A SIMULATION-BASED POST-RELEASE SURVIVAL STUDY FOR BROWN-BANDED BAMBOO SHARKS (*CHILOSCYLLUM PUNCTATUM*) OF THE GULF OF THAILAND.

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A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**Declaration**

I hereby declare this thesis contains no material which has been accepted for a degree or diploma by the Australian Maritime College or any other educational institution and that, to the best of my knowledge this thesis contains no material previously published or written by another person, except where due acknowledgement is made.

Signature

Date
Abstract

Current fishing practices and habitat degradation in most of the world’s oceans pose significant threats to marine fish, especially for sharks which are very susceptible to overfishing due to their $K$-selected (i.e. slow growth and reproduction) characteristics. Sharks are incidentally taken in longlines and other commercial fisheries, resulting in high percentage of caught individuals being discarded (i.e. returned to the ocean either dead or alive). However, little is known about the fate of the released animals. Quantifying post-release survival (PRS) of discarded species is therefore essential to improved management and conservation of these taxa.

There are different approaches to assess PRS (i.e. tagging, on-board observer’s data, etc.). One of the most common approaches to evaluate survival is containment studies (i.e. tanks or cages where individuals are held during a certain period of time and survival rates are estimated). However, and despite the facility of conceiving these experiments in terms of logistics, there is a lack of tools for explicit evaluation of alternative designs.

The present study will use simulations to define efficient (logistics and number of individuals) experimental designs in paired-design (i.e. control and treatment fish in the same tank) containment studies across a range of scenarios defined by sample size (i.e. number of fish per container) and replicates (i.e. number of containers). Also, the effect of condition (i.e. handling) on post-release survival rates for the example of brownbanded bamboo sharks ($Chiloscyllium punctatum$) in the Gulf of Thailand will be assessed using survival analysis based on accurate experimental design of containment studies.

Obtained results showed that proper experimental designs of containment studies and strong survival assessments were dependent upon the control survival and the variability of treatment mortality rates. Therefore, pilot studies should be established to account for a priori survival rates in post-release survival experiments. This is especially important for bottom-
dwelling sharks (i.e. brownbanded bamboo sharks) because PRS for the majority of these species is scarce or null. In addition, precise assessments of survival were obtained for experimental designs with large number of fish (i.e. 360 individuals). Thus, a considerable number of individual is needed for estimations of mortality in containment experiments. These results give a justification for the Animal Ethics Committee concerns about the number of individuals that would have been used for first proposed experiment justifying that large number of specimens is needed to precisely assess survival rates when the target species presents low mortality rates (treatment mortality=40% was assumed to represent mortality of brownbanded bamboo sharks during the simulations).

Assessments of survival with simulated data suggested that condition may affect survival of individuals. However, further research is needed in order to probe these results and take into account other factors (i.e. environmental factors, technical factors, etc.) that may also influence post-release survival of discarded sharks.
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1. Introduction

In the next section, the negative effects of fishing on non-target species with specially emphasis on bycatch and discards, are briefly described. In addition, cryptic fishing mortality and other unaccounted fishing mortalities such as post-release fishing mortality are defined. Negative effects of fishing are also described in relation to shark population’s conservation status worldwide. The volume of shark’s fishing mortality (accounted + unaccounted) is presented along with the consequences that these activities present for this species due to their $K$-selected characteristics. Despite the scarce and poor information available for shark’s population in Thailand, a summary of the situation of these species is presented.

Post-release fishing mortality is described in depth to allow the reader to familiarize with the scope and methodology of the present study. A review of the available methodologies to conduct post-release studies (with special focus on sharks) is also presented. Unfortunately, post-release studies for sharks are very limited and there is no research of this nature available for my studied shark, the brownbanded bamboo shark.
1.1 Negative effects of fishing on non-target species

Bycatch, the incidental fishing mortality of non-target species, is a problem of growing global concern in marine fisheries (Musyl et al., 2009; Watson et al., 2008) and poses significant ecological, social and economic challenges. The incidental catch of individuals in fishing activities can affect biodiversity through the elimination of prey, or the removal of top predators from marine ecosystems (Hall et al., 2000) and might trigger undesirable consequences for other fishery resources (Bonfil, 1994). Some bycatch species such as cetaceans, seabirds, sea turtles, and sharks are particularly sensitive to increased mortality above natural levels due their life history traits. Some economic effects on fisheries derived from bycatch are the imposition of a range of restrictions, closed areas, such as embargos, and possible closures by fisheries management authorities, among others. (Gilman et al., 2008)

The discarding (i.e. returning individuals to the ocean either dead or alive) associated with the incidental catch of non-target species in commercial fishing activities, is a common practice around the world and a cause of concern due to its magnitude (Chopin et al., 1996). In many fisheries, the quantity of marine species discarded from catches is very high (over 60% of the total catch in some trawl fisheries) (King, 2012). In the early 1990s, 25% of the total marine fishing mortality was estimated to be discarded non-target catches (Hall et al., 2000). The magnitude of fish discards is a key problem for marine fisheries management authorities and the broader community because of the waste of the resource and uncertainty of fishing mortality estimates.

In addition to discards, there is a wide variety of other unaccounted fishing mortalities, including non-reporting or under-reporting of landed catch, and cryptic mortality (Fig.1). Cryptic fishing mortality is defined as the unaccounted damage or mortality due to interactions with the fishing gear. It is largely not detectable and comprises of five categories (Chopin et al., 1996): (1) pre-catch losses, where catch dies from the fishing operation but is not brought on-board when the gear is retrieved, (2) ghost-fishing mortality caused by fishing gear that was
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abandoned, lost or discarded, (3) post-release mortality of catch that is retrieved and then released alive but later dies as a result of stress and injury sustained from the fishing interaction, (4) collateral mortalities indirectly caused by various ecological effects of fishing and (5) losses due to synergistic effects of multiple interacting sources of stress and injury from fishing operations, or from cumulative stress and injury caused by repeated sub-lethal interactions with fishing activities.

Often, cryptic components of fishing mortality are not always accounted for in fisheries management because of a lack of adequate data and, for some components, an absence of accurate estimation methods (Chopin *et al.*, 1996). Poor decisions based on this lack of information could cause adverse ecological effects reducing the sustainability of fishery resources and cause errors in stock assessments and population models which may compromise the efficacy of fisheries conservation (Gilman *et al.*, 2013).
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Figure 1: Potential outcomes of total encounters with fishing operations

Includes the five components of unaccounted cryptic mortality (encircled). IUU is for illegal, unreported and unregulated fishing. Adapted from Chopin et al. 1996; Gillman et al. (2013).
1.2 A review of the effects of fishing on shark populations worldwide

Sharks fisheries have historically represented a minor and relatively low-value contribution to the overall fisheries production of most countries, and are often a small and/or seasonal component of multi-species fisheries that include high-profile species such as tuna and swordfish (Keon, 1996). However, there has been an apparent rise in the trade in shark fins in recent years, which has focussed attention on what is in fact a substantial fishery, although little detail is known.

According to Bonfil (1994), the total global level of sharks, rays and chimaeras (elasmobranchs) caught was estimated at 1,350,000 tons. In most cases, due to a general lack of management and research on elasmobranch fisheries, it is not known if the level of harvest is sustainable.

While most of the analyses of trends and patterns in world shark fisheries have focused on the official statistics of shark catches, there are many other sources of fishing mortality that affect these individuals and are usually never accounted for such as bycatch, discarding and Illegal, Unregulated and Unreported (IUU) fishing (Fowler et al., 2002). Particularly, the large-scale fisheries, especially tuna fisheries using purse seines and longlines, incidentally take large numbers of sharks in their operations. Available estimates of bycatch for elasmobranchs in this fisheries suggest that during 1980s and early 1990s about 11-13 million of individuals (230,000 - 300,000 tons) were taken each year in the main open ocean (including EEZ’s-Exclusive Economic Zones) fisheries of the world (Bonfil, 1994). According to these figures, the amount of sharks taken as bycatch represents half of the reported elasmobranch caught in the official statistics (Fowler et al., 2002). By including unreported and illegal catches, Worm et al. (2013) estimated the global fishing mortality of sharks alone at 1.5 million tons, which translates into 100 million individual being killed each year (Afonso and Hazin, 2014). Figure 2 shows reported landings for sharks as derived from FAO data.
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Figure 2: Global shark landing trends

(A) Reported landings of chondrichthians, as derived from FAO data. (B) Reported FAO landings of sharks versus other chondrichthians (rays, skates and chimaeras) and (C) Reported landings of chondrichthians by region. (D) Trade-in shark imports and (E) Exports as reported by FAO. (F) Trade data for shark fin imports for Hong Kong as reported by the Government of Hong Kong Department of Aquaculture and Fisheries. Source: Worm *et al.*, (2013).
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This situation of most shark catches being unregulated, often misidentified and unrecorded when discarded at sea, has resulted in a lack of species-specific landings information (Dulvy *et al.*, 2013) and the available data on shark fishing mortality is considered to be rather limited and questionable (Vanuccini, 1999). This limitation of reliable information is due to the incomplete reporting of shark catches to the United Nations Food and Agricultural Organization (FAO), which tracks the status of fisheries worldwide (Worm *et al.*, 2013). The poor record of sustainability of target shark fisheries is cited as evidence of their vulnerability, but it is also magnified by the fact that only a few countries have any form of management for these resources. Poor baseline data on species identification and landings have been collected because sharks have historically been of low economic value in most countries and the lack of data is fundamental to the concerns (Clarke *et al.*, 2006).

The intense fishing mortality (accounted + unaccounted) coupled with the fact that the exploitation rate remained unknown for most populations of sharks raise serious concerns regarding the sustainability of shark populations. These concerns are intensified by the fact that sharks are susceptible to overfishing resulting from their *K*-selected life-history strategy (i.e. slow growth, late attainment of sexual maturity, long life spans, low fecundity and natural mortality, and a close relationship between the young produced and the size of the breeding biomass) (Stevens *et al.*, 2000). Due to this, shark stocks can be rapidly depleted and may be slow to recover from the effects of overfishing (Clarke *et al.*, 2006). Thus, sharks are among of the greatest threatened marine vertebrates according to the International Union for Conservation of Nature (IUCN) and most of the large coastal and oceanic shark populations have rapidly declined in the last decade (Fig.3) (Baum *et al.*, 2003).
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Sharks are very important to marine and freshwater ecosystems as ‘keystone species’. They are at the top of the trophic level of the aquatic food web, and act as bio-indicators for the health of aquatic environments (Ferretti *et al.*, 2010; Heithaus *et al.*, 2008). Therefore, the continued removal of sharks from the environment may result in severe ecological damage (Cortes, 1999).

The effective management of shark populations and, subsequently, the conservation of marine biodiversity thus require a significant reduction in shark fishing mortality (Bensley *et al.*, 2010).

**Figure 3: Declines in estimated relative abundance for coastal shark species**

(A) Hammerhead, (B) white, (C) tiger and (D) coastal shark species identified from 1992 onward: and oceanic shark species: (E) thresher, (F) blue, (G) mako and (H) oceanic whitetip. For each species, the overall trend (solid line) and individual year estimates (± 95%CI) are shown. Relative abundance initially set to 1, to allow comparison among species. (Baum *et al.*, 2003).
1.2.1 Shark fisheries and the trade in of shark products in Thailand

The general situation of elasmobranch fisheries in Asia is worrying due to the lack of information (i.e. fishery dependent data: landings) and regulations which protect shark species. About one-third of the 13 countries listed in figure 4 show declining trends in shark and ray catches (Taiwan, Hong Kong, Japan and Philippines); two others show slight declines (Thailand and Korea); and four countries have very steep increases in catches (India, Indonesia, Maldives and Pakistan).

<table>
<thead>
<tr>
<th>Country</th>
<th>Recent trend</th>
<th>Catch 1994 (t)</th>
<th>Management plan</th>
<th>Fishery data by species</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>???</td>
<td>???</td>
<td>no?</td>
<td>no?</td>
<td>concern over juvenile catch</td>
</tr>
<tr>
<td>Taiwan</td>
<td>decline</td>
<td>44,000</td>
<td>no</td>
<td>no (partial)*</td>
<td>distant water 85%+: some species in decline (unexplained)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>decline</td>
<td>7-12</td>
<td>no</td>
<td>no (partial)*</td>
<td>catch crash since early 1970s; 50% shark fin trade</td>
</tr>
<tr>
<td>Japan</td>
<td>decline</td>
<td>33,500</td>
<td>no</td>
<td>no (partial)*</td>
<td>declines in dogfish and mako shark CPUE</td>
</tr>
<tr>
<td>Philippines</td>
<td>decline</td>
<td>9,000</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>slight decline</td>
<td>8,500</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>slight decline</td>
<td>17,500</td>
<td>no</td>
<td>no*</td>
<td>distant water 50%: various &quot;reasons&quot; for declines</td>
</tr>
<tr>
<td>Malaysia</td>
<td>slight increase</td>
<td>21,000</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>increase</td>
<td>34,000</td>
<td>no</td>
<td>no (partial)</td>
<td></td>
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<tr>
<td>Pakistan</td>
<td>steep increase</td>
<td>50,000</td>
<td>no</td>
<td>no</td>
<td>drop in 1980</td>
</tr>
<tr>
<td>India</td>
<td>steep increase</td>
<td>84,000</td>
<td>no</td>
<td>no</td>
<td></td>
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<tr>
<td>Indonesia</td>
<td>steep increase</td>
<td>93,000</td>
<td>no</td>
<td>no</td>
<td>catch rates &quot;stable&quot;; reliability of data; localised depletion suspected</td>
</tr>
<tr>
<td>Maldives</td>
<td>steep increase</td>
<td>11,000</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>

* some available from TRAFFIC surveys

Figure 4: Trends and patterns of elasmobranch fisheries in Asia.
(Fowler et al., 2002).

Perhaps the most worrying aspect for the long-term sustainability of elasmobranch fisheries in Asia is that, at this time, none of the countries in this region has implemented, or is known to be planning management of their elasmobranch fisheries. In the light of the prominent position of the Indo-Pacific region as the world centre of elasmobranch biodiversity, this is a major concern (Fowler et al., 2002).
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In the particular case of Thailand, the situation is even more concerning due to the illegal, unregulated and non-compliance characteristics of the fishing activities in the Andaman Sea and the Gulf of Thailand. This situation makes it even harder for the collection of fishery-dependent data, which limits the ability to assess the past and future status of fish stocks in the area especially for sharks and rays since they have become increasingly important because of the profitable fin trade that has developed very quickly in the area (Fowler *et al.* 2002).

Elasmobranch fisheries in Thailand have been documented since the 1960’s, but have been in existence for much longer. The main fishing grounds for sharks are in the Gulf of Thailand and the Adaman Sea. According to the fisheries statistics of Thailand during 2004 – 2011, local shark catches have declined dramatically in last years (Fig. 5) with a total approximate catch of 8,600 metric tons/year during 2004 down to 4,800 metric tons/year during 2011 (Krajangdara, 2014).

![Figure 5: Shark catches in Thailand since 2004 to 2011](The Marine Fisheries Statistics based on The Sample Survey. Department of Fisheries. Thailand. 2002-2012).

Shark fin products are the main target for shark fisheries in Thailand and form the basis of the lucrative shark fin industry. The grading of fins is based on size, colour, species, cut and rendering, and the moisture content of the products (Keon, 1996). In Thailand, there are a few
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Large scale shark fin industries and the number of small locally-owned businesses is unknown. The largest export destination for shark fin products from Thailand is Singapore, followed by Hong Kong and Japan (Fowler *et al.*, 2002).

More than 50 species of elasmobranchs are sold in the fish markets of the Gulf of Thailand. The dominant species of sharks sold here are *Chiloscyllium punctatum* and *Chiloscyllium griseum* (brownbanded and grey bamboo shark respectively) (Krajangdara, 2014). The flesh of sharks and rays is usually processed into sweetened, salted, dried and fishball products and some are cooked fresh (Keon, 1996).

The Department of Fisheries established the *National Plan of Action for the Conservation and Management of Sharks (NPOA-Sharks)* of Thailand in 2005. However, the plan has not been implemented yet. In relation to the *National Policy for Management of Shark and Ray Resources*, the only shark measure in Thailand relates to the whale shark (*Rhincodon typus*). This species is protected in Thailand with a ban on whale shark fishing within Thai waters (*Ministerial Proclamation of the Ministry of Agriculture and Cooperatives* dated 28 March 2000). However, there are no further measures targeted specifically at the other 50 species of shark and rays, which are regularly traded in local markets.

Thailand is now faced with the depletion of their fisheries resources, due in part to overpopulation and to inappropriate uses of the marine environment (i.e. sea-water contamination because of disposal of grey and sewage waters, garbage, etc.). Based on local divers’ reports, shark sightings have declined dramatically on the last three years with most benthic and pelagic species disappearing from the Gulf of Thailand. The causes of the loss of diversity of local shark species are overfishing and habitat loss, as well as pollution (Fowler *et al.*, 2002).
In order to move towards the effective management of shark populations and the conservation of the marine environment, it is important to consider sources of non-accounted fishing mortality or cryptic mortality within stock assessments. An important component of cryptic mortality is post-release fishing mortality. Post-release fishing mortality occurs when individuals within the catch are retrieved and then released alive but stressed and injured to a degree that causes them to die later (Borucinska et al., 2002; Davis, 2005; Davis and Ryer, 2003; Gilman et al., 2008; Gilman et al., 2013; Ryer, 2002; Snoddy and Williard, 2010; Swimmer and Gilman, 2012). The types and severity of the injuries and the fate of the discarded individuals are influenced by multiple factors including environmental conditions (i.e. depth and temperature), biological characteristics of the species (i.e. life history traits: K-selected, r-selected), technical factors (i.e. fishing practices and gear used), and handling (on-deck) and release (Broadhurst et al., 2006; Davis, 2005; Davis and Ryer, 2003). Furthermore, the fate of released individuals is further complicated by the fact that fishing effects can trigger behavioural changes after release (i.e. behavioural impairment) (Ryer, 2004).

Post-release fishing mortality can be divided in three components (Pollock and Pine, 2007):

- **Immediate post-release fishing mortality**: which is defined as mortality caused by injuries or predation resulting from the fish being trapped on the fishing gear.

- **Short-term fishing mortality (24-72 h)**: which can be divided in two components: the first is the mortality resulting from a fishing gear or handling injury; and the second is the additional mortality due to indirect effects including increased predation risk following release. Short-term mortality is often measured in cages or containment studies.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

- **Long-term fishing mortality (>72 h):** Is the percentage of individuals that die due to interactions with the fishing gear after >72 hours of the capture event. It is difficult to estimate and is often assumed to be zero. However, this assumption requires validation. To assess long-term mortality, modifications of tag-return or telemetry survival methodology likely are most appropriate.

Quantifying post-release mortality/survival of discarded catch has been an ongoing challenge for fisheries management. Mortality of discarded fish may have serious economic and ecological consequences, as it represents a waste of natural resources, and exacerbates fishing pressure on commercial fish stocks, as well as on rare endangered species (Crowder and Murawski, 1998; Hall *et al.*, 2000; Harrington *et al.*, 2005). Failure to incorporate post-release mortality into stock assessment models could result in underestimates of fishing mortality, which in turn reduces the accuracy of the abundance and projected catch estimates, and may undermine the efficacy of conservation measures, such as size limits and quotas (Coggins *et al.*, 2007). Thus, our ability to assess the status of a stock depends in part on our knowledge of the fate of discards.

Numerous approaches have been developed to assess post-release mortality/survival of discarded catch. They fall into five general categories:

(a) **Containment methods**

Fish are caught during commercial (or simulated commercial) fishing operations and are handled according to regular practices and then monitored in tanks or sea cages. This approach has provided some measure of survival rates and identified important factors that influence mortality for several commercial and non-commercial species (Neilson *et al.*, 2011). For instance, several studies have reported size-related influence on survival rates, with larger fish having a higher survival rate (Broadhurst *et al.*, 2006; Neilson *et al.*, 1989; Miliken *et al.*, 1999).
However, there are considerable drawbacks associated with this approach. Confinement is not practical for large migratory species targeted by pelagic longline fisheries (Post et al., 1997; Skomal, 2007). As a result, studies using this approach have focussed on relatively small species; particularly those captured using towed gear (Broadhurst et al., 2006). Due to the high costs of vessel time and personnel, confinement studies have been limited to short term examination of post-release mortality precluding factors that could cause delayed mortality such as predation due to post-gear impairment (Neilson et al., 2011). Several studies have shown that confinement itself can cause mortality through the propagation of infection due to overcrowding or additive stress associated with holding fish (Mandelman and Farrington, 2007).

(b) Condition or vitality data from field observations

Post-release mortality/survival estimates are derived from an individual’s condition or vitality data collected by at-sea observers prior to discarding. The number of levels of condition varies, depending largely on the ability to make detailed observations of the specimens caught. In some fisheries (i.e. longline) where fish are assessed for retention/release while the individuals are alongside the vessel, the opportunities to collect detailed information on condition are limited. In other fisheries, where the fish are brought on-board, increased observations are possible. The assessment of the condition of individuals varies from each fishery and country. For example, the International Pacific Halibut Commission (IPHC) has used three categories of condition (excellent, poor and dead; Appendix B), which are defined by a series of criteria related to injuries and physical response to stimuli (Williams and Wilderbuer, 1995). It is important to note that fisheries observers have not set criteria on how to classify the condition of released individuals and therefore, the coding of an individual in a category is subjective.

Researchers have used data from observer programs extensively to estimate bycatch, bycatch/hooking mortality and post-release mortality (Neilson et al., 2011).
(c) Conventional tagging

Views of the utility of conventional tagging for studies of post-release mortality/survival differ quite substantially among authors. Cramer (2004) in a review of data available for the estimation of post-release mortality of large pelagic species, concluded that low conventional tag recaptures (0.4-1.83%) (Ortiz et al., 2003; Prince et al., 2002) are confounded with tag shedding, low exploitation rate, and failure to report recaptured tags. Therefore, they do not provide sufficient information to estimate post-release mortality (Neilson et al., 2011). On the other hand, the IPHC has relied extensively on this methodology to validate the condition grades described in the previous section. The different perspectives might be due to the fact that the IPHC were able to tag a large (14,872) number of individuals over three studies conducted during the 1990s, while obtaining an overall 5% recapture rate (Trumble et al., 2000).

(d) Telemetry studies

Some of the earliest studies of post-release mortality/survival using telemetry studies employed acoustic tagging approaches. Active acoustic tracking studies utilize individual coded acoustic tags that transmit information to a mobile ship-borne receiver. As noted by Cramer (2004), Pepperell and Davis (1999), and Graves et al. (2002), limitations and biases of acoustic tracking study procedures may limit the accuracy of post-release mortality estimates. These procedures include additional handling required to apply the acoustic tag to these animals, which would be expected to increase mortality compared to mortality of animals that were only caught and released. Also, studies using telemetry methods are usually short termed because of the cost in personnel and ship time and the labour intensive nature of the studies. Consequently, only short term survival may be examined (i.e., usually 12 hr) (Neilson et al., 2011).

Conventional satellite tags, or platform transmitter terminals (PTTs), have been widely used on all species of sea turtles (Neilson et al., 2011). As sea turtles spend much time at
the surface, compressed behavioural and environmental data from PTT-tagged animals can be regularly relayed to polar orbiting satellites. PTTs have been integrated into studies of post-hooking mortality, but the results are not conclusive due to several confounding factors. The application of tags to ‘control’ animals required to distinguish natural mortality from post-hooking mortality, has been lacking in some studies. Moreover, it is often not possible to distinguish between PTT tag failure and post-hooking mortality (Chaloupka et al., 2004; Hays et al., 2007; Parker et al., 2003).

Pop-up archival transmitting tags (PAT) are an alternative to PTTs and are well-suited to survival studies of open-ocean animals (i.e. pelagic sharks). PAT tags are programmed to release, or “pop-up”, from subject animals at a pre-set time, then transmit archived data continuously at the surface. Importantly, PAT tags also incorporate premature release features which can detect mortality events, such as when the tag descends beyond the depth range utilized by a live animal or remains at the surface or a depth without periodic surfacing. These additional data can be used to deduce the fate of an animal, based on certain behavioural assumptions, without necessarily recapturing the individual or retrieving the tag (Neilson et al., 2011).

(e) Biochemical methods

These methods consist of correlating the physical or physiological response indicators with mortality. Fish capture results in some physical and/or physiological trauma, regardless of gear employed, and measures of such trauma or the accompanying physiological response can be used as post-release mortality indicators (Farmer et al., 1998; Pronavi et al., 2001; Rose, 1999). For fish, physiological response indicators have commonly included blood concentrations of cortisol, lactate, glucose, chloride, sodium, potassium and haematocrit, while physical indicators include scale loss, bruising or wounds (Neilson et al., 2011).

The benefit of these techniques in terms of providing a tractable relationship with mortality varies considerably: for example, there are key issues associated with their use
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

on larger pelagic species. Determination of a baseline of physiological indicators prior to gear encounters is difficult, as individuals are unlikely to maintain the stress-free state during the collection of biochemical samples (Post et al., 1997; Skomal, 2007). In this respect, biochemical and physical studies are similar, since both could provide cost-effective means of estimating post-release mortality once their predictive accuracy has been tested. However, to derive post-release mortality estimates, it is essential to conduct quantitative studies to determine the fate of released fish. Therefore, in absence of telemetry, biochemical methods yield little information on the fate of released fish (Skomal, 2007) and thus are not recommended for use in large pelagic fishes until appropriate baseline levels and linkages to actual mortality have been developed (Neilson et al., 2011).

The use of an appropriate approach for assessing survival/mortality of released catch depends on the studied species (i.e. sharks, turtles) and their physiological characteristics (i.e., size) and behavioural patterns (i.e., depth, pelagic, benthic, migratory, etc.). In addition, the costs of the different methodologies are very variable and therefore, the availability of resources for a study will determine the use of a certain methodology. Better results have been achieved when combining more than one approaches together (Neilson et al., 2011).
1.4 Post-release survival studies in sharks

Although discarding rates of sharks have been increasingly quantified over the past decades (Braccini et al., 2009; Walker et al., 2005), much less is known about the post-capture survival (PCS) or post-release survival (PRS) of discards which would be an important factor contributing to the overall impact of fishing on shark populations (Braccini et al., 2012).

Several studies examining post-release survival of sharks following release from a commercial pelagic longline fishery have been completed (e.g., Moyes et al. 2006; Musyl 2009). Nevertheless, the most comprehensive study completed to date, examined both capture mortality and post-release survival of blue sharks caught on the North Atlantic commercial longline fishery (Campana et al., 2009). In this study, estimates of capture mortality of 13% and 20% made by observers and scientific staff respectively differed significantly, reflecting differences in shark examination, fishing vessels observed, and the gear that was used. However, the estimates of post-release mortality ranged from 0% for uninjured sharks to 33% of the injured sharks, with an overall mean of 19%. These results contrast with a total mortality rate of 5% reported for uninjured blue sharks caught as part of a research survey in the Hawaiian longline commercial fishery (Moyes et al., 2006). In an exchange of views in the scientific literature, Campana et al. (2009) and Musyl et al. (2009) debated the exact source of the discrepancy, but agreed that the post-release mortality depended on fishery specific features, such as hook type, soak time, and handling of the bycatch during capture and release. A central conclusion of the two studies was that researchers must be mindful of the need to emulate fishery operations so that the estimates of survival obtained using PSAT’s tags are representative of those experienced in the fishery.

More generally, there may also be a strong potential for a cluster effect in the estimated mortality obtained from PSAT studies. If fish are tagged from a small number of fishing sets (possible even during a single trip), estimated mortality may not be representative of discards in the fishery over a greater spatial and temporal range.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

A summary of the reviewed primary scientific literature of post-release studies for different species of sharks is presented in Appendix A. The majority of these investigations used biochemical techniques and conventional tagging as methods for determining PRS rates of different species of sharks.

The studies suggest that the mortality rates of pelagic sharks are species-specific and depend on numerous factors that may affect post-release mortality or survivorship of discarded sharks (Benoît *et al.*, 2010; Davis and Ryer, 2003; Frick *et al.*, 2009). Individual habits appeared to influence post-release survival rates of sharks caught in commercial fisheries. Specimens with bottom-dwelling habits (e.g., Port Jackson sharks and Australian swellsharks) had the highest post-capture survival rates whereas those with pelagic habits (e.g., mako and school sharks) had the lowest post-capture survival rates (Braccini *et al.*, 2012). Also, live individuals caught in longline gear seem to experience negligible post-release mortality if they are adequately released (i.e. handled with care, keep air exposure to a minimum, and treat the animal as gently as possible) thus an eventual mandatory release of live animals could be effective to improve the conservation of pelagic sharks (Afonso and Hazin, 2014).

Evidence suggests that during the fishing and discarding process, the release of non-target sharks is accompanied by a high mortality rate arising from the cumulative effects of physical injury and exercise-induced capture stress (Skomal, 2007). However, Moyes *et al.* (2006) proposed that sharks landed in an apparently healthy condition are likely to survive long term if released (95% survival based on biochemical analyses; 100% based on PSATs).

Based on the conclusions and recommendations of the reviewed studies, knowledge on the post-release mortality of caught sharks is essential to ensure the usefulness and suitability of catch-and-release strategies in any area (Afonso and Hazin, 2014). This measure could improve the survivorship of sharks caught in different fishing gears and may also contribute to reducing the impacts of worldwide fisheries upon shark populations.
2. Identification of the research problem

In the next section, the situation of sharks in Southeast Asia, and particularly Thailand, is described specially in relation to the brownbanded bamboo shark (*Chiloscyllium punctatum*). Dive Tribe’s shark conservation programme and its objectives, which are focussed on this species, are also outlined in this section. A brief introduction of the proposed methodology to assess Dive Tribe’s shark conservation programme is also defined. Due to the concerns of the University of Tasmania Animal Ethics Committee, the approach of the present study had to change considerably. The reasons and the new scope of the present study are commented on this section. Justification is gave for the need to provide an explanation for experimental designs for containment post-release studies, which was the biggest concern of the Animal Ethics Committee about the first proposed study.
2.1 Situation of sharks in Thailand

As mentioned in Chapter 1, shark populations in Thailand have decreased dramatically in last few years due to overfishing, and the destruction and contamination of the marine habitat. In addition, there is another threat for these taxa (to be found in Thailand and in other countries of South East Asia), which is either accounted for or regulated: the “live-trade” of sharks and rays for ornamental, exhibition and fresh consumption purposes. All types of species of live fish and corals could be found on a daily basis in fish markets of South East Asian countries (Fig.6). Here, animals are traded and sold for exhibition in private and public aquariums as well as for fresh consumption in local markets.

There are no records of the volume of sharks and rays that have been through this live-trade and, sometimes the catch of individuals for these purposes can be significant (30 to 50 juvenile specimens in one day of fishing). There is no protection protocol that prohibits or regulates this live-trade of juveniles of sharks and rays in the whole of the Indo-Pacific region (Krajangdara, 2014). The impacts of this activity are potentially large and must be considered as another important source of cryptic mortality for elasmobranchs and clearly should be taken into account in future stock assessments and regulation planning.

One of the most common sharks that form part of this live-trade is the brownbanded bamboo sharks (*Chiloscyllium punctatum*). This species is in high demand and the most commonly kept shark species in captivity (public and private aquaria) (Compagno, 2001). Live specimens of this species are sold in markets around Thailand (for captivity and fresh consumption purposes). Here, live individuals are kept in very poor conditions (i.e., poor quality and limited supply of water, overcrowded, etc.) where stress and trauma influenced negatively the wellbeing and post-release survival of the captive specimens (Fig.7).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 6: Juvenile blacktip sharks found in one of the markets of Bangkok, Thailand 2014
Individuals are sold alive for aquariums and ornamental purposes. Each one of these sharks was worth 160 AUD. Photo by Isabel Timpe, 2014.

Figure 7: Live adult brownbanded sharks kept in poor conditions in Pattaya’s (Thailand) fish market
Photo by Isabel Timpe, 2014.
The brownbanded bamboo shark (Müller and Henle 1838) is a small-sized (maximum total length- MTL- 87 cm), slender and long-tailed, unpatterned (juveniles with dark saddles and transverse bands) shark (Fig. 8). It is distributed throughout the Indo-west Pacific waters including: India (east coast, Andaman Islands), Malaysia, Singapore, Thailand, Indonesia (Java, Sumatra, Sulawesi, Komodo), Vietnam, China, Taiwan (Province of China), Japan, Philippines, south coast of New Guinea (Papua New Guinea and Irian Jaya, Indonesia), and the north coast of Australia (Northern Territory, Western Australia, Queensland) (fig. 9). It is an inshore benthic shark, inhabiting rocks and lagoons with depths between 5 to 80 metres.

Brownbanded bamboo sharks are oviparous and deposit eggs in small oval cases on the sea bed. It feeds mainly on small-to-medium sized invertebrates (i.e. crabs, squid) (Compagno, 2001).

In relation to its conservation status, the brownbanded is classified as Near Threatened (NT) by the IUCN (Fowler and Cavanagh, 2003).

I decided to focus my research on brownbanded bamboo sharks because I had the opportunity of collaboration with a Thai marine conservation association (Dive Tribe) that has been working on the recovery and conservation of these species for the past 3 years. Additionally, the biological and behavioural characteristics of the brownbanded bamboo shark make this species appropriate for captivity experiments according to the Elasmobranch Husbandry Manual (Smith et al., 2004):

- Availability: Readily available South Pacific waters.
- Species compatibility: timid, non-aggressive.
- Hardiness: Adapts rapidly to new environments and have few problems to eat in captivity.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**Figure 8:** Illustration of brownbanded bamboo sharks (*Chiloscyllium punctatum*) juvenile and adult. (Compagno *et al.*, 2002).

**Figure 9:** Geographic distribution of the brownbanded bamboo shark (*Chiloscyllium punctatum*). (Fowler and Cavanagh, 2003).
2.2 Dive Tribe’s shark conservation programme

Growing public and scientific awareness about the conservation situation of sharks in Thailand has resulted in local communities and conservation groups taking action (e.g. Dive Tribe). In an attempt to protect local marine ecosystems from depletion, Dive Tribe (www.divetribe.weebly.com) developed the “Save Our Seas (SOS)” initiative, which has the goal of protecting the unique marine environment of Pattaya’s near islands (Gulf of Thailand) by assessing, managing and conserving the local marine resources. The “shark conservation programme” has been developed under this “SOS” initiative. Established in 2011, the objectives of the project are to reduce fishing mortality that is causing the overexploitation of local shark stocks. Dive Tribe’s strategy is based on live recuperated individuals from markets, which are later tagged and liberated in specific sites around Pattaya reefs (Koh Tao, Koh Lan and Hat Nuan islands). Liberated individuals of shark species include mostly juvenile (20-60cm) and adult (60-85cm) specimens of brownbanded bamboo sharks (Chiloscyllium punctatum).

Tagging experiments have been conducted since the beginning of the project in order to describe the movement patterns of sharks on the reef and evaluate post-release survival rates of sampled individuals. Despite the best efforts by Dive Tribe and local volunteers, the return rate of tags has been low, therefore PRS estimates for the studied species of sharks are not considered accurate. Thus, an assessment of Dive Tribe’s shark conservation programme was needed.

Understanding post-release mortality and behavior of recovered sharks by Dive Tribe’s programme was required to ensure the adequacy of the current release strategies of live individuals employed by the association. The assessment of Dive Tribe’s shark conservation programme would have facilitate the incorporation of reliable estimates of post-release mortality of threatened species (i.e. brownbanded bamboo sharks) into stock assessments and catch projections of Thailand shark populations and would have improve the accuracy in the prediction values for those assessments.


2.3 Proposed methodology to assess Dive Tribe’s shark conservation programme

In order to assess Dive Tribe’s shark conservation programme and its contribution to the recovery of local shark stocks in Pattaya waters, post-release survival rates of brownbanded bamboo sharks (*Chiloscyllium punctatum*) which are part of the live-trade, were going to be evaluated. Immediate PRS rates for brownbanded bamboo sharks were going to be estimated by observing the condition of sampled specimens and short-term (24-48h) and long-term (>72h) PRS rates were going to be evaluated using experimental laboratory holding studies.

For estimating immediate PRS, data on the condition of individual recovered brownbanded bamboo sharks were going to be collected at the three different stages of Dive Tribe’s “shark recovery programme”: market collection, during captivity and prior to release. Records of the condition of sampled sharks were going to be collected by measurements of length and scores of the specimen’s “vitality” on a four level scale (ranging from 1 for the best condition i.e. very healthy; to 4 the worst condition, i.e. close to death) based on a rapid 10s evaluation adapted from Braccini *et al.*, (2013) (Appendix B). The selection of one of the four categorical indices used would have reflect the physical damage and behavioral condition of each shark.

This approach would have provided some estimate of immediate, short and long-term survival rates of the species and may have identified important factors that influence mortality of brownbanded bamboo sharks.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

### 2.4 Outcome of the Animal Ethics application

The proposed study was first orientated to conduct confinement experiments with brownbanded bamboo sharks that inhabit Pattaya’s (Gulf of Thailand) reefs, in order to evaluate their post-release survival rates in relation to the condition or vitality of individuals (i.e. covariate). This was going to be achieved by the assessment Dive Tribe’s shark conservation programme and the evaluation of the immediate, short and long-term PRS during every stage of the programme.

Under Tasmanian Legislation, any investigation held at the University of Tasmania that involves vertebrates or cephalopods which are alive or which are to be killed specifically for the investigation, needs an Animal Ethics Committee application approval from the University of Tasmania Animal Ethics Committee (AEC). This is done in order to ensure that all animal care and use conducted by the University is done in accordance with the “*Australian Code for the Care and Use of Animals for the Scientific Purposes* - 8th Edition 2013”.

Due to the concerns from the University of Tasmania Animal Ethics Committee (AEC) about the proposed number of animals to be used for the proposed experiment and the ability to provide appropriate levels of care to the animals in Thailand, approval for the collection of data in Thailand was not granted and a different approach had to be taken to develop the actual research. The present study will now use simulation (computational) techniques to estimate most appropriate experimental designs to measure the impact of a covariate (i.e. condition) on post-release survival rates taking the brownbanded bamboo shark as an example. The developed approach can be used in future survival studies and will provide statistically significant results without comprising animal’s welfare by having to use too many individuals (i.e. sharks).
2.5 Need to provide justification for experimental designs for estimating post-release survival in containment studies

A common approach to assessing post-release survival/mortality (PRS/PRM) is to capture fish using conventional angling gear and monitor their survival for short time periods (hours to days) in cages, pens, or ponds [termed “containment studies (Pollock and Pine, 2007)]. Although this approach may not always be appropriate because of the size or behaviour of the species (e.g., pelagic billfishes and tunas) (Goodyear 2002), containment studies are conducted for many species. Yet guidance for experimental design of PRS with containers is lacking (Rogers et al. 2014).

Multiple examples of PRS or PRM estimation come from “catch-and-release science” (Cooke and Schraman, 2007), accounting for tagging mortality in mark-and-recapture studies (e.g., Brenden et al. 2010) and incorporating parameters of cryptic mortality into population models (e.g., Coggins et al., 2007). Freshwater catch-and-release (CR) mortality studies have been a large contributor to understanding handling effects (Rogers et al. 2014). These studies have demonstrated that efforts aimed at decreasing unintended mortality (e.g., circle hooks requirements) and accounting for unintended mortality (e.g., tagging studies, fisheries bycatch) need robust PRS experimental designs.

Pollock and Pine (2007) provided guidance for estimating mortality and uncertainty in CR studies and discussed trade-offs among approaches (e.g., containment vs. telemetry). For the most common method, containment studies, recommended that: (1) control fish always be used; (2) individual fish not be treated as replicates, and (3) precision be considered before experimentation to determine if estimates will be informative.

Cowx et al. (2010) stated that a “plethora” of studies have aimed to measure lethal and sub-lethal handling effects. However, I am not aware of any in-depth exploration for careful analysis of field to estimate catch-and-release mortality. Cooke et al. (2013) provided perspectives on
measuring physiological consequences of CR fishing and common limitation, yet gaps remain for quantifying treatment (i.e. tagging, handling, etc.) effects.

For fish tagging studies, in particular, guidance exists for tag selection and ensuring that the marked population is representative of the unmarked population (e.g., Pine et al. 2003) and mark-recapture literature explicitly details the need and methods to accurate estimates of capture probability. So far, methods or quantifying mortality due to handling, tagging and condition of the individual have received less attention and could result in bias in fish tagging studies if the number-at-large is not adequately assessed (Rogers et al. 2014).

I have decided to design a pilot simulation-based study following Pollock and Pine (2007) and Wilde (2002) procedures, which will allow me to formulate the accurate experimental design for future PRS studies of brownbanded bamboo sharks (*Chiloscyllium punctatum*) in the Gulf of Thailand. I also want to extend the results to create a broadly applicable simulation tool to inform future PRS study designs focussed on different shark species.
3. Project goals and objectives

In order to give an answer to the concerns of the Animal Ethics Committee, the present study will conduct simulation-based procedures that will give a justification for experimental designs in containment survival studies. In addition, the efficacy of the achieved design will be validated by assessments of post-release survival using brownbanded bamboo sharks (*Chiloscyllium punctatum*) in the Gulf of Thailand as an example. Therefore, this study aims to:

I. **Review simulation and modelling techniques as analytical tools.**
   A brief review of simulation and modelling processes with special emphasis on the characteristics that make them suitable for the creation and analysis of data sets, will be described. This will provide the reader with the required background in order to fully understand the incoming data-generation processes.

II. **Review available statistical methods for assessing survival (Survival analysis and modelling).**
   *Simulation analysis* is a broad and extent division of statistics. In this section, I will summarize the most important features of available statistical methods for assessing survival, giving special emphasis on the ones that make survival analysis applicable to describe biological and ecological processes. The actual review will describe the variables and methodologies that will be applied later to the data generating processes.

III. **Run simulations in order to:**

   i. **Define efficient (in terms of costs and number of individuals needed) experimental designs of containment studies across a range of scenarios in order to provide precise survival assessments.**
In order to response to the actual concerns of the Animal Ethics Committee about the number of animals needed for the proposed containment survival experiments in Thailand, simulations will be used to evaluate precision in the ability to detect survival in paired-design (control and treatment fish in the same tank) containment experiments. Evaluations will be performed across a range of design scenarios in which sample size (i.e. number if fish per container) and replication (i.e. number of containers) are independent variables. Simulated survival rates for each scenario will be ranging from 20%-80% for treatment mortality or mortality related to the condition of the individual, and 95% for treatment mortality. ANOVA analyses will be applied in order to determine which experimental design (i.e. combination of sample size and replicates) will allow the researchers to achieve precise assessments of survival at 95% of confidence. The obtained results will be applied to the brownbanded bamboo shark example.

ii. **Assess post-release survival rates of brownbanded bamboo sharks** (*Chiloscyllium punctatum*) **in the Gulf of Thailand using survival analysis based on the most accurate experimental design of containment studies.**

Previously performed simulations will allow defining an efficient and accurate experimental design for survival containment studies in relation to costs and number of individuals needed to achieve precise survival assessments. The obtained outputs (i.e. number of fish needed) derived from these simulations will be used to simulate survival times taking brownbanded bamboo sharks as an example. The generated data will follow an *exponential* distribution and estimates of survival (i.e. number of events, coefficients, standard errors and evaluation metrics) will be obtained by nonparametric (i.e. *Kaplan-Meier* estimation) and semiparametric methods (i.e. Cox proportional hazards regression model).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

4. Materials and methods

The next section will described the approach and methods that have been considered appropriate to achieve the aims of this study. First, a review of simulations and modelling procedures is presented, in order to orient the reader towards the using of simulation tools that have been employed along this section. Secondly, a briefly description of survival analysis and the available statistical methods to assess survival is also outlined, which will help the reader to identify the rationale beyond the simulation processes and finally, a detailed explanation of the *R*-code and procedures that were used to performed simulations that allow to generate the desired data. The provided *R*-code can be modified by the reader to address other scenarios.

The reviews, functions, equations, and the necessary steps towards the generation of data will be presented here. The *R*-codes and routines used for the data simulation could be found on the Appendixes C and D.
Objective I: Review of simulation and modelling techniques as an analytical tool

Modelling can be defined as “the process of producing a model”. The explanation of an observed pattern is referred as a model or theory (Ford, 2002), which is a series of statements (or formulae) that explains why the observations have occurred. Model development (i.e. modelling) is also what Peters (1991) referred to as the synthetic or private phase of the scientific method, where the perceived problem interacts with insight, existing theory, belief and previous observations to produce a set of competing models. This phase is clearly inductive and involves developing theories from observations (Chalmers, 1999), the explanatory process of hypothesis formulation. One purpose of a model is to enable the analyst to predict the effect of changes to the system. On the one hand, a model should be a close approximation to the real system and incorporate most of the salient features. On the other hand, it should not be so complex that is impossible to understand and to experiment with. A good model is a judicious trade-off between realism and simplicity. Generally, a model intended for a simulation study is a mathematical model developed with the help of simulation software. Mathematical model classifications include deterministic (input and output variables are fixed values) or stochastic (at least one of the input or output variables is probabilistic) (Anu, 1997).

A simulation of a system is the operation of a model of the system. The operation of the model can be studied, and hence, properties concerning the behaviour of the actual system or its subsystem can be inferred. In its broadest sense, simulation is “a tool to evaluate the performance of a system, existing or proposed, under different configurations of interest and over long periods of real time” (Anu, 1997). In particular, simulation involves creating a computational representation of the underlying theoretical logic that links constructs together within simplified worlds. These representations are then coded into software that is run repeatedly under varying experimental conditions (i.e. alternative assumptions, varied construct values, etc.) in order to obtain results (Davis et al., 2007).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 10 is a schematic of a simulation study. The iterative nature of the process is indicated by the system under study becoming the altered system, which then becomes the system under study and the cycle repeats.

![Simulation study schematic](image)

*Figure 10: Simulation study schematic.*

Adapted from Anu (1997).

Although many statistical questions can be answered directly through mathematical analysis rather than simulations, the complexity of some statistical questions makes them more easily answered through simulation methods. In these cases, simulations may be used to generate datasets that conform to a set of known properties (e.g., mean, standard deviation, etc.) and the accuracy of the model-computed parameter estimates may be compared to their specified values to determine how adequately the model performs under specified conditions (Hallgren, 2013). As several methods may be available for analysing datasets with these characteristics,
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the suitability of these different methods could also be tested with simulations in order to determine if some methods offer greater accuracy than others and identify the required inputs (Estabrook *et al.*, 2012; Luh and Guo, 1999).

Simulation studies typically are designed according to the following steps to ensure that the study can be informative for the researchers` question (Hallgren, 2013):

1. A set of assumptions about the nature and parameters of a dataset are specified.
2. A dataset is generated according to these assumptions.
3. Statistical analyses of interest are performed on this dataset, and the parameter.
4. Estimates of interest from these analyses (e.g., model coefficient estimates, fit indices, *p*-values, etc.) are retained.
5. Steps 2 and 3 are repeated many times with many newly generated datasets (e.g., 1000 datasets) in order to obtain an empirical distribution of parameter estimates.
6. Often, the assumptions specified in step 1 are modified and steps 2-4 are repeated for datasets generated according to new parameters assumptions.
7. The obtained distributions of parameter estimates from these simulated datasets are analysed to evaluate the question of interest.

**Important issues to consider when designing a simulation study**

When planning a simulation study, as with randomized trials, a detailed protocol should be produced giving full details of how the study is to be performed, analysed, and reported. The protocol should document the specific objectives for the simulation study and the procedures for generating multivariate data sets and, if relevant, with censored survival times. The choices for the different scenarios to be considered, for example different sample sizes and the
methods that will be evaluated, should also be included in the protocol together with the number of simulations that will be performed (Burton et al., 2006).

A checklist of the important issues that will require consideration in the design of the present study is provided in table 1.

Table 1: Checklist of important issues that require consideration when designing a simulation study
Adapted from Burton et al. (2006).

<table>
<thead>
<tr>
<th>Detailed protocol of all aspects of the simulation study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Clearly define aims and objectives</td>
</tr>
<tr>
<td>2. Simulation procedures</td>
</tr>
<tr>
<td>a. Level of dependence between simulated data sets</td>
</tr>
<tr>
<td>b. Allowance for failures</td>
</tr>
<tr>
<td>c. Software to perform simulations</td>
</tr>
<tr>
<td>d. Random number generator to use</td>
</tr>
<tr>
<td>e. Specification of the starting seeds</td>
</tr>
<tr>
<td>3. Methods for generating the datasets</td>
</tr>
<tr>
<td>4. Scenarios to be investigated</td>
</tr>
<tr>
<td>5. Statistical methods to be evaluated</td>
</tr>
<tr>
<td>6. Estimates to be stored for each simulation and summary measures to be calculated over all simulations</td>
</tr>
<tr>
<td>7. Number of simulations to be performed</td>
</tr>
<tr>
<td>8. Criteria to evaluate the performance of statistical methods for different scenarios</td>
</tr>
<tr>
<td>a. Assessment of bias</td>
</tr>
<tr>
<td>b. Assessment of accuracy</td>
</tr>
<tr>
<td>c. Assessment of coverage</td>
</tr>
<tr>
<td>9. Presentation of the simulation results</td>
</tr>
</tbody>
</table>
Advantages of simulation studies

It seems clear that analysing real data is always less controversial (in terms of replicating the natural condition in a mathematical environment) than analysing simulated data. However, many authors (e.g., Law and Kelton, 1991; Padilla et al., 2011) have recognised the benefits of simulation studies as an analytical tool.

Simulations provide a powerful technique for answering a broad set of methodological and theoretical questions and provide a flexible framework to answer specific questions relevant to one’s own research. For example, simulations can evaluate the robustness of a statistical procedure under ideal and non-ideal conditions, and can identify strengths (e.g., accuracy of parameter estimates) and weaknesses (e.g., type-I and type-II error rates) of competing approaches for hypothesis testing (Hallgreen, 2013).

In addition, simulations also can be used to estimate the statistical power of many models that cannot be estimated directly through power tables and other classical methods (e.g., mediation analyses, hierarchical linear models, structural equation models, etc.). The procedures used for simulation studies are also at the heart of bootstrapping methods, which use resampling procedures to obtain empirical estimates (that could not be achieved from a single study) of sampling distributions, confidence intervals, and p-values when a parameter sampling distribution is non-normal or unknown (i.e. survival data) (Hallgreen, 2013).

The usefulness and advantages that simulation and modelling techniques provide as analytical tools are recognize in the scientific sector and Elsevier has published its own Journal: Ecological Modelling, which is concerned with the use of mathematical models and systems analysis for the description of ecological processes and for the sustainable management of resources. Several authors have used simulation and modelling process to describe ecological processes in relation to survival or mortality (Cooper and Goggin, 2005; Fox and Kendall, 2006; He and Alfaro, 2000; Ma, 1997, 2008; Tanhuanpa and Ruohhoma, 2001; Zhanshan and Bechinski, 2008). However, only one has been applied to post-release mortality of fish (Rogers et al., 2014).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Despite all the advantages of using simulation and modelling as a tool to transform conceptualizations, one has to recognize that modelling and simulation processes are not always formal and rigorous (Padilla *et al.*, 2011). Furthermore, modelling and simulation processes are not always coherent and consistent or even repeatable and this is why some authors do not view modelling and simulation as a science with its own theories and methodologies. A research question in modelling and simulation reflects the assumptions and constraints that went into transforming that question into a situation or problem that need to be solved. All modelling and simulation problems are purposeful simplifications of a bigger situation. Consequently, truth is relative in modelling and simulation processes and the assignment of truth value depends entirely on the axiomatic structure imposed on the referent (Padilla *et al.*, 2011).

Assumptions that are going to be made during the modelling and simulation procedures used in the actual research include: (1) data sets are fully independent simulated assuming that fish in each container all fully independent from fish in another container and that there is not interaction among individuals from the same tank that may affect survival (i.e. predation), and (2) that brownbanded bamboo sharks present low mortality rates due to their bottom-dwelling habits [based on Braccini *et al.*, (2012) results, were bottom-dwelling studied sharks shown higher survival rates than pelagic sharks].
Objective I: Review available statistical methods for assessing survival (Survival analysis)

Survival analysis has been of interest in population ecology for several decades (Morris, 1959; Caughley, 1977; Southwood, 1978), but it is only recently that the statistical methods used to analyse survival data of patients in clinical trials and reliability of products in engineering have attracted the attention of ecologists (Muenchow, 1986; Pyke and Thompson, 1986; Dixon and Newman, 1991; Fox, 1993; Newman and McCloskey, 1996). Ecologists are progressively realizing that many phenomena in biology and ecology can be described by the “time-until-a-given-event occurs”, which is the basis of survival analysis.

Survival analysis is widely applied in many fields such as biology, medicine, public health and epidemiology. A typical analysis of survival data involves the modelling of time-to-event data, such as time until death (Zhao, 2008).

Special features of survival time

The primary variable in Survival analysis is survival time. Survival time is defined as “a variable that measures the time from a particular start point (e.g., the time at which individuals are caught) to a certain endpoint of interest (e.g., the time until they are released)” (Lee and Go, 1997. pp. 106). In most situations, survival data is collected over a finite period of time due to practical reasons. The observed time-to-event data is always non-negative and can be exact or censored (Zhao, 2008). Exact data, also known as uncensored data, occurs when the precise time until the event of interest is known. Censored data arises when the exact time points at which failures (i.e. deaths) occur are unknown (Lee and Go, 1997). Right-censored data is a common type of data encountered in real life scenarios and occurs when an individual has a failure time after their final observed time. A typical right-censored data includes a variable for an individual’s time to study and an indicator of whether the associated time is an exactly
known or a right-censored survival time. Usually an indicator of 1 is used if the exact survival time is known, and an indicator of 0 for right-censored times (Zhao, 2008).

Survival times often follow a skewed distribution, far from normal. Although transformations can be applied to make distribution more symmetrical, using a different model may be more satisfactory. Some of the distributions known to be appropriate for survival times include exponential, Weibull, lognormal, gamma, Gompertz, and log-logistic (table 2). Due to these special features (i.e. censoring and not-normal distribution), survival times are not amendable to standard statistical methods (Lee and Go, 1997).

Table 2: Commonly used distributions in survival analysis
Adapted from Lee and Go (1997).

<table>
<thead>
<tr>
<th>Name</th>
<th>Functions</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exponential</strong></td>
<td>Parameter $\lambda &gt; 0$</td>
<td>Is characterized by a constant hazard function $\lambda$, independent from the age of the individual. Therefore, failure or death is independent of time.</td>
</tr>
<tr>
<td><strong>distribution</strong></td>
<td>a) Proportional density function: $f(t) = \lambda \exp(-\lambda t)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) Survival function: $S(t) = \exp(-\lambda t)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) Hazard function: $h(t) = \lambda$</td>
<td></td>
</tr>
<tr>
<td><strong>Weibull</strong></td>
<td>Parameter $\lambda, \gamma &gt; 0$</td>
<td>Is characterized by two parameters, $\gamma$ (shape parameter) and $\lambda$ (scale parameter).</td>
</tr>
<tr>
<td><strong>distribution</strong></td>
<td>a) Proportional density function: $f(t) = \lambda \gamma (\lambda t)^{\gamma-1} \exp(-\lambda t)^\gamma$</td>
<td>This distribution may be used to model survival distribution of a population with increasing, decreasing, or constant risk.</td>
</tr>
<tr>
<td></td>
<td>b) Survival function: $S(t) = \exp(-\lambda t)^\gamma$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) Hazard function: $h(t) = \lambda \gamma (\lambda t)^{\gamma-1}$</td>
<td></td>
</tr>
</tbody>
</table>
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

<table>
<thead>
<tr>
<th>Lognormal distribution (Peto and Peto, 1972)</th>
<th>Parameter $\mu, \sigma &gt; 0$; $a = \exp(-\mu)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Proportional density function:</td>
<td></td>
</tr>
<tr>
<td>$f(t) = 1/t\sigma\sqrt{2\pi} \exp[-1/2\sigma^2(log_e at)^2]$</td>
<td></td>
</tr>
<tr>
<td>b) Survival function:</td>
<td></td>
</tr>
<tr>
<td>$S(t) = 1 - G(log_e at/\sigma)$</td>
<td></td>
</tr>
<tr>
<td>c) Hazard function:</td>
<td></td>
</tr>
<tr>
<td>$h(t) = [1/t\sigma\sqrt{2\pi} \exp[-1/2\sigma^2(log_e at)^2] {1 - G(log_e (at)/\sigma)}]^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Comparison of two survival distributions.</td>
<td></td>
</tr>
<tr>
<td>Appropriate for survival distributions whose hazard functions are proportional over time, i.e. the two survival curves do not cross.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gamma distribution</th>
<th>Parameter $\lambda, \gamma &gt; 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Proportional density function:</td>
<td></td>
</tr>
<tr>
<td>$f(t) = \frac{\lambda}{\Gamma(\gamma)}(\lambda t)^{\gamma-1}e^{-\lambda t}$</td>
<td></td>
</tr>
<tr>
<td>b) Survival function:</td>
<td></td>
</tr>
<tr>
<td>$S(t) = t \int_0^\infty f(x)dx$</td>
<td></td>
</tr>
<tr>
<td>c) Hazard function:</td>
<td></td>
</tr>
<tr>
<td>$h(t) = f(t)/S(t)$</td>
<td></td>
</tr>
<tr>
<td>It is also characterized by two parameters, $\gamma$ (shape parameter) and $\lambda$ (scale parameter).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gompertz distribution</th>
<th>Parameter $\lambda, \gamma &gt; 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Proportional density function:</td>
<td></td>
</tr>
<tr>
<td>$f(t) = \exp[(\lambda + \gamma t) - \frac{1}{\gamma(e^{\lambda + \gamma t} - 1)}]$</td>
<td></td>
</tr>
<tr>
<td>b) Survival function:</td>
<td></td>
</tr>
<tr>
<td>$S(t) = \exp[-e^\lambda/\gamma(e^{\gamma t} - 1)]$</td>
<td></td>
</tr>
<tr>
<td>c) Hazard function:</td>
<td></td>
</tr>
<tr>
<td>$h(t) = \exp(\lambda + \gamma t)$</td>
<td></td>
</tr>
<tr>
<td>The Gompertz distribution is also characterized by two parameters, $\gamma$ and $\lambda$. When $\gamma &lt; 0(&gt;0)$, the hazard rate decreases (increases) from $\exp(\lambda)$ and when $\gamma = 0$, the hazard rate is constant, $\exp(\lambda)$.</td>
<td></td>
</tr>
</tbody>
</table>
Survival functions

The distribution of survival times is characterized by three functions: (a) the survival function, (b) the probability density function, and (c) the hazard function.

- The survival function

The Survival function models the probability of an individual surviving beyond a specified time \( t \) (Zhao, 2008). We denote \( T \) as the random variable representing survival time, which is the time until the event of interest. The statistical expression of the survival function, \( S(t) \), is shown in Equation 1:

\[
S(t) = \int_t^\infty f(t)dt = 1 - F(t)
\]

**Equation 1: Survival function**

Adapted from Lee and Go (1997).

Where \( F(t) \) is the distribution function of \( T \). Therefore, \( S(t)=1 \) at \( t=0 \) and \( S(t)=0 \) at \( t=\infty \). The graph of \( S(t) \) is known as the ‘survival curve’ which begins at \( S(0)=1 \) and decreases to \( 0 \) as \( t \) increases to infinity. In this way, the survival curve can be plotted graphically to represent the probability of an individual’s survivorship at varying time points. Many types of curves can be shown, but the important point to note is that they all have the same properties: (a) they are monotone, (b) non-increasing functions, (c) when \( t=0 \), \( S(t)=1 \), and (d) \( S(t)=0 \) as \( t \to \infty \). Their rate of decline, of course, varies according to the risk of experiencing the event at time \( t \).

Figure 11 shows three different survival curves resulting from distributions that are commonly used in survival analysis (Cantor, 2003; Allison, 1995; Andersen et al., 1993). These distributions are the exponential distribution with shape parameter \( \lambda=5 \), the standard lognormal distribution and the Weibull distribution with shape and scale parameters of 2 and 8, respectively (for an extended explanation of survival distributions see next section). In this example, we can see the three distributions, and the probability associated with the Weibull distribution which decreases much more slowly compared to the other two distributions (Zhao 2008).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**survival Curves**

![Exponential, lognormal and Weibull distribution's survival functions.](image)

**Figure 11:** Exponential, lognormal and Weibull distribution’s survival functions.

Survival time (days) is represented in the x-axis. The cumulative survival probability (1 to 0) is represented on the y-axis. Adapted from Zhao (2008).

- **The probability density function (pdf)**

  The probability density function is defined in Equation 2, which is also known as the *unconditional failure rate*:

  \[
  f(t) = \lim_{\Delta t \to 0} \frac{P(t < T < t + \Delta t)}{\Delta t}
  \]

  **Equation 2:** Probability density function or unconditional failure rate

  Adapted from Lee and Go (1997).

  * Where \( \Delta t \) denotes increase in survival time.
• **The hazard function**

On the other hand, the hazard function gives the *conditional failure rate* and it is defined as,

\[
h(t) = \lim_{\Delta t \to 0} \frac{P(T \leq t + \Delta t | T > t)}{\Delta t} = \frac{f(t)}{S(t)} = -\log(S(t))'
\]

Equation 3: Hazard function

Adapted from Lee and Go (1997).

The hazard function is also known as the *instantaneous failure rate*, *age-specific failure rate*, or *conditional mortality rate*. It is a measure of the proneness to failure as a function of the individual’s spent time in the experiment. It is not really a probability since its value can be greater than one. The hazard function plays an important role in survival analysis (Lee and Go, 1997).

The three functions are mathematically equivalent, if one is known, the other two can be derived. For practical purposes, the survival function is most useful because it gives the median survival time and other summary statistics (Lee and Go 1997).

Major applications of survival analysis in biology include the following: (a) estimation of survival distributions, (b) testing hypotheses of equal survival distributions, and (c) identification of risk of prognostic factors. The approach taken may be *nonparametric*, *parametric*, or *semiparametric*. Regardless of the approach selected, the estimation of survival distribution provides estimates of the descriptive statistics such as the median survival time and the probability of surviving longer than a given period of time. In order to compare the survival distributions of two or more groups, two-sample and K-sample tests have been developed (Zhao, 2008).

**Survival estimation models**

In biology research, it is often of interest to know whether certain characteristics of an individual are related to the occurrence of a certain death-related event. In these cases, the
characteristics of the individual are referred as risk factors or covariates. In addition to examining individually each variable’s relationship to the length of survival, multivariate regression analysis is necessary to control for confounding factors between the covariates (Lee and Go, 1997).

Both parametric and nonparametric estimation methods for the survival function will be introduced in this section. For right-censored data, a parametric estimator is sometimes used for the estimation of survival functions. However, in real life scenarios, the exact distribution of the data is usually unknown. In such cases, using a nonparametric method is common practice, since the nonparametric estimators do not assume that the data comes from a specific distribution. The Kaplan-Meier estimator (Kaplan and Meier, 1958) is widely employed as the nonparametric estimator in the presence of right-censored data (Lee and Go, 1997).

Modeling of survival data usually employs the hazard function or the log hazard. For example, assuming a constant hazard, \( h(t) = \nu \), implies an exponential distribution of survival times, with density function \( p(t) = \nu e^{-\nu t} \). Other common hazard models include \( \log h(t) = \nu + \rho t \) which leads to the Gompertz distribution of survival times, and \( \log h(t) = \nu + \rho \log(t) \) which leads to the Weibull distribution of survival times. (See, for example, Cox and Oakes, 1984: Sec. 2.3, for these and other possibilities.) In both the Gompertz and Weibull distributions, the hazard can either increase or decrease with time; moreover, in both instances, setting \( \rho = 0 \) yields the exponential model (Fox, 2002).

A review of the most commonly models used in survival data analysis is presented in table 3:
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**Table 3: Commonly models used in the analysis of survival data**

Adapted from Lee and Go (1997).

<table>
<thead>
<tr>
<th>Nonparametric approaches</th>
<th>Function</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kaplan-Meier</strong> or <strong>Product Limit Method (PLM)</strong> (Kaplan and Meier, 1958)</td>
<td>$S_{KM} = \prod_{t_i &lt; t} \frac{r(t_i) - d(t_i)}{r(t_i)}$</td>
<td>Useful in estimating the survival time distribution, $S(t)$. PL estimates are limited to the time interval in which the observations fall.</td>
</tr>
<tr>
<td><strong>Gehan’s Generalized Wilcoxon test</strong> (Gehan, 1965)</td>
<td>$W = \sum_{i=1}^{n_1} U_i^2$, $Var(w) = n_1 n_2 \sum_{i=1}^{n_1+n_2} U_i^2$</td>
<td>Comparison of two survival distributions. Recommended when logrank cannot be applied.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Semiparametric approaches</th>
<th>Function</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cox proportional hazard model (CPHM)</strong> (Cox, 1972)</td>
<td>a) Cox’s hazard function: $h(t; x) = h_0(t)e^{\beta x}$ where $h_0(t)$ is the hazard function. b) Conditional proportional density function for T: $f(t; x) = \lambda_0(t)e^{\beta x}\exp\left[ e^{\beta}\int_0^t \lambda(u)du \right]$ c) Conditional survival function for T under z: $S(t; x) = [S_0(t)]^{\exp(\beta x)}$ where $S_0(t) = \exp\left[-\int_0^t \lambda_0(u)du\right]$</td>
<td>Useful to obtain information about bias and efficiency of the estimated regression coefficients for a variety of situations, in particular, when fundamental model assumptions are violated</td>
</tr>
</tbody>
</table>
Objective III. Run simulation studies in order to: (i) Define efficient (in terms of costs and number of individuals) experimental designs in containment studies across a range of scenarios in order to achieve precise survival assessments

Following Rogers et al. (2014), simulations will be used to evaluate precision in the ability to detect survival in paired-design (control and treatment fish in the same tank) containment experiments. Evaluations will be performed across a range of design scenarios in which sample size (i.e. number if fish per container) and replication (i.e. number of containers) are independent variables.

A conceptual model for post-release survival mortality (Pollock and Pine, 2007)

The simplest (and ideal) situation to estimate post-release mortality is where there are both “control” and “treatment” fish. Here, treatment fish will be considered as those which have been caught, released and hence show different states of individual condition due this process that may affect their survival (Pollock and Pine, 2007). The control fish are assumed identical to the treatment fish except that they have not been subjected to the catch-and-release process and hence no condition of the individual can be related to their survival. All fish will be monitored for the same period.

An instantaneous-rate mortality model will be used for both groups assuming additivity of the mortality components. The total mortality \((M)\) for the control fish at the time \(T\) \((M_C)\) is defined as:

\[
M_C = M_{HA} + M_{CC}
\]

Equation 4: Total mortality for control fish

Adapted from Pollock and Pine (2007)
and the total post-release mortality ($M_{PR}$) will be defined as:

$$M_{PR} = M_{HA} + M_{RE}$$

Equation 5: Post-release total mortality
Adapted from Pollock and Pine (2007)

where $M_{HA}$ is the handling mortality (for being placed in a cage or being tagged) for control and treatment fish, $M_{CC}$ is the extra mortality resulting from captures of the control fish and $M_{RE}$ is the mortality due to the fish being caught and released. The goal of the simulation process estimate $M_{RE}$ (the extra mortality resulting from the catch-and-release process) in an unbiased manner.

Design of containment-based post-release survival studies

An important issue in containment-study designs is the question of what is the valid experimental unit. It is unfortunately common for fisheries scientist to use, for example, one cage and view individual fish in the cage as the experimental unit. Standard results based on the binomial distributions are then used to obtain standard errors of survival rates (and hence mortality rates) (Pollock and Pine, 2007). However, this measure of variation is incorrect as it ignores cage-to-cage effects. Better experimental designs are either completely random or randomised block designs (Steel et al., 1997) based on the replicate cages.

For a completely random design, I will consider a replicate cage set-up for simulations: paired-designed containment studies.

Paired design containment studies: where we have $r$ cages and in each cage put both control and treatment fish. Under this randomised block design (or paired design if there are only two groups), the treatment mortality (Equation 6), the mean (Equation 7) and the standard error of the mean (Equation 8) will be calculated for each cage using the next equations in the simulation process:
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

\[
\hat{M}_T = 1 - e^{-\hat{M}_T} = 1 - \left\{ \frac{\hat{S}_T}{\hat{S}_C} \right\}
\]

**Equation 6: Mortality rate estimate for treatment fish**
Adapted from Pollock and Pine (2007)

\[
\hat{S} = \sum_{i}^r \hat{S}_i
\]

**Equation 7: Estimate of survival for random-designed containment experiments**
Adapted from Pollock and Pine (2007)

\[
SE(\hat{S}) = \sqrt{\frac{\sum_{i}^r (\hat{S}_i - \hat{S})^2}{r(r-1)}}
\]

**Equation 8: Standard error for control and treatment fish in random-designed containment studies**
Adapted from Pollock and Pine (2007)

**Simulation procedures**

A binomial simulation of survival rates was carried out for individuals for each group (i.e. treatment and control) as a binomial process. Each individual had a probability of surviving determined by treatment-dependent handling and treatment-dependent (i.e. condition) mortality inputs. The number of treatment and control fish in each enclosure was equal, thus the number of total fish per container had to be an even number.

Data sets were fully independently simulated and assumed that fish were fully in each independent from other fish in other containers and that there was no interaction among individuals in the same tank that may affect survival (e.g. predation).

The different scenarios where defined by a combination of number of containers (i.e. sample size) and number of fish per container (i.e. replicates). The number of fish in each simulated container ranged from 2 to 40 by 2, equally divided between control and treatment. I simulated
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

a range of containers from 2 to 20 by 1. Since I’m trying to apply this model to assess PRS of any kind of shark, the scenarios considered encompass a complete range of possible experimental designs that may be used for these studies. The selected scheme would depend on particular characteristics of the studied species that may affect captivity requirements and facilities design (i.e. individual factors such as size, age, behaviour, susceptibility to stress, health status, etc) (Choromanski, 2004) and the logistics and resources available for the research.

Treatment survival was estimated for each group as the difference between control survival and treatment mortality (see Equations 4 and 5) and standard errors (see Equation 8) of the mean treatment survival was calculated for each container to determine 95% confidence intervals.

The different data sets was generated within the R statistical programming environment (R Development Core Team, 2012). The R-syntax that has been used to generate the data was adapted from Rogers *et al.* (2014) and it is described in detail in Appendix C.

Simulation inputs of mortality rates for brownbanded bamboo sharks (*Chiloscyllium punctatum*) were hard to find as there is not mortality/survival studies available for these species so far. However, based on prior personal experience and Braccini *et al.* (2012) conclusions, we assumed that post-release survival rates for this species was relatively high (90-100%). Therefore, the baseline handling survival (constant control survival) considered for simulations was 95%. A range of additive condition (treatment) mortalities were simulated ranging from 5%, 10%, 20%, 40% to 80%. Constant treatment mortality rates of 20% were used to simulate a range of additive control survival rates from 55% to 95% in increments of 10% in order to determine if differences in control and tagging survival affects the precision of mortality estimates under a certain experimental design. The combination of simulated data sets is presented in Table 4.

To determine the average expectation of each scenario, I repeated simulations 1000 times (Rogers *et al.*, 2014).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Table 4: Generated data sets for containment studies’ simulation

<table>
<thead>
<tr>
<th>Simulated data sets</th>
<th>Constant handling mortality</th>
<th>Constant tagging mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (handling survival)</td>
<td>Treatment mortality</td>
<td>95%</td>
</tr>
<tr>
<td>95%</td>
<td>5%</td>
<td>10%</td>
</tr>
</tbody>
</table>

**Evaluation metrics**

The comparison of simulated results with the true values used to simulate the data provides a measure of the performance (i.e. evaluation) and associated precision of the simulation process (Burton *et al.*, 2006). For each of the simulated data sets outlined in Table 4, the next evaluation metrics will be calculated in order to assess efficacy of each scenario to precisely assess survival rates:

1. **Relative bias** in treatment mortality estimates that will be calculated using the formula:

   \[
   bias = (\hat{M}_T - M_T) \times M_T^{-1}
   \]

   **Equation 9: Formula used to calculate relative bias of simulated data**

Adapted from Pollock and Pine (2007)

where \( \hat{M}_T \) is estimated treatment mortality for the \( i \)th iteration and \( M_T \) is the true (observed) treatment mortality level. I then derived the average of the relative departures for all iterations for a given parameter scenario and reported that value as bias.
(2) **Average standard error** in treatment mortality estimate that was calculated using Equation 8.

(3) **Percent of coverage** of treatment mortality estimates that was determined as the number of iterations during which the 95% confidence interval of estimated tagging mortality included the true value of a given parameter se.

(4) **The mean p-value** from simulation replications for an analysis of variances (ANOVA) that will test for differences in survival rates between control and treatment fish with containers as replicates for each iteration.

(5) **Precision** of each experimental design to assess survival rates was determined as a ratio of the standard error and mean of the estimated treatment mortality.

These evaluation metrics helped us to determine the best-case scenario or minimum number of fish needed to define the most accurate experimental design for containment studies which precisely assessed survival for the brownbanded bamboo shark example. In order to achieve this, a “decision matrix” (Table 5) was developed in excel where all the evaluation metrics where combined under the “IF” statement. The next rules were followed to determine significant limits for each of the metrics: (1) Only simulations with ≥90% coverage where considered†; (2) Only simulations with <10% of bias where allowed‡; (3) Only simulations with a p-value ≤0.05 (ANOVA) where accepted§; and (4) only a simulations with ≤10% where took into account**.

† Based on Rogers *et al.*, (2014).
‡ Based on power analysis from Quinn and Keough (2002).
§ This metric reflects statistical power among experimental designs (Hoenig and Heisey, 2001).
** Based on power analysis from Quinn and Keough (2002).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Table 5: Example of the “decision matrix” used in the current simulations to determine the best-case scenario (minimum of fish and tanks needed) of experimental design for containment studies in order to precisely assess survival when treatment mortality=40% and control survival=95%

Decision rule=IF(Coverage!B2<0.9;0;SI(Bias!B2>0.1;0;SI(Bias!B2<-0.1;0;IF(PValue!B2>0.05;0;IF(Precision!B2>10;0;1))))

The light orange cells are the ones that will provide a precise assessment of survival considering number of containers and number of fish per container. In this example, and for our scenario of the brownbanded bamboo shark, minimum numbers of fish that will be needed to accurately determine survival will be 360 (40 fish per container; 9 containers).
Objective III. Run simulation studies in order to: (ii) Assess effect of condition on post-release survival rates of brownbanded bamboo sharks (*Chiloscyllium punctatum*) in the Gulf of Thailand using survival analysis based on accurate experimental design of containment studies.

In the following section, simulation procedures were used to assess PRS rates for the example of brownbanded bamboo sharks in the Gulf of Thailand in relation to one covariate/factor (i.e. condition of the individual††). Assessments of post-release survival rates for individuals were based on the obtained outputs (i.e. number of fish) from previous simulations which had been established as accurate to assess survival precisely. Therefore, a significant influence of condition of individuals on their survival rates is expected.

The logic and steps of the simulation process are outlined in this section. The *R*-code that has been used to generate this data will be similarly described in detail to allow users to replicate the process when similar research is developed (Appendix D).

Generation of data sets follow Fox (2002) and Bender *et al.* (2005) simulation procedures for survival data within the *R* statistical programming environment (R Development Core Team, 2012).

Once the data was generated, the survival estimates and functions were estimated using both nonparametric and semiparametric estimates (*Kaplan-Meir* and Cox proportional hazards, respectively).

**Simulation procedures**

The steps used to generate time-to-event data for brownbanded bamboo sharks are presented below. I implemented *R*-code and procedures (for the detailed *R*-routine see †† It is known that post-release survival of sharks depends on different factors (see Section 1.4). Condition of the individual may reflect the treatment (i.e. handling practices, hauling processes, time spent on deck, etc.) at which animals are exposed prior being discarded.
Appendix D), including \texttt{survfit}, \texttt{Surv}, \texttt{survreg} functions, all included in the \textit{R}-package “survival” (Therneau, 2014). This package allows \textit{R} users to (among other functions): conduct survival analysis using nonparametric (i.e. Kaplan Meier method), and semi-parametric (i.e. Cox proportional hazards model) approaches and the application of parametric distributions (i.e. \textit{Weibull}, \textit{lognormal}, \textit{exponential}, etc.) to the studied data.

Survival rates will be simulated in relation to a condition factor, which is thought to be representative of handling and release practices and can be significant causes of post-release mortality (Gilman et al. 2013). Condition will be generated in a range from 1 to 3, where 1 represents “very good” condition of the individual and 3 is “poor” (See Appendix B for more detail on the categorical criteria that will be used to assess condition of the individuals).

- **Step 1**: Covariates (i.e. \textit{condition of the individual}) were generated randomly between specified values (1-3) for each individual.

- **Step 2**: Survival times (\textit{lifetimes}) for each individual by condition where generated following the \textit{exponential} distributions (See table 2 for survival functions and applications of the distributions).

- **Step 3**: Inputs for the present simulation process will be obtained from previous analyses that will define accurate experimental designs to precisely assess survival of brownbanded bamboo sharks in the Gulf of Thailand. These will include number of individuals or sample size ($N$), Duration of the experiment (half of the duration of monitoring of fish in captivity in days) and estimated survival rate ($\lambda$);

Data sets were fully independently simulated assuming that fish in each container all fully independent from fish in another container and that there is not interaction among individuals from the same tank that may affect survival such as predation), generating a completely different set of data for each method and scenario considered.
Simulated data will follow an exponential distribution (see table 3). The exponential distribution is one of the most important distributional forms in modelling survival data and frequently employed to build up survival parametric models (Jiang et al. 2012).

**Estimates obtained from each simulation**

Non-parametric (Kaplan-Meier), semiparametric (Cox proportional hazards regression model) approaches will be applied to analyse the data in order to obtain the estimates for each simulation.

It is essential to plan how to the estimates will be stored after each simulation. Storing estimates enables consistency checks to be performed and allows for the identification of any errors or outlaying values and the exploration of trends and patterns within the individual simulations that may not be observed from the summary measure alone (Burton et al. 2006).

The estimates of interest, \( \hat{\beta}_i \), for the present study will include: (i) the parameter estimate after fitting regression models with semi-parametric (Cox proportional hazards regression model), (ii) the log-hazard ratio (or log-rank test in R) with its 95% confidence intervals for the Cox proportional hazards model and (iii) a simulation standard error (SE) for the estimate of interest, \( \text{SE}(\hat{\beta}_i) \).

Figure 12 shows an output of the R-procedures used to generate survival data using the Cox proportional hazards model. An example of the obtained estimates of interest [(i),(ii), (iii)] that will be stored after each simulation is provided.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Call:
```r
coxph(formula = Surv(time, censor) ~ factor(condition), method = "breslow")
```

n= 100, number of events= 100

| factor(condition) | coef     | exp(coef) | se(coef) | z    | Pr(>|z|) |
|-------------------|----------|-----------|----------|------|----------|
| factor(condition)2| -0.07312 | 0.92949   | 0.24150  | -0.303 | 0.762    |
| factor(condition)3| -0.01299 | 0.98709   | 0.25241  | -0.051 | 0.959    |

exp(coef) exp(-coef) lower .95 upper .95

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<tr>
<th>factor(condition)</th>
<th>exp(coef)</th>
<th>exp(-coef)</th>
<th>lower .95</th>
<th>upper .95</th>
</tr>
</thead>
<tbody>
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<td>1.076</td>
<td>0.5790</td>
<td>1.492</td>
</tr>
<tr>
<td>factor(condition)3</td>
<td>0.9871</td>
<td>1.013</td>
<td>0.6019</td>
<td>1.619</td>
</tr>
</tbody>
</table>

Concordance= 0.511 (se = 0.045 )
Rsquare= 0.001  (max possible= 1 )
Likelihood ratio test= 0.1  on 2 df,  p=0.9503
Wald test            = 0.1  on 2 df,   p=0.9505
Score (logrank) test = 0.1  on 2 df,   p=0.9505

Figure 12: R-procedures used for survival data simulation using the Cox proportional hazards regression model showing the estimates of interest that will be stored after each simulation

The data was generated following an exponential distribution with λ=0.5, for n=100 individuals and 20 days of captivity. The estimates of interest that will be stored after each simulation are surrounded by green squares and denoted by (i), (ii) and (iii)

It is also important to establish how to summarize these estimates once all simulations have been performed (Burton *et al.* 2006). In the present study we are going to report the average estimate of interest over the *N* simulations performed as a measure of the true estimate of interest:
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

\[ \hat{\beta} = \frac{\sum_{i=1}^{N} \hat{\beta}_i}{N} \]

**Equation 10: Average estimate of interest over the \( N \) simulations**

Adapted from Burton *et al.* 2006).

An assessment of the uncertainty in the estimate of interest between the simulations is necessary. For this purpose we will use empirical SE, calculated as the standard deviation of the estimates of interest in all simulations:

\[ \sqrt{\frac{1 - (N - 1)}{N} \sum_{i=1}^{N} (\hat{\beta}_i - \overline{\hat{\beta}})^2} \]

**Equation 11: Empirical Standard Error**

Adapted from Burton *et al.* (2006).

The empirical SE (i.e. standard deviation) should be close to the average of the estimated within simulation SE if the estimates are unbiased (Schafer *et al.* 2002) and therefore, it may be sensible to consider both estimates of uncertainty.

**Evaluation and efficiency measures for estimates**

Then it is necessary to consider the criteria for evaluating the performance of the obtained results from the different scenarios or statistical approaches being used (Burton *et al.* 2006). To achieve this, after each simulation was completed, the next evaluation measures described by Zhao (2008) will be obtained:

1. **Average bias** as the difference between the true survival probability at time \( t \) and the average of the 1000 simulations estimated survival at time \( t \). This will be denoted as
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

\[
\text{Bias}\{\hat{S}(t)\} = \bar{S}(t) - S(t)
\]

Equation 12: Average bias as an evaluation measure of the performance of used simulation methods
Adapted from Zhao (2008).

(2) **Mean Square Error Estimates (MSE)** will be calculated using Equation 15:

\[
MSE = [\text{Bias}\{\hat{S}(t)\}]^2 + \text{Var}\{\hat{S}(t)\} = \{\bar{S}(t) - S(t)\}^2 + \text{Var}\{\hat{S}(t)\}
\]

Equation 13: Mean Square Error Estimates (MSE)
Adapted from Zhao (2008).

where \( S(t) \) is the true survival probability at time \( t \), and \( \text{Var}\{\hat{S}(t)\} \) is the variance of the 1000 estimates at time \( t \).

(3) **Relatively Efficiency (RE)** for comparing the Kaplan-Meier and parametric estimators:

\[
RE = \frac{MSE(\text{Kaplan-Meier estimation})}{MSE(\text{parametric estimation})}
\]

Equation 14: Relatively Efficiency of parametric and nonparametric methods
Adapted from Zhao (2008).
5. Results

In the next section, results from the simulation procedures and the “decision matrix” are presented. There are two groups of simulations which results are outlined: (1) the simulations following Rogers et al. (2014) which will allow us to define an efficient experimental design of containment studies and achieve precise survival assessments. These results are presented in sets of 4 graphics, to allow the reader to compare the obtained outputs from the four different studied scenarios (i.e. different sample sizes and replicates) and the effect on the variation of treatment and control mortality; and (2) results from the survival assessments for the example of brownbanded bamboo sharks performed by nonparametrical and semiparametrical methods. These results will be linked to the previous simulations in the way that assessments of survival for the brownbanded bamboo sharks will be performed using outputs (i.e. number of fish) determined by efficient experimental designs. Graphics are also presented in sets of four to allow for comparison of different scenarios (i.e. different treatment mortality).
Objective III: Run simulation studies in order to: (i) Define efficient (in terms of costs and number of individuals) experimental designs in containment studies across a range of scenarios in order to achieve precise survival assessments.

The performed simulations allowed for direct comparison of trade-offs in the number of containers (i.e. replicates) versus total number of fish per container for informing experimental designs of post-release survival/mortality studies.

As expected, experiments with few individual fish and little replication were most biased. In experiments with low number of replicates, increasing the number of fish per container was marginally effective for improving bias (reducing bias in 1% to 5%; Fig. 13-15).

As treatment mortality increased, estimates of bias and precision (i.e. standard errors) improved at lower number of replicates and fish per container relative to low treatment mortality (Fig. 16-18).

Based on Rogers et al. (2014), we decided to define suitable coverage levels (at 95% confidence) greater or equal than 90%. For all the evaluated treatment mortality values, acceptable coverage levels (≥90%) were only obtained with >7 containers (Fig. 19-21). However, when differences between treatment mortality and control mortality are small (i.e. treatment mortality=80%), it appears that we can reach 90% of coverage with >7 containers. The number of fish per container (i.e. sample size) had little effect on coverage (Fig.19-21) thereby requiring to use a minimum of >7 replicates when estimating treatment mortality under this survival scenario.

Experiments with low treatment mortality and few replicates had a low ability to detect the effects of condition of the individual on survival (ANOVA). For our brownbanded bamboo shark scenario (i.e. low treatment mortality rates), I concluded that a minimum of 2 replicates with 8 fish per container will allow us to detect additive treatment mortality ≥0.05 (Fig. 22 and 24) when differences between treatment and control mortality are minor (i.e. treatment...
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

mortality=80%; control survival=95%). However, when differences between control and treatment mortality increase (i.e. treatment mortality=5%; control survival=95%), the minimum replicates (containers) and sample size that allowed us to detected additive mortality ≥0.05 increase considerably with a minimum of 8 replicates with 36 fish per container (Fig. 22 and 24).

Results based on a combination of all the examined evaluation measures (i.e. $p$-value, precision, coverage and bias) were obtained by using a “decision matrix”. The matrix helped to determine the best-case scenario where experimental designs of containment studies will assessed post-release survival rates (for the example of the brownbanded bamboo shark) precisely. For control survival 95%, the minimum number of fish needed to provide precise estimates of post-release survival was 90 fish when treatment mortality was 40% (Fig.25). For the same control survival, 360 fish were needed to achieve the same results when treatment mortality was 80% (Fig.25). These results suggested that for individuals with higher mortality rates (i.e. treatment mortality), greater number of fish are needed in the experiments to precisely assess survival. In the example of brownbanded bamboo sharks, which are thought to present low mortality rates (we assumed this based on reviewed papers and personal experience as there is no survival or mortality estimates available for this kind of sharks), 360 individuals (9 fish/400 tanks) should give accurate estimates of post-release survival for containment experiments. These numbers are fairly high and seem impossible to achieve in terms of logistics and costs.

Optimal experimental design was dependent upon mean control fish survival and variability of mean control fish survival across containers. Lower survival of control fish resulted in a need for more experimental effort to maintain precision. For example, the ability to detect a significant difference between handling and no-handing mortality when alpha was equal to 0.05 could be achieved when control survival was 85% and treatment mortality 20% by using 5 containers with 34 fish per container, but required additional 5 containers and 6 fish per container when control survival decreased to 75% with 20% of treatment mortality. Therefore, experimental design requires an expectation of what baseline control mortality may be and careful consideration to minimize among container variation.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**Figure 13:** Simulated relative bias in treatment mortality estimates relative to input value (panels) across a range of scenarios when control (handling) survival=95%.

Lighter colours indicate less biased experiments.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 14: Simulated relative bias in treatment mortality=5% relative to number of containers and number of fish per container across a range of scenarios when control (handling) survival=95%.

Peaks indicate estimated relative bias for each combination of experiments defined by number of containers vs. number of cages. Highest peaks determine more biased experiments.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 15: Simulated relative bias in treatment mortality=80% relative to number of containers and number of fish per container across a range of scenarios when control (handling) survival=95%.

Peaks indicate estimated relative bias for each combination of experiments defined by number of containers vs. number of cages. Highest peaks determine more biased experiments (for treatment mortality=80%, only experiments with <6 cages show high relative biases).
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 16: Precision (SE) in treatment mortality estimates across a range of scenarios when control (handling) survival = 95%.

Darker colours indicate more precise experiments (i.e. experiments with lower standard errors) (e.g. for treatment mortality = 40% the lowest standard errors are obtained for 20 cages and 40 fish/cage. As number of cages and fish starts decreasing, standard error and hence precision, starts to decrease as well.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllum punctatum*) of the Gulf of Thailand.

**Figure 17**: Precision (SE) in treatment mortality estimates relative to number of containers and number of fish per container across a range of scenarios when control (handling) survival = 95% and treatment mortality = 5%

Colours show different values of estimated standard error (SE) for each experiment. As the grid flattened, SE are lower for the considered combination of number of cages vs. number of fish/cage number of cages.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 18: Precision (SE) in treatment mortality estimates relative to number of containers and number of fish per container across a range of scenarios when control (handling) survival=95% and treatment mortality=80%

Colours show different values of estimated standard error (SE) for each experiment. As the grid flattened, SE are lower for the considered combination of number of cages vs. number of fish/cage number of cages.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 19: Percentage coverage of estimated 95% confidence intervals for treatment mortality estimates across a range of scenarios when control (handling) survival=95%.

Lighter colours indicate a higher percentage of coverage for each experiment (i.e. for treatment mortality=40%, highest values of coverage are obtained for >10 cages). The lines show the 90% limit at which experiments were considered to be significant.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 20: Percentage coverage of estimated 95% confidence intervals for treatment mortality estimates relative to number of fish per container and number of containers across a range of scenarios when control (handling) survival=95% and treatment mortality=5%

Different colours show different levels of coverage for the different experiments. An even and flat surface of the grids is shown when coverage levels are close to 90%. As the coverage level starts decreasing, the grid starts dropping down towards zero.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 21: Percentage coverage of estimated 95% confidence intervals for treatment mortality estimates relative to number of fish per container and number of containers across a range of scenarios when control (handling) survival = 95% and treatment mortality = 80%.

Different colours show different levels of coverage for the different experiments. An even and flat surface of the grids is shown when coverage levels are close to 90%. As the coverage level starts decreasing, the grid starts dropping down towards zero.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 22: Average *p*-value resulting from analysis of variance tests for differences in mortality between control and treatment fish across a range of scenarios when control (handling) survival=95%.

Darker colours show significant *p*-values (<0.05) for different experiments in relation to number of cages and number of fish/cage. Grey even surfaces are shown for constant significant *p*-values as opposite as panels, where *p*-value and significance can vary from one experiment to another.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllum punctatum*) of the Gulf of Thailand.

Figure 23: Average *p*-value resulting from analysis of variance tests for differences in mortality between control and treatment fish relative to number of fish per container and number of containers across a range of scenarios when control (handling) survival = 95% and treatment mortality = 5%.

Different colours show different *p*-values for the experiment in relation to number of cages and number of fish/cage. An even and flat surface of the grid is shown when constant significant *p*-values (<0.05) are obtained for the different scenarios. As significance (*p*-values < 0.05) the grid starts to elevate.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**Figure 24:** Average *p*-value resulting from analysis of variance tests for differences in mortality between control and treatment fish relative to number of fish per container and number of containers across a range of scenarios when control (handling) survival=95% and treatment mortality=80%

Different colours show different *p*-values for the experiment in relation to number of cages and number of fish/cage. An even and flat surface of the grid is shown when constant significant *p*-values (<0.05) are obtained for the different scenarios. As significance (*p*-values<0.05) the grid starts to elevate.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

<table>
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<th>Number of fish per container</th>
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<table>
<thead>
<tr>
<th>Number of containers</th>
<th>Number of fish per container</th>
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<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Treatment mortality=40%; Control survival=95%</td>
<td>Treatment mortality=80%; Control survival=95%</td>
<td></td>
<td></td>
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<td></td>
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Figure 25: “Decision matrix” used in the current simulations to determine the best-case scenario (minimum of fish and tanks) of experimental design for containment studies in order to precisely assess survival when treatment mortality=40%; 80% and control survival=95%.

The minimum number of fish needed to provide precise estimates of post-release survival for treatment mortalities of 40% and 80% are outlined by black discontinuous lines in each matrix.
Objective III: Run simulation studies in order to: (ii) Assess post-release survival rates of brownbanded bamboo sharks (*Chiloscyllium punctatum*) in the Gulf of Thailand using survival analysis based on the most accurate experimental design of containment studies

Previously performed simulations allowed us to define the best-case scenario to determine the most accurate experimental design for precise survival assessments. For our brownbanded bamboo sharks example (treatment mortality=40%), accurate assessments of survival were achieved with 360 individuals (9 fish/40 tanks). For the case of species which present higher mortality rates (80%), 90 fish (9 fish/10 tanks) were necessary to attain the same results. Based on these outputs, survival rates were assessed for the two different considered scenarios (90 fish or 360 fish) for treatment mortalities 80% and 40%, respectively.

Survival estimates were obtained applying nonparametric methods (i.e. *Kaplan Meier*) and semiparametric methods (i.e. Cox proportional hazards). Survival data was generated (for the two methodologies) in relation to three treatment groups (dependent on condition of the individual): excellent (condition 1), good (condition 2) and poor (condition 3).

For the case of the two treatment mortalities considered (i.e. 80% and 40%), simulations showed that most of the individuals died within the first 3 days of the experiment (Fig. 22-25). As condition deteriorates, number of individuals at risk increment and fewer events (i.e. deaths) were recorded. Baseline mortality rates influence the variation and distribution of the number of events (in each of the considered conditions) during the captivity period, independently from the defined experimental design (i.e. number of replicates and sample size). For example, in simulations when a treatment mortality rate of 80% was considered, the number of events was randomly distributed along the whole study period (i.e. 5 days). However, for individuals with a lower treatment mortality (i.e. 40%), most of the events took part during the first 3 days of the experiment, allowing to detect differences in survival with shorter periods of captivity (Fig. 23-25). This suggests that mortality rates of each species should be taken into account prior the
condonation of confinement post-release survival experiments. Otherwise, the results could be biased by the duration of the study confounding results.

As expected, simulated survival time decreased as condition of the individual deteriorated and differences in survival rates appeared to be higher when treatment mortality was low (Fig. 26).

Results from the log-rank test (Mantel, 1966) show that there was a significant difference ($\chi^2=7.8; \ p=0.02$) in survival among conditions when alpha=0.05, independently from the experimental design (Table 6). However, for a higher number of fish and lower mortalities (i.e. 40%) the log-rank test was not able to detect significant differences in survival among the considered treatment groups (i.e. conditions).

Table 6: Results of differences in simulated survival times among conditions from log-rank test (Kaplan-Meier estimator).

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Treatment mortality</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 tanks; 10 fish per tank</td>
<td>80%</td>
<td>$\chi^2$=26.5</td>
<td>$p=1.75\times10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>$\chi^2$=41.9</td>
<td>$p=7.88\times10^{-10}$</td>
</tr>
<tr>
<td>9 tanks; 40 fish</td>
<td>80%</td>
<td>$\chi^2$=42.1</td>
<td>$p=7.34\times10^{-10}$</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>$\chi^2$=179</td>
<td>$p&lt;0.05$</td>
</tr>
</tbody>
</table>

Analyses from regression (Cox proportional hazards model) show that condition highly affects survival as the $p$-values from all the simulations are highly significant (Table 7). Influence of condition on survival appeared to be affected by number of fish independently from mortality (highest $p$-value are achieved with 360 fish and 80% or 40% of treatment mortality).

Condition appeared to influenced survival negatively (i.e. $\hat{\beta}_i$ is negative) or in other words, for this simulated data, survival of individuals decreased when condition deteriorates (Table 7). Condition 3 seemed to be the one that most affected survival ($\hat{\beta}_i=-2.365$) suggesting that an
individual that is landed in poor condition may less chances to survive than one that is landed in good condition.

**Table 7: Estimates of survival resulted from Cox regression for experiments with 90 and 360 fish and 40% and 80% treatment mortality when control mortality was 95%**.

Significant p-values are highlighted in orange colour ranging from light orange when slightly significant to brown when highly significant. Parameter are highlighted in purple colour ranging from light purple when condition slightly affects survival (i.e. \(\hat{\beta}_i<1\)) and dark purple when condition highly affects survival (i.e. \(\hat{\beta}_i>1\)). (c n. is for condition; only condition 2 and 3 are shown as regression is calculated with condition 1 against each one of the other conditions).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment mortality</th>
<th>Parameter ((\hat{\beta}_i))</th>
<th>Estimate</th>
<th>Standard Error (95%CI)</th>
<th>p-value</th>
</tr>
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<tr>
<td>360 fish</td>
<td>80%</td>
<td>-0.7637</td>
<td>-1.0784</td>
<td>0.4659 [0.1868, -4.089]</td>
<td>4.34e-5</td>
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<tr>
<td></td>
<td></td>
<td>-1.3238</td>
<td>-2.36575</td>
<td>0.26610 [0.1665; -7.951]</td>
<td>1.89e-15</td>
</tr>
<tr>
<td>40%</td>
<td></td>
<td>-1.2429</td>
<td>-1.4024</td>
<td>0.2885 [0.3211; -3.871]</td>
<td>0.000109</td>
</tr>
<tr>
<td>90 fish</td>
<td>80%</td>
<td>-0.8661</td>
<td>-1.6551</td>
<td>0.4123 [0.4202; -2.109]</td>
<td>0.0349</td>
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<tr>
<td></td>
<td></td>
<td>-1.2429</td>
<td>-1.4024</td>
<td>0.2885 [0.3211; -3.871]</td>
<td>0.000141</td>
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</table>

Simulation results of survival assessments by the Cox proportional hazards method resulted in significant (only for 360 fish and treatment mortality=40% results were not significant according to the log-rank test. See table 6) differences of survival in relation to treatment (i.e. condition) for the example of brownbanded bamboo sharks. In addition, Kaplan-Meier estimations shown that condition was related to survival as poorer condition resulted in lowest survival rates. These outcomes provide a justification for the Animal Ethics Committee concerns in relation to the number of brownbanded bamboo sharks needed (i.e. 120
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

individuals) for my first proposed experiment showing that large number of individuals is needed in order to provided precise survival assessments when the target species have low mortality rates.
Figure 26: Estimated number of events (± SE), number at risk and survival times by condition obtained by the Kaplan Meier estimator when treatment mortality=80% for 9 containers and 10 fish per container.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

**Figure 27**: Estimated number of events (± SE), number at risk and survival times by condition obtained by the Kaplan Meier estimator when treatment mortality=80% for 9 containers and 40 fish per container.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

2014

Figure 28: Estimated number of events (± SE), number at risk and survival times by condition obtained by the *Kaplan Meier* estimator when treatment mortality=40% for 9 containers and 10 fish per container.

<table>
<thead>
<tr>
<th>condition=1</th>
<th>time</th>
<th>n.risk</th>
<th>n.event</th>
<th>survival</th>
<th>std.err</th>
<th>lower 95% CI</th>
<th>upper 95% CI</th>
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<tr>
<td>0.001</td>
<td>35</td>
<td>10</td>
<td>0.714</td>
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<th>n.event</th>
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<th>std.err</th>
<th>lower 95% CI</th>
<th>upper 95% CI</th>
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<td>5</td>
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<th>n.risk</th>
<th>n.event</th>
<th>survival</th>
<th>std.err</th>
<th>lower 95% CI</th>
<th>upper 95% CI</th>
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<td>0.96</td>
<td>0.0392</td>
<td>0.886</td>
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<td>0.761</td>
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A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllum punctatum*) of the Gulf of Thailand.

Figure 29: Estimated number of events (± SE), number at risk and survival times by condition obtained by the *Kaplan Meier* estimator when treatment mortality=40% for 9 containers and 40 fish per container.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

Figure 30: Estimated survival function with Kaplan-Meier estimator by condition for 9 containers and 10 fish per container when treatment mortality=20%.
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

6. Discussion

Developing a tool to assess trade-offs in the number of containers employed and number of fish per container was able to maximize design efficiency for a desired precision.

The simulations confirmed the expectations that increased number of replicates (i.e. containers) and fish per container (i.e. sample size) would provide less biased and more precise estimates of treatment mortality. Much of these results are statistically intuitive in nature, but only one post-release survival investigation (*Rogers et al.*, 2014) has formally quantified the trade-offs in containment-study designs that could be considered along with logistical limitations (e.g., numbers of containers available, number of fish that can be collected or held) in post-release survival studies.

The effectiveness in study designs were largely driven by the interaction of control and treatment survival rates. For an expected control survival, a more intensive experimental effort (i.e. increasing number of containers and fish per container) was required as additive treatment mortality (i.e. mortality due to condition of the individual) decreased. For fisheries management evaluations, the ability to detect strong post-release effects at lower experimental effort may be advantageous because the most deleterious treatments are the most easily to detect. In contrast, research studies that strive to impose minimal post-release effects would be required more intensive efforts to quantify treatment effects (*Rogers et al.*, 2014).

The use of the mean $p$-value from ANOVA illustrated on average the level of significance between differences in mortality between treatment and control fish and this metrics reflects statistical power among experimental designs (Hoenig and Heisey, 2011).

Sensitivity of control-fish survival to estimate treatment mortality can highly influence estimation and assessments of survival. Numerous authors (e.g. Pollock and Pine, 2007) have stated that true controls (i.e. fish with zero effects from the capture process) are practically impossible to obtain for post-release survival studies because there is always some unnatural handling, transport, or containment effects in the experiments (*Rogers et al.*, 2014). Collection
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

of control individuals is always made using a gear that is believed to be harmless for the collected individuals but some species will require collection with a riskier gear (i.e. gill nets) or experiments may take place when environmental conditions (i.e. air and water temperature) that might affect survival of control fish. This strong influence of control fish survival on treatment mortality estimation suggested a need for an a priori expectation of survival of the studied species. Failing on incorporating this data during the design process for containment studies might have negative consequences (i.e. obtaining poor or inconclusive results) and will cause a waste of resources (logistics and individuals used for research). One of the techniques that can be applied to obtain the necessary mortality/survival information for conducting survival research are “pilot studies”: a simple experiment where few individuals from the studied species are held in captivity for a defined short period of time (i.e. 5-10 days) and survival assessments are conducted. There is no need for control or treatment individuals in these experiments because the aim is to define survival rates of the species (not comparing survival among groups) which then will be used as an input for future containment-post-release studies. Unfortunately, there is little evidence that much research has been done in this area and post-release survival rates estimates are scarce for marine fish, especially for sharks. The few available post-release survival studies for sharks are presented in Appendix A. Most of them are focused on important bycatch species from commercial fisheries and other pelagic species (e.g. the blue shark; Campana *et al.*, 2009; Moyes *et al.*, 2009). There are no post-release studies available for brownbanded bamboo sharks and survival studies for other bottom-dwelling sharks are scarce (Frick *et al.*, 2009; Gruber *et al.*, 2001).

The design of post-release survival studies includes several more logistical and biological considerations than the ones that we took into account in our simulations. For example, the duration of the experiments (in order to evaluate immediate, short-term, and long-term survival (Pollock and Pine, 2007)). For the brownbanded bamboo shark example, simulations were based on a captivity period of 5 days (recommended by Wassenberg and Hill, 1993). One limitation of short-term experiments in survival assessments is that results are often treated as finite rates by assuming that all the treatment mortality (i.e. condition, tagging, etc.) was
accounted during the experimental period, while other methods (e.g. telemetry tags or condition/vitality data from field evaluations) may provide estimates that are more representative of the total mortality produced because of the capture process.

Species-specific characteristics may also limit options for experimental design. Dehart (2004) pointed out that there are three key factors that can influence wellbeing of sharks when they are kept in captivity: (1) how well it adapts to the rigors of the captive environment; (2) interspecific inter and intraspecific interaction of the target species with other individuals within the containers; (3) and feeding needs. Container structure and design (dimensions, materials, shape, etc.) and species-specific characteristics may also limit the number of fish per container before crowding starts affecting the wellbeing of the individuals.

Logistics considerations (i.e. availability of space, number of tanks, etc.) will also determine the selection of one or another experimental design in containment studies.

Pollock and Pine (2007) concluded that post-release studies are easy to design. Rogers et al. (2014) agreed in terms that they are easy to conceive, but tools for explicit evaluation of alternative designs were lacking. This lack of evaluation can lead to unsuccessful experiments. I consider that containment studies are useful to determine a priori survival or mortality rates that are needed. However, and due to the strong influence of control fish survival on treatment mortality estimation that has been proved by the actual results, containment experiments may not be suitable for species with low mortality rates. This is why a combination of methods (i.e. containment experiments and tagging) is recommended in order to achieve precise and complete post-release survival rates.

While my simulation procedures were focussed only in one for the available designs for post-release mortality studies (i.e. containment experiments), I recognize that outputs resulting from these experiments may be an underestimate due to the unnatural treatment of experimental fish in natural settings (Rogers et al., 2014).

For our brownbanded bamboo shark scenario, simulations indicated that is feasible to achieve a robust estimate of post-release survival. The generation of different survival curves
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

and estimates based on different condition factors may reflect physical and physiological trauma of individuals due to handling, deck time, hauling processes on-board, etc. Cox proportional analyses suggested that these factors may influence survival of post-released individuals, increasing mortality when condition of the individual was poorer. However, an observational research has to be done in order to demonstrate these outcomes and made appropriate conclusions.
7. Conclusions

I am aware that this study shows some limitations in relation of working with simulated data rather than data collected from observations. Therefore, several assumptions have been made as part of the simulation process (e.g., survival rates of brownbanded sharks, independence of samples, etc.) that may constrain the scope of the achieved results. However, the present study does show a need for a thoughtful planning in the early stages of the experimental design in order to identify the numbers of fish required to achieve significant results and emphasise the usefulness of simulation and modelling techniques as analytical tools, especially for designing experiments.

One of the most difficult aspects of the simulation process was to decide to what extent the evaluation metrics (i.e. precision, coverage, bias, etc.) was required to define a satisfactory experimental design. Although there is plenty of guidance in the coding and simulation process (i.e. how to obtain the metrics), there is a lack of direction in terms of attainment of benchmarks and limits of what could be considered an “acceptable” design for post-release containment studies in order to achieve precise assessment of survival. Therefore, a compilation of evaluation metrics stating the “acceptable” limits to reflect statistical power among experimental designs is needed.

A very large proportion of shark’s global catches is discarded (Bonfil, 1995; Fowler et al., 2002; Worm et al., 2013; Afonzo and Hazin, 2014) though little is known about the fate of discarded individuals. Hence, post-release survival information is rarely considered as part of the strategies addressing the management of shark species. The lack of post-survival studies for sharks is concerning and should be afforded scientific priority. Efficient containment-post-release survival studies can be applied to: (1) the identification of potential mitigation measures to improve the likelihood of successful live release of sharks and hence curtail the impacts of fishing on this species due to discards, and (2) the estimation of otherwise unaccounted fishing mortality which could contribute to improve the species-specific landings and biological
information gathering, especially bottom-dwelling species such as the brownbanded bamboo shark.

Much of the success of live release depends on other circumstances surrounding a fish’s capture and release process. In addition to on-board handling practices, a number of other factors affecting survival can be controlled, such as type and configuration of the gear used, the seasons and depths of fished, among others. The relative impact of these factors on survival potential needs to be quantified to increase the likelihood of achieving the desired management objectives related to post-release survival (Benoit et al., 2010).

The main incentive to developed the actual research came from the concerns of the Animal Ethics Committee about the number of animals that was going to be used for the first proposed experiment. The use of 120 sharks that was proposed in this first study was rejected by the Animal Ethics Committee claiming that it was not justification for the use of that amount of specimens for the experiment. However, and taking into account the present research results, for species with low mortality rates (i.e. brownbanded bamboo sharks) a number of 360 individuals was found to be the minimum number of fish to use if precise assessments of post-release survival rates wanted to be achieved. In this way, the actual research gives a justification for the usage of considerable large numbers of individuals for post-release assessments in containment studies. Conclusions obtained from this study’s results have clearly stated that ‘pilot studies’ are necessary in order to determine a priori survival/mortality rates from the target species as mortality estimates affect considerably experimental designs in containment studies. Thus, trial experiments, as my first proposed study, should be afforded the same importance as the definite experiments.
8. References


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Appendix A

Post-release survival (PRS) studies for different species of sharks that have been reviewed for the actual study.

<table>
<thead>
<tr>
<th>Primary literature</th>
<th>Species</th>
<th>Location</th>
<th>Approaches used for determining PRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Captivity</td>
</tr>
<tr>
<td>(Afonso and Hazin, 2014)</td>
<td>Juvenile tiger sharks</td>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>(Benoît et al., 2010)</td>
<td>Different sp.</td>
<td>US (CA)</td>
<td>X</td>
</tr>
<tr>
<td>(Braccini et al., 2012)</td>
<td>Different sp.</td>
<td>Australia</td>
<td>X</td>
</tr>
<tr>
<td>(Campana et al., 2009)</td>
<td>Blue shark</td>
<td>Hawaiian &amp; North Atlantic longline fisheries</td>
<td>Comparison between Campana, Joyce et al. 2009 and Moyes, Fragoso et al. 2006</td>
</tr>
<tr>
<td>(Campana et al., 2009)</td>
<td>Different sp.</td>
<td>Mexico</td>
<td>Review of post-release live-discard mortality estimates for shark by fishing gear type</td>
</tr>
<tr>
<td>(Davis and Ryer, 2003)</td>
<td>Different sp.</td>
<td>Review</td>
<td>Different factors affecting fish bycatch discard mortality</td>
</tr>
<tr>
<td>(Frick et al., 2009)</td>
<td>Port Jackson sharks and Australian swellsharks</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>(Gruber et al., 2001)</td>
<td>Juvenile lemon shark</td>
<td>Bahamas</td>
<td>PRS rates estimated by mark-depletion experiments</td>
</tr>
</tbody>
</table>
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species/Environment</th>
<th>Location</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Gurshin and Szedlmayer, 2004)</td>
<td>Atlantic sharpnose shark</td>
<td>Mexico</td>
<td>x</td>
</tr>
<tr>
<td>(Hoffmayer and Parsons, 2001)</td>
<td>Atlantic sharpnose sharks</td>
<td>US (the)</td>
<td>x</td>
</tr>
<tr>
<td>(Hueter, 1994)</td>
<td>Different sp.</td>
<td>US (flora)</td>
<td>x</td>
</tr>
<tr>
<td>(Heupel and Simpfendorfer, 2002)</td>
<td>Juvenile blacktip shark</td>
<td>US (flora)</td>
<td>x</td>
</tr>
<tr>
<td>(Kneebone et al., 2013)</td>
<td>Juvenile sand tiger sharks</td>
<td>Jamaica</td>
<td>x</td>
</tr>
<tr>
<td>(Mandelman and Farrington, 2007)</td>
<td>Spiny dogfish</td>
<td>US (MA)</td>
<td>x</td>
</tr>
<tr>
<td>(Moyes et al., 2006)</td>
<td>Blue shark</td>
<td>Hawaiian longline fishery</td>
<td>x</td>
</tr>
<tr>
<td>(Musyl K. Michael, 2009)</td>
<td>Blue shark</td>
<td>Hawaiian &amp; North Atlantic longlines</td>
<td>Comparison between Campana, Joyce et al. 2009 and Moyes, Fragoso et al. 2006</td>
</tr>
<tr>
<td>(Skomal, 2007)</td>
<td>Different pelagic sp.</td>
<td>North Atlantic (US)</td>
<td>x</td>
</tr>
<tr>
<td>(Skomal and Mandelman 2012)</td>
<td>Different sp.</td>
<td>Review</td>
<td>x</td>
</tr>
</tbody>
</table>
Categorical criteria that will be used to assess condition of individuals [adapted from the International Pacific Halibut Commission (IPHC)].

<table>
<thead>
<tr>
<th>Index</th>
<th>Description</th>
<th>Condition category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Excellent (1)</td>
</tr>
<tr>
<td>Activity and stimuli</td>
<td>Physical activity and response to stimuli</td>
<td>Strong and lively</td>
</tr>
<tr>
<td></td>
<td>Active response to external stimuli</td>
<td>Active response to external stimuli</td>
</tr>
<tr>
<td></td>
<td>Tightly clench jaws</td>
<td>If provoked individual can clench jaws</td>
</tr>
<tr>
<td></td>
<td>No stiffness</td>
<td>No stiffness</td>
</tr>
<tr>
<td>Wounds and bleeding</td>
<td>Presence and type of wounds and bleeding</td>
<td>No big cuts</td>
</tr>
<tr>
<td></td>
<td>No small cuts</td>
<td>1-3 small cuts</td>
</tr>
<tr>
<td></td>
<td>No critical cuts or organ exposure</td>
<td>No big cuts or exposed organs</td>
</tr>
</tbody>
</table>
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

<table>
<thead>
<tr>
<th>Category</th>
<th>Condition Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sea lice</strong></td>
<td>No bleeding</td>
</tr>
<tr>
<td></td>
<td>Some bleeding but not flowing profusely</td>
</tr>
<tr>
<td></td>
<td>Some bleeding but not flowing profusely</td>
</tr>
<tr>
<td></td>
<td>Blood flowing freely continuously in large quantities</td>
</tr>
<tr>
<td><strong>Skin damage and bruising</strong></td>
<td>Skin damage by sea lice. Body is intact</td>
</tr>
<tr>
<td></td>
<td>Minor penetration of body by sea lice. Mostly on the cloaca area</td>
</tr>
<tr>
<td></td>
<td>Extensive penetration of body via eyes, cloaca, gills, and/or skin sea lice ate tissue</td>
</tr>
<tr>
<td><strong>Skin damage and surface bruising</strong></td>
<td>0% of skin body damage</td>
</tr>
<tr>
<td></td>
<td>&lt;5% of skin body damage</td>
</tr>
<tr>
<td></td>
<td>5-40% of skin body damage</td>
</tr>
<tr>
<td></td>
<td>&gt;40% of skin body damage</td>
</tr>
<tr>
<td><strong>No bruises or redness</strong></td>
<td>No bruises or redness</td>
</tr>
<tr>
<td><strong>Some bruises or redness</strong></td>
<td>Some bruises or redness</td>
</tr>
<tr>
<td><strong>Prominent bruises or redness</strong></td>
<td>Prominent bruises or redness</td>
</tr>
<tr>
<td><strong>Extensive bruises and redness</strong></td>
<td>Extensive bruises and redness</td>
</tr>
</tbody>
</table>
Appendix C

Detailed R-code for simulating trade-offs in number of experimental containers and number of fish per container. Adapted from Rogers et al. (2014).

```r
# MUST HAVE AN EVEN NUMBER OF 'FISHPERCAGE' DUE TO EXPERIMENTAL DESIGN
CagedFish <- function(FishPerCage, nCages, TagSurv, HandSurv) {
  #START FUNCTION
  #FUNCTION OF VARIABLES:
  #1) 'FISHPERCAGE' (NUMBER OF FISHES IN EACH CAGE);
  #2) 'NCAGES' (NUMBER OF CAGES);
  #3) 'TAGSURV' (SURVIVAL OF TAGGED AND HANDLED FISH);
  #4) 'HANDSURV' (SURVIVAL OF HANDLED, NOT TAGGED FISH)
  phi.mat <- matrix(rep(NA, FishPerCage*nCages), nrow=nCages, ncol=FishPerCage) #Survival matrix. Rows # are individual cages, columns are fish
  for(i in 1:nCages){ #START FIRST FORLOOP. RUNNING THROUGH CAGES FIRST, ONE AT A TIME
    for(j in 1:FishPerCage){ #START SECOND FORLOOP. RUN THROUGH INDIVIDUAL FISH IN EACH CAGE 'I'
      ifelse(j <= FishPerCage/2, phi.mat[i,j] <- rbinom(n=1, size=1, prob=HandSurv), phi.mat[i,j] <- rbinom(n=1, size=1, prob=TagSurv)) #LOGICAL STATEMENT.
    }
  }
}
```

7 # Symbol is used to insert comments in R console without altering the simulation process
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```r
# If you are less than or equal to the halfway point, 'HandSurv' will be used as survival value, as these individuals are only handled. If over halfway point in number of fish, 'TagSurv' will be used as survival value, as these individuals are handled and tagged. 
# End second forloop, which is for individual fish 
) # End first forloop, which is for cages 
return(list(phi.mat=phi.mat))
}
) # End function

#FUNCTION CREATED ##

# PARAMETERIZATION ##

#FISH PER CAGE MUST BE AN EVEN NUMBER
FishPerCage <- seq(10,100,by=2) #This is the cumulative number of tagged and untagged fish per cage
#FISH PER CAGE MUST BE AN EVEN NUMBER
nCages <- seq(2,25,by=1) #Number of cages to simulate over
HandSurv <- 0.95 #Survival of fish only handled
TagMortality <- 0.05 #Additive mortality induced by tagging
TagSurv <- HandSurv-TagMortality #Survival of a fish handled and then tagged
nIterations <- 1000 #Number of iterations for simulation run for single parameter set

# END PARAMETERIZATION ##

# RESULTS STORAGE ##

#1) Storing mortality estimate results from all iterations in a single parameter set
TagMortMeanCI <- matrix(NA,nrow=3,ncol=nIterations)
attributes(TagMortMeanCI)$dimnames<-list(c("MeanMort","LowerClMort","UpperClMort"),c())

#2) Storing the four output metrics from all iterations in a single parameter set
IterationResultsMat<- matrix(NA,nrow=4,ncol=nIterations)
attributes(IterationResultsMat)$dimnames<-list(c("RelativeBias","Coverage","SE","pValue"),1:nIterations)

#3) Storing full output metric results from all FishPerCage/nCages combinations
FishCagesFullData<- array(NA,dim=c(length(FishPerCage),length(nCages),10))
```
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

attributes(FishCagesFullData)$dimnames<-list(FishPerCage,nCages,c("RelBiasMean","RelBias0.025","RelBias0.975","Coverage","SEmean","SEmean0.025","SEmean0.975","pValuemean","pValuemean0.025","pValue0.975"))

############################
## RESULTS STORAGE END ##
############################

############################
## SIMULATION LOOP START ##
###################

for(k in 1:length(FishPerCage)){
  #Start 'k' FishPerCage iteration loop
  for(w in 1:length(nCages)){
    #Start 'w' nCages iteration loop
    CageByGroupSurv <- matrix(NA,nrow=nCages[w],ncol=2)
    attributes(CageByGroupSurv)$dimnames<-list(1:nCages[w],c("Handled","Tagged"))
    for(j in 1:nIterations){
      # 'j' iteration loop start
      trial.sim <- CagedFish(FishPerCage=FishPerCage[k],nCages=nCages[w],HandSurv=HandSurv,TagSurv=TagSurv)
      results <- trial.sim$phi.mat #Storing function results
      for(i in 1:nCages[w]){ #Survival of tagged vs handled fish survived per cage
        CageByGroupSurv[i,1] <- sum(results[i,1:(FishPerCage[k]/2)])/(FishPerCage[k]/2)
        CageByGroupSurv[i,2] <- sum(results[i,(FishPerCage[k]/2+1):FishPerCage[k]])/(FishPerCage[k]/2)
      }
    }
    #Retrieving TagMortality additive term
    TagMortMeanCI[1,j]<-mean(CageByGroupSurv[,1]-CageByGroupSurv[,2])
    #line above defines handled fish survival per cage
    ConfIntTagMort <- qnorm(0.975)*sd((CageByGroupSurv[,1]-CageByGroupSurv[,2]))/sqrt(nCages[w])
    #Using SE to determine each 2.5% tail of CI interval for estimates of 'TagMortality'
  }
}
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

TagMortMeanCI[2,j]<-TagMortMeanCI[1,j]-ConfIntTagMort  #Lower tail of 95% CI
TagMortMeanCI[3,j]<-TagMortMeanCI[1,j]+ConfIntTagMort  #Upper tail of 95% CI

#METRIC 1 Relative Bias of additive mortality effect ('TagMortality')
IterationResultsMat[1,j]<-(TagMortMeanCI[1,j]-TagMortality)/TagMortality
#line above is relative departure of additive tagging mortality estimate vs true value

#METRIC 2 Coverage of 95% CI made from estimates of additive mortality effect ('TagMortality')
if(as.numeric(TagMortMeanCI[2,j])<=(TagMortality)&as.numeric(TagMortMeanCI[3,j])>=(TagMortality)){      #95% CI coverage T/F statement. Is the true TagMortality additive term covered in the 95% CI
  IterationResultsMat[2,j]<-1}else{   #Coverage = 1 if true
  IterationResultsMat[2,j]<-0   #Coverage = 0 if false
}

#METRIC 3 Standard Error of additive mortality effect ('TagMortality')
IterationResultsMat[3,j]<-sd((CageByGroupSurv[,1]-CageByGroupSurv[,2]))/sqrt(nCages[w])
#line above is SE of 'TagMortality' estimate, our measure of variance

#METRIC 4 ANOVA p-value
SurvData<-append(CageByGroupSurv[,1],CageByGroupSurv[,2])    #Formatting survival data
GroupInfo<-c(rep("Handle",nCages[w]),rep("Tagging",nCages[w]))    #Creating group designations
anova.data<-data.frame(GroupInfo=GroupInfo,SurvData=SurvData)  #Creating the dataframe for input to ANOVA
anovaRun<-aov(SurvData~GroupInfo,data=anova.data)   # ANOVA, cages=replicates, groups=treatment
anovaSum<-summary(anovaRun)                  #Summarizing ANOVA
anovaTable<-anovaSum[[1]]        #Saving outputs as table
IterationResultsMat[4,j]<-anovaTable[1,5]    #Extracting p-value from ANOVA
}
 # 'j' iteration loop end

FishCagesFullData[k,w,1]<-mean(IterationResultsMat[1,]) #Mean relative bias of 'TagMortality' estimate
FishCagesFullData[k,w,2]<-quantile(IterationResultsMat[1,],0.025) #Lower quantile of relative bias
FishCagesFullData[k,w,3]<-quantile(IterationResultsMat[1,],0.975) #Upper quantile of relative bias
FishCagesFullData[k,w,4]<-sum(IterationResultsMat[2,])/nIterations  #Coverage

FishCagesFullData[k,w,5]<-mean(IterationResultsMat[3,]) #Mean SE of 'TagMortality' estimates
FishCagesFullData[k,w,6]<-quantile(IterationResultsMat[3,],0.025) #Lower quantile of SE
FishCagesFullData[k,w,7]<-quantile(IterationResultsMat[3,],0.975) #Upper quantile of SE

FishCagesFullData[k,w,8]<-mean(IterationResultsMat[4,],na.rm=TRUE) #Mean pValue of ANOVA
FishCagesFullData[k,w,9]<-quantile(IterationResultsMat[4,],0.025,na.rm=TRUE) #Lower quantile of pValue
FishCagesFullData[k,w,10]<-quantile(IterationResultsMat[4,],0.975,na.rm=TRUE) #Upper quantile of pValue
}
 #End 'w' nCages iteration loop
}
 #End 'k' FishPerCage iteration loop
library(ggplot2) #loading ggplot2 graphing packing

#Manipulating data for GGPLOT2
FigureData<-matrix(NA,nrow=length(nCages)*length(FishPerCage),ncol=6)

#Setting up a vector with the #of cages used repeated by as many different number of fishes we tested.
fake<-rep(nCages,length(FishPerCage)) #This lays out the nCages for all possible combinations
FigureData[,1]<-fake[order(fake)] #First column will be nCages, and we are ordering them by nCages
FigureData[,2]<-rep(FishPerCage,length(nCages)) #Second column will be FishPerCage, which are not # ordered but just repeated

for(i in 1:length(nCages)){ #running a loop through simulation data to fill in FigureData with relative
  # bias, coverage, SE, and p-values for every possible combination
  FigureData[((1-length(FishPerCage)):0)+i*length(FishPerCage)),3]<-FishCagesFullData[1:length(FishPerCage),i,1] #Mean Relative Bias
  FigureData[((1-length(FishPerCage)):0)+i*length(FishPerCage)),4]<-FishCagesFullData[1:length(FishPerCage),i,4] #Coverage
  FigureData[((1-length(FishPerCage)):0)+i*length(FishPerCage)),5]<-FishCagesFullData[1:length(FishPerCage),i,5] #SE
  FigureData[((1-length(FishPerCage)):0)+i*length(FishPerCage)),6]<-FishCagesFullData[1:length(FishPerCage),i,8] #Mean p-value

  #Placing transformed data into a data.frame
  PlotData<- data.frame(nCages=FigureData[,1],FishPerCage=FigureData[,2],RelBias=FigureData[,3],Coverage =FigureData[,4],SE=FigureData[,5],pValue=FigureData[,6])

  #Saving individual ggplots as objects 'p', 'm', 'n', and 'o'
  p <-ggplot(PlotData,aes(x=nCages,y=FishPerCage,z=RelBias))+ geom_tile(aes(fill = RelBias))
p<-p+scale_fill_gradient2(name="Relative Bias",limits=c(-.2,0.2),low="black",high="black")

  m <-ggplot(PlotData,aes(x=nCages,y=FishPerCage,z=Coverage))+ geom_tile(aes(fill = Coverage)) +
    stat_contour()
m<-m+scale_fill_gradient(name="Coverage",limits=c(0.5,1.0),low="black",high="white")

  n <-ggplot(PlotData,aes(x=nCages,y=FishPerCage,z=SE))+ geom_tile(aes(fill = SE))
n<-n+scale_fill_gradient(name="Standard Error",limits=c(0.025,0.1),low="black",high="white")
A simulation-based post-release survival study for brownbanded bamboo sharks (*Chiloscyllium punctatum*) of the Gulf of Thailand.

```r
o <- ggplot(PlotData,aes(x=nCages,y=FishPerCage,z=pValue))+ geom_tile(aes(fill = pValue))
o<-o+scale_fill_gradient(name="P Value",limits=c(0.05,0.4),low="white",high="black")

# below outputs simulation plots
p #Relative bias
m #Coverage
n #SE
o #pValue

####################################################
## END GRAPHING ##
####################################################
```
Appendix D

Detailed R-code for assessing post-release survival rates of brownbanded bamboo sharks in relation to condition of the individual (i.e. condition)

# Generating survival data

n = 100 # number of individuals
c = 20 # half of days of total experiment
r = 0.4 # estimated survival rate
condition=floor(runif(n, min=1, max=4)) # randomly generates conditions between 1 and 3

lifetimes<- rexp(n, rate=r)

censtimes<-c+c*runif(n)
time<-round(pmin(lifetimes,censtimes))
censor<-as.numeric(censtimes>lifetimes)
data= data.frame(condition,censor,time)

# install.packages ("survival")
library(survival)

my.surv<-Surv(time,censor)
my.surv

kmsurvival <- survfit(my.surv~1,conf.int=0.95)
summary (kmsurvival)
plot(kmsurvival, xlab="Time (Days)", ylab="Survival Probability")

# Kaplan-Meier non-parametric analysis by condition

kmsurvivall <- survfit(my.surv~condition,conf.int=0.95)
# To get estimated survival probabilities and confidence intervals
summary (kmsurvivall)
plot(kmsurvivall, col=c(1:4), xlab="Time (Days)", ylab="Survival Probability")

legend(30,1, c("Cond 1","Cond 2","Cond 3"), col=(1:3),lwd=0.5)
# To compare median times of death across levels
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kmsurvivall
# Test of difference between survival functions between treatment levels (=condition)
# Copied from 'http://www.youtube.com/watch?v=oxTiiJaMbK0'
# 3 test with parameter rho. Power of tests difference depend on curves (vague)
# Ho: no difference in survival function between groups
# H1: difference in at least one pair of survival functions
# rho=0 is log-rank test (more weight on higher values of time)
# rho=1 is generalised wilcoxon test (more weight on lower values of time)
# rho=1.5 is Tarone-Ware (in between log-rank and wilcoxon)
# Choice of rho
# Logrank popular - compares hazards across groups. Applicable if hazard functions do not
# cross, eg. if proportional hazard. Crossing of hazard functions across groups suggest
# presence
# Results of different weightings usually lead to similar conclusions
# The best choice is test with most power
# Choice of weighting should be a priori! Not fishing for a
# desired p-value
survdiff(my.surv~condition,rho=0) # log-rank test
survdiff(my.surv~condition,rho=1) # generalised wilcoxon test
survdiff(my.surv~condition,rho=1.5) # Tarone-Ware

# Nelson_Aalen non-parametric analysis
nasurvival <- survfit(coxph(my.surv~1),type = "aalen", conf.int=0.95)
summary(nasurvival)
plot(nasurvival, col=c(1:3), xlab="Time (Days)", ylab="Survival Probability")

# Cox proportional hazard model - coefficients and hazard rates
# rm(list=ls()) # Clears memory
condition.coxph<-coxph(Surv(time,censor)~condition, method = "breslow")
summary(condition.coxph)

#anova to get the overall test of condition #Anova (condition.coxph)
#Kaplan
#to estimate the baseline hazard of the cox fc
#need to specified values for the data un newdata=argument
#to obtain this estimates for each condition:
#1. specify different values or condition in newdata file
#2. plot the results
#3. superimpose KM estimates for comparison
#KM estimates
plot(kmsurvivall, col=c(1:4), xlab="Time (Days)", ylab="Survival Probability")
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```r
legend(30,1, c("Cond 1","Cond 2","Cond 3"), col=(1:3),lwd=0.5)

#separate cox estimates by condition
cox1<-survfit(condition.coxph, newdata=list(condition=1))
cox2<-survfit(condition.coxph, newdata=list(condition=2))
cox3<-survfit(condition.coxph, newdata=list(condition=3))
cox4<-survfit(condition.coxph, newdata=list(condition=4))

names(cox1)

#cox estimates
lines(cox1$time, cox1$surv, lty=2, type='s')
lines(cox2$time, cox2$surv, lty=2, type='s', col=2)
lines(cox3$time, cox3$surv, lty=2, type='s', col=3)
lines(cox4$time, cox4$surv, lty=2, type='s', col=4)

legend("topright", as.vector(sapply(list("Cox:", "KM:"), function(x) paste(x, paste("condition=",
1:4)))), col=c(1:4, 1:4), lty=c(2,2,2,1,1,1), bty="n", cex=.8)

#the proportional hazards assumption can be assessed by plotting
#log(-log(S(t))) against time separately for condition categories
#KM estimate
KMph<-survfit(Surv(time, censor)~factor(condition), data=data)

KMph

condition<-rep(1:3, KMph$strata)

plot(KMph$time, log(-log(KMph$time)), type="n", ylab=expression(log(-log(S(t)))), xlab="t")

#first, modify cox formula so coefficients appear by condition
cox<-coxph(Surv(time, censor)~factor(condition), data=data)

summary(cox)

out.zph<-cox.zph(cox)

out.zph

#produce plots to display how the coefficient is estimated to be changing with time
par(mfrow=c(2,2))

plot(out.zph[1], ylim=c(-2,2))

abline(h=0, col=2, lty=2)

plot(out.zph[2], ylim=c(-1,1))

abline(h=0, col=2, lty=2)

plot(out.zph[3], ylim=c(-.05,.05))

abline(h=0, col=2, lty=2)

#systematic departures from the horizontal line are indicative of non-proportional hazards

coef(cox)

#schoenfeld residuals for each covariate in the model. from Fox 2002

residuals(cox, "scaledsch")
```
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```r
cox.zph(cox) # same as out.zph
par(mfrow=c(2,2))
plot(cox.zph(cox))

# Exponential, Weibull, log-logistic parametric model coefficients
exponential <- survreg(Surv(time,censor)~condition, dist="exponential")
summary(exponential)

weibull <- survreg(Surv(time,censor)~condition, dist="weibull")
summary(weibull)

loglogistic <- survreg(Surv(time,censor)~condition, dist="loglogistic")
summary(loglogistic)
```