The Ecology of Ray Species in an Urbanised Estuary:

Seasonality, Habitat use and Pollutant Exposure in Tauranga Harbour.

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ABSTRACT

Information about the movement, seasonality, and use of habitats by marine animals is vital for the mitigation of potential anthropogenic impacts. Ray species may be particularly at risk as they regularly inhabit coastal and estuarine waters. In New Zealand to-date, there has been scant research on the ecology of native ray species in estuarine habitats. In particular, there is a dearth of knowledge pertaining to the spatio-temporal use of the range of habitats within estuaries. The research detailed in this thesis was aimed at addressing the shortfall of information. First, a review of the methodology utilised in ascertaining movement behaviour in non-shark-like batoid elasmobranch species was carried out, as optimisation of tagging research technique underpins the ability to track behaviour of these organisms for long periods. Most studies reviewed adopted tag anchor techniques used on teleost fishes or sharks. As a consequence, the quality of information pertaining to ray habitat use and movements was, in many circumstances, poor. Synthesis of tag longevity using differing anchor methods and field and aquarium longevity experiments led to a recommendation of nylon umbrella darts for soft-skinned non-shark-like rays such as *Bathytoshia brevicaudata*. Second, seasonality in habitat use within the Tauranga Harbour system was examined using monthly counts of the feeding excavations of *Myliobatis tenuicaudatus.* This study expanded previous estimations of seasonality and feeding habitat choice in estuaries. It determined that temperature-mediated sinusoidal seasonal patterns in feeding behaviour over a period of 24 months, differed in magnitude and peak month across a range of spatial scales. This could suggest some form of sequential habitat use. Unlike previous studies, evidence of ray feeding was found year-round. This behavioural pattern has implications for calculations of sediment turnover and transport. Peak turnover estimates of ray origin from this study doubled previous estimated calculations. In addition, infaunal prey density, and locational aspects of estuary ‘sub-habitats’ characterised as various ‘zones’ as compared to ‘harbour basin’ habitats, were all found to be influential in the prediction of *M. tenuicaudatus* feeding activity. There were inverse seasonal differences in the relationship between densities of large infaunal bivalves (putative prey items) and ray feeding activity, suggesting that during some periods, other prey types (soft bodied organisms) may also be important. Suggestions are made that perceived predator risk and human disturbance may have a role in driving habitat preferences in addition to prey density. This study also found that natural mangrove fringe is preferred by *M. tenuicaudatus* for feeding habitat over areas of ‘fringe’ that had been trimmed to prevent mangrove spread. The implications of this are significant as there is a reduction in ideal feeding habitat with ongoing mangrove trimming regimes. Finally, quantification of metal body burden of *M. tenuicaudatus* identified low levels of some heavy metals in rays from Tauranga Harbour when compared to Porirua Harbour, and that metals in rays from the outer coast of the Bay of Plenty region were likely to be of volcanic origin. Significantly different metal assemblages of estuarine and offshore animals combined with feeding evidence found year-round in Tauranga Harbour, suggests a separation in populations between these areas. Overall however, it is clear that metal content in Tauranga Harbour rays lies below FZANZ levels of concern and the harbour may be classified as relatively unpolluted. However, the behavioural patterns of rays clearly lead them away from shallower sub estuary areas, that are known to be more contaminated by anthropogenic activity.

In conclusion, this thesis provides previously unknown information about the habits and ecology of the important estuarine mesopredator *M. tenuicaudatus* in the context of anthropogenic risk associated with an urbanised harbour ecosystem. The information will allow informed management of harbour activities and developmental options with regard to conservation of an ecologically important species.

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PREFACE

“An understanding of the natural world and what’s in it is a source of not only a great curiosity but great fulfilment.”

- Sir David F. Attenborough

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

GENERAL INTRODUCTION

Large predators play an essential role in regulating the structure and functioning of ecosystems (Estes et al., 2011). However, their natural rarity relative to species at lower trophic levels makes them vulnerable to human exploitation and to changes that may occur in their environments (Estes et al., 2011). Understanding baseline patterns of predator behaviour is essential in order to both understand the risk of anthropogenic change and also to determine their specific position and role within an ecosystem, (Underwood et al., 2000); knowledge that is in turn vital to be able predict and comprehend ecosystem consequences of predator decline (Estes et al., 2011).

In the marine environment, estuarine and coastal dwelling elasmobranchs are predators that may be particularly at risk from anthropogenic stressors (Dulvy et al., 2014). Many batoid (ray) species in particular are not only important mesopredators but also have a key role in the functioning of their ecosystems through bioturbation (Thrush et al., 1991, 1994; O'Shea et al., 2012). Ray species as a group are poorly understood (Last et al., 2016a) and thus to mitigate risk of anthropogenic stressors and associated ecosystem consequenses, there is a worldwide need to address this lack of knowledge.

The New Zealand batoid elasmobranchs are examples of large predatory species whose behaviours and ecological roles are not yet clear. During the Austral summer period, around the coastline, harbours and estuaries of New Zealand, eagle rays

(*Myliobatis tenuicaudatus* Hector 1877), short tail stingrays (*Bathytoshia brevicaudata* Hutton 1875) and long tailed stingrays (*Bathytoshia lata* Garman 1880) are observed in great numbers. Despite the regularity and ease with which these species are sighted and caught, little is known of their ecology and behaviour. This lack of knowledge presents a major challenge in regard to conservation and management of these species, at a time of rapidly increasing urban development and anthropogenic use of their estuarine and surrounding coastal habitats.

Understanding the importance of an area that may be characterised as 'habitat' to a species - specifically, spatial and temporal patterns of occupation and 'use', is essential to the assessment of the effects of anthropogenically generated change. For example, an animal that resides and feeds year-round in a polluted area may possess a higher contaminant burden in their tissues and subsequently may have a higher risk of contaminant related health or reproductive issues than one that is seasonally resident or uses an area infrequently. Therefore, a detailed knowledge of seasonal behaviours, movements, site fidelity and the dynamics of habitat use of animals that inhabit potentially impacted coastal areas is necessary in regard to managing potential risk.

It is commonly thought that *M. tenuicaudatus*, *B. brevicaudata* and *B. lata* are solely summer residents in New Zealand harbours (Hines et al., 1997), however, *M. tenuicaudatus* has been spotted in the Tauranga Harbour during winter months (H. Cadwallader Pers. Obs.), prompting this investigation into the seasonality of this species, and of the larger species *B. brevicaudata*, incorporating potential risks to these species resulting from inhabiting an urbanised harbour system year-round.

1.1. Biology of batoid elasmobranchs

The animals that reside within the Superorder Batoidea, commonly referred to as the ‘rays’, are a large group of cartilaginous fishes that along with their relatives the sharks (Superorder Selachimorpha), are classified within the subclass Elasmobranchii and commonly referred to as the ‘elasmobranchs’. At the most recent count, there are 633 species of batoid elasmobranch globally, contained within 26 families that are arranged into four orders: Torpediniformes (electric rays), Rajiformes (skates and relatives), Rhinopristiformes (shovelnose rays and relatives) and Myliobatiformes (stingrays and relatives) (Last et al., 2016a). Morphologically, rays are commonly distinguished from sharks by their dorsoventrally flattened bodies, enlarged pectoral fins that are fused to the head, dorsally situated eyes and spiracles and ventrally situated gill slits (Last et al., 2016a). In addition, dorsal fins are often largely reduced or absent in this group (Last & Stevens, 2009; Last et al., 2016a). There are, however, a number of species within the order Rhinopristiformes that resemble sharks of the order Pristophoriformes (sawsharks) in that they possess a more torpediform body shape and larger dorsal fins and are commonly referred to as ‘shark-like’ rays (Last & Stevens, 2009; Last et al., 2016a). Rays are found in a wide range of aquatic environments including freshwater (the family Potamotrygonidae) through estuarine, coastal and pelagic waters (Last & Stevens, 2009).

Ray species are often described as being ecologically important. They often appear as mesopredators in trophic investigations and some have a role in regulation of their prey species (Thrush et al., 1991). In addition, the excavatory feeding mechanism of many species has a bioturbation effect, with associated ecosystem functioning outcomes (Thrush et al., 1991; Hines et al., 1997; Needham et al., 2011; O'Shea et al., 2012).

Members of the Batoidea are thought to have evolved from a shark-like ancestor and possess many similarities in physiological characteristics with the more well-known sharks (Carrier et al., 2010; Last et al., 2016a). For example, all elasmobranchs exhibit internal fertilisation with numerous strategies ranging from oviparity to viviparity (Last & Stevens, 2009; Carrier et al., 2010; Frisk, 2010). In addition, the elasmobranchs possess a highly developed sensory system including a pressure sensitive lateral line, electro-sense and magnetic sense (Last & Stevens, 2009; Carrier et al., 2010). Furthermore, rays share many life-history traits with the sharks; many exhibit slow growth, late maturation and long life, often combined with production of relatively few young (Last & Stevens, 2009).

Ray species are poorly studied in comparison to sharks. However, this has been changing over the past few years. Moreover, it has recently been determined that amongst the elasmobranchs, more rays than sharks are classified by the International Union for Conservation of Nature Red list of Threatened species (IUCN Red List) as Data Deficient or Endangered (Dulvy et al., 2014). This lack of research, combined with the fact that coastal species are thought to be under higher risk from the combined pressures of exploitation and habitat degradation than pelagic species (Dulvy et al., 2014) emphasises the need for more research on coastal ray species.

The New Zealand ray species sit within the order Myliobatiformes: *M. tenuicaudatus* (Family Myliobatidae), *B. brevicaudata* and *B. lata* (Family Dasyatidae). These rays are all found in habitats from estuarine sand-flats to offshore reefs (Last et al., 2016a); they all have the potential to be affected by the pressures described by Dulvy et al. (2014). None of these species are currently considered threatened in New Zealand (Duffy et al., 2016; Kyne, 2016; Duffy et al., 2018), and this gives researchers an opportunity to learn about their ecology and habits while populations are arguably in relatively good condition. This latter assumption does however need examination over longer time scales than is possible in this work.

1.1.1. Recent taxonomic changes affecting the study of batoid elasmobranchs

Phylogenetic relationships and taxonomic classifications of the Batoidea were recently reorganised following an in-depth genetic and morphological analysis (White, 2014; Last et al., 2016a, 2016b). Subsequently, the short tail stingray was reclassified from the genus *Dasyatis* to the newly resurrected genus *Bathytoshia*, which now encompasses the three very large species of stingray: *B. brevicaudata* (short tail stingray), *B. centroura* (roughtail stingray) and *B. lata* (long tailed stingray) (Last et al., 2016b). By this molecular analysis, *Dasyatis matsubarai* (found in Japan) is proposed to be synonymous with *B. brevicaudata.* However, this result is at odds with findings of Le Port et al. (2013), stating that the two species are closely related sister lineages with small but distinct genetic and morphological trait differences. In addition, in the most recent analysis, the longtail stingray (*Dasyatis thetidis*) was merged with the Hawaiian stingray (*Dasyatis lata*) (Last et al., 2016b).

Furthermore, the southern eagle ray, *Myliobatis australis,* found in Southern Australian waters has recently been determined by similar taxonomic investigation to be the same species as the New Zealand eagle ray *M. tenuicaudatus* with the senior synonym *M. tenuicaudatus* taking precedence (White, 2014). In addition, several other batoid species have been renamed and a number of genera have been retired or resurrected (Last et al., 2016a). For this study, the updated nomenclature will be used for all species in cited works with the previous name stated for clarity where necessary.

1.2. Spatial ecology of batoid elasmobranchs

Despite rays occurring in a multitude of different aquatic habitats, from riverine to the deep sea (Last et al., 2016a), we know very little about their spatial ecology. Spatial ecology can include multiple levels of temporal movement behaviour, be that hourly, daily (diel), seasonal migrations and/or seasonal change in biophysical habitat character, or changes in habitat ‘preference’ throughout different developmental stages. It can also include site fidelity behaviour, including differing levels of philopatry and home range behaviours where again there can be a dynamic based on age or gender of the individual. Finally, spatial ecology can include the behavioural aspects of habitat use, i.e. what an animal is doing within an area at any particular time of day or season.

1.2.1. Habitat use: Disambiguation of terminology

‘Habitat use’ is a term which has, in elasmobranch behavioural studies to date, most commonly been defined in its most simplistic form, i.e. the use of one or more habitats by a species or individual. Knowledge of home ranges, philopatry, migration patterns and other dynamics of species’ movement can inform marine spatial planning programs (now implicit in coastal urban expansion/management strategies), and in creating policy for fisheries, conservation and coastal development programs. However, the approach where the notion of 'habitat use' has traditionally been a proxy term for the simple 'presence of an animal in a habitat', may be limiting; it is important to determine not only that a species or animal is using/is present in an area, but also what they are actually doing there. Examples of behavioural use of a habitat include foraging/feeding, mating behaviours including lek sites (Márquez & Dunn, In Prep) or aggregations (Le Port et al., 2012) and nursery areas (Heupel et al., 2007; Davy et al., 2015). Examples of the use of the term 'habitat use' when study has only quantified presence/absence include studies where fishing, visual or aerial surveys may be used, determining abundance or confirming use of an area. For example, longline sampling of an atoll location revealed differential abundance patterns of shark and ray species, at different depths, however did not determine what each species was doing there (Pikitch et al., 2005).

The distinction between the presence of an animal or species in an area versus the period of time or behavioural function of an area and the importance of this distinction within a management/policy context, can be illustrated by the following example. A developer has earmarked an area for development as a new marina complex. Trawl surveys determined that a protected or important species is present in two areas, including the one for development. Initially this may seem ideal: development could occur, and the species would still have access to original habitat. However, if one area was a foraging or feeding habitat and the other a mating area, then this could be catastrophic for the species.

Therefore, for the purposes of this study, the term 'habitat use' will refer to the activity that an animal is performing in a given habitat, for example habitat will be characterised as being used for foraging, mating, or as a nursery area. Where this is not known, it will be stated that the species is present or moving between or within a habitat or habitats.

1.2.2. Movement behaviour and habitat use of batoid elasmobranchs

Very little is known about the movement behaviours and habitat uses of batoid elasmobranchs. Previous studies focussing on behaviour of batoid elasmobranchs have mostly concentrated on movement and presence within certain habitats rather than specific habitat use and has revealed a wide variety of behavioural movement strategies. These include various forms of migration behaviour (Schwartz, 1990; Blaylock, 1993; Grusha, 2005; Goodman et al., 2010), cycles of horizontal movement (Cartamil et al., 2003; Lowe et al., 2007; Le Port et al., 2008; Campbell et al., 2012; Le Port et al., 2012; Vaudo & Heithaus, 2012), varying occupational patterns of depth strata (Le Port et al., 2008; Canese et al., 2011), and varying site fidelity and habitat specificity patterns (Topping et al., 2006; Collins et al., 2008; Dewar et al., 2008; Ajemian & Powers, 2011; Tilley et al., 2013). In addition, it is suggested that certain species use nursery areas (Davy et al., 2015) according to the strict criteria suggested for sharks by Heupel et al. (2007) and confirmed for rays by Martins et al. (2018) where young of year individuals are encountered in the area more often than in other areas, remain for long periods and the use of the area is consistently utilised across multiple years. The diverse findings from these studies highlights the need for more investigation into the habitat use dynamics and spatial ecology of this group of ecologically important animals.

To this end, an increasing number of studies are utilising tagging and hi-tech tracking techniques in order to elucidate the movements and behaviours of underwater animals (Block et al., 2011; Hammerschlag et al., 2011). The tag/tracking device attachment techniques in use for batoid species have been largely taken from methods developed in the study of sharks. This may not be universally ideal for rays however, due to differences in body morphology and epidermal physiology. To date, there has been no assessment of the relative usefulness of these attachment technologies on non-shark-like ray species, hence the reliability of seemingly sophisticated tracking technologies is drawn into question for monitoring ray movement and behaviours.

1.3. Anthropogenic impacts on coastal elasmobranch species

In their assessment of the extinction risk of sharks and rays globally, Dulvy et al. (2014) identified species that inhabit coastal areas as potentially under greater threat due to the combined anthropogenic pressures of fishing and habitat degradation present in these areas. Currently, coastal ray species in New Zealand are not considered endangered. *M. tenuicaudatus* and *B. brevicaudata* are currently classified as 'Least concern' (Duffy et al., 2016; Kyne, 2016) and *D. thetidis* was classified 'Data deficient' until it was amalgamated with *B. lata* as a junior synonym (Last et al., 2016b), a species that is classified 'Least concern' (Ebert et al., 2016). All are considered 'Not Threatened' in New Zealand following an examination of the status of New Zealand chondrichthyan species. However, according to a report by Statistics New Zealand, the human coastal population in New Zealand is increasing; the population living within 10 km of the coast increased from 72 to 75 per cent from 1981 to 2006 (Statistics New Zealand, 2016). This increase in population is likely to exacerbate the potential anthropogenic impacts to these species.

In regard to fishing as a potential threat to New Zealand coastal ray species, none are commercially targeted, although they are caught as bycatch by commercial fishing vessels (Duffy et al., 2016; Kyne, 2016; Anderson et al., 2017). Reported total catch of all species in the year 2016-2017 was 242 tonnes. More than 95 per cent of the total *B. brevicaudata* and *B. lata* catch was discarded, whereas 51 per cent of the total *M. tenuicaudatus* catch was landed (Ministry for Primary Industries, 2019). These ray species are occasionally taken by recreational fishers and are considered a game fish target for bow fishers (Dick Marquand and Lucas Allan, Pers. Comm.). Recreationally caught rays may be kept or discarded, although no catch data is available. It is assumed, however, that they are not taken in large numbers.

The greater potential threat to New Zealand coastal ray species is likely to be habitat degradation, due to their direct exposure to the influence of an increasing coastal population. This increase in population is continuing to result in increases of three of the four potential facets of habitat degradation suggested by Dulvy et al. (2014): residential and industrial development, mangrove destruction, and aquatic pollution, omitting the fourth, riverine development. While these animals are able to move from degraded habitats, problems may occur if levels of philopatry or residency behaviour occurs in these species. Urbanisation and industrialisation are thought to have contributed to the loss or reduction in use of nursery areas in several shark species including school shark (*Galeorhinus galeus*) in South Australia (Walker, 1998), sandbar shark (*Carcharhinus plumbeus*) in some areas of the Eastern United States (McCandless et al., 2007) and lemon sharks (*Negaprion brevirostris*) in areas of the Bahamas (Jennings et al., 2008).

1.3.1. Development

Development of coastal and estuarine areas is inevitable with an increasing population, with the negative influence of urban areas on the adjacent aquatic habitat being far and above that of any other type of land-use activity (Paul & Meyer, 2001). Building works and land development can result in an increase in sedimentation and its associated effects including smothering of benthic habitat resulting in alterations in the infaunal assemblage with subsequent knock-on effects on higher trophic levels (Thrush et al., 2004). Furthermore, loss of intertidal and shallow-water habitat can occur with land-reclamation and shoreline hardening by the addition of sea defences or waterside beautification, with the associated changes in biological communities (Bilkovic et al., 2006; Bilkovic & Roggero, 2008).

Development of coastal areas and areas surrounding estuaries is also likely to increase the impervious surface cover (ISC) of a watershed area (Paul & Meyer, 2001). Impervious surface area includes roofs, tar-sealed carparks, roads and any other surface that does not readily allow surface absorption of precipitation (Paul & Meyer, 2001). An increase from 10 to 20 per cent ISC can result in large increases in run-off of precipitation and waste products from industry exacerbating the input of pollution into the adjacent aquatic environment (Paul & Meyer, 2001). At levels of ISC exceeding 20% changes in biological communities have been shown (Holland et al., 2004). The proportion of copper in storm water run-off in an urban area in Christchurch, New Zealand, was 45% road-sourced, 27% from copper roofs and 28% from carparks whereas the proportions of zinc in the same storm water run-off was 81% sourced from galvanised roofs, 12% roads, and 7% carparks (Charters et al., 2016).

1.3.2. Mangrove destruction

The removal of mangrove species, whilst considered problematic worldwide (Laegdsgaard & Johnson, 2001; Walters et al., 2008; Dulvy et al., 2014) is widespread in New Zealand. Popular public opinion considers it a positive step to conserve visual attractiveness, recreational use of the estuarine amenity, and 'perceived' biodiversity of the original estuarine area (where mangroves are a recent arrival or recently expanded in area within an estuary). Whether or not these are scientifically defensible arguments remains in strong debate (De Luca, 2015). Whilst studies have been completed on the benthic biodiversity of New Zealand mangrove (*Avicennia marina* var. *australasica*) areas, the effects of removal on the ecology of estuaries is not well understood and are dependent on the mechanisms of removal (Lundquist et al., 2012; Bulmer et al., 2017). Changes to the ecology of mangrove fringe areas and the effect of removal on biodiversity have been examined by Alfaro (2006, 2010), but the effects on rays have not been specifically examined. It is unknown whether batoid elasmobranch species in New Zealand utilise these areas, and therefore whether removal or trimming would have any consequences for these species. As councils and regulatory bodies are under considerable pressure in some areas for removal and control action (De Luca, 2015), an analysis of the use of these areas by ray species will be beneficial in the decision-making process.

1.3.3. Aquatic pollution

Aquatic pollution has been a global concern for many years (Islam & Tanaka, 2004) and species that reside close to our coastlines may be particularly at risk (Dulvy et al., 2014). Major sources of pollution in estuarine and coastal areas include (but are not limited to); agrichemicals including fertilisers and pesticides, sewage, oil products, heavy metals and other trace elements, synthetic organic compounds and plastics (Islam & Tanaka, 2004; Gelsleichter & Walker, 2010). As predators that reside in coastal areas, with relatively large body size and slow metabolism, many ray and skate species are at risk of negative effects resulting from pollutant exposure, including the bioaccumulation and biomagnification of these harmful chemicals (Gelsleichter & Walker, 2010; Escobar-Sánchez et al., 2013; Türkmen et al., 2013; Šlejkovec et al., 2014; Türkmen et al., 2014). Metals may be a particular risk to marine elasmobranchs partially due to their alternative osmoregulatory strategy, retaining urea as an osmolyte (De Boeck et al., 2010). Radioisotope tracers were utilised in a study that showed metal uptake in three elasmobranch species, including two ray species, had higher rates of metal uptake than teleost fish of comparable size and diet, suggesting that elasmobranch species are potentially more susceptible to and more at risk from accumulation of metals from their environment (Jeffree et al., 2006, 2010). In addition, females that possess high contaminant loads may passively transfer contaminants during mobilisation of lipids from fat stores for egg production (Lyons et al., 2013).

Heavy metals, primarily mercury, lead and cadmium but also including copper, chromium and zinc amongst others are biotoxic in high concentrations, with negative effects to aspects of physiology, including respiratory, reproductive, sensory and musculatory systems (Farrell & Brauner, 2012; Farrell et al., 2012). Therefore, if habitat contamination is elevated, absorption via waterborne and dietary routes (Mathews & Fisher, 2009) may result in alterations to behaviour, fecundity and overall vigour of elasmobranch populations utilising the affected area (Matta et al., 1999; Farrell & Brauner, 2012; Farrell et al., 2012).

Heavy metals in estuarine and coastal systems may be anthropogenically or naturally sourced (Gelsleichter & Walker, 2010). In New Zealand, volcanic activity provides a natural source of many heavy metals (Kulgemeyer et al., 2016), while increasing urbanisation and intensive agriculture are the main anthropogenic sources of metals (Ellis et al., 2013; Huteau, 2017).

1.4. Study site

Tauranga Harbour is a large estuarine lagoon system situated on the east coast of the North Island of New Zealand (Figure 1.1). The harbour spans more than 200 km^2 with entrances at the northern and southern tip of Matakana Island, which separates the harbour from the Pacific Ocean. The majority of the harbour is shallow (less than 3m at high tide), with approximately 66% of the total area consisting of exposed sand flat at low tide (Inglis et al., 2008). The northern harbour basin (Figure 1.1 A) is mostly bounded by agricultural land use including citrus, kiwifruit and avocado orchards while the city of Tauranga and the Port of Tauranga are both situated at the southern reaches of the harbour (Figure 1.1 B). As of 2017, the city of Tauranga is home to 131,500 people and the population is increasing rapidly with a projected population of over 198,000 by 2063 (Tauranga City Council, 2018). The Port of Tauranga is the busiest commercial port in New Zealand with total cargo handled in 2015 reaching more than 20 million tonnes (Port of Tauranga, 2015). The southern harbour entrance and port has recently undergone a large-scale dredging operation to deepen and widen the channel, allowing larger cargo vessels to use the Port of Tauranga.

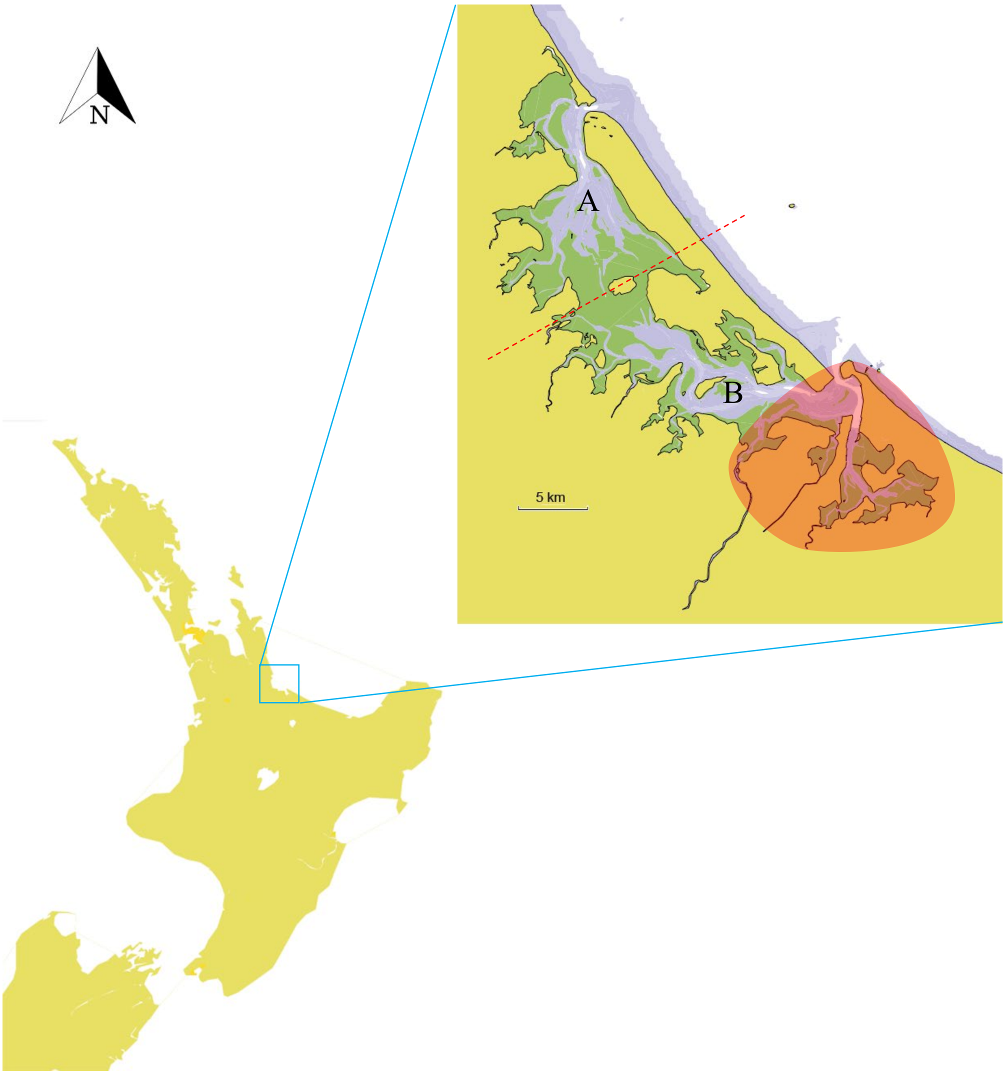


Figure 1.1. The location of Tauranga Harbour on the east coast of the North Island of New Zealand showing the extent of the harbour system. Dotted line indicates approximate boundary between the A) northern harbour and B) southern harbour basins. Shaded area indicates approximate extent of the city of Tauranga.

The harbour is utilised by a number of elasmobranch species. Bronze whaler sharks (*Carcharhinus brachyurus*) and blue sharks (*Prionace glauca*) are regularly observed during the summer months, and the harbour has been identified as a potentially important nursery area for rig (*Mustelus lenticulatus*) (Francis et al., 2012). The New Zealand eagle ray (*M. tenuicaudatus*) can be seen year-round, although more frequently in the summer months and the larger long tailed stingray

(*B. lata*) and short tail stingray (*B. brevicaudata*) can be present in high densities during the warmer months.

1.5. Study species

1.5.1. *Myliobatis tenuicaudatus*

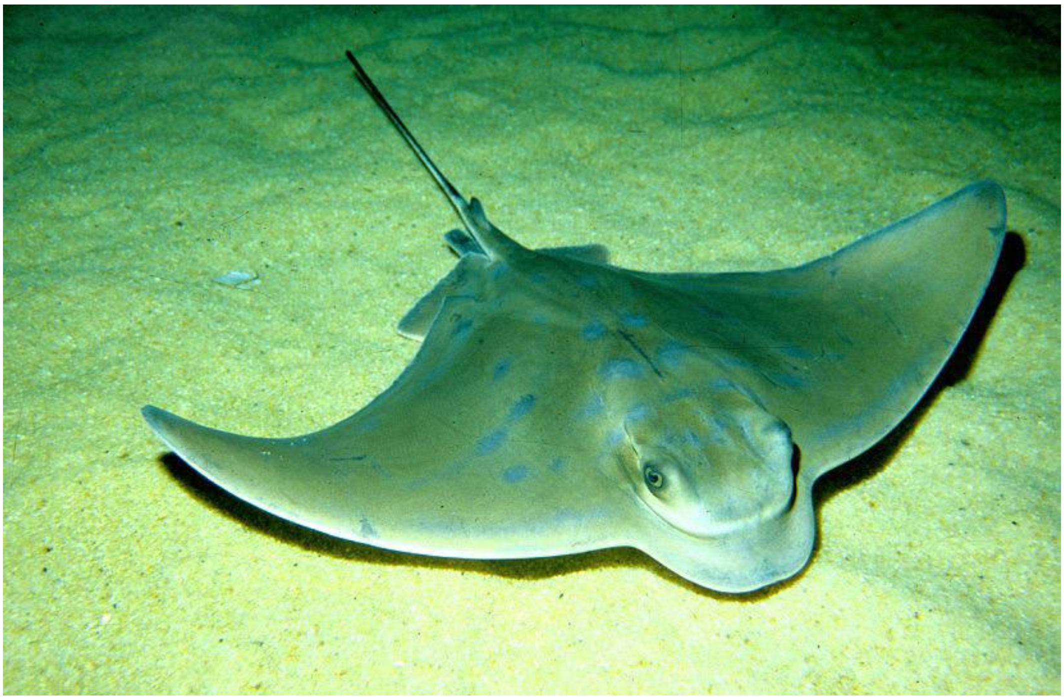


Figure 1.2. Photo of New Zealand eagle ray *Myliobatis tenuicaudatus*. Copyright Malcolm Francis. Reproduced with permission.

Myliobatis tenuicaudatus) (Figure 1.2) is common in coastal and estuarine waters to a maximum of 422 m (although usually shallower than 50 m) throughout the New Zealand mainland and offshore islands including the Kermadec Islands (Last & Stevens, 2009; Last et al., 2016a). Previously considered endemic to New Zealand, White (2014) determined that the southern eagle ray (*M. australis*) is a junior synonym, extending the distribution of *M. tenuicaudatus* to Southern Australia and Norfolk Island (Kyne, 2016).

Myliobatis tenuicaudatus are thought to prey on infaunal molluscs such as *Macomona liliana*, and are also thought to consume crustacean, teleost and polychaete prey (Gregory & Ballance, 1979; Hartill, 1989; Thrush et al., 1991; Hines et al., 1997; Sommerville et al., 2011). Stomach contents analysis during a study on the diets of four elasmobranch species in coastal south-west Australian trawl surveys showed that diet compositions of *M. tenuicaudatus* (named in the publication as *M. australis*) altered towards larger prey species as body size increased and that there was a higher proportion of crustacean prey present overall than bivalve prey (Sommerville et al., 2011). In addition, the only diet analysis of this species in New Zealand waters similarly demonstrated ontogenetic changes in prey choice with a shift towards infaunal prey such as bivalves and polychaetes with increasing size (Hartill, 1989). Stomach contents such as these are valuable and provide quantification of different prey types, however due to the soft nature and difference in digested state of food items, bias can exist in identifying the total breadth of prey species, hence the apparent diet can be skewed towards slower digesting items (Baker et al., 2013; Buckland et al., 2017).

The feeding method of rays by which they excavate buried infaunal prey by using jets of water from their mouth or gills was first described in *M. tenuicaudatus* by Gregory and Ballance (1979). This feeding technique creates visible and distinctive depressions in the sediment (Figure 1.3.) which can prevail over multiple tides and can therefore be used as evidence of feeding occurring in an area. Feeding (pit density) was non-linearly related to densities of the estuarine bivalve *M. liliana* on a sandflat in the Manukau Harbour, northern New Zealand (Hines et al., 1997). In addition, a threshold density of *M. liliana* was found, over which ray feeding was seen, leading to the conclusion that *M. tenuicaudatus* feeding efforts regulate population numbers of this prey species (Hines et al., 1997). Other work on the feeding behaviour of this species has concentrated on the bioturbatory effects of feeding pit excavation on macrobenthic organisms and sediment chemistry (Thrush et al., 1991, 1994).

Other than the work on feeding pit creation and diet, there has been little research on *M. tenuicaudatus.* Marcotte (2014) found that 64 percent of *M. tenuicaudatus* individuals tagged with acoustic transmitters and released at varying distances from Whangateau Estuary, returned to the estuary within 16 days, suggesting a high level of site fidelity in this species although the longevity of this fidelity is unknown. A high level of site fidelity prevailing over long periods would imply a greater level of risk if the area inhabited is impacted or degraded.

Figure 1.3. A distinctive feeding pit created by *M. tenuicaudatus.*

1.5.2. *Bathytoshia brevicaudata*

The short tail stingray (Figure 1.4.) is distributed across much of the temperate southern hemisphere (Duffy et al., 2016). This species has been recorded in southern Africa, from the Zambezi river to Cape Town, in Australia from southern Queensland to Shark Bay in Western Australia and throughout New Zealand including the Chatham and Kermadec Islands, although it is uncommon south of Cook Strait and at the Kermadec Islands (Last & Stevens, 2009; Duffy et al., 2016). In addition, the recent molecular genetic analysis combining the species with *Dasyatis matsubarai* has increased the range of *B. brevicaudata* into a highly dispersed antitropical distribution.

Figure 1.4. Short tail stingray (*Bathytoshia brevicaudata*). Copyright P.T. Hirschfield. Reproduced with permission.

Bathytoshia brevicaudata is classified as ‘Least Concern’ under the IUCN red list as it is not a commercially targeted species and is recorded at relatively high densities across much of its distribution (Duffy et al., 2016). This species is very large, recorded reaching disc widths over 2.1 metres and a total length of over 4.3 metres (Last & Stevens, 2009; Last et al., 2016a) with anecdotally larger sizes still. In New Zealand it is often confused with the long tailed stingray despite its tail being shorter and stouter, often shorter in length than the disc. *Bathytoshia brevicaudata* also possess a line of white pores either side of the disc and sometimes on the top of the skull, markings that are not present on *B. lata* (Last & Stevens, 2009; Last et al., 2016a).

Throughout its range*, B. brevicaudata* occupies a variety of coastal habitats including shallow bays, harbours, estuaries and inlets open coast beaches, rocky reefs, offshore islands and open sea floor (Duffy et al., 2016). Its diet is varied, primarily benthic molluscs, crustaceans and bony fishes (Francis, 2012; Duffy et al., 2016) and like other Dasyatid rays (Cartamil et al., 2003) it is thought to be an opportunistic feeder having been observed taking larger prey such as octopus (D. Herbert, Pers. Comm.) and discarded fish frames (H. Cadwallader, Pers. Obs.). This species is easily habituated to provisioning and is a common visitor at sites where they are either deliberately fed for tourism and at sites where incident feeding occurs, such as at fish cleaning (Pini-Fitzsimmons et al., 2018) or shark cage diving locations (Rizzari et al., 2017). Individuals are often seen during the summer months swimming up and down wharves, breakwaters and beaches of the Tauranga Harbour and associated estuaries.

There has been limited research conducted on this species. For example, we know almost nothing about size and age at maturity, growth rates and other life history parameters. Le Port et al. (2012) examined the seasonal aggregation behaviour of this species at the Poor Knights Islands with a view to examining the efficacy of marine protected areas (MPAs) as a conservation method. They recorded an increase in the number of both males and females in the area during the summer, in particular an increase in the number of large females with mating wounds, corroborating anecdotal evidence that this aggregation has reproductive importance (Le Port et al., 2012). In addition, the majority of the rays reported were juveniles and sub-adults, suggesting that the area is also an important nursery ground for this species (Le Port et al., 2012). Le Port et al. (2008) attempted to elucidate the mesoscale movement behaviour of *B. brevicaudata* after seasonal aggregations in the Poor Knights Islands in northern New Zealand. While their tag attachment and satellite tracking was successful, their sample size of just two immature females does not provide a clear picture of common habits, particularly as each of the tagged rays exhibited different behaviours. However, the study did show that for these individuals at least, the hypothesised long-term migration did not occur, with both individuals showing a seasonal shift to utilisation of deeper waters within 25 kilometres of the aggregation site (Le Port et al., 2008). The Poor Knights Islands is an offshore reserve where fishing is prohibited, and thus further investigation is required in order to ascertain movement behaviours of *B. brevicaudata* within coastal and estuarine areas subjected to a higher level of anthropogenic influence. Rizzari et al. (2017) and Pini-Fitzsimmons et al. (2018) also noted that the assemblages in the North Neptune Islands and at Jervis Bay (Australia), were predominantly female of the 100-150 cm size class, with individuals being present for several months and occasionally multiple years at a time.

Genetic investigation has discovered that male-based gene-flow in this species is five-times that of female-based gene flow, and males are more likely to be immigrants into the area where they were sampled (Roycroft et al., 2019). This supports growing evidence that male-biased dispersal is a common strategy in viviparous elasmobranchs (Roycroft et al., 2019). When applying this more recent information to the previous satellite tag based study by Le Port et al. (2008), it might be expected that these sub-adult females did not undergo a long-distance migration.

1.6. Aims and organisation of this thesis

As mentioned above, Dulvy et al. (2014) highlighted the conservation status and risk of extinction of the world's shark and ray species. This study indicated that a large proportion of ray species were endangered and that those species that inhabit coastal areas were more at risk due to the combined pressures of fishing and habitat degradation. Dulvy et al. (2014) separated habitat degradation into four key categories: residential and commercial development, mangrove destruction, pollution and river engineering. In New Zealand, increases in coastal population resulting in increases in these stressors does not appear to have resulted in declines of local ray species. This provides an opportunity to establish benchmark monitoring combined with more intensive ecological research before potential effects of burgeoning coastal urbanisation occurs. The outcome of such work would be generation of evidence that could inform management measures to mitigate adverse effects on estuarine and coastal ray populations.

This thesis aims to focus in three areas: an overview and review of tagging and attachment methodologies for non-shark-like rays to identify optimal strategies for examining the ecology of ray species; seasonal/spatial patterns in feeding activities of rays and associated habitat use; and assessments of potential anthropogenic effects on habitat quality focusing is on three major degradation processes; urban development, mangrove removal, and pollution.

1) As much of the research reviewed to date comments on difficulties associated with tagging and tracking rays for behavioural assessments, the work begins with an overview and review of current tagging methodologies utilised in the study of batoid elasmobranchs that do not possess shark-like dorsal appendages. Without such body plan attributes application of tags is difficult hence a pilot study of longevity of low-cost acrylic tag attachment is made in captive *B. brevicaudata*, a species that lacks dermal denticles.
2) Following this are studies on the seasonal and spatial patterns in *M. tenuicaudatus* feeding habitat use in the Tauranga Harbour, including an assessment of the use of natural and trimmed mangrove habitat for feeding.
3) Finally, an assessment of heavy metal contamination in *B. brevicaudata* and *M. tenuicaudatus* in the southern Tauranga Harbour compared with the coastal offshore Bay of Plenty area and another, more highly contaminated harbour is made, factoring in the ecology and site fidelity of rays as assessed in the previous component of work. This assessment aims to use the seasonal density of feeding pits in intertidal areas with metal signatures in order to begin to understand the movement behaviour of this species. If metal signatures are similar between Bay of Plenty populations, then there is likely movement between the harbour and the offshore/coastal areas, linking to any pattern in intertidal feeding. If not, then something different is happening.

<u>The hypotheses that will thus be tested are:</u>

H_1: There will be a difference in efficacy of attachment methodologies, and that stainless steel 'shark' tags may not be the optimal choice for soft skinned species.

H_2: There will be a seasonal difference in the magnitude of *M. tenuicaudatus* feeding evidence in intertidal areas.

H_3: Feeding evidence will be positively correlated with density of benthic prey items.

H_4: Feeding evidence will also be affected by site location.

H_5: There will be a difference in heavy metal body burdens between rays inhabiting Tauranga Harbour, and other Bay of Plenty coastal regions.

All portions of this research involving the capture and handling of animals was approved by University of Waikato Animal Ethics Committee. Protocol 975 and addenda (1 and 2) and Protocol 1059.

CHAPTER TWO

A REVIEW OF TAGGING STUDIES OF NON-SHARK-LIKE BATOID ELASMOBRANCHS

H_1: There will be a difference in efficacy of attachment methodologies, and that stainless steel 'shark' tags may not be the best choice for soft skinned species.

2.1. Introduction

Knowledge of animal movement patterns including migration activity, habitat use and behaviour in a spatio-temporal context is increasingly acknowledged as essential for the effective conservation and management of our species and environment (Block et al., 2011; Hammerschlag et al., 2011). Patterns of movement behaviour can include habitat preference, residency, home range or site affinity behaviours, and the types of activities carried out within an area. In addition, the movement response of an animal to environmental cues may be either due to physical phenomena, including but not limited to temperature, salinity and dissolved oxygen, or related to the movement of the Earth or Moon (seasonal, diel and tidal pattern); or biological, including the need to reproduce, avoid predators or to forage. In addition, anthropogenic factors such as habitat alterations, pollution or disturbance by boats may alter movements.

Identification of habitats important to a species or group and connectivity amongst these in an ecosystem sense, together with assessment of the extent of species' site fidelity and the effects of environmental and biological drivers on behaviour, all provide important information on the vulnerability of species to anthropogenic mediated impacts (Hammerschlag et al., 2011). In the marine environment, species that spend part or all of their time in coastal or estuarine waters are under particular pressure from human activities either directly or indirectly. Anthropogenic stressors include fisheries, habitat degradation or development, urban and agricultural contaminant run-off (Halpern et al., 2008; Türkmen et al., 2013; Dulvy et al., 2014; Šlejkovec et al., 2014; Türkmen et al., 2014), climate change (Chin et al., 2010), and possible behavioural alterations due to interactions with humans (Gaspar et al., 2008; Corcoran et al., 2013). To effectively assess the risk associated with these anthropogenic stressors, knowledge of animal movements and an assessment of the time that animals occupy in impacted areas is a critical part of the assessment process. In aquatic environments, the study of movement behaviours and spatial ecology is hindered by the fact that the water obscures visual study of most marine animals (Hammerschlag et al., 2011) and thus, alternative methods of surveillance have needed to be developed. These have included photo-identification, where markings such as spots or scars on individuals are recorded in photographs and then re-sightings of animals are recorded by matching photographs, often with specialised computer software (Mizroch et al., 1990). This technique, commonly used in marine mammal studies, has more recently been applied to rays (Marshall & Pierce, 2012; Flowers et al., 2017). Genetic studies of batoids have exposed varying levels of philopatry and movement (Flowers et al., 2016) and studies of stable isotopes can elucidate movement, diet and resource partitioning and trophic level (Hussey et al., 2011).

However, it is tagging that has become one of the major methodologies utilised for surveillance of marine organisms, including sharks and rays. A number of reviews have covered individual methods of tracking fish behaviour using tagging methodologies. Hammerschlag et al. (2011) reviewed satellite tagging in sharks; Heupel and Webber (2012) reviewed the application of acoustic technology to fish tracking; Kohler and Turner (2001) review conventional tagging of sharks and Jepsen et al. (2015) qualitatively review tagging effects and overall retention of tags in fish species. The methods described each have their own strengths and weaknesses (Table 2.1). While these reviews are comprehensive of their study matter, in most circumstances, members of the Batoidea are either included as 'sharks' or not at all. This lack of distinction is troubling, as rays have very different morphology, skin texture, swimming technique and physiology that would perhaps alter the efficacy of tracking methods. In particular, many batoid elasmobranchs present difficulties with regards to tagging, as they lack (or have significantly reduced) dorsal fins (Le Port et al., 2008). Moreover, many batoid elasmobranchs possess reduced denticles on their dorsal surface (Last & Stevens, 2009; Last et al., 2016a) which may make premature shedding of dart tags more likely. In addition, their rajiform (undulative) or mobuliform (flapping) styles of locomotion may present different forces on attachment points than the front/back locomotive forces of sharks and other fish. As many of the electronic tag technologies are expensive, tag retention is important to gain enough information to justify the cost of the tags.

This review was undertaken due to the lack of information on the longevity and fit-for-purpose of tag attachments on non-shark-like batoid species. For review of other tagging technology see (Musyl et al., 2011) and others (including Hammerschlag et al., 2011; Heupel & Webber, 2012; Hammerschlag et al., 2014). These latter reviews do include some comments on batoid tagging, but commentary is frequently incidental.

Table 2.1. Tag types utilised in shark and ray studies, their strengths and some difficulties. * indicates three tag types that are often combined.

Tag type	Description/use	Other equipment required/ shortcomings	Review/example
PIT	"Passive Integrated Transponder" Identification tag, similar to pet microchip, is implanted into musculature	Scanner for identification.	Kohler and Turner (2001)
Conventional	Identification tag, external, can be dart, disc, streamer	Requires visual sighting or recapture. Can become fouled.	Kohler and Turner (2001)
Acoustic	Utilising ultrasonic signals from the tag to determine presence of a tagged animal. Active telemetry involves following or finding the animal with a portable receiver, while Passive telemetry utilizes arrays of receivers in varying levels of complexity. Simple arrays can determine presence/absence, gated arrays can determine entrance/exit of areas, while complex grid arrays can elucidate fine-scale movement patterns within the array.	Receivers are required, either moored in an array (Passive) or hand/boat held (Active). These can be expensive. Tag cost. Animal cannot be tracked when/if it leaves the array Receivers have differing ranges of detection in different habitats and weather conditions. Requires batteries	Heupel and Webber (2012)
Satellite*	Tags utilizing the ARGOS satellite network in order to provide positions of animals. Can be fixed (requiring animal to break water surface in order to transmit data, or Pop-Up that detaches from animal after a pre-programmed duration and transmits data once antenna is clear of the water's surface.	Requires batteries. Expensive. Some require recovering tag for full information collected (see Datalogger). If towed, can be energetically costly to animal.	Hammerschlag et al. (2011); Musyl et al. (2011)
Datalogger*	Recording devices associated with measurement devices such as accelerometers; salinity, light, pressure sensors; attached to an animal and recovered later. Commonly used on marine mammals.	Requires recovering to gain data. Can be large/heavy. Requires batteries.	Sims et al. (2008)
GPS*	Fine-scale positioning tags, for real-time locations of aquatic animals, towed-float mechanisms are often required in order for antenna to be clear of the water.	Requires batteries. Towed float mechanisms are energetically costly to animals.	Riding et al. (2009)

Therefore, the aim of this chapter is to review the literature where tagging methods have been utilised to study non-shark-like batoid elasmobranchs and to assess the tag attachment methodology for longevity, ease of use, and impact on the subject animals. In addition, aquarium and field experiments were performed in order to test a cheap and easily accessible method, simple acrylic darts, for longevity on a large smooth-skinned stingray, *B. brevicaudata*.

2.2. Methods

2.2.1. Review of batoid elasmobranch tagging studies

In order to assess the technologies utilised for batoid surveillance to-date, a search was conducted of the primary literature utilising Web of Science and Google Scholar databases of peer-reviewed studies published prior to the 10th of July 2019. Search terms used were: ‘batoid’, ‘stingray’, ‘manta ray’, ‘ray’, ‘devil ray’, ‘mobula ray’ ‘eagle ray’, ‘cownose ray’, ‘skate’, ‘electric ray’, ‘myliobatiformes’, ‘rajiformes’, and ‘torpediniformes’. These terms were coupled with the terms ‘tag’, ‘tagging’, ‘telemetry’, ‘acoustic’, ‘satellite’, ‘movement’, ‘diel’, ‘migration’, site fidelity’ and ‘residency’ on an individual basis. Data were collated from each publication on: study location, study purpose and species, type of tag, type of attachment method and duration of attachment (if available). Mention of range testing of acoustic receivers was also noted for acoustic studies. If a publication included more than one species, or more than one method was used, each was counted as a separate study for analysis.

Non-shark-like batoids are defined for the purposes of this study as members of those families of batoids that do not possess (or possess highly reduced) appendages that tags are traditionally affixed to on shark species such as the dorsal fin. This equates to members of the orders Myliobatiformes, Rajiformes, and Torpediformes; but excludes the order Rhinopristiformes, containing the families Pristidae (Sawfishes), Rhinidae (Wedgefishes), Rhinobatidae (Guitarfishes), Glaucostegidae (Giant guitarfishes), and the Trygonorrinidae (Banjo rays) (Last et al., 2016a). Publications that included species in the Rhinopristiformes alongside other batoid elasmobranchs were included, but species in this order were not included in tag longevity analysis.

2.2.2. External tag longevity on ray species

In order to determine differences in the efficacy of different attachment/anchor methods for externally mounted tags on stingrays, the duration of retention of satellite tags from the collected publications was used. Satellite tagging studies were used due to the ability to determine date of tag shedding/pop-off.

Statistical analysis

The percentage success of each tagging event was calculated for pop-off satellite (PSAT) tags by using the pre-programmed pop off duration or the stated maximum battery life of the tag for fixed satellite tags, and the actual number of days a ray was tagged, as reported by the publication. For publications lacking a reported pre-programmed pop-off date or battery life, (and/or where battery life could not be ascertained via the tag manufacturers website) the maximum attachment period achieved was used to calculate percentage success. Tagging events where a tag failed to report were excluded from the analysis and tags that were retained longer than the pre-determined pop-off date (or equivalent, see above), were counted as 100 percent successful for the purposes of this analysis.

The percentage success of each tag attachment method was compared using Kruskal-Wallis analysis, followed by pair-wise Mann-Whitney U tests to determine where any differences lay. The calculated mean proportion success was combined with metrics representing other important factors to consider when selecting an external tag attachment method: whether capture is required, and approximate cost of anchors.

2.2.3. Aquarium experiment

Three adult male short tail stingrays (*Bathytoshia brevicaudata*) were tagged with acrylic tipped dart tags (Figure 2.1 B) (Hallprint, Australia) by aquarists on SCUBA in the main tank at the National Aquarium of New Zealand in Napier during October and November 2017. A standard tag applicator (supplied by tag supplier) fixed into a 1 metre aluminium pole was utilised and tags were applied at a 45 degree angle into the dorsal disc surface. Duration of tag attachment and any necrosis or irritation at the site of attachment was noted by staff of the aquarium during their daily feeding and care of the tank.

2.2.4. Field tagging experiment of Bathytoshia brevicaudata

Thirty-two *B. bathytoshia* individuals were caught and tagged with identification tags between April 2016 and February 2018 in order to (a) develop a capture methodology for a typically very large and difficult to capture and handle stingray, and (b) to determine an effective tagging methodology for a smooth skinned stingray (lacking dermal denticles).

Bathytoshia brevicaudata are a very large and powerful animal. Individuals can reach over 2.3 m disc width (DW) and 350 kg (Last & Stevens, 2009; Last et al., 2016a). A new capture methodology was developed for such a large animal avoiding the use of hooks and nets in order to minimise stress for the animal and maximise safety for participating personnel. This methodology allowed control of the tail and quick release when the work was complete. All rays were caught at Cross Road boat ramp, Tauranga Harbour, New Zealand (37°40'18.3"S 176°10'18.9"E).

A large (3 m wide by 2 m long) stretcher made of water permeable shade cloth material held taut with aluminium poles was placed on the boat ramp submerged in around 45 cm of water. Early events utilised a slightly smaller stretcher with plastic fencing poles, but this was superseded by the stronger and larger apparatus. A berley/chum trail to attract the rays was created by pulling apart fish frames in the water and pieces of bait were placed on the stretcher to entice animals to swim on top of the stretcher surface. When a ray was sufficiently on the stretcher to ensure both ray and personnel safety, the seaward pole ends were gently lifted to an approximately 30-degree angle and the stretcher with the ray was moved up the ramp into water approximately 3 centimetres deep. Stretcher sides were lifted in order to contain the ray. Once the ray was contained within the stretcher, a large wet blanket was thrown onto the tail to slow tail movement before tail and blanket was held firm by an assistant. Thickness of blanket was sufficient to allow no penetration by the barb. After the tail was controlled allowing safe manoeuvring around the ray, the animal was measured and 12 of the animals were biopsied for metals work (*see* Chapter 6). Rays were tagged for identification with both a numbered 3 cm diameter Peterson disc tag (Floytag, USA) through the pelvic fin (Figure 2.1 A) and a nylon tipped dart tag (Hallprint, Australia) (Figure 2.1 B) with a unique pattern of coloured heat shrink tubing, in the dorsal musculature.

Figure 2.1. A) Petersen disc tags and B) nylon tipped dart tags utilised for longevity experiments on *B. brevicaudata*.

Once work was complete, the animal was swiftly released. As there were no nets to untangle or hooks to remove, release was much quicker than would have been possible with these other methods. Rays rarely struggled once the tail was controlled preventing tiring of the animal that can occur with hook and line and net capture.

Over summer and autumn 2017, a number of timed surveys were carried out in order to sight tagged rays in four locations in the Southern Tauranga Harbour where *B. brevicaudata* individuals are regularly sighted close to Tauranga City Centre; Cross Road boat ramp, Bridge Marina, Sulphur Point Marina and The Strand (Figure 2.2). Timed surveys were performed either walking (Marinas and the Strand) or stationary (Cross road ramp) for 60 minutes at varying tidal states. Ray sightings, and whether they were tagged or untagged, were noted and photographs were taken where possible. Incidental sightings were also recorded, and a website was built and combined with public engagement to allow public tag sightings to be recorded.

Figure 2.2. Satellite image of an area of the southern Tauranga Harbour showing locations of walking (red shading) and stationary (yellow arrow) *B. brevicaudata* surveys. A) Cross road boat ramp, B) Bridge marina, C) Sulphur Point marina, D) The Strand. *Image source*: **Google Earth**. June 3, 2016. Accessed July 4, 2019.

2.3. Results and Discussion

2.3.1. *Review of previous work*

A total of 72 studies spanning 56 publications between 1984 and 2019 were examined (*see* Appendix A, Table A.1.). To date, 31 species of non-shark-like batoid elasmobranchs have been studied using a form of tagging methodology. These species represent 7 families (Figure 2.3), from two orders, Myliobatiformes and Rajiformes.

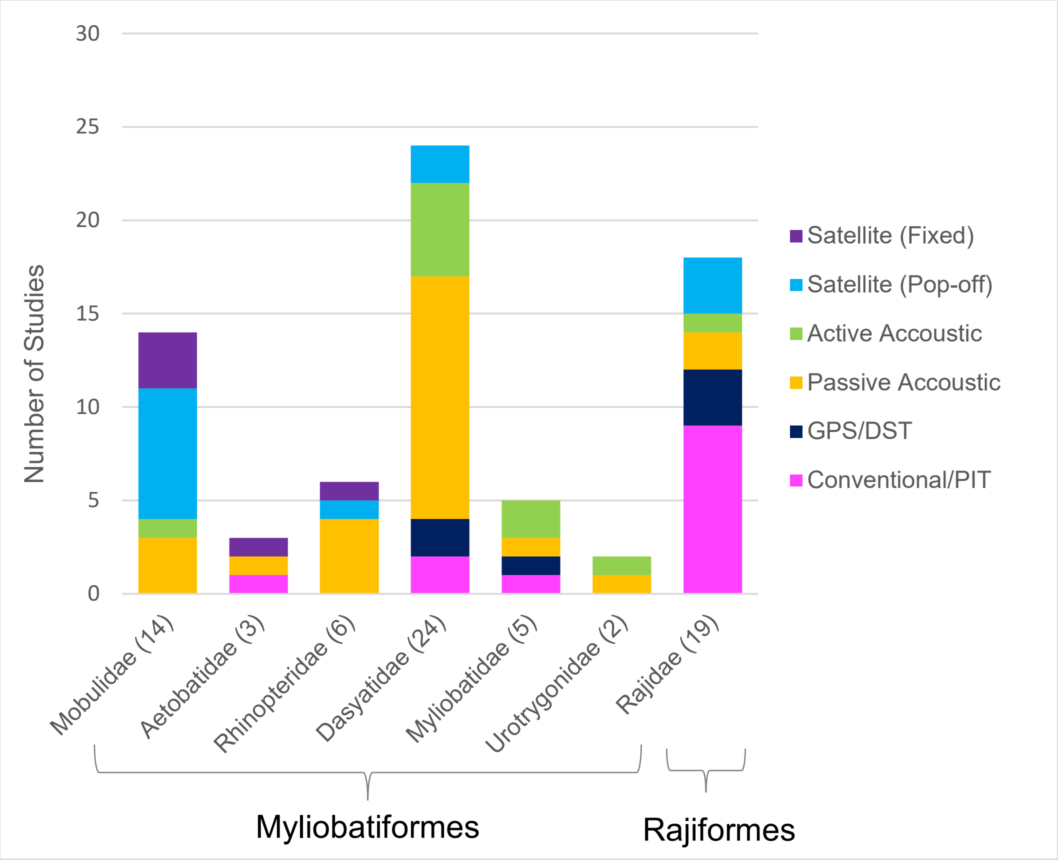


Figure 2.3 Graph showing tagging methodologies used to study non-shark-like batoid elasmobranchs between 1984 and 2019. Numbers in brackets denote number of studies for each family.

Four broad types of tags were used in the studies reviewed. These were; Satellite, Acoustic, GPS/Data storage (DST) and Conventional/identification/Passive integrated transponder (PIT) tags (Figure 2.3). Some publications included more than one species or more than one tag type, creating 72 separate studies for analysis (*see* Appendix A, Table A.1.). Out of these 72 studies, 21 used satellite tagging technology, including 9 fixed tags and 12 PSAT (*see* Appendix 1, Table A.2.), 32 utilised acoustic telemetry with 23 passive acoustic monitoring studies and 9 active acoustic studies (*see* Appendix 1, Table A.3.). Thirteen studies utilised conventional tagging or ID methods, including electronic PIT tags (*see* Appendix 1, Table A.4.) and 6 utilised GPS or data storage tags.

2.3.2. Overview of tagging methodologies used on ray species

Conventional Tagging

The 13 studies that used conventional tagging methods (Kohler & Turner, 2001) utilised either external identification tags such as spaghetti, Petersen discs and dart tags, or the electronic PIT tags, for mainly tag-release-recapture experiments between 1984 and 2018. One study utilised dart tags for identification of individuals to determine returns at a provisioning site (Pini-Fitzsimmons et al., 2018). Of the species studied, 70% were in the family Rajidae, and the families Aetobatidae, Myliobatidae and Dasyatidae equally comprised the remainder. Fifty percent of studies tagged animals with Petersen discs, 20% with electronic PIT tags, 10% with spaghetti tags inserted through the spiracles of the animal, and 20% with dart tags.

Unlike many shark species, batoid elasmobranchs do not tend to be considered as sportfish species. Therefore, there is no network of recreational anglers regularly targeting and catching animals to include them in such a large-scale tagging projects as the National Marine Fisheries Service (NMFS) Cooperative Shark Tagging Program (CSTP) in the United States (Kohler & Turner, 2001). This has meant that the number of studies using conventional tags for this group is well below that of sharks. Neat et al. (2014) utilised recapture of conventionally tagged *Dipturus batis* through the Scottish Shark Tagging Programme. Of the 280 *D. batis* tagged, 74 were recaptured, with 33 additional recaptures being multiple recaptures of these individuals. The majority of conventional tagging studies examined the Rajidae (Ellis et al., 2011; Neat et al., 2014). This is likely to be due to the fact that many

Rajidae species are common fisheries targets or appear in large numbers as bycatch species in trawl fisheries. This technology, while useful for species that are likely to be re-captured or re-sighted, does not provide fine-scale data and may be compromised by the unknown longevity of tags, therefore the use of this method is limited.

GPS Tags and Data-logging Tags

Small data storage tags have allowed conventional tag-release-recapture studies to develop into much more detailed habitat use studies by recording multiple environmental parameters such as pressure (used as a proxy for depth), temperature and salinity (Wearmouth & Sims, 2008). Like conventional or PIT tags and unlike PSAT tags, they require the return of the tag for the data to be accessed. This has meant that the two types are often used together and may be more suited to species that are likely to be recaptured by high fishing effort. Data storage tags have also been used to determine fisheries independent stock distributions and species residency in order to inform policy decisions (Hunter et al., 2005; Neat et al., 2014). Otaki et al. (2015) affixed data logging tags equipped with accelerometers to five *Hemitrygon akajei* individuals in Tokyo Bay, Japan, for between two and four hours each. Tags were attached to a release mechanism, in turn fixed to a net which was surgically mounted on the back of the ray along with an acoustic transmitter (Otaki et al., 2015). These tags have the potential to be very useful and may provide fine-scale behavioural data that other methods cannot.

Acoustic Telemetry

Passive acoustic monitoring has answered some important questions including the discovery of nursery areas due to its ability to determine residence time in a particular area (Davy et al., 2015). Thirty-two studies utilised acoustic telemetry in order to study non-shark-like batoid elasmobranchs (Appendix A, Table A.3.) of which 72% utilised passive acoustic telemetry and 28% active acoustic tracking methodologies. Twelve studies used internal implantation, 10 of which were passive acoustic studies. The remainder of studies utilised Petersen discs (4 studies) stainless steel darts (7 studies), plastic umbrella darts (1), spiracular attachment (4 studies), or an attachment through the tail musculature (1 study). McCauley et al. (2014) used both passive and active telemetry, but the attachment method was not reported.

Passive acoustic telemetry is highly effective for long-term studies to determine fine-scale habitat utilisation using a grid style array (Collins et al., 2007; Davy et al., 2015), entrance and exit rates (Marcotte, 2014) and location within an estuarine or riverine system using gates (Campbell et al., 2012) and gives real-time detections that can be linked with time of day or abiotic factors such as temperature and tidal cycles. However, this technology cannot show the location of an animal when it leaves the area where the receivers are located (Davy et al., 2015). Vaudo and Heithaus (2012) studied habitat use of batoid elasmobranchs at Shark Bay, Western Australia, using passive acoustic telemetry. During this study, high densities of shovelnose rays (*Glaucostegus typus*) and reticulate whiprays (*Himantura uarnak*) were found in shallow sandflat habitats during the summer months. Throughout the winter however, much lower densities were present on the sandflats but were still present in the bay, suggesting a seasonal change in preferred habitat.

Active acoustic tracking has been used to determine activity space, and temperature preference amongst other behavioural traits. Tilley et al. (2013) using repeated active acoustic tracking, found that the diurnal activity space (the area used by the animal) of the Atlantic stingray (*Hypanus americanus*) was much larger than that of the night-time activity space. In contrast, *B. lata* had much larger night time activity spaces than during the day by similar methods (Cartamil et al., 2003). Matern et al. (2000) using active tracking, suggested that the strong tidally independent daily movements found in bat rays (*Myliobatis californica)* are evidence of behavioural thermoregulation.

Satellite Telemetry

Satellite tagging studies, perhaps due to the high cost of tags, typically have low sample sizes: 17 out of 21 studies in this review had sample sizes less than 10 (*see* Appendix A, table A.2.). Studies have been used to determine larger-scale movement and migration of species and post-capture survival. Peklova et al. (2014) tracked five Arctic skate that demonstrated three distinct behavioural strategies and remained within very narrow ranges of water temperature. Le Port et al. (2008) challenged the traditionally held belief that short tail stingrays underwent long-distance (>100km) migrations with the observations from two PSAT tracked juvenile females and discovered that while long-distance migrations were not undertaken by these two individuals, they occupied deeper water during the winter months. Francis and Jones (2017) found survival rates of commercially caught *Mobula mobular* in New Zealand waters were low but obtained migration and depth-use data for surviving individuals.

2.3.3. *Potential Impacts of tagging methodologies on physiology and behaviour*

The effect of tagging on the physiology and behaviour of rays is important not only for the wellbeing of the animals but also for the accuracy of the information gained from the tagging. A damaged or disturbed animal may behave abnormally and thus reduce the value of the study. Capture is stressful for the animals, and as different elasmobranch species respond differently to capture stress (Mandelman & Skomal, 2009) care is required, which may not always occur if the capture method is via a commercial fishery (Francis & Jones, 2017). For example, members of the Rajidae are often caught in trawls, which facilitates tag-release-recapture studies, but may also result in a high rate of post-capture mortality (Enever et al., 2009). External attachment methodology does not always require capture; however, as tag-loss is common it is not as reliable as internal implantation (M. Heupel, Pers. Comm.).

The handling of rays for tagging is varied, as indeed is the attachment methodology. For example, electronic tags are either externally anchored, or internally implanted in the body cavity. The former may cause drag and a variation in movement behaviour if the tag is too large (Blaylock, 1990), while the latter creates a large stitched wound. Four publications evaluated the hydrodynamic effects of transmitter attachment to non-shark-like batoid elasmobranchs (Blaylock, 1990; Grusha & Patterson, 2003; Corcoran et al., 2013; Speed et al., 2013), specifically drag and lift. For internal implantation, some studies use anaesthetic and others rely on tonic immobility, the former may promote the production of stress hormones (Frick et al., 2009) while the latter is unreliable and not possible in large specimens.

There may be behavioural influences of boat noise in active tracking studies – close following with a boat may cause altered movement speeds (refuging or swimming away), or influence direction travelled (for instance away from the boat) (Heupel et al., 2006).

Three studies, by Matern et al. (2000), Ogburn et al. (2018), and Otaki et al. (2015), removed animals from their environment for long periods (up to two months) prior to tag and release (instead of practicing immediate catch-tag-release of animals). Matern et al. (2000) is one of the most commonly cited publications when behavioural thermoregulation and thermal behaviour of rays is discussed (147 citations to date according to Google Scholar). However, the methods as to how tagging took place and the subsequent active tracking method may have influenced the result. The transport and captivity implemented by these studies, including feeding non-natural food whilst in captivity, may alter the animal's natural behaviour significantly and any conclusions from these studies should be taken with caution. For example, Otaki et al. (2015) reported different behaviours by all animals, a result that could have stemmed from their treatment prior to release.

2.3.4. Tagging as a tool for Conservation

In order to effectively preserve species and their critical habitats, we must first determine which habitats are important and why. This can be determined by the movements and behaviours of animals within the ecosystem, and tagging has been used on many occasions to obtain this data.

Neat et al. (2014) used a combination of conventional, passive acoustic and data-logging tags to provide evidence of residency of the critically endangered common skate (*Dipturus batis)* off the west coast of Scotland, in the United Kingdom. They used this information to recommend the establishment of a marine protected area in their study location and suggested that management should consider all depths of the study area and areas beyond the study site.

Hunter et al. (2006) used DSTs on 197 animals to provide a fishery-independent estimation of stock distribution and tidal associated movement of the thornback ray (*Raja clavata*) in the Thames Estuary, UK. Results showed that while 77% of recovered data storage tag indicated seasonal movement outside the estuary, the area is an important habitat for this species. The information collected provided data that was subsequently used to test the effects of differing fishing closure scenarios (Hunter et al., 2006).

Stewart et al. (2016a) utilised pop-off satellite tagging in order to refute the commonly held belief that the oceanic mobula ray, *Mobula birostris,* exhibits long term migratory behaviour. It was demonstrated that individuals tagged at four sites in the Indo-Pacific did not overlap in geographic range. This pattern was further confirmed by complimentary stable isotope and genetic analyses.

The IUCN Red List classifies one of the species studied in the publications reviewed as data deficient, 10 as least concern, 6 as near threatened, 7 as vulnerable, 4 as endangered, and 1 as critically endangered. Of the species studied 41% (n=12) were classified by the IUCN Red List as threatened (in the categories vulnerable, endangered or critically endangered), 55% (n=16) as near threatened or least concern and 3% (n=1) as Data Deficient. With a global number of 83 species of non-shark-like rays identified as vulnerable, there is a long way to go to understand the habits of this group. Understanding movement and habitat use behaviour is essential for conservation science and management, and utilising tagging technologies with appropriate anchor mechanisms will aid in the gathering of this crucial information.

2.3.5. Review of External Attachment Methodology

Attachment methods for external identification or telemetry tags on non-shark-like batoids are varied amongst the literature and many are derived from methodology used for shark tagging studies. Attachment methods include varying darts, Peterson discs, bridle mechanisms, and through-tail attachment (*see* Appendix A, table A.1.). Darts had varying tips, including stainless steel ‘shark tags’, nylon ‘umbrella’ tips often used for big-game fishing and acrylic gamefish dart tips (Figure 2.4).

Figure 2.4. Dart anchor designs. A) Stainless-steel or Titanium, B) Umbrella, C) Acrylic gamefish dart. Image adapted from Hallprint (Australia).

Bridle mechanisms were tested and used by Ajemian and Powers (2014) and included pectoral wing bridle and spiracular bridles. Tail suture involved monofilament line inserted through the musculature at the base of the tail, creating a semi intra-muscular loop around the tail to which tagging apparatus could be fixed (Le Port et al., 2008). Ajemian and Powers (2014) also tested a method that in the publication was entitled ‘tail suture’. This method, that also involved attachment at the base of the tail, was confirmed to be the same as the method developed by Le Port et al. (2008) after communication with the author (M. Ajemian, Pers. Comm.).

Dart tags, either metal or plastic anchored are quick to implant, and do not require animal capture to deploy, for larger species such as Mobulidae and the larger Dasyatidae, these may be preferable. In general, Petersen disc tags have been used for members of the Rajidae, perhaps due to these species often being target or bycatch in trawl fisheries due to their benthic habits. Handling the animals is already necessary to return them to the ocean and so tagging with Petersen discs may be easier.

2.3.6. Longevity of external attachment methodology

Twenty-one studies from 16 publications utilised satellite tags in order to study rays, with nine species studied from five families, utilising nine methods of external tag attachment (Appendix A, Table A.1.). This review identified that an average of 72% of tags were released before their programmed pop-off date, comparing well with the review by Hammerschlag et al. (2011), who found premature releases of pop-up satellite tags averaged 66% over all shark tagging studies. Premature tag loss is not always a negative; in the case of snagging or entanglement a premature loss of tag would be beneficial and may save the life of the animal (Jepsen et al., 2015). However, to record animal movement successfully, tag retention must be sufficient to collect suitable long-term data.

Kruskal-Wallis analysis showed that there was a highly significant difference between percentage success (see p. 30) of each method (χ^2=23.58, d.f.=8, $p = 0.002$; Figure 2.5). The most effective anchor mechanism was the stainless-steel dart often known as ‘shark tags’ (mean proportion success = 0.85 +/- 0.09 SE), followed by the titanium dart (mean proportion success = 0.75 +/- 0.06 SE). Nylon darts had mean success of 0.56 (+/- 0.065) while Petersen discs had mean success of 0.58 (+/-

0.14 SE). The three 'Bridle' techniques, spiracular, disc and dart were less successful, with mean proportion success of 0.26 (+/-0.11 SE), 0.41 (+/-0.21 SE) and 0.16 (+/-0.06 SE) respectively with the dart bridle being the least successful of all the methods. The one study utilising the small dorsal fin of *M. birostris* had one tag reach programmed pop-off and two very unsuccessful deployments, giving this method a mean proportion success of 0.42 (+/-0.28 SE).

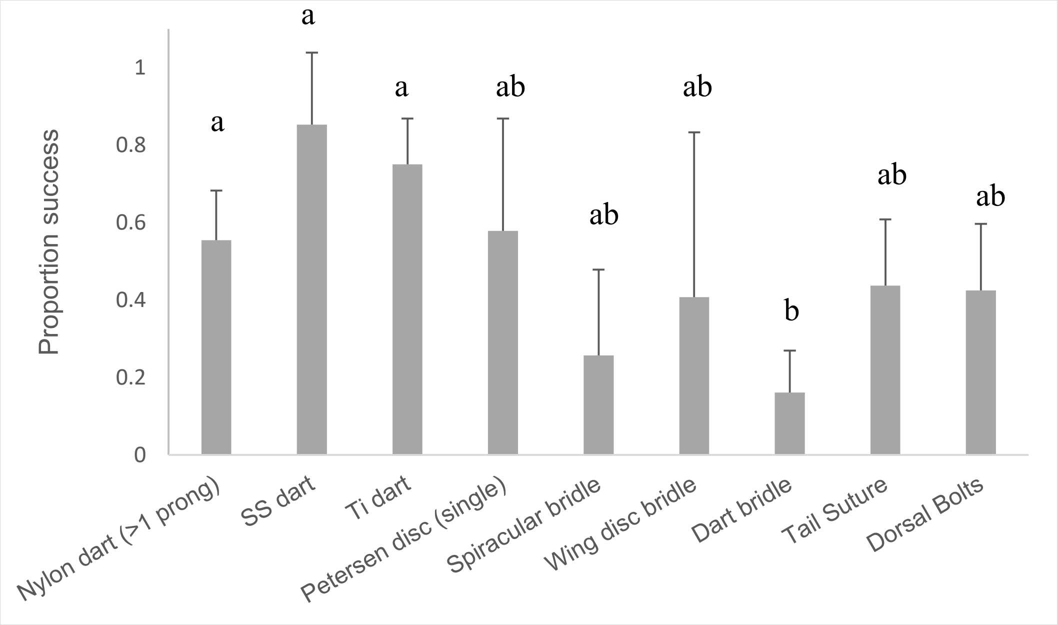


Figure 2.5. Mean proportion success of each tag anchor method used in the study of non-shark-like batoid elasmobranchs. Error bars show 95% confidence intervals. The same letters show no significant difference at $p<0.05$.

When anchor methods were grouped into 'Metal darts', 'Plastic darts', 'Petersen discs', 'Tail Suture' and 'Dorsal', there was no significant difference among the methods. The lack of difference found between single nylon and metallic darts found overall was in contrast to the findings of Musyl et al. (2011) who determined a significantly lower retention rate of PSAT tags by nylon dart anchors over shark and teleost species. However, a slight, but non-significant lower retention success of Nylon tag anchors was found when considering only mobulid animals, perhaps due to mobulid rough skin with often tightly packed denticles being similar to the skin of sharks (Last et al., 2016a). Nylon tags were not used in other families for comparison. For example, the short tail stingray (*B. brevicaudata*) has very few denticles and thus stainless-steel tags may cut their way out of the skin more rapidly, hence a new more suitable and successful method for this species was developed by Le Port et al. (2008). Indeed, stainless steel darts and Petersen discs used in *B. brevicaudata* in Jervis Bay, Australia were both retained for fewer than 365 and 180 days respectively (J. Pini-Fitzsimmons, Pers. Comm).

Due to the low number of studies utilising satellite tagging methods, sample sizes were small for this analysis. It is possible that with larger sample sizes, more differences between anchor methodologies would be found. In addition, the differing programmed deployment periods may mean that some less effective methodologies may be showing a higher success rate due to the completion of the programmed period when in reality it would not have lasted for a longer programmed period.

2.3.7. Aquarium acrylic tag longevity experiment

Two out of three tags (rays 1 and 3) were still attached with no necrosis or irritation at the site of the tag by 15 July 2019 (a total of 645 days and 627 days). The final ray (ray 2) was euthanised in February 2019 due to deterioration from a neurological issue present before tag attachment. The tag on this animal had been lost during December 2018 (approximately 430 days attached). When this tag was applied by the diver, it was applied very close to the distal edge of the disc, rather than close to the body as is usual (Figure 2.6). Immediately post application, this individual swam normally, then settled on the substrate and proceeded to undulate the distal edge of the disc around the tag before wrapping the edge of the disc around the tag, and tugging as if to pull it out. This behaviour was repeated 5 times, before previous behaviour was resumed. This seems to indicate a very high level of dexterity in the disc edge that may not have been observed previously in dasyatid rays.

Figure 2.6. Short tail stingray (*B. brevicaudata*) number 2 at the National Aquarium of New Zealand with sub-optimal tag attachment close to the distal edge of the disc.

2.3.8. Field tagging experiment of *Bathytoshia brevicaudata*

General observations

Thirty-three *Bathytoshia brevicaudata* individuals were caught throughout this project. Nine rays were caught and tagged in April 2016, 19 between January and May 2017 and five in January and February 2018. All individuals were female. Size ranged between 88 and 144 cm disc width. As this species reaches 230 cm disc width (Last et al., 2016a) and size at maturity is unknown, assumed to be ~100 cm (Le Port et al., 2012), most of the individuals are likely to be juvenile or sub-adult

(3 individuals) or small adults (30 individuals). Two individuals (disc width 134 and 144 cm) showed the distended rear body that is characteristic of pregnancy in ray species, although without ultrasound equipment pregnancy cannot be confirmed. The breeding habits of this species is unclear, with annual aggregations reported at the Poor Knights Islands Marine Reserve often considered to be for breeding purposes.

During the course of this project neonate (~35 cm DW) individuals were sighted on a number of occasions, mostly associated with adult females in shallow, intertidal areas of the harbour during the months of February and March (L. Allen, D. Marquand, Pers. Comm) (Figure 2.7)

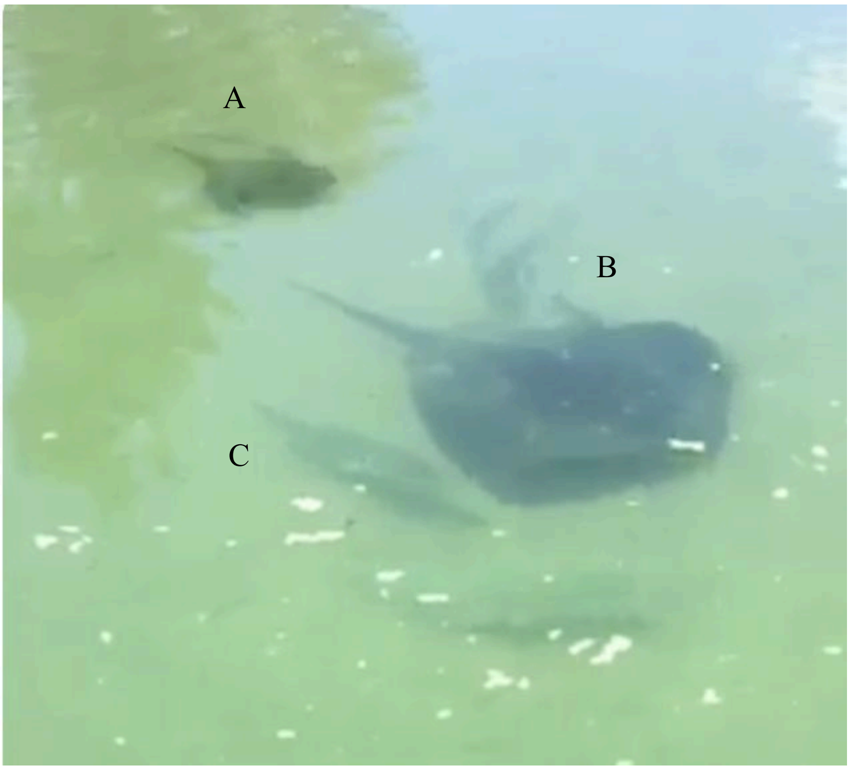


Figure 2.7. (A) Neonate (~35cm disc width) *B. brevicaudata* in the Tauranga Harbour during March 2019 associated with (B) an Adult female *B. brevicaudata*. (C) are yellowtail kingfish (*Seriola lalandi lalandi*), a species often associated with *B. brevicaudata*. Photo taken from a video, Copyright L. Allen, reproduced with permission.

Tagging process

All tagging events lasted no more than 4.5 minutes, from the time the stretcher was lifted. Rays' spiracular ventilation rate increased slightly, as is expected in a capture situation (Charbeneau, 2004), then quickly returned to normal. All rays swam off strongly at the end of the capture procedure and several returned to the capture site for more bait shortly afterwards.

Ray sightings and tag re-sightings

Thirty-eight surveys of 60 minutes were performed between January and March 2017. *B. brevicaudata* individuals were sighted on 19 of those surveys, however only six of the rays sighted were tagged.

Four of the rays re-sighted had retained both tags, however two had lost discs, one after 33 days at liberty and one unknown. One ray was sighted after 603 days at liberty, outside of the observation schedule. The disc tag was lost, and the dart tag was severely fouled. The ray was identifiable due to the short tail (assumed to be cut by a fisherman), and the fact that it was regularly seen in Bridge Marina over several years. Although rays were tagged in 2016, 2017 and 2018, all rays re-sighted during the survey period in 2017 had been tagged that year, and no rays other than the individual mentioned above were re-sighted in subsequent years, perhaps due to tag loss, movement of animals, or insufficient observation effort.

An ongoing study utilising disc tags on the pelvic fin to secure acoustic tags to *B. brevicaudata* in Australia noted that all discs had been lost on returning to the site after less than 6 months (J. Pini-Fitzsimmons Pers. Comm.). A previous study on the same rays found that the rays lost identifying stainless steel dart tags after less than one year; one ray had retained the dart head, but the tag streamer had been bitten off - it is thought by a smaller fish (J. Pini-Fitzsimmons Pers. Comm.).

While this field and aquarium longevity experiment had been intended to be a pre-cursor to an extensive visual and acoustic tagging programme of *B. brevicaudata*, the low number of re-sights combined a number of logistical constraints prevented this from coming into fruition.

2.4. Conclusions

Overall, there have been relatively few methodological studies of tag use and attachment on batoid species, especially those of non-shark-like body form. Due to their different body form, swimming styles, and in many cases, habitats to the majority of species that tagging has been utilised on, a review of the efficacy of tag attachment methodologies on non-shark-like batoid elasmobranchs was required to collate and focus information for future studies.

Certainly, what was clear when comparing anchor mechanisms was that even in the more effective methods such as metallic anchors, a large proportion of tags did not reach their full programmed pop-off dates or battery life. This may be due to tag failure or poor attachment technique. In addition, reporting of effects of tagging events on animals needs to be more consistent between studies, as does reporting of attachment methodology.

Experimental studies have led to the conclusion that simple plastic one-barbed darts are cheap and last well under controlled conditions and tagging sites have healed well with a very small entry wound. However, in field conditions for visual surveys this method was not so successful with fouling after a long period and poor visibility and obstacles making re-sightings difficult. In dense populations that return to an area of good visibility, they may be better than stainless steel darts for soft-skinned animals. However, they may not provide enough anchorage for the drag induced by electronic tags.

This study aimed to test the hypothesis that there would be a difference in efficacy of attachment methodologies, and that stainless steel 'shark' tags may not be the best choice for soft skinned species. Indeed, there was a difference in the efficacy of attachment methodologies, and when all non-shark-like batoid species were considered, traditionally shaped stainless-steel and titanium dart anchors had the highest proportion of success (Summary, Table 2.2).

While there were not any long-term studies utilising nylon umbrella darts on soft-skinned species to allow comparison in this study, it is likely that due to the design, these tags would have a higher rate of success than traditional shaped stainless-steel or titanium anchors. Indeed, nylon umbrella tags have lasted longer than stainless steel darts or pelvic fin disc tags for *B. brevicaudata* in a current field study (J. Pini-Fitzsimmons Pers. Comm) and lasted over 2 months in an acoustic tagging study (although it is not known whether tags detached, or the animal left the array after this point) (Rizzari et al., 2017).

Due to the issues with post-capture behaviour and stress it is recommended that remote tagging methods should be used wherever possible. This study also developed a minimally invasive and safe hook-free capture methodology for a very large, soft skinned species (*B. brevicaudata*) that is recommended for use wherever possible to avoid unnecessary stress to this species.

Table 2.2. Summary of external tag anchor longevity, deployment method and cost. *Cost from D. Hall, M. Francis, K. Lay Pers. Comm. ** Cost of equipment variable for tail suture depending on materials and consumables used.

Anchor type	Mean longevity (proportion success)	Deployment	Approx. Cost /unit (US$) *
Nylon	0.55	Remote	2.5 (Standard)
		Remote	10 (Umbrella)
Stainless-steel	0.85	Remote	3.5
Titanium	0.75	Remote	15
Disc	0.23	Capture	0.75
Tail suture	0.44	Capture	**

When choosing a methodology requiring external tag attachment on a non-shark-like batoid elasmobranch subject it is clear that different species require individual consideration when choosing an anchor. Species differences in skin physiology; breaching, affinity to potential snagging risk habitats such as coral reefs, pelagic versus benthic habit, and mating behaviours need to be considered. For example, ray mating behaviours of males biting female discs are forceful enough to leave mating scars, so may pull out tags (Kajiura et al., 2000). In addition, the benthic or bentho-pelagic nature of many batoid species may create different issues for tag retention such as more obstacles for entanglement or more objects for rubbing.

It is evident that the use of tagging in its many forms is very useful, providing information on movement, behaviour and habitat use that is essential for conservation and management of ray species. This study consolidated information on anchor method longevity, issues with deployment and post-tag behaviour from the literature and other sources that will likely prove useful for future studies utilising external tag methodologies on non-shark-like batoid species.

CHAPTER THREE

SEASONAL FEEDING PATTERNS IN *MYLIOBATIS TENUICAUDATUS* IN A NEW ZEALAND ESTUARY

H_2: There will be a seasonal difference in the magnitude of M. tenuicaudatus feeding evidence in intertidal areas.

3.1. Introduction

Seasonality is a well-documented phenomenon in the animal kingdom. With examples as wide-ranging as migrations of tens of thousands of miles and those of only a few miles (Alerstam et al., 2003), hibernation (Lyman, 2013), seasonal breeding patterns and seasonal changes in population growth (Levy et al., 2016). Such changes in behaviour can be driven by a variety of biotic or abiotic factors, such as temperature, weather patterns (monsoons etc), diurnal light regimes, breeding cycles, or as a knock-on effect from seasonal changes in prey presence or abundance (or condition) (Shaw, 2016).

While studies of seasonal patterns of behaviour in terrestrial animals or birds are often challenging, detecting and gathering evidence for seasonal changes in behaviour and accurately identifying the potential drivers of such patterns in the marine realm can be prohibitively difficult. This is due to limitations of current technologies, high attendant costs and uncertainties of the weather limiting time at sea. With the advent of technological advances such as satellite tagging techniques, the monitoring of location and environmental preferences of large mobile marine species is now more achievable (Hammerschlag et al., 2011).

Understanding seasonal patterns has advantages to both single species and whole ecosystem conservation. For example, knowing whether animals are present in certain areas year-round or at specific times can help to predict degree of exposure to anthropogenic pressures such as pollution and fishing. If individuals occupy a polluted area for long periods or return on a regular basis, then the risk to them may be greater than if they are only present in these areas for short periods. Similarly, if a vessel fishes in the breeding grounds of a species during the breeding season there is the potential for significant impact to breeding age individuals in that region, with serious ramifications for the population into the future. Understanding spatio-temporal behavioural patterns of higher trophic groups will provide information of value to ensuring the sustainability of fisheries or maintaining marine ecosystem services generally. Seasonal patterns can also inform the need and spatial planning of potential marine protected areas (MPAs).

3.1.1. Seasonality in batoid elasmobranchs

From breeding and hormonal or physiological changes, through seasonal presence/absence to long distance migrations, there are a number of examples of seasonal changes in batoid elasmobranchs. Atlantic stingrays (*Hypanus sabinus*) have a distinctly defined breeding season like many mammals and birds, demonstrated by the number of mating wounds on females increasing significantly (Kajiura et al., 2000). In addition, the teeth of the male *H. sabinus* change shape seasonally to facilitate mating behaviour, altering from plate-like molariform to recurved cuspidate during the aforementioned breeding season to increase grip and make holding the females during mating more successful (Kajiura & Tricas, 1996). Seasonal presence/absence of *M. birostris* and *M. alfredi* in southeastern Brazil and the Maldives respectively has been determined by observations of scuba divers and fishers (Luiz et al., 2009; Anderson et al., 2011). *Myliobatis californica* were found to be seasonally present/absent during summer/winter respectively in Tomales Bay, California (Hopkins & Cech, 2003). In New Zealand, *Bathytoshia brevicaudata* is seasonally found in large numbers around the Poor Knights Islands (Le Port et al., 2012). Technological advances to tracking techniques have clarified some presence/absences into seasonal migrations, such as that of the cownose ray (*Rhinoptera bonasus*) migrating long distances along the east coast of the United States between Florida in winter months and estuaries of Long Island, New York for reproductive activities (Ogburn et al., 2018). In addition, 77 percent of thornback rays (*Raja clavata*) tagged moved out of the Thames Estuary during the winter months, returning in spring/summer (Hunter et al., 2005, 2006).

3.1.2. Likely drivers

Temperature is arguably the most important driver of seasonal behaviour and spatial patterns of movement for populations of elasmobranchs, as it correlates with key biophysical attributes of the environment, in turn influencing prey abundance and physiological cycles (Hopkins & Cech, 2003). Like most teleost fish, most elasmobranchs are unable to internally regulate body temperature and so an increase in environmental temperature will affect body processes. Matern et al. (2000) suggested that the strong tidally independent daily movements found in bat rays (*Myliobatis californica)* are evidence of behavioural thermoregulation. In addition, Peklova et al. (2014) while finding three distinct behavioural strategies within the five reliably tracked arctic skate (*Amblyraja hyperborea*), noted that all individuals remained within a very narrow thermal range. In addition, Bassos-Hull et al. (2014) found the number of spotted eagle rays sighted in aerial surveys (*Aetobatus narinari*) had a positive relationship with increasing temperature. Hopkins and Cech (2003) noted that *M. californica* were not present in Tomales Bay, California, when temperatures fell below 10-12 degrees centigrade but returned once temperatures increased. Within the bay, bat rays were most abundant in the areas with the highest temperatures and highest salinity, suggesting salinity may also important. Prey abundance or presence may be regulated by temperature and this may in turn affect the movements or presence of predators as has been shown in zooplanktivorous species such as basking sharks (*Cetorhinus maximus*), whale sharks (*Rhincodon typus*) and *Mobula* species (Notarbartolo-di-Sciara, 1988; Sims et al., 1997; Wilson et al., 2001).

3.1.3. Study species

Myliobatis tenuicaudatus was previously considered endemic to New Zealand and the Kermadec Islands. However, it has recently been confirmed to be a senior synonym of *Myliobatis australis*, known in Australia as the southern eagle ray (White, 2014). This has opened up a much larger (although still limited) library of research for what has been a relatively understudied species. In Australia, this species is often described as undergoing annual 'migrations' either southerly (Potter & Hyndes, 1994; Last & Stevens, 2009; Last et al., 2016a), or inshore (Daley, 2002) during the summer months. The theories of migration behaviours are due to apparent higher numbers of this species appearing in southern Australian and Tasmanian bays and estuaries during the Austral summer (Last & Stevens, 2009). Potter and Hyndes (1994) found that *M. tenuicaudatus* (*M. australis* in study) was present in gill- and seine-net catches in two southern Australian estuaries during summer and autumn months. Animals caught were between 24 and 97 cm disc width therefore would have comprised both juveniles and adults. No sex ratio was mentioned.

The highly identifiable feeding pits of *M. tenuicaudatus* allow non-invasive quantification of feeding effort in intertidal areas. Hines et al. (1997) identified that the number of pits observed decreased during five successive sampling dates throughout February and into March (austral late summer to autumn) in an intertidal sandflat area in the Manukau Harbour estuary system in Auckland, New Zealand. However, such a small number of observations over a typically variable weather season cannot truly be described as seasonality. Hartill (1989) found the highest densities of *M. tenuicaudatus* at shallow depths in the Leigh marine reserve to be during the autumn months, with almost no presence in winter. Hartill (1989) suggested that this could be explained by a migration to greater depths, and this was supported by trawl surveys. As Shaw (2016) suggests, many seasonal presence/absence patterns and migration cycles are due to temperature refuging, with animals moving from an area that may be susceptible to colder (or hotter) conditions. As shallow intertidal sandflats may be cooler than coastal or deeper waters during the winter, this may be the case for *M. tenuicaudatus*. Females of a closely related species *Myliobatis aquila* undertake temporary aggregations during the summer months in a number of locations in the Azores (Alfonso & Vasco-Rodrigues, 2015).

In addition, anecdotal evidence suggests *B. brevicaudata* and *B. lata* are more abundant in Tauranga Harbour in summer (D. Marquand and L. Allan, Pers. Comm.). Similar observations have been made in a range of other species, (Snelson et al., 1988; Hopkins & Cech, 2003; Vaudo & Lowe, 2006; Pierce et al., 2011;

Vaudo & Heithaus, 2012). Consequently, it is hypothesised that a higher density of *M. tenuicaudatus* feeding pits will be present in the summer season, compared to observations during the winter months.

3.1.4. Implications of seasonality in M. tenuicaudatus intertidal feeding

In their creation, the feeding pits of *M. tenuicaudatus* displace a large volume of sediment. This bioturbation likely has a role in broader ecosystem functioning of the habitats where they occur, influencing nutrient cycling and infaunal distribution (Thrush et al., 1991). Thrush et al. (1991) calculated that with 21 fresh pits each day, 1.4 % of an 800 m^2 area of the Manukau estuary was being turned over daily, taking 70 days for complete turnover. If this influence is highly seasonal, this means that the effect on the ecosystem functioning will also be highly seasonal leading to a need to factor in ray bioturbation and predation into any modelling of estuary ecosystem function. There is to-date, no detailed study on ray-pit seasonality in Tauranga Harbour, and no fully quantified study in New Zealand as a whole as the previous study by Hines et al. (1997) only quantified pits during one late summer period.

For the rays, seasonal residency in the harbour rather than full-time residency will reduce exposure to any pollutants that may be present in any particular habitat. Hence to fully assess any risk of contaminants or any other anthropogenic pressures of harbour residence, spatio-temporal patterns of habitat occupation need to be ascertained.

The strongest evidence of feeding pressure of *M. tenuicaudatus* is present on the intertidal sand flat areas, where individuals are regularly seen entering on the incoming tide (D. Marquand, Pers. Comm., H. Cadwallader, Pers. Obs.) and leave at least 2 hours after high tide (D. Culliford, Pers. Comm.). In addition, there is little evidence of feeding in sub-tidal channels (C. Battershill, Pers. Comm.). Intertidal areas in the Tauranga Harbour comprise more than 60 percent of its area and as they are evidently highly important to this species it is here that this study will concentrate. As seasonal movement is often temperature-mediated it is thought that increases in harbour water temperature may drive an increase in feeding evidence. From the available evidence, the hypothesis is that there will be a seasonal difference in the density of *M. tenuicaudatus* feeding evidence in the Tauranga Harbour with higher densities present in the warmer months.

To test this hypothesis the following questions will be asked:

Does the abundance of Myliobatis tenuicaudatus intertidal feeding activity as evidenced by pits, change in a seasonal pattern in the Tauranga Harbour?

Is water temperature correlated with the abundance of pits?

3.2. Methods

The occurrence and density of feeding excavations created by *Myliobatis tenuicaudatus* was quantified monthly over a period of 24 months, from August 2016 to July 2018 over ten sites within Tauranga Harbour. Density of feeding pits was compared across months to determine whether a seasonal pattern was present. Comparison with temperature and weather conditions was also performed to ascertain any identifiable drivers/links.

3.2.1. Survey Methodology

Site location

For the primary study, ten intertidal sites were selected throughout the Tauranga Harbour with similar bathymetric heights using the Land Information New Zealand (LINZ) chart NZ5411, allowing for comparable immersion time, and thus time for rays to feed. Five of these sites were located in the southern basin of the harbour, and five in the Northern basin (Figure 3.1,Table 3.1.). Choice of sites was made from charts so as to not discriminate or bias habitat type on initial selection.

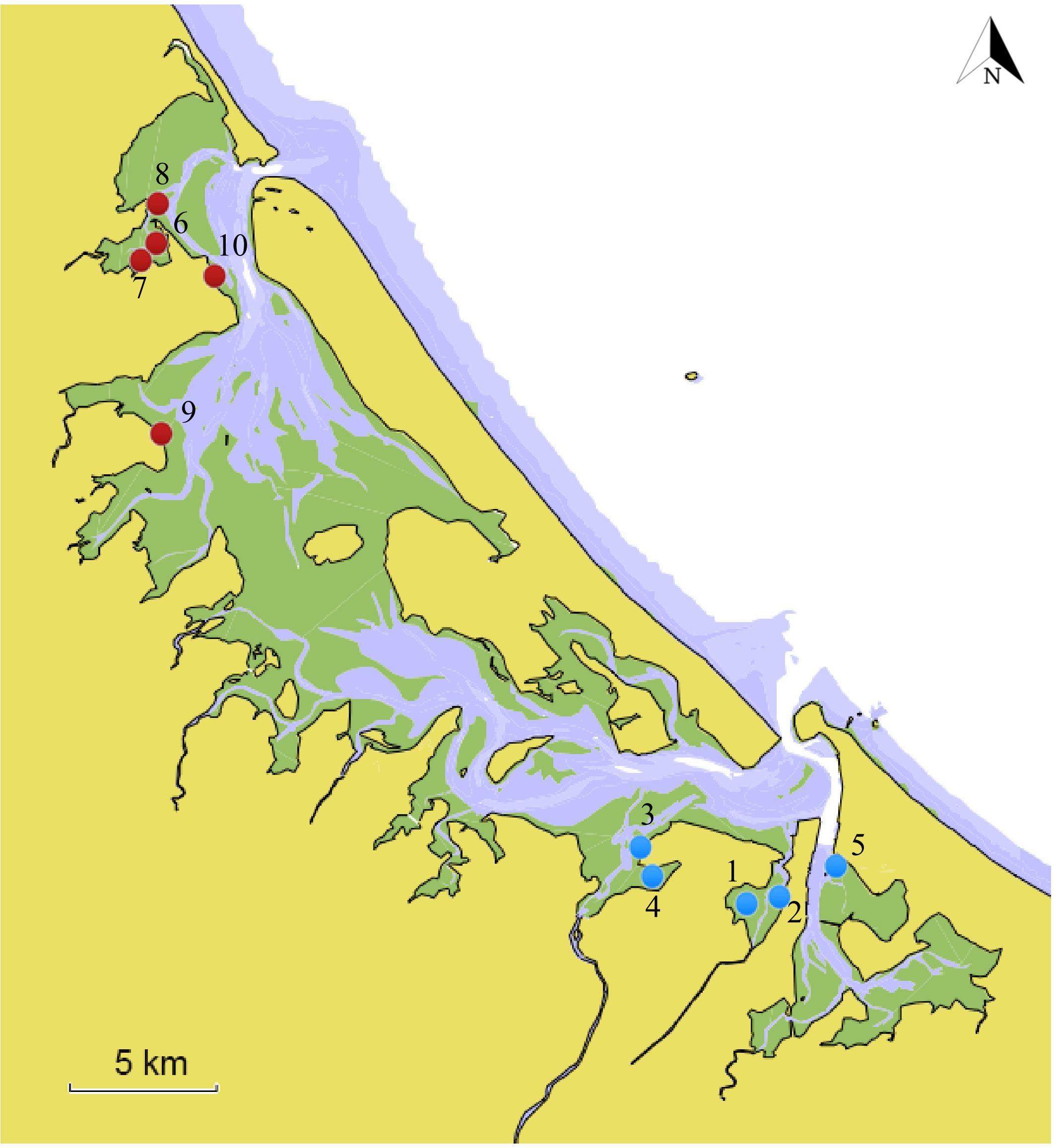


Figure 3.1. Map of Tauranga Harbour, showing sites where *M. tenuicaudatus* feeding pits were quantified monthly between 2016 and 2018.Green areas are exposed at low tide.

Site choice was also limited by access, as much of the land adjacent to the northern harbour is private land occupied by orchards with no or difficult access to sand flats. Sites also had to be close enough for foot access within the low tide timeframe. This meant that all sites were on the western fringes of the harbour basin (Figure 3.1.).

Table 3.1. Central positions and characteristics of the 10 sites at which *M. tenuicaudatus* feeding pit density was quantified in Tauranga Harbour. Sites 1-5 were located in the southern harbour and sites 6-10 were located in the northern harbour.

Site	Latitude	Longitude	Characteristics	Substrate
1	S37° 40.978'	E176° 09.058'	Inner basin Waikareo Estuary, Nearest channel <50cm at low tide	Sandy
2	S37° 40.932'	E176° 09.709'	Outer basin, Waikareo Estuary next to motorway, often a lot of snapper/bird pits present	Sandy
3	S37° 40.140'	E176° 06.879'	30 m wide, 300m long strip along shore. Bordered by shore on one side and seagrass meadow on the other	Sandy
4	S37° 40.493'	E176° 07.121'	Large embayment, mangrove at one edge of bay. Channel >50 cm deep at low tide within 200 m of site centre point	Sandy
5	S37° 40.389'	E176° 10.880'	Close to motorway and airport embayment but channel access to main basin	Sandy
6	S37° 29.453'	E175° 57.016'	Outer embayment, Tuapiro Estuary, bordered on two sides by channel >50 cm deep at low tide	Sandy
7	S37° 29.756'	E175° 56.718'	Inner embayment, Tuapiro Estuary	Muddy
8	S37° 28.896'	E175° 57.336'	Strip of sand ~50 m wide outside of Tuapiro Estuary. Bordered one side by expansive seagrass meadow and the other by deep channel opposite Tanners Point boat ramp.	Sandy
9	S37° 32.765'	E175° 57.115'	Strip of muddy sand ~ 50 m wide bordered one side by seagrass meadow and the other by main harbour basin.	Muddy sand
10	S37° 30.012'	E175° 58.232'	Quiet location in main harbour basin	Sandy

During site reconnaissance, it was discovered that evidence of *M. tenuicaudatus* feeding was more problematic to determine with accuracy in seagrass (*Zostera muelleri*) meadow habitats. *R. bonasus* have been found to feed in seagrass areas, and their feeding style of digging for infaunal prey is similar to that of *M. tenuicaudatus* (Orth, 1975; Collins et al., 2007) so it is likely that this species does use seagrass habitats to some extent. However, there is a difficulty in ascertaining the origin of any pits found in dense seagrass due to the structural nature of the rhizomes preventing the typical shape of pits. It is also known that black swan (*Cygnus atratus)* forages in seagrass habitats (Dos Santos et al., 2012) and their feeding evidence is visually similar to that of *M. tenuicaudatus.* Therefore, for this investigation, during site selection, dense seagrass meadows were excluded from site choice. Patchy, and/or sparse seagrass at sites was tolerated as pits could be distinguished clearly.

Ray feeding pit identification and pit longevity

Feeding excavations (pits) created by *M. tenuicaudatus* are distinctive but often vary in size and shape. The freshest pits are characteristically deep (up to 20cm) and steep sided with a diameter of between 15 and 25cm. Fresh pits are often but not always surrounded by an imprint of the ray body shape, and in some circumstances wingtips and pelvic fins are identifiable (Figure 3.2).

Figure 3.2. New Zealand eagle ray (*Myliobatis tenuicaudatus*) feeding pits in Tauranga Harbour, New Zealand. A) a fresh pit in sandy sediment B) a slightly degraded pit in muddy sediment in a mangrove fringe habitat.

Pits degrade after tidal inundation, and through the action of wind and precipitation. This makes identification more difficult. Degradation is not uniform, suspended debris such as sticks and rocks, and colonisation of pits by burrowing crabs and other infaunal organisms (Thrush et al., 1991, 1994) may exacerbate this process. To determine pit longevity, five fresh pits were identified across the sandflat at Site 4 and marked with a bamboo stake and GPS. A photograph of each pit was also taken. On five successive days, photographs were taken until each pit was indistinguishable from the surrounding sediment.

Other species may create similar excavations in intertidal sandflat regions. These include teleost fishes such as snapper (*Chrysophrys auratus*), black swan (*Cygnus atratus*) and other birds. These excavations are distinguishable from ray-generated pits due to their small size, shallow depth and common appearance in groups of five or more similar pits often in a linear arrangement with highly visible anoxic sediment surrounding the group (Figure 3.3); swan pits often have footprints surrounding the pit. Due to this variability, counts of pits were only performed by experienced personnel and only pits that were considered to be definitely of ray origin were counted.

Over the course of the 24 months of feeding pit survey a small number (<10) pits were identified as potentially created by *B. brevicaudata*. These were very large, often greater than 40 cm width, and were different to *M. tenuicaudatus* pits that had scoured out due to debris (Figure 3.4.). These pits had a very large sand/debris mound to one side of the excavation that was equal to or greater than the width of the pit. In *M. tenuicaudatus* pits that had scoured, this mound was no longer visible. These pits were not counted as part of this investigation.

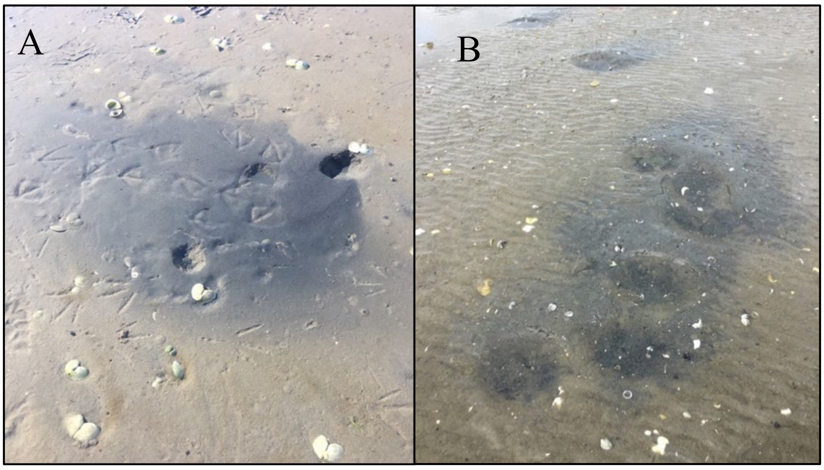


Figure 3.3. Examples of feeding excavations that are not eagle ray (*M. tenuicaudatus*) derived. A) Is likely to be either swan or other sediment investigating seabirds, B) Possible snapper pits.

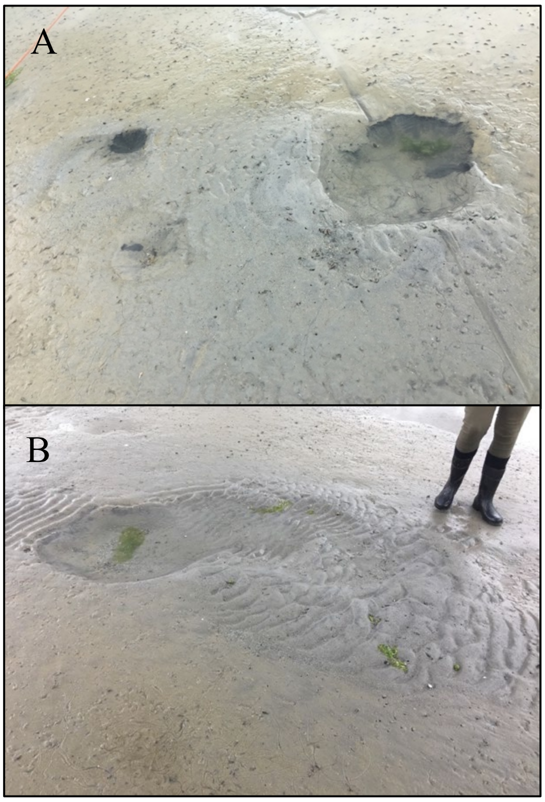


Figure 3.4. Sediment excavations of ray origin that are likely to be *B. brevicaudata* derived and therefore not included in this investigation. A) shows one of these very large excavations (right) next to a typical *M. tenuicaudatus* derived pit, B) shows another large pit with distinctive sediment plume at the rear that is unlikely to have been *M. tenuicaudatus* derived.

Data collection Protocol

At each site, the number of pits present was counted in ten circular plots, each with a radius of 15 metres. This method, adapted from Hines et al. (1997), allows comparison with this previous study and was also the optimal method during pilot studies (Appendix B). Plots were placed using a random number generator to determine compass bearing and distance (between 1 and 150 metres) from the centre point of the site. If this method placed the quadrat outside of habitat boundaries (such as into deep channels or dense seagrass meadows) the compass bearing was re-generated. Data was collected monthly over a two-year period between August 2016 and July 2018, as close to the middle of the month as tides and weather conditions permitted, to allow seasonal patterns to be detected. In an ideal world, all sites would have been sampled on the same day, but due to the time taken for sampling, this was usually carried out over 3 or 4 days. However, during some months, adverse weather conditions e.g. cyclones forced data collection to be postponed until later in the month (e.g. April 2017, *see* Appendix C).

3.2.2. Analysis

All analyses were performed in SPSS Statistics Version 25 (IBM Corp. Armonk, New York) or R version 3.5.0. (R Core Team, 2018).

To identify the presence of any seasonal patterns at the level of whole harbour, harbour basin or site, a number of analyses were used. Firstly, to determine whether there was a difference between the observed pit density in each season and whether this was influenced by site, a Schierer-Ray-Hare analysis (a non-parametric equivalent of a two-way ANOVA) (Dytham, 2011) was performed with number of pits per plot as the response variable and with Season and Site as the predictor variables. Season was taken as the Austral seasons, with pits /plot numbers for September, October and November combined as Spring and so forth.

To determine whether any differences between months picked up in the initial analysis translated to a consistent seasonal pattern, a cosinor analysis (fitted as part of a generalised linear model) was performed with the ‘Season’ package in R (Barnett & Dobson, 2010; Barnett et al., 2018) using a negative binomial distribution for overdispersed count data taking into account the dispersion parameter θ for the count at each site. The cosinor model can also be expressed as:

$$s_t = A\cos\left(\frac{2\pi t}{c} - P\right), \qquad t = 1, \ldots, n$$

where the amplitude (A) is the size of seasonal change, and the phase (P) is the peak of the curve. This analysis also calculated the low point of the seasonal pattern and determined whether the data fitted a sinusoid pattern (seasonal increase is equal to the decrease). To allow cosinor analysis, means of counts per month in each site were converted into integer values. This analysis was performed for whole harbour, basin and site level. For models at the site level, a Bonferroni correction for multiple comparisons was used so that significance was taken at $\alpha = 0.05/10 = 0.005$. For whole harbour and harbour basin models, conservative significance was taken at $\alpha = 0.01$, as only 24 months of data were available.

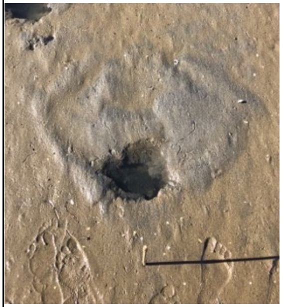
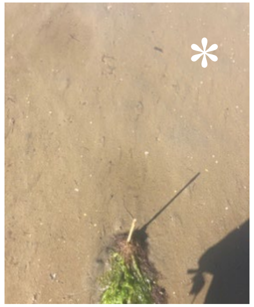
Finally, to determine whether there was a relationship between the temperature of the water inside the harbour and the number of pits, linear regression analyses were performed. Water temperature in Tauranga Harbour was provided by Port of Tauranga Ltd from a logger positioned close to the harbour entrance. Prior to the analysis the minimum, maximum and mean temperature for 6 days prior to data collection was calculated, as the maximum period of time during which the pits would have been created (*see* below). As all sites weren't sampled on the same day, this was repeated for each sampling date and numbers of pits were aligned with corresponding temperature values. Significance was set at $\alpha = 0.0016$ following a Bonferroni correction for multiple comparisons.

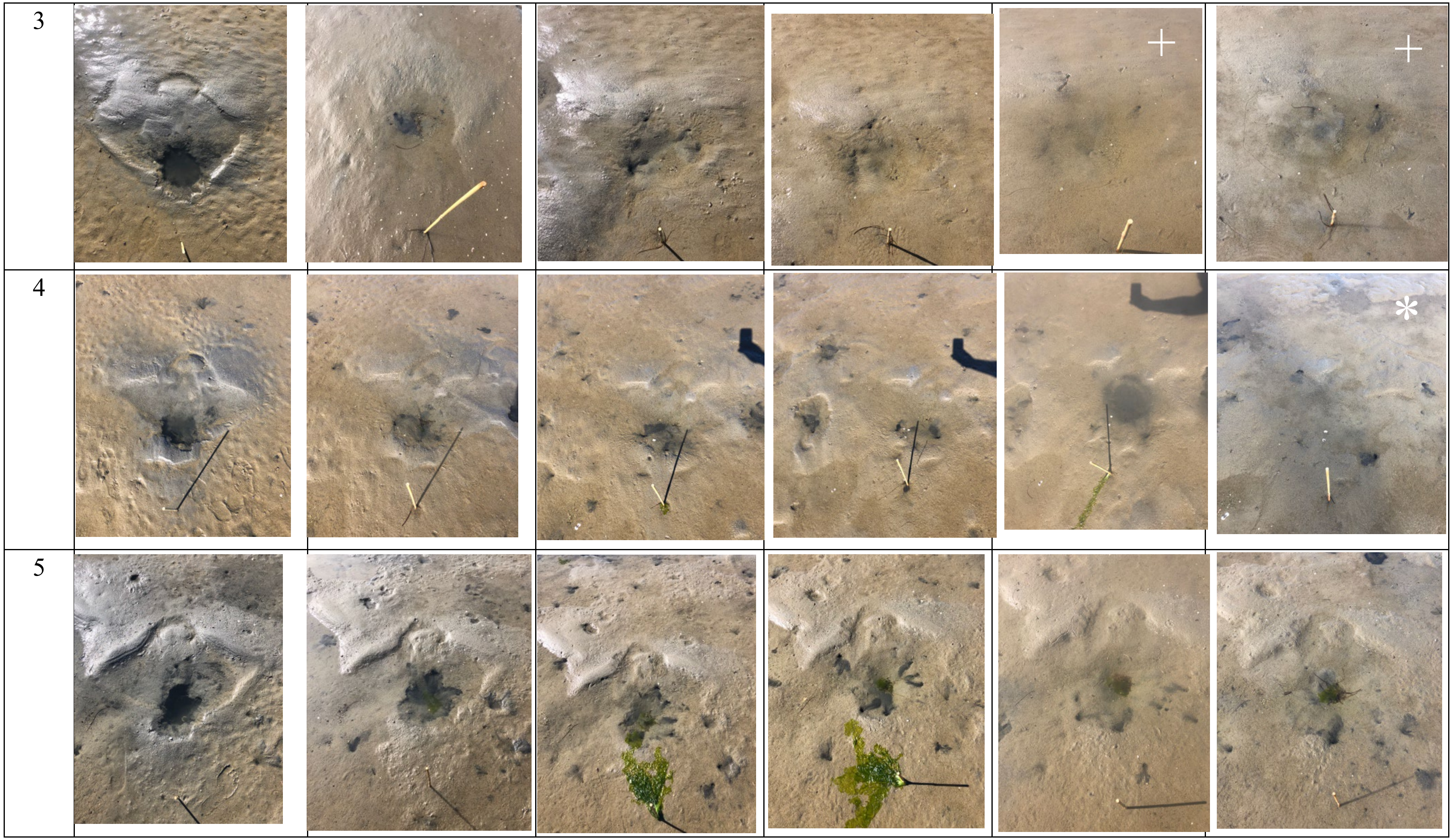
3.3. Results

Pit longevity

Pits were highly variable in their longevity and prevailed identifiably for a minimum of 2 and maximum of 5 days following initial observation (Table 3.2). On the 6th day, one pit was identifiable but was classed as an old pit and would not have been counted in the main investigation (not pictured). During the observation period, the weather was fine and settled with little wind, therefore the durations of prevalence are likely the maximum likely for this site. A further investigation in a very muddy habitat revealed that pit longevity in muddy sediments was a maximum of 3 days (G. Fernihough, Unpub. Data.).

Table 3.2. Photographs of the successive degradation of eagle ray (*Myliobatis tenuicaudatus*) feeding pits over days during fine weather. * indicates no longer identifiable as a ray pit. + indicates identified as an ‘old’ ray pit and not counted. It must be noted that, as it is not always possible to identify pits due to the difficulty of determining depth of excavation in photographs, identification classifications for this study were performed in person.

Pit #	Initial photograph	After 1 day	After 2 days	After 3 days	After 4 days	After 5 days
1						
2						


3
+
+
4
*
5

Seasonal observations

The number of pits per 707 m^2 plot varied considerably over the 24 month period (Figure 3.6 and Figure 3.7), ranging from a minimum of zero observed on several occasions to a maximum of 210 observed at Site 4 during April 2017. Overall, the highest pit densities were observed during Summer and Autumn and the lowest during Winter and Spring (Figure 3.5). However, the interaction between season and site was significant (Table 3.3), indicating that seasonality in feeding intensity was not consistent between sites. Sites had very different overall monthly numbers of pits, and while very few or no pits were observed over the late winter and spring at some sites, at other sites pits were observed throughout the year (Figure 3.6 and Figure 3.7).

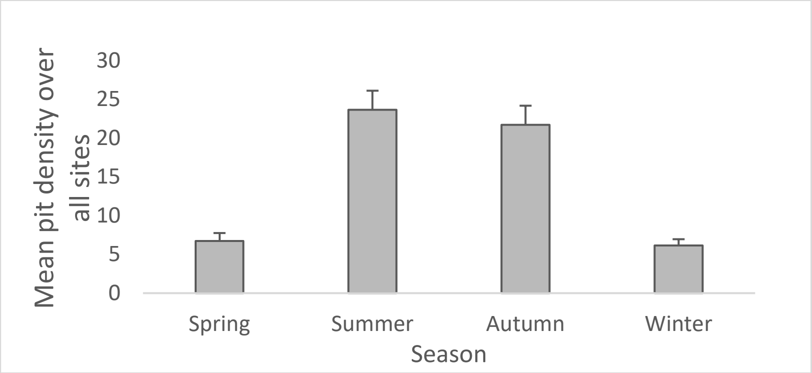


Figure 3.5. Mean pit density (number of pits /plot) over all sites combined into the four Austral seasons. Error bars show 95% confidence intervals

Table 3.3. Results of Scheirer-Ray Hare analysis of the number of pits by Season and Site and the interaction between season and site.

	SS	***SS/MS*_total**	**d.f.**	***p***
Site (Factor)	494183785.373	1066.8	9	<0.001
Season (Factor)	142684508.581	306.9	3	<0.001
Site * Season (Interaction)	120592666.838	261.3	27	<0.001

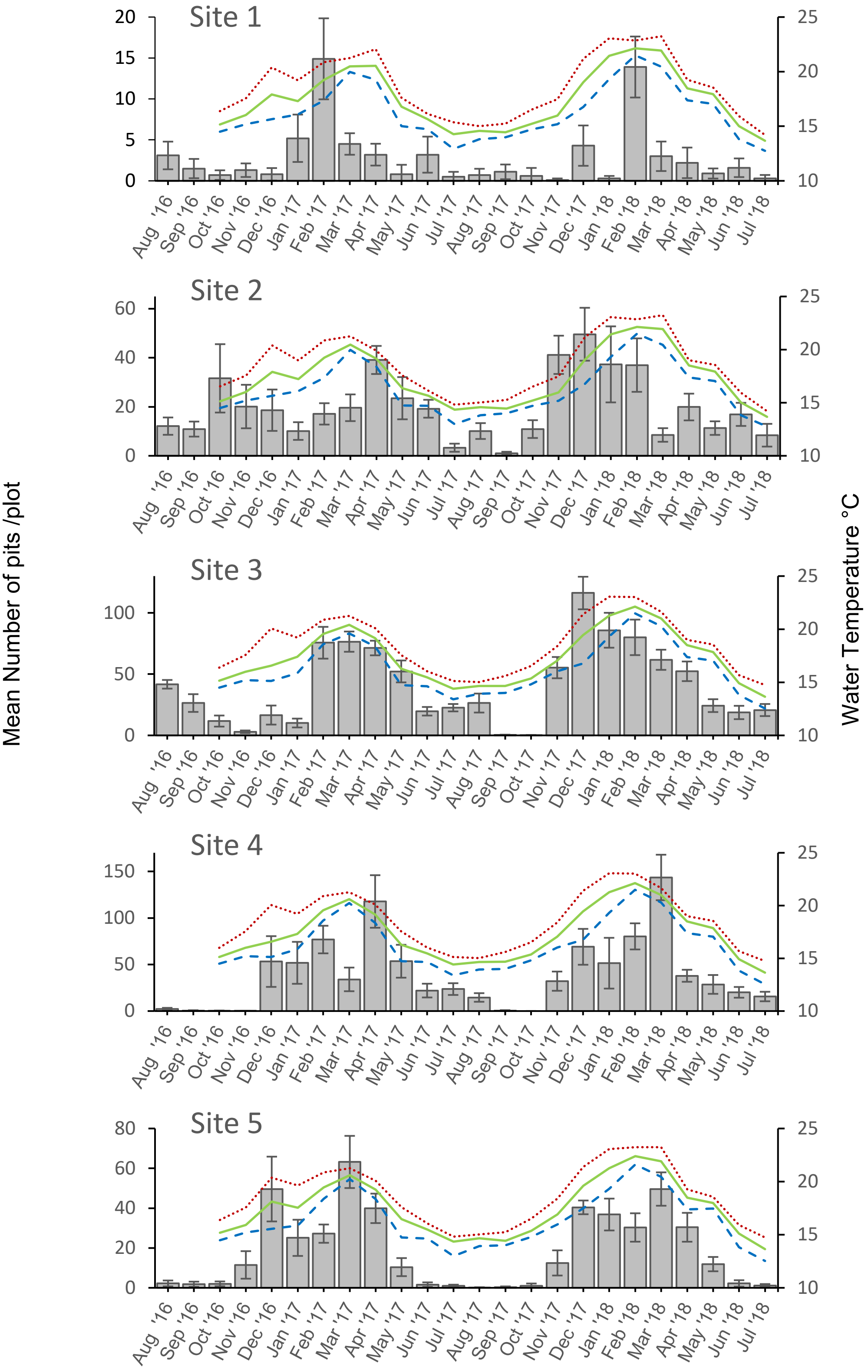


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Figure 3.6. Mean pits /707 m^2 plot in each month during observational period at 5 sites in Tauranga Harbour southern basin. Also showing minimum, maximum and mean water temperature at the harbour entrance over six days, prior to and including day of observation. Error bars show 95% confidence intervals.

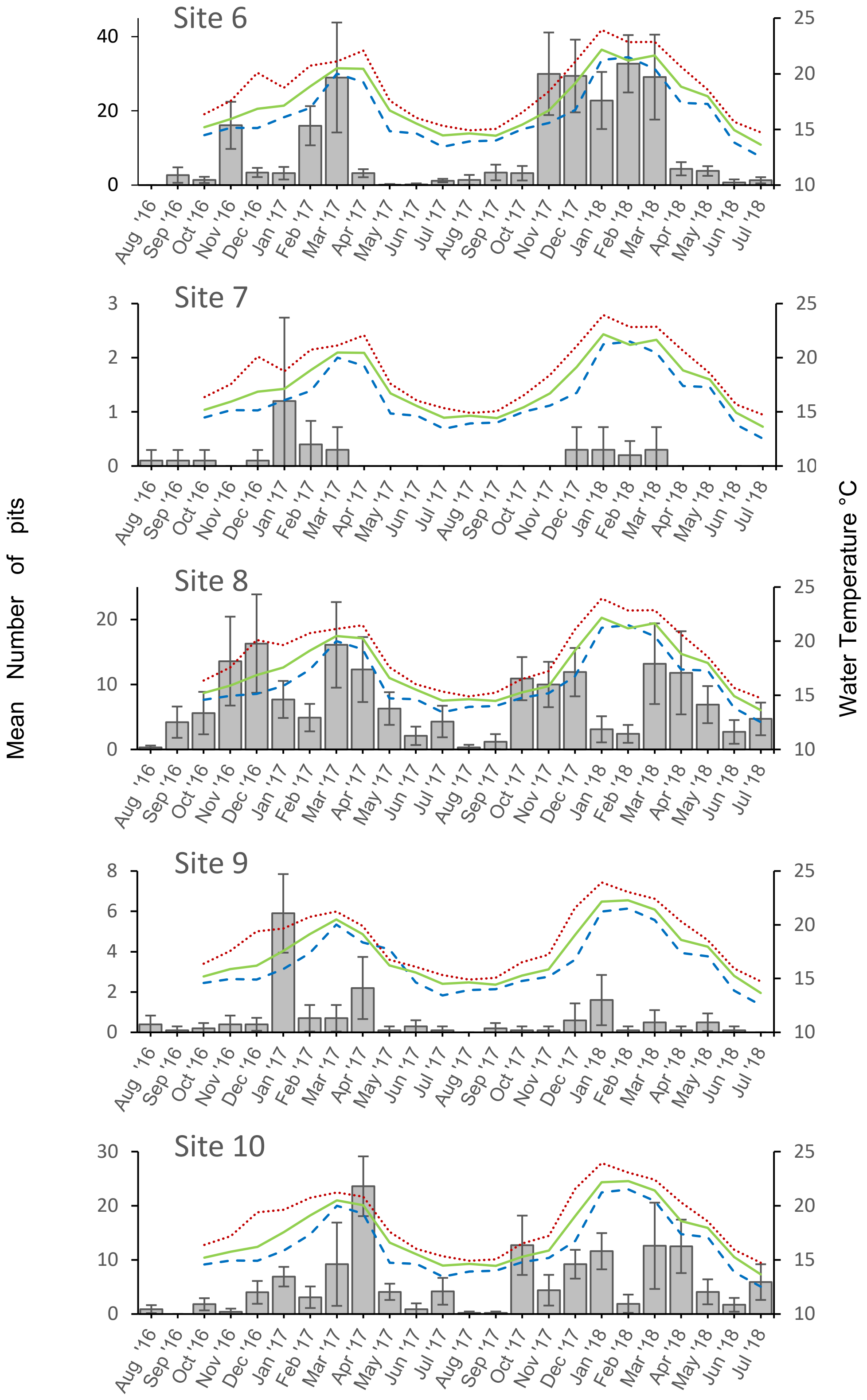

Figure 3.7. Mean pits /707 m^2 plot in each month during observational period at 5 sites in Tauranga Harbour northern basin. Also showing minimum, maximum and mean water temperature at the harbour entrance over six days, prior to and including day of observation. Error bars show 95% confidence intervals.

Overall, the mean number of pits /plot in the northern harbour was lower than in the southern harbour in all seasons (Figure 3.8).

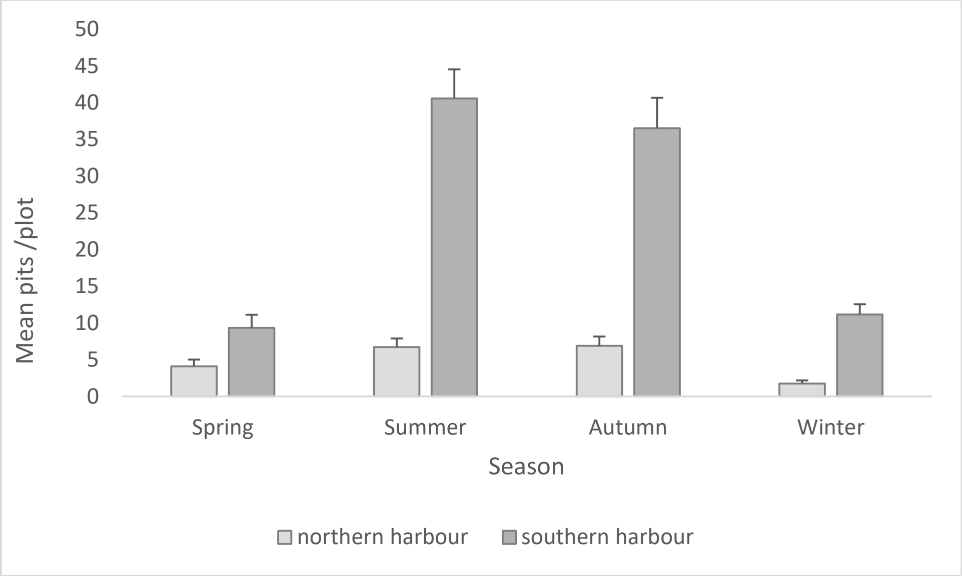

Figure 3.8. Mean number of pits /plot in each harbour basin. Error bars show 95% confidence intervals.

When sites were considered on an individual basis, five out of the 10 sites significantly fit a sinusoidal seasonal pattern at the conservative significance level. Amplitudes ranged between 96.28 and 0.5, peak of all sites was between January and March (Summer/Autumn) and low points were between July and September (Winter/Spring) (Figure 3.6, Figure 3.7, Table 3.4).

There was a significant relationship between site density and harbour entrance water temperature at most locations (Table 3.5). Low R^2 values indicating high variability around the trend for many sites suggests other drivers are also important.

Table 3.4. Table showing results of cosinor analysis on the number of pits per month at 10 sites throughout Tauranga Harbour. Amplitude denotes the size of the seasonal change, Phase and Low Point denote in which month the peak and trough of the sinusoid fall. Significance is given at the corrected level of α = 0.005.

Site	Amplitude	Peak	Low point	Significant
1	3.46	February	August	No
2	13.81	January	July	No
3	37.79	March	September	No
4	96.28	March	September	***Yes***
5	68.37	February	August	***Yes***
6	29.22	January	July	***Yes***
7	0.5	N/A	July	No
8	7.03	February	August	***Yes***
9	2.75	February	August	***Yes***
10	6.07	February	August	No

Table 3.5. Results from linear regression analysis of the number of pits /plot at mean minimum and maximum water temperatures recorded at the harbour entrance in the 6 days prior to pit observations. * indicates significant correlations

	Temperature					
Site	Mean		Minimum		Maximum	
	R^2	*p*	R^2	*p*	R^2	*p*
1	0.18	<0.001*	0.19	<0.001*	0.17	<0.001*
2	0.11	<0.001*	0.09	<0.001*	0.13	<0.001*
3	0.59	<0.001*	0.47	<0.001*	0.38	<0.001*
4	0.38	<0.001*	0.35	<0.001*	0.37	<0.001*
5	0.57	<0.001*	0.5	<0.001*	0.59	<0.001*
6	0.3	<0.001*	0.31	<0.001*	0.24	<0.001*
7	0.02	0.046	0.015	0.08	0.018	0.05
8	0.05	0.002	0.03	0.012	0.08	<0.001*
9	0.04	0.005	0.02	0.05	0.05	0.001
10	0.14	<0.001*	0.14	<0.001*	0.12	<0.001*

Overall there was a distinct seasonal pattern in the numbers of *M. tenuicaudatus* in the Tauranga Harbour, with higher pit densities present in the summer and autumn months than in winter and spring. Harbour basins are very different, with much higher pit numbers over all seasons in the southern harbour than the northern harbour. There were differences in both magnitude of pit densities and timing of peak pit densities among sites suggesting that some sites may be preferred, and that there may be evidence of sequential use of sites.

3.4. Discussion

Visual evidence of *M. tenuicaudatus* feeding effort, the excavations that are made when infaunal prey is extracted from the sediment, is a quantifiable measure of use of an area by this species. Here this measure was used to demonstrate a seasonal pattern in *M. tenuicaudatus* feeding intensity throughout the year that supports the initial hypothesis that there would be higher densities of pits present in the warmer months.

Overall, mean densities of 24 pits per 707 m^2 plot during the summer and 21 pits per plot during the autumn were significantly higher than 7 pits per plot in the spring and 6 pits per plot in the winter. This supports and expands on the previous work on this species (Hines et al., 1997). This prior study concluded that a seasonal pattern existed in pit density, an inference supported by the observations in the current study, however this conclusion was reached with data collected solely through February and into March. In addition, while Hines et al. (1997) state that no rays were present in their study site during winter months, this was not the case in the Tauranga Harbour, with some sites showing evidence of ray presence all year. However, pit density was not quantified during winter in the previous work, therefore some low-level presence may have been missed. Methodology to count pits in 15 m radius plots that was utilised by Hines et al. (1997) was the most efficient in preliminary tests (Appendix B) and thus was employed in this study.

There was a significant interaction between site and season, indicating that this seasonal difference was not consistent between sites. It is likely that this interaction stems from a number of factors, not least that certain sites regularly had upwards of 50 pits per plot during the warmer months, whereas others peaked at much lower numbers (Figure 3.6 and Figure 3.7). This would mean that the magnitude of the seasonal difference would be different between sites. Density of pits differed between harbour basins, with markedly more pits counted in the southern harbour than the northern harbour. The amplitude of sinusoid (size of seasonal change) calculated for the northern harbour was lower than that of the southern harbour, and northern sites have lower overall pit densities. Furthermore, this interaction could be a result of other patterns of use and preference may be occurring, such as a response to prey densities or locational factors, to be examined in the following chapters.

Moreover, when the sites were considered independently, seasonal variations in pit density that fit a sinusoidal pattern (seasonal increase was equal to decrease) were identified at 5 out of 10 sites, sites 4, 5, 6, 8 and 9 (Table 3.6). Peak pit densities varied between January and March, and low points vary between July and September depending on the site.

Pit densities at sites 1, 2, 3, 7 and 10 did not significantly fit a sinusoidal pattern. This could be a result of no seasonal pattern, however there do appear to be higher pit densities during the warmer months in these sites, albeit the patterns may not be as regular (Figure 3.6 and Figure 3.7). A longer period of data collection could elucidate this. For example, Site 3 seems to have had a different pattern each year, starting with a relatively high mean pit density in August 2016 before it declined into summer. Pit densities quickly rose again in February and stayed high until June. In contrast to the previous year densities begin to rise in November, peak in December and then gradually fell (Figure 3.6). This difference between years could have prevented a significant fit to a sinusoidal model.

In addition, rather than an absolute count of pits created per month, these observations are from one day a month sampling effort at any one site; lower than usual counts could be an artefact of an event occurring in the few days before observation that could have disturbed feeding activity. For example, orca, one of the main predators of *M. tenuicaudatus* (Visser, 1999), are regular but uncommon visitors to Tauranga Harbour, and ray behaviour throughout the harbour often changes during these events with exhibitions of refuging or fleeing behaviour (N. Pettigrew, Pers. Comm.). Tauranga Harbour, particularly the southern basin, is also a very busy harbour, with recreational craft of all types frequenting the area, and disturbances from this source could also have altered feeding behaviour. This species is highly mobile, and low observations on some days could be a result of eagle rays moving around to feed on different days, rather than always using the same site. Distance travelled was calculated by Riding et al. (2009) to be an average of 32 km per day or 1.34 km per hour, which supports the ability of this species to travel high daily distances and the potential to use different areas to feed.

A difference in the peak densities at different sites could be evidence of sequential feeding, with rays using particular sites then moving on to another. However, this could not be proven using this methodology. Fine-scale tracking, through a towed-float system or an acoustic array with associated tags could possibly reveal such a pattern with calculations of time spent in each area over sequential months.

Pit longevity was found to be highly variable, between two and six days, even within one site during the same period. This test was carried out during a period of excellent weather, so it is likely that throughout all sites pits do not prevail for more than six days, and that there is a great deal of variability in the longevity. In a second separate study pits in a muddy area were found to prevail a maximum of two days (G. Fernihough, Unpub. Data.). It would have been ideal to have quantified pit longevity at all sites, in a variety of weather conditions, to provide weighting in subsequent analyses, however time constraints prevented this.

Neonates (< 20 cm disc width) of *M. tenuicaudatus* are occasionally sighted in shallow warm bays during late January–February, suggesting that parturition may occur here. The vast majority of individuals caught within this and other harbour systems in New Zealand have been female: 9/10 in the current study; 4/4 in Porirua (Cook-Auckram, 2019), 10/11 in Whangateau (Marcotte, 2014), 6/7 in Whangateau (Le Port, 2003), 4/4 in Taiharuru (Riding, 2009), and 9/9 in Taiharuru (Davis, 2010).

Sex segregation is common in elasmobranchs (Wearmouth & Sims, 2010). This species has been found to segregate at all times of the year on the open coast, with segregation occurring when more than five individuals are present (Hartill, 1989). however, the same study shows females are more common in shallow open coast areas during winter and spring. Other species have similar segregations, for example, adult female *Bathytoshia centroura* are found in shallow water during the summer (Struhsaker, 1969). This may mean that females are using the warmer harbour water temperatures for gestation and birth as warmer water may speed up gestation (Jirik & Lowe, 2012). It is not, however, a fixed nursery area, as it does not meet the strict criteria for nursery areas described by Heupel et al. (2007) and corroborated for batoids by Martins et al. (2018).

Seasonal habitat change is often thought to be a response to temperature and rays have been observed seeking out warmer water (Vaudo & Lowe, 2006). Indeed, there appears to be some positive relationship between increasing temperature and increasing pit numbers. This suggests that where a seasonal pattern does occur, temperature may be one of the driving forces behind it. In addition, warmer temperatures during the summer months may drive an increase in number of pits indirectly by an increase in metabolic rate requiring increased feeding to sustain (Di Santo & Bennett, 2011; Whitney et al., 2016). Low R^2 values for a number of the significant relationships is likely due to the high variation in pit numbers even within sites on the same day.

Thrush et al. (1991) calculated that an 800 m^2 area of sandflat would be turned over by feeding *M. tenuicaudatus* every 70 days at a rate of 1.4% per day. Using the same calculations for ray pit size as that study, and the conservative average longevity from this investigation of 3 days, and the highest mean monthly pits counted in at a single site (Site 4, autumn 2018), assuming equal pit excavation each day, maximum turnover achieved in this study would have been more than double the previous study at 3.2 % daily turnover, taking only 31 days for complete turnover of 800 m^2. This difference could be important in calculations of ecosystem functioning stemming from sediment turnover. In addition, studies of sediment transport should include ray-derived mobile sediment. However, this turnover rate is highly variable with pit density, and this study has shown how this varies with season, and by site so including a seasonal factor to these calculations including ray pit sediment turnover would be pertinent.

The seasonal pattern found may be the result of at least two possible behavioural strategies. First, most animals leave the harbour system during the winter and spring, resulting in fewer pits in these seasons. Second, most rays remain in the harbour, but inhabit the deeper channels rather than the intertidal flats. This seasonal behavioural strategy has been observed in Atlantic stingrays (*Hypanus sabinus*) where individuals utilised both deep and shallow areas within a creek system during the day and the night in the warmer months, however, during the colder months shallow areas were rarely used and only during the daytime (Brinton & Curran, 2017).

Seagrass meadows were deliberately excluded from this analysis, as the pits were difficult to identify. As these areas are likely to be important foraging habitats for *M. tenuicaudatus* (Orth, 1975; Collins et al., 2007). It is recommended that further investigation occurs, perhaps utilizing video or fine-scale tagging techniques to quantify the time spent and the activities occurring in this habitat.

In conclusion, an overall seasonal pattern was found in *M. tenuicaudatus* feeding in Tauranga Harbour intertidal sand flat areas. There was a difference between locations, indicating a degree of site preference that will be explored in the following chapter. Implications of this finding include an associated seasonal difference in bioturbation rates, which could alter ecosystem functioning.

CHAPTER FOUR

HABITAT PREFERENCE IN *MYLIOBATIS TENUICAUDATUS* INTERTIDAL FEEDING

H_3: M. tenuicaudatus feeding evidence will be positively correlated with the density of benthic prey items

4.1. Introduction

In addition to information on whether an animal is present in an area, knowledge of behaviour (what an animal is doing in an area), duration of the use of an area, and ontogenetic stage of users is important for fisheries, conservation, development and management strategies (Block et al., 2011). Furthermore, an understanding of why certain habitats are preferred is valuable in defining the populating dynamics and ecology of not only the species in question, but the entire system in which it is involved (Sims, 2003).

Choice of a habitat is likely to be driven by a number of both abiotic and biotic factors driving motivational or reproductive state (Heithaus et al., 2002; Sims, 2003). In the marine realm, important abiotic factors can include temperature, salinity, and bathymetry of a site (Sims, 2003). Biotic factors can be either extrinsic or physiological; extrinsic factors include prey availability, competition and predator risk; while physiological factors driving motivational or reproductive state may be satiation, or egg production (Sims, 2003). In addition, the relative importance of various behavioural drivers may differ depending on habitat use for example: safety from predators may be important in a nursery area (Davy et al., 2015), while prey presence is important in a foraging habitat (Tregenza, 1995).

At a simplistic level the use of foraging habitats is expected to be related to the density of prey items (Charnov, 1976). When prey has a patchy distribution, the expectation is that predators select the highest density prey patches, thus optimising energetic gain (Charnov, 1976). As a result, high density prey patches are expected to elicit an aggregative response of predator individuals, this may be described as the 'Ideal Free Distribution' (Tregenza, 1995).

For mid-level predators such as rays, choice of foraging patch is thought to be not only a response to food density but also to perceived predation risk in that patch (Ahrens et al., 2012). In equally profitable prey patches with differing perceived predation risk, an individual is expected to choose the patch with the lowest perceived risk (Stephens & Krebs, 1986). Moreover, perceived predation risk is not restricted to actual predators; there has been work, primarily in the realms of ornithology and terrestrial mammalogy, into human disturbance as a source of perceived predation risk (Gill et al., 1996; Frid & Dill, 2002; Beale & Monaghan, 2004). This may result in lower use of areas with increased levels of anthropogenic disturbance including boat traffic.

4.1.1. Habitat choice in sharks and rays

For sharks and rays, the aquatic nature of their habitats makes the identification of habitat preference somewhat difficult. Abiotic factors are most often studied, due to their relative ease of measurement, and their importance within the context of a changing climate (Schlaff et al., 2014). Factors such as temperature and salinity may be the most important drivers of movement and habitat choice in these species (Hopkins & Cech, 2003; Schlaff et al., 2014).

Responses to prey density have been studied in piscivorous sharks (Heithaus et al., 2002; Torres et al., 2006; Goetze & Fullwood, 2013) with increasing prey density resulting in higher predator densities. In addition, cyclical use of different depths by the giant devil ray (*M. mobular*) is thought to be a consequence of varying prey depth distributions (Canese et al., 2011). For benthic feeding rays, this is less well studied. Feeding (pit density) of *M. tenuicaudatus* was found to be positively, but non-linearly related to densities of the tellinid bivalve mollusc *Macomona liliana* of a size greater than 15 mm diameter on a sandflat in the Manukau Harbour, northern New Zealand (Hines et al., 1997). A threshold density of *M. liliana* was found, over which ray feeding was seen, leading to the conclusion that *M. tenuicaudatus* feeding efforts has a regulatory effect on population numbers of this prey species (Hines et al., 1997). Pit densities of the same ray species were also positively related to the densities of the bivalve *Austrovenus stutchburyi* in another study in a different estuary (Le Port, 2003). In contrast, Ajemian and Powers (2011) found while bivalve prey was important to *R. bonasus*, the opportunistic feeding nature of this ray meant that individuals were present even in areas of low bivalve density.

Choice of habitat use with regard to protection from predators has been observed in juvenile mangrove whiprays (*Urogymnus granulatus*) with individuals leaving mangrove stands only during low tide periods (Davy et al., 2015). In addition, predator abundance was considered an important factor in habitat selection of pink whiprays (*Pateobatis fai*), reticulate whiprays (*Himantura uarnak*) and juvenile giant shovelnose rays (*Glaucostegus typus*) in nearshore habitats (Vaudo &

Heithaus, 2013). Indeed, in the latter study, the microhabitat selection of these species did not match the predictions from prey distributions, and perceived predation risk was more influential (Vaudo & Heithaus, 2013).

4.1.2. Potential factors in this study

Many factors may influence the use of a particular area for feeding purposes by *M. tenuicaudatus* in the Tauranga Harbour. Both prey species, *M. liliana* and *A. stuctchburyi,* that were previously determined to have an effect on the feeding pit density of this ray (Hines et al., 1997; Le Port, 2003), are common in the harbour (Ellis et al., 2013), and thus either or both may influence feeding pit density in this location. Hartill (1989) found that ray size determined prey size, with large prey consumed by individuals of a pelvic length over 45 cm. As the majority *M. tenuicaudatus* caught in Tauranga Harbour as part of a later chapter (see Chapter 6 for capture details) were over this size, it seems likely that large prey will have the most influence over pit density.

In addition, aspects of an areas' position within the harbour could have an influence on ray use. For example, whether a site was within an embayment or the main harbour basin could influence ease of access to the area by rays and also accessibility of the area to predators such as orca (*Orcinus orca*). In addition, proximity to the city of Tauranga could, through human disturbance, also influence the use of an area.

In the previous chapter, it was discovered that while the density of *M. tenuicaudatus* feeding pits followed seasonal patterns at a number of sites, how these patterns presented themselves at different sites were intrinsically different. As infaunal density and assemblage varies dramatically between areas in Tauranga Harbour

(Ellis et al., 2013) it is hypothesised that *M. tenuicaudatus* feeding evidence will be positively correlated with the density of large benthic prey items

To test this hypothesis the following questions will be asked:

Does the density of M. liliana or A. stutchburyi affect the pit density in Tauranga Harbour?

Does site position within the harbour have an effect on pit density?

4.2. Methods

Ray pit densities (*see* Chapter 3) at 10 sites throughout Tauranga Harbour (Figure 4.1) were compared with prey densities and two aspects of site location in order to determine whether either of these factors influenced the densities of pits found in these locations. Pit densities among seasons were very different, with the highest

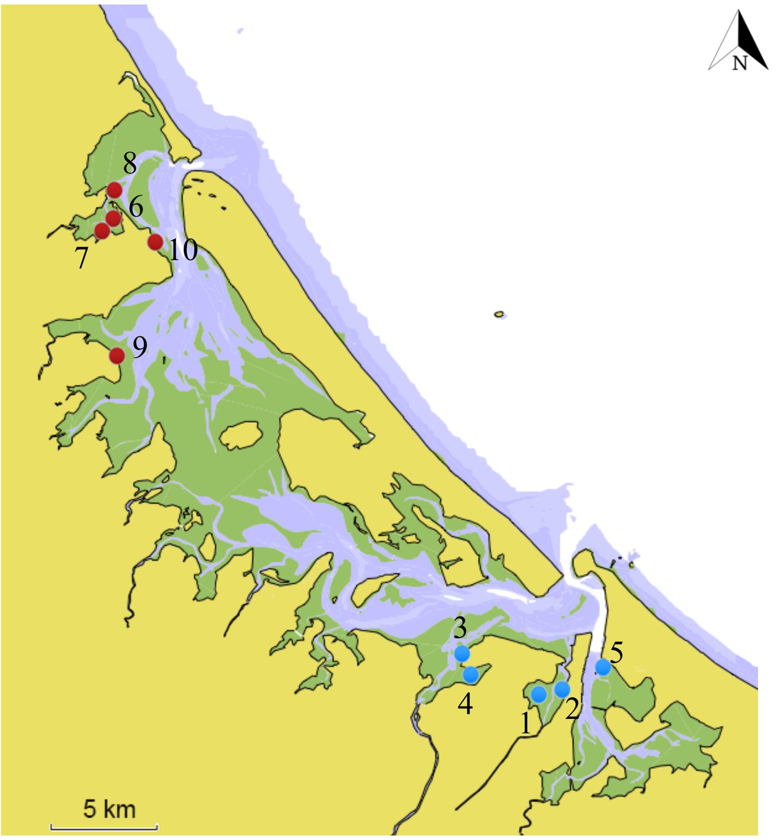

Figure 4.1. Map of Tauranga Harbour, showing sites where *M. tenuicaudatus* feeding pits were quantified with associated infaunal surveys. Green areas are exposed at low tide.

numbers of pits counted during summer and autumn, and much lower numbers throughout winter and spring (Chapter 3), therefore with the aim of determining whether different factors were important in each season, each season was considered independently.

4.2.1. Infaunal survey

In order to determine whether aspects of infaunal species composition had an influence on ray feeding pit density at each site, an infaunal survey was performed in Spring 2016, Summer, Autumn and Winter 2017, corresponding with a pit counting event (see Chapter 3). Although it would have been ideal to obtain data for the full 2-year period, after the first year's data was obtained there was no significant difference between infaunal densities between seasons (see section 4.3.1). In addition, there is a wealth of long term spatial and temporal data on infaunal assemblages in the Tauranga Harbour already in existence (Ellis et al., 2013).

At each site, the number of *M. tenuicaudatus* feeding pits was counted in 10 circular plots of 15 m radius as detailed in Chapter 3. In each plot, after pits were counted, three 25 cm x 25 cm quadrats were haphazardly placed and excavated to 15 cm depth. This corresponds with the depth of feeding pits observed during pilot studies, and the burial depth of *M. liliana.* It is also a similar method used by Hines et al. (1997) and therefore allowed direct comparison with this previous work. For each quadrat, the excavated sediment was sieved with a 4 mm screen and any infauna were retained in plastic bags for identification and quantification. Infauna was counted and measured to allow comparison with the previous work; bivalve molluscs *A. stutchburyi* and *M. liliana* were counted in size classes <15 mm and >15 mm (Hines et al., 1997).

4.2.2. Site locational factors

To determine whether the location of a site within the harbour had any influence on the foraging use by *M. tenuicaudatus*, two factors were considered. Firstly, the position of a site within an embayment, or the main harbour basin was described as 'Zone'. This factor was defined at three levels, 'inner embayment' when a site was in the inner reaches of an embayment or sub-estuary, 'embayment' when a site was within an embayment or sub-estuary but close to the entrance, and 'main basin' when a site was located within one of the main harbour basins (Figure 4.2,Table 4.1). Secondly, the harbour basin in which a site was located was also included as a separate factor 'Basin' of two levels, 'north' and 'south' (Table 4.1).

Figure 4.2. Illustrating the three levels of factor 'Zone'. A) Inner embayment, B) Embayment and C) Main harbour basin.

Table 4.1.Levels of factors 'Zone' and 'Basin' assigned to each observation site.

Site	Zone	Basin
1	Inner Embayment	South
2	Embayment	South
3	Main Basin	South
4	Embayment	South
5	Embayment	South
6	Embayment	North
7	Inner Embayment	North
8	Main Basin	North
9	Main Basin	North
10	Main Basin	North

4.2.3. Analysis

All analyses were performed in R version 3.5.0. (R Core Team, 2018) with the R Studio interface version 1.1.453 (RStudio Team, 2015).

The counted infaunal quadrat samples were compiled and compared with Kruskal-Wallis analyses to determine any differences in large (>15 mm) infauna among seasons, or among sites. Then, the mean number of large *M. liliana* and *A. stutchburyi* for each plot was calculated in order to compare this with the counted number of pits.

In order to determine whether the density of large infaunal species or the position of the site within the harbour had an effect on the number of pits found per plot, negative binomial (NB) regression models were constructed in the package MASS (Ripley & Venables, 2002). As this data was highly over-dispersed (variance much larger than the mean), with a large number of zeros, the use of ANOVA models even with transformation was not possible. Therefore, negative binomial models constructed in a generalised linear model allow extension to the Poisson model for over-dispersed count data by the addition of a parameter allowing the conditional variance to exceed the conditional mean (Hoffmann, 2016). A number of models were constructed with varying factors in order to determine which model fitted best.

Best fit was determined by the Akaike's Information Criterion (AIC) statistic, with the smallest AIC considered the most appropriate (Hoffmann, 2016) as well as the percentage deviance (calculated with the package BiodiversityR) (Kindt & Coe, 2005) to describe how much of the deviance is explained by the model.

4.3. Results

4.3.1. Infaunal densities

The densities of large (>15 mm) *M. liliana* and *A. stutchburyi* did not vary by season (Kruskal-Wallis: *M. Liliana* χ^2=0.22, d.f.=3, p =0.97, *A. stutchburyi* χ^2=0.78, d.f.=3, p = 0.85), however they did significantly differ by site (Kruskal-Wallis: *M. Liliana* χ^2=842.61, d.f.= 9, p <0.001, *A. stutchburyi* χ^2=1048.8, d.f.=9, p <0.001). Sites 3 and 4 had consistently very low densities of both infaunal species, and Site 1 had very low densities of *A. stutchburyi* (Figures 4.3 and 4.4).

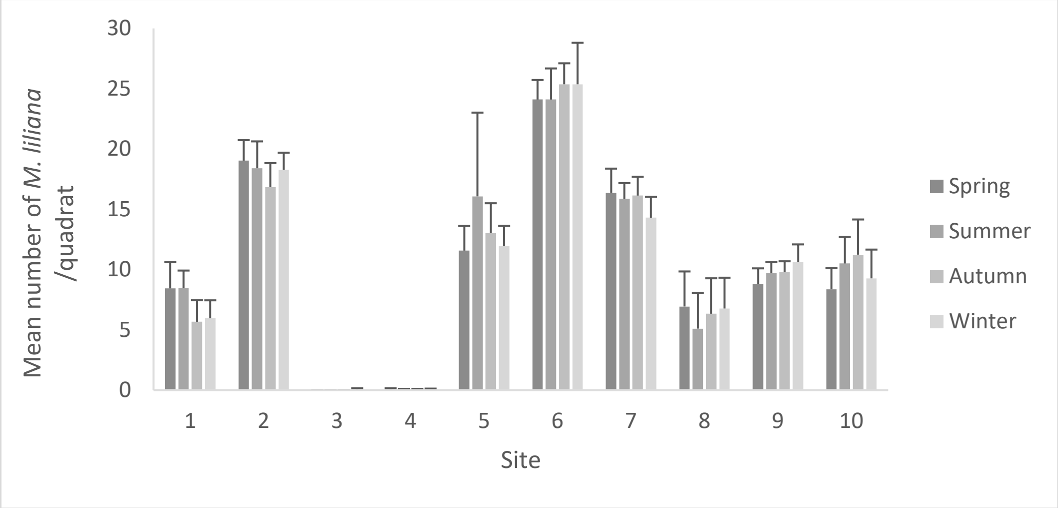

Figure 4.3. Densities of *M. liliana* > 15 mm counted at 10 sites over 4 seasons in the Tauranga Harbour.

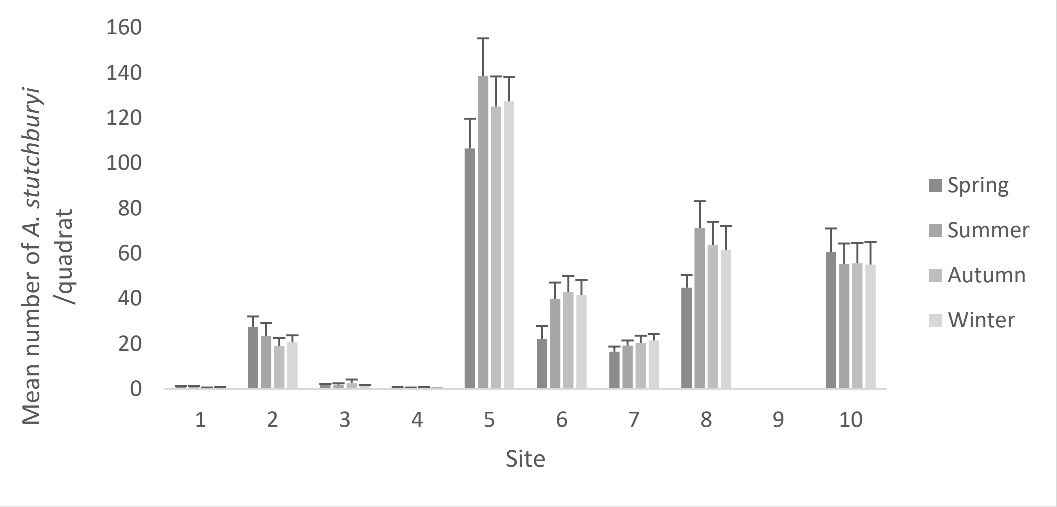

Figure 4.4. Densities of *A. stutchburyi* > 15 mm counted at 10 sites over 4 seasons in the Tauranga Harbour.

4.3.2. Factors affecting density of pits

For each Austral season, multiple step-wise negative binomial regression models were constructed to test the influence of the density of large *M. liliana* and *A. stutchburyi* and the locational factors Zone and Basin on the density of *M. tenuicaudatus* feeding pits. For spring observations, the model (Table 4.2) that best fitted the data was:

$$Number\ pits/plot \sim density\ M.liliana + \ Aspect\ + Basin$$

This model explained 52.3 percent of the deviance in the data and the coefficient of regression demonstrated a positive relationship between the density of large *M. liliana* and the density of pits (Table 4.3, Figure 4.5). Regression coefficients also demonstrate that the expected values of a site in outer embayment and main basin locations are greater than that of inner embayment locations and that the model expects the northern basin to have a lower density of pits than the southern basin (Table 4.3).

Table 4.2. Comparison of Negative binomial regression models of factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in spring 2016. * indicates the best model.

Model	Variables included	AIC
1	Density *M. liliana* >15 mm Density *A. stutchburyi* > 15mm Zone Basin	427.9
2	Density *M. liliana* Zone Basin	425.9 *
3	Zone Basin	453.5
4	Density *M. liliana* Basin	469.4
5	Density *M. liliana* Zone	468

Table 4.3. Regression coefficients of the factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in Spring 2016.

Factor	**Density *M. liliana* >15 mm**	**Zone (Embayment)**	**Zone (Main Basin)**	**Basin (North)**
Regression coefficient (SE)	0.175 (0.026)	1.842 (0.586)	5.025 (0.677)	-3.283 (0.434)

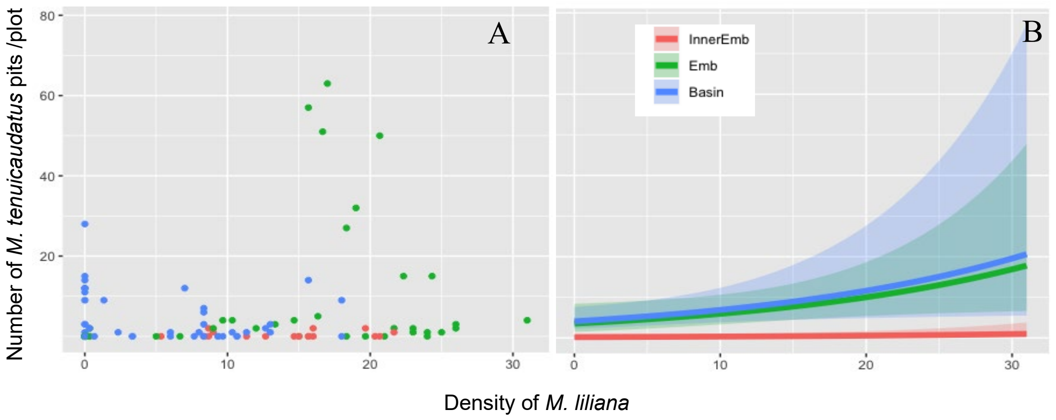

Figure 4.5. Graphs showing A) Observed values of numbers of pits /plot in Spring 2017 with plot mean *M. liliana* >15mm at the three levels of factor Zone. B) Predicted numbers of pits /plot +/- 95% CI with increasing large *M. liliana* at levels of factor Zone with negative binomial model.

For summer, autumn and winter, the model that best fitted the data was the full model:

$$Number\ pits/plot \sim density\ A.stutchburyi\ +\ density\ M.liliana +\ Aspect\ + Basin$$

This model described 70.8%, 77.2% and 79.6% of the deviance in the data for summer, autumn and winter respectively. For the summer model, the addition of *A. stutchburyi* density to the model although it reduced the AIC and resulted in a better fit (Hoffmann, 2016), the reduction was very small indicating the effect of adding this factor to the model was not strong (Table 4.4). In contrast to the spring model, the coefficient of regression for summer shows a negative relationship between the density of *M. liliana* and the density of pits (Table 4.5 and Figure 4.6).

In autumn, the addition of the density of large *M. liliana* only improved the model very slightly indicating the influence of this factor was not strong (Table 4.6). The coefficient of regression showed a slight positive relationship between the density of large *A. stutchburyi* and the number of pits counted (Table 4.7, Figure 4.7). Similarly, in winter, the model was only very slightly improved by the addition of *M. liliana* density, indicating that once again, the influence of this factor was not strong (Table 4.8). However, in this season, the coefficient of regression showed a negative relationship between *A. stutchburyi* density and the number of pits counted (Table 4.9, Figure 4.8).

Regression coefficients for Zone showed that the model expects outer embayment and main basin sites to have consistently higher number of pits than inner embayment sites, with main basin expected to have slightly higher pit densities in the autumn. The models also expect the northern harbour to have consistently lower pit densities than the southern harbour.

Table 4.4. Comparison of Negative binomial regression models of factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in summer 2017. * indicates the best model.

Model	Variables included	AIC
1	Density *M. liliana* >15 mm Density *A. stutchburyi* > 15mm Zone Basin	641.78*
2	Density *M. liliana* > 15mm Zone Basin	642.9
3	Density *A. stutchburyi* > 15mm Zone Basin	658.99
4	Zone Basin	657
5	Density *M. liliana* > 15mm Density *A. stutchburyi* > 15mm Basin	686.85
6	Density *M. liliana* > 15mm Density *A. stutchburyi* > 15mm Zone	692.22

Table 4.5. Regression coefficients of the factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in summer 2017.

Factor	**Density *M. liliana* >15 mm**	**Density *A. stutchburyi* >15 mm**	**Zone (Embayment)**	**Zone (Main Basin)**	**Basin (North)**
Regression coefficient (SE)	-0.045 (0.009)	0.003 (0.002)	1.510 (0.226)	1.599 (0.243)	-1.462 (0.181)

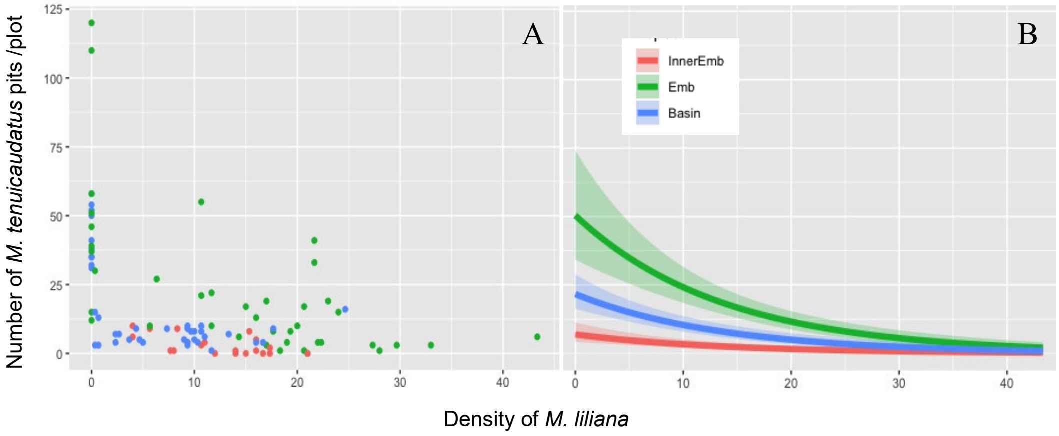

Figure 4.6. Graphs showing A) Observed values of numbers of pits /plot in Summer 2017 with plot mean *M. liliana* >15mm at the three levels of factor Zone. B) Predicted numbers of pits /plot +/- 95% CI with increasing large *M. liliana* densities at levels of Zone with negative binomial model

Table 4.6. Comparison of Negative binomial regression models of factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in autumn 2017. * indicates the best model

Model	Variables included	AIC
1	Density *M. liliana* >15 mm Density *A. stutchburyi* > 15mm Zone Basin	673.54 *
2	Density *M. liliana* > 15mm Zone Basin	698.64
3	Density *A. stutchburyi* > 15mm Zone Basin	674.68
4	Zone Basin	696.73
5	Density *M. liliana* > 15mm Density *A. stutchburyi* > 15mm Basin	763.45
6	Density *M. liliana* > 15mm Density *A. stutchburyi* > 15mm Zone	744.54

Table 4.7. Regression coefficients of the factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in autumn 2017.

Factor	**Density *M. liliana* >15 mm**	**Density *A. stutchburyi* >15 mm**	**Zone (Embayment)**	**Zone (Main Basin)**	**Basin (North)**
Regression coefficient (SE)	-0.02 (0.012)	0.010 (0.002)	2.198 (0.277)	3.275 (0.293)	-2.155 (0.211)

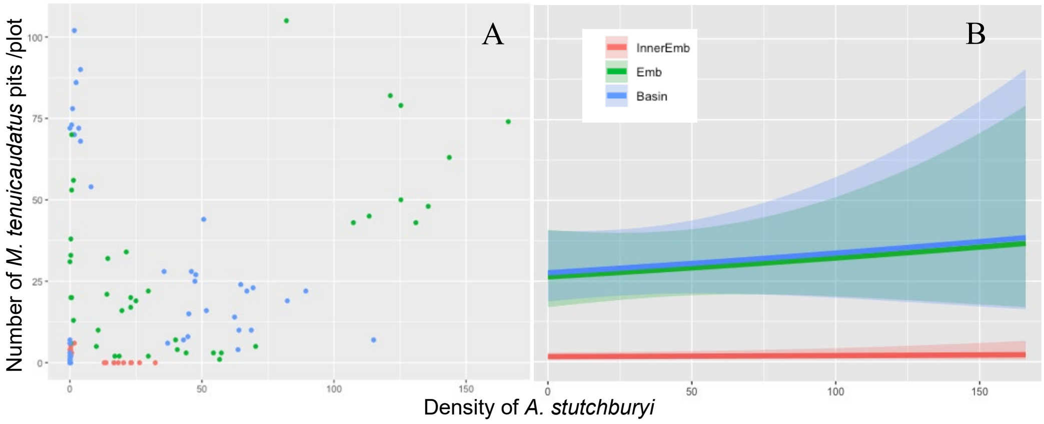

Figure 4.7. Graphs showing Observed pits /plot in autumn 2017 with increasing A) large *A. stutchburyi* B) predicted numbers of pits /plot +/- 95% CI with increasing large *A. stutchburyi* at levels of Zone with negative binomial model

Table 4.8. Comparison of Negative binomial regression models of factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in autumn 2017. * indicates the best model

Model	Variables included	AIC
1	Density *M. liliana* >15 mm Density *A. stutchburyi* > 15mm Zone Basin	344.6 *
2	Density *M. liliana* > 15mm Zone Basin	388.61
3	Density *A. stutchburyi* > 15mm Zone Basin	349.03
4	Zone Basin	388.17
5	Density *M. liliana* > 15mm Density *A. stutchburyi* > 15mm Basin	387.25
6	Density *M. liliana* > 15mm Density *A. stutchburyi* > 15mm Zone	387.7

Table 4.9.Regression coefficients of the factors influencing the density of *M. tenuicaudatus* feeding pits at 10 sites in the Tauranga Harbour in winter 2017.

Factor	**Density *M. liliana* >15 mm**	**Density *A. stutchburyi* >15 mm**	**Zone (Embayment)**	**Zone (Main Basin)**	**Basin (North)**
Regression coefficient (SE)	0.044 (0.017)	-0.036 (0.005)	3.304 (0.466)	3.399 (0.492)	-3.272 (0.363)

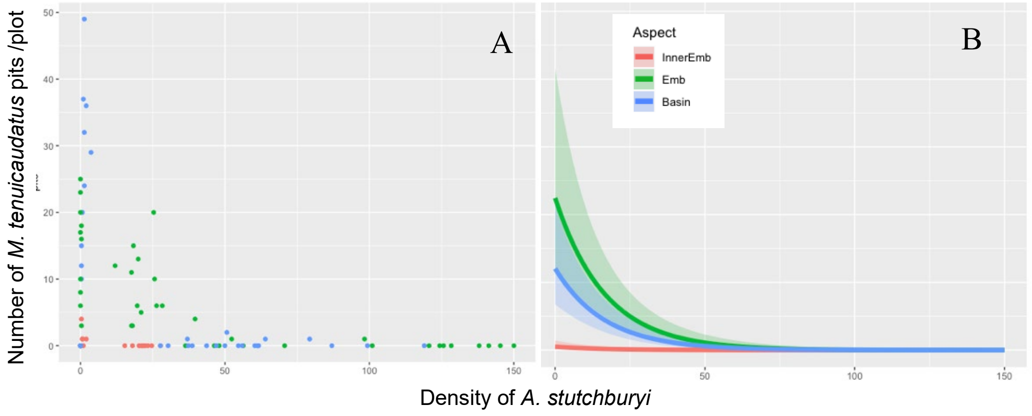

Figure 4.8. Graphs showing Observed pits /plot in winter 2017 with increasing A) large *A. stutchburyi* B) predicted numbers of pits /plot +/- 95% CI with increasing large *A. stutchburyi* at levels of Aspect with negative binomial model

Overall, the influence of the density of large infaunal bivalves varies over seasons. In spring and summer, *M. liliana* appears to be the most influential in terms of driving feeding activity, while in autumn and winter, *A. stutchburyi* appears to be the most important. However, the direction of effect is not always the same. Locational aspects Zone and Basin are highly influential in all seasons with locations in Embayment and Main Basin sites having consistently higher pit densities than Inner Embayment sites and consistently lower pit densities in the northern harbour basin when compared to the southern harbour basin.

4.4. Discussion

This chapter has confirmed that both prey densities and locational factors are correlated with the density of *M. tenuicaudatus* feeding pits and confirms the conclusion from the previous chapter that there are seasonal differences in use.

Macomona liliana density was an important factor in driving feeding behaviours in both the Spring and Summer models whereas *A. stutchburyi* was more influential in autumn and winter. It is unclear why there is a difference between the importance of the two infaunal species in driving feeding patterns among seasons, particularly as there was no significant difference in density of these species among seasons. It could perhaps be due to the higher overall densities of *A. stutchburyi* at sites where it is located, or a relic of site, rather than food species preference.

The spring model showed a positive relationship between *M. liliana* density and pit density, corroborating previous work, even though none of the sampling sites had *M. liliana* densities that reached the threshold (40 per quadrat) determined by for a positive relationship with pit densities (Hines et al., 1997). The autumn model showed a similar positive relationship with *A. stutchburyi* which corroborates previous work in the Whangateau Estuary by Le Port (2003).

In contrast, the summer model found a negative relationship between pit density and density of large *M. liliana*, suggesting that rays may feed preferentially in sites with fewer large bivalve infauna during warmer months. A negative relationship with densities of large infaunal bivalves was echoed in the winter observations. These relationships are in contrast to the commonly held expectation that when prey has a patchy distribution, the predators should select the highest density prey patches, thus optimising energetic gain (Charnov, 1976). This may mean that in sites with lower large bivalve densities, they are feeding on another infaunal prey type, for example worms or crustaceans. These groups have been found to be important in the diet of *M. tenuicaudatus* by previous stomach contents analyses (Hartill, 1989; Sommerville et al., 2011). Although the infaunal survey methodology of this study was not efficient in sampling worm presence or density, a previous large-scale survey of Tauranga Harbour found high worm presence in sites either very close to in location, or analogous to (by sediment grain size) the sites surveyed in this study (Ellis et al., 2013).

Crustaceans such as burrowing crabs *Helice crassa* or snapping shrimp *Alpheus* sp. are also known to be common infaunal species in intertidal sand flat areas such as those in this study (Morrisey et al., 1999). Survey methods to quantify these species reliably are difficult, as burrow morphology is not constant (Nye, 1974; Morrisey et al., 1999). Previous stomach contents analyses on *M. tenuicaudatus* (Hartill, 1989; Sommerville et al., 2011) were undertaken in coastal rather than estuarine locations, where prey type and availability will be different. Therefore, details of diet of this species within estuarine environments by stomach contents analysis is necessary in future work. The stomach of a male *M. tenuicaudatus* found in the southern harbour did indeed contain shrimp (H. Cadwallader Pers. Obs.) and in some ray species male dentition changes in breeding season, which may lead to prey choice differences between sexes (Kajiura & Tricas, 1996). However, individuals caught in this (See Chapter 6) and other New Zealand harbours (Le Port, 2003; Riding, 2009; Davis, 2010; Marcotte, 2014; Cook-Auckram, 2019) have mostly been female, so this is unlikely to explain a change in prey preference in this location.

Due to the possession of 'electrosense', and thus the ability to detect buried prey (Carrier et al., 2010) it is likely that the process of pit excavation is only carried out when prey is detected rather than on an exploratory basis. However high pit numbers were observed in some embayment locations with low bivalve density. This could be explained by the presence of alternate food sources in high enough densities to support a high number of feeding rays (Tregenza, 1995). Alternatively, if refuging from perceived predators in embayment areas is occurring, rather than choice of foraging habitat by prey density, observed high numbers of pits may be due to a small number of rays needing to excavate more pits to obtain the same energetic value of food. In addition, grouping behaviour, sex and size segregation is common in elasmobranchs (Jacoby et al., 2012) and it is possible that grouping behaviour may explain high densities of pits in unlikely, prey-poor areas.

Harbour basin proved to be a significant factor in the density of pits/plot in all seasons, supporting the observation in the previous chapter that overall the northern basin had consistently lower pits/plot than the southern basin. Rather than being a symptom of poorer habitat in the northern harbour, these figures may be due to a larger area of suitable habitat here, with the southern harbour being more urbanised, industrialised and the focus of much of the development in the area. This may result in a lower proportion of the area being suitable for eagle ray feeding, or a higher risk of disturbance from boat traffic, concentrating feeding in a smaller area. Pits may be spread over a larger area in more suitable habitat or habitat with fewer disturbances, and thus show lower densities in the areas sampled. Alternately, there may simply be fewer rays present in this location.

Zone was also a significant factor in all models. Plots sampled within inner embayment locations had consistently fewer pits over all seasons, however, only two sites were in this category, one of which had very few food items present and there were higher pit densities observed in an area outside of the sampling area (H. Cadwallader, Pers. Obs.). The other inner embayment location had a good infaunal density, but the sediment was very muddy, leading to the conclusion that if *M. tenuicaudatus* feeding was indeed occurring here it is uncertain whether pits were lasting long enough to be counted. It is recommended that future investigation into inner embayment use by this species be carried out, possibly using alternate methodologies, as these areas are often the most impacted by pollution (Ellis et al., 2013).

Relationships between pit density and infaunal density were different between outer embayment and main basin locations. This may be due to greater perceived safety from predators in embayments, leading to increased use at low densities of infaunal prey. Vaudo and Heithaus (2013) found that microhabitat selection of three batoid mesoconsumer species did not fit predictions from prey distributions, and although initially behavioural thermoregulation was considered as a counter-hypothesis, at fine scales it was revealed that instead, predator density was a significant driver. In that study it was concluded that selection of shallow near-shore habitats was a response to higher predator density rather than temperature or prey (Vaudo & Heithaus, 2013). Predators of ray species, the orca and larger shark species are common in Tauranga Harbour and, although it is not known how often they occur in this area, these predators would not be able to enter some of the shallower embayments. Harbour basin locations would not provide this protection, however they may serve as transitory foraging locations between embayments and deeper channels where *M. tenuicaudatus* are present at low tide. Indeed, boat-based observations reveal that a high number of *M. tenuicaudatus* individuals pass through an area of main basin sand flat habitat consistently on the outgoing tide, approximately two hours post high (See Chapter 6). Fine-scale tracking using towed-float GPS would elucidate whether this tidal difference in feeding location is occurring.

It is likely that other abiotic factors play a part in habitat choice of this species, including salinity, and temperature at the specific sites (Vaudo & Heithaus, 2013; Schlaff et al., 2014). These were not measured as part of this study as it was decided to focus on large-scale overarching factors such as prey density and location. There was very little unexplained deviation in the models, so these other factors may not be having much effect. However, this could be studied by data logging tags mounted on *M. tenuicaudatus* individuals in future investigations.

In conclusion, large bivalve density is not the major factor in foraging habitat choice in *M. tenuicaudatus* in Tauranga Harbour. Other factors such as disturbance and perceived predator risk may be more important. In addition, presence of other prey types such as worms or crustaceans in areas with low bivalve density may explain use of these habitats. It is clear that the density of feeding pits is complex and cannot be explained or predicted by any one variable.

CHAPTER FIVE

THE ROLE OF MANGROVE FRINGE AREAS IN PROVIDING FEEDING HABITAT FOR THE NEW ZEALAND EAGLE RAY (*Myliobatis tenuicaudatus*)

5.1. Introduction

Mangroves in many tropical areas are declining rapidly as a result of a number of anthropogenic activities, including, but not limited to, coastal development, agriculture and aquaculture (Laegdsgaard & Johnson, 2001; Walters et al., 2008). The ecological and economic benefits of tropical mangroves (ecosystem services) are numerous and well documented, and include carbon sequestration, the provision of fisheries-important nursery habitats and protection of coastlines from erosion (Ellison, 2008; Walters et al., 2008; Polidoro et al., 2010). Less well documented is the importance of mangrove habitat for larger predatory species, such as elasmobranchs (sharks and rays), which inhabit estuaries for part or all of their lives. Given the clear ecological value of mangrove habitat, the observed global decline of this habitat is of particular concern, especially in tropical regions (Dulvy et al., 2014; Rivera-Monroy et al., 2017). In New Zealand, there is some knowledge of the ecological value of mangroves for small species (Dencer-Brown et al., 2018) whereas there is a distinct lack of information regarding the role of temperate mangrove habitat for larger species. This should be of concern given the widespread clearance of mangroves by resource management agencies throughout the northern North Island.

Mangrove forests have the potential to be important for elasmobranchs, both as feeding and protective habitat. The morphology of mangroves, with their close growing structure of often buttressed stems (in tropical regions), aerial roots and pneumatophores, may prevent large predators from accessing these areas, therefore providing protection for small species of fish and juveniles of larger species (Davy et al., 2015). Mangroves often support nurseries of teleost fishes, and therefore have the additional potential to be a productive feeding habitat for small piscivorous elasmobranchs (Robertson & Duke, 1987; Simpfendorfer & Milward, 1993; Kimirei et al., 2013). The sediments below and immediately adjacent to mangroves typically contains a high mud content and has the potential to provide highly productive habitat for benthic and infaunal invertebrate species (Alfaro, 2006).

In New Zealand, mangroves may constitute an important trophic resource for benthic feeding fish species such as flounder, parore, grey mullet and benthic feeding elasmobranch species such as eagle rays. However, our understanding of their importance is limited as there have been few investigations into the use of temperate mangrove areas in New Zealand by fish species (Dencer-Brown et al., 2018).

In addition to the value of mangroves as feeding habitat, batoid elasmobranchs may use mangroves as nursery habitat. Davy et al. (2015), using passive acoustic telemetry found that juvenile mangrove whiprays (*Urogymnus granulatus*) moved into mangrove areas at high tide. It was suggested that this behaviour may have been driven by the threat from large sharks present in the area (Davy et al., 2015). White and Potter (2004) used gill nets in unvegetated areas immediately adjacent to dense and sparse stands of the temperate mangrove *Avicennia marina* in Shark Bay, Western Australia, to quantify numbers of elasmobranchs entering mangrove adjacent areas on the incoming tide. They found that the mean number of elasmobranch species caught, and the mean catch rate at these sites was higher than at an unvegetated site within 200 m. They also found that the majority of individuals caught were juvenile, with umbilical scars, suggesting the area may be used as a nursery area (White & Potter, 2004).

While the loss of mangrove habitat has been relatively rapid in the tropics, in New Zealand, the endemic mangrove species, *Avicennia marina* var. *australasica* (hereafter called *Avicennia marina*), has been steadily expanding its coverage in estuaries within its biogeographic range (De Lange & De Lange, 1994; Park, 2004; Swales et al., 2008; Lovelock et al., 2010; Morrisey et al., 2010; Lundquist et al., 2012). This mangrove expansion has been correlated with an increase in sedimentation associated with changes in land-use from native forest to agriculture, forestry and coastal/catchment urbanisation (De Lange & De Lange, 1994; Lovelock et al., 2007). To a lesser extent, the expansion in mangroves is also linked to nutrient enrichment (Lovelock et al., 2007). Public perception of mangroves is often negative, due to a perceived or real loss of water views, recreational and boating access, or habitat/biodiversity (Green et al., 2003; Morrisey et al., 2007; Harty, 2009; Lundquist et al., 2014; De Luca, 2015). Regional Councils are under increasing pressure to engage in, or support, various forms of mangrove management to prevent further spread; or to restore open sandflat areas that are perceived to be ecologically and recreationally more valuable (Green et al., 2003; Morrisey et al., 2007; Harty, 2009; Lundquist et al., 2012, 2014; De Luca, 2015). Consequently, there has been widespread removal and management (both legal and illegal) of mangroves in recent decades (Lundquist et al., 2012, 2014). The ecological consequences of mangrove management remain largely unknown, with particular paucity of information relating to the effects of mangrove removal on higher trophic levels (De Luca, 2015; Dencer-Brown et al., 2018).

Removal methods can be either manual (chainsaws) or mechanical (tractors with low pressure tyres) with waste material either removed or left *in situ* to degrade (with or without burning) (Lundquist et al., 2014). Management of mangroves in New Zealand is often carried out by Estuary Care groups made up of local volunteers and consists of working parties removing seedlings and pneumatophores with hand-tools (Lundquist et al., 2014). The removal and management of mangroves generally results in a significant disruption to the sediment. Negative impacts on benthic infauna have been recorded immediately after management, followed by possible increases in abundance and diversity depending on sediment properties (Alfaro, 2006, 2010). The recovery of managed areas to the desired sandflat conditions post-removal has been varied. Observations of some sites more than 5 years following a removal event recorded much sandier conditions, however most sites showed minimal recovery, retaining high proportions of muddy sediments (Lundquist et al., 2014). Where cut mangrove material has been left in place, or where the remnants of extensive, formerly well-established root and pneumatophore systems remain, there has been a persistence of anoxic, muddy sediments and low infaunal diversity and biomass (Lundquist et al., 2012). As the ecological value of mangroves in New Zealand is poorly understood (Alfaro, 2010; Dencer-Brown et al., 2018), the consequences of mangrove removal remains largely unknown.

One elasmobranch species known to use estuarine and potentially mangrove habitats in New Zealand is the eagle ray (*Myliobatis tenuicaudatus*). This species of eagle ray is not currently threatened and is considered ‘Least Concern’ by the

IUCN red-list analysis (Kyne, 2016). However, habitat degradation, including mangrove removal, is considered one of the major risk factors for coastal elasmobranchs, particularly batoid (ray) species (Dulvy et al., 2014). Therefore, with the removal and management of mangroves in New Zealand a common occurrence, it is important to assess the use of mangrove habitats by this and other elasmobranch species.

Myliobatis tenuicaudatus is a small to medium sized myliobatid ray that exhibits a highly specialised method of extracting benthic and infaunal prey by using jets of water to excavate a pit (Gregory & Ballance, 1979). In intertidal areas these pits survive over a number of tides and can be used to quantify the feeding effort of this species (Hines et al., 1997). Feeding excavations of *M. tenuicaudatus* have been observed in the pneumatophore habitat immediately fringing *A. marina* areas in several New Zealand estuaries (H. Jones, Pers. Comm.), suggesting that this habitat is a profitable feeding area for this species. It has been shown that the fringing pneumatophore habitat of *A. marina* habitat contains a higher diversity and abundance of organisms than sediments within the stands, although lower than in sandflat areas (Alfaro, 2006), further suggesting that the fringe area is an important habitat.

Mangrove fringe is classified as the 30 m wide strip immediately adjacent to an area of dense *A. marina* trees. In an untrimmed, natural site, this area is often characterised by mangrove pneumatophores, mangrove seedlings, small plants (<1 m in diameter) and mud crab holes at varying densities. A trimmed edge may have as little as 50 cm of pneumatophore spread beyond the dense mangrove forest and seedlings are removed on a regular basis. In Tauranga Harbour, in northern New Zealand, eleven areas of mangrove were consented for management or removal in

2008 and 2009. Approximately 32 kilometres of mangrove edge are now trimmed in an attempt to prevent mangrove expansion (calculated using consent maps, courtesy of Bay of Plenty Regional Council and Google Earth). It was postulated that due to current management (manual removal of seedlings and pneumatophores) likely resulting in sediment disruption, and natural fringe habitat providing an aspect of protection from predators, feeding intensity by eagle rays would be lower in the trimmed fringe habitat compared to the natural (unmodified) fringe habitat.

To test this hypothesis the following questions will be asked:

Are rays are using mangrove fringe habitat for feeding

Does trimming of mangrove fringe alter the way in which rays use this habitat.

5.2. Methods

5.2.1. Study Area

The survey was conducted in the Matua sub-estuary, Southern Tauranga Harbour, New Zealand (37˚E, 175˚S, Figure 5.1). In this sub-estuary, an area of consented management trimming is located adjacent to an area of natural fringe, separated by a persistent channel (>50 cm deep at lowest astronomical tide) (Figure 5.2). This channel provides equal access to both habitats.

Figure 5.1. Satellite image showing Tauranga Harbour (North Island, New Zealand) indicating the location of the Matua sub-estuary study site. Image: Image source: Google Earth. June 3, 2016. Accessed July 4, 2019.

5.2.2. Survey methodology

Two fringe habitats were surveyed between January and August 2017 to compare *M. tenuicaudatus* feeding effort at natural and trimmed mangrove fringe (Figure 5.2). In both managed (trimmed, Site B) and unmanaged (natural, Site A) fringe habitats, counts were performed at approximately two-week intervals between January and August 2017, exact observation dates and times were dependent on tides and weather conditions. In addition, on three occasions, pits were counted at an adjacent outer sandflat site (Site C) and on one occasion at an adjacent inner sandflat site (Site D) in order to determine whether pit densities in mangrove fringe were comparable to those in sandflat habitat, and whether the position within the embayment had any effect on pit densities.

A circular plot counting method designed by Hines et al. (1997) was used to quantify density of ray feeding pits (Figure 5.3). This methodology was also used for the parallel study of *M. tenuicaudatus* feeding in the wider Tauranga Harbour (*see* Chapter 3 and 4). In each habitat, feeding pits were counted in ten circular plots, each with a radius of 15 m and an area of 707 m^2. Plots were placed at varying distances along a 300 m transect of the fringe zone using a random number generator to determine distance from the beginning of the transect. In the sandflat areas, plots were placed using a random number generator to determine compass direction and distance from a central point.

5.2.3. Statistical analysis

To allow ease of comparison with the only previous work of this type, density was described as the number of pits per 707 m^2 plot. A Scheirer-Ray-Hare test (a non-parametric ranked variant of the two-way ANOVA (Dytham, 2011)) was used to compare pit density between habitats. A *post-hoc* pairwise Mann-Whitney U tested where differences between trimmed and untrimmed sites lay. All statistics were performed in SPSS (IBM, version 24). In addition, densities of feeding pits in the two mangrove fringe areas were compared with adjacent outer sandflat habitats (Sites C and D) using the same methodology.

Figure 5.2. Matua sub-estuary, Tauranga Harbour. Shaded overlay shows the area consented for Mangrove (*Avicenna marina*) removal and maintenance. Lines show survey transects, A) Untrimmed mangrove fringe, B) mangrove fringe within trimmed area. C) Adjacent outer sandflat area, D) Adjacent inner sandflat area. Consent area information courtesy of Bay of Plenty Regional Council. Image: Google.

Figure 5.3. New Zealand eagle ray (*Myliobatis tenuicaudatus*) feeding pits in Tauranga Harbour, New Zealand. **A)** a fresh pit in sandy sediment **B**) a slightly degraded pit in muddy sediment in a mangrove fringe habitat.

5.3. Results

Myliobatis tenuicaudatus feeding pits were counted at both trimmed and untrimmed sites on 15 occasions between January and August 2017 (Table 5.1). The maximum observed density of pits was 82 pits per 707 m^2 plot, observed in the untrimmed site on 28th June. Minimum observed density was 0 pits per 707 m^2 plot. Pits were recognisable for a maximum of 6 tides at both sites.

Table 5.1. Dates of and mean pits per plot counted on each sampling event. *Indicates an extra observation 24 hours following trimming event by hovercraft. All observations were >4 days apart, pit-longevity at this site was observed to be maximum 6 tides and so all observational events were deemed independent for analysis

Event	Date	Trimmed	Natural	Sandflat (outer)	Sandflat (inner)
1	14th January	18	43.2	51.8	51
2	25th January	0	0.2		
3	7th February	0.9	6.4		
4	27th February	0.1	11.9		
5	21st March	0.3	4.4	34	
6	2nd April	1.2	24.6		
7	20th April	0	0.2		
8	10th May	11.6	26		
9	28th May	13.7	18.3		
10	7th June*	0.7	8.2		
11	11th June	0.4	3.7		
12	28th June	12.5	52.1		
13	20th July	0	2.5		
14	5th August	0	1.2		
15	19th August	1.1	4.5	14.6	
Mean		**4.03**	**13.83**	**33.47**	**51**

Combining all plots on observational events, a higher total number of pits were observed in the natural fringe zone with 2074 pits counted in the natural zone and 605 pits in the trimmed zone. Density was significantly higher in the natural fringe zone than the trimmed fringe zone (13.82 pits /plot versus 4.03 /plot; Scheirer-Ray-Hare: $p < 0.001$; d.f. = 1, Figure 5.4). Pit density varied between sampling events ($p < 0.001$; d.f. = 14) suggesting a possible seasonal pattern. The time x treatment interaction term was not significant ($p = 0.196$); indicating a consistent relationship in the difference between trimmed and untrimmed sites over the observational period. Post-hoc Mann-Whitney-U analysis showed significantly higher pit density at natural fringe zone than trimmed fringe zone on 11 out of 15 observations (Table 5.2, Figure 5.5)

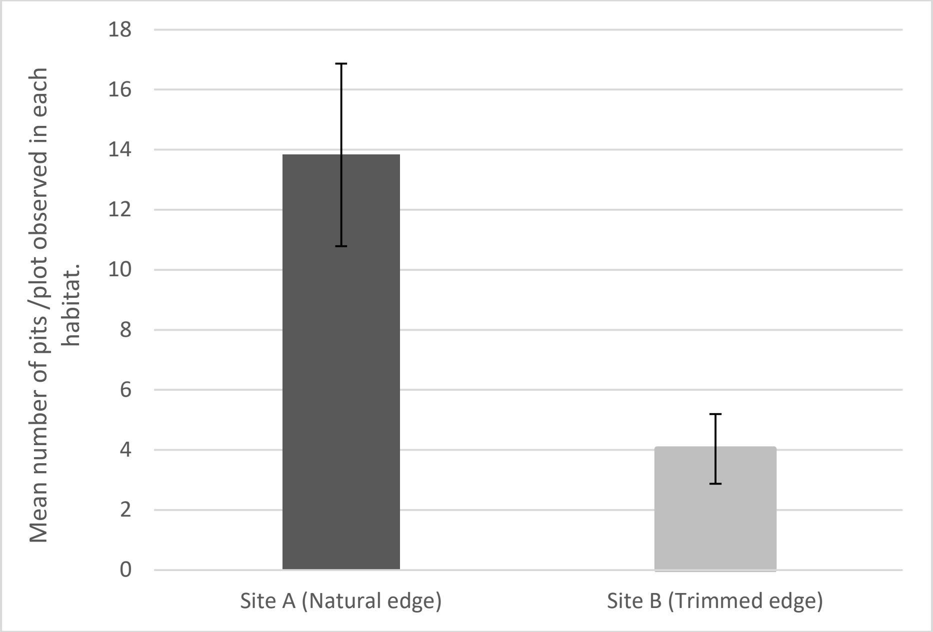

Figure 5.4. Mean number of pits /plot overall in natural edge and trimmed edge mangrove fringe habitats in the Matua sub-estuary, Tauranga Harbour, New Zealand during 15 observational events between January and August 2017. Error bars +/- 95% CI.

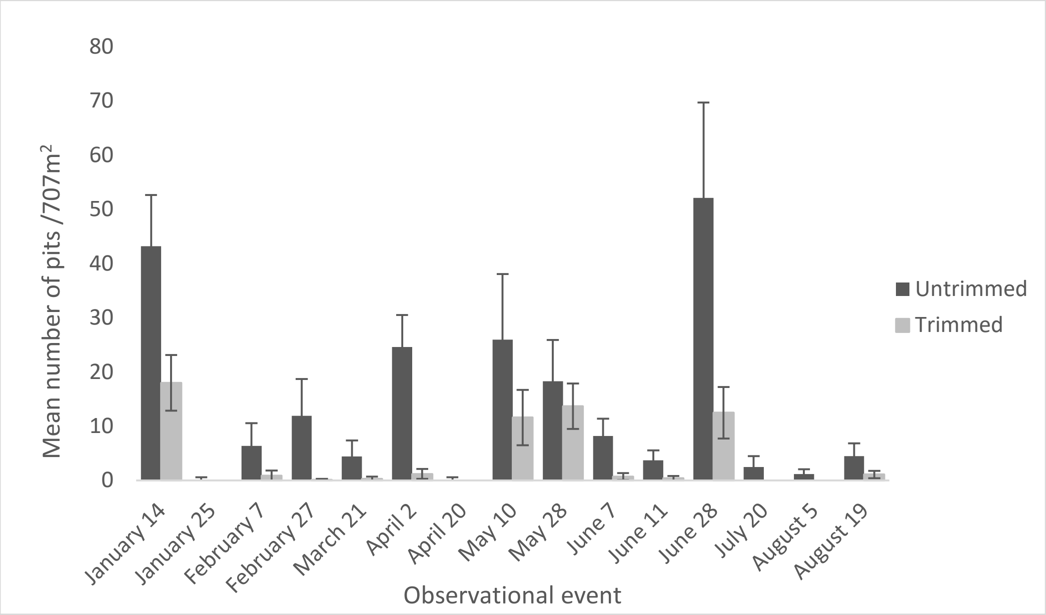

Figure 5.5. Mean number of pits per 707m^2 plot at trimmed and untrimmed mangrove fringe habitats in the Matua sub-estuary, Tauranga Harbour, New Zealand during 15 observational events between January and August 2017. Error bars +/- 95% CI.

Table 5.2. Post hoc Mann-Whitney U test analyses for density of ray feeding pits at a trimmed versus an untrimmed mangrove fringe site in Matua sub-estuary, Tauranga Harbour, New Zealand. * indicates a significant difference between the sites.

Event	**U**	***p***
1	3.5	<0.001*
2	45	0.317
3	27.5	0.063
4	0.5	<0.001*
5	14.5	0.004*
6	0	<0.001*
7	45	0.317
8	22.5	0.037*
9	34.5	0.241
10	1.5	<0.001*
11	19.5	0.014*
12	15	0.008*
13	25	0.013*
14	20	0.005*
15	23.5	0.041*

When compared to feeding pit densities in an adjacent sandflat area (Site C, Figure 5.6), the Summer observation (January 14th) revealed significantly lower densities in the trimmed fringe site (U = 13, p = <0.005), whereas the natural fringe site showed no significant difference in pit density to the adjacent sandflat (U = 46.5, p = 0.791, Table 5.3). In the Autumn and Winter observations (March 21st and August 15th respectively) density of pits at both natural and trimmed mangrove fringe zones were significantly lower than within the adjacent sandflat area (Natural: U = 4.0, p<0.001, Trimmed: U = 0, p<0, Table 5.3). On one occasion, during the summer observation (January 14th) an additional sandflat (Site D) was surveyed in order to determine whether the effect of location within the estuary was a factor. No significant difference in pit density was found between the two sandflat areas C and D (U = 45, p = 0.71), nor between the inner sandflat (Site D) and the natural mangrove fringe zone (U = 40, p = 0.45). However, the density of pits in the trimmed fringe zone was significantly lower than at the inner sandflat area (U = 7.5, p = 0.001, Table 5.3).

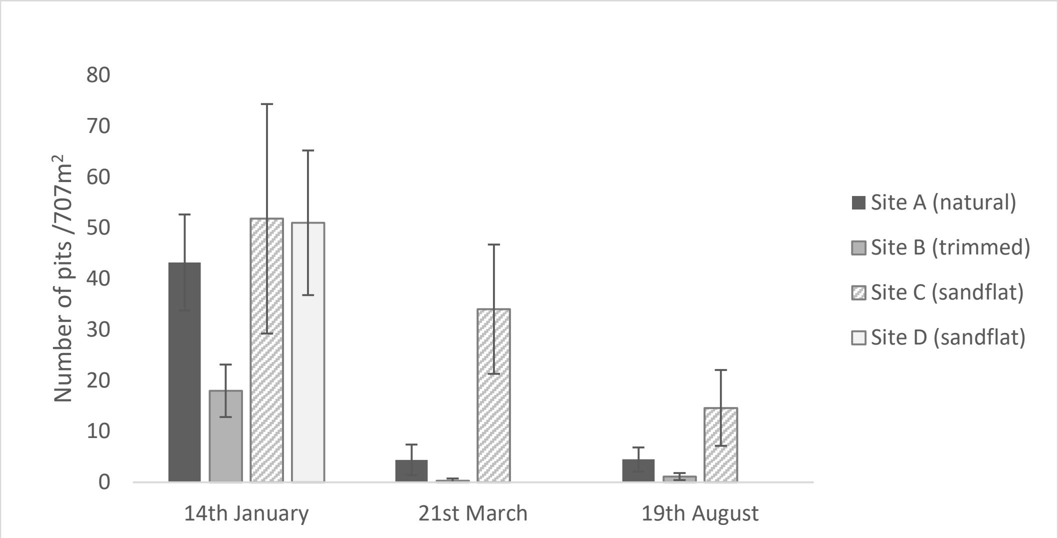

Figure 5.6. Showing mean number of M. tenuicaudatus feeding pits in mangrove (*Avicennia marina*) fringe habitats that have been left natural, been trimmed and two adjacent sandflat habitats. Error bars +/- 95% CI.

Table 5.3. Matrix of Mann- Whitney U comparison of density of *M. tenuicaudatus* feeding pits between Untrimmed (A), Trimmed (B), Sandflat (C) and Inner Sandflat (D) locations in Matua Sub-Estuary, Tauranga Harbour, New Zealand on three occasions during 2017. *denotes a significant result. Note: Inner Sandflat was sampled on one occasion only.

	January			**March**		**August**	
	B	**C**	**D**	**B**	**C**	**B**	**C**
A	$U = 3.5$ $p<0.001$*	$U = 46.5$ $p=0.79$	$U = 40.0$ $p=0.45$	$U = 14.5$ $p = 0.004$*	$U = 4.0$ $p < 0.001$*	$U = 23.5$ $p = 0.041$*	$U = 12.5$ $p = 0.005$*
B		$U = 13$ $p = 0.005$*	$U = 7.5$ $p = 0.001$*		$U = 0$ $p < 0.001$*		$U = 0.5$ $p<00.001$*
C			$U = 45$ $p = 0.71$				

5.4. Discussion

The presence of feeding pits in the vicinity of mangroves on multiple occasions over a relatively long period, shows that *M. tenuicaudatus* are using mangrove fringe areas for feeding. The consistent reduced occurrence of feeding pits in the trimmed fringe zone indicates that this habitat may be of lesser value vs. natural fringe zone. Observation of *M. tenuicaudatus* during foraging periods, where animals swim, then stop, turn, and dig, has led to the conclusion that prey is located using electroreception, much like other ray species feeding on benthic prey (Blonder & Alevizon, 1988; Tillett et al., 2008). This leads to the assumption that feeding pits are likely only dug when appropriate food items are present. It may therefore be the case that trimmed fringe is a lesser quality feeding habitat, with lower infaunal densities or altered species composition relative to a natural fringe, potentially due to the repeated disturbance of the sediment during trimming events (Rossi et al., 2007). However, this was not examined as part of this study. If there is a lower infaunal density or species composition in trimmed areas, then it may be that it is less optimal energetically for animals to forage in these areas, leading to reduced use.

Alternatively, if the infaunal density or species composition in both fringe habitats is similar then the observed difference in pit counts may be that the small (<1m diameter) bushes, seedlings and pneumatophores that characterise the natural fringe habitat may be providing some protection from potential predators. Choice of foraging patch is thought to be not only a factor of food density but also predation risk in that patch (Ahrens et al., 2012). Orca (*Orcinus orca*) are a major predator of small elasmobranch species in New Zealand, particularly rays, (Visser, 1999). The presence of orca in the Tauranga Harbour often results in large aggregations of *M. tenuicaudatus* seeking refuge in shallow protected areas. It may be that the perception by the rays that the natural fringe area provides protection is driving a consistently higher use of this habitat.

The possibility that the lower densities in the trimmed fringe zone may be due to the position of the zone within the embayment, with the natural fringe zone being closer to the embayment mouth, seems to be countered by the summer (January 14th) sampling occasion including an inner sandflat area. There was no significant difference between densities of pits between the inner and outer sandflat areas, and the natural fringe zone, but all densities were significantly higher than that of the trimmed zone.

High variation in pit densities between the observational events is not unexpected. Seasonal differences in *M. tenuicaudatus* feeding pit density was initially suggested by Hines et al. (1997) over a short time period and has been confirmed in the Tauranga Harbour over a two-year period (Chapter 3). Additionally, feeding pit presence in intertidal areas in another New Zealand estuary (Whangateau Estuary) depended on time of day and meteorological conditions: fewer pits were present after high rainfall, and more pits were present during early morning low tides than during afternoon low tides (Le Port, 2003). Similar variation may be occurring in Matua estuary. Furthermore, myliobatid rays can be highly mobile and some species cover large areas on a daily basis. For example, the cownose ray (*Rhinoptera bonasus*) is known to travel up to 50 km per day (Ajemian & Powers, 2014) and have daily core areas of use of up to 9.7 km^2 (Collins et al., 2007). The distance travelled by 10 *M. tenuicaudatus* individuals tracked using buoy-based GPS telemetry in a study by Riding et al. (2009) can be calculated at an average of 1.34 km per hour, or 32 km per day. Therefore, low pit abundance in Matua during some periods of observation is likely due to the animals feeding elsewhere. Variation in pit density between observational events may also be due to presence or absence of large predators such as the Orca. Pods of Orca in the Tauranga Harbour are relatively common but occur irregularly. Their presence may drive large numbers of rays into or out of certain areas within the harbour depending on their location. Despite this variation, the differences between feeding effort at trimmed and natural fringe zones remains consistent over all events with the interaction term effect not showing significance.

The significantly lower pit density in both fringe habitats compared with the adjacent sandflat area in the autumn and winter sampling events may be attributed to a higher abundance of preferred infaunal species in the sandflat habitat. Alfaro (2006) identified that pneumatophore zones in Matapouri Estuary, New Zealand, had an intermediate diversity and abundance of infaunal species, with sandflats higher, and inner mangrove habitat lower.

Barnes (2017) in Moreton Bay, Queensland, Australia, found a similar pattern in study sites with pneumatophore zones that bordered sandflats, but also found that when pneumatophore zones bordered seagrass *(Zostera* spp.) areas the biodiversity in the fringe habitat was comparatively high, similar to that in the seagrass area. The lack of difference between densities of feeding pits between the natural fringe zone and sandflat areas during the summer observation event may be due to a higher number of rays generally over this time period resulting in increased competition for resources and effort was spread over a larger area.

From the results of this investigation, it appears that in terms of ecological value to *M. tenuicaudatus* measured by pit density, some sandflats are more valuable than natural mangrove fringe, which are in turn more valuable than trimmed mangrove fringe. Therefore, if removal events result in increased suitable sandflat then this will in turn increase the availability of valuable feeding habitat for this species. However, this recovery has not been the case in all monitored mangrove removal locations and is reliant on many factors including but not limited to the hydrodynamics of the site and the removal methodology (Lundquist et al., 2012; Bulmer et al., 2017; Lundquist et al., 2017). In addition, the suitability of sandflat areas for *M. tenuicaudatus* is likely to be not purely dependent on abundance of food items. Results presented in Chapter 4 suggested that the density of pits and thus the feeding effort is highly dependent on location, with 'Zone' and 'Harbour Basin' both significant factors explaining the density of pits, and this is likely to be the case for the use of mangrove fringe. However, feeding pits observed in the mangrove fringe for this study as well as others outside of the study area, indicate that mangrove fringe is an important habitat for this species.

This study has confirmed the use of mangrove fringe as feeding habitat for *M. tenuicaudatus.* In addition, it is clear from the difference in pit densities between natural and trimmed mangrove fringe zones that the current management strategy of removal of seedling and pneumatophore to mitigate mangrove spread may be resulting in a lower quality foraging habitat for *M. tenuicaudatus*. When extrapolated to the whole of the Tauranga Harbour, the approximately 32 km of currently trimmed mangrove fringe results in over 95 hectares of degraded habitat assuming a fringe zone diameter of 30 m. Although *M. tenuicaudatus* is currently considered ‘Least concern’ by the IUCN red list (Kyne, 2016), the potential risk to coastal elasmobranch species is high (Dulvy et al., 2014). Therefore, this continued degradation of large areas of habitat for ‘aesthetic consideration’ is troubling and the use of an area by elasmobranch species should be included in considerations for future mangrove removal consents.

In conclusion, this study has confirmed the use of mangrove fringe as feeding habitat for *M. tenuicaudatus*. It is clear from the difference in pit densities between natural and trimmed mangrove fringe zones that the current management strategy of seedling and pneumatophore removal to mitigate mangrove spread may result in a lower quality foraging habitat for *M. tenuicaudatus.* However, it is also clear that sand flat, at least in this location, may be a more valuable habitat for eagle ray feeding. Hence the outcome of a recovery process, post mangrove removal, is critical to establishing whether more or less foraging habitat is available to stingrays. This study therefore indicates that the ecological and hydrodynamic processes that ensue following a mangrove removal campaign (Bulmer et al., 2017) must be investigated thoroughly before further mangrove management action is undertaken.

CHAPTER SIX

HEAVY METALS IN RAY MUSCLE TISSUE IN A NEW ZEALAND URBAN HARBOUR

H_5: There will be a difference in heavy metal body burdens between rays inhabiting Tauranga Harbour, and other Bay of Plenty coastal regions.

6.1. Introduction

Coastal and estuarine species the world over are likely to be experiencing increasing pressure from the rapid changes in use of coastal and catchment land. One of the major consequences of these land-use changes has been an increase in contamination of estuarine and coastal waters with organic and inorganic pollutants (Islam & Tanaka, 2004; Vieira et al., 2009; Gelsleichter & Walker, 2010). In New Zealand, this change has been particularly rapid and since the relatively recent human habitation, native forest has given way to pasture and intensive commercial forestry and horticulture. Coastal regions in many areas have become heavily urbanised and industrialised. Pollution has been determined to be one of the greatest threats to near-shore dwelling elasmobranchs, alongside fishing, development and mangrove removal (Dulvy et al., 2014). Despite this, relatively few studies have determined the concentrations/impact/burden of pollutants, particularly heavy metals, in ray species (Lyons et al., 2017).

Heavy metals and metalloids are among the most well-known estuarine pollutants (Vieira et al., 2009). They are known to have a number of biotoxic effects in high concentrations (Farrell et al., 2012), and enter the marine environment by many anthropogenic mediated pathways in addition to background sources from natural volcanism (Garrett, 2000; Gelsleichter & Walker, 2010). The latter is of particular relevance in the Bay of Plenty region (Kulgemeyer et al., 2016, 2017). Particular metals of interest include chromium (Cr), copper (Cu), cobalt (Co), arsenic (As), nickel (Ni), zinc (Zn), cadmium (Cd), mercury (Hg), and lead (Pb).

6.1.1. Sources of Metals in the Marine Environment

Anthropogenic sources of heavy metals are widespread, most are washed into waterways by storm-water rather than directly discharged. In New Zealand particularly, copper-based sprays have been widely applied as a treatment and prevention for the damaging kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa-V) (Monchiero et al., 2015). In addition, chromated copper arsenate is a wood preservative associated with vine supports in kiwifruit production and viticulture and a source of Cr, Cu and As into the environment (Robinson et al., 2006). Other agricultural sources can include Cd in phosphate-based fertilisers (McGeer et al., 2011), and a further use of Cu (as sulphate) as fertilizer (Grosell, 2011)

Additional sources of Chromium include the steel industry, automotive and aeronautic painting and finishing, coal combustion and the cement industry (Reid, 2011). Also dyes, printing and the pharmaceutical industry (Authman et al., 2015). Copper levels may be elevated in areas with high boating activity due to the use of copper compounds for their biocidal properties in antifouling paint products (Turner et al., 1997; Srinivasan & Swain, 2007; Piola et al., 2009).

Road dust created by brake pad and tire wear in addition to combustion emissions is high in metals such as Zn, Cu, Pb, Ni, Cr and Cd and may be washed into stormwater systems (Rogge et al., 1993; Gunawardana et al., 2012). In areas where storm-water is washed directly into waterways or harbours, this can be a source of these metals into the environment.

Natural sources of heavy metals include volcanic activity, vents and eruptions and weathering of metal containing rocks (Garrett, 2000). Locally to this study, geysers and other vent systems within the Taupo Volcanic Zone have been found with high levels of compounds rich in in Hg and As (Stoffers et al., 1999).

6.1.2. Effects of Metals on Elasmobranchs

Accumulation of metals in fish can be through diet, by consuming contaminated prey, or by absorption from water or sediment through gills or skin tissues respectively (Mathews et al., 2008). Diet is by far the dominant route of uptake for many metals with dietary source of Cd, Mn and Zn accounting for 60–100% of uptake of these metals in both elasmobranch and a teleost (*Schliorhinus canicula* and *Psetta maxima* respectfully) under experimental conditions (Mathews & Fisher, 2009). In addition, the same study showed that aqueous uptake contributed proportionally more to body burden in the elasmobranch than the teleost. This is likely due to the high affinity of elasmobranch placoid scales to these metals (Jeffree et al., 2006). Rate of dietary uptake is highly species-specific and accuracy in calculations can only be obtained through controlled aquarium experimentation (Andres et al., 2000; Mathews & Fisher, 2009; Authman et al., 2015).

Once absorbed, affinity of metals to different tissues is highly dependent on species, in addition to characteristics such as size and sex (Jeffree et al., 2006; Mathews et al., 2008; Jeffree et al., 2010). For example, females may show a lower metal burden than males, due to a transfer of metals during reproduction (Mathews et al., 2008; Barbara et al., 2009). This maternal transfer has been documented in a number of elasmobranch species, particularly regarding Hg, however the process is poorly understood (Lyons & Lowe, 2013; Naidoo et al., 2017). In viviparous species this is possibly due to the use of liver-derived lipids for energy required in the production of live young (Hamlett, 2005).

The vast majority of information available to date on the effects of heavy metals in fish is concentrated on freshwater species (Authman et al., 2015), especially embryonic and larval stages (Barbara et al., 2009). Effects of metals in freshwater teleost fish include low larval motility, spinal deformities and increased susceptibility to infection (Barbara et al., 2009). Examples of observed effects of metal exposure are widespread and include Cu causing cardiovascular deformities in embryonic Japanese rice fish (*Oryzias latipes*) (Barjhoux et al., 2012), Cd causing endocrine disruption affecting the production of steroids, eggs and sperm in juvenile rainbow trout (*Onchorhynchus mykiss*) (Vetillard & Bailhache, 2005) and Pb causing hormone alterations in adult *Cyprinus carpio* (Ramesh et al., 2009).

Extrapolation of these effects to elasmobranchs should be treated with caution however, due to the differences in physiology between teleost and elasmobranch fish (De Boeck et al., 2010), and the chemical differences between fresh and salt water affecting metal physiological processes (Varol et al., 2017). Irrespective of phylogenetic differences in biochemical response, it is highly likely that disruption of physiological processes occurs in response to exposure to heavy metal pollution, and that high levels of metals are deleterious.

Despite dietary uptake being the dominant route (Mathews & Fisher, 2009), most existing studies that have directly studied the effects of metals on elasmobranchs, have been short term exposure to waterborne metals. Effects have been detected such as Cd causing reduction in haemoglobin production in *Scylorhinus canicular* (Tort & Torres, 1988), Pb causing an increased physiological stress response in spiny dogfish (*Squalus acanthius*) (Eyckmans et al., 2013) and Cu and Pb accumulating in the rectal gland, with the possibility of disruption of osmoregulatory processes (De Boeck et al., 2010).

It has been determined that elasmobranchs may be particularly susceptible to silver (Ag) with toxicity of this metal tenfold higher in *S. acanthius* than in comparably sized marine teleosts (Wood et al., 1999). Ag exhibits very high accumulation in gills and other tissues at relatively low concentrations in the water, even at levels up to 1000 times lower than other metals (Webb & Wood, 2000; De Boeck et al., 2001, 2010). However, it has also been determined that accumulation is lower in higher salinities, due to the bioavailability of differing Ag compounds found in the differing conditions (Webb & Wood, 2000).

The high toxicity of Ag observed in these species is related to the fact that it disturbs osmoregulatory processes by loss of urea (De Boeck et al., 2010), urea is an important osmolyte in elasmobranchs (Hazon et al., 2003). Additionally, De Boeck et al. (2010) found that high silver concentrations in tissues did not initiate production of metallothionein, a metal binding protein that is in part, utilised in elasmobranchs to bind non-essential toxic metals such as Cd and Hg (Hidalgo & Flos, 1986; Cho et al., 2005; Walker et al., 2014).

Previous Field Studies on Heavy Metals in Ray Species

Lyons et al. (2017) compared mercury levels in muscle and liver between mature and immature *Urobatis halleri* in one urban and one offshore location in California.

They discovered that in both areas, the concentration of Hg was higher in the mature animals from both areas, but the increase was significantly steeper and higher in the mainland urban rays, leading to the conclusion that location, and ontogeny determined concentration levels (Lyons et al., 2017).

Türkmen et al. (2013, 2014) studied heavy metal levels in a number of ray and skate species in two adjacent locations in the Mediterranean. Rather than comparing levels with another location, these two studies were primarily baseline studies, comparing tissues. Cd levels were very high in these locations, in the muscle as well as in the liver, suggesting that while Cd normally accumulates in the liver (Torres et al., 2016), at high environmental levels it accumulates in the muscle as well. In addition, Torres et al. (2016) studied the bioaccumulation and biomagnification of metals in *Raja clavata* muscle and liver in the Azores, a volcanic area, and found no difference between concentrations in males and females. They also found higher As, Cr and Hg concentrations in muscle, and Cd, Cu, Se and Zn was found mainly in the liver (Torres et al., 2016).

Study Locations

Tauranga Harbour is a large estuarine lagoon of over 200 km^2 on the east coast of the North Island of New Zealand (Ellis et al., 2013). The harbour is highly flushed, with over 60% of the area exposed at low tide (Inglis et al., 2008). Tauranga Harbour opens to the Bay of Plenty Coastal region at Bowentown in the Northern Basin and Mount Maunganui in the Southern basin (Briggs et al., 2005). The hydrodynamics of Tauranga Harbour means that much of the water is flushed tidally twice daily, preventing build-up of run-off chemicals (Wolanski & Elliott, 2015). The main freshwater input into the harbour comes from the Wairoa River, which also discharges sediment from farmland and the Taupo volcanic zone into the southern basin (Park, 2003; Briggs et al., 2005).

The Southern basin of Tauranga Harbour is separated from the Northern basin by a large sand flat with little to no water exchange at high tide (Tay et al., 2013). Tauranga city and the Port of Tauranga, including large swathes of industrial land, border this Southern Basin in a large proportion of its boundaries. Tauranga city is growing rapidly, (Tauranga City Council, 2018) and with this comes increased road usage and industrial developments including land hardening and impervious surface cover increasing storm-water run-off (Paul & Meyer, 2001). The Port of Tauranga is located adjacent to the harbour entrance and is the busiest commercial port in New Zealand with total cargo handled in 2015 reaching more than 20 million tonnes (Port of Tauranga, 2015). The southern harbour entrance and port has recently undergone a large-scale dredging operation to deepen and widen the channel allowing larger cargo vessels to use the Port of Tauranga (Ross, 2018).

Metal levels in Tauranga Harbour surface sediments have demonstrably been lower than those in other measured harbour such as Napier and the Manukau estuary systems (Park, 2003; Ellis et al., 2013; Park, 2014; Huteau, 2017). Metals have been measured highest in the innermost estuaries and rural and urban locations have had different signatures (Ellis et al., 2013; Park, 2014). Storm water runoff has been linked to higher Zn (23 times higher), Cd, Cr, Cu (6 times higher) and As and Pb (4 times higher) concentrations in sediments in commercial areas surrounding Tauranga City than those in a rural location in the harbour (McIntosh & Deely, 2001).

The Bay of Plenty coastal region is a north east facing area bounded by the Coromandel Peninsula at the northern bounds and East Cape to the east. Sediments range from fine/medium sand close to shore and become coarser further offshore (Kulgemeyer et al., 2016, 2017). The seafloor is broken up by a number of reefs and islands, many of which are of volcanic origin (Kulgemeyer et al., 2016, 2017). A major terrestrial sediment input is from the Kaituna River that discharges sediment originating from the Taupo volcanic zone (Kulgemeyer et al., 2017).

There are a variety of possible metal sources in the Bay of Plenty coastal region. The area is in some areas actively volcanic, with White Island and associated vents being the most active (Stoffers et al., 1999; Botz et al., 2002). Elemental Hg and As rich compounds have been found at vents associated with White island and the surrounding area (Stoffers et al., 1999). Another possible metal source is the grounding of the MV Rena in October 2011 on Astrolabe Reef (Otaiti) situated 25 km from Tauranga (Schiel et al., 2016). Following this event, intensive investigation was carried out into the potential impacts and although one of the wrecked containers from the ship contained 21 tonnes of granulated copper clove (Schiel et al., 2016), elevated levels of waterborne Cu were restricted to waters immediately surrounding the wreckage and not control areas nearby (Dempsey et al., 2016). Levels of waterborne Zn showed similar patterns (Dempsey et al., 2016). Metal concentrations in biota were raised in urchins and gastropods from the debris field when compared to control sites however this difference did not translate up the food chain into predatory fishes (Ross et al., 2016).

Porirua Harbour in the southern North Island of New Zealand is used in this study as a contrasting harbour system to Tauranga Harbour. It is relatively small, at only 14 km^2, yet in contrast to Tauranga Harbour, there is significantly less intertidal habitat, with 65% of the harbour considered subtidal. Porirua Harbour is shallow at around 3 m depth (Blaschke et al., 2010) and has been heavily impacted by intensive development and industry within the catchment, partially due to the low level of flushing (Francis, 2013). Sediment metal levels in Porirua Harbour are monitored regularly due to known issues of high metal concentrations around storm water inputs and recent readings of Zn, Cu, Pb, As, and Cr have all been higher than those measured in the Tauranga Harbour (Ellis et al., 2013, Oliver, 2016)

As a high proportion of heavy metal input is from anthropogenic sources, it was hypothesized that rays from estuaries and harbours would have higher concentrations of metals than those from coastal habitats. In addition, Tauranga Harbour rays would show lower levels of metal contamination than those from Porirua Harbour.

To test these hypotheses the following questions will be asked:

How do the metal levels of M. tenuicaudatus from Tauranga Harbour compare with those from the Bay of Plenty coastal region?

How do the metal levels of M. tenuicaudatus from Tauranga Harbour compare with those from Porirua Harbour?

Is there a difference between the metal levels of male and female rays from the same habitat?

Do the metal levels of M. tenuicaudatus and B. brevicaudata differ within the Tauranga Harbour and do they reflect a 'clean' or polluted habitat?

Finally, this chapter also aims to answer the question:

Is non-lethal muscle biopsy suitable for metal level determination in M. tenuicaudatus?

6.2. Methods

To ascertain whether rays within the Tauranga Harbour system had a higher heavy metal burden than those outside of the harbour system, tissue samples from a number of *M. tenuicaudatus* and *B. brevicaudata* were collected and analysed for metal levels. In addition, a small outgroup of rays from an area of typically higher pollution levels than Tauranga, Porirua Harbour (caught as part of another project, Cook-Auckram, 2019), were sampled for comparison.

6.2.1. Sample collection

Muscle and liver samples were taken from a variety of sources (Figure 6.1). Live *M. tenuicaudatus* and *B. brevicaudata* were captured and sampled within the Tauranga Harbour. In addition, samples were also taken from deceased animals either obtained through Fisheries New Zealand or from incidental finds. Finally, a small number of live individuals were sampled from Porirua Harbour, Wellington, as part of an MSc project at the Victoria University of Wellington (Cook-Auckram, 2019). All samples were placed on ice on collection and frozen at -18 °C as soon as possible until processing.

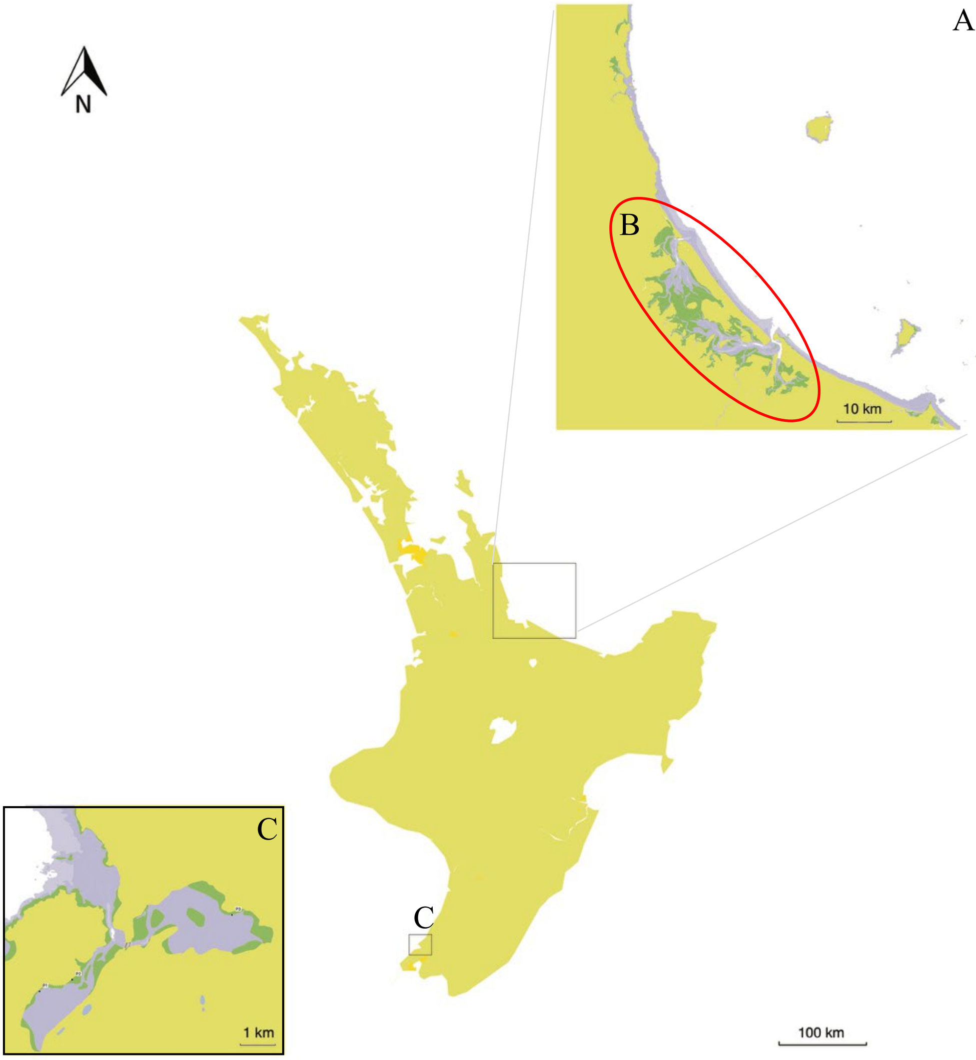

Figure 6.1. Locations of (A) Bay of Plenty coastal region, (B) Tauranga Harbour and (C) Porirua Harbour.

Live animal capture

Myliobatis tenuicaudatus were caught using a 40 m polycotton flounder net with 140 mm mesh in water between 1 metre and 1.5 metres off Fergusson Park, Tauranga Harbour (Figure 6.2). The net was set on the shore-ward side by an aluminium pole held by assistants, and the other was affixed to a small (~6m) boat at anchor. The net was deployed at around 2 hours post-high tide, as this is a time when this species has been seen to pass through reliably in large numbers (D. Culliford Pers. Comm).

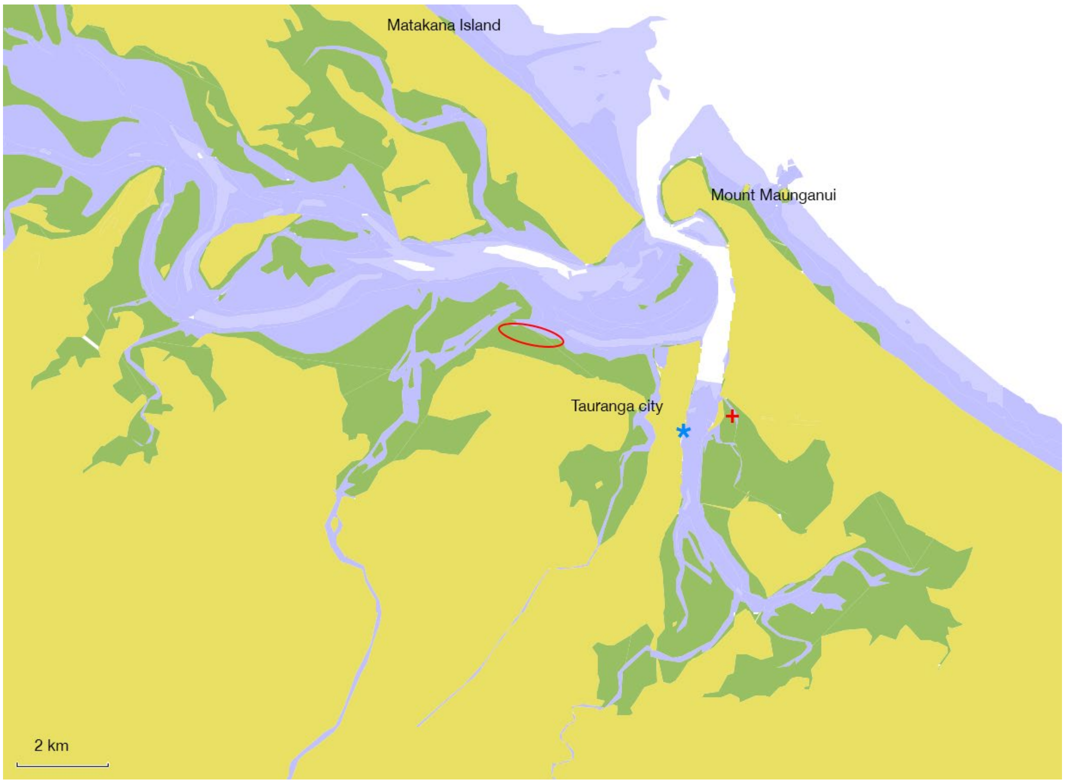

Figure 6.2. Southern basin of Tauranga Harbour showing locations of *Myliobatis tenuicaudatus* capture (red ellipse); incidental *M. tenuicaudatus* find (+); and cross road boat ramp, location of *B. brevicaudata* capture (*)

When an animal was caught in the net, its weight was supported, tail was controlled and was brought closer to the boat. A supporting board was placed under the body and the ray held still in the water. The ray was measured (disc width, body length to pelvic fin and tail length; Figure 6.3) maturity was categorised (maturity taken at

80 cm for females from Last and Stevens (2009) and clasper calcification state for males) , and biopsy samples were taken from the dorsal surface of the wing using biopsy probe (Figure 6.4) (Rob Allen Spearguns, Durban, South Africa). The ray was then untangled from the net before release. The whole process from capture to release was very quick, minimising stress to the animal. Time out of the water was under 2 minutes. All rays swam off strongly.

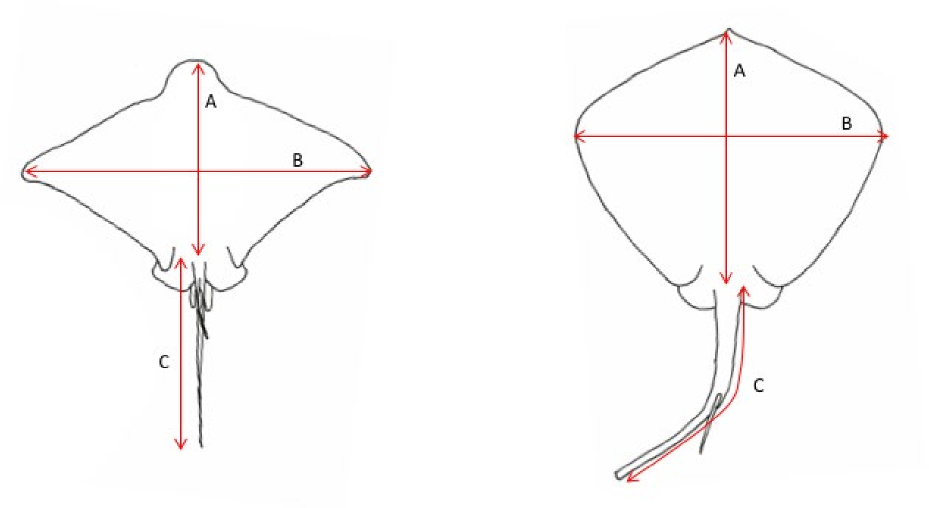

Figure 6.3. Measurements taken of live caught and dissected stingrays. A) Body Length, B) Disc Width, C) Tail Length.

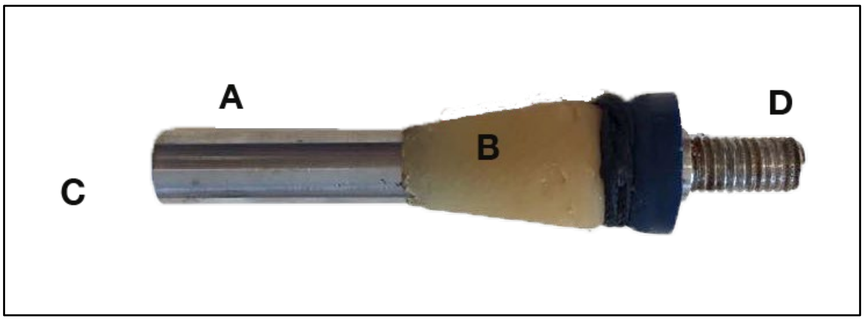

Figure 6.4. Biopsy probe used for taking muscle samples of live rays. A) Stainless steel barrel, B) Rubber stopper, C) Sharpened edge, D) Thread for attachment to spear for speargun use. Not shown are internal barbs to hold onto sample. Probe purchased from Rob Allen Spearguns, Durban, South Africa.

Bathytoshia brevicaudata

Bathytoshia brevicaudata is a very large and powerful animal. Individuals can reach over 2.3 m disc width and 350 kg (Last & Stevens, 2009; Last et al., 2016a). A new capture methodology was developed for such a large animal, avoiding the use of hooks and nets in order to minimise stress for the animal and maximise safety for participating personnel. This methodology allowed control of the tail and quick release when the work up was complete. All rays were caught at Cross Road boat ramp, Tauranga (Figure 6.2) as described in Chapter 2.

Deceased samples

Animals were retained as bycatch from the inshore trawl fisheries in the Bay of Plenty coastal area by observers from the Fisheries New Zealand Observer Programme on board the FV Margaret Phillipa and the FV Kaiti with permission from the skipper (Figure 6.1. (A)). Absolute tow locations were proprietary to fishermen, however are available confidentially on request. Animals were kept frozen onboard and sampled as soon as possible.

Measurements were recorded (Figure 6.3), as was sex, and clasper state (maturity) if male. Muscle samples were taken from the dorsal surface of the wing using a clean scalpel, and liver samples were taken similarly on dissection. Stomach contents were also retained. When a deceased animal was found in the Tauranga Harbour region, samples and measurements were taken when possible using the methodology detailed above.

Samples from Porirua were collected as part of a separate study identifying metal levels in benthic and demersal teleosts and elasmobranchs in Porirua Harbour (Cook-Auckram, 2019). Animals were caught by a monofilament flounder net with stretched mesh size of 118 mm, set at low tide, left to soak and collected after between 6 and 12 hours. Biopsies and measurements were taken following the protocol described above and animals were released immediately. Tissue from all animals was placed on ice in the field and subsequently frozen at -18 °C until further processing.

6.2.2. Analysis

Tissue preparation, digestion and metal analysis

Tissue samples were placed in pre-weighed Falcon tubes and weighed in order to determine sample wet weight. Samples less than 0.5 g in 15 ml tubes and samples 0.5 - 2 g in 50 ml tubes. If samples were greater than 2 g a subsample was taken. Samples were then freeze dried in order to standardise moisture content as longer-term frozen storage may have dehydrated some samples. After freeze drying, samples were re-weighed to determine tissue dry weight.

All of the following volumes of reagents are for the 50 ml Falcon tubes, with volumes for 15 ml tubes in brackets. In addition to tissue samples, four method blanks were prepared (two in 50 ml Falcon tubes and two in 15 ml Falcon Tubes) using the following technique with no tissue.

To each vial, 2 ml (1 ml) of tetramethylammonium hydroxide (TMAH) was added before mixing with a vortex mixer and placing in a 60 °C water bath for two hours, vortex mixing after one and two hours. Samples were then placed in a water-ice slurry to cool. When samples were cool, 0.5 ml of cold hydrogen peroxide was added, vortex mixed and placed in a cold room overnight to oxidise.

The following day, 2 ml (1 ml) of 65% nitric acid was added to each tube, vortex mixed, and placed in a water bath at 90 °C for two hours, again vortex mixing at one and two hours. After allowing tubes to cool to room temperature, samples were diluted to full volume (50 ml or 15 ml) with ultrapure water and mixed.

Samples were filtered through a 0.45 μm syringe filter and 1 ml of the filtered sample was added to a 15 ml pre-labelled Falcon tube with 8.8 ml ultrapure water, 0.1 ml hydrochloric acid and 0.1 ml nitric acid. In addition, three water blank tubes were prepared with 9.8 ml ultrapure water plus 0.1 ml hydrochloric acid and 0.1 ml nitric acid.

Following digestion and dilution, samples were analysed for heavy metal concentrations by Inductively-coupled plasma mass spectrometry (ICP-MS).

Statistical analysis

All statistical analyses in this chapter was performed in PRIMER v7 (PRIMER-e, Auckland, NZ) with PERMANOVA+ (Anderson et al., 2008). In order to test for differences in concentration of metals in *M. tenuicaudatus* muscle tissue among sampling areas (Tauranga Harbour vs Bay of Plenty Coastal vs Porirua Harbour), a permutational analysis of variance (PERMANOVA) using Euclidean similarity resemblance matrices and a covariate of ray disc width was performed (Anderson, 2001, 2014). In addition, a principal coordinate analysis (PCoA) was performed to produce a visual representation of any differences and to determine which metals drive the greatest variance in the data set as a whole (Anderson & Willis, 2003). Following this, univariate PERMANOVA was performed in order to determine whether individual metals showed differences between the three sites (Anderson, 2001). PERMANOVA, followed by PCoA using Euclidean similarity matrices were also used in order to test for differences between male and female body burden of metals using concentrations in *M. tenuicaudatus* liver tissue, to test for differences in metal concentrations between muscle and liver tissue and to test for differences in metal concentrations between muscle tissue of *M. tenuicaudatus* and *B. brevicaudata.*

6.3. Results

6.3.1. Animal samples

Thirteen *M. tenuicaudatus* were caught in the Bay of Plenty coastal fisheries, ten were caught in the Tauranga Harbour, three were found deceased in the Tauranga Harbour, and four were caught in Porirua Harbour. In addition, twelve *B. brevicaudata* were caught in the Tauranga Harbour and one in Porirua Harbour (Appendix E). Sex and maturity demographics were different in different areas; in Tauranga Harbour, only one male was caught, and one sub-adult female, all others caught were mature females. Similarly, all rays caught in Porirua Harbour were mature females. In the coastal Bay of Plenty 7 out of 13 caught were female, three of which were mature, and 5 out of the 6 males were mature (Appendix E).

6.3.2. Metal concentrations

Metal concentrations varied both between tissues in *M. tenuicaudatus*, and between species (Table 6.1). PERMANOVA found a significant difference in *M. tenuicaudatus* muscle metal concentrations among the three sampling areas, Tauranga Harbour, Bay of Plenty Coastal and Porirua Harbour, with the covariate of disc width being non-significant (Table 6.2). Pairwise analysis using the same PERMANOVA design showed a significant difference between all three sites

(Table 6.3). Principal coordinates analysis showed clear differentiation between all three sites with As, Hg, Cu and Cd driving variation in one direction towards the Bay of Plenty coastal samples and Pb, Zn and Cr driving variation towards Porirua Harbour samples (Figure 6.5).

Table 6.1. Mean metal concentrations mg kg^{-1} wet weight in two ray species from three locations. *M.t.*: *M. tenuicaudatus, B. b.*: *B. brevicaudata,* Ms.: Muscle, Lv.: Liver. Cr: chromium, Co: cobalt, Ni: nickel, Cu: copper, Zn: zinc, As: arsenic, Ag: silver, Cd: cadmium, Hg: mercury, Pb: lead. * only one sample

	Location						
	Tauranga Harbour			**Bay of Plenty Coastal**		**Porirua**	
	M. t.		*B. b.*	*M. t.*		*M. t.*	*B. b.**
	Ms.	Lv.	Ms.	Ms.	Lv.	Ms.	Ms.
Cr	0.29 ±0.1	0.03 ±0.02	0.51 ±0.13	0.02 ±0.01	0.02 ±0.005	0.44 ±0.13	0.18
Co	0.0018 ±0.0008	0.1 ±0.06	0.005 ±0.003	0.003 ±0.0003	0.08 ±0.019	0.003* ±0.003	0.04
Ni	0.15 ±0.04	0.09 ±0.06	17.45 ±11.5	0.11 ±0.04	0.08 ±0.023	0.17 ±0.02	0.16
Cu	0.38 ±0.07	1.67 ±0.42	0.5 ±0.11	0.75 ±0.71	2.51 ±0.38	0.15 ±0.04	0.67
Zn	6.27 ±0.33	15.38 ±1.3	8.97 ±0.61	5.6 ±0.49	17.59 ±1.51	15.44 ±0.27	4.01
As	7.78 ±1.44	18.29 ±7.92	9.89 ±0.82	22.73 ±5.43	30.67 ±3.16	2.76 ±0.19	0.7
Ag	0.01	0.03 ±0.002	0	0.001 ±0.001	0.291 ±0.11	0	0
Cd	0.002 ±0.0014	0.49 ±0.32	0	0.003 ±0.002	1.59 ±0.43	0	0
Hg	0.09 ±0.01	0.09 ±0.015	0.5 ±0.04	0.19 ±0.03	±0.56 ±0.21	0.09 ±0.01	0.19
Pb	0.013 ±0.004	0.01 ±0.004	0.06 ±0.017	0.002 ±0.001	0.003 ±0.002	0.095 ±0.029	0.2

Table 6.2. PERMANOVA results showing a significant difference (*) among the *M. tenuicaudatus* muscle tissue concentrations sampled at three sites Tauranga Harbour, Bay of Plenty Coastal and Porirua Harbour. Difference of covariate disc width non-significant.

	d.f.	***SS***	***MS***	**Pseudo-F**	***P(perm)***	**Perms**
Disc Width (Covariate)	1	13.65	13.65	1.75	0.082	9939
Location	2	82.03	41.01	5.32	<0.001*	9913
Residual	27	207.97	7.70			
Total	29	290				

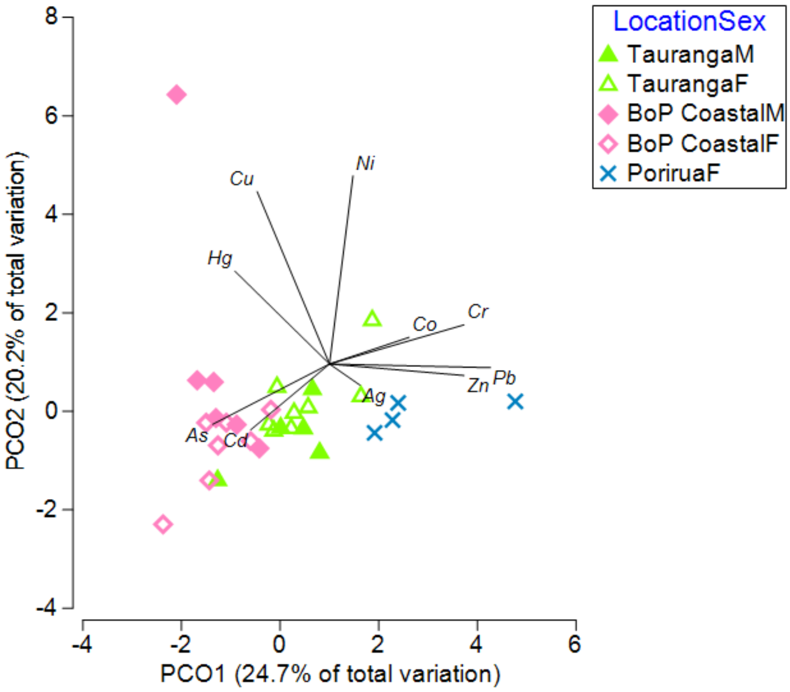

Figure 6.5. Principal coordinates analysis of Euclidian distance similarity matrix illustrating differences in *M. tenuicaudatus* muscle tissue metal concentrations among three different areas in New Zealand. Open shapes denote female, closed shapes denote male. All Porirua samples were female.

Table 6.3. Pairwise PERMANOVA results showing significant differences (*) between *M. tenuicaudatus* muscle metal concentrations among all sites investigated.

	t	***P(perm)***	**Perms**
Tauranga Harbour vs Bay of Plenty Coastal	1.95	0.002*	9940
Tauranga Harbour vs Porirua Harbour	2.73	<0.001*	9942
Porirua Harbour vs Bay of Plenty Coastal	2.38	<0.001*	9944

Pairwise univariate PERMANOVA analyses on the muscle metal concentrations of *M. tenuicaudatus,* revealed significantly higher Cr levels in the two harbours when compared to the coastal area, significantly higher Zn in Porirua Harbour than the other two areas, significantly higher As and Hg in the Bay of Plenty Coastal area when compared to Tauranga Harbour and significantly higher Pb in Porirua Harbour when compared with the other two areas (Figure 6.6).

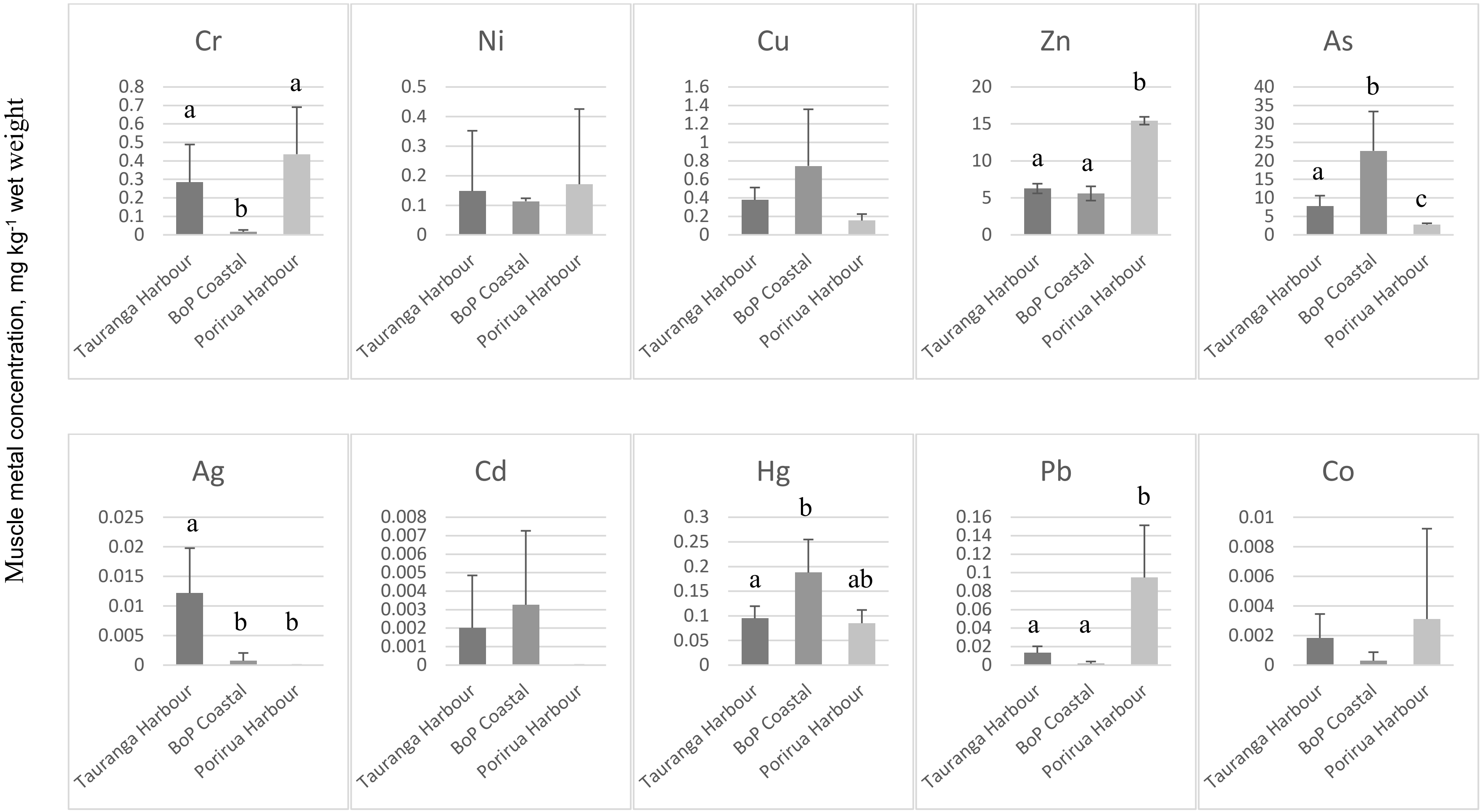

Figure 6.6. Concentrations of metals from *M. tenuicaudatus* muscle tissue from Tauranga Harbour, Bay of Plenty Coastal area and Porirua Harbour, New Zealand. Error bars show 95% confidence intervals. Different letters denote statistical differences. No letters denotes no significant differences.

Although there seems to be a difference between metal liver concentrations of males and females from the PCoA analysis (Figure 6.7) this difference is only significant when only the coastal caught individuals are included (Table 6.4). When individuals found deceased in Tauranga Harbour are included (2 females and 1 male) the difference is slightly non-significant (Table 6.5).

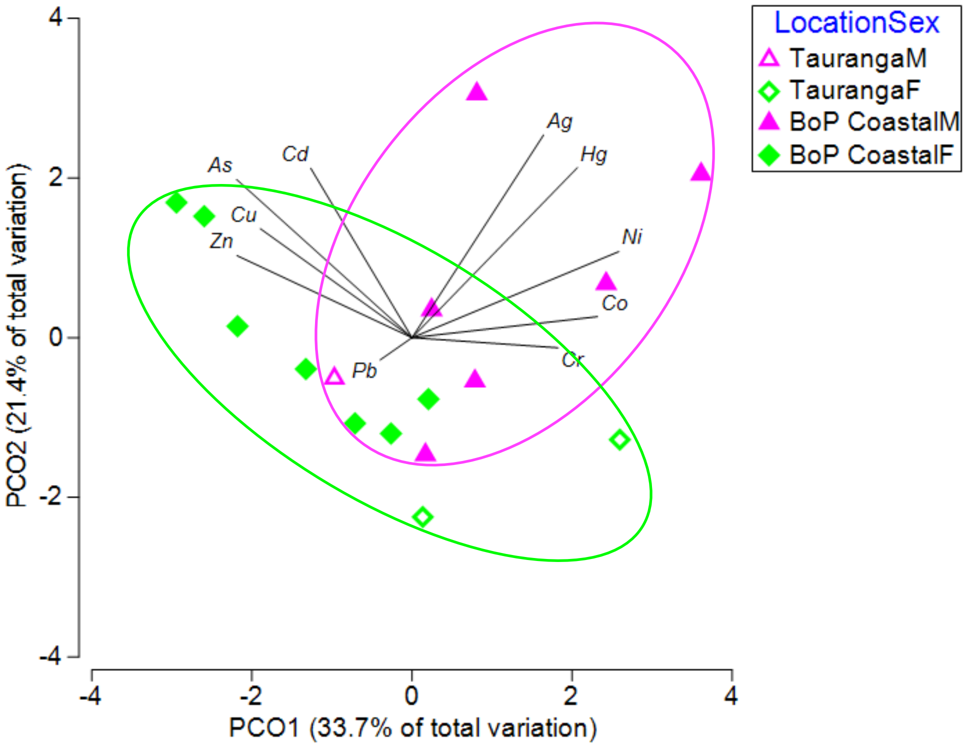

Figure 6.7. Principal coordinates analysis of Euclidian distance similarity matrix illustrating differences between male and female *M. tenuicaudatus* liver tissue metal concentrations. Open shapes denote samples from Tauranga Harbour, closed shapes denote samples from the Bay of Plenty Coastal area.

Table 6.4. PERMANOVA results showing significant difference between liver metal concentrations of male and female *M. tenuicaudatus* caught in the Bay of Plenty Coastal area.

	d.f.	***SS***	***MS***	**Pseudo-F**	***P(perm)***	**Perms**
Sex	2	82.03	41.01	5.32	<0.001*	9913
Residual	27	207.97	7.70			
Total	29	290				

Table 6.5. PERMANOVA results showing slightly non-significant difference between liver metal concentrations of male and female *M. tenuicaudatus* with three Tauranga Harbour deceased samples (1 male, 2 female) included.

	d.f.	***SS***	***MS***	**Pseudo-F**	***P(perm)***	**Perms**
Sex	1	17.78	17.78	5.321.88	0.069	6626
Residual	14	132.22	9.44			
Total	15	150				

PERMANOVA identified a significant difference between the metal concentrations in *M. tenuicaudatus* muscle and liver tissue (Table 6.6) and PCoA indicates that this is driven by higher values of most metals (Figure 6.7).

Table 6.6. PERMANOVA results showing a significant difference between metal concentrations in *M. tenuicaudatus* muscle and liver tissue.

	d.f.	***SS***	***MS***	**Pseudo-F**	***P(perm)***	**Perms**
Tissue	1	64.17	64.17	7.83	<0.001*	9933
Residual	30	245.83	8.19			
Total	31	310				

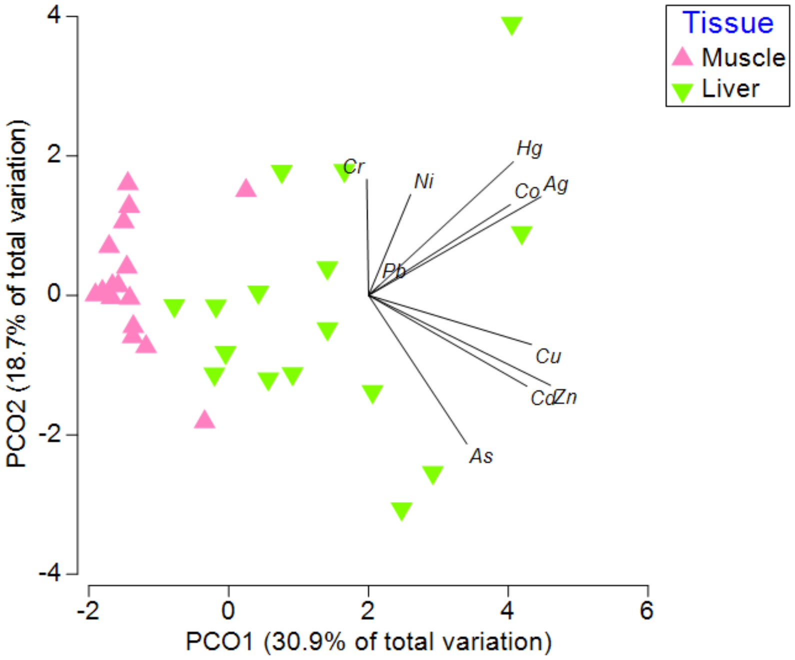

Figure 6.8. Principal coordinates analysis showing difference between metal concentrations in muscle and liver tissue of *M. tenuicaudatus* from the Tauranga Harbour and Bay of Plenty Coastal area.

PERMANOVA also found a difference among metal concentrations in muscle tissue of *M. tenuicaudatus, B. brevicaudata* from the Tauranga Harbour (Table 6.7). Principal coordinates analysis indicates that this difference seems to be driven by *B. brevicaudata* having higher concentrations of most metals, which is indeed the case (Table 6.1, Figure 6.9). Ag or Cd were not found in the *B. brevicaudata* muscle tissue.

Table 6.7. PERMANOVA results showing a significant difference among metal concentrations in muscle tissue of *M. tenuicaudatus* and *B. brevicaudata* in the Tauranga Harbour.

	d.f.	***SS***	***MS***	**Pseudo-F**	***P(perm)***	**Perms**
Species	1	59.07	59.07	8.04	<0.001*	9904
Residual	23	169.06	7.35			
Total	24	228.13				

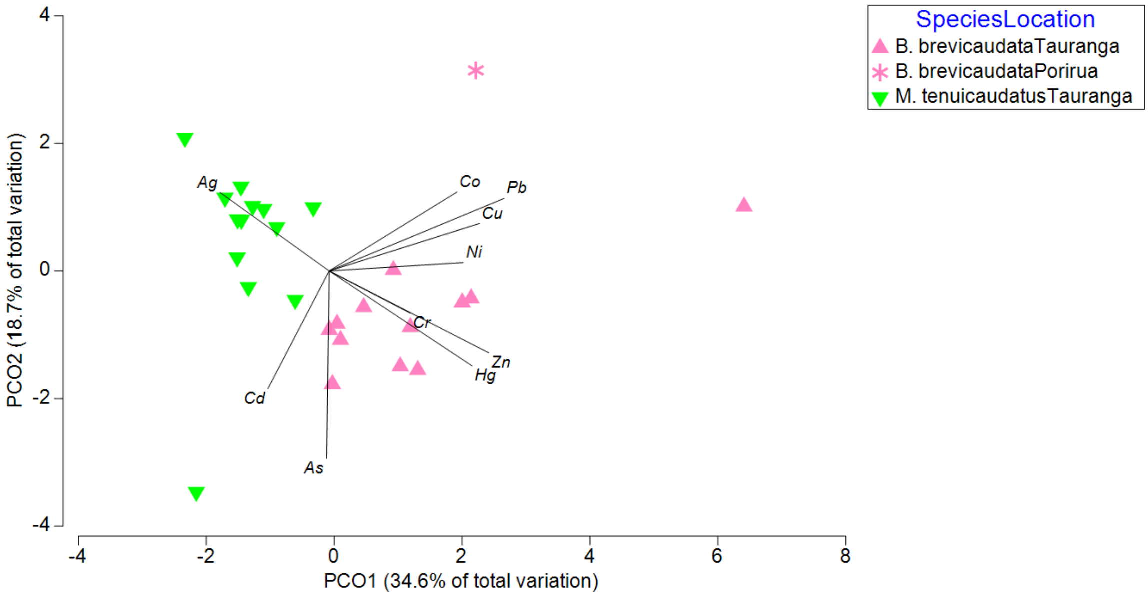

Figure 6.9. Principal coordinates analysis illustrating difference between muscle metal concentrations of *M. tenuicaudatus* and *B. brevicaudata* from the Tauranga Harbour. **B. brevicaudata* from Porirua Harbour was not included in PERMANOVA analysis due to differing location and sex.

In summary, the metal assemblages in *M. tenuicaudatus* muscle tissue varied significantly between locations. In addition, in offshore sampled *M. tenuicaudatus* liver tissue, there was a difference between males and females. As expected, there, was also a difference between concentrations in muscle and liver tissue. Finally, *Bathytoshia brevicaudata* individuals from Tauranga Harbour had higher muscle metal concentrations than *M. tenuicaudatus* individuals from the same location in all metals except Ag.

6.4. Discussion

Muscle metal concentration assemblages were quantified for *M. tenuicaudatus* from the Tauranga Harbour, Bay of Plenty coastal region, and Porirua Harbour and were found to be significantly different in all areas. This suggests that populations inhabiting these areas are somewhat separate. Principal coordinates analysis placed the samples from Porirua Harbour and the Bay of Plenty coastal region furthest apart, with Tauranga Harbour as an intermediate, suggesting that the rays inhabiting the two harbours are subject to similar pollutive pressures.

Arsenic muscle concentration was significantly higher in coastal Bay of Plenty rays than in either Harbour, and Hg was significantly higher in coastal Bay of Plenty than in Tauranga and higher but not significantly so than levels found in Porirua Harbour. This could be due to the coastal Bay of Plenty region being a highly volcanic area, with As and Hg both being found in vents around White island, locally to this region (Stoffers et al., 1999). Suggestion that the slightly higher (although not significantly so) levels of Cu in rays from the Bay of Plenty coastal area may be in part due to residual Cu from the grounding of the MV Rena in

October 2011 is unlikely as Cu contamination did not spread from the debris field in either water or biota (Dempsey et al., 2016; Ross et al., 2016).

In sedimentary analyses, levels of Zn, Cu, Pb, As and Cr were found to be higher in Porirua Harbour than in Tauranga (Ellis et al., 2013; Oliver, 2016). This difference is evident in *M. tenuicaudatus* muscle tissue in the case of Zn and Pb where significantly higher concentrations of these metals were found in the Porirua samples. In addition, a slightly higher concentration of Cr in the Porirua samples was not significant. In contrast to the sedimentary levels Cu was slightly although not significantly lower and As was significantly lower in Porirua Harbour. Chromium was higher in muscle tissues from both harbours than in the Bay of Plenty coastal region, which is to be expected as major sources of Cr in the environment are anthropogenic (Reid, 2011; Authman et al., 2015).

It would have been interesting to compare the Porirua Harbour samples with coastal caught rays from the local areas, to see whether the difference between Tauranga Harbour and Bay of Plenty coastal rays was echoed. Porirua Harbour is much smaller than Tauranga Harbour, with lower intertidal area, therefore rays present may not be resident, and may be transitory. This may lessen any difference between harbour and offshore samples in this area. However, animals caught were all mature females, similar to those caught in harbour and estuarine areas New Zealand wide (Le Port, 2003; Riding, 2009; Davis, 2010; Marcotte, 2014) so they may well have been harbour residents.

There was a significant difference between metal assemblages in liver tissue of *M. tenuicaudatus* males and females from the offshore samples. This could be attributed to a difference in physiology, with females passing certain metals to their young during gestation (Maternal transfer) (Mathews et al., 2008), or a difference in philopatry and movement habits between sexes as is known for *B. brevicaudata* (Roycroft et al., 2019). Female teleosts typically have higher levels of Zn and Cu than males due to differences in metabolic demands (Canli & Atli, 2003) and indeed the PCoA analysis in this study found that these metals were driving some of the difference between females and males.

There was a significant difference between the muscle metal assemblages of *M. tenuicaudatus* and *B. brevicaudata* in the Tauranga Harbour with significantly higher Ni, Hg and As levels in *B. brevicaudata*. These differences may be due to the larger body size of *B. brevicaudata* or a difference in trophic level position, as metal levels are known to increase with both factors (Taguchi et al., 1979; Dietz et al., 2000; Lyons et al., 2017). *Bathytoshia brevicaudata* is usually thought to have a similar diet to *M. tenuicaudatus* (Le Port et al., 2008) and thus should occupy a similar trophic position. However, the *B. brevicaudata* individuals sampled in this investigation were provisioned by commercial fishing operations in their location of capture with fish frames and scraps. This may mean that their dietary source of metals were from an offshore source rather than the harbour dwelling, intertidally feeding *M. tenuicaudatus* samples. If this was the case, then a higher level of As and Hg mirrors that of the Coastal Bay of Plenty *M. tenuicaudatus* samples. In addition, regular cleaning of the boats may have increased Cu levels in the immediate vicinity due to release of antifouling paint, however although a slightly higher level of Cu is present in *B. brevicaudata* muscle tissue, it is not significantly so.

Even though elasmobranchs have an affinity to Ag, only some of the Tauranga Harbour *M. tenuicaudatus* muscle tissue contained Ag and at low levels. None was found in muscle tissue in *B. brevicaudata*, offshore or in the Porirua samples. As Ag affinity is increased in lower salinity water (Webb & Wood, 2000), it may be expected that Ag levels would be higher in harbour samples, however liver tissue of the Bay of Plenty coastal samples had a much higher level of Ag than those from the Tauranga Harbour. In elasmobranchs, it has been found that liver has a higher affinity to Ag than muscle, although lower than that of the gills (Webb & Wood, 2000; De Boeck et al., 2010) which would explain the difference in the tissues.

A significant difference in metal assemblage between muscle and liver tissue was expected, due to the affinity of different metals to different tissues (Webb & Wood, 2000; Jeffree et al., 2006; Mathews et al., 2008; Jeffree et al., 2010). Cd and Co were very low in all muscle samples. This may be due to physiological reasons. Torres et al. (2016) found that Cd was predominantly found in liver tissue in *Raja clavata*. Two previous studies in the Mediterranean found several ray species with Cd present in muscle tissue, however it is likely the study area had a higher contamination level, particularly as it was stated that they were urbanised port areas (Türkmen et al., 2013, 2014). Therefore, muscle tissue in low contamination areas may not pick up all metals present. However, more studies need to be done in contaminated and low contamination areas in order to test this. A difference in the metals found between areas in this study may mean that for some circumstances muscle biopsy is a valid method, particularly as it is non-lethal. There has been some debate over the need for lethal sampling in elasmobranch science, especially when impacts on threatened species are concerned (Heupel & Simpfendorfer, 2010; Hammerschlag & Sulikowski, 2011) and a method of determining metal contamination without euthanising members of a threatened species would be ideal.

Where enough samples exist of both tissues, muscle-liver ratios of metals can be constructed, and therefore in future muscle biopsy could predict levels in liver tissue. This study however did not obtain enough samples for this to be calculated accurately. With future work to do this, muscle biopsy could be a simple and non-lethal method of determining the metal contamination level of this species.

Logistical difficulties throughout this study prevented offshore samples of *B. brevicaudata* from being collected. These samples would have enabled comparison of this larger species between harbour and coastal areas and allowed a comparison between species. The single *B. brevicaudata* sample from Porirua Harbour was not a good comparison with the Tauranga Harbour samples, not only because there was only one sample, but because the individual caught in Porirua was male. It has recently been discovered using genetic methods, that male *B. brevicaudata* are highly transitory, and provide much of the gene-flow between different areas, while female *B. brevicaudata* show a higher degree of philopatry (Roycroft et al., 2019). This may mean that the single male caught in Porirua Harbour had not been resident in the location for long, while the females caught in Tauranga had been present for a while (See Chapter 2).

This study confirms with elasmobranch subjects, that Tauranga Harbour is not particularly polluted with heavy metals. Muscle tissue of both *M. tenuicaudatus* and *B. brevicaudata* were below FSANZ recommended levels for elasmobranch tissue (FSANZ, 2015). This is good news for the New Zealand orca population, members of which regularly enter the Tauranga Harbour in order to feed on *M. tenuicaudatus* and particularly their livers (Visser, 1999; Duignan et al., 2000). In addition, recreational fishers in Tauranga Harbour need not worry about metal levels in muscle tissue of these species.

In summary, metal concentrations in *M. tenuicaudatus* showed a low level of metal contamination in Tauranga Harbour, supporting previous studies on sediments and infaunal species (Ellis et al., 2013; Huteau, 2017). Metal assemblage differences between Tauranga Harbour and Bay of Plenty coastal rays, suggests little mixing between these populations. Volcanic rather than anthropogenic sources are likely for higher As and Hg levels in offshore samples. There was a difference between sexes when liver tissue was compared, indicating sexual segregation and possible physiological differences. Differences between *M. tenuicaudatus* and *B. brevicaudata* indicate a possible impact of proximity to fishing vessel operations for the latter species, causing higher metal levels in this species. Finally, the non-lethal method of muscle biopsy was in general an easy and effective method of determining the levels of some metals studied, however not all, and for it to be used as the only method of metal determination, a muscle-liver ration would need to be calculated.

In conclusion, this study had limitations, with low sample sizes from all areas, but has shown interesting differences and patterns that indicate implications for both ray movement and the contamination levels of different areas.

CHAPTER SEVEN

GENERAL DISCUSSION

The research presented in this thesis aimed to increase our understanding of the spatial ecology, and specifically the foraging behaviour, of the batoid elasmobranchs *Myliobatis tenuicaudatus* present in a New Zealand harbour. It addresses a paucity of information pertaining to the trophic role of batoid elasmobranchs and how they may partition estuarine environments. In addition, research aimed to assess the risk to rays from some aspects of anthropogenic pressure arising from burgeoning development of coastal catchments and urbanisation of harbour and estuarine areas.

An initial review of the literature identified a number of areas for which there was little information, particularly in a New Zealand context. These areas included elements of the ecology of harbour-dwelling stingrays and also details pertaining to methodologies of examining the movement behaviours of non-shark-like rays. Major research questions identified were as follows: 1) Was there a specific methodology of tag attachment to non-shark-like batoid species that was minimally-invasive and that prevailed for a sufficient period so that the tag used delivered information of relevance? 2) Do *M. tenuicaudatus* follow a seasonal pattern in foraging intensity that may indicate periodic presence/absence in harbour habitats? 3) Does this species have preferences in foraging habitat? 4) Finally, is their presence in harbour and estuarine habitats putting them at risk of increased heavy metal contamination? These questions were formulated as hypotheses to be examined as follows:

H_1: *There will be a difference in efficacy of attachment methodologies, and that stainless steel 'shark' tags may not be the optimal choice for soft skinned species.*

H_2: *There will be a seasonal difference in M. tenuicaudatus feeding evidence.*

H_3: *Feeding evidence will be positively correlated with density of benthic prey items.*

H_4: *Feeding evidence will also be affected by site location.*

H_5: *There will be a difference in heavy metal body burdens between rays inhabiting Tauranga Harbour, and other Bay of Plenty coastal regions.*

This chapter therefore aims to consolidate the findings from the entire study and to place them in the context of current knowledge, both in a New Zealand and international setting. Relevance of findings will be discussed in the context of the threats that batoid elasmobranchs face in an urbanised coastal environment. Finally, recommendations will be made for management and for future research.

7.1. Overview of research contributions and associated implications

7.1.1. Tagging anchor methodologies

Chapter Two reviewed the use of tagging technologies and assessed the attachment methodologies for non-shark-like batoid elasmobranchs. Tagging in all its forms is an important tool for ecology research and conservation as it provides information that other methods simply cannot. For these methods to be effective however, the attachment or anchoring methodologies need to be fit for purpose for the species in question to ensure attachment for the correct (planned) period of time. While the use of tag technologies in elasmobranchs has been reviewed a number of times (Kohler & Turner, 2001; Hammerschlag et al., 2011; Heupel & Webber, 2012; Jepsen et al., 2015), rays are either not included or no specific reference is given to them, despite differences in body form from other elasmobranchs and skin physiology for many species,. No synthesis had been performed to-date on tag attachment efficacies specifically for batoid elasmobranchs with non-shark-like body forms.

This study revealed that for the group as a whole, there was little difference between the varying anchor materials and designs. However, as a large proportion of studies have been to-date performed on the Mobulidae with their very tough skin equipped with a denticular surface (Graham et al., 2012; Jaine et al., 2014; Peel et al., 2019), soft-skinned species are not well represented so this should be taken with caution. It was also revealed that while simple one-prong acrylic dart identification tags remain anchored for a long period of time when used on the large soft-skinned species *B. brevicaudata* in an aquarium setting, field trials were less successful due to the poor underwater visibility in Tauranga Harbour. Anecdotal experiences of other researchers found that sharp stainless-steel tags do not remain in place in this species (J. Pini-Fitzsimmons Pers. Comm.). A double-tagging aspect to the aquarium portion would have enhanced this investigation but was unfortunately prevented by logistical constraints. It is recommended therefore that for soft-skinned species, pectoral Petersen discs or traditional sharp stainless-steel/ titanium anchors be eschewed in favour of acrylic designs, with multi-pronged umbrella darts for external electronic tag attachment.

7.1.2 Seasonal ecology of M. tenuicaudatus

Chapter 3 confirmed the hypothesis that there would be a seasonal pattern in *M. tenuicaudatus* feeding evidence in the intertidal habitats studied. The New Zealand eagle ray (*M. tenuicaudatus*) was the first ray species for which the specifics of pit excavation were determined (Gregory & Ballance, 1979) and the oft-cited work by Hines et al. (1997) on *M. tenuicaudatus* suggested a seasonal pattern in feeding effort, as quantified by feeding pit density, however it was not quantified on a meaningful timeframe.

This thesis has extended the previous pattern to include winter presence in some areas, and months of peak intensity of feeding effort differing among sites. The latter may indicate sequential use, and although this cannot be proven by the survey method used in this study, would be an important phenomenon to consider during future studies.

Seasonal increase in feeding effort at most sites seemed to be positively related to harbour entrance water temperature. This could have been incidental; however, temperature is a common aspect of habitat use in a number of elasmobranch species (Hopkins & Cech, 2003; Schlaff et al., 2014). An increase in temperature could also cause an increase in metabolic rate requiring increased feeding to sustain (Di Santo & Bennett, 2011; Whitney et al., 2016) and thus result in the creation of more pits. Given this apparently temperature-mediated change in habitat use, warming seas as a result of climate change may have implications for the duration of feeding in sandflat areas, with knock-on effects on benthic infauna.

Myliobatis tenuicaudatus-mediated sediment turnover calculations by Thrush et al. (1991) determined that an area of sandflat of 800 m2 would be turned over at a rate of 1.4% per day, resulting in complete turnover every 70 days. Calculations using the maximum mean pit density observed during this study (Site 4, autumn 2018) determined that an area of sandflat of the same size would be turned over at a rate of 3.2 % per day, resulting in complete turnover every 31 days. Models of ecosystem functioning including sediment turnover, and sediment transport models should consider the seasonal and site difference in *M. tenuicaudatus* feeding activity in order to accurately predict its effect.

7.1.3. Habitat preference of M. tenuicaudatus

Both prey densities and locational factors were found to be correlated with the density *M. tenuicaudatus* feeding pits, in a different way among seasons. The initial hypothesis was that large infaunal bivalve density would be positively correlated with pit density, following work by (Hines et al., 1997; Le Port, 2003). However, in contrast to this initial hypothesis, density of large infaunal bivalves was only positively correlated with pit density during spring and autumn. Summer and winter observations found negative relationships with the bivalves *M. liliana* and *A. stutchburyi* respectively. This led to the alternate hypotheses that locational factors such as zone, where a feeding site is located within an embayment or the main harbour basin, or whether a feeding site is located in the northern or southern harbour basin, and the associated proximity to anthropogenic disturbance.

Zone was an important factor to predict pit density, with the preference for embayments suggesting an inclination to select feeding sites based on perceived predator risk. This is seen in other ray species (Vaudo & Heithaus, 2013) where areas of low prey density were favoured if there was a lower predator risk. Many embayments in Tauranga Harbour are shallow, and likely out of reach to he larger predators of M. tenuicaudatus such as the orca. Unfortunately, due to access constraints, the analysis was not balanced for this factor, and it is recommended that future balanced studies investigating the importance of habitat location to ray feeding be completed.

Harbour basin was also an important factor to predict pit density in all four seasons, with northern basin sites consistently showing lower pit densities than southern basin sites both in the model and in reality. This could reflect lower *M. tenuicaudatus* numbers in this area, however as large numbers of this species are regularly seen in this area the suggestion is that due to the lower proximity to the city of Tauranga, this area has less human disturbance, both directly and indirectly. This may result in a larger area of suitable undisturbed habitat, and consequently resulting in a lower density of feeding pits.

The infaunal sampling method used was not suitable for determining densities of other infaunal groups that may well have been important, such as worms and crustaceans, which are known to be present at sites low in bivalve density (Morrisey et al., 1999; Ellis et al., 2013) and are important in the diet of *M. tenuicaudatus* on the open coast (Hartill, 1989; Sommerville et al., 2011). Therefore, it would be prudent to include these in future work to obtain a full picture of infaunal species that are important to *M. tenuicaudatus* habitat choice.

7.1.4. The effect of mangrove management on *M. tenuicaudatus* feeding effort

Chapter 5 quantified the use of mangrove (*Avicennia marina*) fringe habitat by *M. tenuicaudatus* with an aim to confirm the use of this habitat by this species, and a secondary aim to determine whether the trimming of mangrove fringe has any impact on the magnitude of use. Mangrove habitat in New Zealand estuaries, in contrast to tropical areas, is increasing in area, and the pressure for Regional Councils in areas that this habitat occurs to manage this increase is in some areas great (Green et al., 2003; Morrisey et al., 2007; Harty, 2009; Lundquist et al., 2012, 2014; De Luca, 2015).

The presence of feeding pits in the vicinity of mangroves on multiple occasions over a relatively long period, shows that *M. tenuicaudatus* are using mangrove fringe areas for feeding. A consistently lower occurrence of feeding pits was observed in the trimmed mangrove fringe zone than an adjacent natural edge, indicating that the natural edge is preferred. This feeding evidence is not present in every natural mangrove fringe area and thus individual observations of sites should be considered prior to making decisions to remove or to trim fringe areas.

A BACI (Before/After, Control/Impact) design would have been preferable for this portion of the work, with the change in ray feeding pit abundance measured pre- and post-removal event. However, all the consented mangrove removals for Tauranga Harbour have already taken place meaning this approach was not possible. Therefore, the design employed here with adjacent natural and trimmed fringe zones with cross checking with inner-embayment locations was the best method available. Replication of the study at other locations within Tauranga Harbour was initially planned, but no suitable areas were found within the harbour.

Despite these limitations, this study provides clear evidence of the preference of natural mangrove fringe when compared to trimmed fringe.

7.1.5. Heavy metal contamination

Chapter 6 presented evidence that the hypothesis that there would be a difference between heavy metal body burdens between Tauranga Harbour, Bay of Plenty coastal region and Porirua Harbour was supported. This indicates a low level of population mixing between these three areas. This is expected for the east coast sites (Tauranga Harbour, Bay of Plenty coastal) when compared with the west coast site (Porirua Harbour), but is somewhat surprising between the two east coast sites. However, this difference was not entirely due to anthropogenic metal input into the harbour systems. As expected, levels of Zn and Pb in *M. tenuicaudatus* muscle tissue were significantly higher corresponding to higher sedimentary levels of these metals in Porirua Harbour (Oliver, 2016). Contrary to sedimentary levels however Cu and As were higher in Tauranga Harbour.

Arsenic and Hg levels were found to be higher in the coastal rays, and it is surmised that these metals have a volcanic source in this region rather than an anthropogenic source, due to both of these metals being found locally in volcanic vent systems (Stoffers et al., 1999). However, Cr was higher in both harbour systems than the coastal rays. This is expected due to the anthropogenic source of much environmental Cr (Reid, 2011; Authman et al., 2015). Unfortunately, there were no coastal ray samples from the region around Porirua. This would have given the study an interesting out-group to compare the volcanic Bay of Plenty region with a less volcanic coastal area. There were differences found between metal levels in liver tissue of male and female *M. tenuicaudatus*, a fact that supports sexual segregation as a strategy in this species, as has been suggested previously (Hartill, 1989).

It was established during this Chapter that while muscle biopsy did not pick up all metals present, as indicated by a difference in metal concentrations between muscle and liver tissue, it may still be a valid methodology to assess contamination in ray species. This is because it is low-impact and does not require lethal sampling. However, it should be used with caution.

7.1.6. Overall synthesis

The findings of Chapter 3 indicated there are seasonal patterns in the intensity of *M. tenuicaudatus* foraging in the intertidal habitats of Tauranga Harbour, with lower observed pit numbers during winter and spring, suggesting that there are fewer rays present during this period. However, during Chapter 6 it was found that *M. tenuicaudatus* sampled in Tauranga Harbour had different muscle metal signatures to those sampled in the coastal Bay of Plenty region. This was despite the fact that the majority of the coastal samples were caught during the period that it was previously thought that rays inhabiting Tauranga Harbour during the warmer months would be inhabiting these open coastal habitats. It is suggested here, that ‘harbour dwelling’ rays may remain in the harbour and use intertidal areas to a lesser extent, instead remaining in deeper channels. If migration out of the harbour during colder months does occur, it is suggested that it is not as far out as previously thought, and the populations may remain relatively separate.

The former theory of a change in use of the available habitat, such as sandflat to deeper channels, during different seasons is supported by the habits of *H. sabina*, which remain in creek environments year-round. However, they only utilise shallow habitats for diurnal periods during the colder months, and during both diurnal and nocturnal periods during the warmer months (Brinton & Curran, 2017). In addition, satellite tagging of two sub-adult *B. brevicaudata* found that long-distance migrations did not occur, and a change in depth use occurred instead, with both individuals spending time in deeper water during winter than during the summer months (Le Port et al., 2008).

Of course, the possibility remains that rays that had been inhabiting the harbour during the summer months were simply not caught or were in different locations to the tows that were part of this study. It is also unknown how long metals remain in tissues of this species, although it is known that they can assimilate waterborne metals within a few weeks (De Boeck et al., 2010). However, the fact that the difference remains when autumn-caught coastal rays were included in the analysis supports the statement. In addition, these autumn-caught coastal individuals were of the same size and sex class as the majority of harbour-caught individuals, and as elasmobranchs tend to segregate by sex and size class (Jacoby et al., 2012), if these individuals were part of the same population, it is likely that they would have been inhabiting a similar location.

In addition, the finding of Chapter 6 that Tauranga Harbour rays did not have high levels of anthropogenically sourced metals supports the work by previous studies in indicating that Tauranga Harbour is relatively clean (Ellis et al., 2013). This previous study and another by Huteau (2017) concluded that inner embayment areas were somewhat more contaminated than other areas in the Harbour. Low contamination levels of these rays may suggest that rays may not be using these inner embayment areas, an observation also suggested by Chapter 4. Whether rays are differentially using uncontaminated areas preferentially, or there are lower quality prey resources in these areas is unclear and requires further investigation.

The evidence that rays preferentially feed in natural mangrove fringe rather than trimmed areas (Chapter 5) may supplement the conclusion from Chapter 4 that an important factor in feeding site preference may be perceived predator risk. The trimmed mangrove edge does not offer any protection from predators if they should appear, whereas the natural edge contains small bushes and less dense trees that may provide some protection if necessary.

7.2. Associated outcomes of the study

This project has led to an increased public awareness of the rays that inhabit Tauranga Harbour and the effect that people may be having on them. A number of public engagement and science-communication events in conjunction with the website that was built, and multiple media articles, have assisted to this end. In addition, the New Zealand Bowhunters association have taken rays off their list of essential game-fish species towards their bow-fishing awards in direct response to contributions by this project and the salt-water fly-fishing community. As *M. tenuicaudatus* and *B. brevicaudata* are so far considered “least concern” by the IUCN (Duffy et al., 2016; Kyne, 2016) support from the community is essential in maintaining this status.

7.3. Future research and management considerations

It is recommended that a future investigation utilising tagging technology would augment the findings of this study. Towed-float GPS tagging would provide fine-scale knowledge of habitat use over the tidal cycle, while satellite tags, programmed to detach during the winter period would provide knowledge of the location of seasonal presence and confirm whether individuals are indeed remaining year-round. It was recommended previously that more tagging studies should be hypothesis driven, rather than 'see what happens' (Hammerschlag et al., 2011) and the current study has provided essential background information to make suitable hypotheses for this species. It is recommended from the review of methods in Chapter 2 that nylon umbrella anchors be used for any tagging programme on the New Zealand ray species.

It is also recommended that a dietary study be done on *M. tenuicaudatus* with both traditional and genetic study of stomach contents to determine which prey groups are important in a harbour/estuarine setting.

In addition, a further investigation into metal signatures, with samples taken not only from the harbour and from several miles out to sea, but immediately outside of the harbour during the colder months, including associated beach locations to determine if individuals found here have similar metal signatures to harbour, or coastal populations.

During the tagging experiment in the aquarium it was noted that one *B. brevicaudata* individual tagged too close to the distal edge of the disc exhibited extraordinary dexterity in attempting to remove the tag. This was previously unknown, and it is suggested that the dexterity of dasyatid ray discs be investigated in more depth.

Management considerations stemming from this research include the recommendation that the current management strategy of removing pneumatophores and seedlings by hand to mitigate mangrove spread may result in a lower quality foraging habitat for M. tenuicaudatus and alternative less-invasive management strategies be explored.

7.4. Conclusions

Coastal ray species are under consistent pressure from fishing, and habitat degradation (Dulvy et al. 2014). In New Zealand, increases in human coastal populations are resulting in increases in three of the key habitat degradation stressors: residential and commercial development, mangrove destruction, and pollution. While these stressors do not appear to have caused declines of local ray species to date, little was known of the rays' habits.

Observation of natural patterns of behaviour is thought to be as important as experimental manipulations (Underwood et al., 2000). The work described in this thesis has elucidated a number of key pieces of information that will inform future research and management strategies of New Zealand ray species. In addition, this observation of patterns of seasonal and spatial behaviour of these large mesopredators inhabiting estuarine systems will inform effective conservation and management of the ecosystems as a whole.

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APPENDICES

APPENDIX A

Table A.1. Studies utilising tagging methodology to study non-shark-like batoid elasmobranchs between 1984 and 2019. IUCN = IUCN red list classification: NT, Near threatened, VU, Vulnerable, EN, Endangered, DD, Data deficient, LC, Least concern, CR, Critically endangered.

#	Reference	Species	Family	Tag type	IUCN	Location (ocean basin)
1	Ajemian and Powers (2014)	*Aetobatus narinari* (spotted eagle ray)	Aetobatidae	Satellite (Fixed)	NT	US (Atlantic)
2	Ajemian and Powers (2014)	*Rhinoptera bonasus* (cownose ray)	Rhinopteridae	Satellite (Fixed)	NT	US (Atlantic)
3	Ajemian et al. (2012)	*Aetobatus narinari* (spotted eagle ray)	Aetobatidae	Acoustic (Passive)	NT	Bermuda (Atlantic)
4	Bassos-Hull et al. (2014)	*Aetobatus narinari* (spotted eagle ray)	Aetobatidae	ID/ PIT	NT	US (Atlantic)
5	Bell et al. (2016)	*Zearaja maugeana* (Maugean skate)	Rajidae	Acoustic (Passive)	EN	Australia (Indian)
6	Branco-Nunes et al. (2016)	*Hypanus americanus* (southern stingray)	Dasyatidae	Satellite (Pop-up)	DD	Brazil (Atlantic)
7	Braun et al. (2015)	*Mobula alfredi* (reef manta)	Mobulidae	Satellite (Pop-up)	VU	Saudi Arabia (Red Sea)
8	Brinton and Curran (2017)	*Hypanus sabinus* (Atlantic stingray)	Dasyatidae	Acoustic (Passive)	LC	US (Atlantic)
9	Campbell et al. (2012)	*Urogymnus dalyensis* (freshwater whipray)	Dasyatidae	Acoustic (Passive)	LC	Australia (Pacific)
10	Canese et al. (2011)	*Mobula mobular* (giant devil ray)	Mobulidae	Satellite (Pop-up)	EN	Italy (Mediterranean Sea)
11	Cartamil et al. (2003)	*Bathytoshia lata* (Hawaiian stingray)	Dasyatidae	Acoustic (Active)	LC	US (Pacific)
12	Cerutti-Pereyra et al. (2013)	*Himantura uarnak* (reticulate whipray)	Dasyatidae	Acoustic (Passive)	VU	Australia (Indian)
13	Cerutti-Pereyra et al. (2013)	*Pastinachus ater* (eastern cowtail stingray)	Dasyatidae	Acoustic (Passive)	LC	Australia (Indian)
14	Cerutti-Pereyra et al. (2013)	*Urogymnus asperrimus* (porcupine ray)	Dasyatidae	Acoustic (Passive)	VU	Australia (Indian)
15	Collins et al. (2007)	*Rhinoptera bonasus* (cownose ray)	Rhinopteridae	Acoustic (Passive)	NT	US (Atlantic)
16	Collins et al. (2008)	*Rhinoptera bonasus* (cownose ray)	Rhinopteridae	Acoustic (Passive)	NT	US (Atlantic)
17	Corcoran et al. (2013)	*Hypanus americanus* (southern stingray)	Dasyatidae	Acoustic (Passive)	DD	Cayman Islands (Atlantic)
18	Croll et al. (2012)	*Mobula mobular* (giant devil ray)	Mobulidae	Satellite (Pop-up)	NT	Mexico (Pacific)
19	Davy et al. (2015)	*Urogymnus granulatus* (mangrove whipray)	Dasyatidae	Acoustic (Active)	VU	Australia (Pacific)
20	Davy et al. (2015)	*Urogymnus granulatus* (mangrove whipray)	Dasyatidae	Acoustic (Passive)	VU	Australia (Pacific)
21	Ellis et al. (2011)	*Raja brachyura* (blonde ray)	Rajidae	ID/ PIT	NT	UK (Atlantic)
22	Ellis et al. (2011)	*Raja clavata* (thornback ray)	Rajidae	ID/ PIT	NT	UK (Atlantic)
23	Ellis et al. (2011)	*Raja microocellata* (small-eyed ray)	Rajidae	ID/ PIT	NT	UK (Atlantic)
24	Ellis et al. (2011)	*Raja undulata* (undulate ray)	Rajidae	ID/ PIT	EN	UK (Atlantic)
25	Farrugia et al. (2016)	*Beringraja binoculata* (Big skate)	Rajidae	Satellite (Pop-up)	LC	US (Pacific)
26	Francis and Jones (2017)	*Mobula mobular* (giant devil ray)	Mobulidae	Satellite (Pop-up)	NT	New Zealand (Pacific)

27	Frisk et al. (2019)	*Leucoraja ocellata* (Winter skate)	Rajidae	Acoustic (Passive)	EN	US (Atlantic)
28	Frisk et al. (2019)	*Leucoraja ocellata* (Winter skate)	Rajidae	ID/PIT	EN	US (Atlantic)
29	Gaspar et al. (2008)	*Pateobatis fai* (pink whipray)	Dasyatidae	Acoustic (Passive)	VU	French Polynesia (Pacific)
30	Graham et al. (2012)	*Mobula birostris* (giant manta)	Mobulidae	Satellite (Fixed)	VU	Mexico (Gulf of Mexico)
31	Hopkins and Cech (2003)	*Myliobatis californica* (bat ray)	Myliobatidae	ID/ PIT	LC	US (Pacific)
32	Hunter et al. (2005)	*Raja clavata* (thornback ray)	Rajidae	DST/GPS	NT	UK (Atlantic)
33	Jaine et al. (2014)	*Mobula alfredi* (reef manta)	Mobulidae	Satellite (Pop-up)	VU	Australia (Pacific)
34	Kessel et al. (2017)	*Mobula alfredi* (reef manta)	Mobulidae	Satellite (Fixed)	VU	Sudan (Red Sea)
35	King and McFarlane (2010)	*Beringraja binoculata* (big skate)	Rajidae	ID/ PIT	LC	Canada (Pacific)
36	Klimley et al. (2005)	*Myliobatis californica* (bat ray)	Myliobatidae	Acoustic (Active)	LC	US (Pacific)
37	Le Port et al. (2008)	*Bathytoshia brevicaudata* (short tail stingray)	Dasyatidae	Satellite (Pop-up)	LC	New Zealand (Pacific)
38	Marcotte (2014)	*Myliobatis tenuicaudatus* (New Zealand eagle ray)	Myliobatidae	Acoustic (Passive)	LC	New Zealand (Pacific)
39	Matern et al. (2000)	*Myliobatis californica* (bat ray)	Myliobatidae	Acoustic (Active)	LC	US (Pacific)
40	McCauley et al. (2014)	*Mobula alfredi* (reef manta)	Mobulidae	Acoustic (Active)	VU	Palmyra Atoll
41	McCauley et al. (2014)	*Mobula alfredi* (reef manta)	Mobulidae	Acoustic (Passive)	VU	(Pacific)
42	Neat et al. (2014)	*Dipturus intermedius*	Rajidae	DST/GPS	CR	UK, (Atlantic)
43	Neat et al. (2014)	*Dipturus intermedius*	Rajidae	ID/ PIT	CR	UK (Atlantic)
44	Ogburn et al. (2018)	*Rhinoptera bonasus* (cownose ray)	Rhinopteridae	Acoustic (Passive)	NT	US (Atlantic)
45	Omori and Fisher (2017)	*Rhinoptera bonasus* (cownose ray)	Rhinopteridae	Satellite (Pop-up)	NT	US (Atlantic)
46	Otaki et al. (2015)	*Hemitrygon akajei* (red stingray)	Dasyatidae	DST/GPS	NT	Japan (Pacific)
47	Peel et al. (2019)	*Mobula alfredi* (reef manta	Mobulidae	Acoustic (Passive)	VU	Seychelles (Indian)
48	Peklova et al. (2014)	*Amblyraja hyperborea* (arctic skate)	Rajidae	Satellite (Pop-up)	LC	Canada (Atlantic)
49	Pini-Fitzsimmons et al. (2018)	*Bathytoshia brevicaudata* (Short tail stingray)	Dasyatidae	ID/PIT	LC	Australia (Pacific)
50	Ramsden et al. (2017)	*Hypanus sabinus* (Atlantic stingray)	Dasyatidae	Acoustic (Passive)	LC	US (Atlantic)
51	Riding (2009)	*Myliobatis tenuicaudatus* (New Zealand eagle ray)	Myliobatidae	DST/GPS	LC	New Zealand (Pacific)
52	Rizzari et al. (2017)	*Bathytoshia brevicaudata* (Short tail stingray)	Dasyatidae	Acoustic (Passive)	LC	Australia (Indian)
53	Semeniuk and Rothley (2008)	*Hypanus americanus* (southern stingray)	Dasyatidae	ID/ PIT	DD	Grand Cayman (Atlantic)
54	Setyawan et al. (2018)	*Mobula alfredi* (reef manta)	Mobulidae	Acoustic (Passive)	VU	Indonesia (Pacific)
55	Simpfendorfer et al. (2008)	*Rhinoptera bonasus* (cownose ray)	Rhinopteridae	Acoustic (Passive)	NT	Palmyra Atoll (Pacific)
56	Speed et al. (2013)	*Pastinachus ater* (eastern cowtail stingray)	Dasyatidae	Acoustic (Active)	LC	US (Atlantic)
57	Speed et al. (2013)	*Urogymnus asperrimus* (porcupine ray)	Dasyatidae	Acoustic (Active)	VU	Australia (Indian)
58	Stewart et al. (2016a)	*Mobula birostris* (giant manta)	Mobulidae	Satellite (Pop-up)	VU	Mexico and Indonesia (Pacific)
59	Stewart et al. (2016a)	*Mobula birostris* (giant manta)	Mobulidae	Satellite (Fixed)	VU	Indonesia (Pacific)
60	Stewart et al. (2016b)	*Mobula birostris* (Giant Manta)	Mobulidae	Satellite (Pop-up)	VU	Mexico (Pacific)
61	Templeman (1984)	*Amblyraja radiata* (thorny skate)	Rajidae	ID/PIT	VU	Canada (Atlantic)

62	Tilley et al. (2013)	*Hypanus americanus* (southern stingray)	Dasyatidae	Acoustic (Active)	DD	Australia (Indian)
63	Treloar et al. (2017)	*Zearaja maugeana* (Maugean skate)	Rajidae	Acoustic (Active)	EN	Australia (Indian)
64	Treloar et al. (2017)	*Zearaja maugeana* (Maugean skate)	Rajidae	ID/PIT	EN	Australia (Indian)
65	Vaudo and Heithaus (2012)	*Pateobatis fai* (pink whipray)	Dasyatidae	Acoustic (Passive)	VU	Belize (Atlantic)
66	Vaudo and Heithaus (2012)	*Himantura uarnak* (reticulate whipray)	Dasyatidae	Acoustic (Passive)	VU	Australia (Indian)
67	Vaudo and Heithaus (2012)	*Pastinachus ater* (eastern cowtail stingray)	Dasyatidae	Acoustic (Passive)	LC	Australia (Indian)
68	Vaudo and Lowe (2006)	*Urobatis halleri* (round stingray)	Urotrygonidae	Acoustic (Active)	LC	Australia (Indian)
69	Vaudo and Lowe (2006)	*Urobatis halleri* (round stingray)	Urotrygonidae	Acoustic (Passive)	LC	US (Pacific)
70	Ward et al. (2019)	*Hypanus americanus* (southern stingray)	Dasyatidae	DST/GPS	DD	Bahamas (Atlantic)
71	Wearmouth and Sims (2008)	*Dipturus batis* (common skate)	Rajidae	DST/GPS	CE	UK (Atlantic)
72	Wearmouth and Sims (2008)	*Dipturus batis* (common skate)	Rajidae	Satellite (Pop-up)	CE	UK (Atlantic)

Table A.2. Satellite tagging studies of non-shark like batoid elasmobranchs. *Programmed date of each tag taken from publication. ** Maximum battery life of SPOT tags taken as 280 days from Ajemian and Powers (2014) as no other studies using this method stated maximum battery life. Although this technology can have batteries lasting well over 1 year, this conservative value was used to prevent under-estimation of attachment efficacy. All deployment length metrics not including tags that failed to report. (^J. Stewart Pers. Comm)

#	Reference	Species	Location	Tag Type	Attachment	Sample Size	Tag Failure (Did not report)	Deployment length (days)		Proportion of programmed deployment* or maximum battery life** achieved	
								Min.	Max	$\bar{x}$	SE
1	Ajemian and Powers (2014)	*Rhinoptera bonasus* (cownose ray)	US (Atlantic)	Fixed	Spiracular Bridle	3	0	27	133	0.26	0.11
2	Ajemian and Powers (2014)	*Rhinoptera bonasus* (cownose ray)	US (Atlantic)	Fixed	Dart Bridle	9	0	1	155	0.20	0.08
3	Ajemian and Powers (2014)	*Rhinoptera bonasus* (cownose ray)	US (Atlantic)	Fixed	Tail Suture	3	0	1	7	0.01	0.01
4	Ajemian and Powers (2014)	*Aetobatus narinari* (spotted eagle ray)	Bermuda (Atlantic)	Fixed	Wing Disc Bridle	5	0	2	349	0.46	0.25
5	Ajemian and Powers (2014)	*Aetobatus narinari* (spotted eagle ray)	Bermuda (Atlantic)	Fixed	Dart Bridle	4	0	2	35	0.07	0.02
6	Braun et al. (2015)	*Mobula alfredi* (reef manta)	Saudi Arabia (Red Sea)	Pop-Up	Stainless Steel Dart	9	2	102	188	0.93	0.04
7	Branco-Nunes et al. (2016)	*Hypanus americanus* (Southern stingray)	Brazil (Atlantic)	Pop-Up	Stainless Steel Dart	2	0	8	60	0.57	0.43
8	Canese et al. (2011)	*Mobula mobular* (giant devil ray)	Italy (Mediterranean Sea)	Pop-Up	Nylon Game-fish Anchor	3	0	60	120	1	-
9	Croll et al. (2012)	*Mobula mobular* (giant devil ray)	Mexico (Pacific)	Pop-Up	Umbrella Dart	13	0	14	188	0.46	0.08
10	Farrugia et al. (2016)	*Beringraja binoculata* (big skate)	United States (Pacific)	Pop-Up	Titanium Dart	8	2	90	354	0.79	0.11
11	Francis and Jones (2017)	*Mobula mobular* (giant devil ray)	New Zealand (Pacific)	Pop-Up	Umbrella Dart	9	2 (+4 deceased)	30	82	0.79	0.21
12	Graham et al. (2012)	*Mobula birostris* (giant manta)	Mexico (Gulf of Mexico)	Fixed	Umbrella Dart	6	0	2	64	0.10	0.03
13	Jaine et al. (2014)	*Mobula alfredi* (reef manta)	Australia (Pacific)	Pop-Up	Umbrella Dart	10	2	0	120	0.83	0.09

14	Kessel et al. (2017)	*Mobula alfredi* (reef manta)	Sudan (Red Sea)	Fixed	Dorsal	3	0	32	366	0.43	0.29
15	Le Port et al. (2008)	*Bathytoshia brevicaudata* (short tail stingray)	New Zealand (Pacific)	Pop-Up	Tail Suture	2	0	62	151	1	-
16	Peklova et al. (2014)	Amblyraja hyperborea (arctic skate)	Canada (Atlantic)	Pop-Up	Peterson Disc	9	2	0	70	0.60	0.17
17	Omori and Fisher (2017)	*Rhinoptera bonasus* (cownose ray)	United States (Pacific)	Pop-Up	Tail Suture	20	0	5	147	0.44	0.09
18	Stewart et al. (2016a)	*Mobula birostris* (giant manta)	Mexico and Indonesia (Pacific)	Pop-Up	Titanium Dart	21	3	0	193	0.83	0.08
19	Stewart et al. (2016a))	*Mobula birostris* (giant manta)	Indonesia (Pacific)	Fixed	Titanium Dart	1	0	64	64	*Unknown*	-
20	Stewart et al. (2016b)	*Mobula birostris* (giant manta)	Indonesia (Pacific)	Fixed	Titanium Dart^	6	0	11	189	0.71	0.18
21	Wearmouth and Sims (2008)	*Dipturus batis* (common skate)	United Kingdom (Atlantic)	Pop-Up	Peterson Disc	1	0	41	41	0.46	-

Table A.3. Studies using acoustic telemetry to study non-shark-like batoid elasmobranchs.

#	Reference	Species	Family	Location	Tracking Method	Attachment	Sample Size	Range test mentioned	Track length (days)		
									Min.	Max	$\bar{x}$ (SE)
1	Ajemian et al. (2012)	*Aetobatus narinari*	Aetobatidae	Bermuda (Atlantic)	Passive	Stainless steel Dart	18	N/A	0	67	
2	Campbell et al. (2012)	*Himantura dalyensis*	Dasyatidae	Australia (Pacific)	Passive	Internal Implant	6	Y	128	440	
3	Cartamil et al. (2003)	*Bathytoshia lata*	Dasyatidae	United States (Pacific)	Active	Petersen Disc	7	N/A	1.29	3	
4	Cerutti-Pereyra et al. (2013)	*Pastinachus atrus*	Dasyatidae	Australia (Indian)	Passive	Internal Implant	6	Y	83	448	
5	Cerutti-Pereyra et al. (2013)	*Urogymnus asperrimus*	Dasyatidae	Australia (Indian)	Passive	Internal Implant	4	Y	162	439	
6	Cerutti-Pereyra et al. (2013)	*Himantura uarnak*	Dasyatidae	Australia (Indian)	Passive	Internal Implant	1	Y	1	179	
7	Collins et al. (2007)	*Rhinoptera bonasus*	Rhinopteridae	United States (Atlantic)	Passive	Spiracle	21	Y	156	457	
8	Collins et al. (2008)	*Rhinoptera bonasus*	Rhinopteridae	United States (Atlantic)	Passive	Spiracle	12	Y	12	234	90
9	Corcoran et al. (2013)	*Hypanus americanus*	Dasyatidae	Cayman Islands (Atlantic)	Passive	Internal Implant	5	Y	1	389	
10	Davy et al. (2015)	*Himantura granulata*	Dasyatidae	Australia (Pacific)	Passive	Internal Implant	15	Y	6	204	
11	Davy et al. (2015)	*Himantura granulata*	Dasyatidae	Australia (Pacific)	Active	Spiracle	4	N/A	7h:25m	7h:55m	
12	Frisk et al. (2019)	*Leucoraja ocellate*	Rajidae	United States (Atlantic)	Passive	Disc + Internal Implant	20 + 38	N/A	3.42	1,122	199
13	Gaspar et al. (2008)	*Himantura fai*	Dasyatidae	French Polynesia (Pacific)	Passive	Internal Implant	14	Y	0	340	
14	Klimley et al. (2005)	*Myliobatis californica*	Myliobatidae	United States (Pacific)	Active	Stainless steel Dart	4	N/A	1.17	3.14	
15	Marcotte (2014)	*Myliobatis tenuicaudatus*	Myliobatidae	New Zealand (Pacific)	Passive	Petersen Disc	11	Y	0.8	16.8	
16	Matern et al. (2000)	*Myliobatis californica*	Myliobatidae	United States (Pacific)	Active	Internal Implant	11	N/A	30	395	
17	McCauley et al. (2014)	*Mobula alfredi*	Mobulidae	Palmyra Atoll (Pacific)	Passive	***None Mentioned***	18	N			

18	McCauley et al. (2014)	*Mobula alfredi*	Mobulidae	Palmyra Atoll (Pacific)	Active	***None Mentioned***	18	N/A	0.7h	2	
19	Ogburn et al. (2018)	*Rhinoptera bonasus*	Rhinopteridae	United States (Atlantic)	Passive	Internal Implant	36	N/A	12	116	
20	Setyawan et al. (2018)	*Mobula alfredi*	Mobulidae	Indonesia (Pacific)	Passive	Titanium Dart	39	Y	1	188	42 (7)
21	Simpfendorfer et al. (2008)	*Rhinoptera bonasus*	Rhinopteridae	United States (Atlantic)	Passive	Spiracle	10	Y	7	42	
22	Speed et al. (2013)	*Pastinachus atrus*	Dasyatidae	Australia (Indian)	Active	Stainless Steel Dart	2	N/A	0.3	0.4	
23	Speed et al. (2013)	*Urogymnus asperrimus*	Dasyatidae	Australia (Indian)	Active	Stainless Steel Dart	1	N/A	1	2	
24	Ramsden et al. (2017)	*Hypanus sabinus*	Dasyatidae	United States (Atlantic)	Passive	Internal Implant	40	Y	279	820	
25	Rizzari et al. (2017)	*Bathytoshia brevicaudata*	Dasyatidae	Australia (Pacific)	Passive	Nylon Umbrella Dart	10	Y	1	76	20.1 (9.5)
26	Tilley et al. (2013)	*Hypanus americanus*	Dasyatidae	Belize (Atlantic)	Active	Tail suture + Dart	12	N/A	22h	32h	
27	Treloar et al. (2017)	*Zearaja maugeana* (Maugean skate)	Rajidae	Australia (Indian)	Passive	Internal Implant	2	N/A	1	2	
28	Vaudo and Heithaus (2012)	*Himantura uarnak*	Dasyatidae	Australia (Indian)	Passive	Stainless Steel Dart	11	Y	17	295	
29	Vaudo and Heithaus (2012)	*Himantura fai*	Dasyatidae	Australia (Indian)	Passive	Stainless Steel Dart	5	Y	7	112	34.8
30	Vaudo and Heithaus (2012)	*Pastinachus atrus*	Dasyatidae	Australia (Indian)	Passive	Stainless Steel Dart	9	Y	4	189	32.9
31	Vaudo and Lowe (2006)	*Urobatis halleri*	Urotrygonidae	United States (Pacific)	Passive	Petersen Disc	25	N	-	635	
32	Vaudo and Lowe (2006)	*Urobatis halleri*	Urotrygonidae	United States (Pacific)	Active	Petersen Disc	10	N/A	5h	72h	

Table A.4. Identification and PIT tagging studies of non-shark like batoid elasmobranchs

#	Reference	Species	Family	Location	Tag Type	Sample Size	Return	Days at liberty	
								Min	Max
1	Bassos-Hull et al. (2014)	*Aetobatus narinari*	Aetobatidae	United States (Atlantic)	PIT	393	19	5	1293
2	Ellis et al. (2011)	*Raja brachyura*	Rajidae	United Kingdom (Atlantic)	Petersen disc	184	41	12	522
3	Ellis et al. (2011)	*Raja microocellata*	Rajidae	United Kingdom (Atlantic)	Petersen disc	521	85	1	754
4	Ellis et al. (2011)	*Raja clavata*	Rajidae	United Kingdom (Atlantic)	Petersen disc	7	1	192	192
5	Ellis et al. (2011)	*Raja undulata*	Rajidae	United Kingdom (Atlantic)	Petersen disc	101	11	9	138
6	Frisk et al. (2019)	*Leucoraja ocellata*	Rajidae	United States (Atlantic)	Acrylic Dart	3,416	51	6	1167
7	Hopkins and Cech (2003)	*Myliobatis californica*	Myliobatidae	United States (Pacific)	Spaghetti (Spiracular)	257	3	106	583
8	King and McFarlane (2010)	*(Beringraja binoculata*	Rajidae	Canada (Pacific)	Dart tag (Acrylic & Stainless steel)	18,180	1238	52	2205
9	Neat et al. (2014)	*Dipturus. intermedius*	Rajidae	United Kingdom (Atlantic)	Acrylic dart	280	74	-	-
10	Pini-Fitzsimmons et al. (2018)	*Bathytoshia brevicaudata*	Dasyatidae	Australia (Pacific)	Stainless-steel dart	-	-	-	-
11	Semeniuk and Rothley (2008)	*Hypanus americanus*	Dasyatidae	Grand Cayman (Atlantic)	PIT	172	-	-	-
12	Templeman (1984)	*Amblyraja radiata*	Rajidae	Canada (Atlantic)	Petersen disc	722	102	73	7300
13	Treloar et al. (2017)	*Zearaja maugeana*	Rajidae	Australia (Indian)	PIT	82	1	-	1

APPENDIX B

OPTIMISATION OF METHODOLOGY FOR RAY PIT QUANTIFICATION OVER LARGE AREAS

In a previous study utilising large scale quantification of ray pit presence in the Manukau Harbour, New Zealand, Hines et al. (1997) used 15 m radius circular quadrats in order to quantify ray pit density. In order to determine the most effective method to quantify ray feeding effort for the current investigation, an optimisation of methods exercise was carried out comparing the efficiency, accuracy and precision of this method with alternatives. Efficiency was determined by the time taken to complete necessary observations, as observations are tidally constrained. It was anticipated that the method would need to be accurate over areas with both high and low pit densities.

Firstly, an area measuring 1500 m^2 (50 m by 30 m) with a large number of ray pits present (Site A) was marked out with measuring tapes and a map of all ray pits within the marked area was created by walking 2-metre-wide transects (Figure B.1). The total number of pits was used to calculate the true density of pits in the measured area and subsequently the density of pits per 100 square metres to allow for methodology comparison (Table B.1.).

Linear transects and quadrats of varying sizes and shapes were overlaid onto the map in random locations and directions, using a numbered grid and the RANDBETWEEN function on Excel (Version 15.24 for Mac, Microsoft Inc.), in order to determine the accuracy and precision of each method and the number of replicates needed before the true density was represented. Small quadrats proved ineffective, possibly due to the patchy occurrences of pits. The two most accurate methods trialled were 30 m x 4 m transects (Figure B.2.A) and 15 m radius circular quadrats (Hines et al., 1997) (Figure B.2.B). The transects however were less accurate, reaching a plateau above the true density (Figure B. 2.A).

To determine whether these methods were as effective in an area with a low number of pits an area (Site B) was mapped, this time using a handheld GPS (Garmin GPSmap 60CSx). Due to the low number of pits a much larger area of 150 metres by 90 metres was mapped to ensure sufficient pits for accurate calculations. The total number of pits was counted and the true density of pits per 100 square metres was calculated as for Site A above (Table B.1).

Table B.1. The area, dimensions and number of pits counted for the optimisation of methodology exercise

Location	**Dimensions**	**Area (m^2)**	**Total pits**	**Pit density /100 m^2**
Area A	50 x 30	1500	109	7.27
Area B	150 x 90	13500	19	0.14

At this site, the two most effective sampling methods at site A, 30 x 4 metre transects, and 15 metre radius circular quadrats were carried out in situ at random locations and directions within the marked area (Figure B.3. A and B respectively). Probably due to the low number of pits, the transects consistently overestimated the density of pits within the area (Figure B.3.A) whereas the circular quadrats were much more accurate and reached accuracy with fewer replicates (Figure B.3.B). In addition, the quadrats took less time to complete. For these reasons, in addition to ease of comparison to the previous study by Hines et al. (1997) this method of 15 metre radius circular quadrats was chosen for the study, with a replicate number of 6.

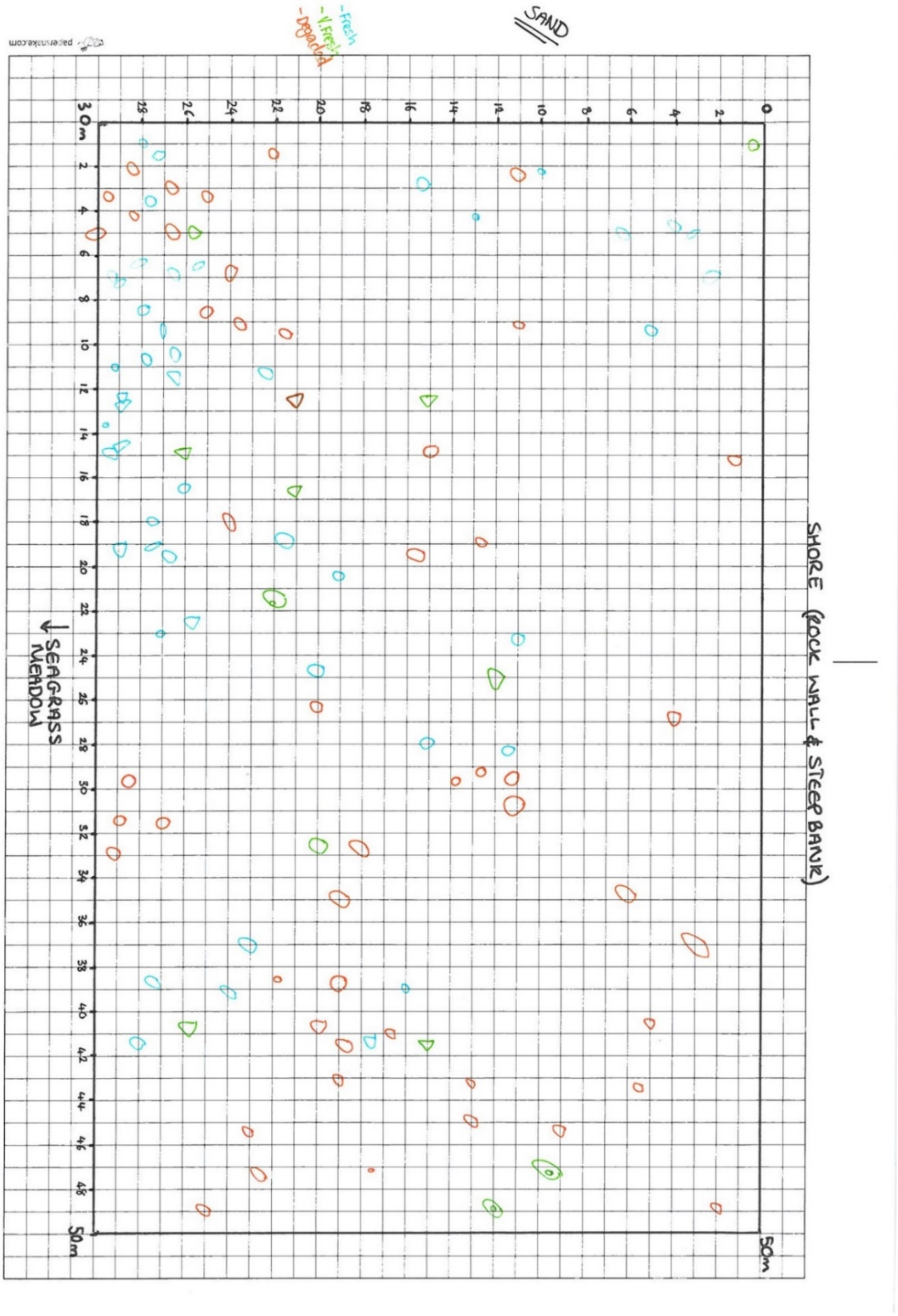


Figure B.1. Map of ray feeding pits created at Site A for method optimisation exercise

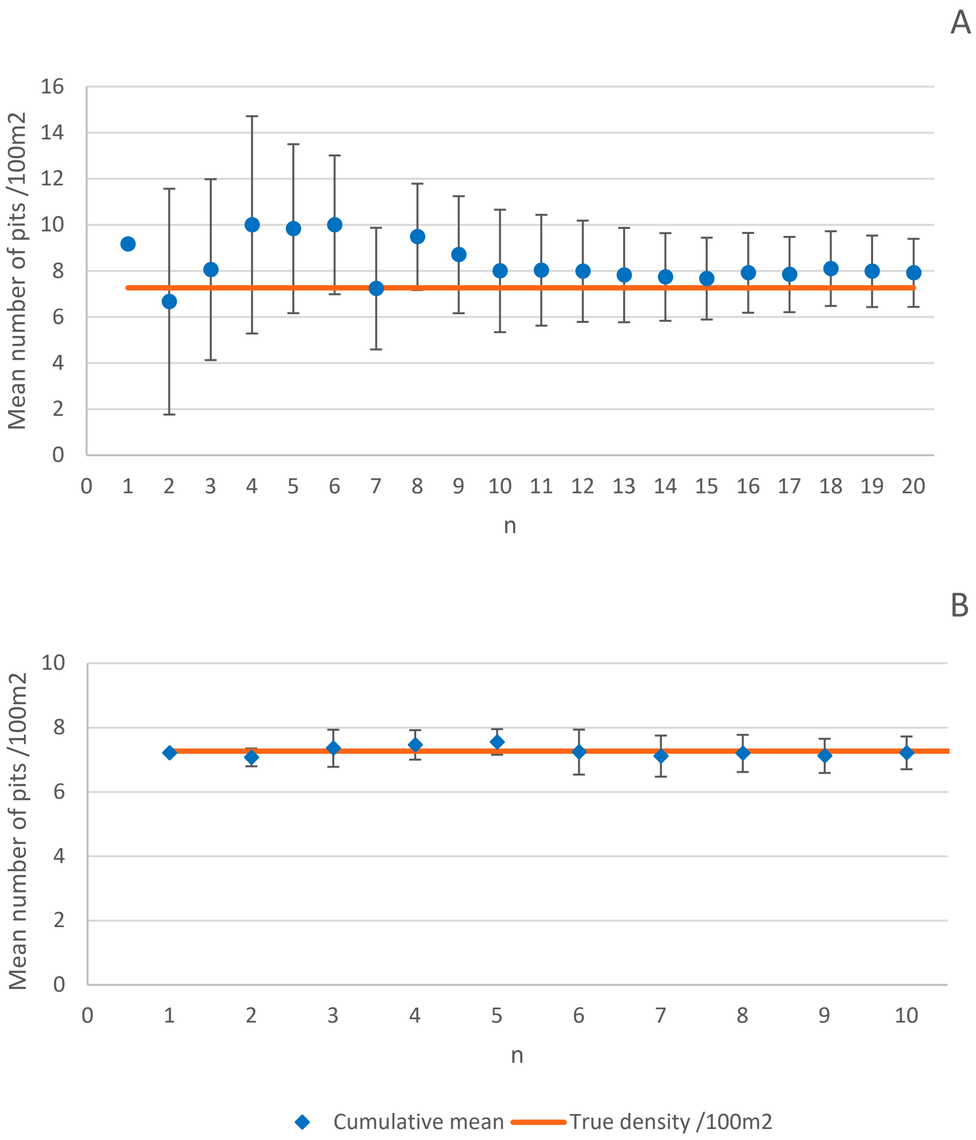
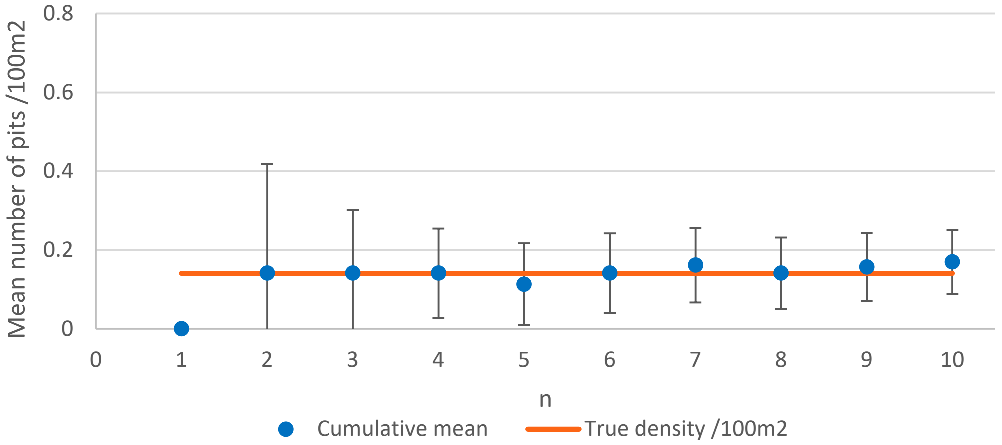


Figure B.2. The cumulative mean number of pits per 100 square metres and the number of replicates (n) of A: 30 metre x 4 metre transects at Site A, B: 15 metre radius circular quadrat at Site A. Error Bars show 95% confidence intervals. Solid line shows true density of ray pits at this site on the day sampled (7.27 per 100m^2).



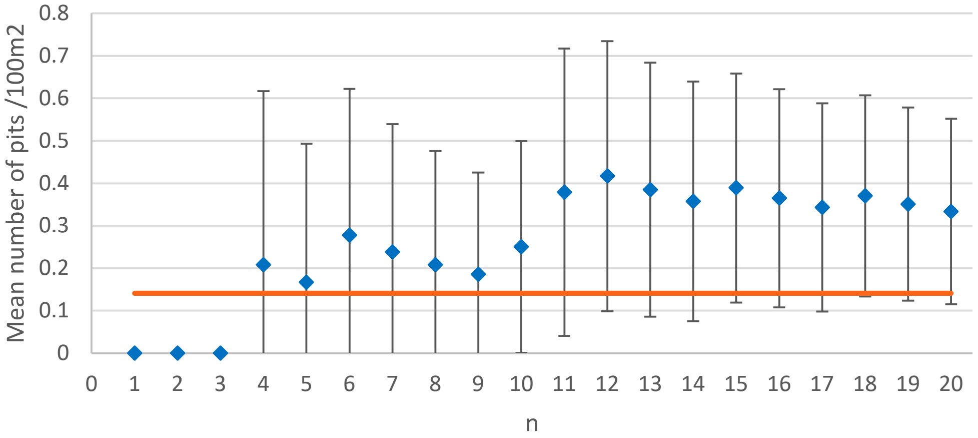


Figure B.3. The cumulative mean number of pits per 100 square metres and the number of replicates (n) of A: 30 metre x 4 metre transects at Site B, B: 15 metre radius circular quadrat at Site B. Error Bars show 95% confidence intervals. Solid line shows true density of ray pits at this site on the day sampled (0.14 per 100m^2).

APPENDIX C

Sampling dates, minimum, maximum and mean water temperature over 6 days (previous 5 days and sampling date) and total and mean rainfall over 6 days (previous 5 days and sampling date) for all sites over the 24-month sampling period between August 2016 and July 2018. No temperature data was available for August and September 2016.

			Water Temp °C		
Month	Site	Date	Min.	Max.	Mean
August 2016	1	15/08/16			
	2	15/08/16			
	3	16/08/16			
	4	16/08/16			
	5	17/08/16			
	6	14/08/16			
	7	14/08/16			
	8	14/08/16			
	9	19/08/16			
	10	19/08/16			
September 2016	1	12/09/16			
	2	12/09/16			
	3	13/09/16			
	4	13/09/16			
	5	16/09/16			
	6	14/09/16			
	7	14/09/16			
	8	15/09/16			
	9	15/09/16			
	10	15/09/16			
October 2016	1	13/10/16	14.49	16.38	15.16
	2	10/10/16	14.49	16.53	15.14
	3	13/10/16	14.49	16.38	15.16
	4	11/10/16	14.49	16.00	15.11
	5	14/10/16	14.49	16.38	15.19
	6	15/10/16	14.49	16.38	15.20
	7	15/10/16	14.49	16.38	15.20
	8	16/10/16	14.59	16.38	15.21
	9	16/10/16	14.59	16.38	15.21
	10	16/10/16	14.59	16.38	15.21
November 2016	1	12/11/16	15.20	17.56	16.02
	2	12/11/16	15.20	17.56	16.02
	3	13/11/16	15.20	17.56	16.00
	4	13/11/16	15.20	17.56	16.00
	5	11/11/16	15.20	17.56	15.92
	6	15/11/16	15.16	17.56	15.94
	7	15/11/16	15.16	17.56	15.94
	8	16/11/16	14.96	17.56	15.89
	9	16/11/16	14.96	17.56	15.89
	10	17/11/16	14.94	17.19	15.75

December 2016	1	12/12/16	15.64	20.38	17.91
	2	12/12/16	15.64	20.38	17.91
	3	15/12/16	15.13	20.06	16.56
	4	15/12/16	15.13	20.06	16.56
	5	11/12/16	15.55	20.38	18.11
	6	14/12/16	15.13	20.10	16.86
	7	14/12/16	15.13	20.10	16.86
	8	14/12/16	15.13	20.10	16.86
	9	16/12/16	14.92	19.40	16.19
	10	16/12/16	14.92	19.40	16.19
January 2017	1	13/01/17	16.09	19.20	17.29
	2	9/01/17	16.09	18.98	17.22
	3	15/01/17	15.87	19.20	17.40
	4	14/01/17	15.87	19.20	17.30
	5	16/01/17	15.87	19.64	17.55
	6	11/01/17	16.09	18.74	17.14
	7	11/01/17	16.09	18.74	17.14
	8	18/01/17	15.87	19.64	17.56
	9	18/01/17	15.87	19.64	17.56
	10	18/01/17	15.87	19.64	17.56
February 2017	1	13/02/17	17.35	20.87	19.24
	2	13/02/17	17.35	20.87	19.24
	3	15/02/17	18.57	20.88	19.56
	4	15/02/17	18.57	20.88	19.56
	5	14/02/17	18.38	20.87	19.45
	6	11/02/17	16.94	20.73	18.86
	7	11/02/17	16.94	20.73	18.86
	8	12/02/17	17.35	20.73	19.11
	9	12/02/17	17.35	20.73	19.11
	10	12/02/17	17.35	20.73	19.11
March 2017	1	22/03/17	19.98	21.24	20.48
	2	22/03/17	19.98	21.24	20.48
	3	21/03/17	19.61	21.24	20.42
	4	27/03/17	20.23	21.26	20.59
	5	27/03/17	20.23	21.26	20.59
	6	25/03/17	20.01	21.12	20.49
	7	25/03/17	20.01	21.12	20.49
	8	25/03/17	20.01	21.12	20.49
	9	23/03/17	20.01	21.24	20.50
	10	23/03/17	20.01	21.24	20.50
April 2017	1	7/04/17	19.26	22.07	20.53
	2	27/04/17	18.36	19.90	19.13
	3	26/04/17	18.36	20.09	19.17
	4	26/04/17	18.36	20.09	19.17
	5	25/04/17	18.36	20.09	19.25
	6	8/04/17	19.26	22.07	20.44
	7	8/04/17	19.26	22.07	20.44
	8	9/04/17	19.26	21.47	20.26
	9	28/04/17	18.36	19.90	19.14
	10	10/04/17	19.26	20.83	20.04

May 2017	1	21/05/17	15.01	17.60	16.80
	2	25/05/17	14.73	17.55	16.37
	3	26/05/17	14.73	17.55	16.29
	4	26/05/17	14.73	17.55	16.29
	5	24/05/17	14.73	17.60	16.47
	6	22/05/17	14.84	17.60	16.71
	7	22/05/17	14.84	17.60	16.71
	8	23/05/17	14.73	17.60	16.59
	9	28/05/17	17.73	16.74	16.23
	10	23/05/17	14.73	17.60	16.59
June 2017	1	21/06/17	14.72	16.13	15.65
	2	21/06/17	14.72	16.13	15.65
	3	25/06/17	14.64	6.03	15.48
	4	25/06/17	14.64	16.03	15.48
	5	26/06/17	14.64	16.05	15.47
	6	24/06/17	14.64	6.03	15.53
	7	24/06/17	14.64	6.03	15.53
	8	24/06/17	14.64	6.03	15.53
	9	22/06/17	14.64	16.08	15.58
	10	24/06/17	14.64	6.03	15.53
July 2017	1	19/07/17	12.91	15.36	14.27
	2	21/07/17	12.99	14.81	14.34
	3	23/07/17	13.39	15.12	14.40
	4	23/07/17	13.39	15.12	14.40
	5	21/07/17	12.99	14.81	14.34
	6	24/07/17	13.44	15.34	14.46
	7	24/07/17	13.44	15.34	14.46
	8	25/07/17	13.44	15.34	14.51
	9	25/07/17	13.44	15.34	14.51
	10	24/07/17	13.44	15.34	14.46
August 2017	1	17/08/17	13.82	15.02	14.58
	2	17/08/17	13.82	15.02	14.58
	3	19/08/17	13.93	15.02	14.66
	4	19/08/17	13.93	15.02	14.66
	5	19/08/17	13.93	15.02	14.66
	6	20/08/17	13.93	14.91	14.63
	7	20/08/17	13.93	14.91	14.63
	8	20/08/17	13.93	14.91	14.63
	9	20/08/17	13.93	14.91	14.63
	10	20/08/17	13.93	14.91	14.63
September 2017	1	23/09/07	14.00	15.25	14.44
	2	23/09/71	14.00	15.25	14.44
	3	19/09/17	14.00	15.59	14.66
	4	19/09/17	14.00	15.59	14.66
	5	23/09/17	14.00	15.25	14.44
	6	22/09/17	14.00	15.06	14.44
	7	22/09/17	14.00	15.06	14.44
	8	24/09/17	14.00	15.25	14.49
	9	22/09/17	14.00	15.06	14.44
	10	22/09/17	14.00	15.06	14.44

October 2017	1	18/10/17	14.70	16.51	15.22
	2	18/10/17	14.70	16.51	15.22
	3	16/10/17	14.80	16.53	15.37
	4	16/10/17	14.80	16.53	15.37
	5	16/10/17	14.80	16.53	15.37
	6	15/10/17	15.01	16.53	15.44
	7	15/10/17	15.01	16.53	15.44
	8	17/10/17	14.78	16.51	15.28
	9	17/10/17	14.78	16.51	15.28
	10	17/10/17	14.78	16.51	15.28
November 2017	1	15/11/17	15.18	17.48	15.97
	2	15/11/17	15.18	17.48	15.97
	3	20/11/17	16.03	18.40	17.04
	4	20/11/17	16.03	18.40	17.04
	5	19/11/17	15.97	18.40	16.91
	6	18/11/17	15.59	18.40	16.70
	7	18/11/17	15.59	18.40	16.70
	8	14/11/17	15.18	17.22	15.85
	9	14/11/17	15.18	17.22	15.85
	10	14/11/17	15.18	17.22	15.85
December 2017	1	17/12/17	16.76	21.16	19.02
	2	17/12/17	16.76	21.16	19.02
	3	19/12/17	16.76	21.40	19.44
	4	19/12/17	16.76	21.40	19.44
	5	20/12/17	17.49	21.40	19.61
	6	18/12/17	16.76	21.07	19.14
	7	18/12/17	16.76	21.07	19.14
	8	18/12/17	16.76	21.07	19.14
	9	16/12/17	16.76	21.58	19.06
	10	16/12/17	16.76	21.58	19.06
January 2018	1	16/01/18	19.32	23.06	21.45
	2	16/01/18	19.32	23.06	21.45
	3	15/01/18	19.32	23.06	21.26
	4	15/01/18	19.32	23.06	21.26
	5	15/01/18	19.32	23.06	21.26
	6	21/01/18	21.23	23.94	22.17
	7	21/01/18	21.23	23.94	22.17
	8	21/01/18	21.23	23.94	22.17
	9	21/01/18	21.23	23.94	22.17
	10	21/01/18	21.23	23.94	22.17
February 2018	1	13/02/18	21.49	22.84	22.13
	2	13/02/18	21.49	22.84	22.13
	3	12/02/18	21.49	23.03	22.12
	4	12/02/18	21.49	23.03	22.12
	5	16/02/18	21.61	23.24	22.40
	6	14/02/18	21.49	22.84	21.18
	7	14/02/18	21.49	22.84	21.18
	8	14/02/18	21.49	22.84	21.18
	9	15/02/18	21.51	23.06	22.28
	10	15/02/18	21.51	23.06	22.28

March 2018	1	14/03/18	20.45	23.24	21.92
	2	14/03/18	20.45	23.24	21.92
	3	19/03/18	20.29	21.65	21.00
	4	19/03/18	20.29	21.65	21.00
	5	14/03/18	20.45	23.24	21.92
	6	15/03/18	20.45	22.87	21.65
	7	15/03/18	20.45	22.87	21.65
	8	15/03/18	20.45	22.87	21.65
	9	16/03/18	20.45	22.41	21.40
	10	16/03/18	20.45	22.41	21.40
April 2018	1	17/04/18	17.38	19.26	18.48
	2	18/04/18	17.38	18.99	18.49
	3	18/04/18	17.38	18.99	18.49
	4	18/04/18	17.38	18.99	18.49
	5	17/04/18	17.38	19.26	18.48
	6	15/04/18	17.38	20.63	18.83
	7	15/04/18	17.38	20.63	18.83
	8	15/04/18	17.38	20.63	18.83
	9	16/04/18	17.38	20.32	18.59
	10	16/04/18	17.38	20.32	18.59
May 2018	1	12/05/18	17.04	18.56	17.93
	2	12/05/18	17.04	18.56	17.93
	3	11/05/18	17.04	18.56	17.86
	4	11/05/18	17.04	18.56	17.86
	5	15/05/18	17.45	18.56	17.98
	6	14/05/18	17.29	18.56	17.99
	7	14/05/18	17.29	18.56	17.99
	8	14/05/18	17.29	18.56	17.99
	9	13/05/18	17.08	18.56	17.96
	10	13/05/18	17.08	18.56	17.96
June 2018	1	15/06/18	13.82	15.93	15.02
	2	15/06/18	13.82	15.93	15.02
	3	13/06/18	13.82	15.68	14.92
	4	13/06/18	13.82	15.68	14.92
	5	16/06/18	13.82	15.93	15.12
	6	14/06/18	13.82	15.68	14.94
	7	14/06/18	13.82	15.68	14.94
	8	14/06/18	13.82	15.68	14.94
	9	17/06/18	13.87	15.93	15.25
	10	17/06/18	13.87	15.93	15.25
July 2018	1	10/07/18	12.75	14.20	13.67
	2	10/07/18	12.75	14.20	13.67
	3	12/07/18	12.53	14.74	13.64
	4	12/07/18	12.53	14.74	13.64
	5	12/07/18	12.53	14.74	13.64
	6	14/07/18	12.53	14.74	13.63
	7	14/07/18	12.53	14.74	13.63
	8	14/07/18	12.53	14.74	13.63
	9	13/07/18	12.53	14.74	13.66
	10	13/07/18	12.53	14.74	13.66

APPENDIX D

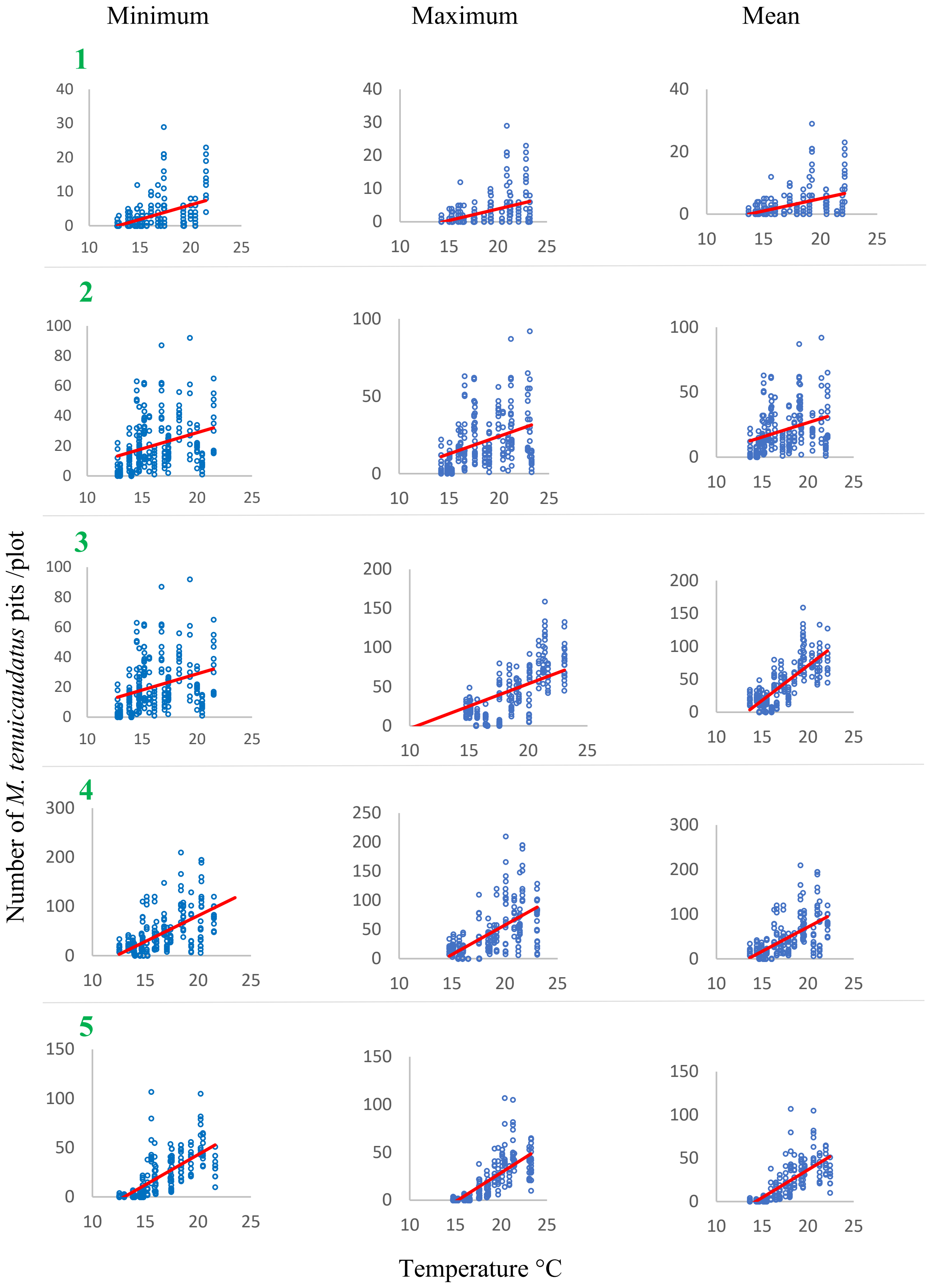


Figure D.1. Plots showing regressions of number of *M. tenuicaudatus* pits /plot compared to water temperature. Solid red lines indicate significant relationship by linear regression.

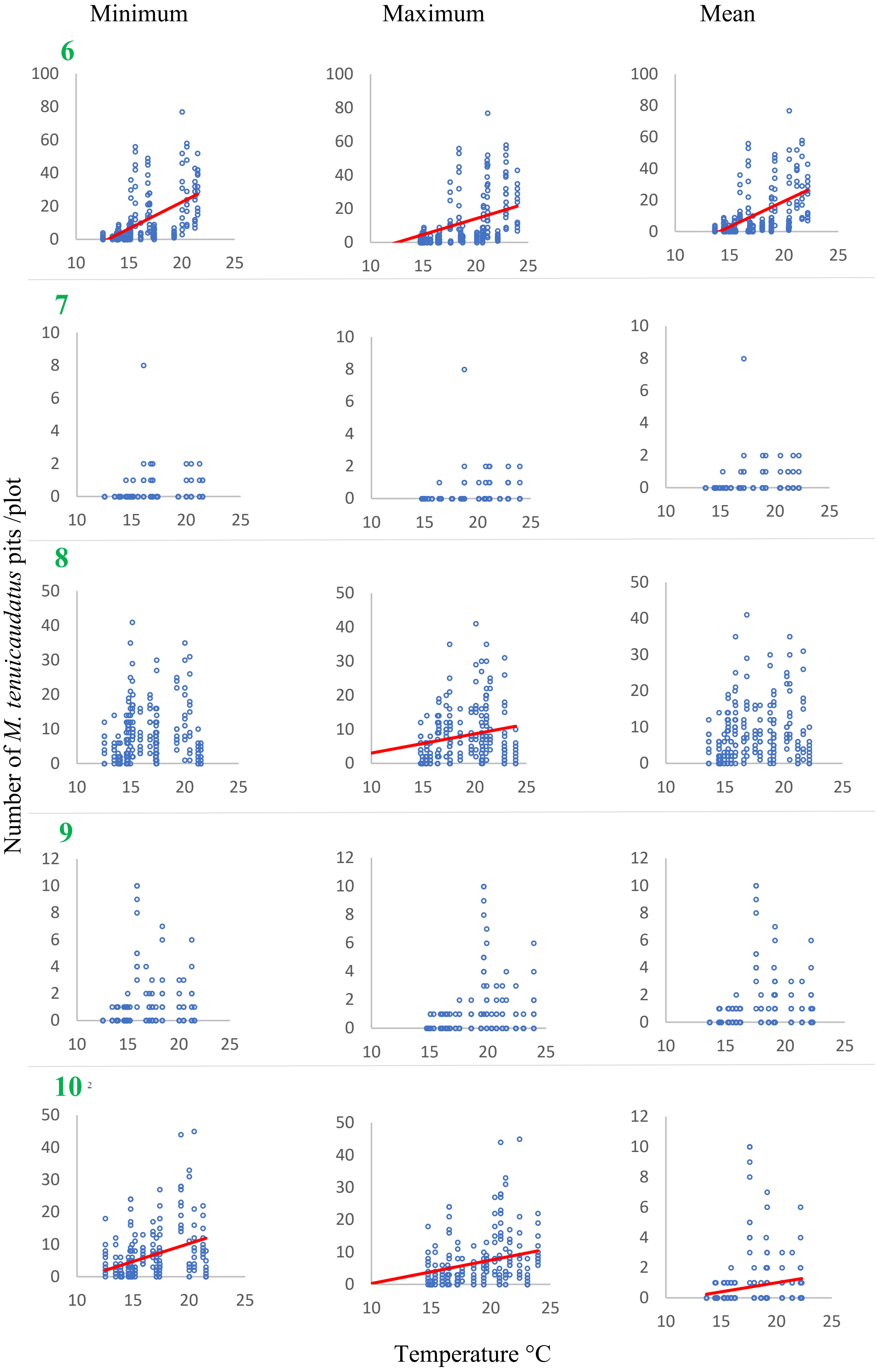


Figure D. Plots showing regressions of number of *M. tenuicaudatus* pits /plot compared to water temperature. Solid red lines indicate significant relationship by linear regression.

APPENDIX E

Table D.1. Rays caught or found and sampled for heavy metals. Locations include Tauranga Harbour, Coastal Bay of Plenty, Porirua Harbour.

LOCATION	SPECIES	SEX (M/F)	DISC WIDTH (CM)	MATURITY*
TAURANGA	*M. tenuicaudatus*	M	68	Y
TAURANGA	*M. tenuicaudatus*	F	88.0	Y
TAURANGA	*M. tenuicaudatus*	F	112.0	Y
TAURANGA	*M. tenuicaudatus*	F	110.0	Y
TAURANGA	*M. tenuicaudatus*	F	107.0	Y
TAURANGA	*M. tenuicaudatus*	F	110.0	Y
TAURANGA	*M. tenuicaudatus*	F	106.0	Y
TAURANGA	*M. tenuicaudatus*	F	100.0	Y
TAURANGA	*M. tenuicaudatus*	F	98	Y
TAURANGA	*M. tenuicaudatus*	F	75	N
TAURANGA	*M. tenuicaudatus*	F	110	Y
TAURANGA	*M. tenuicaudatus*	M	63	Y
TAURANGA	*M. tenuicaudatus*	F	81.0	Y
BOP COASTAL	*M. tenuicaudatus*	M	55.5	N
BOP COASTAL	*M. tenuicaudatus*	M	80.5	Y
BOP COASTAL	*M. tenuicaudatus*	F	59.0	N
BOP COASTAL	*M. tenuicaudatus*	F	73.0	N
BOP COASTAL	*M. tenuicaudatus*	M	72.5	Y
BOP COASTAL	*M. tenuicaudatus*	F	77.5	N
BOP COASTAL	*M. tenuicaudatus*	F	77.5	N
BOP COASTAL	*M. tenuicaudatus*	F	85.5	Y
BOP COASTAL	*M. tenuicaudatus*	M	80.0	Y
BOP COASTAL	*M. tenuicaudatus*	M	74.0	Y
BOP COASTAL	*M. tenuicaudatus*	M	76.0	Y
BOP COASTAL	*M. tenuicaudatus*	F	94.0	Y
BOP COASTAL	*M. tenuicaudatus*	F	82.0	Y
PORIRUA	*M. tenuicaudatus*	F	84	Y
PORIRUA	*M. tenuicaudatus*	F	96	Y
PORIRUA	*M. tenuicaudatus*	F	93	Y
PORIRUA	*M. tenuicaudatus*	F	108	Y
TAURANGA	*B. brevicaudata*	F	98	N
TAURANGA	*B. brevicaudata*	F	119	Y
TAURANGA	*B. brevicaudata*	F	108	Y
TAURANGA	*B. brevicaudata*	F	111	Y
TAURANGA	*B. brevicaudata*	F	122	Y
TAURANGA	*B. brevicaudata*	F	125	Y
TAURANGA	*B. brevicaudata*	F	122	Y
TAURANGA	*B. brevicaudata*	F	119	Y
TAURANGA	*B. brevicaudata*	F	118	Y
TAURANGA	*B. brevicaudata*	F	111	Y
TAURANGA	*B. brevicaudata*	F	119	Y
TAURANGA	*B. brevicaudata*	F	122	Y
PORIRUA	*B. brevicaudata*	M	84	?