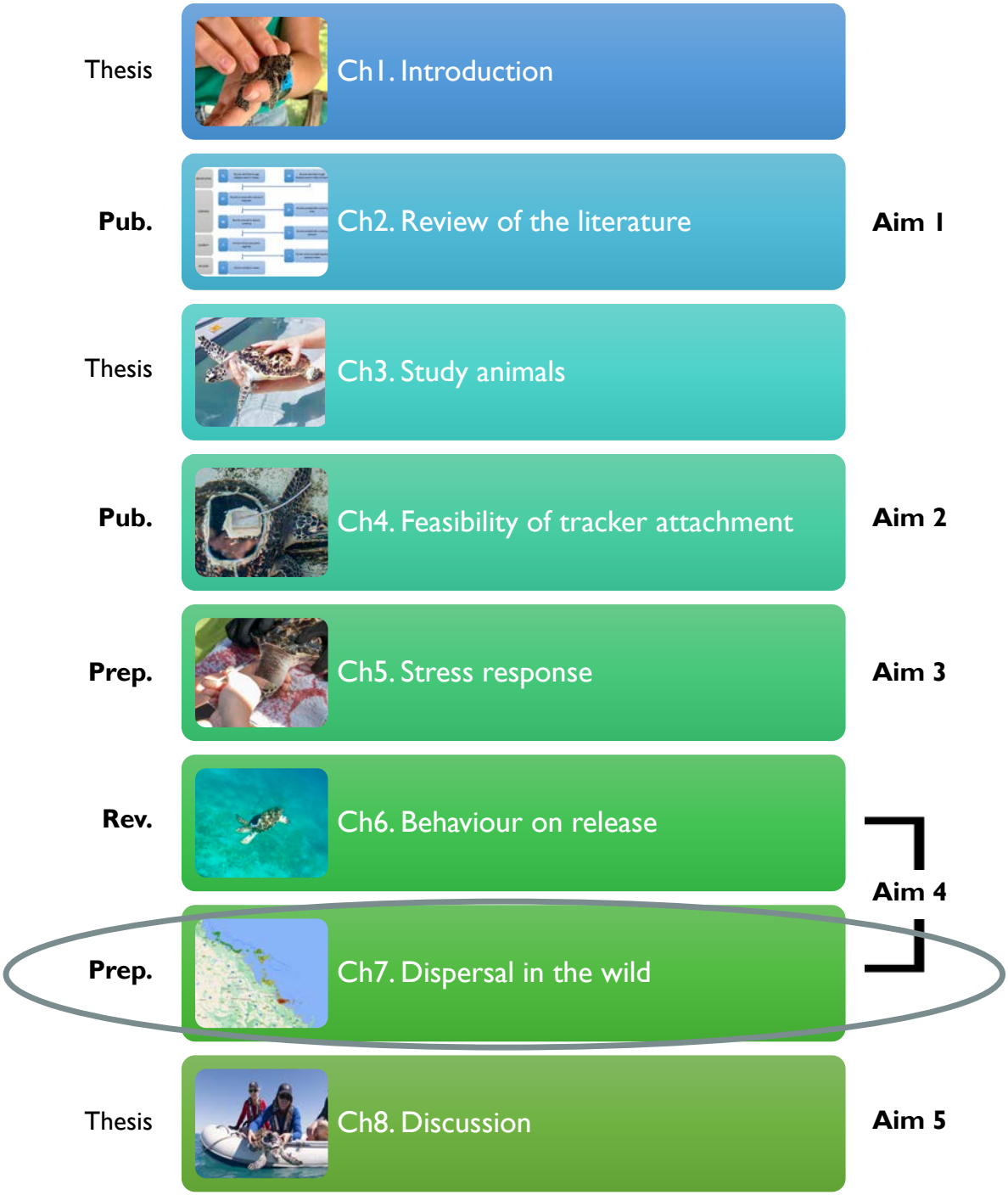
## Chapter aim 2

- Compare in-water behaviours of recently released juvenile hawksbill turtles with free-living hawksbill turtles reported in published studies.

Locomotive behaviours combined were comparable to published studies of free-living turtles (33.4% – 78.9%). Feeding was the only commonly recorded behaviour in free-living turtles that was not observed in these study turtles. All other commonly reported behaviours of free-living hawksbill turtles were captured in this study of captive-raised hawksbill turtles. Dive profiles of the study turtles were also found to be comparable with published data from free-living hawksbill studies for depth and dive time. These results indicate that the captive-raised study turtles maintained their innate ability to navigate depth, diving and breath taking, despite being reared in a relatively constricted environment. Thus, this in-water observational case study showed that captive-raised juvenile hawksbill turtles demonstrated naturalistic behaviours key to survival upon immediate release into the ocean. Adoption of detailed behavioural descriptions in future studies would facilitate better comparisons between other head-started cohorts and their wild-raised counterparts.

# CHAPTER 7: DISPERSAL IN THE WILD

## Thesis structure



# Background and aims of this chapter

## Rationale

Some sea turtles are removed from the wild as eggs or hatchlings and raised under human care for conservation or research purposes. Sea turtles naturally disperse away from their natal beaches after hatching, and decades later migrate to neritic areas to forage and develop. It is not yet fully understood how removal from the wild of turtles in their very early life-stages affects their orientation, dispersal, and migration between foraging grounds once released into the ocean months or years later. This study provided the opportunity to track juvenile hawksbill turtles from release into the ocean after being raised for more than 2 years under human care to assess. It was unknown how the study turtles would behave when released into a comparable ecological setting to those transited by their free-lving conspecifics; whether they would disperse, and if so, what migration patterns (if any) they would follow. Releasing the turtles with satellite trackers enabled validation that the tracker attachment method worked in the ocean as well as in a research setting and gave an insight into whether the turtles were able to survive in the wild.

## Thesis aim 4

Determine whether juvenile hawksbill turtles maintain naturalistic behaviours after 2 years under human care.

### Chapter aim

- Document the dispersal of captive-raised juvenile hawksbill turtles on release using satellite telemetry.

## Research outputs

**Diggins** RL, Hamann M, Smithers S, Jones K, Mendez D, Bell I, and Ariel E (*in preparation*). Dispersal of captive-raised juvenile hawksbill turtles *(Eretmochelys imbricata)* upon release into the ocean.

# Dispersal of captive-raised juvenile hawksbill turtles (*Eretmochelys imbricata*) upon release into the ocean

## Introduction

Sea turtles have inhabited the Earth for over 120 million years and are fundamental to the health of the marine and coastal ecosystems in which they live (Bjorndal & Jackson, 2002). Yet, six out of the seven remaining extant species are threatened with extinction (Abreu-Grobois & Plotkin, 2008; Casale & Tucker, 2017; Mortimer & Donnelly, 2008; Seminoff, 2004; Wallace et al., 2013; Wibbels & Bevan, 2019). Conservation efforts are required to prevent sea turtle extinction, and these efforts must be carefully targeted to maximise the impact of often limited management resources (Hamann et al., 2010; Lascelles et al., 2014; Rees et al., 2016). A comprehensive understanding of the biology and ecology of each species of turtle at each life stage is required for effective conservation due to inter and intra-species differences (Bolten, 2003; Heppell et al., 2002; Wyneken, 2017). Sea turtles are migratory animals, that at different life stages inhabit different geographic locations and undertake lengthy migrations attached to their ontogenic development (Bowen et al., 1992; Meylan et al., 2011; Miller et al., 1998). In general, sea turtles hatch from eggs laid on a beach, enter the ocean and disperse for several years (the "lost years") before settling in neritic foraging grounds as new recruits where they typically remain until adult-sized. Once reproductively mature, the turtles will periodically migrate back to their natal nesting grounds to reproduce before returning to their foraging grounds to rebuild their fat reserves and prepare for another nesting season (Heppell et al., 2002).

As sea turtles progress through their life cycle and grow in size, likelihood of predation related mortality decreases (Tetzlaff et al., 2019a). Turtles in their early life-stages (egg incubation, hatchling emergence, and post-hatchling dispersal) are thus at greatest risk of predation. Head-start organisations aim to assist turtles in these early life-stages by raising them ex-situ until they reach later stages of development where predation pressure is lessened due to their increased size (Mullin et al., 2023). At head-start facilities, nests are protected from predators and monitored to try and increase hatching success. Once hatchlings emerge from the nest, they are raised in captivity for a period and released into the wild when they are larger and less vulnerable to predation (Mullin et al., 2023). The size and life-stage at which the head-started turtles are released varies between facilities but should determine the location and process for release. To determine where to release head-started turtles, it is necessary to understand the natural dispersal, behaviour, and movements of free-living turtle populations. For example, oceanic stage

head-started turtles should be released where those turtles would be found naturally, whereas larger, new recruit sized turtles should be released in neritic areas with other new recruits of the same species.

Satellite trackers are tools commonly used to study animal movement and behavioural ecology of a multitude of aerial, terrestrial, and aquatic species, including sea turtles (Godley et al., 2008; Hays & Hawkes, 2018; Jeffers & Godley, 2016). They work by emitting a messaging signal when the antenna breaches the surface as the turtle surfaces to breathe, and this signal is received by satellites. Marine turtles have been successfully tagged with both acoustic and satellite telemetry tags to study inter-nesting (Robinson et al., 2017), foraging (Shimada et al., 2016), and diving (Blumenthal et al., 2008) movements and behaviours, to determine home-range (Hart et al., 2012), and to track reproductive migrations (Hoenner et al., 2016). While most studies have focused on adult turtles, particularly green (*Chelonia mydas*), loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) turtles (Godley et al., 2008; Hays & Hawkes, 2018; Jeffers & Godley, 2016), fewer studies have tracked juvenile turtles, and fewer still have tracked post-hatchling dispersal though some have modelled possible dispersal (Madden Hof et al., 2023a). Ease of access to nesting females has historically skewed the research towards the study of mature females (Godley et al., 2008). However, validated research techniques exist for the capture of all sea turtle size-classes, including juveniles (León & Diez, 1999; Limpus & Reed, 1985; Makowski et al., 2006). Another limiting factor to tracking smaller turtles is availability of size appropriate trackers and adapted methods for attachment. Consequently, more studies focusing on early sea turtle life stages are needed. This is currently a developing area of research (Diggins et al., 2023; Mansfield et al., 2017; Mansfield et al., 2021; Mansfield et al., 2014; Mansfield et al., 2012; Seney et al., 2010). Using these emerging techniques to fit neonatal and post-hatchling sized turtles with satellite trackers would help to bridge the knowledge gap of the movements and ecological behaviours of turtles during their first oceanic dispersal. The outcome of such studies would inform management and conservation efforts targeting sea turtles and help guide both rehabilitation and head-start release procedures for juvenile (neonatal to new-recruit) sea turtles (Pabón-Aldana et al., 2012; Robinson et al., 2020).

Hawksbill turtles (*Eretmochelys imbricata*) are globally critically endangered (Mortimer & Donnelly, 2008), which is only one category above 'extinct in the wild'. Conservation programs should be targeted at this species to assist with population growth, and head-start facilities are a plausible option, though few exist globally at present (Evans et al., 2022; Nasiri et al., 2023). Whilst several studies have been conducted on dispersal of head-started loggerhead (Abalo-Morla et al., 2018;

Nagelkerken et al., 2003) and Kemp's Ridley (*Lepidochelys kempii*) turtles (Klima & McVey, 1995; Shaver & Rubio, 2008), only one has compared five head-started versus five wild hawksbill turtles in Japan (Okuyama et al., 2010) and another tracked a single head-started hawksbill turtle in Colombia (Pabón-Aldana et al., 2012). The paucity of data studies on this species highlights that more research is needed to determine the effects of captive-raising on hawksbill turtles and potential influence on their post-release survivability. This is particularly important given hawksbill turtle populations are identified by distinct management units and should therefore be studied on a local as well as global scale (Vargas et al., 2016). In Australia, little is known about wild versus head-started hawksbill turtle dispersal. Therefore, this study aimed to increase knowledge in this area by conducting a preliminary analysis of the post-release dispersal of juvenile North Queensland hawksbill turtles after being captive raised from hatchlings for 2 years. The intention of this study was to further validate the success of the release and identify indicators of post-release survivability, such as inhabitation of environmentally suitable areas. Detailed analysis of their movements was beyond the scope of this thesis, but this study will serve as a base for future studies to build on.

## Materials and methods

### Study animals and permits

Eleven hawksbill turtles (H01 – H11) hatched naturally in the wild and were then raised at the James Cook University (JCU) Turtle Health Research facility in Townsville, Australia (Diggins et al., 2023). This purpose-built facility enables health and behavioural studies to be conducted on several species of sea turtles during their first few years of development in near-natural conditions. The turtles were raised according to the facility's own husbandry manual and standard operating procedures (WLD-16) (Turtle Health Research, 2021), with intentional minimisation of handling and disassociation of food from humans. The hawksbill turtles were raised in the facility for just over 2 years and released when they were of a size comparable with free-living juvenile hawksbill turtles that have newly recruited to neritic foraging grounds (Bell & Pike, 2012b; Chaloupka & Limpus, 1997). The study turtles weighed 4,445 – 7830 g (median 5760 g; IQR 5,014 – 6,926 g) and had a curved carapace length (CCL) of 357 – 444 mm (median 389 mm; IQR 374 – 405 mm) at release.

Hatchling collection was authorised and facilitated by the Department of Environment and Science (DES; permit WA0012830) and rearing conditions were monitored under JCU Animal Ethics (permit A2586). The juvenile turtles were released onto the Great Barrier Reef under the

authority of the Great Barrier Reef Marine Park Authority (Marine Parks Permit: G20/44009.1) and in conjunction with Gudjuda and Girringun Rangers and Manbarra people of Palm Island.

## Release site and protocol

John Brewer Reef (JBR) in the Great Barrier Reef, Australia (18.633°S, 147.067°E) was selected as the release site (Chapter 3). This site was found to be suitable because of its food availability and provision of shelter with its moderately large (~5 km x 2 km) platform reef and protected interior lagoon. JBR was also the site used for a previous small, juvenile turtle release event in which captive-raised green turtles were observed as they entered the ocean for the first time (Department of Environment and Science et al., 2019). This reef is also in close proximity to the JCU Turtle Health Research facility, thereby reducing dry-docking time and travel-induced stress to the turtles during transportation to the release site (Chapter 5). Release of the hawksbills onto JBR was a collaborative effort with the Department of Environment and Science and Gudjuda and Girringun rangers. Manbarra Elder, Allan Palm Island, also attended the release, with financial support from the World Wide Fund for Nature (WWF).

Five days prior to release, all 11 turtles were fitted with a SPOT-387 satellite tracker produced by Wildlife Computers (59 x 29 x 23 mm, 39 g) per a modified version of the published attachment protocol detailed in Chapter 4 (Diggins et al., 2023). Some small changes were made to this published protocol: 1) the neoprene covered fewer scutes because the turtles were much larger at the time of release; 2) a larger amount of silicone was used to line the scutes; 3) less epoxy was used to increased drying speed; and 4) anti-foul was painted onto the tracker and antenna, but not covering the two communication ports. Each turtle also had a standard titanium flipper tag (Stockbrands Company, Pty. Ltd., Perth, Western Australia) attached to the trailing edge of their flipper on the morning of their release. Flipper tags were provided by DES. Six turtles were released on 6 May 2021 and the remaining five turtles were released on 7 May 2021.

## Data analysis

Data summaries are presented as median with interquartile range due to the small sample size. Kruskal–Wallis chi-square tests were conducted with R version 4.3.1 2023-06-16 (R Core Team, 2023) in R Studio 2023.09.0 Build 463 (RStudio Team, 2023) to test for differences in data transmission days between groups.

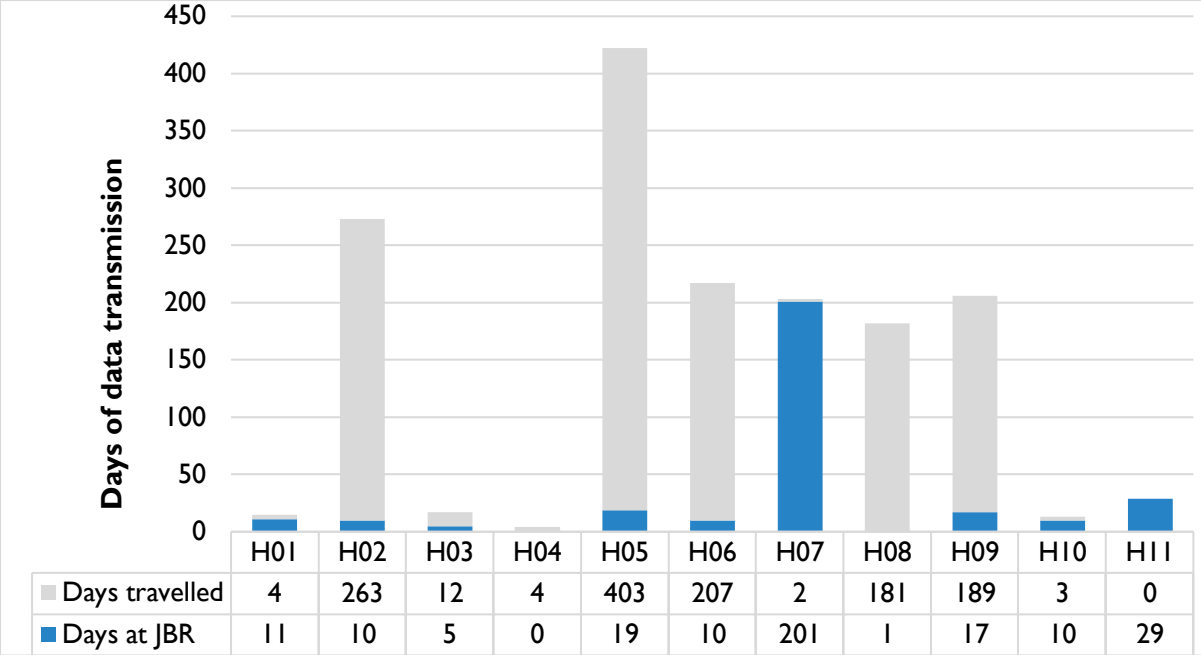
## Results

All 11 study turtles were tracked, and data were received for 4 – 422 days (median 182 days, IQR 16 – 212 days). Data was received from the trackers up to the fifteenth month following release (Table 7.1), indicating that captive-raised hawksbill turtles can survive post-release into the wild for more than a year. The length of time for which each turtle transmitted data was not different ($p>0.05$) between the two release dates, between morning and afternoon release times, or between being followed and filmed on release for a "short" period (<2 minutes) or a "long" period (>20 minutes) (Chapter 6).

*Table 7.1. Number of captive-raised, juvenile hawksbill turtles (Eretmochelys imbricata) still transmitting satellite data each month post release into the ocean.*

| **Month post release** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Number of turtles transmitting** | 11 | 7 | 7 | 7 | 7 | 7 | 6 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 1 |

Upon release into the ocean for the first time, the hawksbill turtles had a varied response. The 11 turtles remained at the release site (JBR) for different lengths of time (Fig. 7.1) before dispersing in non-uniform directions within the Great Barrier Reef. The number of days that each turtle remained at JBR varied from 0 – 201 days (median 10 days; IQR 7.5 – 18 days) and represented 0 – 100% of their transmitting time (median 8%; IQR 4 – 75%). Only one of the turtles (Fig. 7.2) remained resident on JBR for the entirety of its transmission period (29 days). Three more turtles spent the majority of their transmission period on JBR (73 – 99%), including one turtle that remained at JBR for 201 days post-release. The remaining seven hawksbill turtles spent proportionally less time at JBR than dispersing (0 – 29%), including one turtle that travelled away from JBR within the first day of release.




| | H01 | H02 | H03 | H04 | H05 | H06 | H07 | H08 | H09 | H10 | H11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Days travelled | 4 | 263 | 12 | 4 | 403 | 207 | 2 | 181 | 189 | 3 | 0 |
| Days at JBR | 11 | 10 | 5 | 0 | 19 | 10 | 201 | 1 | 17 | 10 | 29 |

*Figure 7.1. Days spent at (blue) and dispersing away from (grey) the release site, John Brewer Reef (JBR), per juvenile hawksbill turtle (Eretmochelys imbricata; H01 – H11). The complete bar (both blue and grey sections combined) represents the total number of days of transmitted data per turtle.*

The most northernly point reached was 15.545°S, 147.129°E (offshore from Cooktown) and the most southernly was 23.630°S, 151.223°E (offshore from Gladstone). Four turtles travelled north, four travelled south, and three remained in the vicinity of the release site. Three turtles travelled among the outer reefs, four travelled along the coast or inner reefs, and three turtles travelled between the coast, inner and outer reefs. The final data transmission locations of the turtles varied from 0 km to approximately 715 km from the release site (straight distance), with a median straight distance of 122 km (IQR 62 – 312 km). The length of time for which data were transmitted was not dependent ($p>0.05$) on direction travelled, nor the number of days remaining at the release site before dispersing elsewhere. However, turtles that transmitted their final location from inshore areas (<10 km of the mainland) transmitted for significantly more days ($W=30$, $p=0.004329$) than those that transmitted their final location further offshore (Fig. 7.3.).

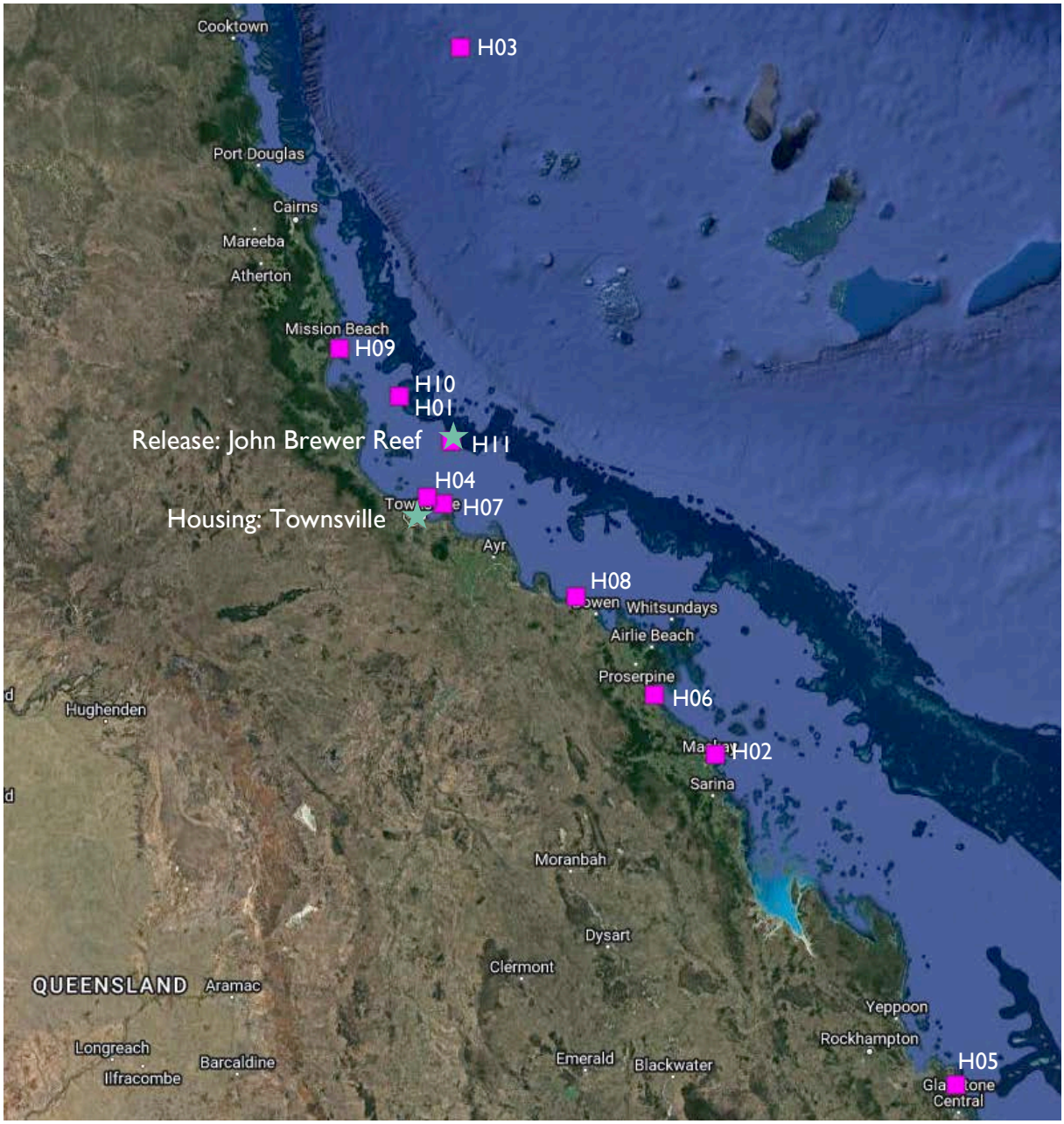



*Figure 7.2. Final snapshot of data transmission location (pink squares) for captive-raised juvenile hawksbill turtles (Eretmochelys imbricata; H01 – H11) retrieved from the Wildlife Computers data portal. Turtles H0 and H10 are sharing one square as their last locations were transmitted from neighbouring reefs. Stars indicate site locations of turtle housing and release. Straight distance between Townsville and John Brewer Reef is approximately 74 km.*

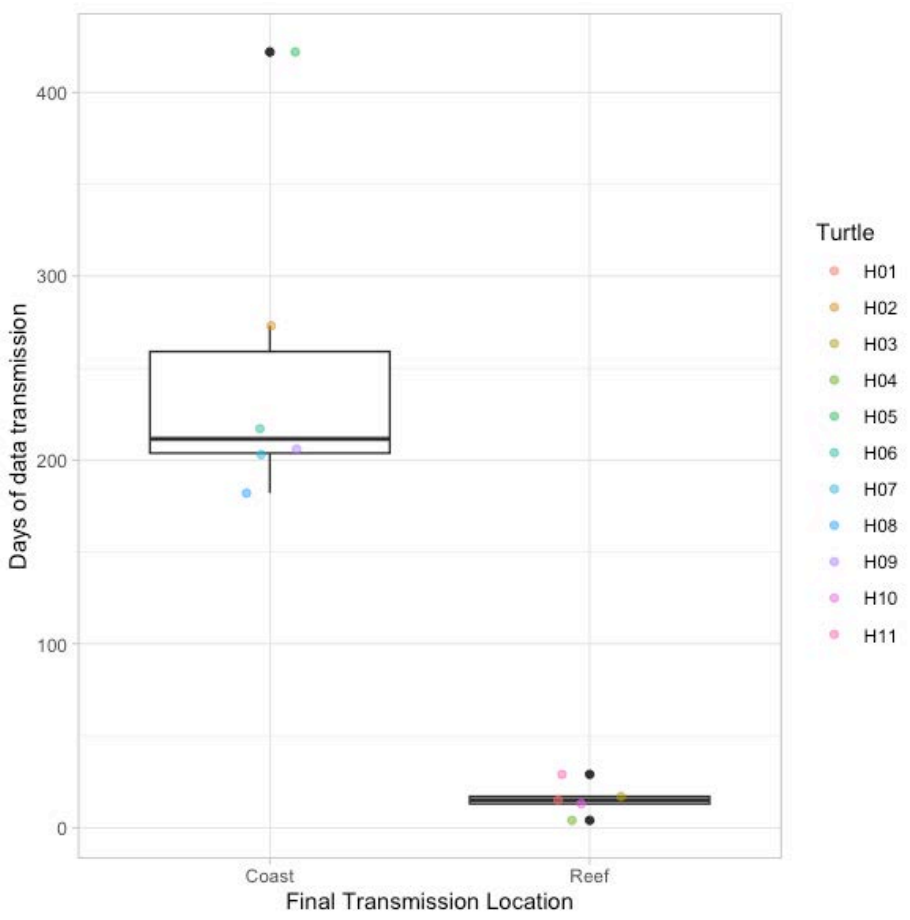



*Figure 7.3. Number of days of data transmission received from trackers fitted to 11 captive-raised juvenile hawksbill turtles (Eretmochelys imbricata; H01 – H11) in relation to their final transmission location. Coast: coastal; Reef: offshore in the Great Barrier Reef; black dots represent data outliers.*

## Discussion

This study showed that captive-raised juvenile hawksbill turtles can be satellite tracked for up to 422 days post-release, which is the longest published record. Previously released head-started hawksbill turtles were tracked for 64 days in Colombia (Pabón-Aldana et al., 2012) and 88 days in Japan (Okuyama et al., 2010). Reasons the satellite trackers typically stop transmitting data include drained battery, mortality of the turtles, tracker detachment, algal fouling over the sensors, and broken antenna (Hays et al., 2007). Having previously tested the attachment methodology used in this study (Chapter 4), trackers were unlikely to have detached in the first few months (Diggins et al., 2023). However, antenna breakage is a possibility because of hawksbill turtle preference for coral reef habitats and their use of coral ledges and rocks to rest under (Carrión-Cortez et al., 2013; Scales et al., 2011). Additionally, algal fouling over the sensors was likely given the propensity

to algivory found in Australian hawksbill turtles (Bell, 2013) and observed in the study turtles whilst at the research facility (RD personal observation).

Turtles in this study remained at the release site for a median of 10 days before dispersing (IQR 7.5 – 18 days). This result is comparable with other studies of satellite tracked head-started turtles (Klima & McVey, 1995; Okuyama et al., 2010). On dispersal, one of the captive-raised study turtles travelled a maximum distance of approximately 715 km from the release site, settling at an established foraging ground for immature hawksbill turtles in the southern Great Barrier Reef (Chaloupka & Limpus, 1997; Limpus, 1992b). This maximum distance travelled is less than the 1463,66 km travelled by a Colombian head-started hawksbill (Pabón-Aldana et al., 2012) but is within reported nesting migration distances of adult hawksbill turtles from the northern Great Barrier Reef (Miller et al., 1998) and in the Caribbean Sea (Maurer et al., 2024), as well as movement ranges of immature hawksbill turtles in the Caribbean Sea (46 – 900 km) (Meylan, 1999) and the Indian Ocean (Hays et al., 2021).

Although distance travelled was comparable with free-living hawksbill turtles, there were no wild-captured hawkbill turtles to release and track alongside the captive-raised turtles for comparison of dispersal direction. Furthermore, small sample size did not allow determination of a dominant dispersal pattern as turtles dispersed non-uniformly. However, one published study has compared dispersal of wild-captured and head-started hawksbill turtles and found the wild-captured turtles showed more directionality than the head-started turtles (Okuyama et al., 2010). Less directionality in head-started turtles could be problematic for survivability if turtles spend longer locating appropriate foraging grounds or do not have suitable habitats in which to shelter from predation. Dispersal studies for head-started turtles of other species also found variability in direction and use of inshore and offshore areas (Abalo-Morla et al., 2018; Klima & McVey, 1995). Notwithstanding, dispersal is likely to be species and life-stage specific (Bolten, 2003) and to be largely influenced by the region in which they inhabit, for example, based on spatial distribution of resources and presence or absence of currents (Abalo-Morla et al., 2018; Abalo-Morla et al., 2023; Levy et al., 2017). Therefore, more spatial studies of Australian, juvenile, foraging hawksbill turtles are required to determine whether the captive-raised turtles in this study dispersed as their free-living counterparts would have and what drives their dispersal patterns.

Limited access to turtles at different points in their life cycle has been overcome due to improved ability to capture different species at different life-stages, and technological advances providing smaller trackers for use on early life-stages (Diggins et al., 2023). However, there remains a bias in the literature toward satellite tracking of adult green, loggerhead, and leatherback turtles, which

should be corrected to gain a full understanding of the behaviour of all seven species across all life-stages (Hays & Hawkes, 2018). Furthermore, not all tracking data have been published in peer-reviewed journals (Godley et al., 2008), which is hindering the advancement of this field and the ability to translate data into conservation outcomes (Jeffers & Godley, 2016). Availability of data across species, regions, and life-stages would allow wider comparisons between populations, within populations, and among species to further determine how specific these dispersal behaviours are to each group. Finally, hawksbill turtles have been included in relatively few tracking studies, particularly at the juvenile stage, which limits our inference of how being raised in captivity may have affected the dispersal behaviour of these study turtles and other captive-raised hawksbill turtles generally.

### Conclusion

When released in an area known to be suitable for juvenile hawksbill turtles, 10 of 11 captive raised hawksbill turtles dispersed away from the release site. The 11 turtles in the study dispersed non-uniformly and transmitted data for 4 – 422 days. Although short transmission times may reflect tracker damage and do not necessarily signify mortality of released turtles, the continued transmission of one tracker for more than 14 months confirms that captive-raised turtles can survive for extended periods post release. This indicates that captive-raised hawksbill turtles have the capacity to survive in the wild (survivability), despite spending more than 2 years under human care in an artificial environment. Furthermore, the turtles were found to migrate similar distances to free-living juvenile hawksbill turtles in other regions and to disperse to habitats suitable for their species and size-class. This is a good indication that turtles were able to adapt to life in the wild after being raised under human care for conservation or research purposes.

## Summary of thesis and chapter aims

### Thesis aim 4

Determine whether juvenile hawksbill turtles display naturalistic behaviours after 2 years under human care.

#### Chapter aim

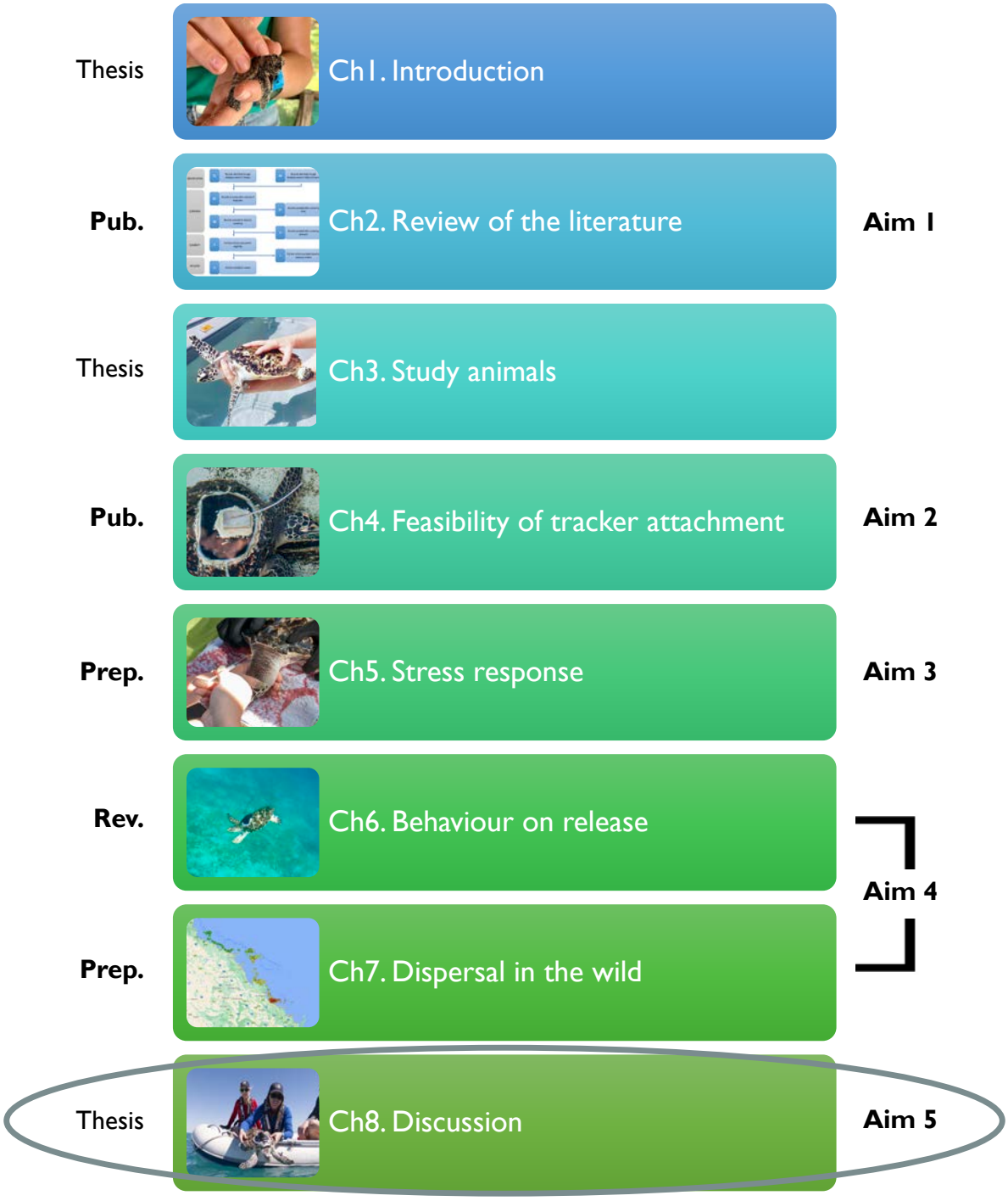
#### Chapter aim 1

- Document the dispersal of captive-raised juvenile hawksbill turtles on release using satellite telemetry.

The juvenile hawksbill turtles were released into an ecologically relevant reef lagoon in the Great Barrier Reef after being raised under human care for more than 2 years. All but one of the turtles dispersed away from the release site. Dispersal was non-uniform; some turtles migrated north, some south, and some stayed near the release site. Turtles transmitted data for up to 422 days, validating in-situ effectiveness of the attachment method and confirming that captive-raised turtles are able to adapt to life in the wild. Although it was not directly observed, the extended transmission from some tracked turtles infers that they were able to forage and feed in the wild. Turtles whose last transmission location was coastal transmitted for more days than those whose last location was offshore on the reef. Turtles migrated varying distances, similar to those documented for free-living conspecifics, and furthermore, the captive-raised turtles dispersed to areas that were species and life-stage appropriate. This showed that captive-raised turtles can survive post-release for a period of time and have positive outcomes when released.

# CHAPTER 8: DISCUSSION

## Thesis structure



# Juvenile hawksbill turtles (*Eretmochelys imbricata*) fare well with good welfare: evaluating welfare of captive-raised turtles to inform best practices of rearing and release

## Turtles under human care for conservation purposes

Sea turtle species are threatened with extinction, especially the hawksbill turtle (*Eretmochelys imbricata*), which is considered critically endangered on a global scale (Mortimer & Donnelly, 2008). Conservation strategies aim to increase recruitment into the population or increase survivorship and can be achieved by addressing key threats (direct take, by-catch, coastal development, pollution, climate change; see Chapter 1). Numerous conservation strategies exist, ranging from large-scale international agreements to small-scale local interventions (De la Cruz-González et al., 2018; Fernández-Llamazares et al., 2021). Some conservation strategies involving removal of individuals from the wild to be held under human care (ex-situ). These interventions do not directly mitigate threats at the population level, but they serve to increase survival of individuals who can then be returned to the population. Examples include, but are not limited to, hatcheries and head-start facilities that protect eggs and hatchlings from predation, poaching, and climate change-derived damage to nests (Blumenthal et al., 2021; Burke, 2015; Nasiri et al., 2023); and rehabilitation centres that care for turtles of all life-stages that are sick or injured often as a result of pollution and pathogens, boat strike, and cold stunning (Flint et al., 2017). Furthermore, researchers that study turtles ex-situ at different life-stages gain insight into their biology to inform conservation and management practices (Hall et al., 2018; March et al., 2019), and aquaria that permanently house animals to educate the public and gain community and financial support for conservation initiatives (Ballantyne & Packer, 2016). However, human interventions that modify natural lifecycles (ex-situ care, i.e. captive-raising programs) also present potential risks to the health and fitness of the turtles with implications for post-release survival (Mullin et al., 2023).

The general goal of conservation strategies that temporarily hold turtles under human care is to keep turtles alive and healthy long enough that they can be released into the wild in an improved state of fitness (e.g. healthier or larger for reduced chance of predation and reach maturation). This is theoretically achieved by protection from predation and provision of suitable husbandry conditions, nutrition and environmental stimulation (Mullin et al., 2023). For the North Queensland management unit of hawksbill turtles, terrestrial predation has been noted as one of the biggest threats to the population (Department of Environment and Science, 2021; Hamann et

al., 2021). In the wild, hatchling mortality on the run from nest to ocean has been recorded at 7.6% in Florida loggerhead turtles (Erb & Wyneken, 2019) and up to 75% in loggerheads on Boa Vista Island (Martins et al., 2021a). Furthermore, 6.9% mortality of hawksbill turtles was reported in Barbados during their initial swim from shore (Harewood & Horrocks, 2008). Thus, protection from predation during this early stage, such as in a head-start program, has the potential to increase recruitment into the population.

However, despite provision of food and protection from predators, captive-rearing does not necessarily result in 100% of turtles surviving to release (Orós et al., 2020). Turtles studied for this thesis research were collected as hatchlings, captive-raised, and subsequently released into the wild; similarly to those in a head-start program. Of the 12 hawksbill turtle hatchlings collected from the wild, 11 were raised at the JCU research facility to the point of release. The twelfth hawksbill turtle from this study cohort was euthanised for ethical reasons due to failure to thrive from undetermined cause (Chapter 3). Mortality has been reported from a few head-start and head-start type research programs (Table 8.1). Although cause of death is sometimes inconclusive and not always investigated, often bacterial and fungal infection are suspected alongside lesions caused by cohabitation of the turtles (Orós et al., 2020). Lesions and infections were also found during necropsy of hatchling hawksbill turtles under rehabilitative care (Rodríguez et al., 2023). This further highlights the importance of suitable housing and husbandry practices during captive rearing to accommodate the environmental and behavioural needs of the turtles and to ensure their welfare and survival (Phillott, 2023; Tetzlaff et al., 2019a).

*Table 8.1. Reports of mortality for sea turtles whilst being raised at research and/or head-start facilities.*

| **Study** | **Species** | **Year** | **Mortality %** (Proportion included where available) | **Cause** |
|---|---|---|---|---|
| This study | Hawksbill | 2019 to 2021 | 8.3% (1/12) | Undetermined failure to thrive: euthanised |
| Orós et al. (2020) | Loggerhead (*Caretta caretta*) | 2008 to 2009 | 88.5% (100/113) | Suspected low water temperatures, lesions from biting and subsequent infection |
| Miller et al. (2009) | Leatherback (*Dermochelys coriacea*) | 2005<br>2006<br>2007 | 15.6% (5/32)<br>22.2% (10/45)<br>75.0% (30/40) | Suspected mixed bacterial infections (muscle degeneration also detected) |
| Robertson and Cannon (1997) | Loggerhead<br>Kemp's ridley (*Lepidochelys kempii*) | 1984 to 1996 | 8.8%<br>5.8% | Infection: *Salmonella* spp., *Aeromonas* spp., and *Pseudomonas* spp. |

## Fitness, welfare, and survivability

Increasing survival to the point of release is only the first step for ex-situ conservation. Such strategies can only be effective if the captive turtles are fit for release, i.e. they have equal post-release survivability compared with their free-living conspecifics (Mullin et al., 2023). "Survival of the fittest" is a well-established concept that highlights the importance of fitness for survival. Therefore, if fitness of the sea turtles is diminished whilst under human care, it follows that their ability to survive when released into the wild might be jeopardised, which highlights the importance of monitoring and measuring fitness to the point of release. As discussed in Chapter 1, biological fitness typically refers to reproductive potential, which is necessary to sustain the population. However, in long-lived, migratory species such as sea turtles, it would be largely unfeasible for conservation and research groups to measure this type of fitness unless the initiative had been running for several decades (Bell et al., 2005; Blumenthal et al., 2021; Shaver & Rubio, 2008). Furthermore, this measure gives no insight into the fitness of the turtles at the point of release and their potential to survive following release. There are several measures of physical fitness that relate to survivability, such as good musculoskeletal and cardiovascular health; however, survival requires more than just physical health (Deem & Harris, 2017). Post-release survival requires physiological capability and behavioural traits that could be assessed more holistically using the Five Domains Model of welfare (Mellor, 2017), which encompasses physical health, nutrition, environmental health, behaviour, and mental state. Methods to assess welfare under each domain could then be used to determine whether a turtle is fit for release and to infer fitness of the turtles in relation to their potential ability to survive following release (survivability).

By removing animals from the wild, a degree of positive welfare is already achieved by temporarily reducing major threats to survival such as predation, starvation, storms, marine pollution, direct take, and bycatch that they can experience in the wild. However, keeping wild animals under human care also has the potential to reduce their overall fitness and ultimately their post-release survivability if turtles are not appropriately reared and prepared for release (Orós et al., 2020; Tomillo et al., 2021). For strategies involving temporary ex-situ care of turtles, reduced post-release survivability would mean conservation aims have not been met. Therefore, conservation and research organisations temporarily keeping wild animals have an obligation to promote positive welfare for those animals (Englefield et al., 2019), including minimising captivity impacts on their post-release survivability. Unfortunately, comparison of head-started turtle survivability with free-living conspecifics is hindered by the paucity of data surrounding survivorship of free-living individuals (Mullin et al., 2023). However, despite this lack of comparative data, it remains

critical to assess post-release survivability of head-started turtles to address key criticisms of head-start type programs. Namely, unknown effects of captive rearing, including: risk of injury and disease through competition and poor housing systems or water quality (Orós et al., 2020); possibility of altered physiological state such as changes to growth rate, muscle development or stress response (Miller et al., 2009; Usategui-Martín et al., 2021); and likelihood of unfavourable behavioural changes including habituation to humans, inability to interact naturally with their environment once released (i.e. sheltering and foraging) (Tetzlaff et al., 2018), and difficulties with post-release navigation to suitable foraging grounds and, if they survive to adulthood, to suitable nesting grounds (Abalo-Morla et al., 2018). Depending on the length of time under human care and their life-stage, these concerns may also apply to turtles in rehabilitation, research, aquaria, farms, and captive breeding settings.

Although the Precautionary Principle precludes conservation actions with unknown consequences (Kriebel et al., 2001), the severity of population declines arguably calls for an adaptive management protocol based on best available scientific evidence (Bennett, 2016). Therefore, it is imperative that these knowledge gaps be addressed. For decades there has been much debate reviewing the role of head-starts in conservation (Allen, 1992; Burke, 2015; Frazer, 1992; Mullin et al., 2023; Phillott, 2023; Seigel & Dodd Jr, 2000; Tomillo et al., 2021; Woody, 1990). However, relatively few empirical experimental studies have been conducted to test these concerns and better understand the impact of captive rearing on sea turtles. Research has been conducted on post-release survivorship of head-started freshwater turtles (Mullin et al., 2020; Mullin et al., 2023) and loggerhead turtles (Abalo-Morla et al., 2018); welfare of farmed green turtles (Chelonia mydas) (Arena et al., 2014); nesting of head-started green and Kemp's ridley turtles (Bell et al., 2005; Blumenthal et al., 2021; Shaver, Lamont, et al., 2016; Shaver, Rubio, et al., 2016); stress of sea turtles (Usategui-Martín et al., 2021); and growth (Sarmiento-Devia et al., 2018). Much research has been conducted on behaviour of freshwater turtles whilst under human care (Carlson & Tetzlaff, 2020; Tetzlaff et al., 2018, 2019b; Tetzlaff et al., 2019c; Tetzlaff et al., 2019a) but little for captive sea turtle behaviour (Kawazu et al., 2022; Usategui Martín, 2020). However, a few studies have recorded post-release behaviour of captive raised sea turtles with regards to feeding, swimming behaviour, and dispersal (Abalo-Morla et al., 2018; Nagelkerken et al., 2003; Okuyama et al., 2010; Shaver & Rubio, 2008). Only four studies have pertained to head-started hawksbill turtles; two studies (Sarmiento-Devia et al., 2018; Whitman, 2009) were based in the Caribbean (Colombia and Nevis) and two in Japan (Kawazu et al., 2022; Okuyama et al., 2010) but none in Australia.

Consequently, there are still many knowledge gaps regarding captive rearing of early life-stage sea turtles for how it may affect their fitness, and subsequently their post-release survivability. This is partially due to the cryptic nature of sea turtles at this stage in their life cycle, often referred to as the 'lost years', as post-hatchling turtles may migrate for many years via offshore oceanic currents and not be readily observed and studied without specialist equipment (Mansfield et al., 2021). Hence, limited scientific literature exists detailing their habits and habitat requirements, which are often species, life-stage, and spatio-temporally dependent (Mansfield et al., 2017). It may also be partially due to lack of available resources and scientific training in conservation settings as well as an absence of standardised research protocols. One way to explore these requirements in a measurable and consistent way for research purposes, could be to hold the young animals in a captive environment (Hall et al., 2018; Miller et al., 2009). However, owing to the level of protection often given to these species as a result of their threatened status, it is difficult to conduct research on a sufficient number of individuals to reach statistically sound conclusions. Hence, there is a need to develop protocols that allow collection of data in a simple and affordable manner to ensure collection of quality data that can then be replicated and compared between facilities. This thesis is concerned with addressing these gaps by answering the following key questions, identified in Chapter 1: 1) How do we determine whether a turtle is fit to be released; and 2) How do we infer survivability following release?

## Situating the thesis findings

### Rearing: welfare under human care (readiness for release)

In summary, question 1 was addressed by assessing the welfare of the captive-raised study turtles under the Five Domains Model (Mellor, 2017) using appropriate methods identified in Chapter 2 (Diggins et al., 2022). The welfare assessment used in this thesis was tailored to juvenile hawksbill turtles and outcomes were compared with wild conspecifics where appropriate (Chapter 3). All assessment methods used could be applied in other similar research or conservation settings. The only exception to this was the additional blood sampling for stress response analyses (Chapter 5), which may be beyond the capacity of some smaller community-based head-start conservation initiatives. Blood sampling experimentation was included in this thesis to provide extra depth to the welfare assessment, and also to understand how the welfare of the turtles might be affected by attachment of a satellite tracker (Chapter 4), which is a common method of assessing sea turtle behaviour and movement in the wild. The attachment methodology itself was also tailored specifically to small juvenile hawksbill turtles (Diggins et al., 2023).

Firstly, methods for assessing welfare of turtles undergoing rehabilitation were reviewed in Chapter 2 following the Five Domains Model (Mellor, 2017). The review was targeted at rehabilitation studies due to the emphasis on health and the link between health, fitness, welfare, and survivability. The review revealed that there are several methods to assess health under each of the five welfare domains. These methods were subsequently tailored specifically to juvenile hawksbill turtles (see Chapter 3). In particular, visual and behavioural observations can be used to infer several health aspects relatively cheaply and without specialist skills and equipment. Additionally, these types of observations potentially carry less risk of inducing high stress response in the animals during assessment compared with invasive physical examinations such as blood or buccal sample collection (Diggins et al., 2022). Therefore, behaviour should be incorporated into welfare assessment including potential use of environmental enrichment devices (EEDs) to support naturalistic behaviours. These methods of welfare assessment can also be applied in a head-start or research setting.

Previous research showed that aggressive behaviours observed in cohabiting, head-started hawksbill turtles were reduced by the introduction of rocks as EEDs (Kawazu et al., 2022), although this intervention did not affect corticosterone concentrations, an indicator of physiological stress. Additionally, naturalistic habitats (e.g. burrowing and hiding structures) were found by Tetzlaff et al. (2018) to be preferred by juvenile box turtles (Terrapene Carolina). However, use of EEDs was found to delay growth (Tetzlaff et al., 2019c) and be less important for post-release success than time spent in captivity (Tetzlaff et al., 2019a). These are just a few behaviours relevant to welfare of captive-raised turtles. Further research is needed to clearly determine and define the best behavioural measures of welfare to account for the large individual variation observed in reptiles (Benn et al., 2019; Burghardt, 2013; Lambert et al., 2019; Moszuti et al., 2017). Improving the welfare of the turtles whilst under human care will ultimately aid in improving conservation outcomes by improving post-release survivability (Escobedo-Bonilla et al., 2022; Michaels et al., 2014), hence the emergence of conservation welfare as a developing discipline (Beausoleil et al., 2018). It is also important to note that positive welfare may look different for turtles remaining permanently under human care (i.e. in aquaria) compared with those due to be released to the wild (Diggins et al., 2022). For example, turtles in aquaria would benefit from habituation to human-turtle interactions to reduce stress induced by health checks and visitor presence. Turtles intended for release into the wild, however, require disassociation from humans. Maintaining this disassociation is necessary for post-release survival so the turtles will not approach humans or boats for food (Hayes et al., 2017; Wright et al., 2020). Such behaviours could result in incidental capture from fishing gear and/or injury from boat strike.

Furthermore, one key concern of keeping animals under human care is that their stress response may be reduced from either too much stress (poor housing and husbandry) or too little (absence of predators and habituation to humans) (Johnstone et al., 2012). Long-term exposure to stressors has the potential to cause chronic stress and should preferably be tested for in captive settings (Fischer & Romero, 2019). The hawksbill turtles studied throughout this thesis were not observed displaying stereotypic behaviours indicative of chronic stress (Diggins et al., 2023); however, this was not tested for physiologically. Testing of additional blood parameters was beyond the scope of this research but could have allowed the development of a summarised health index (Li et al., 2015) or allostatic load index (Seeley et al., 2022). An allostatic load index, which is usually determined from a combination of different biochemical and haematological parameters, tracks the general 'wear and tear' of an individual over time. "Wear and tear" is the degradation of welfare for an individual subjected to several cumulative stressors, resulting in a new homeostasis, i.e. allostasis (Korte et al., 2007). Both indices could be used to monitor long-term stress-related health and welfare (Edes et al., 2018), with implications for survivability if index values drop over time from inability to fully recover from stressors.

The ability to respond physiologically and behaviourally to an acute (short-term) stressor is necessary for survival, for example it enables prey animals like turtles to avoid predation (Boonstra, 2013; Preston et al., 2020). The physiological and behavioural stress responses of reptiles have been found to be independent and not necessarily correlated as expected (Preston et al., 2020). In some conservation settings, physiological stress testing may not be feasible due to funding, skills, and equipment requirements, nor advisable if it risks causing additional stress to the turtle pre-release (Diggins et al., 2022). However, it is still an important aspect of physical welfare of captive-raised sea turtles, and a research area in need of development. Therefore, in Chapter 5, the systemic stress response of juvenile hawksbill turtles was assessed by measuring concentrations of corticosterone and lactate in their blood before, during, and after stress events. Corticosterone has been commonly used to assess stress in captive and free-living animals, including sea turtles, with time to peak concentration potentially different among species (Jessop et al., 2004a; Usategui-Martín et al., 2021). Variation may be due to different times of day, seasons, and stages in the life-cycle, and between sick and healthy individuals (Flower et al., 2015). This further highlights the need to study each species at each life-stage and consider temporal effects when analysing the data (Gormally & Romero, 2020). Lactate was also studied as it plays an important role in reptile stress response and metabolism, though it is less commonly studied (Molinaro et al., 2022).

For the captive-raised juvenile hawksbill turtles studied in this thesis, the basal range upper limit of corticosterone was lower than their free-living counterparts (Jessop et al., 2004a). Basal range of lactate concentrations were also low but there are limited data of free-living hawksbill turtles available for comparison (Muñoz-Pérez et al., 2017). Although comparison data are limited for corticosterone and lactate response to stressors in small, juvenile hawksbill turtles, these captive-raised turtles did show an ability to respond to both known (human presence) and unknown (acute stressor and trial tracker attachment) stressors. The turtles were also observed returning to within basal ranges during the trials, suggesting that the turtles were able to regulate their stress responses. This is a requirement in the wild where a good and immediate response to stress can mean survival, while a return to baseline is also important for long-term health (Cann et al., 2021).

Whilst it may not be advisable to record the physiological stress levels of every sea turtle pre-release, general knowledge of how captive-raised turtles respond to satellite tracker attachment and dry-docking (for adhesive drying and transportation purposes) is useful for informing future conservation and research protocols. However, for this thesis it was first necessary to determine a suitable attachment method. The challenges of tracker attachment to small juvenile hawksbill carapaces and the approach used in this study to mitigate these challenges have been presented in Chapter 4. Briefly, morphological variations of the carapace call for specifically adapted methodologies to be applied for suitable attachment of the trackers (Diggins et al., 2023; Mansfield et al., 2012; Seney et al., 2010). The novel methodology tested for this thesis was developed for small, juvenile hawksbill turtles that have high growth rates and whose carapaces are convex, ridged, and imbricated. Determination of the success of tracker attachment methods to any life-stage of any species should consider not only the level of adhesion but also welfare of the turtles (Diggins et al., 2023).

The approach developed in this thesis to address this knowledge gap was an adaptation of the Seney et al. (2010) protocol for small, juvenile loggerhead turtles, involving additional application of silicone between scutes. In this study, welfare of the turtles did not appear to be compromised, as confirmed by an assessment of morphometric data collection over time, visual observations of behaviour, and physical examination of the attachment site following trial tracker removal. The turtles' carapaces remained undamaged from the tracker attachment and detachment process and their scutes were not misshapen by the end of the trial. Morphometric analysis showed no changes to the growth trajectory of the turtles in weight or curved carapace length as a result of the trial trackers being attached. Neither were there any observable changes in their daily appetite and

defecation, nor displays of unusual behaviours indicative of stress (Diggins et al., 2022) after an initial adjustment period. Therefore, the optimised tracker attachment method and welfare assessments applied here were demonstrably effective and suitable for use by conservation or research organisations wanting to track small, juvenile hawksbill turtles post-release. Despite not observing any behavioural stress indicators throughout the trial period, an investigation into this welfare indicator is warranted, particularly since behavioural changes caused by presence of the tracker might lead to unusual post-release behaviour. Other aspects of turtle welfare such as physical health, ability to feed, and mobility appeared to be unaffected by presence of the tracker (EA, RD personal observation), which is a good indication of no reduced post-release survivability in those areas.

## Release: welfare in the wild (indicators of survivability)

Question 2, inferring survivability post release, was addressed by looking for indications that the turtles had traits necessary to survive, ie. evidence/indicators of post-release survivability. A comparison was then made, where possible, between the traits and behaviours observed for the captive-raised turtles following release and those recorded for free-living juvenile hawksbill turtles. In-water observation during the release event (Chapter 6) and post-release tracking of the turtles (Chapter 7) provided additional data about the effects of captive rearing on sea turtle survivability.

The hawksbill turtles studied in this thesis were deemed ready for release after assessing their welfare holistically, following the Five Domains Model (Mellor, 2017) as a framework and including their physiological stress response. Feasible options for assessing post-release survivability-related welfare included recorded visual observation of behaviours immediately after release and use of satellite telemetry to infer short-term dispersal and migration behaviours. Diversity of behaviours in captive animals has been linked to positive welfare (Miller et al., 2020). Construction of an ethogram was based on direct observation of the captive-raised turtles at the time of release (Liu et al., 2009). The ethogram was then used to assess whether the captive-raised turtles displayed desired naturalistic behaviours, i.e. behaviours that are key to survival and routinely observed in free-living juvenile hawksbill turtles. Such behaviours include swimming, resting, surfacing to breathe, grooming, investigating, and feeding (Proietti et al., 2012). All but one of the abovementioned naturalistic behaviours were observed in the group of captive-raised hawksbill turtles, despite a limited observation time (Chapter 6). The behaviour not observed was feeding; however, the turtles had been observed grazing on algae in their tanks (Bell, 2013) and several of the turtles were tracked for many months, inferring they had the ability to forage and feed in the wild (Chapter 7).

Approximately half of the turtles swam off quickly within the first 2 minutes of release and the other half swam around slowly within the lagoon and were monitored for more than 20 minutes. Individual variation in turtle behaviour and personality has been previously documented and their "boldness" and "shyness" has been studied (Allard et al., 2019; Carlson & Tetzlaff, 2020; Waters et al., 2017). The escape behaviour of the short-observed turtles ("shy") could also be interpreted as a stress response since it involved increased activity away from stressors (divers and boats). Furthermore, evasive manoeuvres were also observed during the release event whereby turtles turned and flashed their carapace as free-living turtles do to avoid predation (Asada et al., 2021). Overall, this period of observation during the release event gave a snapshot indication that the turtles had the ability to perform natural behaviours necessary for survival in the wild, including predator evasion, despite being raised in captivity for more than 2 years. This is an important finding that adds to the debate on possible negative effects of head-starting turtles since innate behaviours in the study turtles were maintained throughout an extended time in captivity. Many head-starting facilities have a shorter rearing time and, as such, those turtles might also be expected to have maintained innate behaviours key for post-release survivability. Other studies of head-started turtles have also concluded that shorter time in captivity confers greater post-release survivability to the turtles and is better in terms of the effort to yield ratio (Mullin et al., 2023).

A slightly longer indication of the survival and survivability of the study turtles in the wild was assessed using satellite telemetry to track their dispersal and migration after release (Chapter 7). It was unknown whether the turtles would remain at the release site, a suitable habitat for hawksbill turtles, or whether they would migrate away. Unsurprisingly, the turtles did not behave in a uniform manner; most travelled in different directions and one spent almost it's entire transmission time at the release site. Again, there are a lack of data of free-living counterparts with which to compare the dispersal behaviour of the captive raised hawksbill turtles (Okuyama et al., 2010). However, the study turtles did remain in areas known to be inhabited by and environmentally suitable for juvenile hawksbill turtles (Limpus, 1992b). Furthermore, the longest transmission lasted 422 days, which is the longest recorded transmission for captive-raised hawksbill turtles. Although it is difficult to determine the reason for transmission ending (including battery, antenna, and sensor failure), it is likely that at least some were predated. However, annual survivorship of sea turtles is lower in their early years and increases as they outgrow the target size of many predators and become more experienced at foraging and evading predation (Tetzlaff et al., 2019a). Therefore, it is a more significant observation that captive-raised turtles had the ability to survive for several months post-release than to postulate if any will survive to adulthood.

Long-term assessment of captive-raised turtles was not feasible for this thesis. However, decadal monitoring of head-started green and Kemp's ridley turtles has documented head-started turtles surviving to adulthood and successfully nesting (Bell et al., 2005; Shaver, 2007; Shaver et al., 2016). Furthermore, satellite tracked head-started Kemp's ridley turtles displayed similar inter-nesting movements to wild turtles (Shaver & Rubio, 2008), thereby disputing the argument that head-started turtles would not be able to exhibit natural behaviour. The findings from this thesis confirms the ability of captive-raised turtles to display naturalistic behaviours upon release into the ocean with inference of longer-term survivability.

## Limitations and future research

This thesis did not directly test whether welfare is a good proxy for survivability, rather it used the framework to assess overall health and wellbeing. However, future research could seek to quantify each parameter and test them with repeatability (Bell et al., 2009; Kamel & Mrosovsky, 2005) and rank analysis (Davy et al., 2014) to determine which specific metrics are statistically most sound for inferring post-release related survivability. Furthermore, future development of a standardised scoresheet and assessment protocol, adapted to each species and life-stage (Diggins et al., 2022), could enable wider comparison amongst organisations. Implementation of a standardised scoresheet across multiple conservation or research facilities would help account for any issues with small sample size, as experienced throughout this thesis. Widespread use of such a scoresheet would enable quick assembly of a larger database to better understand and enhance welfare for turtles under human care and, therefore, improve conservation outcomes. Post-release conservation outcomes could be further enhanced through a better understanding of the welfare status of free-living turtles (Beaulieu, 2024; Hecht, 2021). Welfare of free-living animals is an evolving topic within the conservation welfare discipline (Harvey et al., 2020) and has not yet been scored for sea turtles, but must be addressed in the future.

Specific limitations and future direction are detailed within each data chapter (Chapters 2 and 4 – 7). In general, conservation strategies that temporarily hold turtles under human care (e.g. rehabilitation and head-start facilities) can be contentious because the ratio of effort to yield may not be favourable (Phillott, 2023). Many resources are required for these interventions with potentially limited output, particularly for head-starts which target a life-stage with low elasticity (Heppell et al., 1996; Heppell et al., 1999). This was found to be the case in an elasticity analysis of the European pond turtle (*Emys orbicularis*), which found that protection of adult turtles has greater effect on population size (Mitrus, 2008). Elasticity analysis of North Queensland hawksbill turtles was beyond the scope of this thesis but would add valuable insight. Regardless of elasticity,

the mitigation efforts needed to address the threats targeting later life-stages require legislative efforts, large budgets, and are generally slow to initiate, for example implementation of fishing restrictions or gear alterations (Chapter 1). It is, therefore, more feasible to concentrate efforts locally, at the nesting and early life-stages. Although high predation in early life-stages is expected for sea turtles, unnaturally high mortality of embryos and hatchlings is unsustainable for the population and so is a key concern for researchers and conservation practitioners (Fuentes et al., 2023). As such, conservation strategies aimed at the early life-stages may still benefit the population (Donlan et al., 2010; Smithers & Dawson, 2023). Feasibility of head-start type conservation strategies in terms of cost to benefit ratio requires more research. Determination of the required number of eggs collected and turtles released to affect population dynamics will be specific to species and region as it will vary based on the specific ecology and threats of each population. The incorporation of threats to the population is particularly important because head-starting does not mitigate threats encountered when turtles are released into the ocean (Mullin et al., 2020).

A common theme expressed throughout this thesis is the paucity of comparable data, which hinders the ability to interpret results in the conservation setting. This is due in part to the cryptic nature of some life-stages and the small population sizes available to sample. However, more effort must be made to fill these knowledge gaps, particularly for comparison between captive and free-living individuals. This will assist with adapting management strategies but also aid in improving our understanding of welfare and how it can be optimised for animals under human care, depending on their species, region, and life-stage, as well as the context of their captivity.

## Promoting positive survivability-related welfare: informing best practice

Differences exist in sea turtle biology and ecology amongst species and populations across ontogenetic stages. Some examples include somatic growth rate, size at each life-stage, location of foraging grounds, diet, and migration behaviours (Hamann et al., 2021). Understanding the needs of individuals kept or raised under human care in relation to their biology and ecology will inform and allow for a more specific care plan (housing and husbandry requirements), likely resulting in better pre-release welfare (Diggins et al., 2022) and increased post-release survivability. The outcomes of this thesis research form the basis for recommendations to inform any changes to housing, husbandry, and welfare that may assist conservationists and researchers to ensure better welfare for future cohorts of turtles, and therefore better survivability on release (Allard et al., 2019). Furthermore, head-start and rehabilitation programs are often community

driven so recommendations should be as widely applicable across research and conservation settings as possible.

Welfare and survivability post-release into the wild can be enhanced whilst under human care through provision of species and size-class appropriate housing and care, including suitable nutrition, environmental enrichment, and opportunity to exhibit desired naturalistic behaviours (Wood, 2022). Species and size-appropriate housing conditions and husbandry protocols are required to reduce likelihood of stress attenuation from prolonged exposure to poor welfare, which can result in a reduced capacity to respond appropriately to post-release stressors such as for predatory evasion. EEDs should also be introduced to encourage foraging, investigation, resting, and grooming where possible (Diggins et al., 2022). Foraging and investigation of the hawksbill turtles studied in this thesis could have been further encouraged through the use of feeding-based environmental enrichment devices designed to simulate algae and sponges on the bottom of the tank (Diggins et al., 2022; Kanghae et al., 2021; Whilde et al., 2021). Increased use of such devices in the weeks or months pre-release could help encourage this natural feeding style and reduce potential food-dependency on humans. Additional stimulation for the turtles could be potentially provided through social and visual enrichment via semi cohabitation (Gaos et al., 2021). In the JCU research facility, turtles were housed individually but within view of another turtle (i.e. semi cohabitation). This prevented injury risk and stress caused by aggressive interactions as has been noted in other cohabiting sea turtles (Kawazu et al., 2022; Usategui-Martín et al., 2021).

It is imperative that turtles kept temporarily under human care retain adequate stress response and recovery mechanisms required to survive in the wild (Usategui Martín, 2020). This might be achieved through short but regular handling for some acclimation to husbandry, and periodic exposure to novel stressors such as unfamiliar sights, sounds, and smells to prevent under-stimulation of their stress response systems (Tetzlaff et al., 2019b). To reduce the effects of extended stress from satellite tracker attachment, turtles should be kept as calm as possible and prevented from being able to cause any injury to themselves (i.e. restrict flipper flapping) during and after attachment. Keeping the turtles in a small, enclosed area with a padded floor and appropriate heat and hydration should allow them to regulate their stress responses and return to basal range of stress hormones whilst the epoxy dries. For transportation, again providing comfort and keeping them safe and calm (as during tracker attachment) will assist with reducing and regulating the stress response during the extended stress period. Releasing the turtles as close to the holding facility as practically possible may also be advisable to reduce transportation-induced stress prior to release.

Furthermore, turtles should be released in an environment that is ecologically and biologically relevant to their species and life-stage, which should be considered when planning for collection, housing, and release protocols. Soft release is one suggested possibility to reduce stress and ease transition from captivity to the wild via the use of a pool (Hunt et al., 2019) or enclosed lagoonal area prior to release to the wild (Whitman, 2009). This could theoretically allow the turtles to acclimatise to their new surroundings whilst safe from predators. Acclimatisation in a secure lagoon may potentially benefit the turtles overall and would allow for additional behavioural observation. However, feasibly this may be difficult to set-up and manage for sea turtles. Furthermore, soft-release was tested for head-started Blanding's turtles (*Emydoidea blandingii*) and found to have no effect on survival or growth rate (Wijewardena et al., 2023).

In general, another argument against use of head-starting practices is the interference with nature. Artificially incubating nests risks manipulation of the sex ratio within each clutch, resulting in production of too many males, which has negative effects on the population decades later when turtles reach maturity (Phillott, 2023). Additionally, ex-situ protection of nests and the removal of eggs and hatchlings from the ecosystem risks disrupting the food chain and other ecosystem processes (Fuentes et al., 2023). Although this was not directly tested for in this thesis, turtles were only collected after emergence and crawling down the beach (prior to entering the water), which meant there was no risk of artificial temperature manipulation or removal of excess nutrients from the nest. However, programs conducting ex-situ nest care should seek to prevent excessive shading or watering of nests (unless temperatures are above thermal tolerance of the embryos) (Tomillo et al., 2021), and remnants of eggshells and any undeveloped or deceased hatchlings should be buried back in the beach from which they were collected. Further research is warranted regarding the possible consequences of removing turtle eggs and hatchlings from the ecosystem.

## Conclusions

Head-start and rehabilitation programs are not a panacea for the recovery of endangered sea turtle populations; however, they can still play a role in conservation. Furthermore, whilst turtles are being held and raised under human care it is important to assess and promote positive welfare and to ensure best practice through adaptive management and evidence-based protocols. The Five Domains Model of welfare was used as a framework to assess readiness for release and likely survivability of captive-raised hawksbill turtles. Following this model, turtle fitness was holistically assessed in relation to their physical health, nutrition, environmental health, behaviour, and mental wellbeing. The intention was to use this holistic approach to ensure the best welfare for the turtles

whilst under human care and during release to increase post-release survivability. The hawksbill turtles were assessed as having good welfare at the point of release and had demonstrated behaviours, skills, and physiological responses required to survive post-release. At the release event, they were documented exhibiting desired naturalistic behaviours, and subsequent satellite tracking showed survival of at least several months in many turtles.

Implementation of enhanced protocols will benefit future cohorts of captive-raised juvenile hawksbill turtles through improved welfare and post-release survivability. Sea turtles raised temporarily under human care should receive the best husbandry, housing, and care possible so that they have optimal welfare before release and the highest chance of survival after release. Welfare assessments should also be tailored to the species, population, and life-stage of the individual as well as being feasible to conduct in conservation settings for replicability. The findings from this thesis provide data-driven practice recommendations for the husbandry and release of small juvenile hawksbill turtles, which are globally critically endangered. These recommendations can also be applied by community-based conservation groups to improve the outcome of their interventions for the benefit of their population and the species overall.

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