Comparative analysis of snapper (*Chrysophrys auratus*) otolith microchemistry to ascertain early life fine-scale movement patterns

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2023

Thesis submitted to Auckland University of Technology in partial fulfilment for the degree of Master of Science (MSc)

Abstract

Understanding the early life habitat interactions and movements of fish is vital for fisheries management because it provides vital information such as their post/pre-settlement and nursery habitat use. Currently, there is a lack of high-resolution information on early life habitat dynamics of snapper (*Chrysophrys auratus*) stocks around New Zealand. Snapper are one of the most abundant coastal reef fish in New Zealand belonging to the sea bream family *Sparidae.* Their prevalence in nearshore waters has been evident since human arrival in New Zealand, making up over 70% of fish remains found in Māori middens dating as far back as 700 years. Snapper also have significant economic and social value as they support one of New Zealand's largest and most valuable inshore commercial and recreational fisheries. Otoliths, because of their accretionary nature can act as a biological chronometer and record valuable chemical information from ambient waters across the lifetime of an individual. As such, otolith microchemistry information can be used to provide high-resolution information on the ontogenetic growth and movement of fishes. Our objective was to use the recently developed otolith time-series analysis in Sabetian et al. (2021) to compare patterns in the temporal habitat use of early life stage snapper recorded in their otolith chemistry profile. We collected both contemporary and historical snapper otoliths from a variety of locations around the upper North Island of New Zealand. Our modern-day samples included assemblages from the Hauraki Gulf and Doubtless Bay, while our historical samples included an assemblage from 1975 Hauraki Gulf, and an archaeological midden from Long Bay (Hauraki Gulf) dated between 1430 and 1485 CE. Otoliths were sectioned and prepared for laser ablation inductively coupled mass spectrometry (LA-ICP-MS). The collected time-series data were then subjected to Behavioural Change Point Analysis (BCPA) coupled with k-means clustering in order to infer residency and migration behaviour between different environments based on the elemental concentrations of Barium (138Ba) and Strontium (88Sr). Our results for habitat residency times showed that all groups spent the most amount of time in the marine state, averaging 46.5%, followed by estuarine at 35.8%, and the least amount of time in riverine states, averaging 17.8%. There was no significant difference in the amount of time spent in different habitats between different sites and assemblages. The number of transitions between habitats showed the most common transitions were between estuarine and riverine across all groups with an average of 26.4 transitions. The lowest number of transitions between environments was between riverine and marine with an average of 2.6. There was no statistical difference in the number of switches in states between the sites. Results for movements between different aquatic

habitats showed marine dominant signatures very early on, confirming hatching at sea, followed by a short period of estuarine residency which transitions into a riverine environment. The riverine habitat then becomes the predominant environment for a short period before the transition back to estuarine environment begins. The estuarine environment signatures start to become stronger with some minor riverine transitions. Marine signatures then become prevalent post-settlement, gradually becoming the dominating habitat after 1 year of age. These results show that time-series analysis using tools such as BCPA can unlock fine-scale information which can be used to infer pre- and post-settlement life-history movement patterns of snapper. The novel nature of our methodological approach has also uncovered essential information in the largely unexplored comparison of laser ablation technique, which will help the methodological advancement of otolith microchemistry.

Acknowledgments

First and foremost, I would like to thank my supervisor Dr. Armagan Sabetian. Without his guidance and tireless support this thesis would not have been possible. His calm and approachable manner meant I never felt uncomfortable asking for advice. He constantly provided sound advice that put any of my worries at ease. Throughout my duration at AUT he has gone above and beyond and exposed me to countless opportunities within the world of research which ultimately inspired me to conduct my Masters.

I would like to give a huge thanks to my secondary supervisor Jinjing Zhang and additional supervisor Julian Lilkendey for their constant dedication, guidance and support throughout this thesis. I am indebted to Jingjing for her expertise in statistical analysis which was vital for this thesis. I owe my deepest gratitude to Julian for his thorough and invaluable feedback which helped shape this thesis.

I am extremely thankful to thank Hamish Allen, MPI and the recreational fisherman of Doubtless Bay for providing me the samples for this thesis.

I extend my deepest gratitude to the team from the laser ablation lab in Otago University for making this thesis possible by ablating the samples.

Lastly, I would like to give a special thanks to my partner Grace Ivory, friends and family for their constant encouragement, love and support.

Contents

List of Figures

List of Tables

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signature:

Date: 27/04/2023

Chapter One – General Introduction

1.0 Introduction

Fish, like other organisms, undergo a metabolic-mediated process where chemical substances from the ambient environment are actively transported into their body (Campana, 1999; Schäfer et al., 2015; Proc et al., 2021). In the marine environment, these chemicals can originate from a variety of sources including seawater, sediment, suspended particles and the food chain (Bryan et al., 1979; Hüssy et al., 2020). Fish uptake these chemical substances through absorption, ingestion and respiration (Alexander, 1999). Bioaccumulation occurs when fish take up chemicals at a faster rate than they are excreted or metabolised (Bryan et al., 1979). Biomineralisation is a type of bioaccumulation where living organisms produce minerals such as silicates, carbonates, calcium and phosphates (Dhami et al., 2013). When fish uptake elements from the ambient environment, they absorb and incorporate them as biominerals. Fish then utilise these biominerals to form essential biomineralised structures such as bones, scales, fins and otoliths (Loewen et al., 2016).

Otoliths are biomineralized structures comprised by any or a combination of three calcium carbonate polymorphs: aragonite, calcite and vaterite (Wood et al., 2022). Otoliths are located within the inner ear of teleost fishes (Thorrold and Hare, 2002; Fablet et al., 2011) and are physiologically important for vestibular and auditory functions (Arai et al., 2005; Schulz-Mirbach et al., 2019). Most Osteichthyes (bony fish) share a similar inner ear structure which consist of three semi-circular canals, the ampullae and three otolithic end organs. The inner ear of fish serves three main functions: the semi-circular canals detect angular accelerations, whilst the otolith end organs detect linear accelerations and sound (Schulz-Mirbach et al., 2019). The orientation of the otolith end organs is thought to be partly responsible for sound-source location. There is a high diversity of otolith shapes in teleost fishes (Schulz-Mirbach et al., 2019). Differences in shape and size can be species, population or stock specific. There is no strong correlation between otolith size and fish size because large fish can have small otoliths and vice versa (Popper et al., 2005). Asymmetry in shape and size between the otolith end organs likely affects hearing and balance within fish (Schulz-Mirbach et al., 2019). Studies investigating the relationship between otolith size and auditory and vestibular responses have generally failed to achieve consensus

(Popper et al., 2005; Inoue et al., 2013; Kéver et al., 2014; Schulz-Mirbach et al., 2015, 2019).

It is thought that the solid otoliths found in actinopterygians today may have evolved from loose aggregates of otoconia that are seen in the endolymphatic sac of shark and ray species (Gauldie, 1996). There are three different pairs of otoliths, namely sagittae, lapilli and asterisci (Weisler, 1993). In most species, the sagittal otoliths are the largest of the three pairs, their shape varies greatly and is species and/or genera specific, thus allowing for identification down to the species level (Disspain et al., 2016). Otoliths have a relatively pure composition compared to other biological and mineralogical structures, as they are primarily composed of calcium carbonate in a non-collagenous organic matrix (Cook et al., 2016). Otoliths grow in increments from the core to the edge in periodic growth bands (Campana and Neilson, 1985; Thorrold and Hare, 2002). These growth bands form daily and are protein and mineral rich (Thomas and Swearer, 2019). There are two main types of growth bands: the mineral rich band which is calcium carbonate dominated and is referred to as the incremental zone and the protein rich band which is referred to as the discontinuous zone (Thomas and Swearer, 2019). The growth of otoliths is continuous and is maintained even when somatic growth has halted (Secor and Dean, 1989). Otoliths therefore display continuous growth from before the fish has hatched until its death, which means an elemental profile has been recorded for the entire lifetime of the fish (Campana and Neilson, 1985). Hence, in terms of utility, otoliths are considered a biological chronometer of a fish's life history.

1.1 Otolith as a fisheries biological tool

Otoliths are extremely useful biological structures for two reasons; their microstructure and chemical profile can provide a range of biological, physiological, ecological and environmental information. Their microstructure has been used as an ageing and growth estimate tool by marine biologists for more than a century. The first age estimation of a fish from an otolith was conducted in 1899 (Campana, 1999). The relationship between age and length of fish can then be analysed to assess various early and asymptotic growth parameters (Sabetian et al., 2015).

The chemical profiles of otoliths are equally valuable. Otoliths are acellular and metabolically inert, which means elements and compounds which are deposited onto

its growing surface are not reworked or resorbed and are permanently retained, thus maintaining an excellent record of environmental conditions experienced by the fish (Campana and Neilson, 1985; Thorrold and Hare, 2002). This is why the analogy of a biological clock is often used to describe the utility of otoliths. Otolith chemistry is a field that has been rapidly evolving due to technological advances. In 1980, there were only 6 papers published that had discussed otolith chemistry, this number grew to 157 papers by the end of 1998 (Campana, 1999). Detecting trace elements and isotope analysis can provide information for several avenues of research such as stock identification, reconstruction of environmental histories, determination of migration pathways and uncovering archaeological information (Campana, 1999; Hüssy et al., 2020; Sabetian et al., 2021). To date, a total of 50 elements have been detected within fish otoliths, including major elements such as carbon (C), oxygen (O) and nitrogen (N) and minor elements such as barium (Ba), sodium (Na), strontium (Sr), phosphorus (P), magnesium (Mg), potassium (K), chloride (Cl) and sulphur (S) (Hüssy et al., 2020). Because otoliths infuse elements from the ambient environment, they can be influenced by climatic variations such as temperature and salinity. They can also be influenced by non-environmental effects such as diet, genetics and physiological processes such as reproduction and growth (Sturrock et al., 2015; Grønkjær, 2016; Izzo et al., 2018; Martino et al., 2020). In Sabetian et al. (2021) they addressed these issues by using a novel time series analysis to analyse relative changes in the concentrations of elements rather than absolute values.

A stock of fish is an intraspecific group of reproductive individuals with identical life history traits (Ihssen et al., 1981). However, in population ecology, the term stock can be context specific as there is no widely accepted universal definition. Instead, scientists tend to use more specific terms such as ‘genetic’, ‘phenotypic’ and ‘reproductive’ stocks (Avigliano, 2021). Stock identification is an extremely important tool for fisheries management. Within a single fish species, there can be several different breeding populations which do not mix (Edmonds et al., 1989), therefore stock identification and management at a stock level is crucial for effective fisheries management. An example of this is snapper (*Chrysophrys auratus*) in Western Australia. The hotspot of the Australian snapper fishery is Shark Bay, where heavy fishing pressure in this area has resulted in management problems for the fishery, thus sparking an investigation into the stock dynamics of the area. Population dynamics, tagging and electrophoretic studies showed that there may be at least three different non-mixing stocks of snapper within Shark Bay (Edmonds et al., 1989; Jackson et al., 2010). Elemental concentrations within otoliths can be used as a biological tag to distinguish between different fish stocks according to the characteristics of the

environments within which they inhabit (Campana, 1999; Arai et al., 2005). Elements such as Ba, Sr, Mg, Manganese (Mn), zinc (Zn), and copper (Cu) can act as biological tags for spatial analysis of stocks (Campana et al., 2000; Arslan and Secor, 2005; Halden and Friedrich, 2008; Fowler et al., 2017). Studies on these elements can provide important information about populations that is not necessarily available from standard analyses of otolith micro-structure such as growth band (Hüssy et al., 2020). Stock identification on the basis of otolith microchemistry is relatively accurate because you can observe the relative concentrations of elements against each other. Therefore, it is not necessary to deconstruct how variables such as temperature, salinity and ambient elements may affect absolute chemical incorporation into the otolith (Elsdon and Gillanders, 2003). Because the elemental composition of otoliths reflects ambient conditions experienced by the fish during the time of deposition, elemental fingerprints will differ among groups of fish that have experienced different life histories (Kerr and Campana, 2013; Campbell et al., 2021; Sabetian et al., 2021). When differences are found in the elemental concentrations of groups of fish with similar ages it implies that they have different environmental histories (Campana et al., 2000). However, further supporting information is usually required from water chemistry profiles of different habitats (Elsdon et al., 2008), and other information such as DNA analysis (Feyrer et al., 2007) to distinguish if groups come from different stocks or populations. Differences in the elemental composition of otoliths cannot be used to infer the length of time that fish were separated, this is because when fish spend negligible amounts of time in different environments it can still result in a detectable difference in the elemental composition of their otolith (Campana et al., 2000).

Stock discrimination using otolith chemistry is not without challenges. If there are no detectable differences in the otolith elemental composition between fish it does not necessarily mean they come from the same origin. Therefore, elemental fingerprints can be a great tool for stock identification where differences exist, however, they are not useful when differences cannot be detected (Campana et al., 2000). This is further complicated by the fact that not all fish from the same population will have similar elemental fingerprints, in particular, fish of different size classes within the same population can have varying fingerprints due to age-related differences in elemental exposure history and ontogenetic effects (Campana, 2005). Differences in the chemical composition of otoliths do not automatically imply genetic differences, therefore several assumptions must be met to adequately use otolith chemistry as a method for stock discrimination (Campana et al., 2000; Kerr and Campana, 2013). There must be characteristic and reproducible elemental fingerprints that can be used as markers for each group, all individuals that contribute to the group mixture must be characterised,

and the markers must remain stable during the period between characterization and mixing (Campana et al., 2000).

Studying fish movement allows us to understand a variety of information such as the spatial and temporal habitat use of juveniles and adults as well as mixing processes in a spawning stock (Elsdon and Gillanders, 2003; Hamer and Jenkins, 2007; Jackson et al., 2010). The most frequently used method for determining fish movement and population connectivity is manual tagging where fish are tagged and released and later recaptured. However, this method only tells you where fish were tagged and found again (Hamer and Jenkins, 2007), and nothing about their journey. Migration analysis using otolith chemistry provides a much more detailed chronology of habitat journey, thus allowing the reconstruction of migration pathways by age or date (Campana, 1999). The most common elements used to reconstruct fish environmental histories are Sr, Ba, Mn, and Mg. Sr, Ba, and Mg are good environmental tracers as they are a substitute for calcium and reflect environmental parameters either linearly or nonlinearly (Hüssy et al., 2020), while Mn is initially absorbed at a high level by larvae immediately post-hatching in some species (Brophy et al., 2004; Ruttenberg et al., 2005). The incorporation of these elements into otoliths is primarily dependent on water chemistry and ambient temperature (Cook et al., 2016). Ba is an elemental marker that is more bioavailable for fish in freshwater environments and is thus absorbed in waters with higher freshwater input (i.e., lower salinity) (Elsdon and Gillanders, 2005), while Sr is best absorbed in higher salinity waters (i.e., lower freshwater input) (de Vries et al., 2005; Macdonald and Crook, 2010). The reconstruction of the migration patterns using otolith chemistry better suits fish species with movement life histories that involve travelling between areas with different chemical properties (Hamer and Jenkins, 2007). For example, Sabetian et al. (2021) used concerted shifts in Sr and Ba to infer the habitat use of snapper across different historical and present-day temporal scales.

Chemical signatures from the core of otoliths can also be used to ascertain natal origins (Ruttenberg et al., 2005). A study from Thorrold et al. (2001) examined geochemical signatures in otoliths of weakfish (*Cynoscion regalis*) in eastern North America to determine their natal sources. The study used maximum likelihood estimates of the proportion of spawning fish in each of the five study estuaries. They found high rates of homing in spawning weakfish to natal locations ranging from 60 to 81%. Fish which strayed from natal locations were still largely localised in areas adjacent to natal estuaries. Another study by Barnett-Johnson et al. (2008) traced the natal origins of Chinook salmon (*Oncorhynchus tshawytscha*) using Sr isotope ratios (^{87}Sr:^{86}Sr). They were able to locate the natal regions of Chinook in California with an accuracy of 82%. Zimmerman et al. (2013) identified the natal origin of Chum salmon

(*Oncorhynchus keta*) and Coho Salmon (*Oncorhynchus keta*) in Alaska using element: Ca ratios for Ba, Sr, Mg, Mn and Zn as well as $^{87}Sr:^{86}Sr$ ratios. They located natal origin watersheds with an accuracy of 80% for Coho Salmon and 68% for Chum salmon.

Examining otolith chemistry is also extremely useful for identifying the impacts of anthropogenic factors such as habitat destruction (Sabetian et al., 2021). Otoliths can also be used to monitor levels of pollution as they provide a permanent record of trace metals from the water environments inhabited by the fish over its lifetime (Arslan and Secor, 2005; Halden and Friedrich, 2008). A study from Herrera-Reveles et al. (2013) examined trace metal concentration within the otoliths of a common Caribbean damselfish (*Abudefduf sacatilis*) as an indicator of pollution on the reefs of Mochima National Park and La Tortuga Island, Venezuela. The different trace metals analysed were californium (Cf), Cu, mercury (Hg), lead (Pb) and Zn. The trace metals were detected in the majority of the otoliths that were tested, the most frequently detected of which were Pb and Hg. Pb is thought to be a good proxy trace metal to use when investigating pollutants in an area as its uptake into otoliths accurately reflects that of ambient concentrations. The detection of trace metals is important as it can spark further investigation into the sources of the pollutants. These pollutants can come from a variety of sources. The upwelling of deeper nutrient-rich waters can also be enriched with a variety of trace metals leading to high concentrations of trace metals (Herrera-Reveles et al., 2013). However, the use of absolute chemical values in otoliths as indicators of relative pollution is thought to be problematic as the absorption of elements such as heavy metals is diet and physiologically dependent (Campana, 1999; Ranaldi and Gagnon, 2008; Sturrock et al., 2015; Martino et al., 2020).

Isotope analysis is a common application used to extract environmental conditions from fish otoliths. The most widely used are oxygen and carbon isotopes. Isotope analyses can help infer useful information on a variety of factors such as site usage, seawater temperature, paleoenvironmental conditions, fish migrations, trophic level, predation and habitat alteration. In recent years there have been great advances in the fields of mass spectrometry and microsampling, thus enabling the recovery of high-resolution isotope profiles (Higuchi et al., 2019; Madgwick et al., 2021).

Chemical profiles of pre-historic otoliths can also illuminate various environmental and anthropogenic factors (Campbell et al., 2021). Otolith chemistry from an archaeological standpoint is not only beneficial for gaining information on prehistoric fish species and their marine environment, it can also be used to gain a greater understanding of the historical anthropogenic activities of the site such as fishing methods, trade routes and seasonality (Disspain et al., 2016). Historical otoliths are also an extremely important

tool that can aid fisheries management by determining ecological baselines which is essential for the restoration of native ecosystems (Disspain et al., 2016). The elements and isotopes of ancient otoliths are now frequently studied for numerous applications such as past fish migrations, paleoclimate and paleoenvironmental conditions from the Jurassic to the Holocene periods, as well as past anthropogenic fishing practices (Long et al., 2014; Cook et al., 2016; Campbell et al., 2021; Sabetian et al., 2021).

1.2 Snapper *Chrysophrys auratus*

Snapper are an abundant coastal reef fish in New Zealand belonging to the sea bream family Sparidae (Crossland, 1981). Snapper are the predominant fish found in Māori middens dating as far back as 700 years ago (Leach and Davidson, 2000). They also have significant economic and social value today as they support one of New Zealand's largest and most valuable inshore commercial and recreational fisheries (Paul, 1977; Parsons et al., 2014). Historically, the snapper genus has undergone numerous name changes. Originally, snapper in New Zealand were known as *Chrysophrys auratus,* whilst the Australian stocks were known as *Chrysophrys unicolor* and *Chrysophrys guttulatus,* and the Japanese stock as *Pagrus major*. Further investigation discovered that the morphological and biochemical differences separating the stocks were undetermined, thus prompting the genus of all stocks to be changed to *Pagrus auratus*. However, mitochondrial DNA analysis showed that the Australian and Japanese stocks display genetic differences and are not part of the same monophyletic group as other *Pagrus* species (Chiba et al., 2009). Therefore, the name *C. auratus* was reinstated for snapper in New Zealand and Australia.

Snapper are mesopredatory fish that are generalist feeders, feeding on whatever food is locally abundant. Small variations in feeding ecology have been observed within different size classes of snapper (Godfriaux, 1969; Crossland, 1981). Smaller snapper favour small crustaceans and polychaetes, whilst larger snapper incorporate a wide range of food groups such as brachyurans, echinoderms, molluscs and teleosts (Godfriaux, 1969). Snapper are diurnal feeders, smaller snapper feed consistently during the day, whilst larger snapper feed less frequently, starting at dawn and feeding mostly during the morning (Crossland, 1981).

Most of the New Zealand snapper catch is caught in the Hauraki Gulf (Paul, 1977; Crossland, 1981), as they are the most widespread and common demersal fish species

in the region (Paul, 1977). As a result of heavy commercial and recreational fishing pressure, habitat degradation, and siltation, snapper stocks have decreased dramatically (Hauraki Gulf Forum, 2020). In the Hauraki Gulf, snapper biomass has declined by 83% since human arrival (Hauraki Gulf Forum, 2020). Snapper are long-lived and large fish which can live up to 55-60 years old, grow up to 100 cm in fork length and weigh up to 17 kg (Horn, 1986; Parsons et al., 2014). Snapper growth rate is directly related to temperature, where the growth of juvenile snapper tends to be highest from spring to autumn and lowest during winter (Francis, 1994).

Post-hatching, snapper drift as larvae for approximately 4 weeks before settlement in nursery habitats (Parsons et al., 2014). Snapper are born as sexually immature females, some of which continue to develop into mature females whilst others undergo protogynous sex inversion into males from immature females. Although the female-to-male sex change makes them technically protogynous hermaphrodites, the fact that they change from non-functioning sexually immature females functionally makes them gonochors (Francis and Pankhurst, 1988). In the Hauraki Gulf, all snapper are thought to have reached sexual maturity when they are 30 cm long, with most fish reaching sexual maturity at 25 cm and some as small as 23 cm (Crossland, 1981). Snapper are serial broadcast spawners meaning they release many batches of eggs over several months, they spawn in aggregations in large bays between 20-70 meters during September to January when sea surface temperatures range from 15 to 23°C (Crossland, 1981; Parsons et al., 2014).

Snapper are primarily distributed around the North Island of New Zealand; they are abundant along the east coast from North Cape to East Cape with a major population in the Hauraki Gulf. The southern most major population is located in Hawkes Bay with snapper becoming sparser further south. Snapper are also abundant along the west coast from Ninety Mile Beach to Taranaki with major populations located in the Kaipara and Manukau Harbours. Like the east coast, snapper numbers decline further south along the west coast. Snapper can also be found in the north of the South Island with a significant population in Tasman Bay and smaller numbers in the Marlborough Sounds and down to Greymouth on the west coast (Crossland, 1981).

There are two predominant genetic stocks of snapper in New Zealand; one extends along the west coast of the North Island down to the northern part of the South Island, and the other along the east coast of the North Island. There is also a third genetically different stock located in Hawkes Bay which shows more similarity to the west coast stock (Smith et al., 1978; Bernal-Ramírez et al., 2003). Snapper in New Zealand are managed by the quota management system (QMS). There are four predominant areas for snapper management within the quota management system: SNA 1 – east coast of

the North Island from North Cape to East Cape; SNA 2 – East Cape to the bottom of the North Island; SNA 7 – west coast of the South Island including Marlborough Sounds and Tasman Bay; and SNA 8 - west coast of the North Island (Figure 1.1). Each management area has a different total allowable catch (TAC) derived from different stock assessments and commercial and recreational fishing pressures (Ministry for Primary Industries, 2013).

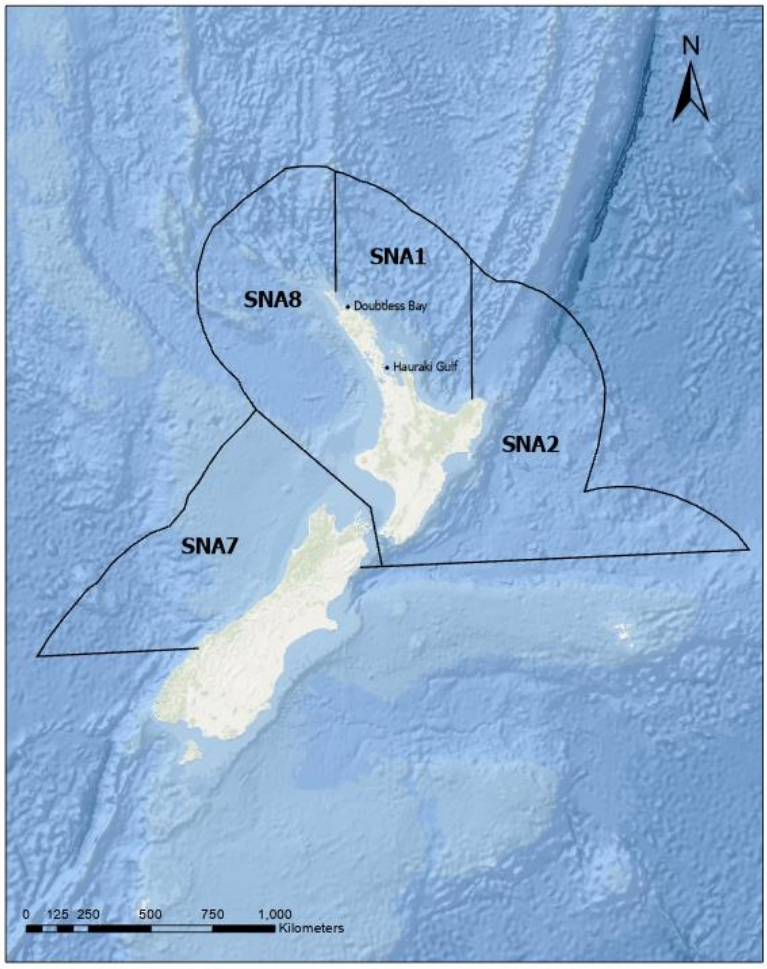

Figure 1. 1. Map displaying the SNA1, SNA2, SNA7 & SNA8 management zones in New Zealand.

Parsons et al. (2016) surveyed post settlement snapper using remote underwater video across coastal habitats within Whangarei Harbour. They found that structured habitats, such as sponge, seagrass and mussel beds, had a much greater abundance of post settlement snapper. A study by Radford et al. (2012) showed that snappers prefer seagrass water to other types of seawater based on olfactory characteristics. Many coastal marine fish species such as snapper require brackish estuarine habitat as nurseries because these habitats have very high rates of primary and secondary production and support a great diversity and abundance of fish and invertebrates (Beck

et al., 2001). A contributing factor as to why these environments provide good nursery habitats is their ability to export large quantities of nutrients such as carbon, nitrogen and phosphorus into coastal food webs, thereby creating favourable environmental conditions (Heck Jr. et al., 2003; Whitfield, 2017; Stewart, 2018).

Despite the important functional roles of coastal nursery areas, they are also one of the most threatened ecosystems due to increasing anthropogenic pressures (Vasconcelos et al., 2007). The nursery role hypothesis, developed by Beck et al. (2001), states that nursery habitats can produce, on average, more recruiting individuals per unit area than other habitats where juveniles are present. Therefore, nursery habitats must contribute more than other habitats for a combination of either the density, growth, survival or movement of juveniles to adult habitats. Rapidly growing coastal populations are putting a huge strain on brackish estuarine environments through habitat alteration, coastal development, and landscapes changes, which bring excessive nutrients sewage and chemical contaminants into these environments (Kennish, 2002). In New Zealand snapper nurseries in are generally under threat. Since the arrival of Europeans, New Zealand's landscape has changed dramatically, which has led to changes in the nursery habitat use of snapper (Sabetian et al., 2021). Sabetian et al. (2021) found that historic juvenile snapper from the Hauraki Gulf spent the majority of their time in estuarine environments, whereas their modern conspecifics do not do this anymore. Historical snapper also displayed synchronised ontogenetic movement patterns, whereas modern snapper exhibited a synchronised movement and shift more chaotically between nursery and adult habitat types.

An example of accurate stock discrimination for snapper can be seen in a study by Hamer et al. (2003), where they recorded spatial variation in the otolith chemistry of snapper collected from inlets along the coast of Victoria, Australia using the elements Sr, Ba and Mg. Their results showed that juveniles from Port Phillip Bay had an elemental tag which was particularly high in Ba. This elemental tag allowed for accurate discrimination between snapper from Port Phillip Bay and other nursery areas within Victoria with an accuracy of 98% in 2000 and 85% in 2001. The lower accuracy level of 2001 was due to lower Ba concentrations within Port Phillip Bay during 2001.

Gillanders (2002) matched up the elemental fingerprints of juvenile snapper caught in 15 different estuaries to adult snapper caught by the commercial fishery around greater Sydney in order to investigate the connectivity between juvenile and adult fish populations. They found that 89% of the fish caught by the commercial fishery originated from local recruitment estuaries around Sydney. These results suggest that local estuaries are vital for the recruitment of the open water commercial snapper

fishery around greater Sydney. Martino et al. (2020) investigated chemical markers in snapper otoliths and their physiological and environmental influence. They analysed carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes, and the elemental Ca ratio for Lithium (Li), Mg, Mn, Sr, and Ba. They found that $\delta^{13}C$ and Mg were both strongly influenced by physiological factors, whilst Sr and Li were weakly influenced. $\delta^{18}O$, Ba and Mn were all influenced by the environment.

Hamer et al. (2006) investigated the suitability of using Ba concentrations in snapper otoliths as a proxy for life history exposure to ambient Ba. To investigate how temperature and growth might affect Ba variation they conducted a laboratory experiment where they maintained snapper in tanks for 3.5 years. They found that annual cycles of temperature and growth in snapper did not have a significant effect on Ba variation. Ba levels recorded from wild juvenile snapper otoliths had a strong positive correlation with ambient Ba levels across 17 different sampling locations. Port Phillip Bay has high ambient Ba concentrations, approximately double that of coastal waters. This was mirrored by otolith Ba concentrations recorded in the study as otolith Ba concentrations from snapper caught in Port Phillip Bay were approximately double that of snapper caught in coastal waters. They also found that amount of Ba incorporation was similar across all life stages. These results suggest that Ba concentrations from snapper otoliths would be suitable for tracking life-history movements. They then used water chemistry data and otolith Ba concentrations to track the movement of snapper between Port Phillip and coastal waters in south-east Australia. They found that large Ba peaks were deposited on snapper otoliths during the spring and summer months which is when snapper migrate from coastal waters to Port Philip for spawning. These results show that snapper otolith Ba variation can be used as a proxy for tracking fish movements.

A study from Fowler et al. (2005) investigated the stock structure of snapper in South Australia by analysing age related differences in otolith elemental concentrations. They found that Sr and Ba levels were similar amongst fish for the first three years of their life, then diverged when the fish were 3 to 5 years old and remained different until they were around 9 years old. This shows that most snapper from South Australia come from only a couple different nursery areas and then spread out across the region in later years. Fowler et al. (2017) investigated the stock structure and movement of snapper in eastern South Australia using Ca profiles. They found that the usage of Ba:Ca ratios were the most informative for investigating the stock structure and movement of snapper. Their results showed that the six regional populations of snapper within South Australia originate from three different stocks. The southern populations are supported by a nursery over 600 km away at Port Phillip Bay in Victoria

and the northern populations are supported by two separate recruitment areas at Gulf St. Vincent and the Spencer Gulf. These findings support South Australia's fishery being divided into three separate stocks.

With the more recent advances in analytical technology, one of the most important aspects of otolith chemistry has been its ability to provide high resolution information about the ontogenetic growth of fishes. In particular, focusing on early life history can benefit fishery scientists two-fold; firstly, it can provide information about nursery habitat use, and secondly it can be used to ascertain spawning or natal origins. However, there is currently a lack of high-resolution information to clearly distinguish nursery habitat dynamics of snapper stocks around New Zealand. This stems from the status quo of otolith chemistry data analysis. Otoliths, because of their accretionary nature, record chemical information across the lifetime of an individual. This provides scientists with chronological data that contains data values (ambient elemental concentrations) with a temporal record. However, the current popular approach in studying such data often does not consider the richness of the otolith records, and the details of temporal autocorrelated structure in the values of elements. To date, only Sabetian et al. (2021) have attempted to unlock the hidden shifts in the auto-correlation nature of snapper otolith chemistry. In this study, I will attempt to contribute more to the knowledge base of the early life history of snapper on the east coast of New Zealand through larger sample size, spatially comparative analysis, and investigating aspects of habitat use, all of which were lacking in said study.

1.2 Aim and Objective

The aim of this study is to use otolith microchemistry data to infer early life-history habitat information on snapper. Specifically, the objective is to use the recently developed time-series analysis in Sabetian et al. (2021) to compare patterns in the temporal habitat use of early life stage Snapper recorded in their otolith chemistry profile.

In Chapter Two, I outline the methodological, analytical and statistical approaches. I will discuss the location and data collection procedures, sample size and preparation, otolith sectioning, laser ablation and statistical analysis. In Chapter Three, I provide the results by analysing early life residency, movement patterns and transitions between environments. Finally, in Chapter Four, I bring together all the information in this study

to discuss the relevance of the findings, the methodological approaches, and what we can infer from the conclusions in relation to early life history habitat use of snapper.

Chapter Two – Materials and Methods

2.1 Location & Data Collection

This study was conducted in the upper regions of the east coast of the North Island of New Zealand; specifically in Doubtless Bay (173,4557580°E 34,9242284°S) and the Hauraki Gulf (174,8990241°E 36,4658122°S). Doubtless Bay is located on the east coast of the Northland region, where there are relatively moderate levels of coastal development. The Hauraki Gulf is a much larger coastal embayment located to the east of New Zealand's most populous city, Auckland, which has the highest levels of coastal development. Sea surface temperature in both regions averages around 20°C over the summer months and 14°C during August-September (*Seatemperature.info*, no date). It should also be noted that in terms of commercial and recreational fishing, the entire upper east coast region of New Zealand, which includes the Hauraki Gulf and Doubtless Bay, fall within the snapper 1 fishery (SNA1) management regime (Figure 2.1). This means that the Ministry for Primary Industries (MPI) considers snapper within this location as a single stock and manages it as such (Fisheries New Zealand, 2022).

Due to the success of Sabetian *et al.*, (2021) in unlocking both present-day and historical (from archaeological data) habitat movement behaviour from snapper in the Hauraki Gulf, we decided to expand our assemblage locations and focus. Firstly, we decided to increase the sample size of otoliths from the Hauraki Gulf, from the 10 examined in Sabetian *et al.*, (2021), to 63 in this study. Secondly, we decided to separate the Long Bay archaeological midden otolith assemblage examined in Sabetian *et al.*, (2021) into early (Layer 1), mid (Layers 4 and 5), and late (layers 7 and 10) human occupations in order to see if the movement behaviour of snapper had changed across the 50-year human occupation of that site (1430 and 1485 CE). The lead author, Associate Professor Armagan Sabetian, provided ten samples to be analysed for each layer, totalling 50 altogether. Thirdly, we decided to sample from a less populated area within SNA1, ultimately agreeing on Doubtless Bay as it is a known spawning location for snapper, ultimately collecting 73 samples from this location. Finally, we were provided with the very first historical otolith samples collected in New Zealand by MPI as part of their snapper management monitoring programme, which began in the early 1970s. I chose 10 samples from the 1975 collection year within the Hauraki Gulf. The Long Bay archaeological otoliths were excavated during the summer of 2015-2016 from the Long Bay Restaurant site, which was located on the lee of the

low foredune in Long Bay. Bayesian modelling was used to radiocarbon date the otolith samples from the Long Bay Restaurant site between 1430 and 1485 CE (Campbell et al., 2021). Snapper from Doubtless Bay were caught by recreational fishers throughout 2021, providing us with the fish heads only. Therefore, we do not have any length or reproductive information available for this assemblage. The otolith samples from the Hauraki Gulf were sourced from a former snapper reproductive study by Allen (2017) which were acquired between 2015 and 2016.

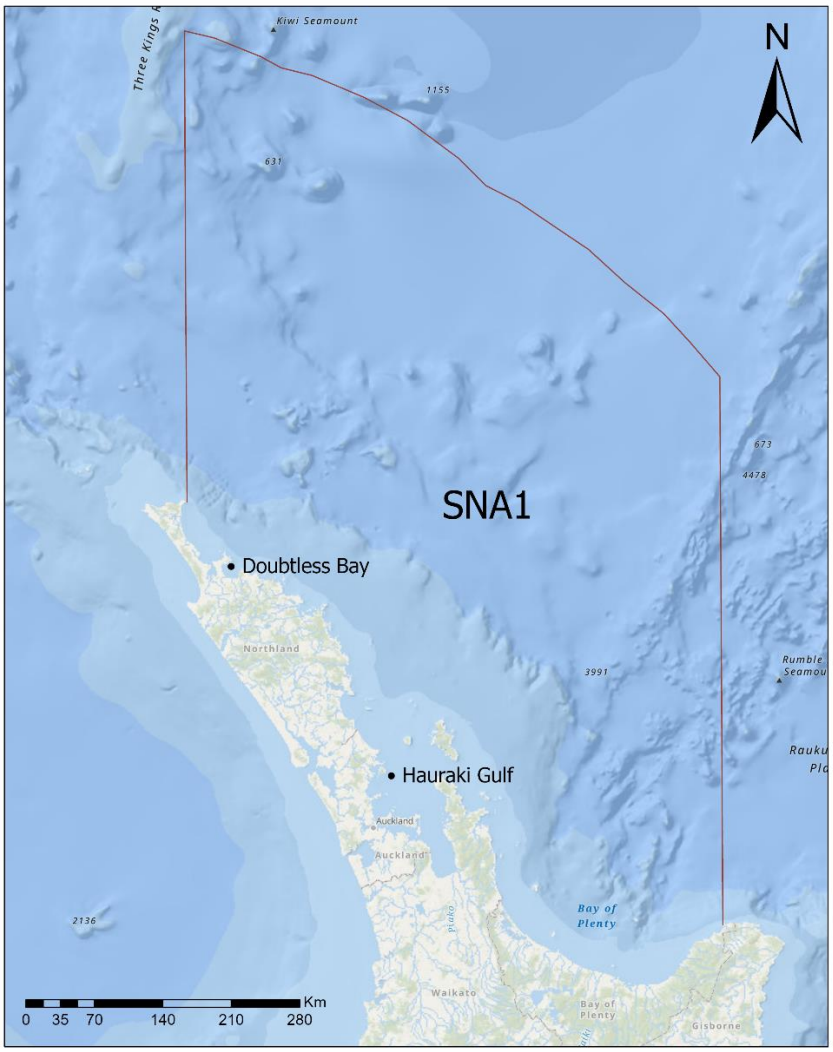

Figure 2. 1. Map of the upper North Island of New Zealand displaying the SNA1 management zone and the locations of Doubtless Bay and the Hauraki Gulf.

2.2 Otolith sectioning and preparation

To extract the otoliths, we first made a transverse incision with a knife across top of the neurocranium to reveal the brain. After the brain tissue was removed the otic capsule was revealed, after which we carefully removed the two sagittal otoliths using forceps. Both otoliths were then cleaned with water, dried in room temperature and weighed (mg). One otolith was used for analysis while the other was kept as spare. Archaeological and historical (Hauraki Gulf 1970s) otoliths were cleaned thoroughly using alcohol and dried in room temperature.

For the purpose of thin sectioning, we placed two glass slides on a hot plate to heat up the sides. We then put Crystalbond™ glue at the end of one slide and in the middle of the other. Next, we took the slide with the glue and mounted an otolith onto it ensuring that the core of the otolith was centred over the end of the slide (Figure 2.2). If need be, we would add more glue to the slide to support the otolith and ensure it was sitting horizontally flush with the slide. Once the otolith had firmly set on the glass slide, a 3000-grit diamond-encrusted disc was used to grind each otolith down to the edge of the slide. We then heated the slide up again until the glue melted in order to remove the otolith and transferred it to the slide with the glue. On this slide, we vertically mounted the otolith with the freshly ground flat section facing down (Figure 2.3). Once the glue had hardened, we again ground the otolith down until it was thin enough for light to penetrate so we could age them but not too thin, so they didn't break when transferring them off the slide for laser ablation (Figure 2.4). Finally, we prepared the transverse-sectioned otoliths for laser ablation by polishing them with a 5 µm lapping film in ultrapure water. The polished transverse-sectioned otoliths were then mounted on a geological slide for laser ablation.

We used a compound microscope to age the otoliths under magnification. To age the otoliths, we would count the number of opaque annual growth bands from the core to the edge of the otolith. These growth bands appear as dark bands under the compound microscope (Figure 2.5). We aged each otolith twice using different examiners to avoid bias. If there were any discrepancies between the estimated age, we would examine the otolith again and reach a consensus. After ageing was completed, the otoliths were transported for laser ablation.

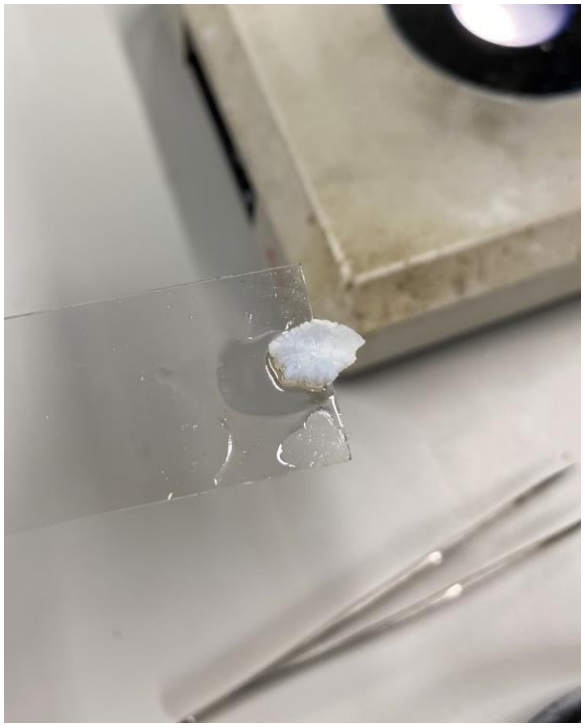

Figure 2. 2. Photograph of the otolith preparation sequence depicting an otolith glued hanging over the edge of a slide ready for the initial grind.

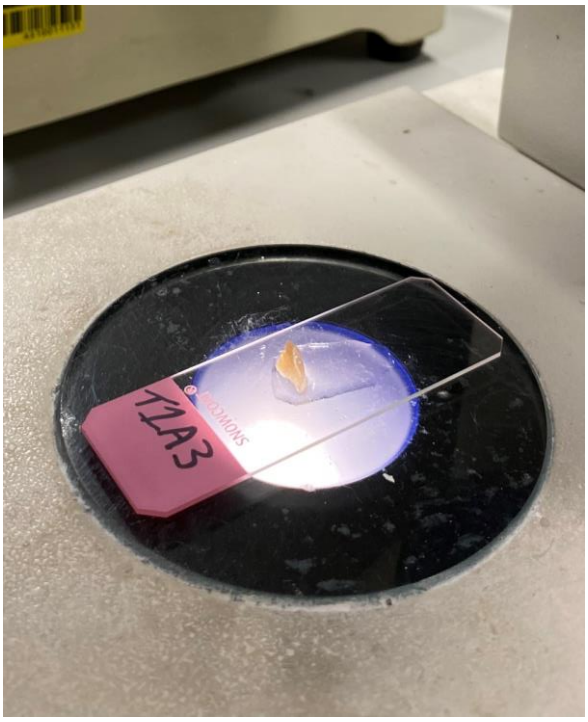

Figure 2. 3. Photograph of the otolith preparation sequence depicting an otolith vertically glued, flat side down, to a slide ready for the second grind.

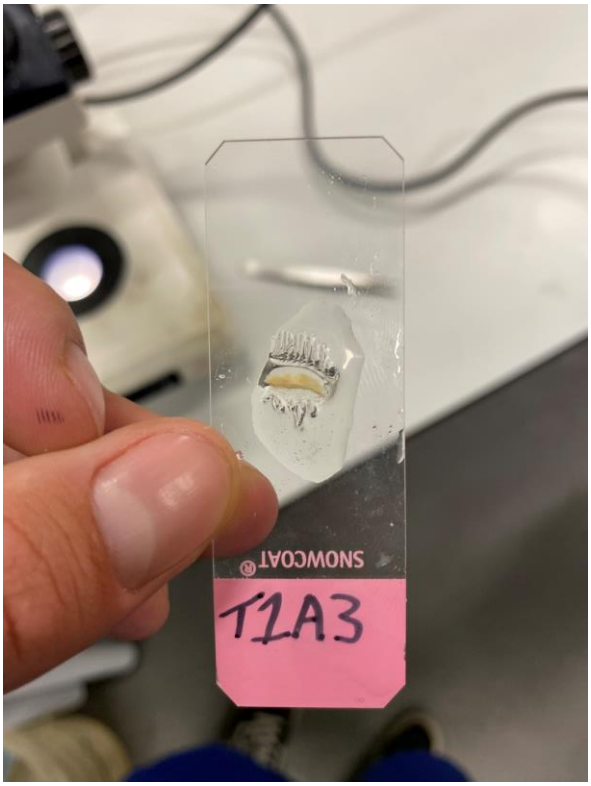

Figure 2. 4. Photograph of the otolith preparation sequence depicting the transverse-sectioned otolith ready for aggregation onto the laser ablation slide.

2.3 Laser ablation

Otolith microchemical profiles were analysed using laser ablation inductively coupled mass spectrometry (LA-ICP-MS) at the Centre for Trace Element Analysis in the Department of Chemistry, University of Otago (Dunedin, New Zealand). LA-ICP-MS allowed for measuring minor and trace element concentrations along an ablation path from the nucleus core to the proximal edge of the otolith (Figure 2.5). The otolith elemental composition provided us with trace element profiles of each fish examined through ablation transects that spanned their life history from birth (core) to death (edge). Instrumentation employed was an Applied Spectroscopy RESOlution M-50 laser ablation system powered by a Coherent 193 nm ArF excimer laser and an Agilent 7900 quadrupole ICP-MS (Figures 2.6 and 2.7). After mounting the otoliths on standard glass slides (75 mm x 25 mm) data for 13 different elements (Li, B, Mg, P, S, Mn, Fe, Cu, Zn, Sr, Sn, Ba and Pb) were collected, reduced and reported as ratios to Ca.

The laser ablation methodology follows the same as Sabetian et al. (2021), specifically; slides with the mounted otoliths (Figure 2.4) were placed in an ablation cell in an

atmosphere of pure helium to minimize any possibilities of experiencing re-condensation of ablated materials and elemental fractionations (Eggins et al., 1998). The video imaging system had suitable magnification to identify the core and was used for mapping transect pathways. Prior to obtaining measurements the 75 mm diameter transects were pre-ablated from core to the edge of the otolith to remove surface contaminants. The spot size employed for the transects was selected as a compromise between spatial sensitivity and detection power of the overall system (Taddese et al., 2019). The ablation with a laser firing frequency of 10 Hz and an on-sample fluence of 2.5 J/cm^2 was operated along the pre-ablated transects with the sample stage moving at 10 mm/s, for determining elemental concentrations in correspondence to life cycle of the fish. The ICP-MS instrument was tuned to minimize oxide formation, double charge formation and mass fractionation. Signal intensities of ^{138}Ba and ^{88}Sr were maximized after carrying out gas tuning processes on software-controlled gas flows of He and N_2 along with ICP-MS controlled Ar. Standards were run regularly with NIST610, NIST 612 and MACS3 used for instrument calibration, verification and matrix matched quality control, respectively. Data reduction of the raw count data to molar ratios (element of interest/Ca) was conducted using Iolite 3.63 (School of Earth Sciences, University of Melbourne) which subtracts gas backgrounds and corrects for any drift in instrument response (Paton et al., 2011). Accuracy and precision of the analyses were assessed using NIST 612 and the MACS-3 otolith reference material (United States Geological Survey - USGS). For the glass control precision was excellent RSD <3% and the accuracy was within ± 5% for all elements.

Sabetian *et al.*, (2021) used a linear ablation path (Figure 2.5) starting from the otolith core and moving vertically up and parallel alongside the sulcul groove. Because we wanted to focus our analysis on the very early life movement of snapper, the settlement regions (Figure 2.5) became the focus. Therefore, a different laser ablation path was used to Sabetian *et al.*, (2021). In this study, we employed an oblique ablation path, starting from the otolith core and moving horizontally across the settlement region of the otolith, before an oblique turn at the settlement point moving up and towards to the proximal tip of the otolith (Figure 2.5). All otoliths were aged (ranging from 1 to over 40 years old) and ablated from their core to the proximal tip. However, for the purpose of focusing our analysis on early life history, only the first 2000 µm of the ablation path data were used. This was based on visual analysis of every single otolith sample under a compound microscope, which revealed that at 2000 µm from the core, all otoliths had reached and surpassed age of one years old. In other words, we did not analyse the entirety of the ablation transect from core to edge, but rather from core to age one.

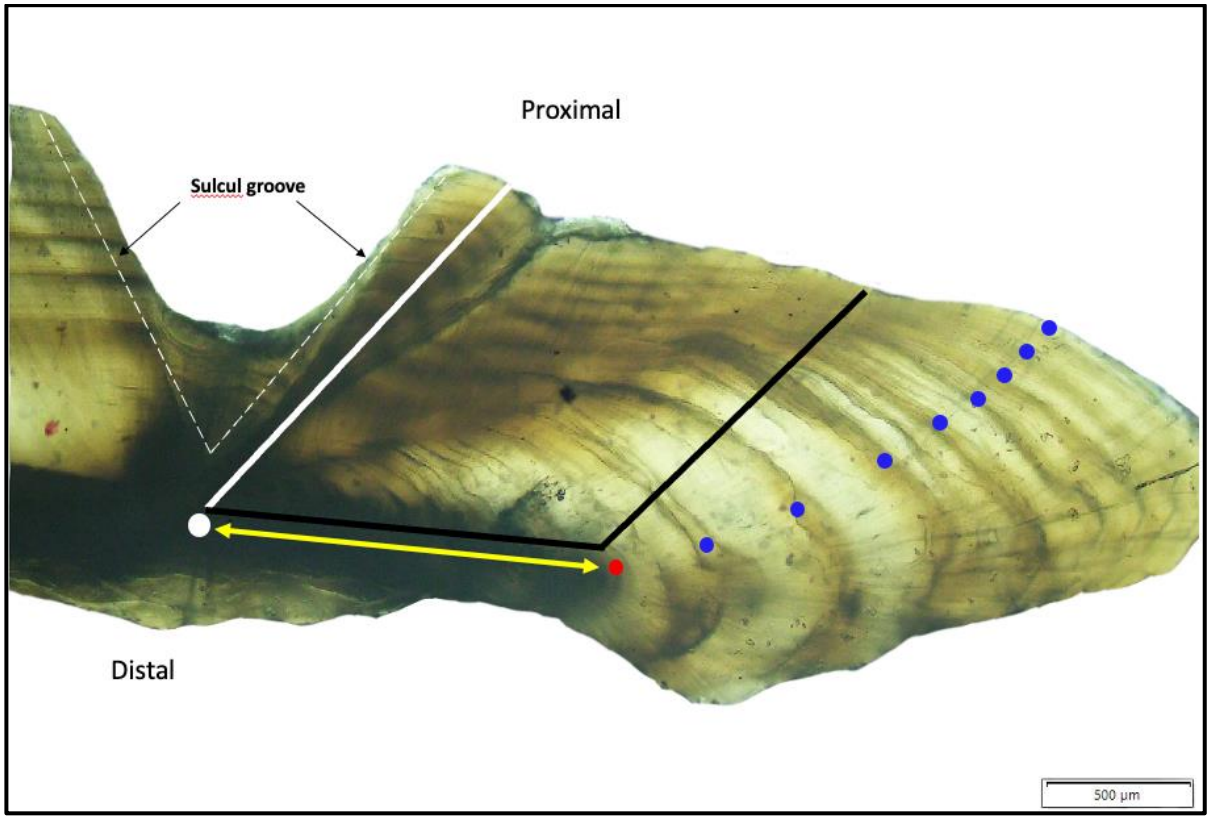

Figure 2. 5. Transverse cross section of an 8-year-old snapper *Chrysophrys auratus*, indicated by annual rings (blue dots), preceded by the settlement (red dot) after approximately 28 days (Parsons et al., 2014) of larval drifting both birth (white dot representing otolith core). The black line indicates the oblique laser ablation path employed in this study, while the white line indicates the linear ablation path employed in Sabetian et al., (2021). Yellow arrows indicate otolith growth representing the hatching to settlement period.

Figure 2. 6. Picture depicting the Applied Spectroscopy RESOlution M-50 laser ablation instrument.

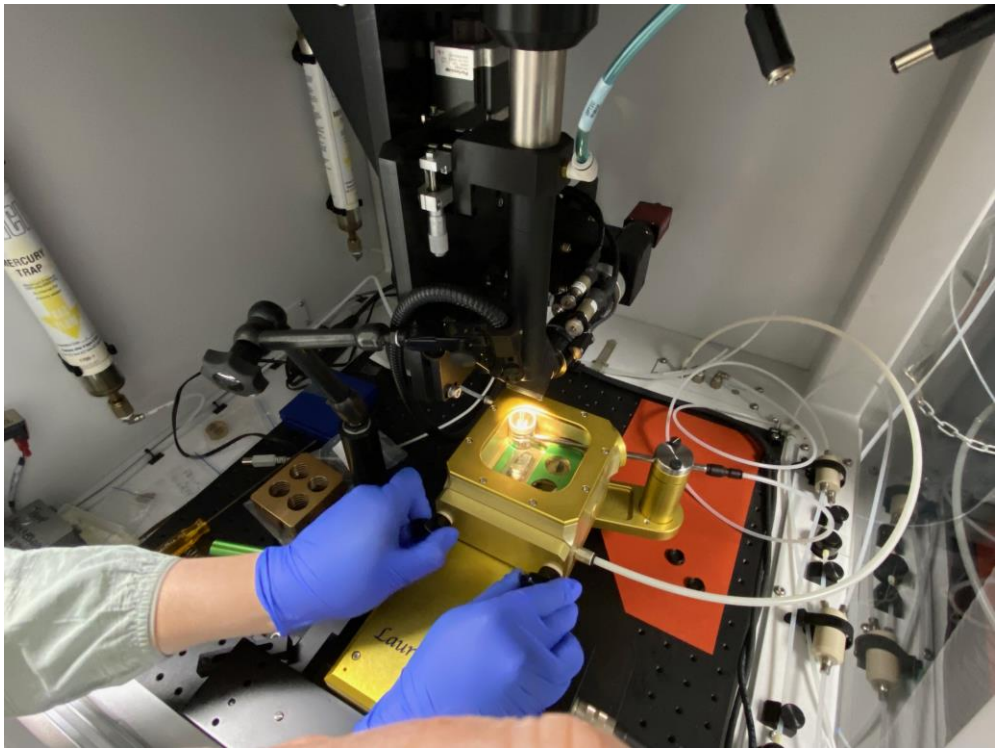

Figure 2. 7. Picture depicting the Applied Spectroscopy RESOlution M-50 laser ablation instrument in operation.

2.4 Statistical Analysis

Following Sabetian *et al.* (2021), we also used Behavioural Change Point Analysis (BCPA) with k-means clustering to describe different aquatic environments according to microchemical information of elements embedded in snapper otoliths. This approach allows us to infer residency and migration behaviour between different environments based on the elemental concentrations of Barium (138Ba) and Strontium (88Sr). Specifically, Ba and Sr can reveal freshwater (Elsdon and Gillanders, 2005) and saltwater residency (Secor and Rooker, 2000; de Vries, Gillanders and Elsdon, 2005; Macdonald and Crook, 2010).

The methodology used in this study employs the approach adopted by (Zhang et al., 2015) to determine transitions between foraging behaviour, with R programming language code (R Core Team) provided by Zhang et al. (2015) adopted and modified to execute the BCPA with k-means clustering analysis. Briefly, we will identify changes in autocorrelation structures within the standardised elemental values/concentrations at the smallest temporal scale possible for BCPA (window size = 30). Segments of trajectories between 'change points' identified by BCPA are referred to as 'bouts'. The means of elemental output metrics are calculated and used as the units for k-means clustering analysis to further classify behavioural states.

Prior to the k-means clustering analysis, the number of distinct 'states' for the otolith time-series will be determined through within-group sums of squares and serial classification of bouts, following the hierarchical cluster method of Krzanowski and Lai, (1988). Individual bouts of same-state behaviour will be classified into one of three mutually exclusive states based on combinations of elemental values (Barium and Strontium), using the k-means clustering algorithm of Hartigan and Wong, (1979) in the statistical software R with the 'cluster' package (Maechler *et al.*, 2016). Thus, bouts identified by BCPA will be assigned to behavioural states based on similarities of 'movement' patterns in the two-dimensional space of Ba and Sr concentrations. Subsequently, the time shares, represented by the distance on the otolith ablation path, that the snapper spent in each environment and the number of changes between the environments that occurred during the entire sample transect will be calculated. To describe the frequency of switches between the behavioural states, we will calculate the transition rate of each individual transect. The proportion of time spent in each behavioural state and the mean

values of behavioural state switches (both ways) will be compared between samples of different locations (Welch's Heteroscedastic F Test).

To evaluate the similarities and differences of the sequence of states in the otoliths, both within and between region groups, the proportion of individuals in each state at all distances measured on the otolith will be calculated. For each regional group, a new time-series will be created for a visual comparison of the consistency in the behavioural sequences among individuals. If the timing for the appearance of a certain state is consistent among the individuals within a region group, then the proportion of this state will be high at that distance/time when the state occurs.

Chapter Three – Results

3.1 Early life residency in different aquatic habitats

Using Behavioural Change Point Analysis (BCPA) with K means clustering, we were able to reconstruct three distinct "behavioural" clusters, which based on diverging Barium (Ba) and Strontium (Sr) concentrations were denoted as Riverine, Estuarine and Marine states; *Riverine*: High Ba (9.96 ± 0.07 ppm) and low Sr (1,767.22 ± 1.55 ppm); *Estuarine*: Low Ba (7.13 ± 0.04 ppm) and medium Sr (1,862.37 ± 1.45 ppm); *Marine*: Low Ba (7.17± 0.08 ppm) and high Sr (2,028.34 ± 3.03 ppm). The figures below depict the residency of individual snapper from different locations and historical periods in different aquatic habitats. The Y-axes represent the relative concentrations of Ba or Sr, while the X-axes represent ablation path distance from the otolith core. Although each otolith was ablated from core to edge, we chose to focus our analysis on the first 2000 µm of the ablation path as visual analysis showed this region to include the first year of life in all the samples analysed (see Chapter Two for explanation). Also, due to the large sample size from each location, the figures below represent a token sample, followed by the entire dataset in the Appendix section.

Below I present the analysis chronologically from oldest to most recent, starting with the present-day samples from Doubtless Bay and Hauraki Gulf, followed by samples from the 1975 Hauraki Gulf assemblage, and finally, the archaeological samples from Long Bay. As described in our methodology (see Chapter Two), the archaeological data from the Long Bay midden were divided into early (layer 1), mid (layers 4&5), and late (layers 7&10) human occupations in order to see whether continued fishing pressure had any impact on movement patterns of snapper.

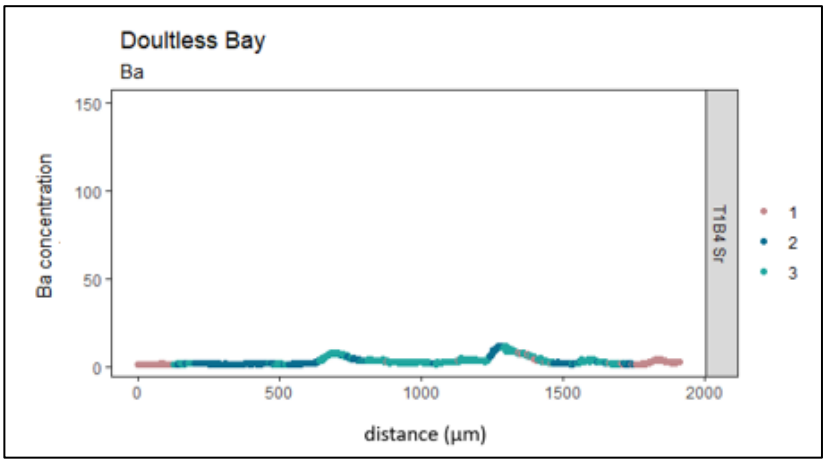

Figure 3.1. 1 Barium (Ba) concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Doubtless Bay. Colours represent habitat types (Estuarine = Green, Riverine = Blue, Marine = Light Red) as derived by behavioural change point analysis with k-means clustering through changing trajectories in barium and strontium concentrations along the ablation path. From here on, the following legends will be used; 1 = marine, 2 = riverine, 3 = estuarine.

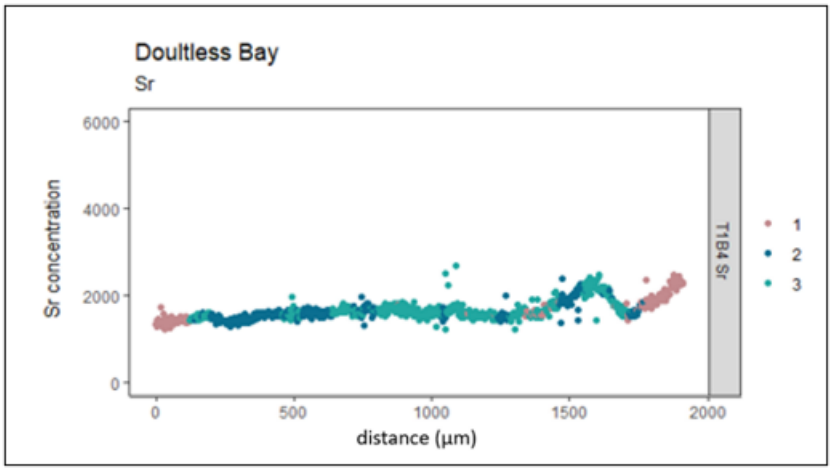

Figure 3.1. 2. Sr concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Doubtless Bay. 1 = marine, 2 = riverine, 3 = estuarine.

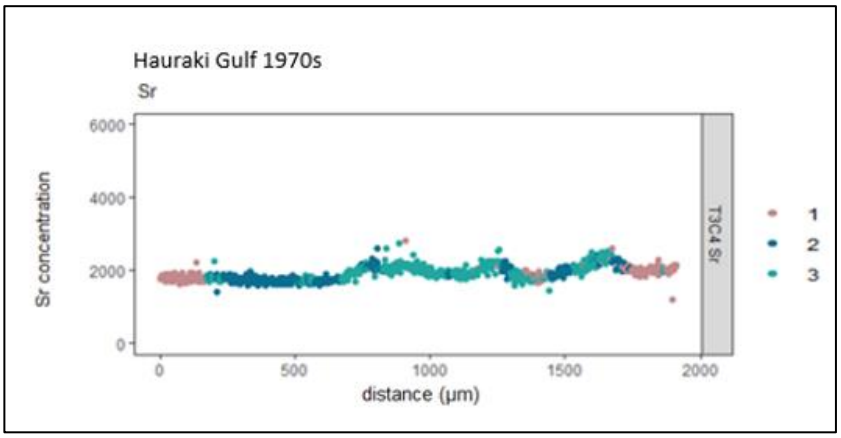

Figure 3.1. 3. Sr concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Hauraki Gulf-1970s. 1 = marine, 2 = riverine, 3 = estuarine.

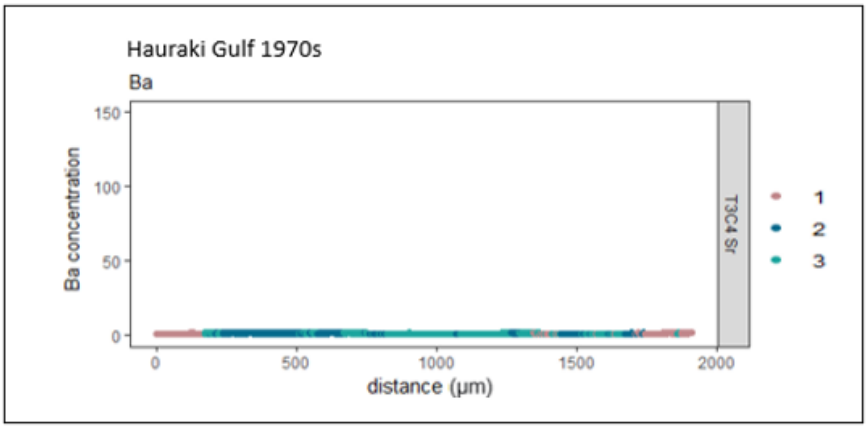

Figure 3.1. 4. Ba concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Hauraki Gulf-1970s. 1 = marine, 2 = riverine, 3 = estuarine.

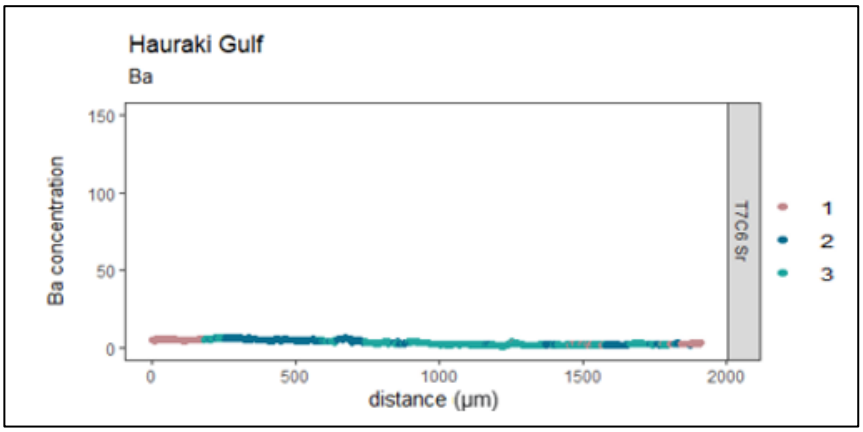

Figure 3.1. 5. Ba concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Hauraki Gulf. 1 = marine, 2 = riverine, 3 = estuarine.

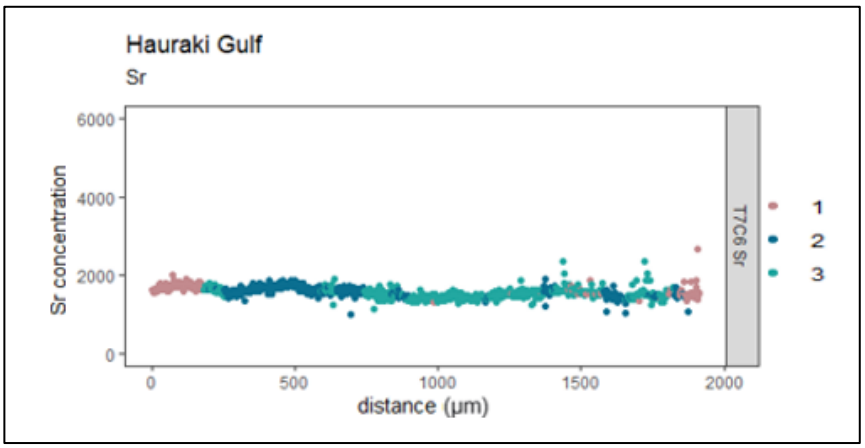

Figure 3.1. 6. Sr concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Hauraki Gulf. 1 = marine, 2 = riverine, 3 = estuarine.

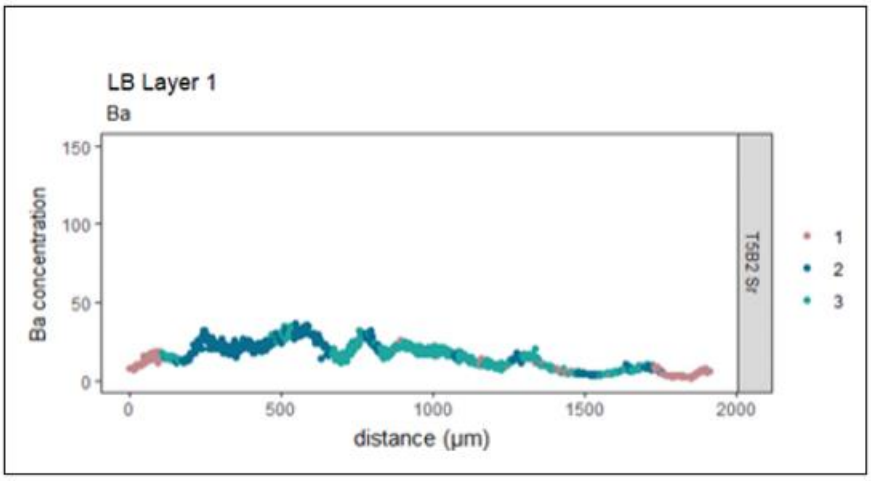

Figure 3.1. 7. Ba concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layer 1, representing early-human occupation 1 = marine, 2 = riverine, 3 = estuarine.

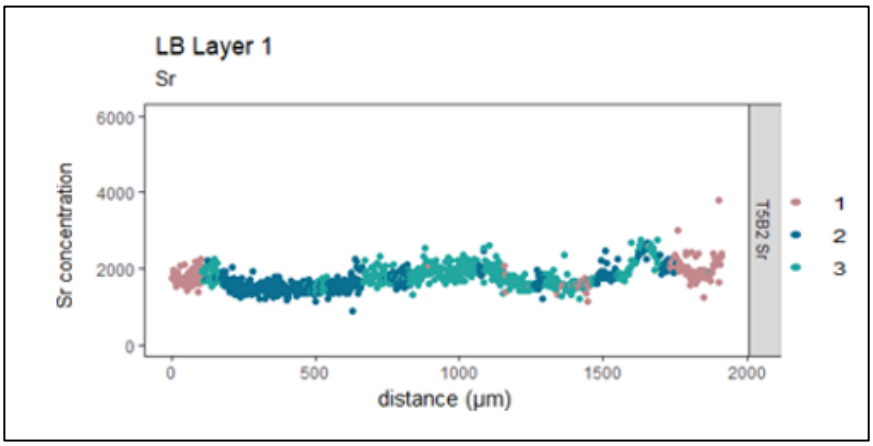

Figure 3.1. 8. Sr concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layer 1, representation early-human occupation. 1 = marine 2 = riverine 3 = estuarine.

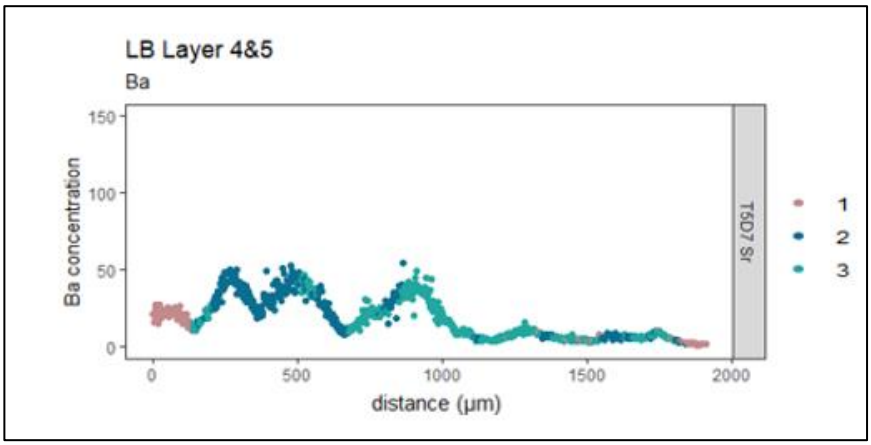

Figure 3.1. 9. Ba concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 4&5, representing mid-human occupation. 1 = marine 2 = riverine 3 = estuarine.

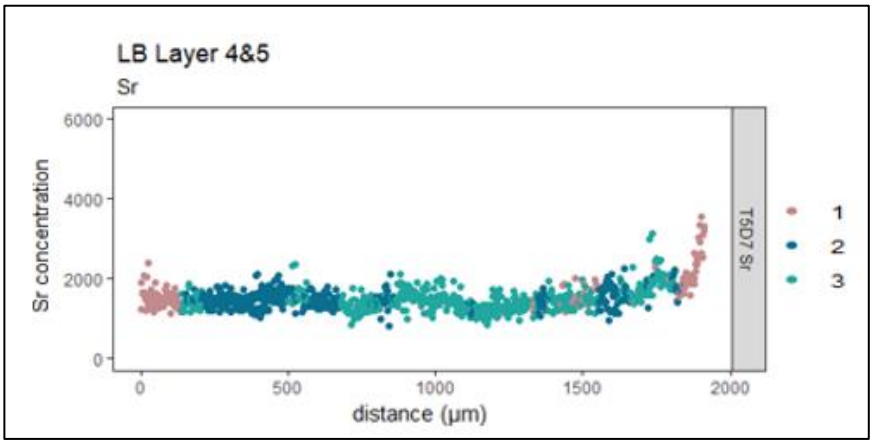

Figure 3.1. 10. Sr concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 4&5, representing mid-human occupation. 1 = marine 2 = riverine 3 = estuarine.

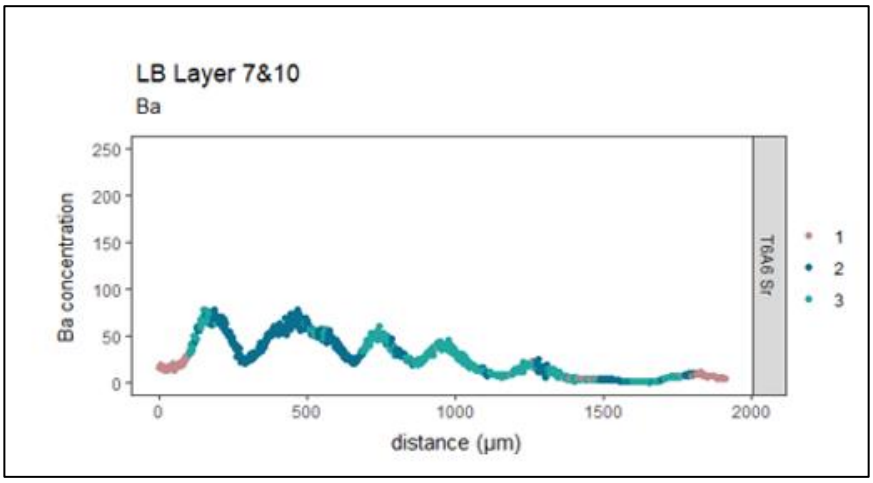

Figure 3.1. 11. Ba concentrations along the laser ablation path (distance µm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 7&10, representing late-human occupation. 1 = marine 2 = riverine 3 = estuarine.

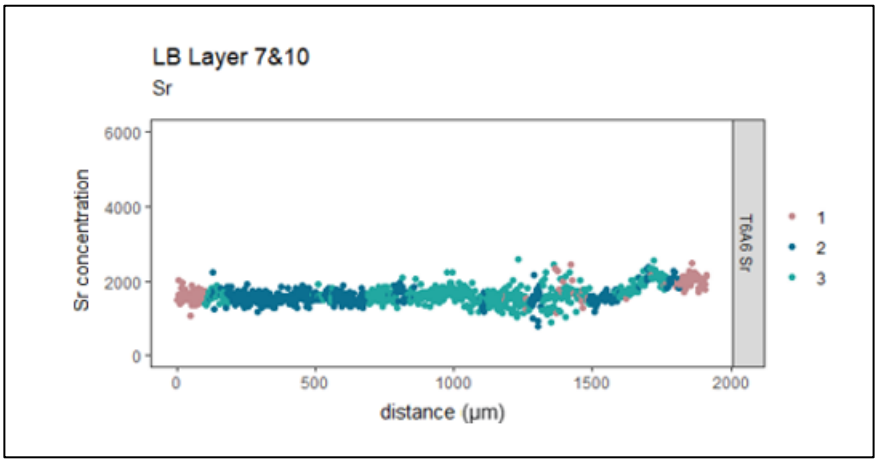

Figure 3.1. 12. Sr concentrations along the laser ablation path (distance µm) of New Zealand snapper *Chrysophrys auratus* in Long Bay layers 7&10, representing late-human occupation. 1 = marine 2 = riverine 3 = estuarine.

3.2 Movement patterns between different aquatic habitats

The table below represent overall mean residency time (as a percentage) in each habitat, plus or minus standard error, calculated form the entire sample assemblage of each location.

Table 3. 1. Mean residency time (± SE) of New Zealand snapper *Chrysophrys auratus* in different habitats (represented as a %) from each location.

Assemblage	Marine	Riverine	Estuarine
Hauraki Gulf 1975 (n=20)	46.2±0.4%	18.1±0.5%	35.7±0.3%
Doubtless Bay (n= 62)	46.5±0.3%	18.0±0.4%	35.6±0.2%
Hauraki Gulf (n=52)	46.5±0.3%	17.9±0.3%	35.6±0.2%
LB Layer 1 (n=10)	47.0±0.5%	17.5±0.5%	35.5±0.4%
LB Layers 4&5 (n=20)	46.4±0.4%	17.7±0.6%	36.1±0.3%
LB Layers 7&10 (n= 18)	46.2±0.5%	17.8±0.4%	36.0±0.3%

The succession of behavioural states between locations was very similar as all groups spent similar amounts of time in each habitat state. All groups spent most amount of time in the marine state, averaging 46.5%, followed by estuarine at 35.8%, and the least amount of time in riverine states, averaging 17.8%.

Multivariate analysis of variance (mANOVA) showed that there were no significant differences in the amount of time spent in different habitats between different sites and assemblages.

Figure 3.2.1 is a graphical representation of habitat residency data in Table 1, depicting a timeline visualisation of where snapper were residing at different stage of their early life. For this graph, the early-mid-late Long Bay assemblages were combined.

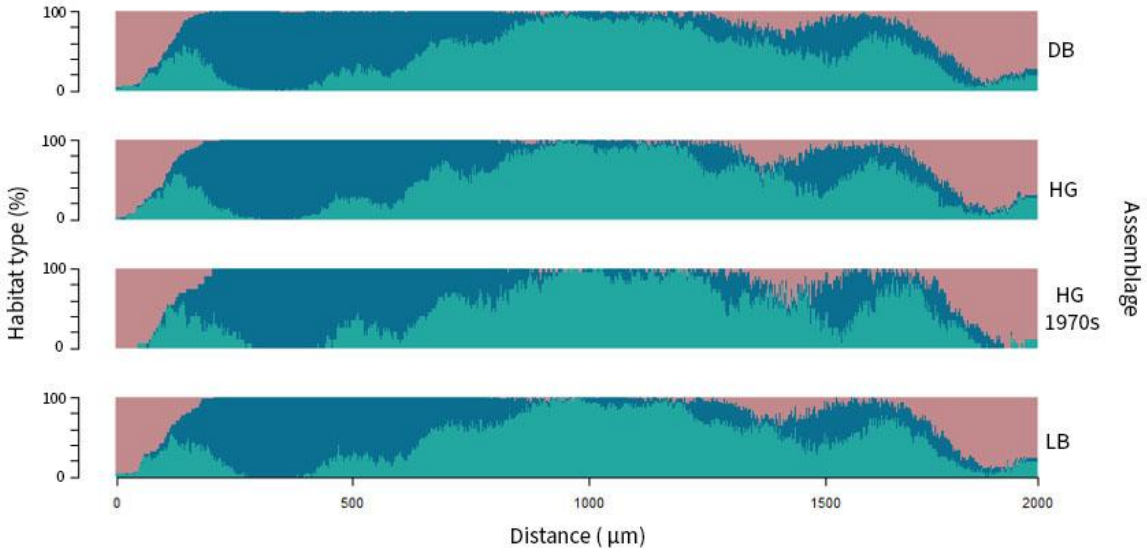

Figure 3.2. 1. Mean proportion of habitat types (riverine = blue, estuarine = green, marine = red) identified by k-means clustering along the laser ablation path (distance µm) on otoliths from New Zealand snapper *Chrysophrys auratus* by assemblage (DB = Doubtless Bay, HG = Hauraki Gulf, HG 1970s = Hauraki Gulf 1970s, and LB = Long Bay).

All groups showed very similar signatures over the core-to-2000 µm laser ablation path. Specifically, we see marine dominant signatures very early for the first 100 µm of transect confirming hatching at sea, followed by a short period of estuarine residency which transitions into a riverine environment. The riverine habitat then becomes the predominant environment for a short period before the transition back to estuarine environment begins. The estuarine environment signatures start to become strong between 500-1000 µm, with some minor riverine transitions. Marine signatures are picked up shortly after 1000 µm, gradually becoming the dominating habitat after 1700 µm.

3.3 Transitions between different aquatic habitats

Below I present analysis of transitions (switching) between the three habitats by fish from each assemblage.

Table 3. 2. Mean number of switches (± SE) of New Zealand snapper *Chrysophrys auratus* in different habitats (E = estuarine, R = riverine, M = marine). To = the total number of switches from one state to another, & = the total number of switches between two states.

Group	Total Switches	E to R	E to M	R to E	R to M	M to E	M to R	E & R	E & M	R & M
1970's Hauraki Gulf	43.4±0.9	13.9±0.4	6.6±0.3	13.25±0.3	1.5±0.1	7.3±0.3	0.9±0.2	27.1±0.7	13.9±0.6	2.4±0.3
Doubtless Bay	43±0.7	13.6±0.3	6.45±0.2	13±0.3	1.65±0.1	7.23±0.3	1.1±0.2	26.6±0.5	13.7±0.5	2.74±0.3
Hauraki Gulf	42.5±0.7	13.4±0.2	6.4±0.3	12.8±0.2	1.62±0.1	7.19±0.3	1.08±0.1	26.2±0.5	13.6±0.5	2.69±0.2
LB Layer 1	42.4±1.2	13.7±0.4	6.2±0.4	12.8±0.3	1.6±0.2	7.3±0.4	0.8±0.3	26.5±0.7	13.5±0.5	2.4±0.4
LB Layers 4&5	41.9±1.2	13.3±0.4	6.15±0.4	12.8±0.4	1.6±0.2	6.85±0.4	1.15±0.2	26.1±0.9	13±0.8	2.75±0.3
LB Layers 7&10	42.9±1.2	13±0.3	7.11±0.5	12.5±0.3	1.5±0.2	7.67±0.5	1.11±0.2	25.6±0.6	14.8±0.7	2.61±0.3

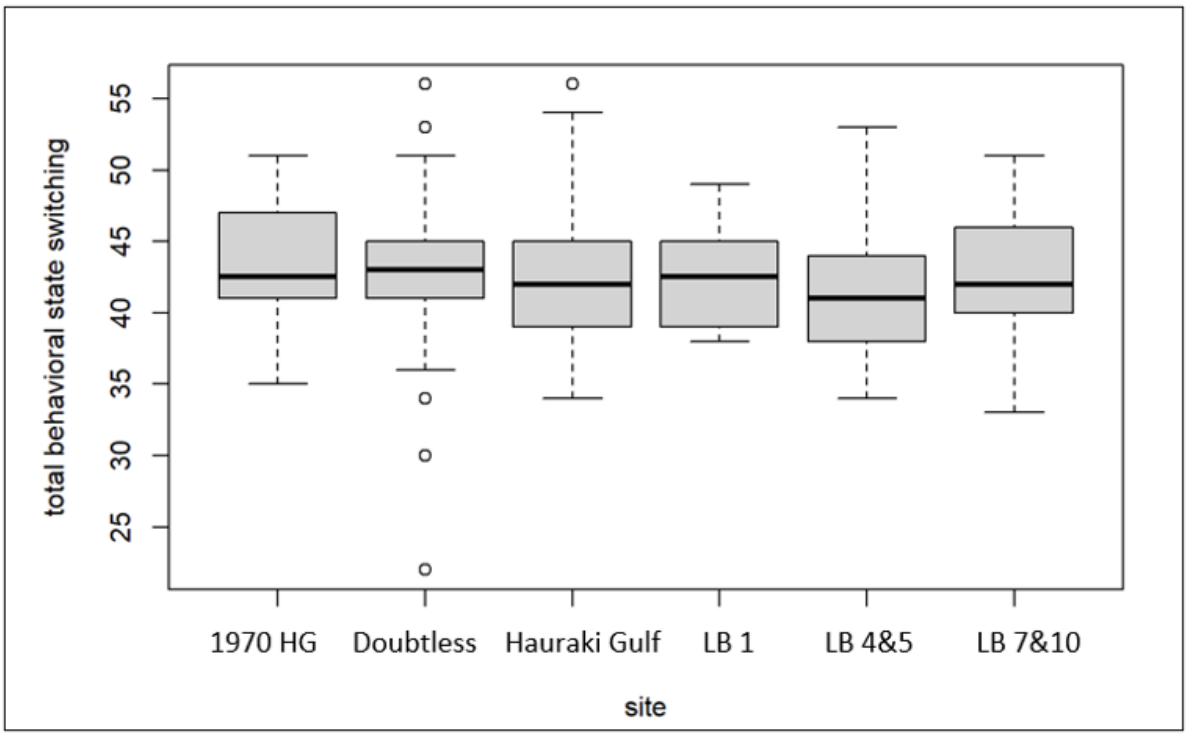

Figure 3.2. 2. Boxplot displaying the total number of switches (± SE) of New Zealand snapper *Chrysophrys auratus* at different sites (1970 HG = 1970's Hauraki Gulf, Doubtless = Doubtless Bay, LB 1 = Long Bay layer 1, LB 4&5 = Long Bay layers 4&5, LB 7&10 = Long Bay layers 7&10).

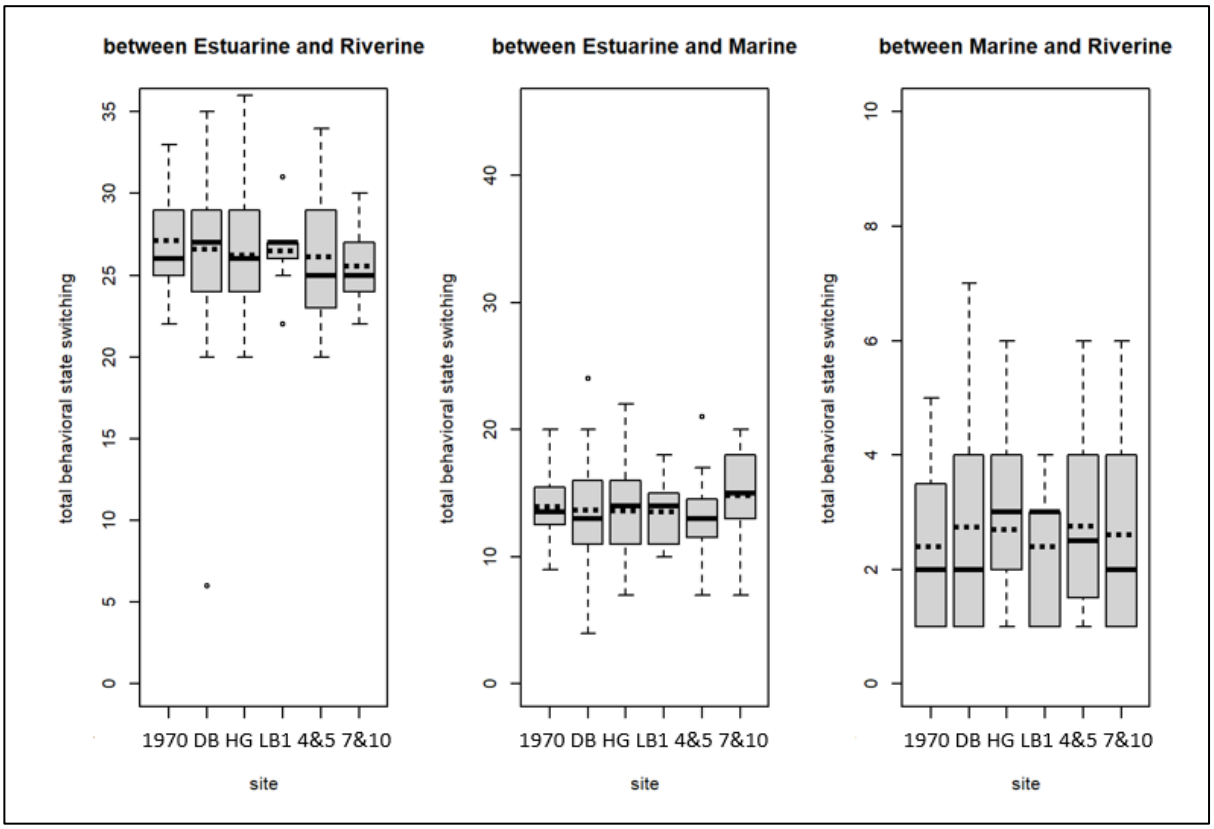

Figure 3.2. 3. Boxplots displaying the total number of behavioural state switches (± SE) of New Zealand snapper *Chrysophrys auratus* at different sites (1970 = 1970's Hauraki Gulf, DB = Doubtless Bay, HG = Hauraki Gulf, LB1 = Long Bay 1, 4&5 = Long Bay 4 & 5, 7 & 10 = Long Bay 7 & 10) between estuarine and riverine, estuarine and marine, and marine and riverine. Note the varying y-axes for total behavioural state switching.

The most common transitions were between riverine and estuarine habitats across all groups with an average of 26.4 transitions. The lowest number of transitions between two environments was between riverine and marine with an average of 2.6. The average number of transitions between estuarine and marine was 13.8. All groups showed a similar pattern in switches between different habitat states. Hauraki Gulf 1970s showed the largest number of switches between states at 43.4 whilst long bay layers 4&5 showed the smallest number of switches at 41.85. Hauraki Gulf 1970s has the most amount of switches between estuarine and riverine at 13.85 whilst long bay layers 7&10 had the least at 13. Long bay layers 7&10 had the most amount of switches from estuarine to marine at 7.11 whilst long bay layers 4&5 had the least at 6.15. Hauraki Gulf 1970s had the most amount of switches from Riverine to Estuarine at 13.25 whilst Long Bay 7&10 had the least at 12.56. Doubtless Bay had the most amount of switches from Riverine to Marine at 1.65 whilst Hauraki Gulf 1970s and Long Bay layers 7&10 had the least at 1.5. Long Bay layers 7&10 had the largest number of

switches from marine to estuarine 7.67 whilst Long Bay layers 4&5 had the least amount at 6.85. Long Bay layers 4&5 had the most switches between marine and riverine at 1.15 whilst Long Bay layer 1 had the least at 0.8. Hauraki Gulf 1970s had the most amount of switches between estuarine and riverine environments at 27.1 whilst Long Bay layers 7&10 had the least at 25.56. Long Bay layers 7&10 had the most switches between estuarine and marine environments at 14.78 whilst Long Bay layers 4&5 had the least at13. Long Bay layers 4&5 had the most switches between riverine and marine environments at 2.75 whilst Hauraki Gulf 1970s and Long Bay layer 1 had the least at 2.4.

Overall, the Welch's Heteroscedastic F-Tests showed that there was no statistical difference in the number of switches in states between the sites.

Chapter Four – General Discussion

This study aimed to use otolith microchemistry data to infer early life-history movement information of snapper in New Zealand. The specific objective was to use the recently developed time-series analysis in Sabetian *et al.*, (2021) to compare patterns in the spatial and temporal habitat use of early life stage snapper recorded in the elemental profile of their otoliths. It is hoped that this information can further be used as a proxy to reveal fine-scale aspects of habitat interactions during the nursery life stage of snapper in New Zealand.

The various types of spatial assemblages examined in this study allowed for a very unique temporal analysis, ranging from 15th Century archaeological specimens, to late 20th and early 21st Century specimens. BCPA with K means clustering enabled us to reconstruct three distinct clusters based on diverging Barium (Ba) and Strontium (Sr) concentrations. These were used to allocate states as Riverine, Estuarine and Marine based on the following concentrations*: Riverine as* High Ba (9.96 ± 0.07 ppm) and low Sr (1,767.22 ± 1.55 ppm); *Estuarine*: Low Ba (7.13 ± 0.04 ppm) and medium Sr (1,862.37 ± 1.45 ppm); *Marine*: Low Ba (7.17± 0.08 ppm) and high Sr (2,028.34 ± 3.03 ppm). Higher Ba concentrations are generally an indicator of freshwater input and can be used to infer freshwater residency (Elsdon and Gillanders, 2005), while higher Sr concentrations are an accurate indication of oceanic residency as Sr is absorbed at higher concentrations into the otolith matrix at higher salinities (Secor and Rooker, 2000; de Vries, Gillanders and Elsdon, 2005; Macdonald and Crook, 2010). For all samples at each location, Ba had a much tighter spread than Sr, indicating that there was less noise in the Ba signals. This was expected as previous studies (Hamer, Jenkins and Coutin, 2006; Fowler, Hamer and Kemp, 2017) show that Ba incorporation into snapper otoliths is similar across all life stages of snapper. Studies have also shown that otolith Sr concentrations show more variation with changes in temperature than otolith Ba concentrations (Bath *et al.*, 2000; Elsdon and Gillanders, 2004).

BCPA with k-means clustering provided us with a powerful tool to infer early life-history information, namely residency, movement patterns, and transitions between different aquatic environments. Across all locations snapper residency at 0 μm (i.e., core) started in the marine environment for nearly 99% of the samples. This is encouraging, because it indicates that our laser ablation technique was precise at identifying the otolith core, as we would expect hatching to start immediately post-spawning at sea, followed by a four-week larval stage before settlement (Parsons et al., 2016). For the

majority of the samples, this short period of oceanic residency was followed by estuarine and riverine residency, in that order, where snapper followed a similar pattern across all locations. They started in a marine environment before moving into an estuarine environment then to a riverine environment where they dwelled exclusively until around to 500 μm on the laser ablation transect before moving back and forth between estuarine and riverine environments. The age timeline that the first 1000 μm of the otolith ablation represents is open to interpretation based on the oblique ablation approach employed in this study (see Chapter Two). As described in chapter two, we examined every otolith under a light microscope, measuring the distance between core and settlement, which showed on average the first 1000 μm of the transect represented the pre-settlement period. Briefly, the dark settlement region on the transverse section of an otolith (see yellow arrows in Figure 2.5) essentially represents approximately 4 weeks of larval drifting in snapper's life history post-hatching. Visual analysis of all the otoliths analysed shows the settlement region to horizontally extend between 800 and 1200 μm either side from the core for the majority of the samples. Given that our oblique ablation path followed this region before turning sharply and moving toward the proximal tip at the point of settlement (see red dot in Figure 2.5), we are confident that in general, the first 1000 μm on the ablation transect represents the first 4 weeks of life before settlement. Therefore, our analysis of residency during this time must consider the fact that during this stage, larvae are drifting at the behest of the prevailing currents and have little control over their movement. Conversely, visual analysis of all the samples revealed the region between 1000-2000 μm represents settlement to the first year of life, where snapper are now associated with a nursery habitat and are moving on their own volition.

The Long Bay midden was divided into early (layer 1), mid (layers 4 & 5), and late (layers 7 & 10) human occupation. At the point of settlement (1000 μm), all samples are in a predominantly estuarine environment, transitioning occasionally between estuarine and riverine. There is some interaction with the marine environment during this stage, but exclusive marine residency appears to happen after 1750 μm, by which time they have already reached the first year of life. The 1975 Hauraki samples, present-day Hauraki samples, and the Doubtless Bay samples, showed the same marine-estuarine-riverine signatures in the first month of life, before settling in a predominantly estuarine/riverine environment. There appears to be more early marine interaction after settlement for these assemblages, however there was no significant difference in residency between the regions.

The movement between different environments were very similar in terms of residency times. Fish spent the majority of their time in marine environments at around 46.5%,

the least amount of time in riverine environments at 17.8% and 35.8% in estuarine. MANOVA showed that there was no significant difference in residency times between the different sites. This pattern observed in the residency times, concurs with literature, as snapper spend the short juvenile stage of their life in nearshore water habitats (Compton et al., 2012). However, when juvenile snapper reach around 70 mm in fork length, they leave nearshore brackish and estuarine waters move into coastal waters (Parsons et al., 2013, 2014). Once snapper mature into adults, they tend to reside in deeper water, as they are no longer depended on structured habitats. Adult snapper also prefer areas with greater water movement so often occur in channels between islands and the waters surrounding islands (Compton et al., 2012). Based on the findings of Sabetian et al., (2021) we would have expected greater residency times in riverine and estuarine environments for the historical assemblages, but this turned out not to be the case.

In terms of transitions between habitats, the lowest number of transitions between two environments was between riverine and marine, with an average of 2.6 transitions. This is likely because travel from a marine to a riverine environment and vice versa, would usually involve passing through an estuarine environment. The highest number of transitions was between riverine and estuarine, with an average of 26.4 transitions. This is likely because these environments neighbour one another and often don't have large gradient shifts in salinity. The average number of transitions between estuarine and marine was 13.8. This is higher than marine and riverine because estuarine and marine habitats neighbour one another. However, it is lower than that of estuarine and riverine, as there are larger gradient shifts in salinity between estuarine and marine environments. All groups showed a similar pattern in switches between different habitat states. Overall, Welch's Heteroscedastic F Tests showed that there was no statistical difference in the number of switches in states between the sites.

All groups showed very similar signatures over the laser ablation path (Figure 2.5). Specifically, we see marine dominant signatures for the first 100 μm of data, followed by riverine dominance with estuarine influence from 200 μm to 700 μm, after which estuarine habitat becomes the dominating habitat type until around 1600 μm. After 1700 μm the habitat dominance shifts toward marine. Marine signatures in first 0-100 μm of all transects confirms that snapper eggs did hatch at sea followed by larval drifting into the estuarine and riverine environment. We can surmise from this data that snapper spawning happens very close to the coast. This concurs with finding from Crossland (1981), who states that snapper spawning takes place at a depth of 20-70 m in large bays or along the open coast and does not occur in low salinity areas such as estuarine environments. Visual observations have shown that snapper are aggregate

broadcast spawners and lay eggs in the marine environment, followed by approximately 4 weeks of larval drifting post-hatching (Parsons *et al.*, 2014). We then see the predominant habitat residency briefly change to riverine, followed by estuarine dominance after 500 μm, which becomes almost 100% residency at 1000 μm. This is likely the settlement stage, as after the drifting period, snapper settle in nursery habitats, largely comprised of estuarine and brackish water, due to their high rates of primary and secondary production which transport vital nutrients throughout the food web (Heck Jr., Hays and Orth, 2003; Whitfield, 2017; Stewart, 2018), thus creating conditions which support diverse fish and invertebrates assemblages (Beck *et al.*, 2001). These findings are promising as they suggest that our analysis is indeed accurate at inferring pre-settlement life-history movement information of snapper.

However, the fact that the residency patterns of all assemblage are very similar across the 0-2000 μm transect, and there are no significant differences between residency times, movement patterns, and transitions is troubling. We did not see the expected fine scale differences between the assemblages that were shown in Sabetian et al. (2021). We suspect the main reason for this is the fact that our laser ablation path differed to that of Sabetian et al. (2021). In our objective, to target the early life history movement patterns of snapper, we employed an oblique ablation path which extensively covers the settlement region on the otolith (Figure 2.5). Essentially what this means is that the first 2000 μm of the ablation transect does not represent a gradual depiction of life post-birth. The first 1000 μm represent the first 4 weeks of life as a drifting larva, while the second 1000 μm represent settlement to one year of life. Therefore, in effect our analysis is confounded by a very narrow and hyper focus on the pre-settlement life history of snapper, and a relatively macro focus on second 1000 μm of the ablation data. As a result, it is not surprising that we were not able to unlock more detailed fine-scale movement patterns.

Ironically, in terms of advancing time series analysis, the oblique ablation approach would be perfectly suited for assessment of natal origins using Dynamic Time Warping (DTW), which we plan on doing in the future. DTW is a distance measure which measures the similarity of two sequential data sets, it can compare sequential data despite temporal offsets that confound other methods. DTW can also detect subtle behavioural patterns within sequential data sets which other methods cannot (Hegg and Kennedy, 2021). DTW, in combination with the discriminate functional analysis (DFA), can be used to distinguish between natal origins and migration patterns in juvenile snappers (Hegg and Kennedy, 2021). In effect, we can cluster different specimens together based on the similarity/difference of elemental profiles, which could be used to infer different nursery habitats. Specifically, multivariate discriminate

functional analysis (DFA) can be performed with chemical tracers to group individuals and identify potential hatchery origin and juvenile habitats. The results of DFA will provide preliminary information on whether fish captured at one study sites are geographically separated from individuals captured at the other sites. Next, we can compare the patterns in the time-series of the chemical tracers. DTW is a useful tool for extracting maximal information from the temporally-autocorrelated structures of the time-series data, to help distinguish the groupings of individual fish in geochemical transitions that are represented by the continuous otolith life-history data. In this way, the patterns in the temporal information of the early life stage of snapper juveniles recorded in the otoliths can be compared. The similarities between measured values of the concentration of chemicals in the otoliths can be assessed, without requiring significant transformation of the time-sequence chemical profiling data as in discriminate functional analysis. The results from the DTW analysis will reveal whether individuals collected from different study sites originate from the same or different natal habitats, as well as the hierarchical migration patterns of the juvenile snappers (Hegg and Kennedy, 2021). That information will indicate which snappers settle in similar areas, and thus as proxy can reveal nursery habitats.

The linear ablation path employed in Sabetian et al. (2021) is best suited for unlocking early life habitat interactions post-settlement using BCPA. This is because identifying the otolith core is not important as long as the laser ablation starts anywhere in the settlement region and then proceeds to very quickly leave the region and move towards the proximal tip of the otolith. This way, the ablation transect data focuses predominantly on the post-settlement timeline, thus making unlocking fine scale habitat movement relatively easier. In order to test this hypothesis, we would need to re-ablate a selection of samples from each assemblage using the linear approach and compare the analysis with what is presented in this study.

Overall, this research was able to confirm several early life characteristics for snapper. Firstly, after hatching at sea, larvae drift immediately towards the coast where they are exposed to a short estuarine environment, followed by an extended riverine and estuarine environment, in that order. The fine-scale transition and movement of snapper larvae prior to settlement was not known till now. At the point of settlement snapper appear to be in a predominantly estuarine environment. This is followed by transitions with the marine environment, which becomes exclusive thereafter. This study has contributed to the knowledge base of snapper microchemistry by advancing our understanding of time-series analysis using BCPA. Specifically, we have learnt that laser ablation methodology is important for fine-scale data analysis depending on the study objective. However, even with the shortcomings of our methodological approach,

we were able to confirm fine-scale pre-settlement movement patterns for snapper, which to date had not been established.

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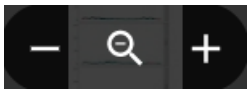
Appendix

Due to the size of the following images, they have been presented as hyperlinks for ease of viewing. Please use the toggle buttons (See Image below) to zoom in rather than a mousepad, using the toggle buttons will ensure that the image will not pixelate.



The figures below (See hyperlinks) depict the residency of individual snapper from different locations and historical periods in different aquatic habitats. The Y-axes represent the relative concentrations of Ba or Sr, while the X-axes represent ablation path distance from the otolith core. Colours represent habitat types (Estuarine = Green, Riverine = Blue, Marine = Light Red) as derived by behavioural change point analysis with k-means clustering through changing trajectories in barium and strontium concentrations along the ablation path. From here on, the following legends will be used; 1 = marine, 2 = riverine, 3 = estuarine.

Ba concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Doubtless Bay:

https://drive.google.com/file/d/1chSkylpGRjlvCiwqKvEFjqICCT5liugy/view?usp=share_link

Sr concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Doubtless Bay:

https://drive.google.com/file/d/1x74a9ARZ-YDrQopIdzsfqRcybzRSb9Ll/view?usp=share_link

Ba concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Hauraki Gulf 1970s:

https://drive.google.com/file/d/1coYJul1yADIWKMdpL9E9HnbwL2wUoG3-/view?usp=share_link

Sr concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Hauraki Gulf 1970s:

Hauraki Gulf 1970s Sr:

https://drive.google.com/file/d/1mJ4-9s_6yY8RXTvPWkLWYWtr9Yim4Ue0/view?usp=share_link

Ba concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in the Hauraki Gulf:

https://drive.google.com/file/d/1vBGeVJ4V1fnMnQQH3hY5JjrA3kC0AF1w/view?usp=share_link

Sr concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in the Hauraki Gulf:

https://drive.google.com/file/d/1pgyYBy_VvsJW1l76hD2MT9Cx7NOQn-dl/view?usp=share_link

Ba concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layer 1, representing early human occupation:

https://drive.google.com/file/d/1zQmMV28JRRX393DDuVh2aTNGohk8aQMX/view?usp=share_link

Sr concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layer 1, representing early human occupation:

https://drive.google.com/file/d/1zUFWqWGlByKSvV8F1D9_q68PSHCxbser/view?usp=share_link

Ba concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 4&5, representing mid human occupation:

https://drive.google.com/file/d/1F03hXfc4pTJHT61fGVHmRjfmcsDSpJRm/view?usp=share_link

Sr concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 4&5, representing mid human occupation:

https://drive.google.com/file/d/1UjYpN4MURE9LrzSM5eYhFeu_xYEAsv_T/view?usp=share_link

Ba concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 7&10, representing late human occupation:

https://drive.google.com/file/d/1txo0t8UrGKTGNkAjMmP01oTU88EDHEus/view?usp=share_link

Sr concentrations along the laser ablation path (distance μm) of a New Zealand snapper *Chrysophrys auratus* in Long Bay layers 7&10, representing late human occupation:

https://drive.google.com/file/d/1akPneaW1GAkkqmBr1VcoJ3bdYLGURVVP/view?usp=share_link