Kahawai Phylogeny and Phylogenetics

A genetic investigation into commercial and recreational fisheries management and practice

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Abstract

Globally, commercially exploited fish species are coming under more and more pressure as the population of humans grow. Protein from the sea has traditionally been available to coastal communities throughout history. In modern times however, traditional artisanal fisheries have been replaced by commercial fishing industries. It is estimated by some authorities that these modern fisheries have led to decreases in pre-exploitation biomass of desirable species of up to 90%. As desirable species decline, secondary species become more valuable and subject to exploitation. An issue with this exploitation is that management decisions of fish stocks are often based on political or commercial concerns rather than sound science focussed on preserving stocks, and ultimately, fishing industries.

To investigate phylogenetic and phylogeographic relationships of fish, kahawai (*Arripis trutta*) was used as a proxy species. *A. trutta* is one of only four members of the genus *Arripis*, which in turn is the sole member of the family Arripidae.

It was found that a single, highly connected population of *A. trutta* inhabit New Zealand waters, and approximately 15 migrants per generation make the journey between New Zealand and Australia, genetically linking these populations.

A phylogeny of *A. trutta* was resolved using mitochondrial DNA, and while COX1 data supported the hypothesis that *A. trutta* forms a monophyletic clade within the Stromateoids (medusa fish, squaretails and drift fish) and the Scombrids (tuna, mackerel and their allies) suggesting a common ancestor, other data collected during the investigation does not support this hypothesis.

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A work of this nature is never solely the work of one person. To complete this thesis, I have had input, advice and assistance from people in several countries.

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Finally, I'd like to thank my family and friends for putting up with me as I worked through this process. You have endured stories of fish caught and lost, sagas of PCRs and sequencing gone wrong and endless tales of why kahawai need to be studied, and why I was doing it. To my friends at the Wellington Improvisation Troupe, my hunting and fishing buddies and especially to my sons Joshua and Jeffery and my partner Alison, thank you.

This thesis is dedicated to you all.

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Chapter 1. Introduction

1.1 The physical ocean environment and factors that impact gene flow

The open oceans provide both barriers to and conduits for marine organism gene flow. Gene flow relies on the transport of propagules between geographic locations. Abiotic factors that can impact on this gene flow include currents (refer to Figure 1-1 for major New Zealand currents), winds, temperature, salinity levels and physical barriers. Biotic factors that can impact on propagule migration include food sources, presence (or more correctly absence) of predators, presence or absence of potential mates, time spent in the plankton and species specific migratory behaviour (Moyle and Cech, 2004). Carr et al (2003) discuss the "openness" of marine systems – the facility and rates of dispersal of nutrients, materials, and organisms and how these factors work together to expand the scales of connectivity of communities and ecosystems. Transport of nutrients into, and organisms out of, marine systems is generally far easier than it is in terrestrial systems, leading to more diffuse population signals.

Historically, gaps in the taxonomic record have confounded investigation into family relationships (see for example, Platnick, 1978) and abundances and distributions of species must be drawn from many and varied sources, such as fishing vessel reports and scientific surveys. Time lines for such surveys may span decades (Ward and Myers, 2005). This is especially so in deep water environments, where pressures of many atmospheres hinder investigation of biological systems (Akyildiz et al, 2005).

Traditionally, models to explain ecological and evolutionary systems have been built up by looking at mainly near shore systems and extrapolating to wider systems (Hutchings and Baum, 2005). Indeed, much of the early work in ecology was undertaken on rocky shores (e.g. Connell, 1961, Paine, 1966 and Dayton, 1971), and even today these classic pieces of work have a great deal of applicability in terrestrial and marine systems. But to fully draw out evolutionary patterns, better tools were needed. Today, molecular techniques (McDonald et al, 1992) are used to determine the evolutionary relationships and

population connectivity through analysis of the genetic variation (Hellberg, 2009). New models (such as the microsatellite evolution modelling of Durrett and Kruglyak, 1999 and molecular phylogeny population modelling of Nee et al, 1992) have been constructed to provide insight into evolutionary relationships of species.

Figure 1-1: New Zealand, showing major ocean currents (after Carter et al, 1998)

1.2 Pelagic Larval Duration (PLD) and fish migration

While not exhaustive, and grades of variation exist (e.g., Allen and Pernet, 2007 discuss the significance of intermediates), there are two main types of marine larvae, lecithotrophic and planktotrophic, which are based on feeding strategies of the larvae and the environment they inhabit in the larval form. Lecithotrophic

species are hatched with large egg sacs attached to the fry and normally spend less time in the plankton (Nybakken and Bertness, 2004). Feeding of such organisms is primarily via the nutrition contained in the egg sacs and when this finite resource is exhausted, the fish changes character to more resemble that of the adult fish. While this might seem to suggest that such a strategy might lead to high levels of population structure Todd et al (1998) found that there is some plasticity in the settling behaviour of lecithotrophic species, and that some species are quite capable of significant migrations and have highly connected populations.

Planktotrophic species are species whose larval form spends some considerable time living and feeding in the plankton (Probst and Crawford, 2008). At hatching, such species are small, do not have obvious egg sacs and need to feed almost immediately. In this stage of their life cycle, they are unable to swim against currents and tides. Riginos and Victor (2001) found that the length of time in the plankton was a good predictor of connectedness of some fish populations. Fish species spending short periods of time (18 days) in the plankton had highly structured populations, fish of intermediate time (24 days) showed less differentiation between populations, and fish with long planktonic periods (50 days) very little structure among populations.

Pelagic Larval Duration (PLD) provides a potential predictor into the genetic structure of populations and levels of genetic connectivity. However, there are many exceptions. Using mitochondrial COX1 data, Kelly and Palumbi (2010) compared genetic patterns of 50 different invertebrate species, and found that PLD was negatively correlated with Φ_{ST}, but when they removed non-pelagic species from their sample set, found that this correlation no longer held. This suggests that while PLD has some utility as a predictor of connectivity of populations, it is a far from universal rule, not the least because marine species have other mechanisms of migration, including migration by adults When combined with current quantitative methods of measuring gene flow however, PLD theory provides some interesting insights into how some species might migrate, and what the implications of this migration are for fisheries managers. By determining, for example, that a specific species primary mode of migration

is larval, fishing quotas and closed areas can be set to protect breeding aggregations of that species.

1.3 Fishery stocks and pressures

The commercial fishing industries of many nations, especially the industrialised nations, have followed similar development pathways. Originally, fishing was an artisanal activity, where fishers merely caught food for themselves, their families and members of their local community. Over time, these activities evolved into commercial fishing enterprises where fishers sold or traded the majority of their catch. Eventually, this activity became a competitive business, with fishers vying for the best catches and the best locations to maximise profits. As this commercial activity continued to evolve and competition for catches intensified, governments and regulatory bodies began to take notice and introduced catch and gear limits to fishing to ensure ongoing viability of stocks and to ensure jobs in the industry were safe and secure. (Roberts, 2007)

While there are some exceptions, many activities undertaken to protect stocks have been largely ineffective as such efforts often failed to account for the rapacious nature of some fishers and stocks continued to decline. Myers and Worm (2005) estimate that less than 10% of virgin biomass of predatory fish species remains worldwide. These are the species that are most regularly targeted by fishers, and are most highly prized at market. The FAO (1999) estimates that the "*great majority (69%) of important marine fish stocks are either fully exploited, overfished, depleted, or recovering*". For this situation to improve, for fishing industries to be preserved and for stocks to recover (or at least stabilise), new management models are required.

To create these management models, significant information is needed in terms of historical biomass levels, current biomass levels and recovery time of stocks where degradation has occurred. Furthermore, knowledge on the genetic structure of stocks is important to ensure breeding groups are clearly identified. But gaps in understanding of true stock sizes, genetic makeup of populations and the connectedness of populations, coupled with politically determined (rather than science based) exploitation decisions and lack of appropriate regulatory controls, meant that many such management efforts were ineffective.

Fish numbers and catches continued to decline. Such declines have most famously been observed in the Atlantic blue fin tuna stocks (see for example Dalton, 2005) or Canadian stocks of Atlantic cod (e.g. Myers et al, 1996)

Countries such as New Zealand have a national Quota Management Systems (QMS) where fishers own specific and tradeable quotas for target species. Theoretically, a QMS relies on accurate information of stock size and population make up to manage fish stocks. However, the reality of fisheries management is that such information is sketchy at best and a number of proxies have been developed to quantify stock sizes. Another issue that has been identified with QMS is that that no clear way has yet been devised to accurately target fish of a single species where nets, trawls or longlines are employed, to ensure only marketable size fish are captured in the case of all of the net-based capture methods, or to ensure no habitat destruction occurs where damaging fishing gears such as bottom trawling are employed. By-catch, dead loss, wastage and cheating are endemic in even the best managed and scrutinised fisheries (Roberts, 2007).

In New Zealand today, there are 628 stocks managed under the Quota Management System (QMS). Of these, there was only enough information to report status on 117 stocks in 2009 (MFish, 2009a), and of these 117, 38 (32%) were known to be below target stock levels. However, for 82% of stocks, insufficient data is held to even make an estimate of stock health (MFish, 2009a). This issue is compounded by the observation that fisheries managers do not make good use of all available stock assessment models. Johnson et al (2009) argued that the findings of molecular ecology are not actually used for ecological purposes very often while Magnuson (1991) describes the gaps in the management of fish species while undertaking studies of fisheries ecology. Heino and Godø (2002) discuss the role of fisheries as a selection pressure, and that the responses to fishery pressures include phenotypic variability in populations and this variability is linked to genetic changes. Law (2000) reports behaviour (especially fishing gear avoidance behaviour) and size and maturation changes in populations and also discusses that these changes can be measured genetically. Such rapid evolution has been observed in the lab (Reznick and Ghalambor, 2005) and the wild in some cases, most famously that

of heavily exploited Atlantic cod (*Gadus morhua*) populations, such changes have been linked to commercial and even local extinction of the species (Olsen et al, 2004). This situation is not totally lacking in hope, however. Conover and Munch (2002) report that when properly managed, many fish populations contain enough genetic variability to allow recovery to occur.

1.4 The Arripidae family

The Arripidae are a family of perciform fish confined to the Australian and New Zealand region. The family Arripidae is comprised of a single genus, *Arripis*, which has four species. Three of these species are found in Australian waters and two are found in New Zealand waters (Paulin, 1993). Approximate distributions are shown in Figure 1-2.

Arripis trutta (kahawai) and *A. xylabion* (Kermadec kahawai) were both added to the New Zealand QMS in 2004 (MFish, 2009b), and treated as a single species for management purposes. There are sound management reasons for this, as the species are very similar in appearance, character and habitat.

The New Zealand Ministry of Fisheries, in its most recent stock assessment analysis for the northern kahawai management region, KAH1 (Hartill, 2009) states:

> *"Based on the scenarios examined, it is likely that current spawning biomass is above B_{MSY}, but it is **uncertain how far above**. Current **assumed** removals are lower than almost all estimates of deterministic MSY. Combining this with the result that most estimates of current biomass are well above B_{MSY} it is **unlikely** that the stock will decline below B_{MSY} at current assumed catch levels, **given the model recruitment assumptions**."*(Emphasis added)

This paper, released in May of 2009, was an assessment of the stock status of *Arripis trutta* and *Arripis xylabion*, for the year 2006. Hartill's paper discusses the methods of estimating population sizes, for the purpose of assigning Total Allowable Commercial Catch (TACC), and finds that the biomass of the species was likely to be above maximum sustainable yield (B_{MSY}), but it was not known by how much. Another issue is that of the number of distinct populations of the

species. The 2009 fisheries plenary report states that "*on balance it seems possible that there are at least two stocks of kahawai (A. trutta) with New Zealand waters with centres of concentration around the Bay of Plenty and northern tip of the South Island. These two areas could be assumed to be separate for management purposes*". As will be shown in **Chapter** 3, there is good genetic evidence to suspect that this statement is incorrect.

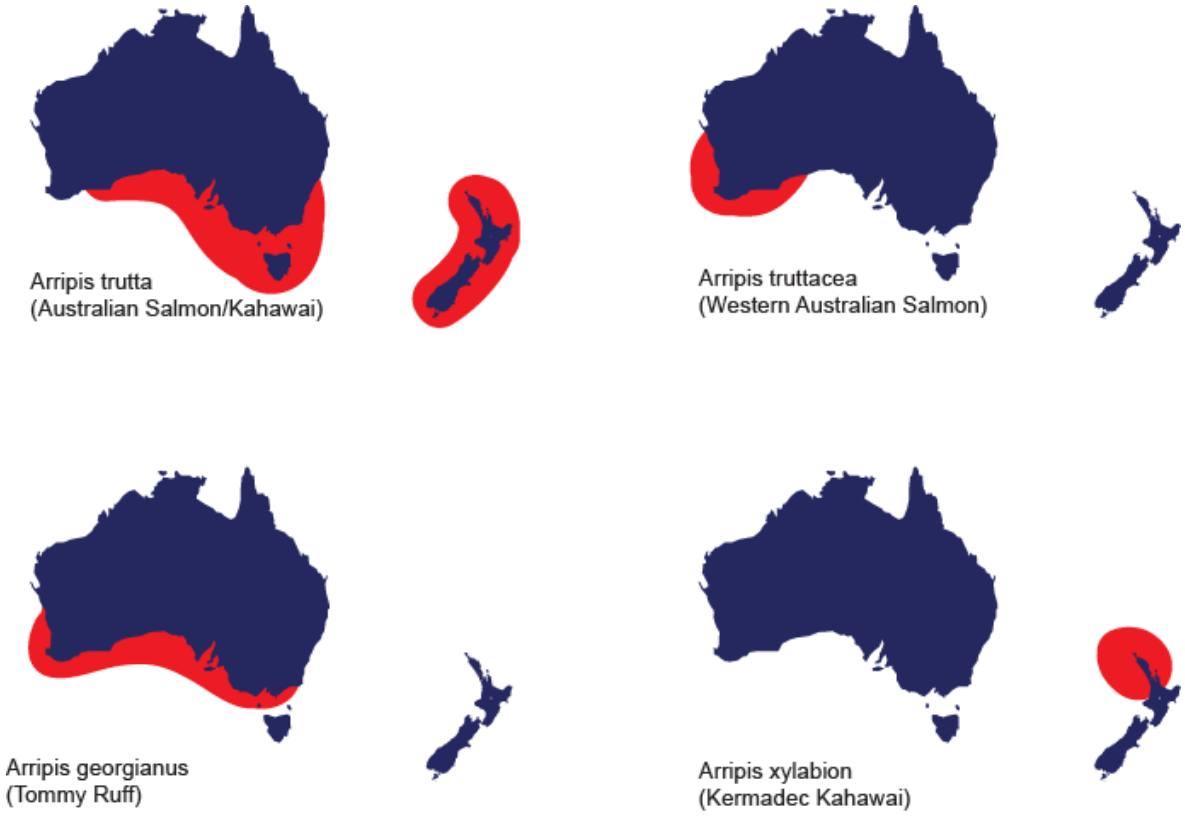


Figure 1-2: Approximate Arripidae Distribution

(Shaded areas show distributions)

A. trutta is the most well known species in both countries and is distributed all around New Zealand and through southern Australia, from Western Australia through to southern Queensland (Kingsford, 1989 and Paulin, 1993.).

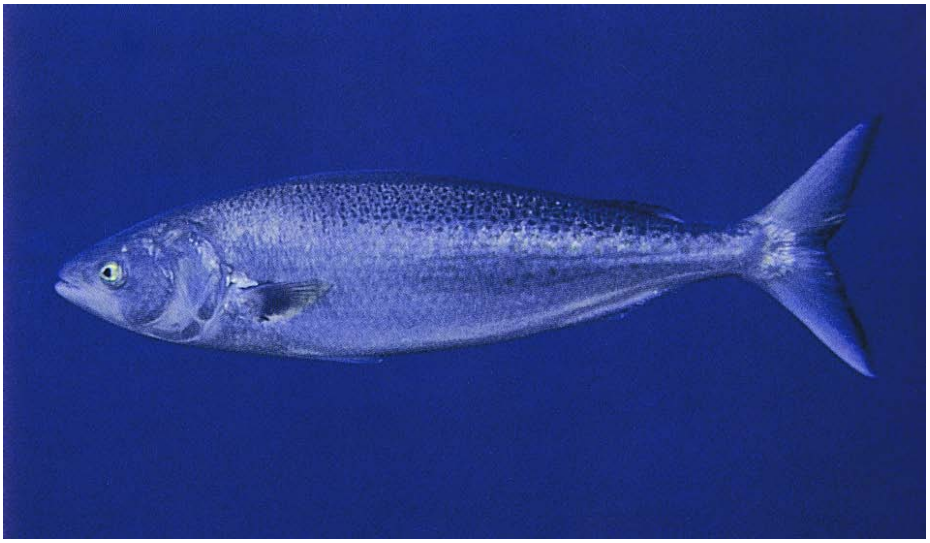


Figure 1-3: *Arripis trutta* (Francis, 2001)

A. xylabion, which has only been recorded from the northern parts of New Zealand, is the largest member of the genus, with anecdotal reports of fish of up to 900 mm being recorded. The most obvious physical difference between the two species is that the tail length of *A. xylabion* is > ⅓ of the body length of the fish. In *A. trutta*, this length is <⅓ of the body length. Other morphological differences are described in Paulin (1993). It was not possible to collect samples of this species during this study, and no sequences available in Genbank.

Figure 1-4: *Arripis xylabion* (Francis, 2001)

The third and smallest species of the family, *A. georgianus* is found throughout southern Australia. The maximum estimated length is 410 mm. The range of this species overlaps the southern Australian range of *A. trutta* and *A. truttacea* (Paulin, 1993. WA Dept of Fisheries, 2008).

Figure 1-5: *Arripis georgianus* (public domain)

The final species, *A. truttacea*, is confined to southern Western Australia. The maximum estimated fork length of this species is 850 mm (Paulin, 1993, WA Dept of Fisheries, 2008)

Figure 1-6: *Arripis truttacea (*WA Dept of Fisheries, 2008*)*

All species are morphologically very similar and are somewhat cryptic. In areas where more than one species is present misidentifications are common, especially when dealing with juvenile specimens (Hutchins and Swainston, 1986, Paulin, 1993, WA Dept of Fisheries, 2008, WA Dept of Fisheries, 2009a and WA Dept of Fisheries 2009b)

There are commercial fisheries in Australia and New Zealand for Arripidae (refer to Table 1-1), but all species are more sought after by recreational anglers for their fighting ability than their table qualities. *A. trutta*, and *A. xylabion* in New Zealand, and *A. trutta*, *A. truttacea* and *A. georgianus* in Australia are sought commercially and are primarily sought for the pet food and

fish meal markets (Lenanton and Potter, 1987), as the flesh loses quality rapidly after capture, making it unappealing at market. In each of the areas Arripidae are fished commercially, species are treated by fisheries managers as single contiguous populations. In New Zealand, *A. trutta* and *A. xylabion* are treated as a single species for commercial exploitation purposes (NZ Ministry of Fisheries, 2009) and appear to be included more in an effort to legitimise by-catch due to indiscriminate fishing methods such as purse trawling, than as an actual commercial venture to target the species. No genetic data is available to support these management decisions and decisions appear to be based on geographical and regulatory management considerations rather than observed biology of the fish. This behaviour of fisheries managers is not unique, as evidenced by the work of Reiss et al, 2009.

Historically, the species were an important food species for coastal Maori and early settlers in New Zealand (Anon, 1890). All members of Arripidae are popular recreational species, though more for their fighting ability (WA Dept of Fisheries, 2009) than their market desirability. While palatable when fresh, the flesh degrades quickly after capture and does not respond well to freezing. However, more desirable species are becoming more difficult to catch commercially internationally, so it is likely that demand for secondary species such *A. trutta* and the other members of the family will rise over the coming years (Roberts, 2007).

While very little information is held on the breeding and spawning behaviour of kahawai in New Zealand, it is known that they spawn in near shore areas or surf areas (Kailola et al, 1993, Neira et al, 1997, NSW DPI 2007), and data from Australia (Smith and Suthers, 1999, NSW DPI 2007) suggests they are serial spawners in the summer through to autumn months. In their larval stage, the fish exhibit a clearly planktotrophic form, which is shown in Figure 1-7 (Neira et al, 1997).

Growth rates are not known (Bradford, 2001) but it is known that fish mature at approximately 4 years of age and 390mm fork length (NSW DPI 2007) and can attain body lengths of 790 mm and body weights of 6.91kg in New Zealand, though larger fish have been reported anecdotally (Duffy and Petherick, 1999).

A. trutta are believed to live at up to 24 years of age, though fish recorded at this age show signs of senescence (Gauldie, 1998).

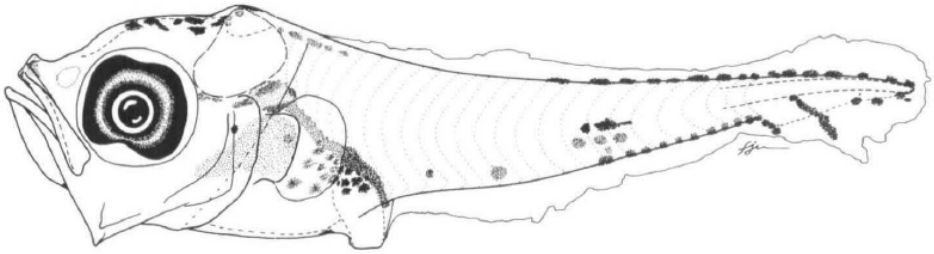


Figure 1-7: Larva of *A. trutta*. Body length = 3.7 mm (Neira at el, 1997)

Early in their life, *A. trutta* are planktivores, becoming active hunters as they grow larger (NSW DPI 2007). Kahawai are themselves predated on by other species at different stages in their life; planktivores when they are in their larval stage and by pelagic feeders including open water pelagic species such as marlin when mature (Baker, 1966). This suggests that kahawai migration is bimodal to some extent, with larvae following currents and adults actively travelling between feeding and breeding grounds.

Arripis trutta range from western South Australia, around the southern coast of Australia as far as southern Queensland and around the whole coast of New Zealand (NSW DPI, 2007, MFish 2009b), though the species is more common from the central South Island to the far north of the North Island. Intersecting the range of *A. trutta* are the three other members of the Arripidae family. *A. xylabion* or Kermadec kahawai (MFish 2009b, Paulin, 1993) is present from the Kermadec Islands, south to approximately Hawke's Bay, (Paulin, 1993). *A. georgianus* is present from central Western Australia, through to southern New South Wales (Paulin, 1993). The final member of the family, *A. truttacea* (also known as *A. truttaceus*), the Western Australian salmon, is present throughout temperate Western Australia to approximately the South Australian border (Paulin, 1993)

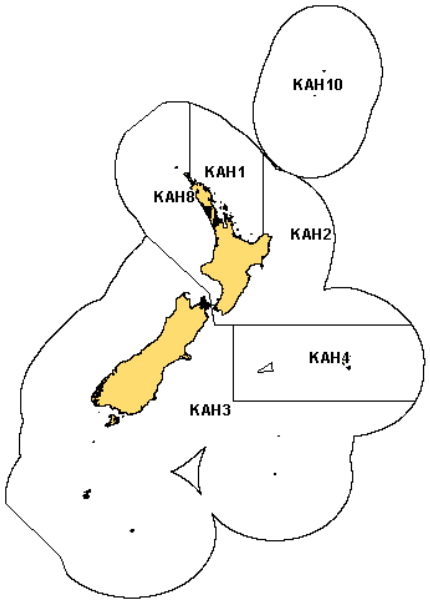


Figure 1-8: New Zealand Kahawai Management Areas (Hartill, 2009)

Like the rest of the Arripidae, *A. trutta* are exploited commercially and recreationally in Australia and New Zealand, and in New Zealand, there is also a customary take. In New South Wales (Australia), approximately 1,000 tonnes of *A. trutta* are caught per year (NSW DPI 2007). Current catch limits in New Zealand for *A. trutta* are shown in Table 1-1

Table 1-1: NZ *Arripis trutta* 2009 catch limits (New Zealand)

Total Allowable Commercial Catch (tonnes)	Recreational Catch (tonnes)	Customary Catch (tonnes)	Other mortality (e.g., by catch) (tonnes)	Total Allowable Catch (tonnes)
1,653	3,073	912	135	5,808

Source: MFish 2009b

Even though commercial catch limits for kahawai are small relative to other commercial species and when compared to recreational and customary catch limits, competition for the resource is contentious. The New Zealand Recreational Fishing Council challenged the right of the Fisheries Minister to increase the commercial take for kahawai in 2004 and 2005. This challenge was denied, which lead to a successful appeal (Court of Appeal, 2008). Further legal action by the Minister of Fisheries and commercial fishing interests led to the Supreme Court overturning this ruling in 2008. The Supreme Court found that the Minister did have the right to determine catch limits (Supreme Court,

2008). It is interesting to note that court records discuss the sustainable harvest of the species but make no mention of the biology of the species, or how sustainability is actually quantified. However, Ministry of Fisheries records (MFish 2009b) indicated that estimated spawning biomass of the species was in decline at the time of the decision. This is shown in Figure 1-9.

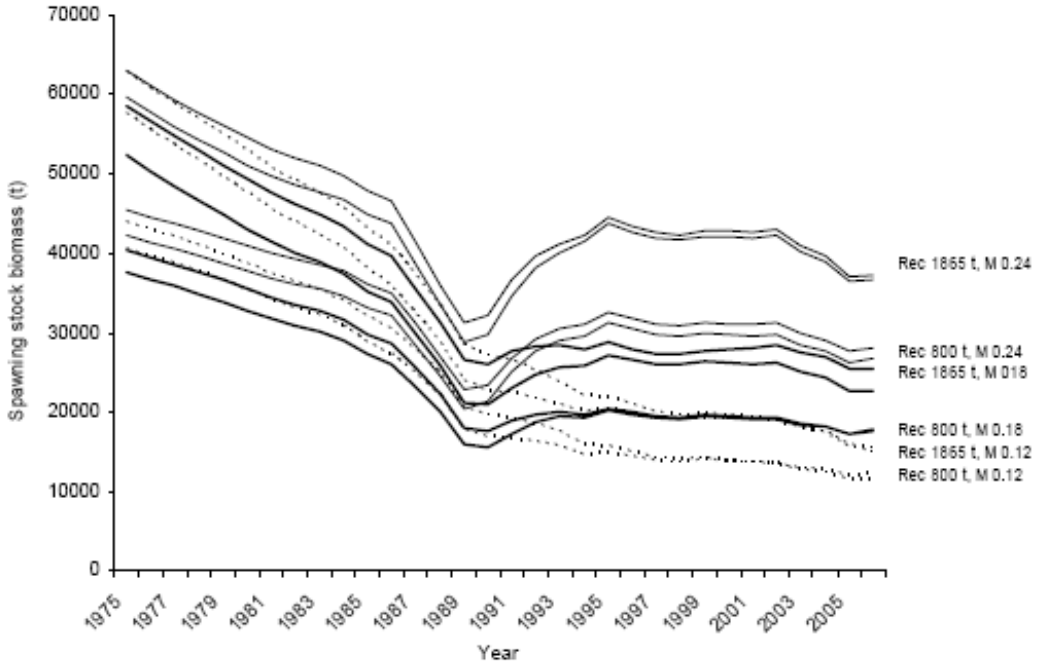


Figure 1-9: *A. trutta* spawning stock biomass projections (MFish, 2009b)

1.5 An introduction to tarakihi (*Nemadactylus macropterus*)

The tarakihi is a silver grey fish with a distinctive black band just behind the head. The fish grows to a maximum length of approximately 700 mm, though fish of 500 – 600 mm are far more common. The fish matures at about 4-6 years old and is believed to live to over 40 years in the wild. Tarakihi are found throughout New Zealand in water of between 100 and 500 metres. The current TACC for tarakihi is 6,439 tonnes and a combined traditional and recreational limit of 840 tonnes, though it is not known how much of this limit is actually taken. (MFish, 2010)

Figure 1-10: *Nemadactylus macropterus* (Froese and Pauly, 2010)

1.6 Aims of this thesis study

The aims of this study are to use mitochondrial DNA sequences to investigate the phylogenetic relationships of the Arripidae family in general and the phylogeographic structure of the species *A. trutta*. The family is something of a taxonomic orphan and even today, there is some degree of controversy over the proper taxonomic place of the family.

Using the genetic data gained, it is also my aim to provide some information on the stock(s) size and recent population history of *A. trutta* in New Zealand. The overall goal is to use population genetic data to better inform fisheries managers and fishery planning.

1.7 References

Akasaki, T, Yanagimoto, T, Yamakami, K, Tomonaga, H and Sato, S. *Species Identification and PCR-RFLP Analysis of Cytochrome b Gene in Cod Fish (Order Gadiformes) Products*. **Journal of Food Science**. 71, C190-C195. 2006.

Akyildiz, IF, Pompili, D and Melodia. T. *Underwater acoustic sensor networks: research challenges*. **Ad Hoc Networks**. Vol 3. Iss. 3. May. Pp 257-279. 2005.

Allen, JD and Pernet, B. *Intermediate modes of larval development: bridging the gap between planktotrophy and lecithotrophy*. **Evolution and Development**. Vol. 9. No. 6. Pp. 643 – 653. 2007

Anon. *The Fisheries of New Zealand*. **Science**. Pp. 140 - 142. Feb. 1890

Baker, AN. *Food of Marlins from New Zealand Waters*. **Copeia**. Vol. 1966. No. 4. Pp. 818-822. 1966

Bernales, SO, Burzio, LO, Engel, E, Martinez, R, Miquel, A, Villegas, J and Valenzuela, PDT. *The complete nucleotide sequence of the mitochondrial DNA genome of chinook salmon, Oncorhynchus tshawytscha.* **Unpublished** (accessed via Genbank)

Bertram, Douglas F., and Richard R. Strathmann. 1998. *Effects of Maternal and Larval Nutrition on Growth and Form of Planktotrophic Larvae*. **Ecology** 79:315–327

Borsa, PJC and Hoarau, G. *Global population structure of the deep-sea fish Beryx cf. splendens*. **Unpublished** (accessed from Genbank)

Brendtro, KS, McDowell, JR, Collette, BB and Graves, JE. *Molecular Phylogeny of the Trichiuroidea*. **Unpublished**. (Accessed from Genbank)

Burridge, CP. *Molecular phylogeny of Nemadactylus and Acantholatris (Perciformes: Cirrhitoidea: Cheilodactylidae), with implications for taxonomy and biogeography.* **Molecular Phylogenetics and Evolution**. 13 (1), 93-109. 1999.

Burridge, CP and Smolenski, AJ. *Molecular phylogeny of the Cheilodactylidae and Latridae. (Perciformes: Cirrhitoidea) with notes on taxonomy and biogeography*. **Molecular and Phylogentic Evolution**. 30 (1), 118-127. 2004.

Cardenas, L, Hernandez, CE, Poulin, E, Magoulas, A, Kornfield, I and Ojeda, FP. *Origin, diversification, and historical biogeography of the genus Trachurus (Perciformes: Carangidae)*. **Molecular Phylogenetics and Evolution**. 35 (2), 496-507. 2005.

Cardenas, L., Silva, AX, Ojeda, P, Magoulas, A and Poulin, E. *Genetic population structure in the Chilean jack mackerel, Trachurus murphyi (Nichols) across the South-eastern Pacific Ocean*. Unpublished

Catanese, G, Manchado, M and Infante, C. *Evolutionary relatedness of mackerels of the genus Scomber based on complete mitochondrial genomes: strong support to the recognition of Atlantic Scomber colias and Pacific Scomber japonicus as distinct species*. **Gene**. 452 (1), 35-43. 2010.

Carr, MH, Neigel, JE, Estes, JA, Andelman, S, Warner, RR, Largier, JL. *Comparing Marine and Terrestrial Ecosystems: Implications for the Design of Coastal Marine Reserves. Ecological Applications*. Vol. 13, No. 1, Supplement: **The Science of Marine Reserves**. February. Pp. S90-S107. 2003.

Carter, L, Garlick, RD, Sutton, P, Chiswell, S, Oien, NA and Stanton, BR. *Ocean circulation New Zealand*. **NIWA Chart Miscellaneous Series** No. 76. 1998

Chang, YS, Huang, FL and Lo, TB. *The complete nucleotide sequence and gene organization of carp (Cyprinus carpio) mitochondrial genome*. **Journal of Molecular Evolution**. 38 (2), 138-155. 1994

Chopelet, J, Waples, RS, and Mariani, S. *Sex change and the genetic structure of marine fish populations*. **Fish and Fisheries**. Vol. 10. Iss. 3. Pp 329–343. Sep. 2009.

Clover, C. *The End of the Line: How Overfishing is Changing the World and What We Eat*. **University of California Press, Berkley and Los Angeles**. 2006.

Coltman, DW. *Molecular ecological approaches to studying the evolutionary impact of selective harvesting in wildlife*. **Molecular Ecology.** Vol. 17. No. 1 Pp. 221 – 235. Jan. 2008.

Connell, JH. *Effects of competition, predation by Thais lapillus and other factors on natural populations of the barnacle Balanus balanoides*. **Ecological Monographs**. Vol. 31. Pp. 61-104. 1961

Conover, DO and Munch, SB. *Sustaining Fisheries Yields over Evolutionary Time Scales*. **Science**. Vol. 297. Pp. 94-96. 2002.

Craig, MT and Hastings, PA. *A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini*. **Ichthyological Research**. 54 (1), 1-17. 2007.

Dalton, R. *Satellite tags give fresh angle on tuna quota*. **Nature**. 434, 1056-1057. 28 April 2005.

Dayton, PK. *Competition, disturbance and community organization: the provision and subsequent utilization of space in a rocky intertidal community*. **Ecological Monographs**. Vol. 41. Pp. 351-389. 1971.

Deagle, BE, Jarman, SN, Pemberton, D and Gales, NJ. *Genetic screening for prey in the gut contents from a giant squid (Architeuthis sp.)*. **Journal of Heredity**. 96 (4), 417-423. 2005.

Diaz de Astarloa, JM, Zhang, J and Hanner, R. *DNA Barcoding Marine Fishes of Argentina*. **Unpublished**. (Accessed from Genbank)

Doiuchi, R. *Direct Submission*. Submitted 01-MAR-2005. (Accessed from Genbank)

Doiuchi, R and Nakabo, T. *Molecular phylogeny of the stromateoid fishes (Teleostei: Perciformes) inferred from mitochondrial DNA sequences and*

compared with morphology-based hypotheses. **Molecular Phylogenetics and Evolution**. 39 (1), 111-123. 2006.

Donnellan, SC, Barclay, A, McGlennon, D and Birrell, JA. *Population structure of Snapper, Pagrus auratus, in South Australian waters: evidence from allozymes and mitochondrial DNA*. Unpublished

Duartea, CM. *Marine ecology warms up to theory*. **Trends in Ecology & Evolution**. Vol. 22. Issue 7. July. Pp 331-333. 2007.

Duc, M, Karlsen, BO and Moum, T. *The mitochondrial genome of Salmo trutta, intraspecific variation, and comparison to other salmonid mitochondrial genomes*. Unpublished

Durrett, R and Kruglyak, S. *A New Stochastic Model of Microsatellite Evolution*. **Journal of Applied Probability**. Vol. 36, No. 3. Pp. 621-631. September. 1999

Ellis, R. *Tuna. Love, Death and Mercury*. Vintage Books. New York. 2008.

Espineira, M, Gonzalez-Lavin, N, Vieites, JM and Santaclara, FJ. *Development of a method for the genetic identification of flatfish species on the basis of mitochondrial DNA sequences*. **Journal of Agricultural and Food Chemistry**. 56 (19), 8954-8961. 2008.

Faber, JE and Stepien, CA. *The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae*. **Molecular Systematics of Fishes**. Academic Press, San Diego. Pp. 129 - 140. 1997.

FAO (Food and Agriculture Organization of the United Nations). *The state of world fisheries*. 1999 and aquaculture 1998. Food and Agriculture Organization of the United Nations, Rome. 1999

Francis, M. *Coastal Fishes of New Zealand. An identification guide*. Third Edition. Reed Publishing. Auckland. New Zealand. 2001

Froese, R. and Pauly D. Editors. 2010. *FishBase*. World Wide Web electronic publication. **www.fishbase.org**, version (05/2010).

Froese, R. and Pauly D. Editors. 2010. *FishBase*. World Wide Web electronic publication. **www.fishbase.org**, version (05/2010).

Fromentin J-M and Powers, JE. *Atlantic bluefin tuna: population dynamics, ecology, fisheries and management*. **Fish and Fisheries**. Vol. 6. Iss. 4. Pp 281–306. Dec. 2005.

Galtier, N, Nabholz, B, Glémin, S and Hurst, GDD. *Mitochondrial DNA as a marker of molecular diversity: a reappraisal*. **Molecular Ecology**. Vol. 18. Iss. 22. Pp. 4541 - 4550. Nov. 2009.

Gauldie, RW. *Vertebral, scale and otolith characteristics of senile kahawai, Arripis trutta: Implications for age estimation*. **Aquarium Sciences and Conservation**. Vol 2. Pp 53 - 66 (1998)

Gonzalez-Sevilla, R, Perez, S, Gonzalez, JA, Jimenez, S, Diez, A and Bautista, JM. Consortium PescaBase. *Pescabase: Genetic catalog of Spanish marine fish*. **Unpublished** (accessed from Genbank)

Gonzalez-Sevilla, R, Perez-Benavente, S, Biscoito, M, Diez, A. and Bautista, JM. *FishTrace: Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes (www.fishtrace.org)*. **Unpublished** (Accessed from Genbank)

Hartill, B. *Assessment of the KAH 1 fishery for 2006*. **New Zealand Ministry of Fisheries**. 2009. Accessed online at http://fs.fish.govt.nz/Doc/22265/Kahawai%20stock%20assessment.pdf.ashx, 24 May, 2010

Heino, M and Godø, OR. *Fisheries-induced Selection Pressures in the Context of Sustainable Fisheries*. **Bulletin of Marine Science**. Vol 70. No. 2. Pp. 639-656. 2002.

Hellberg, ME. *Gene flow and isolation among population of marine animals*. **Annual Review of Ecology, Evolution, and Systematics**. 40. 291 – 310. December 2009.

Hellberg, ME. Gene flow and isolation among population of marine animals. Annual Review of Ecology, Evolution, and Systematics. 40. 291 – 310. December 2009.

Horne, JB van Herwerden, L, Choat, JH, and Robertson, DR. *High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners*. **Molecular Phylogenetics and Evolution**. Vol. 49. Iss. 2. Nov. Pp 629-638. 2008.

Hsieh, CC, Tsai, CL, Chang, HW, Gwo, JC and Chou, YC. *Direct Submission*. Submitted 10-OCT-2006. (Accessed via Genbank)

Huckstorf, V, Lewin, W-C, Mehner, and Wolter, C. *Performance level and efficiency of two differing predator-avoidance strategies depend on nutritional state of the prey fish*. **Behavioral Ecology and Sociobiology**. Vol 63. No. 12. Pp 1735-1742. 2009.

Hutchings, JA and Baum, JK. *Measuring Marine Fish Biodiversity: Temporal Changes in Abundance, Life History and Demography*. **Philosophical Transactions: Biological Sciences**. Vol. 360, No. 1454. Pp.315-338. February. 2005.

Ishiguro, NB, Miya, M and Nishida, M. *Basal euteleostean relationships: a mitogenomic perspective on the phylogenetic reality of the 'Protacanthopterygii'*. **Molecular Phylogenetic Evolution**. 27 (3), 476-488. 2003.

Jerome, M, Lemaire, C, Bautista, JM, Fleurence, J and Etienne, M. *Molecular phylogeny and species identification of sardines*. **Journal of Agricultural and Food Chemistry**. 51 (1), 43-50. 2003.

Johnson, JB, Peat, SM and Adams, BJ. *Issues with molecular ecology and tying molecular findings back to ecology - Where's the ecology in molecular ecology?* **Oikos** Vol. 118: Pp. 1601 - 1609. 2009

Kadoyama, K, Ishizaki, S, Usui, K, Nagashima, Y, Shiomi, K and Watabe, S. *Complete mitochondrial DNA sequence of Xiphiidae and Istiophoridae species*. **Unpublished** (Accessed from Genbank)

Kailola, P Williams, MJ, Stewart, PC, Reuchelt, RE, McNee, A and Greeve, C. *Australian fisheries resources*. Bureau of Resource Sciences. Canberra. 1993

Kelly, RP and Palumbi, SR. *Genetic Structure among 50 Species of the Northeastern Pacific Rocky Intertidal Community*. **PLos One** 5(1): e8584. 2010

Kibbe WA. OligoCalc*: an online oligonucleotide properties calculator*. **Nucleic Acids Research**. 35 (webserver issue): May 25. 2007

Kingsford, M. *Distribution patterns of planktivorous reef fish along the coast of northeastern New Zealand*. **Marine Ecology Progress Series**. Vol. 54. Pp 13 – 24. Jun. 1989

Kocher, TD, Thomas, WK, Meyer, A, Edwards, SV, Pääbo, S, Villablanca, FX and Wilson, AC. *Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers*. **Proceedings of the National Academy of Sciences of the United States of America**. Vol 86. No. 16. Pp. 6196 - 6200. 1989.

Kona, T, Yoshinob, T, Mukaic, T and Nishidaa, M. *DNA sequences identify numerous cryptic species of the vertebrate: A lesson from the gobioid fish Schindleria*. **Molecular Phylogenetics and Evolution**. Vol 44. Iss. 1. Jul. Pp 53-62. 2007.

Law, R. *Fishing Selection and Phenotypic Evolution*. **ICES Journal of Marine Science**. Vol. 57. Pp. 659–669. 2000.

Lenanton, RCJ and Potter, IC. *Contribution of estuaries to commercial fisheries in temperate Western Australia and the concept of estuarine dependence*. **Estuaries**. Vol. 10. No. 1. Pp. 28 - 35. 1987

Lockridge Mueller, R. *Evolutionary Rates, Divergence Dates, and the Performance of Mitochondrial Genes in Bayesian Phylogenetic Analysis*. **Systematic Biology**. Vol. 55. No. 2. Pp. 289 - 300. 2006

Lowe, WH and Allendorf, FW. *What can genetics tell us about population connectivity?* **Molecular Ecology**. Vol 19. Iss. 15. Pp 3038–3051. Aug. 2010

Makris, NC, Purnima Ratilal, P, Jagannathan, S, Gong, Z, Andrews, M, Bertsatos, I, Godø, OR, Nero, RW, Jech, JM. *Critical Population Density Triggers Rapid Formation of Vast Oceanic Fish Shoals*. **Science**. March. Vol. 323. No. 5922. pp. 1734 – 1737. 2009

Magnuson, JJ. *Fish and Fisheries Ecology*. **Ecological Applications**. Vol. 1, No. 1. Pp. 13-26. Feb. 1991

Manchado, M, Catanese, G, Crespo, A and Infante, C. Direct Submission. 2003

Manchado, M, Infante, C and Catanese, G. *Complete mitochondrial DNA sequence of skipjack (Katsuwonus pelamis)*. **Unpublished**. (Accessed via Genbank)

Manchado, M, Catanese, G and Infante, C. *Complete mitochondrial DNA sequence of albacore (Thunnus alalunga)*. **Unpublished**. (Accessed from Genbank)

Martinez Ibarra, C, Ishizaki, S and Nagashima, Y. *Development of a PCR-based method for differentiation of tuna and related species in canned products*. **Unpublished**. (Accessed from Genbank)

McDonald, MA, Smith, MH, Smith, MW, Novak, JM, Johns, PE and De Vries AL. *Biochemical systematics of notothenioid fishes from Antarctica*. **Biochemical Systematics and Ecology**. 20:233-241. 1992.

MFish (2009a). New Zealand Ministry of Fisheries. *Stock Status Report*. 29 October, 2009. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=16, 24 May 2010.

MFish (2009b). New Zealand Ministry of Fisheries. *Kahawai 2009 Plenary Report KAH09*. June 2009. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=113&dk=21825, 24 May 2010

MFish 2010. *Tarakihi species description*. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=7&tk=153&sc=TAR, 10 September, 2010

Minegishi, Y, Aoyama, J, Inoue, JG, Miya, M, Nishida, M and Tsukamoto, K. *Molecular phylogeny and evolution of the freshwater eels genus Anguilla based on the whole mitochondrial genome sequences.* **Molecular Phylogenetics and Evolution**. 34 (1), 134-146. 2005.

Ministry of Fisheries (New Zealand). *www.fish.govt.nz*. Accessed online May, 2010.

Miya, M, Kawaguchi, and A Nishida, M. *Mitogenomic exploration of higher teleostean phylogenies: a case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences*. **Molecular Biological Evolution**. 18 (11), 1993-2009. 2001.

Miya, M, Takeshima, H, Endo, H, Ishiguro, NB, Inoue, JG, Mukai, T, Satoh, TP, Yamaguchi, M, Kawaguchi, A, Mabuchi, K, Shirai, SM and Nishida, M. *Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences*. **Molecular Phylogenetics and Evolution**. 26 (1), 121-138. 2003.

Moyle, PB and Cech, JJ. **An Introduction to Ichthyology**. 5th Edition. Pearson Benjamin Cummings. San Francisco. 2004.

Murakami, M and Takase, Y. *Direct Submission*. Submitted 09-JUN-2003. (Accessed via Genbank)

Myers RA, Hutchings, JA, Barrowman, NJ. *Hypothesis for the decline of cod in the North Atlantic*. **Marine Ecology Progress Series**. Vol 138: 293 -308. 1996.

Myers, RA and Worm, B. *Rapid worldwide depletion of predatory fish communities*. **Nature**. 423, 280-283. 15 May 2003.

Nee, S, Mooers, AO and Harvey PH. *Tempo and Mode of Evolution Revealed from Molecular Phylogenies*. **Proceedings of the National Academy of**

Sciences of the United States of America. Vol. 89, No. 17 pp. 8322-8326. September. 1992

Neira, FJ, Miskiewicz, AG and Bruce, BD. *Larvae of five fish families with pattern 10 of the Ramus Lateralis Accerrorius nerve (Arripidae, Girellidae, Kyphosidae, Microcanthidae and Scorpidae): Relevence to relationships*. **Bulletin of Marine Science**. Vol 60. No. 1. Pp. 117 - 138. 1997

New Zealand Court of Appeal. Court of Appeal Decision CA163/07 [2008] NZCA 160

New Zealand Supreme Court 2008. Supreme Court Decision SC 40/2008 [2008] NZSC 74

NSW DPI. New South Wales Department of Primary Industry. *Australian Salmon*. Status **of Fisheries Resources in NSW, 2006 / 2007**. December 2007

Nybakken, JW and Bertness, MD. *Marine Biology. An Ecological Approach*. Sixth Edition. Benjamin Cummings, San Francisco. 2004.

Olsen, EM, Heino, M, Lilly, GR, Morgan, MJ, Brattey, J, Ernande, B and Dieckmann, U. *Maturation trends indicative of rapid evolution preceded the collapse of northern cod*. **Nature**. Vol 428. 2004.

Oohara, I, Murata, Y, Kobayashi, T, Takashima, Y, Saruwatari, T and Hoshino, K. *Comprehensive determination of the partial base sequences of mitochondrial DNA cytochrome b based on the fish species name guideline*. (in) Yano, H. (Ed.); **DNA Polymorphism**. Vol. 15. Pp. 189 - 194. TOYO Shoten CO., LTD, Tokyo. 2007

Orrell, TM, Carpenter, KE, Musick, JA and Graves, JE. *Phylogenetic and Biogeographic Analysis of the Sparidae (Perciformes: Percoidei) from Cytochrome b Sequences*. **Copeia** 2002 (3), 618-631. 2002

Paine, RT. *Food web complexity and species diversity.* **American Naturalist**. Vol. 100. Pp. 65-75. 1966

Paulin, C. *Review of the Australian fish family Arripididae (Percomorpha), with the description of a new species*. **Australian Journal of Marine and Freshwater Research**. Vol. 44. No. 3. Pp. 459 - 471. 1993.

Peat, M and Adams, BJ. *Where's the ecology in molecular ecology?* **Oikos**. Vol. 118. Pp 1601 – 1609. 2009

Perez, M and Presa, P. *Validation of a tRNA-Glu-cytochrome b key for the molecular identification of 12 hake species (Merluccius spp.) and Atlantic Cod (Gadus morhua) using PCR-RFLPs, FINS, and BLAST*. **Journal of Agricultural and Food Chemistry**. 56 (22), 10865-10871. 2008.

Platnick, NI. *Gaps and Prediction in Classification*. **Systematic Biology**. Vol. 27, Iss 4. pp. 472-474. 1978.

Probst and Crawford. *Population Characteristics and Planktonic Larval Stage of the New Zealand Screwshell Maoricolpus roseus*. **Journal of Molluscan Studies**. Vol. 74. Pp. 191 – 197. 2008

Quince, C, Abrams, PA, Shuter, BJ and Lester, NP. *Biphasic growth in fish I: Theoretical foundations*. **Journal of Theoretical Biology**. Vol. 254. Iss. 2. Pp 197-206. 2008.

Reaka-Kudla, ML. *Biodiversity II. Understanding and Protecting our Biological Resources*. Chapter 7 Reaka-Kudla, ML, Wilson DE, Wilson EO editors. Joseph Henry Press, Washington DC. 1997

Reiss, H, Hoarau, G, Dickey-Collas, M and Wolff, WJ. *Genetic population structure of marine fish: mismatch between biological and fisheries management units*. **Fish and Fisheries**. Vol 10. Pp. 361 - 395. 2009.

Reznick, DN and Ghalambor, CK. *Can commercial fishing cause evolution? Answers from guppies (Poecilia reticulata)*. **Canadian Journal of Fisheries and Aquatic Sciences**. Vol. 62. Pp. 791–801. 2005.

Riginos, C and Victor, BC. L*arval Spatial Distributions and Other Early Life-History Characteristics Predict Genetic Differentiation in Eastern Pacific*

Blennioid Fishes. **Proceedings: Biological Sciences**. Vol. 268, No. 1479. Pp. 1931-1936. 2001

Ritchie, PA, Bargelloni, L, Meyer, A, Taylor, JA, Macdonald, JA and Lambert, DM. *Mitochondrial phylogeny of trematomid fishes (Nototheniidae, Perciformes) and the evolution of Antarctic fish*. **Molecular Phylogenetics and Evolution**. 5 (2), 383-390. 1996.

Roberts, C. *The Unnatural History of the Sea*. Island Press, Washington DC. 2007.

Roberts, C. *The Unnatural History of the Sea*. **Island Press, Washington DC**. 2009.

Robinson, NA, Skinner, A, Sethuraman, L, Mcpartlan, HC, Murray, N, Knuckey, I, Smith, DC, Hindell, J and Talman, S. *Gene flow of blue-eye trevalla (Hyperoglyphe antarctica) and the warehous (Seriolella brama and Seriolella punctata) around south-eastern Australian and New Zealand waters*. **Unpublished** (Accessed from Genbank)

Rozen, S and Skaletsky, HJ. *Primer3 on the WWW for general users and for biologist programmers*. In: Krawetz S, Misener S (eds) **Bioinformatics Methods and Protocols: Methods in Molecular Biology**. Humana Press, Totowa, NJ, pp 365-386. 2000. Source code available at http://fokker.wi.mit.edu/primer3/

Sevilla, RG, Amalia, D, Norén, M, Mouchel, O, Jérôme, M, Verrez-Bagnizm V, Van Pelt, H, Favre-Krey, L, and Krey, G. *Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes*. **Molecular Ecology Note**s. Vol 7. Iss. 5. Pp 730–734. September. 2007

Silva, A, Cardenas, L, Ojeda, FP and Poulin, E. *Cytochrome b sequence of Chilean jack mackerel, Trachurus murphyi*. Unpublished

Smith, KA and Suthers, IM. *Displacement of diverse ichthyoplankton assemblages by a coastal upwelling event on the Sydney shelf*. **Marine Ecology Progress Series**. Vol. 176. Pp. 49 - 62 Jan. 1999

Smith, WL and Wheeler, WC. *Venom evolution widespread in fishes: a phylogenetic road map for the bioprospecting of piscine venoms*. **Journal of Heredity**. Vol. 97 No. (3). Pp 206 - 217. 2006

Smith, WL and Craig, MT. *Casting the percomorph net widely: The importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes*. **Copeia**. Vol. 1. Pp. 35 - 55. 2007

Snoke Smith, M, Zigler, KS and RA Raff. *Evolution of direct-developing larvae: selection vs loss*. **BioEssays**. Vol. 29. Iss. 6. Pp 566–571. Jun. 2007

Steinke, D, Zemlak, TS, Gavin, H and Hebert, PDN. *DNA barcoding fishes of the Canadian Pacific*. **Marine Biology**. Vol. 156 No. 12. Pp. 2641 - 2647. 2009

Takashima, Y, Namikoshi, A, Morita, T and Yamashita,M. *Molecular identification of yellowtail species*. **Unpublished** (Accessed from Genbank)

Takashima, Y, Morita, T and Yamashita, M. *Complete mitochondrial DNA sequence of Atlantic horse mackerel Trachurus trachurus and molecular identification of two commercially important species T. trachurus and T. japonicus using PCR-RFLP*. **Fisheries Science**. 72, 1054-1065. 2006

Te Papa Tongarewa Museum of New Zealand. *Biosystematics of New Zealand Fishes Project*. Available at: http://www.tepapa.govt.nz/ResearchAtTePapa/Research/NaturalEnvironment/Fish/ResearchProjects/EEZFishes/Pages/default.aspx. Collected online, 25 August 2010.

Todd, CD, Lambert, WJ and Thorpe, JP. *The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae "for" dispersal?* **Journal of Experimental Marine Biology and Ecology**. Vol. 228. Iss. 1. Pp. 1-28. Oct. 1998

Turcotte, MM, Pires, MN, Vrijenhoek, RC and Reznick, DN. *Pre- and post-fertilization maternal provisioning in livebearing fish species and their hybrids (Poeciliidae: Poeciliopsis)*. **Functional Ecology**. Vol. 22. Iss. 6. Pp 1118–1124. Dec. 2008

Vandeperre, F and Methven, DA. *Do bigger fish arrive and spawn at the spawning grounds before smaller fish: Cod (Gadus morhua) predation on beach spawning capelin (Mallotus villosus) from coastal Newfoundland*. **Estuarine, Coastal and Shelf Science**. Vol. 71. Iss. 3-4. Pp 391 – 400. Feb. 2007.

Wang, S, Bao, Z, Zhang, L, Li, N, Zhan, A, Guo, W, Wang, X and Hu, J. *A new strategy for species identification of planktonic larvae: PCR–RFLP analysis of the internal transcribed spacer region of ribosomal DNA detected by agarose gel electrophoresis or DHPLC*. **Journal of Plankton Research**. Vol. 28. Iss. 4. Pp. 375-384. 2006.

Wang, C, Chen, Q, Lu, G, Xu, J, Yang, Q and Li, S. *Complete mitochondrial genome of the grass carp (Ctenopharyngodon idella, Teleostei): insight into its phylogenic position within Cyprinidae*. **Gene** 424 (1-2), 96-101. 2008.

WA Dept of Fisheries. *How to identify salmon and herring*. Western Australian Department of Fisheries. July 2008.

WA Dept of Fisheries 2009a *Recreational Fishing Guide, South Coast Region*. Western Australian Department of Fisheries. January 2009.

WA Dept of Fisheries 2009b. *Species Identification Guide. West Coast Region*. Western Australian Department of Fisheries. 2009.

Ward, P and Myers, RA. *Shifts in Open-Ocean Fish Communities Coinciding with the Commencement of Commercial Fishing*. **Ecology** 86:835–847. 2005.

Ward, RD, Costa, FO, Holmes, BH and Steinke, D. *DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence*

for most taxa but Zeus faber and Lepidopus caudatus each probably constitute two species. **Aquatic Biology**. 3, 71-78. 2008.

Ward, RD and Holmes, BH. *An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (cox1) in fishes*. **Molecular Ecology Notes**. 7 (6), 899-907. 2007.

Ward, RD and Holmes, BH. Direct Submission. 2007.

Ward, RD, Zemlak, TS, Innes, BH, Last, PR and Hebert, PDN. Direct Submission. 2005

Ward, RD, Zemlak, TS, Innes, BH, Last, PR and Hebert, PD. *DNA barcoding Australia's fish species*. **Philosophical Transactions. Royal Society of London. B Series. Biological Science**. 360 (1462), 1847-1857. 2005.

Westneat, MW and Alfaro, ME. *Phylogenetic relationships and evolutionary history of the reef fish family Labridae*. **Molecular and Phylogenetic Evolution**. 36 (2), 370-390. 2005.

Wilcox, TP, Garcia de Leon, FJ, Hendrickson, DA and Hillis, DM. *Convergence among cave catfishes: long-branch attraction and a Bayesian relative rates test*. **Molecular Phylogenetic Evolution**. 31 (3), 1101-1113. 2004.

Yancy, HF, Zemlak, TS, Mason, JA, Fry, FS, Randolph, SC and Herbert, PD. *Potential Use of DNA Barcodes in Regulatory Science: Applications of the Regulatory Fish Encyclopedia*. Unpublished

Chapter 2. Phylogeny of the Arripidae

2.1 Abstract

A number of studies have been undertaken in recent years that have placed Arripidae with either Khyphosidae, Stromateoidei or Scombridae. These studies have been based on physical characteristics such as the RLA10 facial nerve as well as genetic characteristics. However, molecular studies based on the ND2 gene have provided an alternative taxonomy for the family, placing it in a clade encompassed by Stromateoidei and Scombridae alone. In this study, 12S, 16S and COX1 sequence data was compared with other species to test the phylogenic relationships of Arripidae.

While inconclusive due to experimental issues, some support for the Scombridae – Stromateoidae – Arripidae clade was found.

2.2 Introduction

The Arripidae family presents something of an enigma to taxonomists. The family consists of a single genus, *Arripis*, which contains four species, *Arripis trutta*, *A. xylabion*, *A. georgianus* and *A. truttacea.* The evolutionary relationships of the family to other members of the Perciformes are not entirely clear. Arripidae had been placed in a clade encompassed by the Khyphosidae (Nelson, 1994 and Johnson and Fritzsche, 1989), based on the unique pattern 10 of the ramus lateralis accessorius (RLA 10) facial nerve. It was believed that this pattern was so unique that it was unlikely to have evolved more than once, and therefore supported a single ancestor.

Recent molecular work has challenged these earlier morphological phylogenies, however. Yagishita et al (2002) used the mitochondrial ND2 gene and have demonstrated that this monophyly can not be supported on genetic grounds. Still later work by Yagishita et al (2009) has placed the Arripidae in a clade encompassed by Scombridae and Stromateoidei (shown in Figure 2-1) based on their 12n3RTn dataset, a finding they described as "unexpected". They resolved the earlier, apparent conflict of monophyly with the Khyphosidae by proposing that the RLA 10 facial nerve pattern had in fact evolved at least twice,

with a common ancestor for Khyphosidae, Terapontidae, Kuhliidae and Oplegnathidae and a second common ancestor for Arripidae and Stromateoidei.

In this study, I investigated phylogenies in the manner of Yagishita et al (2002) and Yagishita et al (2009), but instead of ND2, my study was based on the mitochondrial COX1, 12S and 16S genes. To undertake these tests, “universal” primers, targeting highly conserved areas of these genes, were used.

Mitochondrial genes were selected as they are relatively easy to extract, amplify and sequence in the laboratory (Kocher et al, 1989) and the use of these genes is near-ubiquitous in studies of this nature. Mitochondrial DNA is typically clonal and under neutral selection pressure and offers regions that are both slowly and rapidly evolving (Faber and Stepien, 1997) and it has been described as "clock-like", providing the opportunity to estimate the time of divergence from a most recent common ancestor.

Galtier et al (2009) point out that the very ubiquity of MtDNA use leads to potential bias in studies as all genes sequences have not proven to be as "clock-like" as originally thought, less clonal and "far from neutrally evolving" (Galtier et al, 2009). With these limitations in mind, the MtDNA genes selected for this study were the COX1, 12S and 16S regions of the mitochondrial genome.

2.3 Materials and methods

Use of published sequences

Published 12S, 16S and COX1 sequences for 20 marine fish species were downloaded from Genbank. Accession details for these sequences are shown in Table 2-1. Published data exists for each of the species of interest for COX1, 12S and 16S, however no 12S data has been published for a small number of species. Where it was necessary to generate additional 12S sequences in the laboratory, DNA was extracted from flesh or fin samples from fish captured by the author or donated by recreational or commercial fishers. Table 2-1 identifies such extracted samples with the term “This study” in the Accession Number and Reference columns. Where recreational anglers volunteered to collect samples, instructions were provided to ensure good sample preservation. After tissue

was excised from the fish, it was immediately placed in 70% ethanol and then stored at 4°C.

Primer generation, DNA extraction, mitochondrial DNA amplification and sequencing

DNA was extracted from muscle tissue and fin clippings using the proteinase K / phenol chloroform method. After extraction, DNA was suspended in TE buffer and stored at 4°C.

The 12S rRNA mitochondrial DNA gene was amplified using the polymerase chain reaction (PCR). One microlitre of DNA extract was added to a PCR master mix that consisted of 2.5 µL of 10x reaction buffer, 1 µL of BSA, 1 µL of dNTPS, 1 µL of forward primer (12SB (H1478). TGA CTG CAG AGG GTG ACG GGC GGT GTG T), 1 µL of reverse primer (12SA (L1091). AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT), 0.75 µL of $MgCl_2$, 16.55 µL of ddH_2O and 0.2 µL of BioTAQ. PCR cycler conditions were an initial denaturing for two minutes at 95°C followed by 38 cycles of 20s at 95°C, 60s at 54°C, 60s at 72°C, with a final extension step of five minutes at 72°C.

Following PCR, the amplified products were run on an agarose gel then stained with ethidium bromide to confirm that the amplification had been successful. Successful PCR products were then purified using ExoSAP following the manufacturer's instructions and sent to Massey University Genome Service (MUGS) for sequencing using the Big Dye system.

After sequencing, the resultant sequence files were inspected and read errors repaired by eye. Alignments were then performed using ClustalW (Higgins et al, 1994), in Mega4.

Phylogeny

Phylogenetic analysis was performed using MEGA4 at three loci; COX1, 12S and 16S. Sequence information was collected from Genbank (refer to Table 2-1 below) for the other species encompassing the known Stromateoidei / Arripidae Scombridae clade and from sequence data obtained in the laboratory as part of this study. As 12S and 16S sequences vary across species, direct alignment via

CLUSTAL were not practical, therefore after an initial alignment via CLUSTAL, gaps were removed heuristically using the deletion function in Mega4.

Table 2-1: Species Data from Genbank and Laboratory

Species	Loci used	Accession Number	Pubmed / publication
Dactyloptena peterseni	COX1, 12S, 16S	NC_003194	11606696
Dactyloptena tiltoni	COX1, 12S, 16S	NC_004402	12470944
Acanthogobius hasta	COX1, 12S, 16S	NC_006131	15246526
Rhyacichthys aspro	COX1, 12S, 16S	NC_004414	12470944
Eleotris acanthropoma	COX1, 12S, 16S	NC_004415	12470944
Scomber scombrus	COX1, 12S, 16S	NC_006398	Takashima et al, 2006
Arripis trutta	COX1 16S 12S	AB205452 AB205430 This study	16314116 16314116 This study
Arripis georgianus	COX1 12S 16S	EF609289 DQ533158 DQ532841	Ward and Holmes, 2007 Smith and Wheeler, 2006 Smith and Wheeler, 2006
Arripis truttacea	COX1 16S 12S	EF609291 EU848429 This study	Ward and Holmes, 2007 19317847 This study
Hyperoglyphe japonica	COX1, 12S, 16S	NC_013149	19540351
Cubiceps pauciradiatus	COX1, 12S, 16S	NC_013150	19540351
Psenes cyanophrys	COX1, 12S, 16S	NC_013144	19540351
Thunnus alalunga	COX1, 12S, 16S	NC_005317	Unpublished
Thunnus thynnus	COX1, 12S, 16S	NC_004901	1670579
Auxis rochei	COX1, 12S, 16S	NC_005313	18464037
Auxis thazard	COX1, 12S, 16S	NC_005318	18464037
Euthynnus alletteratus	COX1, 12S, 16S	NC_004530	Unpublished
Katsuwonus pelamis	COX1, 12S, 16S	NC_005316	Unpublished
Nemadactylus macropterus	COX1 12S 16S	AF092153 This study EU848457	15022763 This study 19317847
Polymixia japonica (Outgroup)	COX1, 12S, 16S	NC_002648	11133198

2.4 Results

Nucleotide composition and average pairwise distances

Nucleotide compositions are shown in tables 2-2, 2-3 and 2-4 below. Average pairwise distances were 0.200 0for COX1, 0.791 4 for 12S and 1.233 6 for 16S.

Table 2-2: COX1 Nucleotide Composition

Species	T (%)	C (%)	A (%)	G (%)
D. peterseni	28.5	29.4	22.5	19.6
D. tiltoni	26.9	30.7	23.9	18.5
A. hasta	30.7	27.1	24.1	18.1
R. aspro	29.4	28.2	23.8	18.6
E. acanthopoma	28.0	29.6	23.9	18.5
S. scombrus	29.1	30.0	23.4	17.6
H. japonica	30.2	28.2	24.3	17.4
C. pauciradiatus	29.8	28.9	24.1	17.2
P. cyanophrys	30.3	26.9	25.6	17.2
T. alalunga	29.6	28.3	23.9	18.1
T. thynnus thynnus	29.6	28.3	23.9	18.1
A. rochei	30.2	28.5	23.0	18.3
A. thazard	29.4	29.3	23.2	18.1
E. alletteratus	29.8	28.9	22.7	18.6
K. pelamis	31.1	27.6	23.0	18.3
A. trutta	31.8	26.9	25.2	16.1
A. truttacea	31.8	26.9	25.6	15.7
A. georgianus	30.0	29.6	22.5	17.9
P. japonica	30.5	27.4	24.1	17.9

Table 2-3: 12S Nucleotide Composition

Species	T (%)	C (%)	A (%)	G (%)
D. peterseni	22.7	23.8	32.5	21.0
D. tiltoni	23.0	22.4	32.8	21.8
A. hasta	21.7	22.0	34.4	22.0
R. aspro	21.0	24.6	33.1	21.3
E. acanthopoma	20.6	25.3	33.4	20.6
S. scombrus	21.7	25.2	31.9	21.2
H. japonica	21.9	26.3	31.2	20.5
C. pauciradiatus	20.7	26.0	31.8	21.5
P. cyanophrys	20.5	25.8	31.6	22.2
T. thynnus thynnus	21.8	25.7	30.7	21.8
A. rochei	23.5	25.9	26.9	23.7
A. thazard	23.5	25.6	27.2	23.7
E. alletteratus	23.7	25.6	27.2	23.5
K. pelamis	23.2	26.1	26.9	23.7
T. alalunga	23.5	25.4	27.5	23.5
A. georgianus	25.1	21.7	28.6	24.6
A. trutta	22.8	24.3	29.4	23.5
P. japonica	21.2	24.9	31.4	22.5

Table 2-4: 16S Nucleotide Composition

Species	T (%)	C (%)	A (%)	G (%)
D. peterseni	26.0	29.7	28.3	16.0
D. tiltoni	26.7	28.8	28.1	16.5
A. hasta	28.5	28.5	26.5	16.5
R. aspro	25.8	31.6	25.3	17.4
E. acanthopoma	25.5	31.6	26.2	16.7
S. scombrus	26.5	31.8	25.8	16.0
H. japonica	27.1	31.1	25.1	16.7
C. pauciradiatus (	30.2	27.8	26.7	15.3
P. cyanophrys	28.1	30.2	25.8	16.0
T. thynnus thynnus	26.0	32.5	25.3	16.2
A. rochei	22.0	25.8	30.4	21.8
A. thazard	22.0	25.8	30.2	22.0
E. alletteratus	22.0	25.5	30.9	21.6
K. pelamis	22.5	25.5	30.4	21.6
A. georgianus	22.3	24.4	31.1	22.3
A. truttacea	22.3	25.3	30.6	21.8
A. trutta	22.3	25.3	30.6	21.8
T. alalunga	21.8	25.5	31.1	21.6
P. japonica	22.0	25.3	31.8	20.9

Sequences resolved

As shown in Table 2-1, a 385bp sequence of the 12S region was resolved for three species and downloaded from Genbank for a further 17 species. Sequences of lengths of 547bp and 550bp were downloaded from Genbank for the COX1 and 16S regions respectively, for all 20 species investigated.

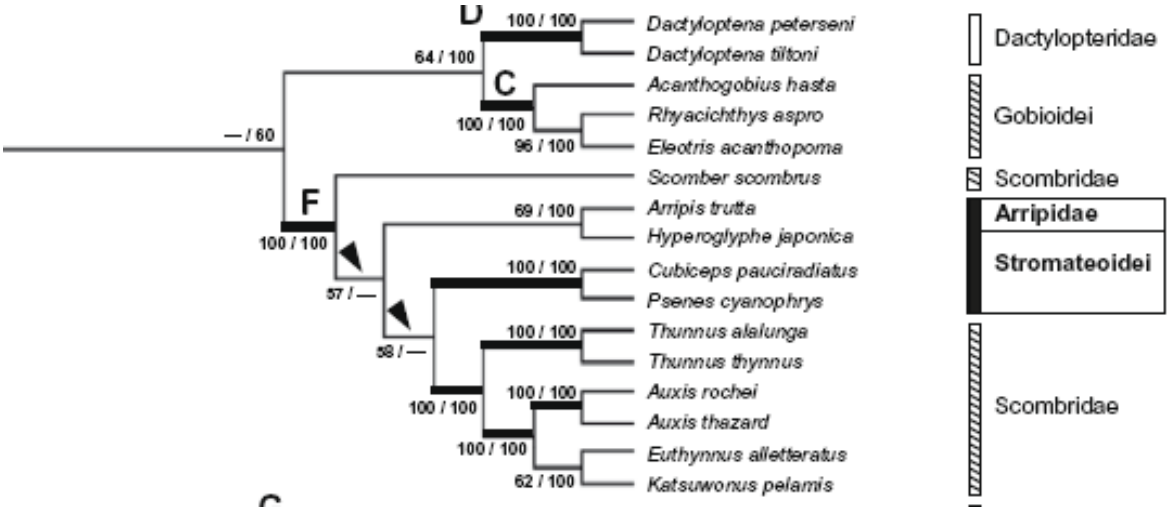


Figure 2-1: ND2 Phylogeny of *Arripis trutta* (from Yagishita et al, 2009)

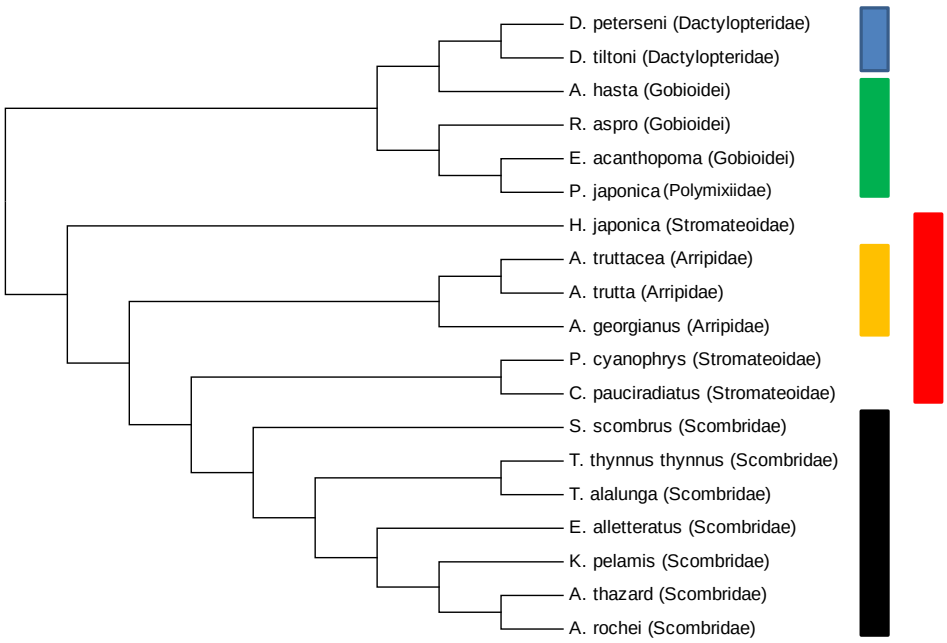


Figure 2-2: Maximum likelihood phylogeny of *Arripis trutta*, COX1 data
Monophyly demonstrated

The resolved COX1 phylogeny shown in Figure 2-2 suggests that Arripidae form a monophyletic clade with Scombridae and Stromateoidae. This supports the findings of Yagishita et al (2002) and Yagishita et al (2009). Divergence time from Arripidae to the most recent common ancestor with Scombridae / Stromateoidae was estimated to be 2.8 million years (Tamura and Nei, 1993 and Bowen et al, 2006).

However, the situation is a little less clear when 12S and 16S data was reviewed. Neighbour joining trees were created for 12S and 16S, and while the *Arripis* species tested formed a clade in each case, when Scombridae and Stromateoidae were included monophyly could not be demonstrated. These additional results are shown in Figure 2-3 and Figure 2-4. Even after a number of heuristic deletions were performed; it was not possible to obtain a clearer indication of the taxonomic relationship of the genus.

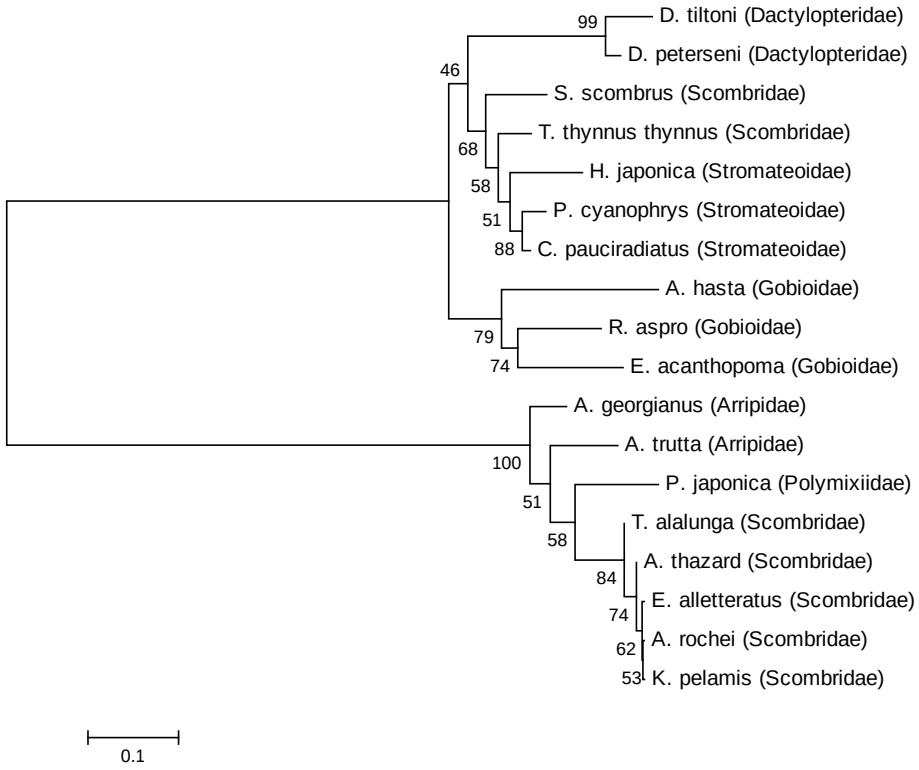


Figure 2-3: 12S Phylogeny of *Arripis trutta*,

Neighbour joining, bootstrap, 1000 replicates

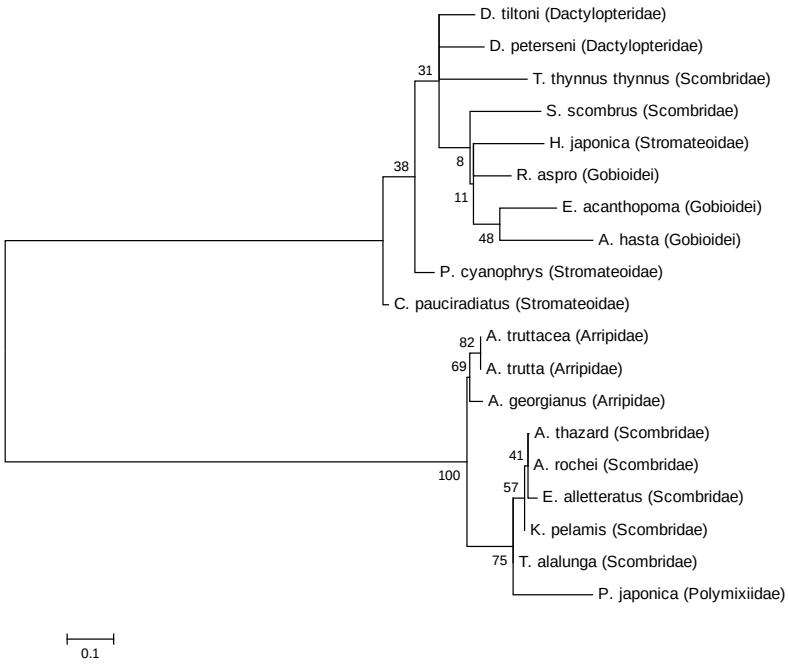


Figure 2-4: 16S phylogeny of *Arripis trutta*

Neighbour joining, bootstrap, 1000 replicates

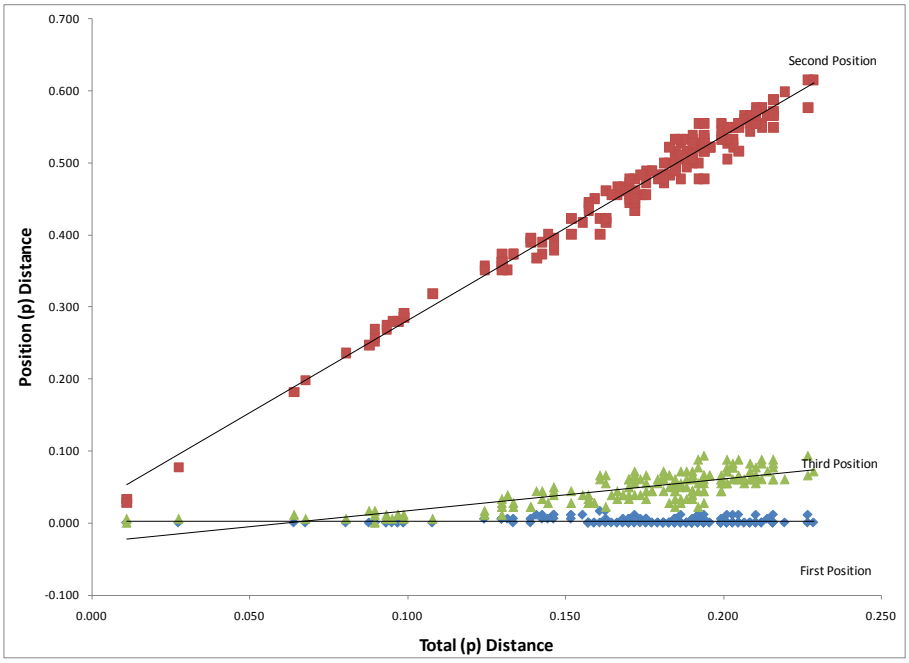


Figure 2-5: Uncorrected pairwise COX1 distances. Partitioned by codon position (182 codons analysed). Each point represents one pair of taxa appearing in the phylogeny.

Uncorrected genetic distances (P) between pairs of COX1 sequences for all taxa ranged between 1.1% and 22.9%, suggesting some degree of saturation (Perkins and Schall, 2002). This was tested by plotting total (P) distance against position (P) distance (refer to Figure 2-5) While the second and third positions show linear increases relative to total divergence, the first position does not.

This provides some evidence of base saturation, suggesting the estimated of divergence time of 2.8 million years is likely to be an underestimate (Jansa et al, 2006).

2.5 Summary

The taxonomic position of the Arripidae has been examined by a relatively small number of researchers in recent years, and that almost incidentally. Morphological studies such as those of Nelson (1994) and Johnson and Fritzsche (1989) focused on the unique pattern 10 of the RLA facial nerve, a finding that was challenged by Yagishita et al (2002) and Yagishita et al (2009). The finding of these studies was that the RLA10 pattern had most likely evolved more than once. This finding meant that the phylogenetic position of the Arripidae was known with more certainty, but that the family most likely formed a monophyletic clade with the Scombrids and the Stromateoids. Seeking support (or otherwise) for this finding was the main goal of this chapter. However, no strong result could be demonstrated.

This was partially because the “universal” primers did not deliver expected results in this series of experiments. Sequences were not able to be generated with Cytochrome B and D-loop (control) region primers at all, and *A. truttacea* sequences were not able to be generated utilising 12S primers. A number of possible reasons exist for this failure, from reagent contamination to experimental error. Efforts to determine the cause and resolution of the issue were not successful and applicability of universal primers to New Zealand species could not be demonstrated.

The resolved COX1 phylogeny did agree with the ND2 results obtained by Yagishita et al (2002) and Yagishita et al (2009), providing some support for the taxonomy advanced in these two studies, however it is clear from the conflicting 12S and 16S results that questions remain over the actual taxonomic

relationship of Arripidae to its putative sister families, and the evolutionary history of the family.

The finding that the estimated divergence time of 2.8 million years is likely to be an underestimate was not surprising given the uncertainty over the relationship of the family to other Perciformes. Even at a gross morphological level, while all of the Arripidae are alike to the point of near-crypsis, they are quite unlike any of their supposed near relatives in most respects. Clearly far more work needs to be done on this family of marine fish to settle the question of taxonomic relationships.

2.6 References

Aris-Brosou, S and Excoffier, L. *The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism*. **Molecular Biology and Evolution**. Vol. 13. Iss. 3. Pp. 494 - 504. 1996

Broughton, RE and Reneau, PC. *Spatial covariation of mutation and nonsynonymous substitution rates in vertebrate mitochondrial genomes*. **Molecular Biology and Evolution**. Vol. 23. No. 8. Pp. 1516 – 1524. 2006

Burridge, CP and Smolenski, AJ. *Molecular phylogeny of the Cheilodactylidae and Latridae (Perciformes: Cirrhitoidea) with notes on taxonomy and biogeography*. **Molecular Phylogenetics and Evolution**. Vol. 30. No. 1. Pp. 118 – 127. 2004

Catanese, G., Infante, C and Manchado, M. *Complete mitochondrial DNA sequences of the frigate tuna Auxis thazard and the bullet tuna Auxis rochei*. **DNA Sequencing**. Vol. 19. No. 3. Pp. 159 – 166. 2008

Deagle, BE, Kirkwood, R and Jarman, SN. *Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces*. **Molecular Ecology**. In press. 2009

Doiuchi, R and Nakabo T. *Molecular phylogeny of the stromateoid fishes (Teleostei: Perciformes) inferred from mitochondrial DNA sequences and compared with morphology-based hypotheses.* **Molecular Phylogenetics and Evolution**. Vol. 39. No. 1. Pp. 111 - 123. 2006

Ehrich, D, Fedorov, VB, Stenseth, NC, Krebs, CJ and Kenney, *A. Phylogeography and mitochondrial DNA (mtDNA) diversity in North American collared lemmings (Dicrostonyx groenlandicus)*. **Molecular Ecology**. Vol. 9. Iss. 3. Pp. 329 – 337. Mar. 2000

Excoffier, L and Lischer, HEL. *Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows*. **Molecular Ecology Resources**. In press. 2010

Fu, YX. *Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection*. **Genetics**. Vol. 147. Pp. 915 – 925. 1997

Fu, YX and Li, WH. *Statistical Tests of Neutrality of Mutations*. **Genetics**. Vol. 133. Pp. 693 – 709. 1993

Ghedotti, MJ and Grose, MJ. *Phylogenetic Relationships of the Fundulus nottii Species Group (Fundulidae, Cyprinodontiformes) as Inferred from the Cytochrome b Gene*. **Copeia**. Vol. 1997. No. 4. Pp. 858 – 862. 1997

Harpending, HC, Batzer, MA, Gurven, M, Jorde, LB, Rogers, AR, Sherry, ST. *Genetic traces of ancient demography*. **Proceedings of the National Academy of Science of the United States of America**. Vol. 95. No. 4. Pp. 1961 – 1967. Feb. 1998

Hutchings, JA and Fraser, DJ. *The nature of fisheries- and farming-induced evolution*. **Molecular Ecology**. Vol 17. Pp. 294 – 313. 2008

Hutchins, B, Swainston, R. 1986. *Sea Fishes of Southern Australia. Complete Field Guide for Anglers and Divers*. Swainston Publishing.

Infante, C, Catanese, G and Manchado, M. *Complete mitochondrial DNA sequence of little tunny (Euthynnus alletteratus)*. Unpublished

Jansa, SA, Barker, FK and Heaney LR. *The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences*. Systematic Biology. No. 55. Pp 73-88. 2006

Johnson, GD and Fritzsche, RA. *Graus nigra, an Omnivorous Girellid, with a Comparative Osteology and Comments on Relationships of the Girellidae (Pisces: Perciformes)*. **Proceedings of the Academy of Natural Sciences of Philadelphia.** Vol. 141. Pp. 1 - 27. 1989.

Kim, IC, Kweon, HS, Kim, YJ, Kim, CB, Gye, MC, Lee, WO, Lee, YS and Lee, JS. *The complete mitochondrial genome of the javeline goby Acanthogobius hasta (Perciformes, Gobiidae) and phylogenetic considerations*. **Gene**. Vol. 336. No. 2. Pp. 147 - 53. 2004

Kingsford, MJ. 1989. *Distribution patterns of planktivorous reef fishalong the coast of northeastern New Zealand*. **Marine Ecology Progress Series**. Vol. 54: 13-24, 1989

Kuparinen, A and Merila, J. Detecting *and managing fisheries induced evolution*. **TRENDS in Ecology and Evolution.** Vol.22. No.12. 2007

McDonald, MA, Smith, MH, Smith, MW, Novak, JM, Johns, PE and De Vries AL. *Biochemical systematics of notothenioid fishes from Antarctica*. **Biochemical Systematics and Ecology**. 20:233-241. 1992.

Manchado, M, Catanese, G and Infante, C. *Complete mitochondrial DNA sequence of albacore (Thunnus alalunga)*. Unpublished

Manchado, M, Infante, C and Catanese, G. *Complete mitochondrial DNA sequence of skipjack (Katsuwonus pelamis)*. Unpublished

Miya, M, Kawaguchi, A and Nishida, M. *Mitogenomic exploration of higher teleostean phylogenies: a case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences*. **Molecular Biology Evolution**. Vol 18, No. 11. Pp. 1993 - 2009. Nov. 2001

Miya, M and Nishida, M. *Use of mitogenomic information in teleostean molecular phylogenetics: a tree-based exploration under the maximum-parsimony optimality criterion*. **Molecular Phylogenetics and Evolution.** Vol. 17. No. 3. Pp. 437 – 455. 2000

Miya, M, Takeshima, H, Endo, H, Ishiguro, NB, Inoue, JG, Mukai, T, Satoh, TP, Yamaguchi, M, Kawaguchi, A, Mabuchi, K, Shirai, SM, Nishida, M. *Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences*. **Molecular Phylogenetic Evolution**. Vol. 26. No. 1. Pp. 121 - 138. 2003

New Zealand Ministry of Fisheries. 2009. *Kahawai Stock Assessment document 41 KAH 09*. Collected online at http://fs.fish.govt.nz/Page.aspx?pk=113&dk=21825 on 25 May, 2010.

New Zealand Ministry of Fisheries 2010a. TAR2 Catch report. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=8&stock=TAR2 06 October 2010.

New Zealand Ministry of Fisheries 2010b. TAR3 Catch report. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=8&stock=TAR3 06 October 2010.

Paulin, C. *Review of the Australian fish family Arripididae (Percomorpha), with the description of a new species*. **Australian Journal of Marine and Freshwater Research**. Vol. 44. No. 3. Pp. 459 – 471. 1993

Rogers, AR and Harpending, H. *Population growth makes waves in the distribution of pairwise genetic differences*. **Molecular Biology and Evolution**. Vol. 9. Iss. 3 Pp. 552 - 569. 1992

Roques, A and Negro, JJ. *MtDNA genetic diversity and population history of a dwindling raptorial bird, the red kite (Milvus milvus)*. **Biological Conservation**. Vol. 126. Iss. 1. Pp. 41 – 50. Nov. 2005

Sevilla, RG, Amalia, D, Norén, M, Mouchel, O, Jérôme, M, Verrez-Bagnizm V, Van Pelt, H, Favre-Krey, L, and Krey, G. *Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes*. **Molecular Ecology Note**s. Vol 7. Iss. 5. Pp 730–734. September. 2007

Smith, WL and Wheeler, WC. *Venom evolution widespread in fishes: a phylogenetic road map for the bioprospecting of piscine venoms*. **Journal of Heredity**. Vol. 97. No. 3. Pp. 206 – 217. 2006

Tajima, F. *Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism*. **Genetics**. Vol. 123. Pp. 585 – 595. 1989

Takashima, Y, Morita, T and Yamashita, M. *Complete mitochondrial DNA sequence of Atlantic horse mackerel Trachurus trachurus and molecular identification of two commercially important species T. trachurus and T. japonicus using PCR-RFLP*. **Fisheries Science**. Vol. 72 Pp. 1054 – 1065. 2006

Tamura, K and Nei, M. *Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees*. **Molecular Biology and Evolution**. Vol. 10. Iss. 3. Pp. 512 - 526. 1993

Ward, RD and Holmes, BH. *An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (cox1) in fishes*. **Molecular Ecology Notes**. Vol. 7. No. 6. Pp. 899 – 907. 2007

Yagishita, N, Kobayoshi, T and Nakobo, T. *Review of monophyly of the Kyphosidae (sensu Nelson, 1994), inferred from the mitochondrial ND2 gene*. **Ichthyological Research**. Vol. 49. No. 2 .Pp 103-108. 2002

Yagishita, N, Miya, M, Yamanoue, Y, Shirai, SM, Nakayama, K, Suzuki, N, Satoh, TP, Mabuchi, K, Nishida, M and Nakabo, T. *Mitogenomic evaluation of the unique facial nerve pattern as a phylogenetic marker within the perciform fishes (Teleostei: Percomorpha)*. **Molecular Phylogenetics and Evolution**. Vol. 53. No. 1. Pp. 258 – 266. 2009

Chapter 3. Marine Phylogeography and Applying DNA Markers to *Arripis trutta* and *Nemadactylus macropterus*

3.1 Abstract

Arripis trutta is managed in NZ as a single population with commercial fishing areas based on geographic or politically devised borders rather than for reasons of population biology. To test if these boundaries are reasonable, 178 samples of *Arripis trutta* flesh were collected from nine locations around New Zealand and Australia. Mitochondrial DNA was extracted and examined and the COX1 gene was amplified and analysed. Analysis of the 547 bp sequence revealed 22 haplotypes. Φ_{ST} values for the species were low, typically less than 0.08. Estimated migration rates amongst populations was high, with "infinite" migrants per generation calculated for some populations within New Zealand and approximately 15 migrants per generation between Australia and New Zealand. Australian samples were indistinguishable from New Zealand samples at the locus tested. From these findings, it is concluded that *Arripis trutta* is genetically a single population within New Zealand and the presently management regime is appropriate for this population structure.

A. trutta and *N. macropterus* from the Wellington region were then compared to investigate differences between the species. All haplotype diversity indices were higher for the *N. macropterus* samples than those of *A. trutta*, suggesting that *A. trutta* is a more homogeneous species. Mismatch distributions for both species were unimodal and best fit an expanding / contracting population model. Tajima's D values were negative for both species, indicative of a recent population decline. Taken together, these findings suggest that exploitation is at a greater level than both species can support through natural recruitment, and that any greater exploitation of the species should be carefully considered to ensure stock health.

3.2 Introduction

Arripis trutta is a pelagic species that inhabits near-shore environments in Australia and New Zealand (Davidson et al, 1997), where they are known by the common names "Australian salmon" and "kahawai", respectively. While a popular recreational species, and one with customary significance in New

Zealand, the flesh of the fish is rather strongly flavoured and degrades quickly after capture. This means that the fish does not command a high price in the market, nor it is subject to high levels of commercial exploitation compared to other near shore species such as snapper (*Pagrus auratus*) and tarakihi (*Nemadactylus macropterus*). However, as the global demand for protein increases, the very real possibility exists that markets and storage techniques could be found that will increase the commercial desirability of this species.

As a species with planktotrophic larvae that broadcast spawns into open water (Johnson, 2000), *A. trutta* is a good candidate species for phylogeographic study. Previous studies (e.g. McDonald, 1980) indicate very low levels of structure within the population in Australia, and very little is known of rates of gene flow between the two areas or around the coast of New Zealand. Also, as the species is present in both countries, several potential barriers to larval migration exist. The most obvious of these barriers is the Tasman Sea. Within New Zealand where the majority of the study samples were collected, ocean currents and land masses further serve to break up populations. However, it is also known from earlier studies that genetic structure is not present in the Australian populations of Arripidae fish (McDonald, 1980).

For this study, mitochondrial DNA was examined to determine the level of population structure. The techniques for collecting, extracting, sequencing and analysing samples are well established, (e.g., Chabot and Allen, 2009) although it must be recognised that MtDNA studies are not without issues or detractors. Galtier et al (2009) suggest that the very ubiquity of such studies hides the issues associated with them, such as inaccurate estimates for mutation rates. COX1 was selected for this study as work such as that undertaken by Taylor and Hardman (2002) suggests that COX1 is useful for studies of this nature due to relatively high substitution rates at the third codon position, providing a “clock” with a reasonably short “tick”.

It was hypothesised for this study that low levels of genetic structure would be apparent within regions, but that higher levels of structure would be observed between regions and across geographical boundaries such as strong currents

or on opposite sides of New Zealand. It was further hypothesised that Australian fish samples would not group with New Zealand samples.

3.3 Materials and Methods

Sample collection

Samples of *A. trutta* were collected by commercial and recreational anglers, who donated material to the study. Material collected was either flesh samples from dead fish, or fin clippings from live fish that were then returned to the water. Collection instructions were provided to anglers who had volunteered to provide material to the study, to ensure that all samples collected would be usable and that sufficient material would be collected for DNA extraction. Collection instructions are shown in Appendix 1 : Collection Instructions.

After removal from the fish, samples were immediately placed in 70% ethanol and stored at 4°C as soon as practical.

Kahawai were sampled from nine geographical regions from New Zealand and Australia. These are shown in Table 3-1 and in diagrammatically in Figure 3-1.

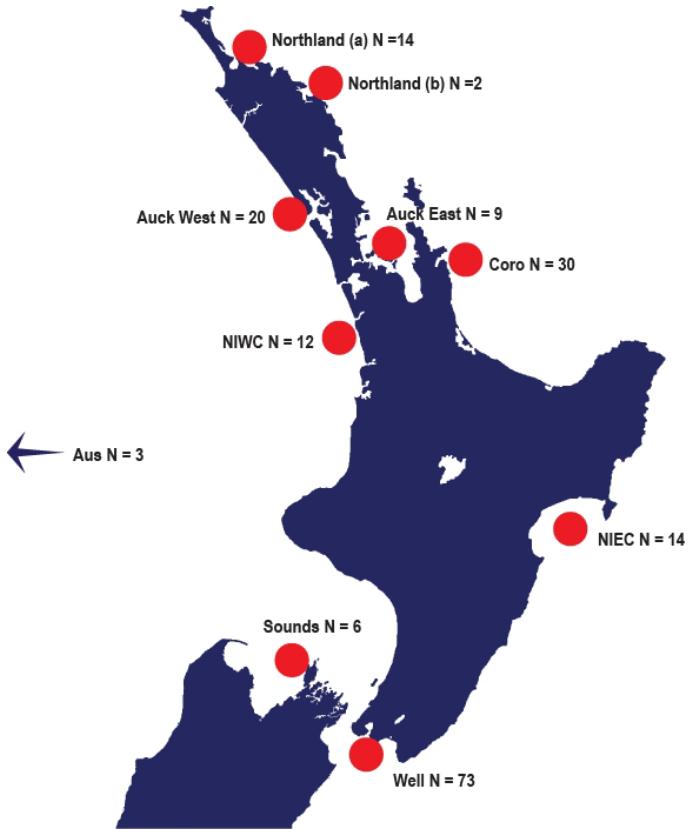


Figure 3-1 : Locations within NZ where *A. trutta* samples were collected

Table 3-1: *Arripis trutta* capture locations

Region	Latitude	Longitude	Sample Size
Northland (a)	34°25'S	172°47'E	6
	34°30'S	172°55'E	6
	35°12'S	174°00'E	2
Northland (b)	35°36'S	174°31'S	1
	35°20'S	174°21'E	1
Coromandel	36°11'S	175°25'E	3
	37°02'S	175°56'E	3
	37°01'S	175°51'E	4
	36°57'S	175°52'E	3
	37°00'S	175°52'E	11
Auckland West Coast	37°02'S	174°43'E	20
	36°13'S	174°07'E	8
Auckland East Coast	36°47'S	174°51'E	9
Central North Island West Coast	38°03'S	174°46'E	12
Central North Island East Coast	39°29'S	176°55'E	14
Wellington	41°23'S	174°48'E	73
Marlborough Sounds	41°14'S	174°07'E	6
Australia	38°25'S	145°09'E	2
	41°03'S	146°26'E	1

An additional 51 samples of tarakihi (*Nemadactylus macropterus*) from the Wellington area were donated to this thesis study by a commercial fish processor.

DNA Extraction, mitochondrial DNA amplification and sequencing

DNA was extracted from muscle tissue and fin clippings using the proteinase K / phenol chloroform method. Samples were digested in Invitrogen proteinase K in accordance with the manufacturer's instructions. DNA was purified in phenol / chloroform and then rehydrated. After extraction, DNA was suspended in TE buffer and stored at 4°C.

COX1 DNA was amplified using the polymerase chain reaction (PCR). One microlitre of DNA extract was added to a PCR master mix that consisted of 2.5 µL of 10x reaction buffer, 1 µL of BSA, 1 µL of dNTPS, 1 µL of forward primer (Fish F2 5'-TCG ACT AAT CAT AAA GAT ATC GGC AC), 1 µL of reverse primer (Fish R2 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA), 0.75 µL of $MgCl_2$, 16.55 µL of ddH_2O and 0.2 µL of BioTAQ. PCR cycler conditions were an initial denaturing for two minutes at 95°C followed by 38 cycles of 30s at 95°C, 60s at 50°C, 60s at 72°C, with a final extension step of five minutes at 72°C. The PCR products were then run on an agarose gel electrophoresis then stained with ethidium bromide to confirm that amplification had been successful. Successful PCR products were then purified using ExoSAP following the manufacturer's instructions and then sent to Massey University Genome Service (MUGS) for sequencing using the Big Dye system.

Following sequencing, resultant sequence files were inspected and read errors repaired by eye. Alignments were then performed using ClustalW (Higgins et al, 1994), in Mega4.

Population structure

DNAsp version 5 (Librado and Rozas, 2009) was used to generate relative nucleotide composition, number of polymorphic sites, haplotype diversity (*h*), nucleotide diversity (π) and number of pairwise differences between populations. In order to estimate levels of genetic divergence among populations of *A. trutta*, the diversity measure Φ_{ST} was calculated using AMOVA (Excoffier et al 1992, Weir and Cockerham, 1984, Weir, 1996) under the parameters of Tamura & Nei (1993) nucleotide substitution model. Φ_{ST} estimates were tested nonparametrically (1000 bootstrapped replicates) by Arlequin 3.5 (Excoffier and Lischer, 2010). COX1 Sequence divergence comparisons were performed between *A. trutta*, *N. macropterus* and published Kimura 2-parameter values (Kimura, 1980) for 35 inshore and offshore fish species (Zemlak et al, 2009). Gene genealogy of *A. trutta* was assessed using TCS (Clement et al, 2000)

Effective population size and migration

To estimate the effective female population size, N_{ef}, which is equal to $\Theta_S = 2\mu k$ (where μ is the mutation rate and k is the number of nucleotides), was calculated for each population using the mutation rate of 0.8 sequence divergence per million years (Bowen et al, 2006)[1]. Θ_S was estimated by Arlequin 3.5 and based on the number of segregating sites, sample size and Θ for a sample of non-recombining DNA. Migrants per generation, M (where M=N*m* for haploid data, with N the effective population size and *m* is the migration rate) was calculated with Arlequin 3.5.

Demographic history and comparison with *Nemadactylus macropterus*

Single populations of *A. trutta* and *N. macropterus* were examined in DNAsp version 5, to compare mismatch distributions, following the procedure of Roques and Negro (2005). When graphed, the mismatch distribution of pairwise differences is generally multimodal for populations at demographic equilibrium and unimodal for populations that have passed through recent demographic expansion (Rogers and Harpending, 1992, Harpending et al, 1998). Overall validity of the estimated demographic model is tested by obtaining the distribution of the test statistic SSD (the sum of the squared differences) between the observed and the estimated mismatch distribution. A significant SSD value is taken as evidence for departure for the estimated demographic model of sudden population expansion (Roques and Negro, 2005). Tajima's D (Tajima, 1989) and Fu's F_S (Fu and Li, 1993; Fu, 1997) statistics were used to test whether loci data conform to expectations of neutrality, considering that departures from neutrality could also be due to factors other than selective effects, such as population bottleneck, expansion, or heterogeneity of mutation rates (see Aris-Brosou and Excoffier, 1996). F_S differences were tested for significance with a coalescent simulation program (1000 simulations), as implemented in ARLEQUIN version 3.5 (Excoffier and Lischer, 2010).

Comparisons with *N. macropterus* were undertaken using techniques outlined by Ehrich et al (2001) and Roques and Negro (2005). Number of haplotypes

[1] Values in Bowen et al (2006) were for mitochondrial D-loop mutation, however Denver et al (2000) and Nabholz et al (2009) point out that calculated mutation rates may be in error by several orders of magnitude.

and the standard gene diversity indices of haplotype diversity, nucleotide diversity and mean number of pairwise differences were compared. Mismatch distributions (Roques and Negro, 2005) were tested in DNAsp version 5. Additionally, nucleotide divergence between the two species was performed using the Tamura-Nei model (Tamura and Nei, 1993), using the procedure described by Ghedotti and Grose (1997)

3.4 Results

Nucleotide and haplotype diversity

One hundred and seventy eight sequences were resolved for the COX1 region. Length of resolved sequences was 547 nucleotides, with an average nucleotide composition (relative values) of 26.68% C, 32.01% T, 25.22% A and 16.10% G. A total of 22 haplotypes were identified from the available New Zealand (refer to Figure 3-2) and Australian samples. As numbers of samples collected was somewhat low in some areas, data was aggregated to test for north / south and east / west variations. A neighbour joining cladogram showing all 22 haplotypes were resolved for *A. trutta* using COX1 sequences (refer to Table 3-2 and Figure 3-3). Note that Haplotype 5 is represented by a single individual from Australia. *A. georgianus* was included as an outgroup.

Table 3-2: *A. trutta* haplotypes resolved

Location	Haplotype Number																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	`7	18	19	20	21	22
Northland		14														1						
Auckland East		2		1					1	1												
Auckland West	1	18		1		1	2	1									2	1	1			
Coromandel	1	19								1	1	1										
North Island East Coast		10	1										2									1
North Island West Coast		9	1							1										1		
Wellington	1	55	7	2						2				2	1			1				
Marlborough Sounds		5	1																			
Australia		2			1																	

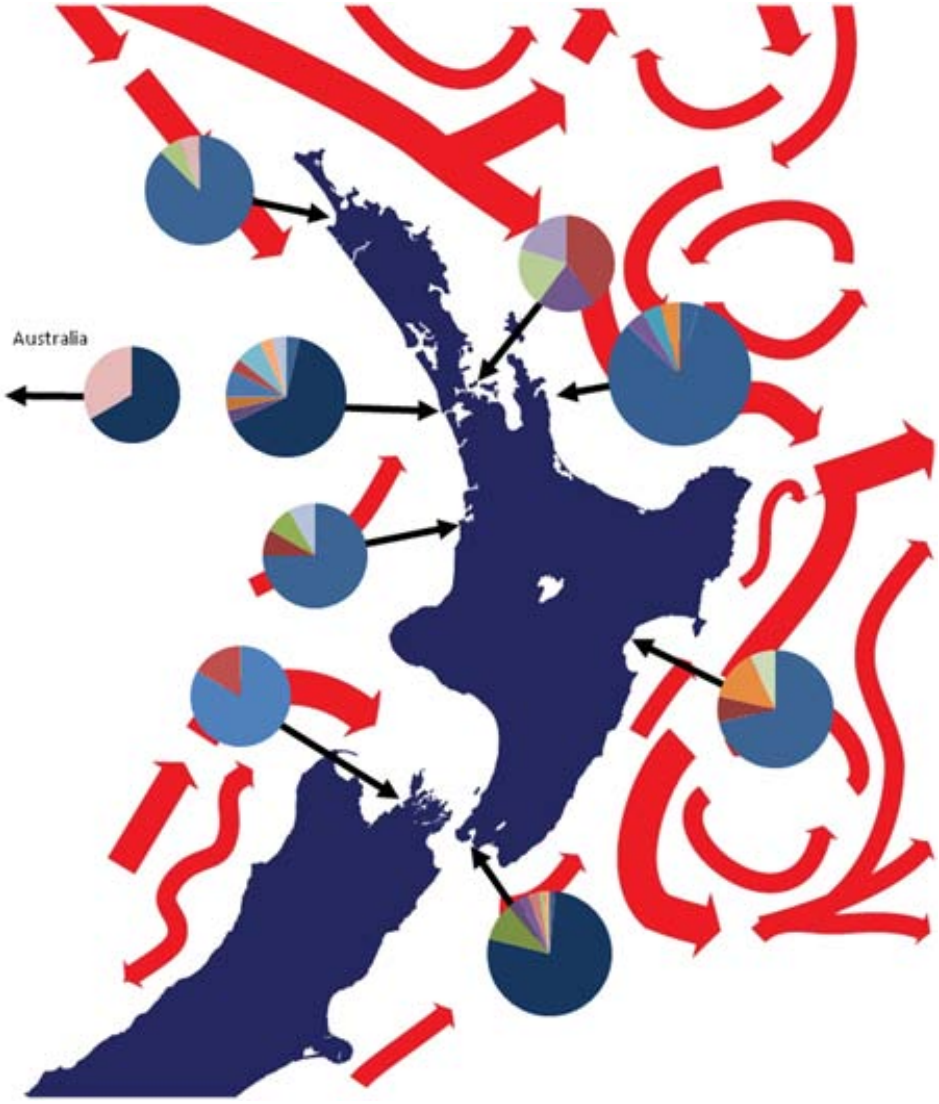


Figure 3-2 :*A. trutta* haplotype map

(Note: Arrowed lines indicate ocean currents)

Overall, 28 polymorphic sites (23 transitions, 5 transversions) were observed in the COX1 sequences defining 22 haplotypes with an overall diversity (*h*) of 0.43 ± 0.00223. Values varied from 0.242 to 0.900. Overall nucleotide diversity was very low (π = 0.00123). A neighbour joining phylogenetic tree was generated (Figure 3-3). This tree demonstrates that that haplotypes were distributed throughout the sampling range, with no geographic stratification apparent from the samples. One haplotype was only observed in a sole Australian sample; however the other two Australian samples were not different to samples collected in the Wellington, New Zealand region.

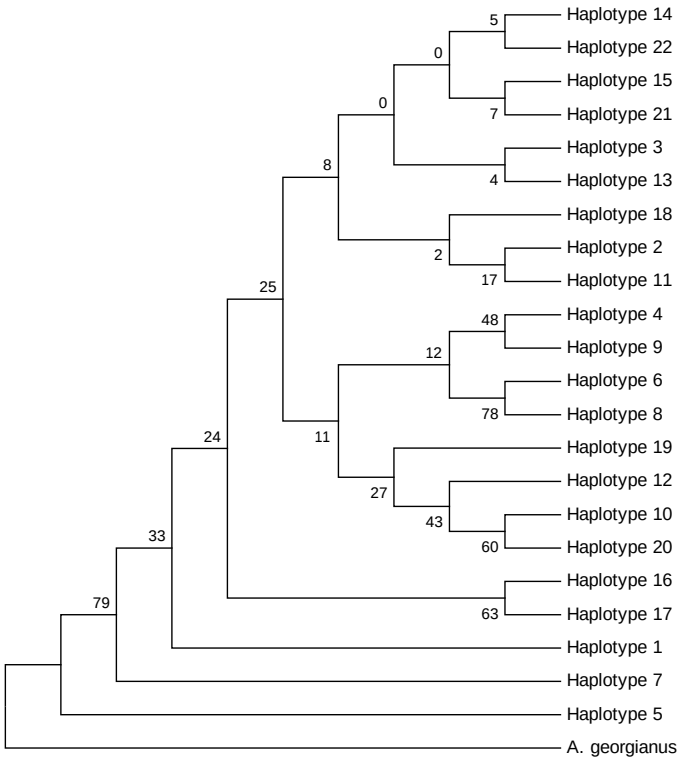


Figure 3-3: *Arripis trutta* phylogeny

Neighbour Joining Tree, bootstrap, 1000 replicates

Population divergences

Sequence divergences of *A. trutta* were very low in this study, with Australia showing the greatest intrapopulation divergence of 0.49% and estimated effective female population size ranges approximately equal between the east and west coasts of the North Island. These data are shown in Table 3-3 and Table 3-4. The estimated number of migrants is very high between New Zealand populations, and moderate between New Zealand and Australian populations, with the highest estimated value for trans-Tasman populations of 4.6 migrants per generation between Australia and Auckland East sub-populations. These values are shown in Table 3-7.

Table 3-3: Population diversity indices and distribution of haplotypes (all regions)

N = Number of individuals sampled, *h* = haplotype diversity, *π* = mean number of pairwise differences between haplotypes, Pi = nucleotide diversity.

Location	No. of samples	No. of haplotypes	*h*	*π*	Mean pairwise difference	Θ_s
Northland	16	3	0.242	0.0005 ± 0.0006	0.2500 ± 0.2966	0.6027
Auckland West	28	9	0.590	0.0020 ± 0.0015	1.1085 ± 0.7467	2.5697
Auckland East	5	4	0.900	0.0026 ± 0.0022	1.4000 ± 1.0188	1.4400
Coromandel	23	5	0.324	0.0014 ± 0.0012	0.7747 ± 0.5866	2.1675
North Island West Coast	12	4	0.455	0.0012 ± 0.0011	0.6364 ± 0.5322	0.9934
North Island East Coast	14	4	0.495	0.0010 ± 0.0010	0.5495 ± 0.4796	0.9434
Wellington	71	8	0.394	0.0008 ± 0.0008	0.4596 ± 0.4093	1.6553
Marlborough Sounds	6	2	0.333	0.0006 ± 0.0008	0.3333 ± 0.3801	0.4380
Australia	3	2	0.667	0.0049 ± 0.0044	2.6667 ± 1.9190	2.6667

Table 3-4: Population diversity indices and distribution of haplotypes (Aggregate values)

N = Number of individuals sampled, h = haplotype diversity, π = mean number of pairwise differences between haplotypes, Pi = nucleotide diversity.

Location	No. of samples	No. of haplotypes	h	π	Mean pairwise difference	Θs	Nef
East Coast Aggregate	58	12	0.400	0.0012 ± 0.0010	0.6479 ± 0.5102	3.0244	63,891
West Coast Aggregate	117	15	0.442	0.0012 ± 0.0010	0.6338 ± 0.4994	3.1864	67,314
North Aggregate	84	17	0.456	0.0015 ± 0.0012	0.8170 ± 0.5924	3.7984	80,243
South Aggregate	91	10	0.400	0.0008 ± 0.0008	0.4640 ± 0.4107	1.9675	41,564
All samples	178	22	0.430	0.0012 ± 0.0010	0.6733 ± 0.5182	4.8643	102,760

Populations of *A. trutta* showed very little genetic structure among populations, with the greatest percentage of variation 95.39% (P < 0.005) attributed to within population differences using Φ_{ST} statistics (refer to Table 3-6). Pairwise population comparisons revealed low levels of heterogeneity with pairwise Φ_{ST} of between 0.00 and 0.41; however only four of the values recorded in Table 3-5 can be considered significant with p-values of < 0.05. Overall Φ_{ST} was 0.046 (Table 3-6).

Table 3-5 Pairwise population (Φ_{ST}) and P values

Location	Northland	Auckland East	Auckland West	Coromandel	North Island East Coast	North Island West Coast	Wellington	Marlborough Sounds	Australia
Northland	--	**0.27***	-0.01	-0.00	0.03	0.04	0.01	0.02	0.39
Auckland East	0.05	--	0.07	0.11	**0.18***	0.10	**0.24***	0.13	0.10
Auckland West	0.60	0.12	--	0.01	0.02	0.02	**0.05***	-0.04	0.17
Coromandel	0.50	0.16	0.25	--	0.02	-0.02	0.02	-0.04	0.24
North Island East Coast	0.14	0.02	0.15	0.14	--	0.02	0.01	-0.05	0.26
North Island West Coast	0.15	0.12	0.19	0.70	0.31	--	0.01	-0.05	0.23
Wellington	0.28	0.00	0.00	0.13	0.21	0.29	--	-0.03	**0.41***
Marlborough Sounds	0.70	0.14	0.62	0.64	0.86	0.86	0.99	--	0.18
Australia	0.07	0.27	0.12	0.54	0.355	0.28	0.03	0.23	--

Pairwise population (Φ_{ST}) values above diagonal, P values below. *Significant (P<0.05) Φ_{ST} values are shown in **BOLD**.

Table 3-6 Φ_{ST} values of *Arripis trutta*

Φ_{ST} Statistics Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	8	4.767	0.0157	4.61
Within populations	169	54.817	0.3244	95.39
Total	177	59.584	0.3400	
Fixation index (Φ_{ST})	0.04611			

Table 3-7 Estimated number of migrants per generation (M) and average pairwise nucleotide differences between populations of *Arripis trutta*.

Location	Northland	Auckland East	Auckland West	Coromandel	North Island East Coast	North Island West Coast	Wellington	Marlborough Sounds	Australia
Northland	--	1.34291	inf	inf	15.72020	10.69430	46.68988	29.71429	0.77100
Auckland East	0.27131	--	6.75973	4.24429	2.21922	4.37500	1.55174	3.37113	4.58955
Auckland West	-0.01004	0.06887	--	45.60714	25.81172	23.34839	10.35027	inf	2.36501
Coromandel	-0.00209	0.10539	0.01084	--	29.52223	inf	21.97904	inf	1.60989
North Island East Coast	0.03083	0.18388	0.01900	0.01665	--	19.94245	25.82275	inf	1.35584
North Island West Coast	0.04467	0.10256	0.02097	-0.01777	0.02446	--	46.62912	inf	1.64444
Wellington	0.01060	0.24370	0.04608	0.02224	0.01899	0.01061	--	inf	0.70990
Marlborough Sounds	0.01655	0.12916	-0.03691	-0.03625	-0.05085	-0.05051	-0.07883	--	2.25000
Australia	0.39339	0.09824	0.17452	0.23698	0.26942	0.23316	0.41326	0.18182	--

Note: M Values above diagonal and average pairwise differences below

Overall mismatch distribution was unimodal in character (refer to Figure 3-4) and most closely fits the expected values for an expanding / contracting population. Tajima's D for the overall *A. trutta* population is -2.469, with a P-value of < 0.01, suggesting the population is contracting (Pichler, 2002).

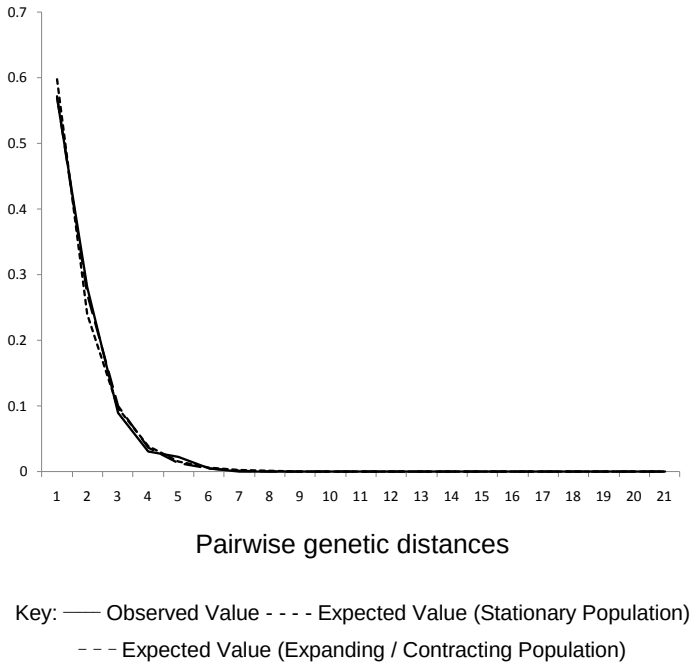


Figure 3-4: Mismatch distribution observed *A. trutta* samples

Nucleotide divergences of *A. trutta* and *N. macropterus* were compared to 35 inshore and offshore species (Zemlak et al, 2009) using the Kimura 2 parameter model (Kimura, 1980). Calculated divergences for both species was low, and while both tarakihi and kahawai would seem to be considered inshore or inshore / offshore species had they been available to Zemlak et al (2009), results were consistent with the values published for off-shore species. Values are shown in Table 3-8 and displayed graphically in Figure 3-5.

Table 3-8: Calculated Kimura 2-parameter nucleotide diversity values from a range of marine fish species

Species	Number of individuals tested	Inshore / Offshore	Kimura 2-p value ± S. E. (%)	Source
Lutjanus rivulatus	4	Inshore	0.05 ± 0.05	Zemlak et al, 2009
Carcharhinus amboinensis	3	Inshore	0.08 ± 0.08	Zemlak et al, 2009
Cephalopholis miniata	4	Inshore	0.09 ± 0.05	Zemlak et al, 2009
Caranx ignobilis	3	Inshore	0.16 ± 0.16	Zemlak et al, 2009
Lethrinus rubrioperculatus	3	Inshore	0.40 ± 0.06	Zemlak et al, 2009
Chanos chanos	5	Inshore	0.48 ± 0.01	Zemlak et al, 2009
Lutjanus argentimaculatus	7	Inshore	0.59 ± 0.11	Zemlak et al, 2009
Parupeneus indicus	6	Inshore	0.60 ± 0.14	Zemlak et al, 2009
Chelidonichthys kumu	8	Inshore	1.12 ± 0.15	Zemlak et al, 2009
Epinephelus rivulatus	7	Inshore	1.95 ± 0.28	Zemlak et al, 2009
Cephalopholis sonnerati	8	Inshore	2.00 ± 0.18	Zemlak et al, 2009
Ariomma indica	6	Inshore	3.39 ± 0.09	Zemlak et al, 2009
Argyrops spinifer	7	Inshore	5.30 ± 0.09	Zemlak et al, 2009
Lethrinus nebulosus	7	Inshore	5.68 ± 0.04	Zemlak et al, 2009
Scomberoides tol	5	Inshore	8.70 ± 0.05	Zemlak et al, 2009
Priacanthus hamrur	5	Inshore	8.91 ± 0.08	Zemlak et al, 2009
Rhabdosargus sarba	8	Inshore	10.01 ± 0.03	Zemlak et al, 2009
Platycephalus indicus	6	Inshore	11.03 ± 0.07	Zemlak et al, 2009
Bodianus perditio	6	Inshore	12.48 ± 0.14	Zemlak et al, 2009
Parupeneus heptacanthus	6	Inshore	16.00 ± 0.04	Zemlak et al, 2009
Otolithes ruber	3	Inshore	16.24 ± 0.37	Zemlak et al, 2009
Mean Value for inshore species			5.10 ± 0.37	Zemlak et al, 2009
Carcharhinus obscurus	4	Inshore / offshore	0	Zemlak et al, 2009
Galeocerdo cuvier	5	Inshore / offshore	0	Zemlak et al, 2009
Hoplostethus mediterraneous	8	Inshore / offshore	0.13 ± 0.04	Zemlak et al, 2009
Carcharhinus limbatus	8	Inshore / offshore	0.26 ± 0.02	Zemlak et al, 2009

Species	Number of individuals tested	Inshore / Offshore	Kimura 2-p value ± S. E. (%)	Source
Pristipomoides filamentosus	6	Inshore / offshore	0.34 ± 0.06	Zemlak et al, 2009
Carcharodon carcharias	3	Inshore / offshore	1.00 ± 0.03	Zemlak et al, 2009
Pomatomus saltatrix	6	Inshore / offshore	1.35 ± 0.00	Zemlak et al, 2009
Sphyrna lewini	3	Inshore / offshore	3.54 ± 0.00	Zemlak et al, 2009
Scomberomorus commerson	6	Inshore / offshore	3.55 ± 0.10	Zemlak et al, 2009
Mean value for inshore / offshore species			0.84 ± 0.15	Zemlak et al, 2009
				Zemlak et al, 2009
Thunnus albacares	10	Offshore	0.08 ± 0.02	Zemlak et al, 2009
Euthynnus affinis	8	Offshore	0.09 ± 0.03	Zemlak et al, 2009
Xiphias gladius	8	Offshore	0.31 ± 0.05	Zemlak et al, 2009
Coryphaena hippurus	10	Offshore	0.46 ± 0.05	Zemlak et al, 2009
Lampris guttatus	6	Offshore	0.53 ± 0.09	Zemlak et al, 2009
Mean value for offshore species			0.26 ± 0.03	Zemlak et al, 2009
Tarakihi *(N. macropterus)* Wellington	51	Inshore / Offshore	0.16 ± 0.12	This study
Arripis trutta (Northland)	16	Inshore / Offshore	0.05 ± 0.06	This study
Arripis trutta (Auckland West)	20	Inshore / Offshore	0.20 ± 0.15	This study
Arripis trutta (Auckland East)	9	Inshore / Offshore	0.26 ± 0.22	This study
Arripis trutta (Coromandel)	30	Inshore / Offshore	0.14 ± 0.12	This study
Arripis trutta (North Island East Coast)	14	Inshore / Offshore	0.10 ± 0.10	This study
Arripis trutta (North Island West Coast)	12	Inshore / Offshore	0.12 ± 0.11	This study
Arripis trutta (Wellington)	73	Inshore / Offshore	0.08 ± 0.08	This study
Arripis trutta (Marlborough Sounds)	6	Inshore / Offshore	0.06 ± 0.08	This study
Arripis trutta (Australia)	3	Inshore / Offshore	0.49 ± 0.44	This study
Arripis trutta (Average)	178	Inshore / Offshore	0.12 ± 0.10	This study

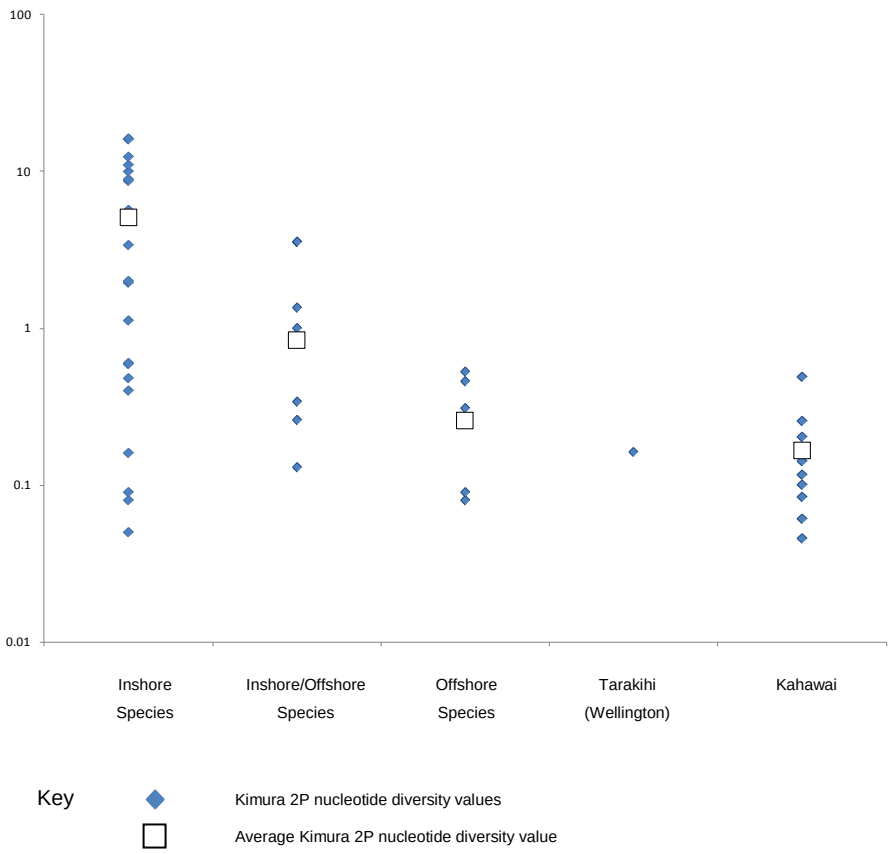


Figure 3-5: Kimura 2 Parameter Comparisons.
A. trutta vs other inshore and offshore species
(After Zemlak et al, 2009)

TCS analysis (refer to Figure 3-6) revealed a maximum of 8 mutational steps between any individual samples collected, with no clear pattern of radiation apparent, which suggests that there were no strong patterns between sample locations, and that genetic drift (Rogell et al, 2010) is the primary evolutionary force shaping present day populations of *A. trutta*.

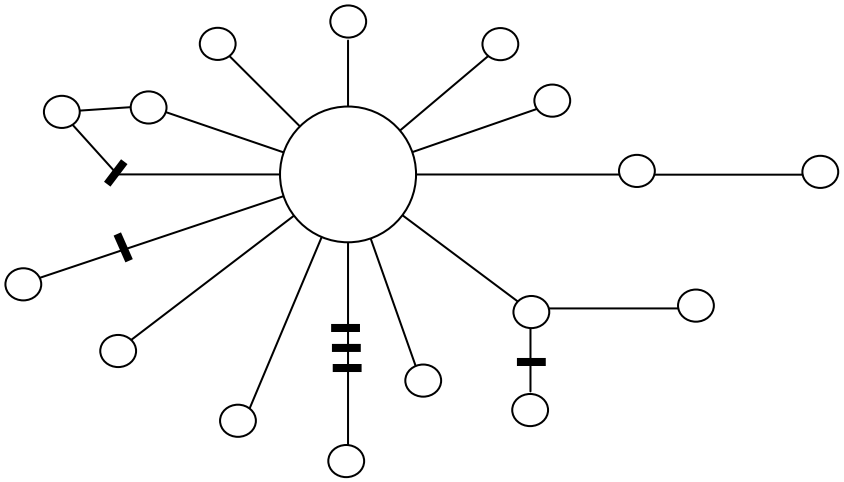


Figure 3-6: Kahawai TCS Network

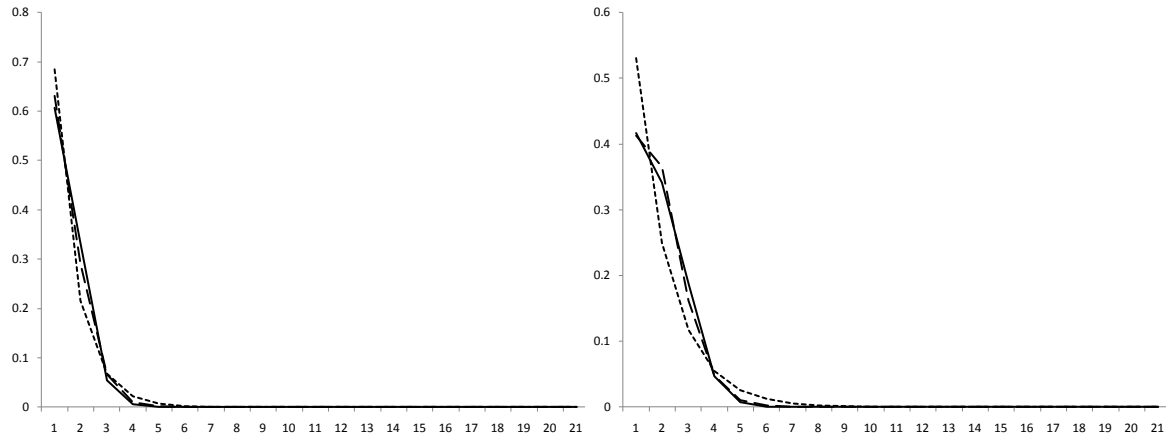
Demographic history (Wellington comparisons)

For the data held, eight haplotypes were resolved for *A. trutta* and 16 haplotypes for *N. macropterus* in the Wellington region. Mismatch distribution graphs (refer to Figure 3-7) are both unimodal and both best fit the expanding / contracting model suggesting recent population bottlenecks. In the case of *A. trutta*, the difference between the stationary model and expanding / contracting model is very slight. Tajima's D values are -1.85116 ($P < 0.05$) for *A. trutta* and -2.31836 ($P < 0.01$) for *N. macropterus*. Both values show significance at the 5% level, suggesting selection neutrality of the COX1 gene.

Table 3-9: Population diversity indices and distribution of haplotypes

n = Number of individuals samples, h = haplotype diversity, π= mean number of pairwise differences between haplotypes, SSD = Sum of Squared Differences (between estimated mismatch distributions) τ = 2ut (where u = mutation rate per sequence per generation and t = time in generations)

Species	n	*h*	*π*	SSD (P value)	τ	Tajima's D (P value)	Fu's F_S (P value)	Population in Equilibrium?
A. trutta	71	0.393 ± 0.00512	0.001 ± 0.001	0.001 (P>0.1)	0.5	-1.851 P < 0.05	-6.099 P>0.1	No
N. macropterus	51	0.583 ± 0.00692	0.002 ± 0.001	0.001 (P>0.1)	1.0	-2.318 P < 0.01	-17.089 P<0.02	No



A. trutta *N. macropterus*

Pairwise genetic distances.

Key: —— Observed Value - - - - Expected Value (Stationary Population). – – – Expected Value (Expanding / Contracting Population)

Figure 3-7: Mismatch distributions observed in Wellington-captured *A. trutta* and *N. macropterus* samples

3.5 Discussion

Population metrics and comparisons between *A. trutta* and *N. macropterus*

There is very little evidence of population genetic structure in the samples of *A. trutta* collected in New Zealand or Australia. Φ_{ST} values are low, suggesting highly connected populations of fish, compared to other near shore pelagic species such as *Pagrus auratus* or snapper (see Hauser et al, 2002). Nucleotide diversity values were also low when compared with other species. *A. trutta* is generally described as a near shore species (Davidson et al, 1997), but the Kimura 2 parameter results indicate the nucleotide diversity pattern has much more in common with offshore species.

Migration calculations based on mutation rates were performed using mitochondrial mutation values suggested by Bowen et al (2006), however Denver et al (2000) demonstrated that mitochondrial mutation rates in *Caenorhabditis elegans* are up to two orders of magnitude higher than those previously calculated by indirect means. Nabholz et al (2009) demonstrated that mitochondrial mutation rates between species are more variable than within species. This leads to the inescapable conclusion that any parameters based on improperly calibrated mutation rates or by comparison with the rates known

from other species, even closely related species, can not be relied upon. *A. trutta*, indeed, the wider Arripidae family is something of a taxonomic orphan. While the family groups monophyletically (refer to Chapter 1) with the Scombrids and Stromateoids, no work can be found that provides calibrated mitochondrial mutation rates for Arripidae. This means that all derived values shown in the above tables are, necessarily, approximations.

However, the migration rates derived, even accepting a large uncertainty, are very high. Even a single migrant per generation (Lowe and Allendorf, 2010) can provide enough gene flow to link populations. The migrant rates estimated for *A. trutta* populations are many times higher than this, suggesting that significant migration does indeed occur between populations.

This in turn leads to further conclusions about the populations of *A. trutta* and has implications for the effective management of the species and to ensure that fishing effort is held at a level that ensures stocks remain healthy.

The first of these implications is that trans-Tasman management agreements be made to ensure the ongoing health of stocks. Modern fisheries are characterised by serial depletion of stocks as high value and more desirable fish are exploited below commercially sustainable levels. While *A. trutta* is not currently a high value species nor particularly targeted, a small shift in fishery economics would change this. This is also linked to the growing protein needs of the world, especially that of the developing world. Sharing of information on stocks and capture rates, and agreed quotas between Australian and New Zealand fisheries managers is seen as an important future step in maintaining the health of the species.

The second implication is that the current management philosophy within New Zealand, that a single (effective) breeding population of *A. trutta* exists, is most likely correct and that local management of stocks on a geographical basis is appropriate. Temporal and spatial variations in local “populations” exist, and this is reflected in the allowable catch limits seen in each of the management areas within New Zealand.

The final implication arising from the estimated migration rates is that fish are potentially travelling between New Zealand and Australia either in the plankton or as adults, and perhaps regularly. It is not known if tagging studies have or are being conducted or planned for *A. trutta*, but such a study could lead to interesting information on this species and provide insight into both planktonic movement and migration by adult fish.

Resolved TCS networks and phylogenetic trees show no obvious population structure between the *A. trutta* populations tested, suggesting random mutation is the primary driver of diversity in the species. This is not at odds with the other findings of the study.

When compared directly, the populations of *A. trutta* and *N. macropterus* demonstrate some interesting differences. *N. macropterus* is a more heterogeneous population, with 16 haplotypes with the values of Fu's F_S and Tajima's D being approximately twice as those values estimated for *A. trutta*. This suggests that the population of *N. macropterus* has experienced a genetic bottleneck more recently than *A. trutta*, a finding not unexpected given the much higher level of exploitation on the species. Many studies demonstrate that high levels of exploitation can affect the genetics of affected species, such as Hutchings and Fraser (2007), Heino and Gordo (2002) and Kuparinen and Merila (2007).

In New Zealand, fisheries management policy is that species are to be managed sustainably, however it is clear from the data presented above that this is not the case. The genetic signals observed in both species are of fish that are being exploited at levels greater than can be maintained by the species. This finding leads to a consideration of the paradox of the "sweepstake hypothesis" (Hedgecock et al, 2007) – that a small number of individuals are responsible for most of the reproductive success (and therefore genetic makeup) of a population. Most fish species produce prodigious numbers of eggs and mortality amongst offspring is also very high. This would suggest that all fish contribute equally to a population. But this is apparently not the case.

Considering just one management area of *A. trutta*, KAH1 in New Zealand's north east, catch limits for the area are in the order of 2.2 million kilograms

annually. At an average of 3 kg per fish, this is roughly equivalent to 733,000 fish. However, the calculated N_{ef} for this region was approximately 90,000 fish, suggesting that only one in 4 mature females contribute reproductively to the population. Hedgecock et al (2007) suggest that inbreeding depression is an outcome of the sweepstakes hypothesis, leading to decreased fitness of the population, which leads inexorably to the question “what effect does high levels of exploitation have on these populations?”

3.6 References

Bowen, BW, Muss, A, Rocha, LA, Grant, WS. *Shallow mtDNA Coalescence in Atlantic Pygmy Angelfishes (Genus Centropyge) Indicates a Recent Invasion from the Indian Ocean*. **Journal of Heredity**. 97(1):1–12. 2006

Clement, M, Posada, D, Crandall, KA. *TCS: a computer program to estimate gene genealogies*. **Molecular Ecology**. No. 9. Iss. 10. Pp. 1657 – 1660. 2000

Davidson, GW, Thorarensen, HT, Lokman, M, Davie, PS. *Stress of capture and captivity in kahawai Arripis trutta (Bloch and Schneider) (Perciformes: Arripidae)*. **Comparative Biochemistry and Physiology Part A: Physiology**. Vol 118. Iss 4. 1405-1410, 1997

Denver, D, Morris, K, Lynch, M, Vassilieva, L, Thomas, WK. *High Direct Estimate of the Mutation Rate in the Mitochondrial Genome of Caenorhabditis elegans*. **Science**. No 289. 2000

Excoffier, L and Lischer, HEL. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. In press. 2010

Excoffier, L, Smouse, P, and Quattro, J. *Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data*. **Genetics** 131:479-491. 1992

Galtier, N, Nabholz, B, Glémin, S, Hurst, GDD. *Mitochondrial DNA as a marker of molecular diversity: A reapprisal*. **Molecular Ecology**. Vol 18. Iss. 22. 4541 – 4550. 2009

Hauser, A, Adcock, GJ, Smith, PJ, Bernal Ramírez, JH, Carvalho, GR. *Loss of Microsatellite Diversity and Low Effective Population Size in an Overexploited Population of New Zealand Snapper (Pagrus auratus).* **Proceedings of the National Academy of Sciences of the United States of America**. Vol. 99, No. 18, Sep. 3. 11742-11747. 2002

Hedgecock, D, Launey, S, Pudovkin, AI, Naciri, Y, Lapègue, S and Bonhomme, F. *Small effective number of parents (Nb) inferred for a naturally*

spawned cohort of juvenile European flat oysters Ostrea edulis. **Marine Biology**. No. 150. Pp.1173 – 1182. 2007

Higgins DG, Thompson JD, Gibson TJ. *CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice*. **Nucleic Acids Research**. 22:4673-4680. 1994

Jennings, RM, Shank, TM, Mullineaux, LS and Halanych, KM. *Assessment of the Cape Cod Phylogeographic Break Using the Bamboo Worm Clymenella torquate Reveals the Role of Regional Water Masses in Dispersal*. **Journal of Heredity**. Vol 100(1). Pp 86–96. 2009

Johnson, MS. *Measuring and interpreting genetic structure to minimize the genetic risks of translocations*. **Aquaculture Research**. 31. 133-143. 2000

Kimura MJ. *A simple method of estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences*. **Molecular Evolution**. No. 16. Pp. 111–120. 1980

Librado P and Rozas J. *DnaSP v5: A software for comprehensive analysis of DNA polymorphism data*. Bioinformatics 25: 1451-1452. 2009

Lowe, WH and Allendorf, FW. What can genetics tell us about population connectivity? Molecular Ecology. Vol 19. Iss. 15. Pp 3038–3051. Aug. 2010

McDonald CM. *Population Structure, Biochemical Adaptation and Systematics in Temperate Marine Fishes of the Genera Arripis and Chrysophrys (Pisces: Perciformes)*. PhD Thesis, Australian National University. 1980. Cited in Johnson 2000.

Nabholz, B, Glémin, S and Galtier, N. *The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals*. **BMC Evolutionary Biology**. 9: 54. 2009

New Zealand Ministry of Fisheries 2010a. TAR2 Catch report. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=8&stock=TAR2 06 October 2010.

New Zealand Ministry of Fisheries 2010b. TAR3 Catch report. Accessed online at http://fs.fish.govt.nz/Page.aspx?pk=8&stock=TAR3 06 October 2010.

Pauly, D, Christensen, V, Dalsgaard, J, Froese, R, Torres, F Jr. *Fishing Down Marine Food Webs*. **Science**. Vol. 279. No. 5352. Pp 860 – 863 February. 1998.

Pichler, FB. *Genetic assessment of population boundaries and gene exchange in Hector's dolphin*. **DOC Science Internal Series 44**. New Zealand Department of Conservation. 2002.

Rice, WR*. Analyzing tables of statistical tests*. **Evolution**. 43. 223–225. 1989

Tamura K and Nei M. *Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees*. **Molecular Biology and Evolutio**n. Number 10 (3) 512-26. 1993

Taylor, CA and Hardman, M. *Phylogenetics of the Crayfish Subgenus Crockerinus, Genus Orconectes (Decapoda: Cambaridae), Based on Cytochrome Oxidase I*. **Journal of Crustacean Biology**. Vol. 22. No. 4. Nov. Pp. 874-881. 2002.

Weir, BS. *Genetic data analysis II: Methods for discrete population genetic data*. Sinauer Associates Inc. Sunderland, USA. 1996

Weir, BS and Cockerham, CC. *Estimating F-statistics for the analysis of population structure*. **Evolution**. Vol. 38. Pp. 1358 - 2370. 1984

Zemlak, TS, Ward, RD, Connell, AD, Holmes, BH, Hebert, PD. *DNA barcoding reveals overlooked marine fishes.* **Molecular Ecology Resources**. Special Issue on Barcoding Life. Vol. 9. Pp. 237 – 242. 2009

Chapter 4. **Discussion**

4.1 Summary and conclusions

New Zealand controls one of the largest marine fisheries in the world today and operates a Quota Management System that is the envy of many countries. Stocks are managed via an Individual Transferable Quota (ITQ) system that delivers virtual ownership of the fishery to quota holders, and this system is acknowledged as one of the best run and sustainable fisheries in the world. However the reality is that many gaps in still exist in the knowledge of stock size, stock composition and the impacts that fishing, including the unintended consequences of fishing using destructive fishing gears (Griffith, 2008), has on stock structure and health.

As ITQ is a species - centric philosophy, the effects of fishing on the habitat of the target species, the effect of unintended capture of non-target species and the economic value of non-target species are downplayed to some extent (Soykan et al, 2008) . Some researchers (e.g., Law 2007) advocate working within ITQ models and suggest that producers focus on methods of targeting smaller, but still marketable, fish. Other researchers such as Grafton et al (2007) argue that any species-centric approach is flawed, and discuss the inherent difficulties of species centric management and calls for ecosystem-centric fisheries. While rights-based fishery management models (Beddington et al, 2008) work, the method and focus needs to change if stocks are to remain healthy and fisheries industries to be more than a purely exploitative activity.

One aspect of fisheries management that should change to improve the health of fish stocks is that of research, coupled with research-informed fisheries policy. Present fisheries research in New Zealand leaves significant gaps in our knowledge of targeted species, including, or perhaps especially, our knowledge of the genetic make up of species.

Hauser and Carvalho (2008) argue that molecular genetics has led to fundamental changes in "*our understanding of marine ecology*" and has allowed us to examine genetic population structure, how exploitation affects population structure (Gårdmark, 2003) and has demonstrated that effective population size

is several orders of magnitude lower than census sizes. They and other researchers remind us that species such as Atlantic cod (Gadus morhua) have suffered economic and local extinction in parts of their range where formerly they were the basis of massive industries (Hauser and Carvalho, 2008, Roberts, 2007)

In this study, these techniques have been applied to local fisheries, and have demonstrated that small numbers of fish and relatively simple genetic testing can provide a wealth of information on the structure and even health of fish populations. Unfortunately, one of the findings of the study was that both A. trutta and N. macropterus exhibit signs of passing through recent population bottlenecks, and in the case of A. trutta, the population is still in decline, despite management efforts to reverse this trend.

While only 178 Arripis trutta were sampled, the calculated levels of migration between locations suggests strongly that the species forms a single, highly connected population in New Zealand, though it must be acknowledged that P-values associated with calculated Φ_{ST} values are high, reducing the statistical significance of the findings.

Also, while numbers of samples from Australia were small, the finding of 15 migrants per generation also suggests that migration of the species, either as larva drifting with ocean currents, or as adults actively migrating, may serve to connect populations across the Tasman Sea.

What was unexpected was that the New Zealand populations showing the highest levels of genetic similarity were not necessarily geographically contiguous, which suggests that either pelagic larval dispersal or adult migration serve as a population structure mechanism. The slight discontinuity observed between some populations was possibly an artefact of the sampling process, but could also point to oceanic currents being the main method of migration, especially if Australian *A. trutta* larvae are regularly seeding New Zealand populations. Unfortunately, insufficient data is available for this to be any more than speculation.

What is clear from the data is that areas as far apart as Marlborough Sounds and Coromandel regions have “infinite” numbers of migrants commuting between them, while for Auckland East and the Coromandel, only separated by the Firth of Thames, only 4 migrants per generation was recorded. Again, it is possible that this is an artefact of the sampling process, which offers intriguing possibilities for future researchers.

These findings of similarity between populations, or perhaps more correctly, a single population of *A. trutta* in New Zealand (and perhaps Australia) have management implications and even fisheries treaty implications for both countries. *A. trutta* is currently managed in New Zealand as a single population, though this is for political and fisheries management reasons rather than an understanding of the biology of the species. However, it appears that the current management regime is the most appropriate method; that a single population does in fact exist. Looking further afield to Australia, the very small amount of evidence available suggests that again, a single population exists and may be shared between both countries.

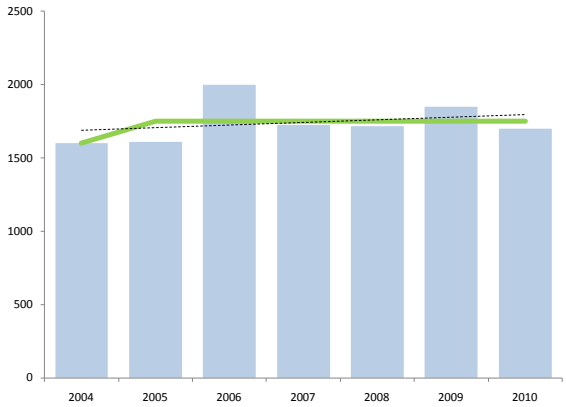
The primary goal of the phylogenetic section of this study was to test the phylogenetic relationship of the Arripidae family, and to test the ND2 derived findings of Yagishita et al (2002), that Arripidae forms a monophyletic clade with the Scombridae and the Stromateoidae. While COX1 data supports this hypothesis, 12S and 16S data did not.

Available data offers a divergence age of 2.8 million years for Arripidae, but saturation at the first position suggested that this is an underestimate. Recalling the caution noted by Galtier et al (2009), the likelihood of this value being in error is probably quite high.

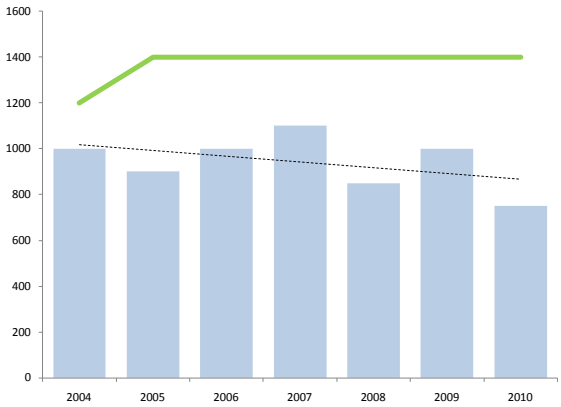
4.2 Implications of this study

The observed difference in mismatch distributions between *A. trutta* and *N. macropterus* was an interesting observation and provides a possible window into the genetic effects of overfishing on a commercially exploited species in New Zealand waters, and provides a potential tool for fisheries managers.

While *N. macropterus* (and all other commercially fished species in the New Zealand EEZ) are managed with the objective of maintaining B_{MSY}, as was discussed in Chapter 1, significant gaps exist in our knowledge of the genetic state of the species we harvest in New Zealand.



Tarakihi Region 2 (TAR 2) TACC and landings
New Zealand Ministry of Fisheries 2010a)



Tarakihi Region 3 (TAR 3) TACC and landings
(New Zealand Ministry of Fisheries 2010b)

Figure 4-1*: N. macropterus* TACC and Landings

To illustrate the impact of such gaps, Figure 4-1 shows the Total Allowable Commercial Catch (TACC) (solid line) versus the actual landings (vertical bars) in tarakihi area 2 (TAR2) on east coast of the North Island and tarakihi area 3 (TAR3), an adjacent area on the east coast of the South Island. The dotted lines on the graphs are trends of landings over the last 6 years. Values are in tonnes. These graphs, sourced from Ministry of Fisheries data, show that TACC in both areas are static, but landings are not. In TAR2, landings are increasing very slightly, while in TAR3 landings are falling. Catch limits and actual landings vary for a number of reasons such as market price of fish, (Roberts, 2007), price of fuel, catch per unit effort, availability of higher value species, etc. TAR2 appears to be a population managed right at the limit of sustainability, while TAR3 appears suggestive of a falling population of fish, unable to respond to excessive fishing pressure.

When taken together, all of these factors suggest that New Zealand's fisheries are not managed sustainably, and that the metrics currently employed by fishery managers do not paint an accurate picture of the health of the stocks. Each population of fish examined in this chapter showed signs that exploitation levels were greater than those needed to maintain stocks at self sustaining levels.

4.3 Opportunities for future work in this area

While the issues noted above generated considerable frustration throughout this study, they also offer opportunities for future study. The literature examined suggests that the universal primers employed should be suitable for the species tested. Determining why they were not successful could be useful in developing student laboratory programmes.

Another opportunity identified was that of the number of samples collected. Good numbers of *A. trutta* samples were collected from the North Island of New Zealand; however attempts to secure adequate numbers of samples from the South Island of New Zealand and from Australia were largely unsuccessful. Only six and three samples were collected from these areas, respectively. It is believed that greater sampling success in these areas could have provided better statistical support for the tests performed. Any further work in this area should make collecting South Island and Australian samples something of a priority, especially with the goal of determining genetic linkage of Australian and New Zealand populations of *A. trutta*.

Related to this, it is believed that gaining significant numbers of samples from the other members of the Arripidae family would be very valuable. While three of the four species (*A. trutta*, *A. georgianus* and *A. truttacea*) were collected, it was not possible to collect any samples of the final member of the genus, *A. xylabion*. Again, future work on this genus should be considered lacking if samples of this species were not obtained.

Finally, the findings for *N. macropterus* and *A. trutta* suggest that both species remain under considerable fishing pressure, to the degree that some populations may currently be experiencing exploitation levels above the capacity of the species to recover. This is despite both species being managed

under what is widely acknowledged as one of the most successful quota management systems in the world. Further work on these species should be initiated to determine if this is the case, and what can be done to improve the situation. The wider implication is that many other species managed in this way in New Zealand may also over-fished, and that current management and monitoring activities are not sufficient to identify or act upon instances of species decline.

The tools are available. They are relatively easy and relatively inexpensive to deploy, and provide insights into species health that are not available from traditionally employed stock assessment models.

Molecular ecology tells us much about the health and history of populations. As ecologists, it is our role to act upon this information wisely.

4.4 References

Beddington, JR, Agnew, DJ and Clark, CW. *Current Problems in the Management of Marine Fisheries*. **Science**. Vol. 316. No. 5832. Pp. 1713 - 1716. Jun. 2007

Bowen, BW, Muss, A, Rocha, LA, Grant, WS. *Shallow mtDNA Coalescence in Atlantic Pygmy Angelfishes (Genus Centropyge) Indicates a Recent Invasion from the Indian Ocean*. **Journal of Heredity**. Vol. 97. No. 1. Pp. 1 – 12. 2006

Davidson, GW, Thorarensen, HT, Lokman, M, Davie, PS. *Stress of capture and captivity in kahawai Arripis trutta (Bloch and Schneider) (Perciformes: Arripidae)*. **Comparative Biochemistry and Physiology Part A: Physiology**. Vol 118. Iss 4. 1405-1410, 1997

Denver, D, Morris, K, Lynch, M, Vassilieva, L, Thomas, WK. *High Direct Estimate of the Mutation Rate in the Mitochondrial Genome of Caenorhabditis elegans*. **Science**. No. 289. 2000

Excoffier, L and Lischer, HEL. *Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows*. **Molecular Ecology Resources.** In press. 2010

Excoffier, L, Smouse, P, and Quattro, J. *Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data*. **Genetics** 131:479-491. 1992

Galtier, N, Nabholz, B, Glémin, S, Hurst, GDD. *Mitochondrial DNA as a marker of molecular diversity: A reapprisal*. **Molecular Ecology**. Vol 18. Iss. 22. 4541 – 4550. 2009

Gårdmark, A, Dieckmann, U and Lundberg, P. *Life-history evolution in harvested populations: the role of natural predation*. **Evolutionary Ecology Research**. Vol. 5. Pp. 239 – 257. 2003

Grafton, RQ, Arnason, R, Bjørndal, T, Campbell, D, Campbell, HF, Clark, CW, Connor, R, Dupont, DP, Hannesson, R, Hilborn, R, Kirkley, JE, Kompas,

T, Lane, DE, Munro, GR, Pascoe, S, Squires, D, Steinshamn, SI, Turris, BR and Weninger, Q. *Incentive-based approaches to sustainable fisheries*. **Canadian Journal of Fisheries and Aquatic Science**. Vol. 63. No. 3. Pp. 699 – 710. 2006

Griffith, DR. 2008. *The ecological implications of individual fishing quotas and harvest cooperatives*. **Frontiers in Ecology and the Environment**. Vol. 6: Pp. 191 – 198. 2008

Hauser, A, Adcock, GJ, Smith, PJ, Bernal Ramírez, JH, Carvalho, GR. *Loss of Microsatellite Diversity and Low Effective Population Size in an Overexploited Population of New Zealand Snapper (Pagrus auratus)*. **Proceedings of the National Academy of Sciences of the United States of America**. Vol. 99, No. 18, Sep. 3. 11742-11747. 2002

Heino, M and Godø, OR. *Fisheries-induced selection pressures in the context of sustainable fisheries*. **Bulletin of Marine Science**. Vol 70. No. 2. Pp. 639 – 656. 2002

Higgins DG, Thompson JD, Gibson TJ. *CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice*. **Nucleic Acids Research**. Vol. 22. Pp. 4673 - 4680. 1994

Hutchins, B, Swainston, R. 1986. *Sea Fishes of Southern Australia. Complete Field Guide for Anglers and Divers*. Swainston Publishing.

Johnson, MS. *Measuring and interpreting genetic structure to minimize the genetic risks of translocations*. **Aquaculture Research**. 31. 133-143. 2000

Kingsford, MJ. 1989. *Distribution patterns of planktivorous reef fishalong the coast of northeastern New Zealand*. **Marine Ecology Progress Series**. Vol. 54. Pp. 13 - 24, 1989

McDonald CM. *Population Structure, Biochemical Adaptation and Systematics in Temperate Marine Fishes of the Genera Arripis and Chrysophrys*

(Pisces: Perciformes) PhD Thesis, Australian National University. 1980. Cited in Johnson 2000.

Nabholz, B, Glémin, S and Galtier, N. *The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals*. **BMC Evolutionary Biology**. Vol. 9. No. 54. 2009

New Zealand Ministry of Fisheries. 2009. Kahawai *Stock Assessment document 41 KAH 09.*Collected online at http://fs.fish.govt.nz/Page.aspx?pk=113&dk=21825 on 25 May, 2010.

Paulin, C. *Review of the Australian fish family Arripididae (Percomorpha), with the description of a new species.* **Australian Journal of Marine and Freshwater Research.** Vol. 44. No. 3. Pp. 459 – 471. 1993

Rice, WR. *Analyzing tables of statistical tests*. **Evolution**. Vol. 43. Pp. 223 – 225. 1989

Roberts, C. *The Unnatural History of the Sea*. Island Press, Washington DC. 2007.

Soykan, CU, Moore, JE, Zydelis, R, Crowder, LB, Safina, C and Lewison, RL. *Why study bycatch? An introduction to the Theme Section on fisheries bycatch*. **Endangered Species Research**. Vol. 5. Pp. 91 - 102. 2008

Tamura K and Nei M. *Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees*. **Molecular Biology and Evolution**. Number 10. Vol. 3. Pp. 512 - 526. 1993

Yagishita, N, Miya, M, Yamanoue, Y, Shirai, SM, Nakayama, K, Suzuki, N, Satoh, TP, Mabuchi, K, Nishida, M, Nakabo, T. *Mitogenomic evaluation of the unique facial nerve pattern as a phylogenetics marker within the perciform fishes (Teleostei: Percomorpha)*. **Molecular Phylogenetics and Evolution**. Vol. 53. Pp. 258 – 266. 2009

Appendix 1. Collection Instructions

Introduction
I am a student at Victoria University of Wellington, studying for an MSc in Marine Biology. In my study, I am collecting DNA from kahawai (*Arripis trutta*) with the goal of determining how many genetic stocks exist in NZ waters. From the material collected in the field, I will be extracting DNA and amplifying it using the Polymerase Chain Reaction, or PCR method. This technique involves creating many millions of copies of DNA by controlled heating and cooling of the sample in the presence of free amino acids. This allows tiny samples to create measurable amounts of DNA within an hour or so, which is then "read" in a computer controlled sequencer. This machine provides a readout of the actual sequence of DNA in the sample.

In addition to investigating the stock structure of kahawai, I am interested in determining the limits of the four members of the family Arripidae. These are:

Species	Details
Kahawai or Australasian salmon (*Arripis trutta*)	Believed to extend right around NZ and around the southern coast of Australia. Grows to approx 80cm.
Kermadec kahawai (*Arripis xylabion*)	Range is from the Kermadecs south to approximately the Bay of Plenty. *A. xylabion* can be identified by measuring the top lobe of the caudal (i.e., tail) fin. If this is >30% of the length of the body of the fish, the specimen is *A. xylabion*. If this fin lobe is <30%, the specimen is *A. trutta*. Can grow larger than *A. trutta*.
Western Australian Salmon (*Arripis truttacea*)	Confined to southern Western Australia. Can grow larger than *A. trutta*.
Tommy Ruff or Australian herring (*Arripis georgianus*)	From southern Australia. The smallest member of the family.

It is possible that each of these fish visit NZ, albeit very rarely in the case of *A. truttacea* and *A. georgianus*. It is also possible that *A. xylabion* and *A. truttacea* are actually sub-species rather than full species.

General Instructions
Only small samples are needed for the study. Clippings need only be approx ½ cm x ½ cm. After collecting the sample and placing it in the tube, please complete the columns in the collection sheet. Measure or estimate the fork length[2] of the fish, and record this in the row of the collection sheet that corresponds with the tube number. Record additional details such as where on the fish the sample was taken. Record the location the fish was captured on the return sheet. This need not be specific. Something like "4nm offshore from Whakatane" is more than sufficient. You can also record any other details you feel are pertinent. There is also a column for "assumed species" on the return sheet. If you are unsure which member of the Arripidae family you have, just record "kahawai".

Live fish – to be returned to the water
If you are taking a clipping from a live fish that you intend to release, take a small clipping, approx ½ cm x ½ cm, from the trailing edge of the dorsal or anal fin. The fin rays are soft in these areas, and the material can be easily clipped off with a pair of scissors.

Record which fin the material was collected from as this will allow me to determine which fin gives better results after I run the genetic tests on the samples.

[2] Fork length is measured from the nose of the fish to the centre of the V of the caudal (tail) fin. This is the easiest and most consistent length measurement to perform.

Dead fish
If the fish you are collecting the material from is dead, it is still perfectly acceptable to take a fin clip as outlined for live fish. However, there is a much better chance of collecting viable DNA from muscle tissue. Cut a small section of meat from the gut cavity or from a fillet, preferably with a small piece of skin attached. Again, this only needs to be very small. Additionally, collect a couple of scales and place them in the collection tube with the flesh sample. I can use these to age the fish, and when combined with the fork length information, can build up and idea of how fish age relates to length.

Sample Record Sheet

Tube No.	Date Collected	Collected by	Location	Assumed Species	Fork Length	Notes
A0001	22nd Nov 08	Brenton	Wellington Harbour	Kahawai (*A. trutta*)	37cm	Dead fish. Sample taken from filet. Two scales also collected for aging.
A0002	"	"	Wellington Harbour	Kahawai	27cm	Live fish, returned to water. Fin clip taken from trailing edge of anal fin
A0003	25th Nov 08	Brenton	90 Mile Beach	Kermadec kahawai (*A. xylabion*)	29cm	Live fish, returned to water. Fin clip taken from trailing edge of anal fin.

Please note: "Ditto" marks are fine on the return sheet, as are estimates of location and fork length.

After you have finished collection
While the tubes need no special treatment other than keeping them out of the sun as much as possible, they do store better if kept cool. Please keep them in the fridge until you are ready to send them back to me. Also, the tubes contain 70% ethanol. While the volume is tiny, it is flammable. Avoid smoking near the tubes while they are open….

After you have a sample in each of the tubes, simply place them back in the ziplock bag, re-wrap them in the bubble wrap, place them in the courier bag and send them back to me.

And thank you so much for your assistance. I am working on putting a website together so I can provide regular updates to everyone who has helped with this project.

Also, if you think these instructions could be clearer, please feel free to let me know! My home email address is brenton.hodgson@clear.net.nz

Regards,

Brenton Hodgson
MSc candidate,
Victoria University of Wellington

Appendix 2. *Arripis trutta* COX1 Variable Nucleotides

Positions	1	2	5	63	77	131	137	150	227	236	281	308	317	371	416	422	425	443	458	461	463	474	501	521	529	539	542
Haplotype 1	C	T	A	A	T	T	A	C	C	T	C	G	G	C	C	A	T	T	T	A	G	G	A	T	A	T	T	C
Haplotype 2	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 3	-	-	-	-	-	-	-	-	T	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 4	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-
Haplotype 5	-	-	-	-	-	-	-	-	-	-	T	A	-	-	-	-	C	C	-	-	-	A	-	-	-	-	-	-
Haplotype 6	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	A	-	-	-	G	-	-	A
Haplotype 7	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-
Haplotype 8	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	T	-	-	-	G	-	-	-
Haplotype 9	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	G	-	-	-	-	G	-	-	-
Haplotype 10	-	-	-	-	-	-	-	-	-	-	-	A	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 11	G	C	T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-
Haplotype 12	-	-	-	-	-	-	-	-	-	-	-	A	A	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-
Haplotype 13	-	-	-	-	C	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 14	-	-	-	-	-	-	-	-	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 15	-	-	-	G	-	C	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 16	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-
Haplotype 17	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	G	-	-	-	-	-	-	-	C	-	-	-	-
Haplotype 18	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-
Haplotype 19	-	-	-	-	-	-	G	-	-	-	-	A	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 20	-	-	-	-	-	-	-	-	-	-	-	A	-	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplotype 21	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-
Haplotype 22	-	-	-	-	-	-	-	T	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 3. *Arripis trutta* COX1 Haplotype List

Appendix 3-Table 3-1 Reference Sequence Data

Sample Number	Location Sample Collected	Sequence Data
1	Wellington	CTTTAAGCCTACTTATTCGAGCTGAACTTAGCCAACCAGGAGCCCTTCTTGGAGACGACCAAATCTACAATGTAATTGTTACAGCTCACGCTTTCGTAATAATCTTCTTCAT AGTTATACCAATTATGATTGGAGGATTTGGAAACTGACTAATCCCTCTAATAATTGGGGCTCCTGATATAGCATTCCCTCGAATAAATAATATAAGCTTCTGACTCCTCCCT CCCTCATTTCTTCTACTCCTAACTTCTTCTGGAGTAGAAGCTGGCGCCGGAACTGGCTGAACCGTTTACCCCCCCTCTAGCCGGGAACCTGGCGCATGCTGGAGCTTCCGT TGACCTAACCATTTTCTCCTTACATCTAGCAGGTATCTCCTCTATCTTAGGGGCCATCAATTTTATTACAACAATTATCAACATGAAACCTACAGCTGTATCCCAATATCAGA CCCCTTTATTTGTATGGGCTGTATTAATTACTGCCGTTTTACTTCTTCTATCTTTACCAGTCCTTGCCGCTGGGATTACAATGCTTCTAACTGACCGCAA

Haplotype Assignments

Haplotype 1: COR140, WELL1. **Haplotype 2**: AKE156, AKE168, AKW80, AKW81, AKW82, AKW84, AKW89, AKW127, AKW128, AKW129, AKW130, AKW131, AKW133, AKW134, AKW157, AKW158, AKW161, AKW163, AKW164, AKW166, AUS181, AUS183, COR90, COR91, COR92, COR93, COR94, COR95, COR96, COR97, COR98, COR99, COR100, COR135, COR139, COR141, COR142, COR144, COR146, COR149, COR150, SOU201, SOU202, SOU203, SOU205, SOU206, NIE5, NIE7, NIE11, NIE13, NIE14, NIE120, NIE121, NIE122, NIE123, NIE126, NIW65, NIW66, NIW67, NIW69, NIW70, NIW71, NIW74, NIW75, NIW76, NOR8, NOR9, NOR38, NOR39, NOR40, NOR41, NOR42, NOR44, NOR45, NOR46, NOR47, NOR64, NOR136, NOR137, WELL3, WELL4, WELL15, WELL17, WELL18, WELL19, WELL20, WELL21, WELL22, WELL24, WELL25, WELL26, WELL27, WELL32, WELL33, WELL34, WELL35, WELL36, WELL55, WELL77, WELL78, WELL79, WELL101, WELL103, WELL104, WELL105, WELL107, WELL108, WELL109,

WELL110, WELL111, WELL112, WELL113, WELL116, WELL117, WELL118, WELL119, WELL169, WELL170, WELL171, WELL172, WELL173, WELL174, WELL175, WELL176, WELL189, WELL191, WELL192, WELL193, WELL194, WELL196, WELL197, WELL198, WELL199, WELL200. **Haplotype 3**: SOU204, NIE12, NIW68, WELL6, WELL16, WELL28, WELL30, WELL37, WELL114, WELL190. **Haplotype 4**: AKE155, AKW132, WELL2, WELL195. **Haplotype 5**: AUS182. **Haplotype 6**: AKW165. **Haplotype 7**: AKW160, AKW162. **Haplotype 8**: AKW159. **Haplotype 9**: AKE153, **Haplotype 10**: AKE138, COR148, NIW73, WELL29, WELL115. **Haplotype 11**: COR147. **Haplotype 12**: COR145. **Haplotype 13**: NIE124, NIE125. **Haplotype 14**: WELL31, WELL106. **Haplotype 15**: WELL102. **Haplotype 16**: AKW88, NOR43. **Haplotype 17**: AKW86, AKW87. **Haplotype 18**: AKW85, WELL23. **Haplotype 19**: AKW83. **Haplotype 20**: NIW72. **Haplotype 21**: NOR48. **Haplotype 22**: NIE10

Appendix 4. 12S Sequence Data

Sample Number	Collection Location	Species	Species Common Name	Sequence Data
184	Australia	*Arripis georgianus*	tommy ruff	CCGTAACACTGATAGAAAGCACTTAATCTATTCGCCTGGGTACTACGAGCGGCAGCTTGAAACCCAAAGGACTTGGCGGTTCTTTAGATCCACCTAGAG GAGCCTGTTCTATAATTGATAATCCCCGTTTAACCTCACCCTCCCTTGCTAAACCCGTCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGTCCTATA GTTAGCAAAATTGGTTCAGCCCAGAACGTCAGGTCAAGGTGTAGCGTATGAAAGGGGAAGAGATGGGCTACATTCGTTAAGATTAACGAACTACGGAAG GTGTAATGAAACCGCACCCAGAAGGAGGATTTAGCAGTAAGTGGGAATTAGAATAGTTCCACTGAATATTGGCTCTGAAGTGCGCACACA
185				CGTAACACTGATAGAAAAGCACTTAATCTATTCGCCTGGGTACTACGAGCGGCAGCTTGAAACCCAAAGGACTTGGCGGTTCTTTAGATCCACCTAGAG GAGCCTGTTCTATAATTGATAATCCCCGTTTAACCTCACCCTCCCTTGCTAAACCCGTCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGTCCTATA GTTAGCAAAATTGGTTCAGCCCAGAACGTCAGGTCAAGGTGTAGCGTATGAAAGGGGAAGAGATGGGCTACATTCGTTAAGATTAACGAACTACGGAAG GTGTAATGAAACCGCACCCAGAAGGAGGATTTAGCAGTAAGTGGGAATTAGAATAGTTCCACTGAATATTGGCTCTGAAGTGCGCACACA
186				CCGTAACACTGATAGAAAGCACTTAATCTATTCGCCTGGGTACTACGAGCGGCAGCTTGAAACCCAAAGGACTTGGCGGTTCTTTAGATCCACCTAGAG GAGCCTGTTCTATAATTGATAATCCCCGTTTAACCTCACCCTCCCTTGCTAAACCCGTCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGTCCTATA GTTAGCAAAATTGGTTCAGCCCAGAACGTCAGGTCAAGGTGTAGCGTATGAAAGGGGAAGAGATGGGCTACATTCGTTAAGATTAACGAACTACGGAAG GTGTAATGAAACCGCACCCAGAAGGAGGATTTAGCAGTAAGTGGGAATTAGAATAGTTCCACTGAATATTGGCTCTGAAGTGCGCACACA
187				GGTAAACACTGATAGAAAGCACTTAATCTATTCGCCTGGGTACTACGAGCGGCAGCTTGAAACCCAAAGGACTTGGCGGTTCTTTAGATCCACCTAGAG GAGCCTGTTCTATAATTGATAATCCCCGTTTAACCTCACCCTCCCTTGCTAAACCCGTCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGTCCTATA GTTAGCAAAATTGGTTCAGCCCAGAACGTCAGGTCAAGGTGTAGCGTATGAAAGGGGAAGAGATGGGCTACATTCGTTAAGATTAACGAACTACGGAAG GTGTTATGAAACCGCACCCAGAAGGAGGATTTAGCAGTAAGTGGGAATTAGAATAGTTCCACTGAATATTGGCTCTGAAGTGCGCACACA
188				CGTAACACTGATAGAAAAGCACTTAATCTATTCGCCTGGGTACTACGAGCGGCGCTTGAAACCCAAAGGACTTGGCGGTTCTTTAGATCCACCTAGAGG AGCCTGTTCTATAATTGATAATCCCCGTTTAACCTCACCCTCCCTTGCTAAACCCGTCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGTCCTATAG TTAGCAAAATTGGTTCAGCCCAGAACGTCAGGTCAAGGTGTAGGGTATGAAAGGGGAAGAGATGGGCTACATTCGTTAAGATTAACGAACTACGGAAG GTGTTTTGAAACCGCACCCAGAAGGAGGATTTAGCAGTAAGTGGGAATTAGAATAGTTCCACTGAATATTGGCTCTGAAGTGCGCACACAC
5	New Zealand	*Arripis trutta*	kahawai	TGTAACACTGATAGAAAAACACTTAACCTATCCGCCCGGGTACTACGAGCTTCAGCTTGAAACCCAAAGGACTTGGCGGTACTTTAGACCCACCTAGAG GAGCCTGTTCTATAATTGATAATCCCCGTTCAACCTCACCCTCCCTTGCTAAACCCGTCTATATACCGCCGTCGTAAGCTTACCCTCTGAGGGACTTATA GTAAGCAGAATTGGTACAACCCAGAACGTCAGGTCAAGGTGTAGCGCATGGGAGGGGAAGAGATGGGCTACATTCACTAAGTTCTAGTGAATCACGGA AGGTGTCATGAAAACCACACTCTCAAAGGAGGATTTAGTAGTAAGCAGGAAATAGAGTGTCCTGCTGAAGCTGGCTCTAAAGTGCGCACAC
52	New Zealand	*Chelidonichthys kumu*	gurnard	CTAAACATTGATAGTACTCTACACCCACTATCCGCCCGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAG GAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTCATA GTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGGAAAGGGAAGAAATGGGCTACATTCCCTATAATTAGTGAATACGGACGA TGTCCTGAAAGAGACATCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGCGTTCCGCTGAAATTGGCCCTGAAGCGCGCACACACCG

Sample Number	Collection Location	Species	Species Common Name	Sequence Data
56				CCTAACATTGATAGTACTCTACACCCACTATCCGCCCGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAG GAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCTTTTCTTGTTTTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTCATA GTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGGAAAGGGAAGAAATGGGCTACATTCCCTATAATTAGTGAATACGGACGA TGTCCTGAAAGAGACATCTGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGCGTTCCGCTGAAATTGGCCCTGAAGCGCGCACACACCG
290	New Zealand	*Helicolenus percoides*	scarfie	CCTAAACCTTGGCATATATCACATACCCTGCCCGCCTGGGAACTACGAGCATCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCCCCTAGAG GAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTTCCTTGTTTATCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCTAAAA GTAAGCACAACTGGCAAAACCCAAAACGTCAGGTCGAGGTGTAGCGCATGGAGGGGGAAGAAATGGGCTACATTCCCTACAATAGGGAACACGAAAG GTGCACTGAAATACGCACCTGAAGGAGGATTTAGTAGTAAGCGGGAAATTGCGTGTTCCGCTGAAATCGGCCCTGAAGCGCGCACACACCGC
54	New Zealand	*Latridopsis ciliaris*	blue moki	CCTAAAATCGATAGTGCACTACACTCACTATCCGCCCGGGTACTACGAGCGTCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAG GAGCCTGTTCTGGAACCGATACCCCCCGTTCAACCTCACCCCTCCTTGTTTTTCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCTTGT AGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCATGGAAGGGGAAGAAATGGGCTACATTCCCTACTGTAGGGAACACGAACG ATGTCTTGAAACATTCATCCGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGAGTTCCGCTGAAACCGGCCCTGAAGCGCGCACACACCGC
180	New Zealand	*Lepidoperca aurantia*	orange perch	CGTAACATCGATAGCACACTACGCCCGCTATCCGCCTGGGAACTACGAGCGCCAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGATCCCCCTAGA GGAGCCTGTCCTAGAACCGATAATCCCCGTTCAACCTCACCTTTTCTTGTTCTCCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGTCCTAT CGTAAGCAAAGTTAGTACAACCCAAAACGTCAGGTCGAGGTGTAGCGTATGAAAAGGGAAGAGATGGGCTACATTCACTAATGTAGTGAATTACGAATG GCACATTGAAACTCTGTGCCCGAAGGAGGATTTAGCAGTAAGCAGGGAGCAAAGTGCCCCGCTGAATTTGGCCCTGAAGCGCGCACACACC
58	New Zealand	*Nemadactylus macropterus*	tarakihi	TCCAACATCGATAGTGCATTACATTCACTATCCGCCCGGGTACTACGAGCGTCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAG GAGCCTGTTCTGGAACCGATACCCCCCGTTCAACCTCACCCCTCCTTGTTTTTCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGTCTTGT AGTAAGCAAAATTGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCGCATGGAAGGGGAAGAAATGGGCTACATTCCCTATCGCAGGGAATACGAACG ATGTCTTGAAACATACATCCGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGAGTTCCGCTGAAACCGGCCCTGAAGCGCGCACACACCGC
49	New Zealand	*Notolabrus celidotus*	spotty	CTAAACATTGATGATACACTACCTATATTATCCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAG GAGCCTGTTCTAGAACCGATAATCCTCGTTTAACCTCACCTTTTCTTGTCTTGTCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGACCCCATA GTAAGCAAAATCGGCACAGCCAAAAACGCCAGGTCGAGGTGTAGCGAATGAGAAGGGAAGAAATGGGCTACATTCAATAACCTTTAATGAACACGAAT GGTATTCTGAAAAGCATGCCTGAAGGAGGATTTAGAAGTAAGCGGGGAACAGAGTGTCCTGCTGAAATTGGCCCTGAAGCGCGTACACACC
50				CTAAACATTGATGATACACTACCTATATTATCCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAG GAGCCTGTTCTAGAACCGATAATCCTCGTTTAACCTCACCTTTTCTTGTCTTGTCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGACCCCATA GTAAGCAGAATCGGCACAGCCAAAAACGCCAGGTCGAGGTGTAGCGAATGAGAAGGGAAGAAATGGGCTACATTCAATAACCTTTAATGAACACGAAT GGTATTCTGAAAAGCATGCCTGAAGGAGGATTTAGAAGTAAGCGGGGAACAGAGTGTCCTGCTGAAATTGGCCCTGAAGCGCGTACACACC

Sample Number	Collection Location	Species	Species Common Name	Sequence Data
51	New Zealand	*Pagrus auratus*	snapper	CGTAACATTGACAGTTGAATACATTTTCTGTCCGCCTGGGTACTACGAGCATTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCACCTAGAG GAGCCTGTTCTAGAACCGATAATCCCCGTTCAACCTCACCCTTCCTTGCTTGTCCCGCCTATATACCACCGTCGCCAGCTTACCCTGTGAAGGGTAAAA AGTAAGCGAAATTGGCACTGCCCAAAACGTCAGGTCGAGGTGTAGCGAATGGAAGGGGAAGAAATGGGCTACATTCCCTTTTTAAACATAGGGTACTAC GAAAGATGCACTGAAACCGTGCCTCTGAAGGAGGATTTAGCAGTAAGCGGAAAGTAGAGCGTTCCACTGAAACCGGCTCTTAAGCGCGCAC
60	New Zealand	*Polyprion oxygeneios*	groper	CCTAACATCGATAGTGCACTACACCTACTATCCGCCTGGGAACTACGAGCATCAGCTTGAAACCCAAAGGACTTGGCGGTGCTTTAGATCCACCTAGAG GAGCCTGTTCTAGAACCGATAACCCCCGTTTAACCTCACCCTTCCTTGTTTATCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCTAATA GTAAGCAAAATTGGCACAACCCAAAACGTCAGGTCGAGGTGTAGCGTATGGAGGGGGAAGAAATGGGCTACATTCCCTAATGCAGCGAATACGAACGA TGCACTGAAATGTACATCCGAAGGAGGATTTAGCAGTAAGCAGGAAATAGAGTGTCCCGCTGAAATCGGCCCTGAAGCGCGCACACACCGC
178	New Zealand	*Pseudocaranx dentex*	trevally	CTTAACTTTGATTACCTATCACATCAAACATCCGCCCGGGGATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTAACATCCACCTAGAGG AGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTCTCTAGTTTATTCCGCCTATATACCACCGTCGTCAGCTTACCCTGTGAAGGTCTAACAG TAAGCACAATTGGTACAACCCAAAACGTCAGGTCCAGGTGTAGTGTATGAGAGGGGAAGAAATGGGCTACATTCGCTGTCTGTCAGCGAATAACGAATG ATGCATTGAAACATGCAACTGAAGGAGGATTTAGCAGTAAGCAGAAAGTAGAGCGTTCCGCTGAAACCGGCTCTTAAGCGCGCACACAC
177	New Zealand	*Pseudophycis bachus*	red cod	TATTAACCCTGATAGCTTGATACTAAGCCATCCGCCAGGGGACTACGAGCAATAGCTTAAAACCCAAAGGACTTGGCGGTGCTTTAGACCCCCCTAGAG GAGCCTGTTCTAGAACTGATAACCCCCGTTTAACCTCACCATCTCTTGTTTAACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGGAAAAAA GTAAGCAAAGTAGGTTAAACCAAAAACGTCAGGTCGAGGTGTAGTGTATGAGATGGGAAGAAATGGGCTACATTCTCTGACACAGAGAATACGGAAAGT GGATTGAAAAATTCACCTGAAGGAGGATTTAGTAGTAAGTAGGGACTAGAGAGCCCTACTGAAAATGGCCCTAAAGCGCGCACACACCGC
63	New Zealand (fresh water)	*Salmo trutta*	brown trout	CCGTAACTTTGATGAAACATACAACTGACATCCGCCAGGGAACTACAAGCGCCAGCTTAAAACCCAAAGGACTTGGCGGTGCCTCAGACCCACCTAGA GGAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCACCTCTTGTTTCCCCCGCCTATATACCACCGTCGTCAGCTTACCCTGTGAAGGCCTTA TAGTAAGCAAAATGGGCAAAACCCAAAACGTCAGGTCGAGGTGTAGCGCATGGGGTGGGAAGAAATGGGCTACATTCTCTAAATTAGAGCACTACGAA CCACGCTGTGAAACCAGCGTCCAAAGGTGGATTTAGCAGTAAATAGAAAATAGAGAGTTCTCTTGAAACTGGCTCTGAGGCGCGCACACACCG
53	New Zealand	*Sardinops sagax*	pilchard	CGTAACTTAGATATCTCAGTACAATAGATATCCGCCAGGGGACTACGAGCGCTAGCTTAAAACCCAAAGGACTTGGCGGTGCTTCAGACCCCCCTAGAG GAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACTACTCCTTGCTTTTCCCGCCTATATACCACCGTCGCCAGCTTACCCTGTGAAGGCACTAC AGTAAGCAGGATGAGCATTGCTCAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTAGGAAGAAATGGGCTACATTATCTGAACCAGATCATTCACGGAA AGTTGTCTGAAACGACCACTCGAAGGTGGATTTAGCAGTAAAGGGGGAATAGAGCGCCCCCTTGAAGCCGGCTCTGAAGCGCGCACACACC

Sample Number	Collection Location	Species	Species Common Name	Sequence Data
59	New Zealand	*Seriola lalandi*	kingfish	AGACCTTTACCACCACTTACTTTGTTTAAGTCCGCCTGAGTACTACAAGCGCTAGCTTAAAACCCAAAGGACTTGGCGGTGCCCCAGACCCCCCTAGAG GAGCCTGTTCTATAACCGATAATCCACGTTAAACCTTACCACTTCTTGCTTTTACCGCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGGCACAGAA GTAAGCATAACGGACTTCCTCCAAAACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGCTACATTTTCTCTAAAGAAAACACGGACAGTA AATGAAAAATCACTTATAAGGTGGATTTAGCAGTAAGAAGAACTTAGGATATTTTTCTGAAATCGGCTCTGAGGCGCGCACACACCGCCC
62	Australia	*Thunnus alalunga*	longfin tuna	CGTAACATTGATAGAATTTTACACCCTCTATCCGCCTGGGTACTACGAGCATTAGCTTGAAACCCAAAGGACTTGGCGGTACTTTAGATCCCCCTAGAGG AGCCTGTTCTATAACCGATGACCCCCGTTCAACCTCACCCTCCCTTGTTTCTCCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGTCTAATAG TAAGCAAAATTGGCACCGCCCAGAACGTCAGGTCGAGGTGTAGCGCATGAGAGGGGAAGAAATGGGCTACATTCGCTAACATAGCGAATACGAACGAT GCACTGAAAACGCTCATCTGAAGGAGGATTTAGCAGTAAGTGGAAAATAGAGTGTTCCACTGAAATCGGCTCTGAAGTGCGTACACACCG
179	New Zealand	*Thyrsites atun*	barracouta	CGTAAATTGATAGGAAATTCACCCCCCTATTCCGCCTGAGTACTACGAGCACCAGCTTAAAACTCAAAGGACTTGGCGGTACTTTAGACCCCCCTAGAG GAGCCTGTTCTATAACCGATAATCCCCGTTAGACCTCACCCTCCCTTGTTCACCCCGCCTATATACCGCCGTCATCAGCTTACCCTGTGAGGGATCCAT AGTAAGCAAAATTGGTACAACCCAGAATGTCAGGTCCAGGTGTAGCGCATGGGAGGGGAAGAAATGGGCTACATTCGCTAATTTAGCGAACACGAACG ACGTAATGAAAAAAACATCCGAAGGAGGATTTAGCAGTAAGTGGAAAACAGAGTGTTCCACTGAAGTCGGCTCTAAAGTGTGCACACACCGC
57	New Zealand	*Trachurus murphyi*	jack mackerel	CTTAACATTGATTATTTATTACATCAAACATCCGCCCGGGAATTACGAACATTAGTTTAAAACCCAAAGGACTTGGCGGTGCTTAACATCCACCTAGAGGA GCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTCCCTAGCTTTTTCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTAATAGT AAGCGCAATTGGTACAACCCAAAACGTCAGGCCGAGGTGTAGTGCATGAGAGGGGAAGAAATGGGCTACATTCGCTGCTCACCAGCGAATAACGAATG ATGCATTGAAACTATGCAGCTGAAGGAGGATTTAGCAGTAAGTGGAAAGTAGAGTGTCCCACTGAAACCGGCTCTTAAGCGCGCACACA

Appendix 5. Cyt B Sequence Data

Sample Number	Species	Species Common Name	Sequence Data
52	*Chelidonichthys kumu*	red gurnard	TTGCTAACGACGCCTTAGTAGACCTCCCCGCCCCCTCGAACATCTCCGTTTGATGAAACTTTGGCTCTCTTCTTGGCCTCTGTTTAATTGCACAAA TTCTAACAGGCCTCTTCCTGGCCATACATTACACCTCAGATATCGCCACAGCTTTCTCATCCGTTGCCCACATCTGTCGGGATGTGAACTACGGA TGGCTCATCCGAAACCTCCATGCTAACGGCGCCTCTTTCTTCTTTGTATGTCTCTACATACACATCGGCCGAGGCCTTTACTATGGCTCATATCTA TACAAAGAAACCTGAAACATCGGAGTCATCCTCCTCCTACTAGTTATAGCAACTGCCTTTGTAGGATACGTCCTGCCATGGGGTCAGATATCCTTT TGAGGCGCTACCGTCATTACCAACCTCTTCTCCGCCATTCCCTACATCGGAAACGATCTTGTCCAATGAATTTGAGGCGGCTTCTCAGTAGACAA CGCCACCCTCACCCGCTTTTTTGCATTTCACTTCCTCTTCCCTTTCATTGTCGCAGGGGCCACCCTCATCCACCTCATCTTCCTACACGAGACCG GGTCAAACAACCCCCTCGGATTAAATTCAGACGCAGACAAAATCTCGTTTCACCCCTATTTCTCCTACAAAGACCTTCTAGGATTTGCAGCCCTAC TCATCGCACTCACATCCCTAGCCCTATTTTGCCCCTAACCTCTTAGGGGATCCAGACAACTTCACCCCCGCCAACCCCTTAGTCACACCCCCTCA TATTAAACCCGAATGATACTTCCTATTTGCCTATGCCATCCTACGTTCAATCCCMAACAAGCTCGGCGGGGTTTAAGCCCTTTTTGCCTCAATCAT TGTTCTCATGGTCGTCCCTGTTCTTCACACCTCAAAACAAGGAGGCCTCACCTTCCGCCCCCTCACCCAACTCCTCTTCTGACCCTGATCGCCAA CGTCGCTTTTTCTTGCCTGAATCGGGGGCATGCCTGTCGACCATCCCTTTAATTATTATTGGGCAAGTCGCATCACTCCTGTACTTCCTTCCCTAC TAGTCCTCA
60	*Polyprion oxygeneios*	groper	TTGCAAACAGCGCACTAGTAGACCTCCCCGCCCCTTCTAACATTTCAGTCTGATGAAATTTTGGCTCCCTCCTAGGCCTCTGCTTAATTACCCAAA TCCTCACAGGACTATTCCTCGCAATACACTACACCTCAGATATTGCCACAGCCTTCTCGTCTGTAGCACACATCTGCCGAGATGTAAACTACGGA TGACTTATTCGAAACATTCACGCCAACGGCGCATCCTTCTTTTTCATCTGTATTTATATACACATCGGCCGAGGGCTCTATTACGGCTCCTACCTC TATAAAGAAACATGAAACGTTGGAGTCGTTCTTCTTCTCCTAGTAATAATAACTGCCTTCGTGGGCTACGTCCTCCCCTGAGGCCAAATATCTTTC TGAGGGGCCACCGTCATCACCAACCTCCTATCTGCCGTCCCATATGTAGGTAACACCCTGGTTCAATGGATCTGAGGGGGCTTCTCAGTAGACA ACGCTACTCTCACCCGCTTCTTTGCCTTCCACTTCCTATTCCCCTTTGTCATCGCAGGTGCAACCTTCATTCATCTGCTTTTCCTCCACGAAACAG GGTCAAACAACCCCCTTGGCCTAAACTCAGACGCAGACAAAATCTCCTTCCACCCATACTTCTCATATAAAGACCTATTAGGTTTCGCAGCCCTC CTCATTGCACTTGCTTCATTAGCACTGTTTTCCCCCAACCTTCTGGGCGACCCAGACAACTTCACCCCCGCCAACCCCTTAGTCACACCCCCACA TATCAAACCCGAATGATACTTCCTATTTGCATACGCCATTCTCCGATCAATCCCCAACAAACTGGGAGGCGTACTAGCCCTACTATTCTCTATCCT TGTTCTTATACTAGTCCCCATCCTCCACACATCAAGCAACGAAGTTTAACATTTCGACCCCTCACCCAATTCCTATTTTGAACTCTCATTGCAACCG TGCCCATTCTTACTTGAATCGGAGGCATGCCGTTGAACACCCCTTTATTATTATTGGACAGTTGCGTCTCTTGTTTTATTTCCTTCTCTTCCTAGTT TTATTCC