

New Zealand Fisheries
Assessment Report
2008/20
March 2008
ISSN 1175-1584

Stock structure of kahawai, *Arripis trutta*

P. J. Smith
B. Hartill
P. Hamer
A. McKenzie

Stock structure of kahawai, *Arripis trutta*

P. J. Smith¹
B. Hartill²
P. Hamer¹
A. McKenzie¹

¹NIWA
Private Bag 14901
Wellington

²NIWA
P O Box 109695
Auckland

**Published by Ministry of Fisheries
Wellington
2008**

ISSN 1175-1584

©
**Ministry of Fisheries
2008**

Smith, P.J.; Hartill, B.; Hamer, P.; McKenzie, A. (2008).
Stock structure of kahawai, *Arripis trutta*.
New Zealand Fisheries Assessment Report 2008/20. 42 p.

This series continues the informal
New Zealand Fisheries Assessment Research Document series
which ceased at the end of 1999.

EXECUTIVE SUMMARY

Smith, P.J.; Hartill, B.; Hamer, P.; McKenzie, A. (2008). Stock structure of kahawai, *Arripis trutta*. *New Zealand Fisheries Assessment Report 2008/20*. 42 p.

This project was undertaken to review data and potential methods for separating biological stocks of kahawai (*Arripis trutta*) in New Zealand waters, and, specifically, to assess whether otolith microchemistry could be used to improve understanding of kahawai stock structure and to determine the natal origin and movements of kahawai around New Zealand. Otoliths from 0-group kahawai from Okahu Bay, Waitaemata Harbour, and from Hakahaka Bay, Port Underwood, were selected for multi-element chemistry and for stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$). Fin ray counts were undertaken on the same specimens.

Inductively coupled plasma mass spectrometry detected 11 trace elements in all of the 40 otolith pairs analysed. There was considerable site overlap in the concentrations of the 11 elements, and a cluster analysis coupled with a non-metric Multi-Dimensional Scaling ordination of the multi-elemental data indicated weak discrimination among samples from the two collection sites. A randomisation test indicated that an a posteriori grouping of otoliths according to their site was not due to chance, implying site differences in trace element composition, but that the differences were not sufficient to clearly delineate the two sites.

A multivariate test to determine the contribution that each element made to between-site differences in multi-elemental composition suggested that magnesium and barium had greater explanatory power than the remaining elements. A classification analysis (discriminant function analysis) involving magnesium and barium was used to determine the probability of correctly assigning individual fish to their collection site based on concentrations of these elements in their otoliths. This analysis indicated that individuals could be correctly assigned to their collection site with an average probability of 75%. Discrimination was largely driven by otolith barium levels, which would indicate that otoliths were recording different environmental histories of the fish. Further information on water chemistry (including barium concentrations) variation among estuaries, and between estuaries and coastal waters, would be valuable for assessing the potential of otolith chemistry for studying natal/juvenile origins of adult kahawai. Stable isotope analyses of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ showed almost complete overlap of values among fish from the two sites. The isotope ratio data were therefore of little value for discriminating between fish from the two collection sites.

Fin rays were counted under a low power stereo microscope in 37 specimens from Okahu Bay and 40 specimens from Hakahaka Bay. There was no significant difference between the two sites for dorsal fin ray counts and anal fin ray counts.

A review of the five stock discrimination tools used for pelagic fishes (phenotypic markers, life history parameters, genetic markers, acquired markers, and tagging), concluded that no single stock discrimination tool is likely to be useful for determining the stock structure of kahawai. The high cost of undertaking genetic, parasite, or chemical analyses of kahawai samples throughout the range of the species in New Zealand waters is not likely to be justified. There is no new information that would warrant a change to the present five management units (KAH 1, KAH 2, KAH 3, KAH 8, and KAH 10) recognised for kahawai. Biological data on kahawai age structure in the regional fisheries, and ad hoc opportunities on observations of spawning areas and times (e.g. presence of running ripe fish), should continue to be collected and periodically reviewed in relation to the stock structure of kahawai.

1. INTRODUCTION

The family Arripidae contains one genus, *Arripis* Jenyns, with four species endemic to the temperate waters of Australia and New Zealand. Kahawai (or eastern Australian salmon), *Arripis trutta* (Forster, 1801), are found in coastal waters off eastern Australia, Victoria, Tasmania, and New Zealand. Australian herring, *Arripis georgianus* (Valenciennes, 1831), are found throughout cool temperate waters around Australia; *Arripis truttaceus* (Cuvier, 1829) off Western Australia and South Australia, Victoria, and Tasmania; and northern kahawai, *Arripis xylabion* Paulin, 1993, around northern New Zealand, Lord Howe Island, Norfolk Island, and the Kermadec Islands (Paulin 1993).

Kahawai are considered as one New Zealand wide stock that has been subdivided into five units for management (Figure 1: KAH 1, KAH 2, KAH 3, KAH 8, and KAH 10). *A. trutta* and *A. xylabion* were introduced into the Quota Management System (QMS) in October 2004 under a single species code (KAH), with six Quota Management Areas (QMAs). *A. trutta* is the most important commercial, recreational, and customary species and makes up most of the kahawai landings. About half of the commercial, recreational, and customary catch is taken in KAH 1 Auckland east (TAC 3685 t), with smaller catches from KAH 2 Central East (TAC 1705 t), KAH 3 South-East (TAC 1035 t), and KAH 8 Auckland west (TAC 1155 t) with only minor catches in KAH 4 Chatham Rise (16 t) and KAH 10 Kermadec (16 t). The total catch has declined from a peak of more than 8000 t in 1990–91 to under 3000 t in 2003–04.

The biology of kahawai in New Zealand waters has been reviewed by several authors (Jones et al. 1992, Drummond et al. 1993, Hartill et al. 2005) and only key results relevant to stock structure are highlighted here. Kahawai are pelagic predators found in coastal waters throughout New Zealand, but are more common around the North Island and northern South Island. Spawning is believed to occur on the sea floor in about 60–100m in late summer/autumn and the eggs are pelagic (Crossland 1982); running ripe fish have been caught off the east coast of the North Island and kahawai eggs have been collected in the outer Hauraki Gulf (Hartill et al. 2005). The juveniles (0+ and 1+) occur in shallow coastal bays and estuaries around the North Island and northern South Island, and are common in the surf zone on sandy beaches, and rarely found in depths >30 m (Webb 1972, Jones et al. 1985, Gerring et al. 1998).

Adults are known to make extensive movements, with individuals tagged off the east coast South Island being recaptured off east and west Northland and vice versa, although most recaptures of tagged fish have been within 50 nautical miles of the release site (Wood et al. 1990, Griggs et al. 1998). However, the levels of exploitation differ considerably between areas and many of the reported tag returns have been from the recreational fishery, for which there are no historical data on recreational harvest and effort (Hartill et al. 2005), making it difficult to infer stock relationships from the tag return data.

There are few published studies on stock structure in *Arripis* species. The Australian herring, *A. georgianus*, is a commercially and recreationally important near-shore species, with fisheries managed independently in each state (Ayvazian et al. 2004). Three techniques have been used to examine stock structure in *A. georgianus*: tagging, allozyme electrophoresis, and stable isotope analyses of otoliths. The combined results were viewed as supporting a single stock structure, with evidence for a westward migration before spawning (Ayvazian et al. 2004). An earlier allozyme study of 570 specimens of western salmon (*A. truttaceus*) also indicated the presence of only one breeding population, from Tasmania to Western Australia (MacDonald 1983).

This project was undertaken to review data and potential methods for separating biological stocks of kahawai (*A. trutta*) in New Zealand waters, and specifically to assess whether otolith microchemistry could be used to improve understanding of kahawai stock structure and to determine the natal origin and movements of kahawai around New Zealand. Fin ray counts were made on the same specimens to evaluate meristic counts for identifying the natal origin of kahawai. Two widely separated nursery sites in different water masses (one close to the largest urban centre in New Zealand and one remote) were sampled for this preliminary assessment of otolith chemistry in order to maximise the chances of detecting between site differences.

2. METHODS

2.1 Data and literature review

Biological and oceanographic information relevant to kahawai, were summarized. Stock discrimination studies of coastal pelagic species employing morphometric, genetic, life-history, and parasite methods were briefly reviewed and considered in relation to stock discrimination of kahawai.

2.2 Collection of specimens

Specimens of 0-group kahawai *A. trutta*, were collected by beach seine from two widely separated locations in the eastern North Island and the northern South Island during July and August 2006.

Kahawai beach sampling completed in 1997 indicated that catches of 0-group kahawai were greater in Golden Bay (Pohara, Paton's Pock, Ligar Bay) than in the Tasman Bay stations (Parker's Cove, Rabbit Island, Ruby Bay). The more recent "Fish in Harbours" project also suggested that kahawai juveniles were more common in Golden Bay than Tasman Bay. Sampling in the Auckland region indicated greater success over open white/yellow sandy beaches, adjacent to, but not inside estuaries, and within 2 hours either side of high tide.

Only 0-group kahawai were collected at each site, because larger 1-group kahawai may have moved between nursery sites. Whole kahawai specimens were stored in individual plastic bags, frozen, and stored at -20°C prior to dissection.

2.3 Meristic counts

Whole specimens of 0-group kahawai were thawed and fork length was recorded. Meristic counts were made on the thawed specimens and focused on characters that had shown intra-specific variation in adult kahawai (Paulin 1993): the numbers of dorsal fin rays (15–16), anal fin rays (9–10), and pectoral fin rays (16–18). The gill rakers (total 29–37 in adults) were not well formed in the juvenile fish and consequently could not be counted. Lateral line scales (49–53 in adults) were not considered because of likely counting errors in small specimens, some of which had been damaged during capture.

Fin rays were counted under a low power stereo-microscope. The paired fins were counted on the left side. However, the smallest fin rays in the pelvic and pectoral fins were difficult to distinguish

from connective tissue, and initial double blind counts failed to give consistency in counts; consequently these counts were not used. Therefore, meristic counts were restricted to the dorsal and anal fin rays that could be easily displayed under low magnification, and were counted in 40 specimens from Port Underwood and 37 specimens from Okahu Bay. Differences in meristic counts between samples were tested by a Student t-test, with the variances assumed to be equal for the two areas. In addition, because meristic characters are influenced by water temperature, data on water temperature were extracted from the NIWA database and the summer monthly water temperatures for January and February 2006, the peak spawning period for kahawai, were compared between the two areas sampled for 0-group kahawai.

2.4 Otolith dissection and preparation

Otoliths were removed from the thawed specimens by dissection under a low power stereo microscope using plastic-coated forceps. Otoliths were individually rinsed in de-ionised water, dried on lint free microscope tissue, and stored dry in individual Eppendorf tubes. The otoliths were weighed to the nearest 0.0001 g at the Marine and Freshwater Systems (Department of Primary Industries, Queenscliff, Victoria). Due to the small otolith size (mean weight = 0.0022 g), both otoliths from each fish were required for inductively coupled plasma mass spectrometry (ICP-MS). Therefore, 20 otolith pairs from each sampling site were randomly selected for elemental chemistry and a further 20 otolith pairs from each site were sent to Iso-Analytical (UK) for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses.

2.5 Otolith trace elements

The selected otolith pairs were cleaned of surface contamination by a 3 min immersion in 0.2% ultra-pure HNO_3 in an ultrasonic bath, followed by four rinses in Milli-Q water and drying in a laminar flow cabinet. This procedure resulted in an average weight loss of about 10%. The cleaned otolith pairs were digested in ultra-pure nitric acid (15%) and diluted to provide final sample dilutions of 1:1000 (w/w) (in 2.5% HNO_3). Internal standards of $10 \mu\text{g L}^{-1}$ Sc and In were included in otolith solutions for later use in drift correction.

Sample solutions were analysed with a ThermoFinnigan Element2 high resolution inductively coupled plasma mass spectrometer (ICP-MS) at Marine and Freshwater Systems, DPI, Queenscliff.

The isotopes analysed and the resolution modes (LR - low resolution Δm 300, MR - medium resolution Δm 3000-4000, HR - high resolution Δm 9000-10000) used for each isotope were: (LR) Li^7 , (HR) Na^{23} , (LR) Mg^{25} , (HR) Ca^{43} , (MR) Mn^{55} , (MR) Fe^{56} , (MR) Cu^{63} , (MR) Zn^{66} , (LR) Rb^{85} , (HR) Sr^{88} , (LR) Ba^{138} , and (LR) Pb^{207} . Sc^{45} and In^{115} were analysed in all resolution modes for use in drift corrections. The analytical sequence consisted of four procedural blanks, followed by the calibration standards, followed by the FEBS-1 certified reference otolith powder (National Research Council Canada), followed by the first block of 10 otolith samples. The FEBS-1 CRM and $10 \mu\text{g L}^{-1}$ spiked multi-element standard were re-analysed about every 10 otolith samples to assess precision and recovery/accuracy. The certified reference otolith material (FEBS-1) was prepared following the same methods as for the sample otoliths (i.e. 1:1000 w/w dilution in 2.5% HNO_3 with internal standards Sc, In).

Trace elements in otolith solutions were quantified by the method of addition calibration involving a series of 8 matrix (*A. trutta* otolith) matched standards; unspiked matrix, matrix with

spikes 0.5, 1, 5, 10, 30, 50 and 100 $\mu\text{g L}^{-1}$. Due to the much higher levels of Na, Ca and Sr in otoliths, these elements were quantified with non-matrix matched standards (i.e., 2.5% nitric with spikes similar to levels expected in otolith solutions; Na: 1000, 2000, 3000 $\mu\text{g L}^{-1}$; Ca: 200 000, 300 000, 380 000 $\mu\text{g L}^{-1}$; Sr: 1400, 2000, 2800 $\mu\text{g L}^{-1}$).

Raw counts were initially drift adjusted to blank levels based on the internal standards, followed by blank subtraction (mean blank levels) and then converted to concentrations based on the regression coefficients derived from the calibration standards. Concentration data were adjusted to concentrations in the otoliths based on the dilution factors determined for each sample.

Limits of detection (LOD) were determined as three times the standard deviation of the concentrations in the blanks, and were as follows ($\mu\text{g L}^{-1}$): Li – 0.028, Na - < 1, Mg – 0.312, Ca < 5, Mn – 0.005, Fe – 0.351, Cu – 0.027, Zn – 0.123, Rb – 0.001, Sr < 1, Ba – 0.065, and Pb – 0.090.

The elemental signatures of the 20 otolith pairs collected from each site were compared using the statistical package PRIMER (Clarke et al. 1994). Initially, the concentrations of each of the 11 trace elements were normalised, to equalise the contribution of each variable to the multivariate analysis. This procedure transformed concentration estimates for each element so that they were expressed within two standard deviations of their mean concentration. A Euclidian similarity matrix was then derived from these data, which were used to:

- cluster the data to identify groupings of otoliths with similar trace element compositions (CLUSTER), over which the site of origin of each otolith was indicated;
- ordinate otoliths on the basis of their trace element concentrations using non-metric Multi-Dimensional Scaling (nMDS), over which the site of origin of each otolith was indicated;
- perform a randomisation test to determine the probability that the trace elements in otoliths collected from each site differed, given the overall similarity in the signatures of otoliths collected across both sites (ANOSIM). The results given were based on 5000 randomly generated permutations.

Univariate plots of the concentrations of each trace element, relative to fish length, suggested regional differences were apparent only for some elements. The degree to which each element contributed to between-area differences in multi-elemental composition was, therefore, assessed using the procedure SIMPER. Two elements were found to have greater explanatory power, and further analyses were performed on concentration data for these elements alone, using the PRIMER (Clarke et al. 2001) procedures nMDS, CLUSTER, and ANOSIM.

Linear discriminant function analysis (LDFA) was used to determine the probability of correctly assigning individual fish to their collection site (Quinn et al. 2002). The elements chosen as predictor variables in the LDFA were those with significant ($P < 0.05$) differences between regions based on univariate ANOVA. This selection criterion resulted in only Mg and Ba being used in the LDFA. Because otolith weight was correlated with Mg levels ($r = -0.38$, $P < 0.05$), this effect was removed from the data for LDFA by adjusting Mg data to the overall mean otolith weight using the common pooled slope from the regression of Mg concentration on otolith weight. Classification accuracies were determined using the leave-one-out approach (i.e., the observation being classified is removed from the data set). Because only two sample sites were used in the analysis, only one discriminant function could be extracted. Canonical scores for this function were plotted to display separation among fish from the two collection sites. Assumptions of univariate normality, homogeneity of variances, and homogeneity of within group variance-

covariance matrices were checked by comparing box plots of univariate data and canonical scores (Quinn et al. 2002). Data for Ba were $\log_{10}(x+1)$ transformed to improve univariate normality.

2.6 Otolith stable isotopes $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

Stable isotope analyses were undertaken by IsoAnalytical (UK). Before analysis, the selected otoliths and the standard reference materials were stored in a drying oven for at least 24 hours to ensure a true result for $\delta^{18}\text{O}$ analysis by removing any moisture that might be present.

One whole otolith from each selected pair was placed in an individual clean glass septum capped vial. Due to the small size of the otoliths they were used whole, to avoid losing material during grinding. The vials were sealed and their headspaces flushed with pure helium (99.995%). After flushing, about 0.5 ml of pure phosphoric acid was injected into the vials and mixed with the sample. The phosphoric acid was prepared for isotopic analysis of carbonate samples following standard procedures (Coplen et al. 1983).

Samples were allowed to react over about 48 hours. Suitable aliquots of standard reference materials were prepared in the same manner as the otolith samples (this removes the temperature dependent fractionation of carbonate as it is converted to carbon dioxide). The CO_2 gas was analysed by continuous flow isotope ratio mass spectrometry. In brief, the CO_2 was flushed from the septum vial using a double-holed needle and resolved on a packed column gas chromatograph. The carbon dioxide then entered the ion source of a Europa Scientific 20-20 IRMS and was ionised and accelerated. The gas species of different mass were separated in a magnetic field and simultaneously measured using a Faraday cup collector array at m/z 44, 45, and 46.

The reference material used for kahawai otolith analyses was the laboratory calcium carbonate standard IA-R022 ($\delta^{13}\text{C}_{\text{V-PDB}} -28.63$ ‰ and $\delta^{18}\text{O}_{\text{V-PDB}} -22.69$ ‰), which is traceable to NBS19 Limestone ($\delta^{13}\text{C}_{\text{V-PDB}} +1.95$ ‰ and $\delta^{18}\text{O}_{\text{V-PDB}} -2.2$ ‰). During analysis NBS-18 (Calcite, $\delta^{13}\text{C}_{\text{V-PDB}} -5.00$ ‰ and $\delta^{18}\text{O}_{\text{V-PDB}} -23.00$ ‰), NBS-19, and IA-R022 were analysed as check samples. The results of these analyses are included in the results summary. The International Atomic Energy Agency, Vienna, distributes NBS-18 and NBS-19 as international reference standards.

Multivariate analyses were not performed on the isotope data as only two elements were considered. Univariate plots of isotope concentration relative to fish length are presented.

3. RESULTS

3.1 Collection of specimens

North Island: About 50 0-group kahawai were collected by beach-seine at Okahu Bay, Waitemata Harbour (Figure 2) on the high tide of 18 July 2006.

South Island: Eighty 0-group kahawai were collected by beach-seine in Hakahaka Bay, Port Underwood, on 1 August. Sampling on 26 July in Golden Bay, at Paton's Rock, Ligar Bay, and Pohara Beach, where kahawai had previously been taken, caught only juveniles of yellow-eyed mullet and sand flounder. Likewise, sampling on 27 July at Collingwood, Totara Ave (Ruataniwha Inlet), two locations off Puponga Beach, and at Kina Beach and Ruby Bay in Tasman Bay, and on 28 July at sites in inner Tasman Bay, the beach adjacent to the Waimea Estuary, and two sites at Rabbit Island, adjacent to the Mapua River mouth, also failed to catch juvenile kahawai.

The kahawai collected from the Waitemata Harbour were about 10 mm shorter than those collected from Port Underwood, but this small difference is unlikely to have much influence on otolith microchemistry composition (Hamer and Jenkins 2007; Hamer et al. 2006).

3.2 Meristic counts

Results of fin ray counts for the anal and dorsal fins are summarised in Table 1. There was no significant difference between the two sites for dorsal fin ray counts (Student t test = 1.105, df = 74, $P = 0.273$) and anal fin ray counts ($t = 0.470$, df = 73, $P = 0.64$).

Mean monthly sea surface temperatures from January to March 2006 were 2–3°C warmer in the Waitaemata Harbour than Port Underwood, so that larvae hatched in these water masses would have been exposed to different temperature regimes.

Table 1: Fin ray counts in 0-group kahawai from Waitemata Harbour and Port Underwood.

Area	Waitemata Harbour	P. Underwood	Waitemata Harbour	P. Underwood
Fin	Dorsal fin	Dorsal fin	Anal fin	Anal fin
Number fish	37	40	36	40
Mean	23.3	23.1	13.0	13.1
SD	0.80	0.92	0.70	0.68

3.3 Otolith trace elements

Levels of the selected elements (Li, Na, Mg, Ca, Mn, Fe, Cu, Zn, Rb, Sr, and Ba) were above the limit of detection in all otolith samples. For Pb, most otolith samples were below LOD and many were below or similar to levels in the blank (these data are not presented). The elemental concentrations for the 20 otolith pairs analysed by ICP-MS and the quality control data are presented in Appendix 1 (in $\mu\text{g g}^{-1}$). The repeat analyses of the FEBS-1 and 10 $\mu\text{g L}^{-1}$ indicated acceptable accuracy (recovery ranged from 91 to 107% for FEBS-1 CRM, and 94 to 104% for the

10 $\mu\text{g L}^{-1}$ standard), and precision (RSD – relative standard deviation FEBS-1: 2-10%, mean = 5%, 10 $\mu\text{g L}^{-1}$ standard: 1-7%, mean = 5%).

Univariate plots of trace element concentrations, relative to fish length, suggested that there was very little between, site difference in the concentration of each of the 11 elements consistently detected (Figure 3). When a cluster analysis was performed on the multi-elemental composition of all 40 otolith pairs, most clusters contained otoliths from both sites (Figure 4a), regardless of the clustering technique used (single linkage, complete linkage or Group linkage – which are presented here). Poor site resolution was also evident in the non-metric Multi-Dimensional Scaling ordination (Figure 5a), which displayed the relative position of each otolith so that the distance between the position of any two samples reflected the similarity in their multi-elemental composition. The associated Stress test statistic of 0.14 suggested that the ordination provides a “potentially useful 2-dimension picture” (Clarke et al. 2001) of the relative association of the microchemistry of the 40 otolith pairs.

Although the elemental compositions of otolith from the two sites overlapped, randomisation testing suggested that the a posteriori grouping of otoliths according to their site was not due to chance (Figure 6a). The ability to detect between site differences in the elemental composition of otoliths is reduced when only one or two elements appear to contribute to any difference in otolith composition. The PRIMER procedure SIMPER was, therefore, used to determine the percentage of dissimilarity that each element contributed to between site multi-elemental differences in the data set. These percentages are given in Figure 3. Two elements, magnesium and barium, explained a greater percentage of the between-site difference than the remaining elements, and the multivariate tests were repeated for this reduced 2-element data set.

Cluster analyses suggested a clearer separation of samples from the two sites, although most clusters contained fish from both areas (Figure 4b). When these clusters were superimposed on an nMDS ordination, the groupings also suggested a greater degree of separation between the two sites, but not independence (Figure 5b). The Stress statistic indicated that this ordination provided “an excellent representation with no prospect of misinterpretation” of the relative similarities of samples. The distribution of similarity test statistics generated by the randomisation procedure ANOSIM, relative to the observed value of 0.327, suggested that the dissimilarity in the concentrations of magnesium and barium from the two sites was not due to chance.

Linear discriminant function analysis based on Mg and Ba classified individual fish to the collection region with an average accuracy of 75% (Table 2). For Port Underwood, 80% of samples were correctly assigned to their collection site and for Waitemata Harbour samples, 70% were correctly assigned to their collection site. Discrimination power was driven largely by Ba (F-to-remove Ba = 12.9, Mg = 1.4). The canonical scores plot showed some overlap among fish from the two groups, consistent with 20–30% misclassification rate (Figure 7, Table 2).

Table 2: Results of linear discriminant function analysis showing classification rates of individual fish to collection regions based on otolith chemistry (Mg, Ba).

Collection region	Number of fish		% correct
	Port Underwood	Waitemata Harbour	
Port Underwood	16	4	80
Waitemata Harbour	6	14	70
Total classified to region	22	18	75

3.4 Otolith stable isotopes

Isotope ratios for all otoliths, along with results of the check samples NBS-18 (Calcite, $\delta^{13}\text{C}_{\text{V-PDB}}$ -5.00 ‰ and $\delta^{18}\text{O}_{\text{V-PDB}}$ -23.00 ‰), NBS-19, and IA-R022, are given in Appendix 2. The results of the check samples NBS-18 (Calcite, $\delta^{13}\text{C}_{\text{V-PDB}}$ -5.00 ‰ and $\delta^{18}\text{O}_{\text{V-PDB}}$ -23.00 ‰), NBS-19, and IA-R022 are also included in the results summary.

Univariate plots of stable isotope concentration (Figures 8 and 9) clearly show that the stable isotope ratios for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ overlap considerably among samples from each site. Although most data overlapped, the highest values of $\delta^{18}\text{O}$ occurred for the Port Underwood samples, which is consistent with the expectation of lower water temperature at this location. In some samples, for which both otoliths were analysed separately, the between otolith differences in isotope concentration were significant, particularly for $\delta^{18}\text{O}$. These significant differences in $\delta^{18}\text{O}$ between otolith pairs from the same fish are difficult to explain given that accuracy and precision for quality control and reference standards were acceptable. These differences would have added to unexplained variation in the $\delta^{18}\text{O}$ data, possibly reducing the power to detect differences between sampling locations.

4. DISCUSSION

4.1 Stock discrimination of marine fish

There is no universal method for determining stock relationships in marine fish, rather management advice is drawn from different and often independent sources. In recent years stock discrimination studies have moved towards an holistic or comparative approach (Begg and Waldman 1999) applying multiple techniques to determine stock relationships (Smith et al. 2002). The Ministry of Fisheries have adopted this approach and projects undertaken by NIWA have used multiple techniques to determine stock relationships of black and smooth oreos (Smith et al. 2002a), alfoncino and cardinalfish (Smith et al. 2001), orange roughy (Smith et al. 2002b), kingfish (Smith et al. 2004), and blue mackerel (Smith et al. 2005). A similar approach was adopted for a stock discrimination study of Australian herring (*Arripis georgianus*) (Ayvazian et al. 2004). The different methods applied to stock discrimination of marine fishes define isolation at different temporal scales; for example, parasite markers provide a tool for discriminating feeding aggregations; meristic and microchemistry traits for discriminating stocks from different water masses; tagging studies for movement of adults and sub-adults; and genetic techniques for discriminating evolutionary significant units. The common element in applying any discrimination technique is the null hypothesis of no differentiation. Discrete stocks are identified when the null hypothesis is rejected. The smaller the spatial scale in question, the less likely that there will be separate genetic or ecological stocks, and no technique will allow rejection of the null hypothesis.

The technical methods currently used in stock discrimination studies fall into five categories, that measure:

- **phenotypic** variation, such as meristic and morphometric characters which have a genetic basis, but expression of the character is determined by the biotic and physical environment experienced by individuals;

- *life history parameters*, such as growth rate, and size and age at first maturity, that may have an underlying genetic basis, but are modified by the biotic and physical environment experienced by individuals;
- *genetic markers*, such as allozymes and DNA markers, inherited characters that are passed down through generations and not modified by the environment, but may be subject to selection;
- *acquired* markers, such as metal ions and elements in otoliths, or body parasites in soft tissues, that accumulate during an individual's life;
- *movement* of adults and sub adults by physical tagging.

Biological data on the distribution of spawning grounds, juveniles, and adults contribute to the development of a stock structure model. The number of larval and juvenile retention areas contained by hydrological barriers can determine the stock "richness" of marine fishes (Sinclair et al. 1988).

4.2 Phenotypic variation: morphometrics

Morphological characters describe the shape of the fish based on distances between landmarks, such as tip of snout to fin origins. Morphological and meristic data are often collected together as the characters are easy to measure in whole specimens (Junquera et al. 1993). However, the characters are determined by different biological processes and provide different information on variation in populations.

Shape may vary through an individual's life, associated with allometric growth. Morphological changes in fishes can be extreme, for example pelagic and demersal morphs of the Pacific armourhead (*Pseudopentaceros wheeleri*) were originally described as two species (Martin et al. 1992). Morphometric differences have been reported among fish stocks when other techniques have shown no differentiation, e.g., capelin (*Mallotus villosus*) off the east coast of Canada (Sharp et al. 1978), European anchovy (*Engraulis encrasicolus*) (Junquera et al. 1993, Tudela 1999), and horse mackerel (*Trachurus trachurus*) off North Africa (Murta 2000). A multivariate analysis of 13 morphometric characters, measured in five population samples of the spotted mackerel (*Scomber australasicus*) in the China Sea revealed three phenotypic groups (Tzeng 2004).

In some small pelagic species, such as anchovy and sardine, a high degree of morphological variation has been found without a geographical pattern (Spankias et al. 1989, Kinsey et al. 1994, Nelson et al. 1994,). Morphometric characters are influenced by growth rate and can be affected by differences in food availability (Waldman et al. 1997). It is likely that intra-specific morphological variation is non-adaptive and reflects local feeding conditions and spawning times, and can be used as an indicator of short-term population associations, rather than reproductive isolation of stocks (Pepin et al. 1992, Kinsey et al. 1994, Waldman et al. 1997). Thus, morphometrics alone would not be recommended as a tool for determining stock relationships of kahawai.

4.3 Phenotypic variation: meristics

Meristic characters, such as the number of vertebrae and numbers of fin rays, were among the first biological markers used to determine stock relationships of marine fish (Heincke 1898), but were largely replaced by direct genetic methods developed in the 1970s. Meristic characters are determined early in the life cycle. For example the number of vertebrae is determined during the embryonic stages (Fahy 1972, Lindsey 1988, Taning 1946). The meristic characters have a genetic basis (Christiansen et al. 1988), but the differences in vertebral numbers and fin rays are modified by environmental factors, such as water temperature, so that stock differences are due largely to environmental rather than genetic variation (Taning 1952, Fahy 1972, Brander 1979, Hulme 1995).

There are cautions to be considered in applying meristic data to determine stock relationships. Some meristic characters exhibit year class variation within fish stocks (Blouw et al. 1988), which reflect variation in the physical environment. Meristic variation measured in adults might reflect environmental variation among year classes rather than stock relationships.

Meristic data for kahawai taken from the literature showed that there is variation in the number of dorsal soft rays, and number of anal soft rays (e.g. FishBase: www.fishbase.org), but there was no indication of numbers or size range of fish analysed. An analysis of 34 specimens from New Zealand reported intra-specific variation in the numbers dorsal fin rays (15–16), anal fin rays (9–10), pectoral fin rays (16–18), lateral line scales (49–53), and gill rakers (9–13 + 20–24) (Paulin 1993). The preliminary meristic counts undertaken on 0-group kahawai from two widely separated sites in different water masses indicate that meristic characters show no significant differences, and are not likely to be useful in determining the natal origin and movements of kahawai.

4.4 Life history traits

Life history parameters, such as growth rate and age at first maturity, often show regional variation in the commercially important marine fishes, and provide complementary data sets to meristic data sets. Meristic characters are determined in the larval stages and provide a tool to test the stock relationships of juvenile production areas. In contrast, the life history traits are determined post recruitment and allow testing of adult stock relationships. Even if adults recruit from a common juvenile pool (a single unit stock) there may be regional differences in growth rate and age at maturity in response to local environmental conditions in the absence of extensive adult movement between areas. Life history parameters can vary temporally within, as well as between stocks; for example, in the Atlantic fisheries for cod, haddock, and yellowtail flounder there have been significant changes in life history parameters (growth rate and age at first maturity) as exploitation has increased (Begg et al. 1999).

In the sardine (*Sardinops sagax*) patterns in the distribution of eggs off Western Australia have revealed a continuous, but uneven, distribution of eggs, with spawning centres linked by intervening areas of less spawning activity (Gaughan et al. 2002). The egg distribution data, together with regional differences in mean gonadosomatic indices (GSI), and age compositions, indicated a lack of wide-scale mixing of mature age classes between regions off Western Australia. These non-mixing assemblages were termed Functionally Distinct Adult Assemblages (FDAAs), which constitute distinct reproductive units within a single breeding population (Gaughan et al. 2002). When fisheries exploit spatially limited but disjunct parts of a single

breeding population, the exploited portions of the stock may be considered as distinct units for the purposes of management (Gaughan et al. 2002).

Life history data for kahawai are available only for KAH 1, and these data are subject to ageing error and inter-annual growth variability. Within the KAH 1 Auckland east fishery there are regional differences in age structure, with 2 to 4 year old fish predominant in the Hauraki Gulf, 3 to 8 year olds in the east Northland fishery, and a wider distribution of 3 to 10 year olds in the Bay of Plenty (Armiger et al. 2006). Consequently the existing life history data are not useful for separating kahawai stocks in New Zealand waters. Given that kahawai make extensive movements it is unlikely that there would be consistent regional differences in growth patterns.

4.5 Genetic markers

In general, genetic studies of large pelagic species such as tunas have shown little or no genetic differentiation within ocean basins (Appleyard et al. 2002). However, genetic studies of some coastal pelagic fishes have found genetic differentiation between sea areas. Separate genetic stocks of king mackerel (*Scomberomorus cavalla*) have been reported in the western Atlantic and Gulf of Mexico (Johnson et al. 1993, Gold et al. 1997); separate stocks of school mackerel (*Scomberomorus queenslandicus*) are associated with large embayments off the east and north coasts of Australia (Begg et al. 1998); separate stocks of scad mackerel (*Decapterus macrosoma*) have been identified between adjoining sea areas in the Sundra Strait and Java Sea (Arnaud et al. 1999); genetic differentiation has been reported among populations of the sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) in the Aegean and Ionian Seas (Spankias et al. 1989) and among populations of the sardine (*Sardinella aurita*) in the Atlantic and Alboran Sea in the Straits of Gibraltar (Atarhouch et al. 2007). Microsatellite markers developed for Spanish mackerel (*Scomberomorus commerson*) showed that most genetic variation was within populations, but allowed the identification of two genetic stocks, one restricted to one locality (Dhofar), the other widespread around the Arabian Peninsula (Herwerden et al. 2006). In the Atlantic mackerel (*Scomber scombrus*), DNA markers have revealed genetic differentiation among three geographically isolated spawning areas off western Europe (Nesbo et al. 2000). In contrast, DNA studies of other small pelagic fishes have shown low differentiation among regions (Donato et al. 2005). Recent DNA studies of blue mackerel (*Scomber australasicus*) around New Zealand, although revealing high levels of genetic diversity, found no evidence for regional differentiation (Smith et al. 2005). In general genetic differentiation in small pelagic species is found between and not within seas. However, many molecular polymorphisms have been applied on the premise that the markers are selectively neutral, and the patterns of spatial differentiation are due to drift and restricted gene flow. Loci under selection or linked to genes under selection may reveal greater regional differentiation (Pogson et al. 2004, Canino et al. 2005, Hemmer-Hansen et al. 2007).

An allozyme study of the Australian herring (*Arripis georgianus*) included 646 fish collected over 4800 km around southern Australia and showed low genetic differentiation among 11 sites with no evidence for isolation by distance (Ayvazian et al. 2004). It was noted that other coastal species, tailor (*Pomatomus saltatrix*) and mullet (*Mugil cephalus*), also exhibited low genetic differentiation over a similar range (Ayvazian et al. 2004). The lack of genetic differentiation in (*A. georgianus*) found with allozyme markers was consistent with results from otolith stable isotope and tagging studies, and the combined results interpreted as supporting a single stock model for this species around southern Australia (Ayvazian et al. 2004). An early allozyme study of 570 specimens of western salmon (*A. truttaceus*) also indicated the presence of only one breeding population, from Tasmania to Western Australia (Macdonald 1983). Allozyme markers

have been largely replaced by more variable DNA markers for stock discrimination studies (see references to pelagic species the previous paragraph), which have revealed finer-scale population subdivision in some species. In the Atlantic cod (*Gadus morhua*) genetic differentiation among stocks varies with the class of marker and geographic region. Only a few markers, e.g. *PanI* (Pogson et al. 1995, Pogson et al. 2004), *Gmo132* (Bentzen et al. 1996), and *Hb* (Frydenberg et al. 1965, Sick 1965) indicate differentiation between neighbouring stocks. In general, coastal species with high dispersal potential, either through adult or juvenile movement, show little genetic differentiation within sea areas. In kahawai the large population size and adult mobility (see summary of tagging studies below) are likely to act against population differentiation.

4.6 Acquired characters: parasites

Parasites have been applied as natural tags or markers to discriminate stocks of marine fishes for more than 50 years. The prevalence and abundance of parasite species can support stock structure models, but the data are often weak and dependent upon other techniques to confirm stock relationships. Fish acquire parasites through their diet, through inoculation via a tissue feeder, or when ecto-parasites attach to skin or gills. If the parasites (or intermediate hosts) have a restricted distribution compared with the host distribution then only some groups of fish will be exposed to the parasite. As fish move and grow they acquire parasites which reflect the habitats that they have occupied and their diet. The ideal situation occurs when a parasite is present in one region and absent in another: for example, changes in the prevalence of parasite markers were used to infer long distance movement of albacore tuna (*Thunnus alalunga*) from tropical spawning areas to temperate feeding areas in New Zealand (Jones 1991).

Knowledge of the intermediate hosts and their distribution can be critical for interpreting the geographical patterns of parasites in the fish host. The spatial abundance of the cestode *Grillotia angeli* in blue mackerel (*Scomber scombrus*) in the northeast Atlantic is limited by the adult worm's definitive host the monkfish, which is confined to a much smaller geographic range than mackerel (Mackenzie et al. 1984).

In the narrow-barred Spanish mackerel (*Scomberomorus commerson*) differences in the parasite fauna between samples taken off northern Australia and one site in Indonesia led to the conclusion that there was a lack of movement of fish between these two regions. At smaller spatial scales differences among samples in northern Australia led to the conclusion that adult Spanish mackerel were relatively sedentary (Moore et al. 2003), a result supported by allozyme differentiation (Shaklee 1987). However, the similar parasite faunas among mackerel samples from Australian west coast sites were indicative of host movement along this coast (Lester et al. 2001).

In the blue mackerel (*Scomber australasicus*) around New Zealand significant differences in the prevalence of larval *Anisakis* sp. in three fishery areas were indicative of stock differences (Smith et al. 2005). Additional differences in the prevalence of a short lived acanthocephalan, were interpreted as an indication of a lack of short-term (seasonal) movement of hosts between areas (Smith et al. 2005).

Parasite studies of kahawai in New Zealand waters have been limited to simple checklists (Hewitt et al. 1972, Webb 1973). A new species of copepod *Caligulus kahawai* was described from kahawai, but this external parasite is easily dislodged during collection and consequently not a useful stock marker (Jones 1988). A preliminary report on parasites in Australian populations of kahawai noted a tapeworm *Callitetrarhynchus speciosus* as a possible stock marker (Cappo 1992).

Recent studies on parasite loads in New Zealand fishes have reported complex variation in parasite abundance and even tow to tow variation in long-lived species such as black oreo (Smith et al. 2000), orange roughy (Gauldie et al. 2000), and alfonsino (Smith et al. 2001). Given these complex patterns in parasite abundance, and the mobility of some kahawai as revealed by tag returns, any differences in prevalence, abundance, and intensity of parasites in kahawai would need to be interpreted cautiously and stock differences supported by additional techniques that measure separation at different time scales.

4.7 Acquired characters: otolith microchemistry

Analyzing trace elements and stable isotopes deposited in fish otoliths can provide insights into the environmental variation that individual fish experience throughout their life history, and the data can be used to infer stock structure (Begg et al. 2001). The application of otolith microchemistry for stock discrimination has been most successful with estuarine species and coastal species exposed to variable salinities (Bastow et al. 2002). An early review of otolith microchemistry studies concluded that the technique was inappropriate for stock discrimination of pelagic and open ocean species because the oceanic environment is relatively homogeneous, and acquired differences, if any, in otolith composition were likely to be very small (Thresher 1999, Thresher et al. 1999). Indeed, no consistent area differences in otolith chemistry have been reported among New Zealand populations of orange roughy (Thresher et al. 1999), oreos (Smith et al. 2000) and hoki (Kalish et al. 1996).

Advances in otolith chemical techniques and interpretations, including the use of elemental ratios and stable isotopes, have provided more powerful tools for determining movement of fish and for inferring stock relationships (Begg et al. 2005, Gillanders 2005). Stable isotopes hold considerable promise as a tool to discriminate stocks of marine fishes and in (*Thunnus thynnus*) at least, may be a more reliable predictor of nursery origin than trace elements in otoliths (Rooker et al. 2004). Analyses of stable isotopes of oxygen and carbon ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) in the otoliths of snapper (*Pagrus auratus*) (Bastow et al. 2002), the red emperor (*Lutjanus sebae*), and the Rankin cod (*Epinephelis*) (Stephenson et al. 2001), have been used to determine relationships among stocks in Western Australia. Similar otolith studies have been used to infer stock relationships in bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea and the western Atlantic (Rooker et al. 2004), and haddock (*Melanogrammus aeglefinus*) in the northwest Atlantic Ocean (Begg et al. 2001). In the small pelagic pilchard (*Sardinops sagax*) there are location specific signatures for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in fishing areas off Western Australia (Edmonds et al. 1997). However, from the figures given in their paper it would appear unlikely that individual fish could be assigned to a specific sampling location based on their $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signal.

Trace element analyses have also been used successfully to determine stock relationships in coastal species, e.g., the black rockfish (*Sebastes melanops*) (Miller et al. 2005), and in bluefin tuna (Rooker et al. 2003). Differences in trace elements between areas have been reported in the Atlantic cod (*Gadus morhua*) sampled on spawning sites around Iceland (Jonsdottir et al. 2006), and around Scotland (Wright et al. 2006); in jack mackerel (*Trachurus mediterraneus*) in the Black and Aegean Seas (Turan 2006); and in the Japanese anchovy (*Engraulis japonicus*) in the Seto Inland Sea and the Pacific Ocean (Zenitani et al. 2007). One notable result is that there does not appear to be consistency in the elements which demonstrate area differentiation (Gillanders et al. 2003, Turan 2006).

Trace elements in otoliths of juvenile snapper (*Pagrus auratus*) have been used to infer relationships among North Island west coast nursery sites (M. Morrison, NIWA, unpublished

data). A preliminary feasibility study using otolith microchemistry to determine the stock structure of kahawai detected only two elements, Ca and Sr, and concentrations did not differ between fish sampled from two sites in northern and central New Zealand (Francis et al. 1997). This led the authors to conclude that otolith microchemistry was not an appropriate tool for distinguishing stocks of kahawai in New Zealand waters. However, Kalish had earlier reported additional elements in samples of kahawai otoliths collected in Australian waters (Kalish 1989, 1992). Life-history transects of Sr/Ca, S/Ca, Na/Ca, and K/Ca ratios made with a wavelength dispersive electron microprobe showed that there were differences in otolith trace element chemistry between a group of kahawai trapped in a stream, with a low salinity and high temperature, and a parallel group collected in an adjacent coastal area (Kalish 1992).

A multi-technique stock delineation study of the Australian herring (*Arripis georgianus*) included stable isotopes (and allozymes and tagging) to investigate stock relationships around southern Australia (Ayvazian et al. 2004). Oxygen isotope ratios of the otolith carbonate revealed that, in general, the fish had not spent their entire life in the waters where they were captured. The mean values for $\delta^{18}\text{O}$ for all sites fell within a narrow range, apart from two samples taken from areas with either a high evaporation or high freshwater input that had altered the isotopic composition of the local waters. Exclusion of these two sites from the total data set resulted in no significant area differences (Ayvazian et al. 2004). The mean values for $\delta^{13}\text{C}$ showed greater variation across sites, and even excluding the two extreme environmental sites, there were significant between area differences. Overall, the results from the multi-technique study supported the single stock hypothesis and were interpreted as evidence that Australian herring is a migratory species, with a westward migration along southern Australia to the lower west coast of Western Australia before spawning (Ayvazian et al. 2004).

Two widely separated nursery sites in different water masses (one close to the largest urban centre in New Zealand and one remote) were deliberately sampled for this preliminary assessment of otolith chemistry for determining the natal origin and movements of kahawai, in order to maximise the chances of detecting between site differences. Although overall differences between sites were statistically insignificant, the highest $\delta^{18}\text{O}$ values occurred for Port Underwood samples which would be predicted based on the lower water temperatures (2–3 °C lower) for this site and the negative relationship between $\delta^{18}\text{O}$ values in otoliths and water temperature (Ayvazian et al. 2004). It is likely that geographically intermediate nursery sites would be intermediate, at least in terms of water temperature, therefore, given the negligible variation in $\delta^{18}\text{O}$ between the distant sites compared in this study it is unlikely that stable isotope analyses of $\delta^{18}\text{O}$ will be a useful tool for determining the natal origin and movements of kahawai around New Zealand. Variation in $\delta^{13}\text{C}$ ratios in otoliths is not closely linked to physico-chemical properties of the water due to the strong influence of endogenous processes, such as metabolism, on carbon isotopic fractionation between water and otolith (Campana 1999). It is therefore also unlikely that variation in $\delta^{13}\text{C}$ will be useful for determining the natal origin and movements of kahawai around New Zealand.

The mechanisms that generate differences in trace elements between nursery sites area are not well known, but are likely to be influenced by local geology and land-use that influence levels of elements in the water, as well as temperature and salinity of the water (Gillanders et al. 2003). Recent studies have indicated that incorporation of at least some elements into otoliths can be influenced directly by variation in ambient water chemistry (Bath et al. 2000, Elsdon et al. 2003, Kraus et al. 2004). In particular, variation in otolith barium levels has now been demonstrated for a variety of species to be closely related to ambient barium levels of the water (Bath et al. 2000, 2006, Milton et al. 2001, Elsdon et al. 2003, Walther et al. 2006). Variation in otolith barium can therefore be used as a proxy for variation in ambient barium and vice versa (Hamer et al. 2006). The

fact that barium was the major contributor to discrimination among samples from the two sampling areas is promising and most likely indicates water chemistry variation among the Port Underwood and Waitemata Harbour sites. Similar variations might be expected among other estuaries, and between estuaries and coastal waters (Hamer et al. 2006). Comparison of water chemistry among estuaries, and between estuaries and coastal waters, would be a valuable extension to the current project. Results from the current project indicate that $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are likely to be of limited value, but trace elements such as barium show some promise to study linkages between juvenile kahawai nursery areas and adult populations.

4.8 Movement of adults and sub-adults from tagging

Two major tagging programmes have been undertaken on kahawai. Some adults have made extensive movements, although most recaptures of tagged fish have been within 50 nautical miles of the release sites (Wood et al. 1990, Griggs et al. 1998). Results from the kahawai tagging projects in 1981 and 1984 were reviewed by Wood et al. (1990) and showed that some adult kahawai make extensive movements between the east coast South Island and west coast North Island, with tag recaptures showing movements of fish in both directions. Griggs et al. (1998) summarised the movement of tagged kahawai from two locations in the Bay of Plenty and Tasman Bay in 1991. Kahawai tagged in the Bay of Plenty (788 recaptures) generally moved as far north as Whangaroa and south to the Tukituki River in southern Hawke Bay. Around 7% of kahawai were recaptured more than 50 n. miles from their release site, with about 4% moving more than 100 n. miles, including one fish recaptured at Mana Island (450 n. miles from the release site).

Kahawai tagged in Tasman Bay (704 recaptures) showed less extensive movement with about 1% moving over 50 n. miles, including movement to Greymouth and North Cape on the west coast. Nearly 30% of the recaptures were from one purse-seine shot in Tasman Bay, 9 months after the release date, indicating that some fish at least remained in the same schools. The Tasman Bay tagged fish were smaller than those tagged in the Bay of Plenty (average lengths 37 and 46 cm respectively), which may account for fewer fish travelling from the tagging site in Tasman Bay (Griggs et al. 1998).

There are problems in using the tagging data to infer stock relationships. The levels of exploitation differ considerably between areas and many of the reported tag returns have been from the recreational fishery (59% for the 1981–84 tagging data set and 39% of the 1991 data set) for which there are no historical data on recreation harvest and effort (Hartill et al. 2005). Wood et al. (1990) noted that areas with the highest return rates were usually those with an intensive recreational fishery, and that recaptures were also influenced by the seasonal nature of some commercial fisheries. For example, fishers target skipjack tuna in the Bay of Plenty during summer.

In Australian waters, tagging studies combined with age distributions have shown that the eastern species *Arripis trutta* move northwards from juvenile nursery areas around Tasmania and Victoria to New South Wales, with a return movement of adults (Stanley 1978). The western salmon (*A. truttaceus*) migrates over much greater distances from eastern nursery sites to spawning grounds off Western Australia (Cappo et al. 2000). *A. georgianus* is also a migratory species, with a westward migration along southern Australia to the southwest coast of Western Australia before spawning (Ayvazian et al. 2004).

4.9 Biological models of stock structure in kahawai

The major oceanic features which potentially restrict movement of juvenile kahawai around the North Island and northern South Island are shown in Figure 10. Off the east coast, the southward flowing East Auckland Current (EAC) brings subtropical water down the east coast of the North Island, and diverges near East Cape with some water flowing north and east, and the remainder flowing southwards as the East Cape Current (ECC), until it reaches the Chatham Rise and is deflected to the east. The Wairarapa Coastal Current (WCC) transports cooler water north along the south-east coast of the North Island (Figure 10). The WCC is a mix of waters from the Southland current, flowing north along the east coast South Island, and the D'Urville Current, flowing east through Cook Strait (Chiswell 2000). Most of the west coast is influenced by the northward drift of the Tasman Current, and is hydrologically more uniform than the east coast, although the West Auckland Current (WAC) influences Ninety Mile Beach off the northwest coast of the North Island (Roberts et al. 1978).

There are three potential stock models for kahawai to be considered in relation to the biological data on kahawai.

1. A single biological stock with multiple spawning areas and extensive larval/juvenile dispersal from the spawning areas, and extensive adult movement between spawning areas (a unit stock model).
2. Isolated "eastern" and "western" stocks with larvae/juveniles contained in separate water masses off the east (EAC, ECC, Figure 10) and west (WEC, DUC, TAC, Figure 10) coasts, and limited adult movement between regions (an island recruitment model).
3. Multiple spawning stocks and juvenile nursery areas, with limited juvenile dispersal, but wide adult dispersal (a stepping stone recruitment model).

No differentiation would be expected under model 1 with any stock discrimination method, but differentiation in some environmentally determined characters (meristics, parasites, trace elements, stable isotopes) would be expected under models 2 and 3, with clinal variation under model 3. Under model 3, juveniles derived from local spawning events may acquire a regional character that can be detected even if the adults subsequently mix on common spawning grounds.

There is evidence from tagging studies for extensive adult movement, but no evidence that this movement is seasonal and linked to movement to and from spawning areas (Griggs et al. 1998, Wood et al. 1990), unlike *Arripis* species in Australian waters which show long distance migrations to spawning areas (Stanley 1978, Cappo et al. 2000, Ayvazian et al. 2004). Kahawai tagged off the east coast of New Zealand have been recaptured on the west coast, and vice versa, which may mask potential spawning site fidelity. There is no direct information on juvenile movement, but the small size and location of 0- and 1-groups in shallow coastal bays and estuaries and the surf zone of sandy beaches (Webb 1972, Jones et al. 1985, Gerring et al. 1998) suggest that movement is restricted, favouring stock structure models 2 and 3. Juveniles are widely distributed in the Manukau and Kaipara Harbours on the west coast, from Golden Bay to Port Underwood in the northern South Island, the Wellington west coast, and in the Hauraki Gulf and Bay of Plenty off the east coast (Jones et al. 1985, Gerring et al. 1998). Spawning has been reported from fish in deep water (about 60–100m), but spawning areas around the North Island and northern South Island are unknown. Running ripe fish have been caught off the east coast of the North Island and kahawai eggs collected in the outer Hauraki Gulf (Hartill et al. 2005). The eggs are pelagic (Crossland 1982), promoting the passive dispersal of eggs and larvae before

recruitment into shallower water. The lack of meristic differentiation in the preliminary analyses undertaken in this project are not informative for stock differentiation, and may indicate recruitment from common spawning sites or that the temperature differentiation in discrete spawning areas is insufficient to promote meristic differences between areas.

Of the 11 trace elements detected in juvenile kahawai otoliths only barium and magnesium varied significantly between the two sites, and there was negligible variation between sites for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. Any environmental differences among sites were therefore insufficient to generate significant variation in otolith chemistry for most elements, and for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. The differences in barium are likely to be indicative of differences in barium concentration of the water between the sample sites (Bath et al. 2000, Milton & Chenery 2001, Elsdon & Gillanders 2003, Bath & Wuenschel 2006, Walther & Thorrold 2006). Differences in otolith magnesium between sites are unlikely to be related to differences in ambient water chemistry but may have been indicative of growth differences among regions (Hamer et al. 2007). The differences in otolith chemistry may have been influenced by the periods of time fish had spent in open coastal versus bay waters. If fish from both areas had spent most of their time before capture in coastal waters, it is possible that the whole otoliths from the 0-group kahawai sampled for microchemistry analyses reflected a coastal waters signal, rather than that of the inshore capture location. This may have been partly responsible for the similarity in elemental chemistry between the sites, and it is possible that otolith chemistry may provide higher discrimination among sampling areas in bays and estuaries for fish sampled at 12–18 months age. Alternatively, laser ablation sampling of recently deposited otolith material may provide a more powerful tool than whole otolith analyses for detecting variation in otolith chemistry specific to different bay and estuary nursery areas. Future work aimed at identifying estuary specific chemical ‘tags’ or ‘signatures’ in juvenile kahawai otoliths would benefit from switching to a laser-based sampling approach.

Gaughan et al. (2002) introduced the term Functionally Distinct Adult Assemblages (FDAAs) to describe stock structure in *Sardinops sagax* off Western Australia, where it exhibits a continuous but uneven distribution of eggs (= spawning aggregations), along with regional differences in mean gonadosomatic indices (GSI) and age compositions. FDAAs were recognised where fisheries exploited spatially limited, but disjunct parts of a single breeding population, and were considered as distinct units for management (Gaughan et al. 2002). The FDAA concept provides a tool for managing pelagic species that show limited biological differentiation among geographically discrete fisheries. Currently there are limited data on the spawning and larval distributions for kahawai; tagging studies have shown long distance movements, but most tagged fish have been recaptured within 50 n. miles of the release site, with some evidence for short term (about 9 months) cohesiveness of schools of kahawai (Griggs et al. 1998), suggesting that there may be FDAAs of kahawai.

There is no single stock discrimination tool that is likely to be useful for determining the stock structure of kahawai, and for distinguishing between stock structure models 2 and 3. The high cost of undertaking genetic or parasite or chemical analyses of kahawai samples throughout the range of the species in New Zealand, coupled with limitations of these techniques for discriminating stocks at relatively small spatial scales, is not justified. There is no new information that would warrant a change to the present five management units (KAH 1, KAH 2, KAH 3, KAH 8, and KAH 10) recognised for kahawai.

5. CONCLUSIONS

Eleven selected elements (Li, Na, Mg, Ca, Mn, Fe, Cu, Zn, Rb, Sr, and Ba) were above the limit of detection in all otolith samples taken from 0-group kahawai in Waitemata Harbour and Port Underwood. Apart from barium and magnesium there was very little between-site difference in the concentration of each of the 11 elements. Discriminant function analysis, based on barium and magnesium levels, assigned individuals to their collection site with 75% probability of correct assignment. The stable isotope analysis found almost complete overlap between the two sampling sites for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. Although variation between sites was insignificant, the highest $\delta^{18}\text{O}$ values occurred in samples from Port Underwood, which would be predicted based on the lower water temperatures for this site and the negative relationship between $\delta^{18}\text{O}$ in otoliths and water temperature.

Trace element chemistry has some potential for determining the nursery origin and movements of adult kahawai, but stable isotopes are unlikely to be useful. It is possible that juvenile kahawai (1- and 2-group) that have spent longer in bays and estuaries may acquire a regional trace element signal that would allow identification of the juvenile as opposed to natal origin of adult kahawai. Laser ablation analyses of marginal otolith material may increase the power to detect variation in otolith chemistry among juveniles from different bays and estuaries.

The preliminary meristic counts undertaken on 0-group kahawai from two widely separated sites in different water masses indicate that meristic characters are not likely to be useful in determining the natal origin and movements of kahawai.

Biological data on kahawai age structure in the regional fisheries and ad hoc opportunities on observations of spawning areas and times (e.g., presence of running ripe fish) should continue to be collected and periodically reviewed by the Pelagic Working Group in relation to the stock structure of kahawai.

6. ACKNOWLEDGMENTS

Cameron Walsh, Ron Blackwell, and Colin Sutton collected 0-group kahawai. Caoimhghin O'Maolagain dissected the otoliths and Margaret McVeagh assisted with the meristic counts. Members of the Pelagic Fisheries Working Group provided feedback on the project, which was supported by the Ministry of Fisheries, Project KAH2006-01.

7. REFERENCES

- Appleyard, S.S.; Ward, R.D.; Grewe, P.M. (2002). Genetic stock structure of bigeye tuna in the Indian Ocean using mitochondrial DNA and microsatellites. *Journal of Fish Biology* 60: 767-770.
- Armiger, H.; Hartill, B.; Tasker, R.; Smith, M.; Griggs, L.H. (2006). Length and age compositions of recreational landings of kahawai in KAH 1 in January to April 2003-04 and 2004-05. *New Zealand Fisheries Assessment Report 2006/57*. 37 p.
- Arnaud, S.; Bonhomme, F.; Borsa, P. (1999). Mitochondrial DNA analysis of the genetic relationships among populations of scad mackerel (*Decapterus macarellus*, *D. macrosoma*, and *D. russelli*) in Southeast Asia. *Marine Biology* 135: 699-707.

- Atarhouch, T.; Rami, M.; Naciri, M.; Dakkak, A. (2007). Genetic population structure of sardine (*Sardina pilchardus*) off Morocco detected with intron polymorphism (EPIC-PCR). *Marine Biology* 150: 521–528.
- Ayvazian, S.G.; Bastow, T.P.; Edmonds, J.S.; How, J.; Nowara, G.B. (2004). Stock structure of Australian herring (*Arripis georgiana*) in southwestern Australia. *Fisheries Research* 67: 39–53.
- Bastow, T.P.; Jackson, G.; Edmonds, J.S. (2002). Elevated salinity and isotopic composition of fish otolith carbonate: stock delineation of pink snapper, *Pagrus auratus*, in Shark Bay, Western Australia. *Marine Biology* 141: 801–806.
- Bath, G.; Thorrold, S.; Jones, C.; Campana, S.; McLaren, J.; Lam, J. (2000). Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* 64: 1705–1714.
- Bath, M.; Wuenschel, M. (2006). Effect of temperature and salinity on otolith elemental incorporation in juvenile gray snapper *Lutjanus griseus*. *Marine Ecology Progress Series* 324: 229–239.
- Begg, G.; Keenan, C.; Sellin, M. (1998). Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters. *Journal of Fish Biology* 53: 543–559.
- Begg, G.; Waldman, C. (2001). Stable delta super(13)C and delta super(18)O isotopes in otoliths of haddock *Melanogrammus aeglefinus* from the Northwest Atlantic Ocean. *Marine Ecology Progress Series* 216: 223–233.
- Begg, G.; Waldman, J. (1999). An holistic approach to fish stock identification. *Fisheries Research* 43: 35–44.
- Begg, G.A.; Campana, S.E.; Fowler, A.J.; Suthers, I.M. (2005). Otolith research and application: current directions in innovation and implementation. *Marine and Freshwater Research* 56: 477–483.
- Begg, G.A.; Hare, J.A.; Sheehan, D.D. (1999). The role of life history parameters as indicators of stock structure. *Fisheries Research* 43: 141–163.
- Bentzen, P.; Taggart, C.; Ruzzante, D.; Cook, D. (1996). Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 2706–2721.
- Blouw, D.M.; Saxon, S.D.; Chadwick, E.M.P. (1988). Temporal variation of meristic traits within an Atlantic salmon (*Salmo salar*) stock and implications for stock identification. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 1330–1339.
- Brander, K.M. (1979). The relationship between vertebral number and water temperature in cod. *Journal du Conseil* 38: 286–292.
- Campana, S.E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* 188: 263–297.

- Canino, M.F.; O'reilly, P.T.; Hauser, L.; Bentzen, P. (2005). Genetic differentiation in walleye pollock (*Theragra chalcogramma*) in response to selection at the pantophysin (PanI) locus. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2519–2529.
- Cappo, M. (1992). Australian salmon - parasites as biological fish tags. *SAFISH* 17(2): 7–10.
- Cappo, M.; Walters, C.J.; Lenanton, R.C. (2000). Estimation of rates of migration, exploitation and survival using tag recovery data for western Australian "salmon" (*Arripis truttaceus*: Arripidae: Percoidae). *Fisheries Research* 44: 207–217.
- Chiswell, S.M. (2000). The Wairarapa Coastal Current. *New Zealand Journal of Marine and Freshwater Research* 34: 303–315.
- Christiansen, F.B.; Nielsen, V.H.; Simonsen, V. (1988). Genetics of *Zoarces* populations XV. Genetic and morphological variation in Mariager Fjord. *Hereditas* 109: 99–112.
- Clarke, K.R.; Warwick, R.M. (1994). Change in marine communities: An approach to statistical analysis and interpretation. *Plymouth Marine Laboratory*. 144p.
- Clarke, K.R.; Warwick, R.M. (2001). Change in marine communities: An approach to statistical analysis and interpretation. 2nd edition. *Plymouth Marine Laboratory*: 144 p.
- Coplen, T.B.; Kendall, C.; Hopper, J. (1983). Comparison of stable isotope reference samples. *Nature* 302: 236–238.
- Crossland, J. (1982). Distribution and abundance of fish eggs and larvae from the spring and summer plankton of north-east New Zealand. *Fisheries Research Bulletin* 24. 59 p.
- Donato, M.D.; Lorono, I.M.D.; Ramirez, I.; Marin, B. (2005). Low genetic differentiation among sardine populations, *Sardinella aurita*, from eastern Venezuela. *Ciencias Marinas* 31: 529–535.
- Drummond, K.L.; Wilson, A.L. (1993). The biology and purse-seine fishery of kahawai (*Arripis trutta* Bloch and Schneider) from central New Zealand. Unpublished report held by MFish, Nelson. 42 p.
- Edmonds, J.S.; Fletcher, W.J. (1997). Stock discrimination of pilchards *Sardinops sagax* by stable isotope ratio analysis of otolith carbonate. *Marine Ecology Progress Series* 152: 241–247.
- Elsdon, T.S.; Gillanders, B.M. (2003). Relationship between water chemistry and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series* 260: 263–272.
- Fahy, W.R.E. (1972). Influence of temperature change on number of vertebrae and caudal fin rays in *Fundulus majalis* (Walbaum). *Journal du Conseil International pour l'Exploration de la Mer* 34: 217–231.
- Francis, M.; Mulligan, K.; Coote, G. (1997). Use of otolith microchemistry to determine kahawai (*Arripis trutta*) stock structure: a feasibility study. Unpublished report held by MFish, Wellington: 7 p.

- Frydenberg, O.; Møller, D.; Nævdal, G.; Sick, K. (1965). Haemoglobin polymorphism in Norwegian cod populations. *Hereditas* 53: 257–272.
- Gaughan, D.J.; Fletcher, W.J.; Mckinlay, J.P. (2002). Functionally distinct adult assemblages within a single breeding stock of the sardine, *Sardinops sagax*: management units within a management unit. *Fisheries Research* 59: 217–231.
- Gauldie, R.W.; Jones, J.B. (2000). Stocks or geographically separated populations of the New Zealand orange roughy *Hoplostethus atlanticus* in relation to parasite infestation, growth rate, and otolith shape. *Bulletin Marine Science* 67: 949–971.
- Gerring, P.; Bradford, E. (1998). Juvenile kahawai recruitment index feasibility study. *NIWA Technical Report 36*. 38 p.
- Gillanders, B.M. (2005). Otolith chemistry to determine movements of diadromous and freshwater fish. *Aquatic Living Resources* 18: 291–300.
- Gillanders, B.M.; Kingsford, M.J. (2003). Spatial variation in elemental composition of otoliths of three species of fish (family Sparidae). *Estuarine Coastal and Shelf Science* 57: 1049–1064.
- Gold, J.R.; Kristmundsdottir, A.Y.; Richardson, L.R. (1997). Mitochondrial DNA variation in king mackerel (*Scomberomorus cavalla*) from the western Atlantic Ocean and Gulf of Mexico. *Marine Biology* 129: 221–232.
- Griggs, L.; Bradford, E.; Jones, B.; Drummond, K. (1998). Growth and movement of tagged kahawai in New Zealand waters. *NIWA Technical Report 10*: 37 p.
- Hamer, P.A.; Jenkins, G.P. (2007). Comparison of spatial variation in otolith chemistry of two fish species and relationships with water chemistry and otolith growth. *Journal of Fish Biology in press*.
- Hamer, P.A.; Jenkins, G.P.; Coutin, P. (2006). Barium variation in *Pagrus auratus* (Sparidae) otoliths: a potential indicator of migration between an embayment and ocean waters in south-eastern Australia. *Estuarine Coastal and Shelf Science* 68: 686–702.
- Hartill, B.; Walsh, C. (2005). Characterisation of the kahawai fisheries of New Zealand and review of biological knowledge. Final Research Report for Ministry of Fisheries Research Project KAH2004/01. 160 p.
- Heincke, D. (1898). Naturgeschichte des herrings. *Abhandlungen der deutschen Seefischereivereins* 12: 128–223.
- Hemmer-Hansen, J.; Nielsen, E.; Frydenberg, J.; Loeschcke, V. (2007). Adaptive divergence in a high gene flow environment: *Hsc70* variation in the European flounder (*Platichthys flesus* L.). *Heredity* 99: 592–600.
- Herwerden, L.V.; Mcilwain, J.; Al-Oufi, H.; Al-Amry, W.; Alejandro, R. (2006). Development and application of microsatellite markers for *Scomberomorus commerson* (Perciformes; Teleostei) to a population genetic study of Arabian Peninsula stocks. *Fisheries Research* 79: 258–266.

- Hewitt, G.; Hine, P. (1972). Checklist of parasites of New Zealand fishes and their hosts. *New Zealand Journal of Marine and Freshwater Research* 6: 69–114.
- Hulme, T.J. (1995). The use of vertebral counts to discriminate between North Sea herring stocks. *ICES Journal of Marine Science* 52: 775–779.
- Johnson, A.; Fable, W.; Grimes, C.; Trent, L.; Perez, J. (1993). Evidence for distinct stocks of king mackerel *Scomberomorus cavalla* in the Gulf of Mexico. *Fishery Bulletin* 92: 91–101.
- Jones, J.B. (1988). New Zealand parasitic Copepoda; genus *Caligus* Mueller, 1785 (Siphonostomatoida: Caligidae). *New Zealand Journal of Zoology* 15: 397–413.
- Jones, J. B. (1991). Movements of albacore tuna (*Thunnus alalunga*) in the South Pacific: evidence from parasites. *Marine Biology* 111: 1–9.
- Jones, J.; Cresswell, P.; Drummond, K.; Mckenzie, J. (1992). Kahawai. *Unpublished report held in NIWA library, Wellington* 92/2: 27 p.
- Jones, J.B.; Hadfield, J.D. (1985). Fishes from Porirua and Pauatahanui inlets: occurrence in gill nets. *New Zealand Journal of Marine and Freshwater Research* 19: 477–484.
- Jonsdottir, I.G.; Campana, S.E.; Marteinsdottir, G. (2006). Stock structure of Icelandic cod *Gadus morhua* L. based on otolith chemistry. *Journal of Fish Biology* 69: 136–150.
- Junquera, S.; Perez-Gandaras, G. (1993). Population diversity in Bay of Biscay anchovy (*Engraulis encasicolus* L. 1758) as revealed by multivariate analysis of morphometric and meristic characters. *ICES Journal of Marine Science* 50: 383–391.
- Kalish, J. (1989). Otolith microchemistry: validation of the effects of physiology, age, and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology* 132: 151–178.
- Kalish, J. (1992). Formation of a stress-induced chemical check in fish otoliths. *Journal of Experimental Marine Biology and Ecology* 162: 265–277.
- Kalish, J.; Livingston, M.; Schofield, K. (1996). Trace elements in the otoliths of New Zealand blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. *New Zealand Journal of Marine and Freshwater Research* 47: 537–542.
- Kinsey, S.T.; Orsoy, T.; Bert, T.M.; Mahmoudi, B. (1994). Population structure of the the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. *Marine Biology* 18: 309–317.
- Kraus, R.T.; Secor, D.H. (2004). Incorporation of strontium into otoliths of an estuarine fish. *Journal of Experimental Marine Biology and Ecology* 303: 85–106.
- Lester, R.J.; Thompson, C.; Moss, H.; Barker, S.C. (2001). Movement and stock structure of narrow-barred Spanish mackerel as indicated by parasites. *Journal of Fish Biology* 59: 833–842.
- Lindsey, C.C. (1988). Factors controlling meristic variation. *Fish physiology XIB* Hoar, WS; Randal DJ (eds): 179–274.

- MacDonald, C. (1983). Population, taxonomic and evolutionary studies on marine fishes of the genus *Arripis* (Perciformes: Arripidae). *Bulletin of Marine Science* 33: 780–796.
- Mackenzie, K.; Mehl, S. (1984). The cestode parasite *Grillotia angeli* (Cestoda Trypanorhyncha) as a biological tag for mackerel in the eastern North Atlantic. *ICES CM 1984/H:52*. 8p.
- Martin, A.; Humphreys, R.; Palumbi, S. (1992). Population genetic structure of the armourhead *Pseudopentaceros wheeleri* in the North Pacific Ocean: application of the polymerase chain reaction to fisheries problems. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 2386–2391.
- Miller, J.; Banks, M.; Gomez-Uchida, D.; Shanks, A. (2005). A comparison of population structure in black rockfish (*Sebastes melanops*) as determined with otolith microchemistry and microsatellite DNA. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2189–2198.
- Milton, D.A.; Chenery, S.R. (2001). Sources of uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). *Journal of Experimental Marine Biology and Ecology* 264: 47–65.
- Moore, B.R.; Buckworth, R.C.; Moss, H.; Lester, R.J.G. (2003). Stock discrimination and movements of narrow-barred Spanish mackerel across northern Australia as indicated by parasites. *Journal of Fish Biology* 63: 765–779.
- Murta, A.G. (2000). Morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic: implications for stock identification. *ICES Journal of Marine Science* 57: 1240–1248.
- Nelson, K.; Hutchinson, E.S.; Li, G.; Sly, F.L.; Hedgecock, D. (1994). Variation in life history and morphology in Northern anchovies (*Engraulis mordax*). *CalCoFi Report* 35: 108–120.
- Nesbo, C.L.; Rueness, E.K.; Iverson, S.A.; Skagen, D.W.; Jakobsen, K.S. (2000). Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. *Proceedings of the Royal Society of London B* 267: 281–292.
- Paulin, C. (1993). Review of the Australian fish family Arripidae (Percomorpha), with the description of a new species. *Australian Journal of Marine and Freshwater Research* 44: 459–471.
- Pepin, P.; Carr, S.M. (1992). Morphometrical, meristic, and genetic analysis of stock structure in juvenile Atlantic cod (*Gadus morhua*) from the Newfoundland shelf. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1924–1933.
- Pogson, G.; Mesa, K. (2004). Positive Darwinian Selection at the Pantophysin (Pan I) Locus in Marine Gadid Fishes. *Molecular Biology and Evolution* 21: 65–75.
- Pogson, G.; Mesa, K.; Boutilier, R. (1995). Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics* 139: 375– 85.

- Quinn, G.P.; Keough, M.J. (2002). *Experimental design and data analysis for biologists*. Cambridge University Press: 537 p.
- Roberts, P.E.; Paul, L.J. (1978). Seasonal hydrological changes in continental shelf waters off the west coast, North Island, New Zealand and comments on fish distributions. *New Zealand Journal of Marine and Freshwater Research* 12: 323–339.
- Rooker, J.R.; Secor, D.H. (2004). Stock structure and mixing of Atlantic bluefin tuna: Evidence from stable delta super(13)C and delta super(18)O isotopes in otoliths. *Collective Volume of Scientific Papers of the International Commission for the Conservation of Atlantic tunas* 56: 1115–1120.
- Rooker, J.; Secor, D.; Zdanowicz, V.; Metrio, G.D.; Relini, L. (2003). Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. *Fisheries Oceanography* 12: 75–84.
- Shaklee, J.B. (1987). Patterns of genetic variation and subpopulation structure in narrow-barred Spanish mackerel. *Stock Identification Workshop, Panama City, Florida, November 1985*.
- Sharp, J.C.; Able, K.W.; Leggett, W.V.C.; Carscadden, J.E. (1978). Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian waters. *Journal of the Fisheries Research Board of Canada* 35: 124–130.
- Sick, K. (1965). Haemoglobin polymorphism of cod in the Baltic and Danish Belt Sea. *Hereditas* 54: 19–48.
- Sinclair, M.; Iles, T.D. (1988). Population richness of marine species. *Aquatic Living Resources* 1: 71–83.
- Smith, P.J.; McMillan, P.; Bull, B.; Mcveagh, S.M. (2002a). Genetic and meristic variation in black and smooth oreos in the New Zealand EEZ. *New Zealand Journal of Marine and Freshwater Research* 36: 737–750.
- Smith, P.J.; Robertson, S.; Horn, P.; Bull, B.; Anderson, O.; Stanton, B.A.; Oke, C. (2002b). Multiple techniques for determining stock relationships between orange roughy, *Hoplostethus atlanticus*, fisheries in the eastern Tasman Sea. *Fisheries Research* 58: 119–140.
- Smith, P.J.; Diggles, B.; Bull, B.; Benson, P.G. (2001). Stock relationships of alfonso and cardinalfish in New Zealand waters. *Final Research Report. New Zealand Ministry of Fisheries Research Project DEE1999/03*.
- Smith, P.J.; Diggles, B.; Kim, S. (2005). Stock structure of blue mackerel, *Scomber australasicus*. *New Zealand Fisheries Assessment Report 2005/43*: 38 p.
- Smith, P.J.; Diggles, B.; McKenzie, J.; Kim, S.; Maolagáin, C.Ó.; Notman, P.; Griggs, L.H. (2004). Stock structure of kingfish. *New Zealand Ministry of Fisheries Final Research Report, Project KIN2002/01 Sept 2004*.
- Smith, P.J.; Proctor, C.; Robertson, S.; McMillan, P.; Bull, B.; Diggles, B. (2000). Final research report on stock relationships of black oreo in New Zealand waters. *Report to New Zealand Ministry of Fisheries, Project DEE9801 Nov 2000*.

- Spankias, E.; Tsimenides, N.; Zouros, E. (1989). Genetic differences between populations of sardine, *Sardina pilchardus*, and anchovy, *Engraulis encrasicolus*, in the Aegean and Ionian Seas. *Journal of Fish Biology* 35: 417–437.
- Stanley, C.A. (1978). Area of distribution, movements, age composition and mortality rates of the Australian salmon population in Tasmania, Victoria and New South Wales. *Australian Journal of Marine and Freshwater Research* 29: 417–433.
- Stephenson, P.C.; Edmonds, J.S.; Moran, M.J.; Caputi, N. (2001). Analysis of stable isotope ratios to investigate stock structure of red emperor and Rankin cod in northern Western Australia. *Journal of Fish Biology* 58: 126–144.
- Taning, A.V. (1946). Stage of determination of vertebrae in teleostean fishes. *Nature* 157: 594–595.
- Taning, A.V. (1952). Experimental study of meristic characters in fishes. *Biological Reviews* 27: 169–193.
- Thresher, R.E. (1999). Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research* 43: 165–204.
- Thresher, R.E.; Proctor, C.; Evans, K.; Mackinnon, C. (1999). Completion of an analysis of stock structure of orange roughy, based on otolith chemical composition. *Fisheries Research and Development Corporation Project, held in CSIRO library, Hobart 1996/111*.
- Tudela, S. (1999). Morphological variability in a Mediterranean genetically homogeneous population of the European anchovy *Engraulis encrasicolus*. *Fisheries Research* 42: 229–243.
- Turan, C. (2006). The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). *Journal of Fish Biology* 69: 165–180.
- Tzeng, T.D. (2004). Morphological variation between populations of spotted mackerel (*Scomber australasicus*) off Taiwan. *Fisheries Research* 68: 45–55.
- Waldman, J.R.; Richards, R.A.; Schill, W.B.; Wigrin, I.; Fabrizio, M.C. (1997). An empirical comparison of stock identification techniques applied to striped bass. *Transactions of the American Fisheries Society* 126: 369–385.
- Walther, B.D.; Thorrold, S.R. (2006). Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. *Marine Ecology Progress Series* 311: 125–130.
- Webb, B.F. (1972). Fish populations of the Avon-Heathcote Estuary. I. General ecology, distribution, and length-frequency. *New Zealand Journal of Marine and Freshwater Research* 6: 570–601.
- Webb, B.F. (1973). Fish populations of the Avon-Heathcote Estuary. 4. Parasites. *New Zealand Journal of Marine and Freshwater Research* 7: 301–305.

Wood, B.A.; Bradstock, M.A.; James, G.D. (1990). Tagging of kahawai, *Arripis trutta*, in New Zealand, 1981-84. *New Zealand Fisheries Technical Report 19*. 16 p.

Wright, P.J.; Neat, F.C.; Gibb, F.M.; Gibb, I.M.; Thordarson, H. (2006). Evidence for metapopulation structuring in cod from the west of Scotland and North Sea. *Journal of Fish Biology 69*: 181–199.

Zenitani, H.; Kimura, R. (2007). Elemental analysis of otoliths of Japanese anchovy: trial to discriminate between Seto Inland Sea and Pacific stock. *Fisheries Science 73*: 1–8.

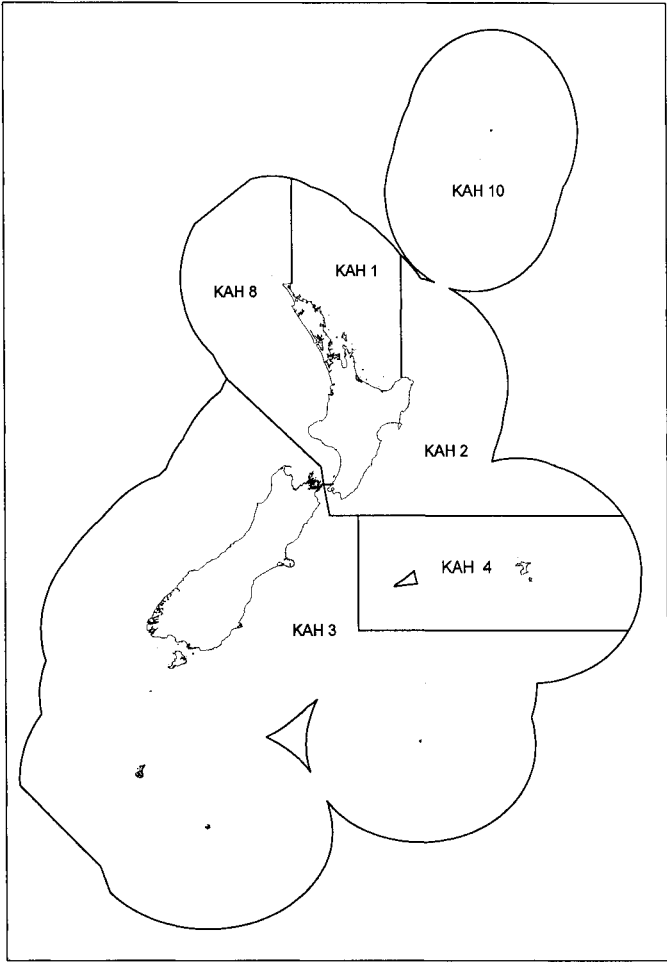


Figure 1: Kahawai stock management areas.

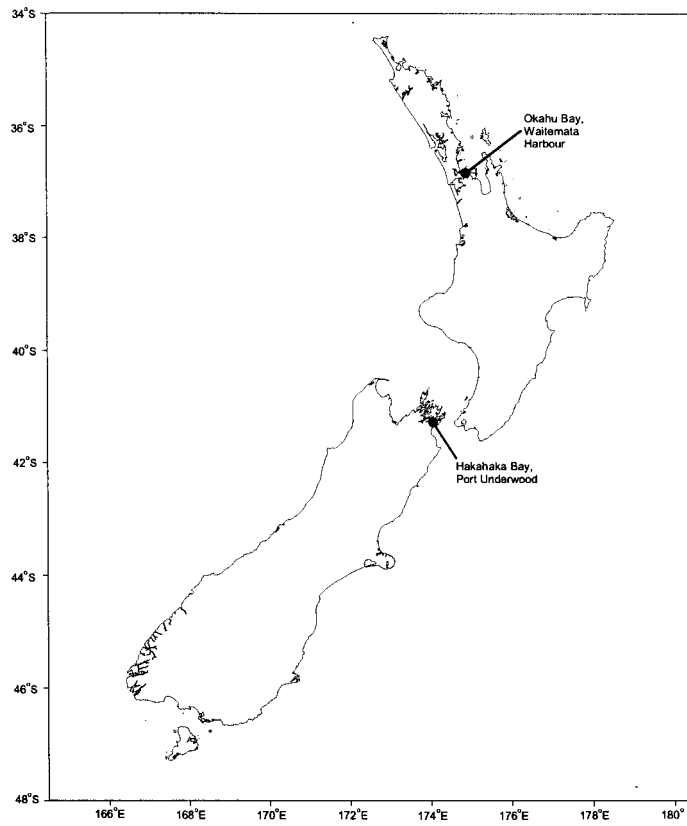


Figure 2: Collection sites for 0-group kahawai.

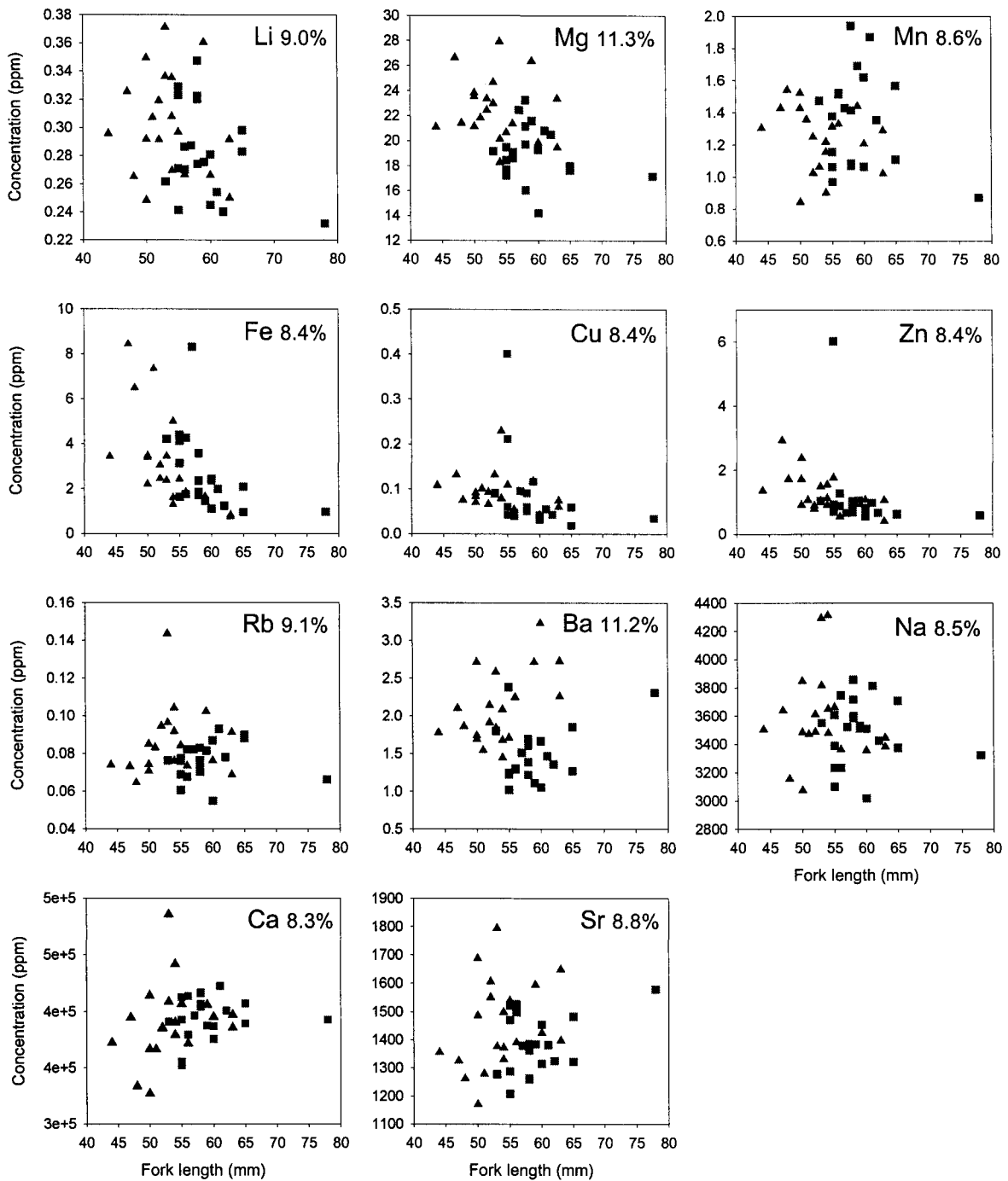
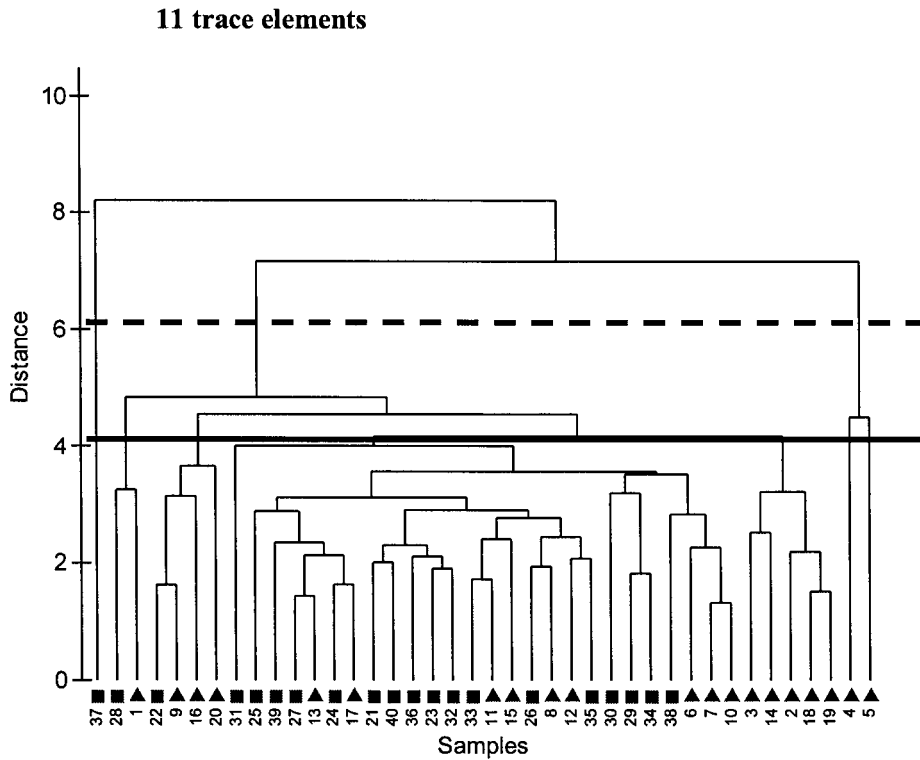
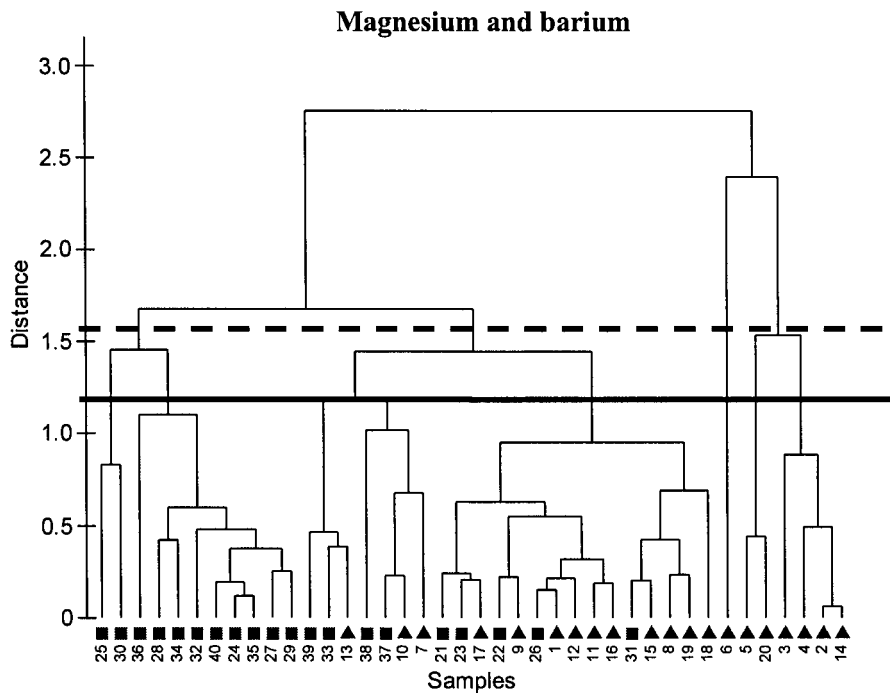


Figure 3: Univariate plots of the concentration of consistently detectable trace elements, relative to fish length. Triangles denote fish from the Waitemata Harbour and squares denote fish from Port Underwood. The percentage given for each element is the contribution that each element makes to the multi-elemental between-site difference, as determined by the PRIMER procedure SIMPER.

a)



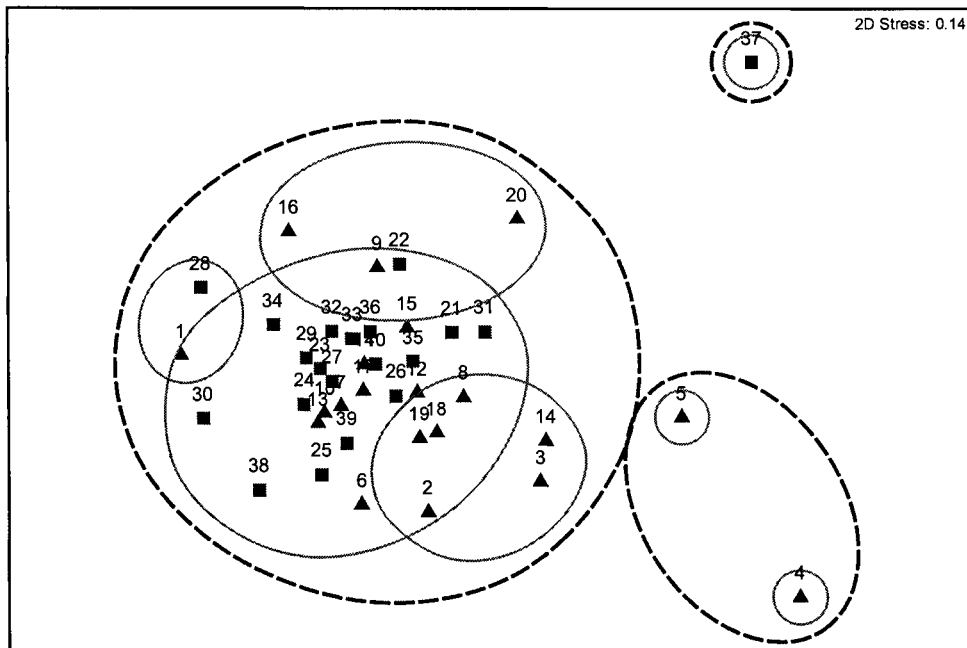
b)



Figures 4a and 4b: Dendrogram of the elemental compositions of 40 otolith pairs, using group average clustering. Triangles denote fish from the Waitemata Harbour and squares denote fish from Port Underwood. 4a gives linkages based on all 11 detectable trace elements, and 4b gives linkages based on just magnesium and barium concentrations. Solid and dotted horizontal lines denote levels of clustering used in Figures 5a and 5b.

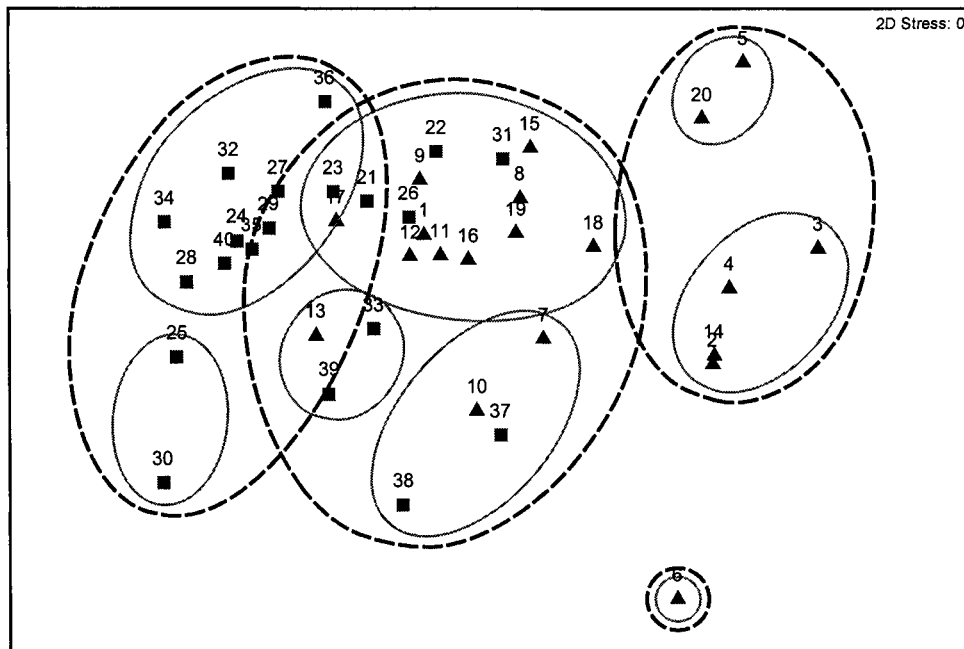
a)

11 trace elements

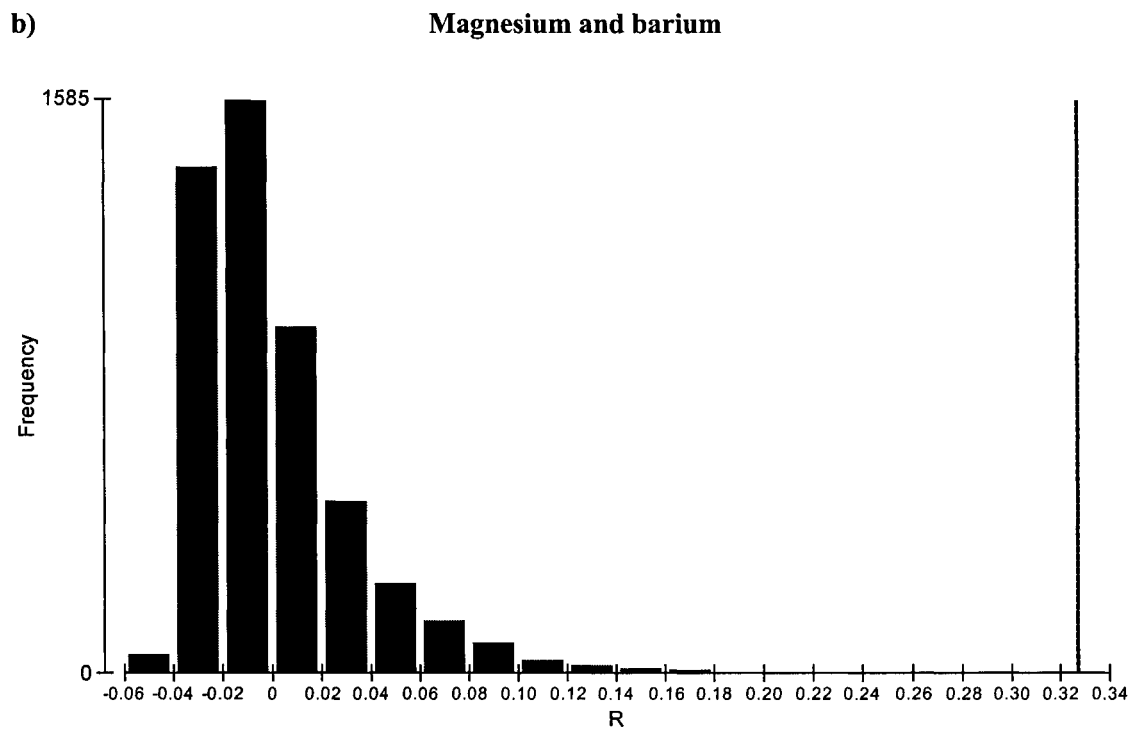
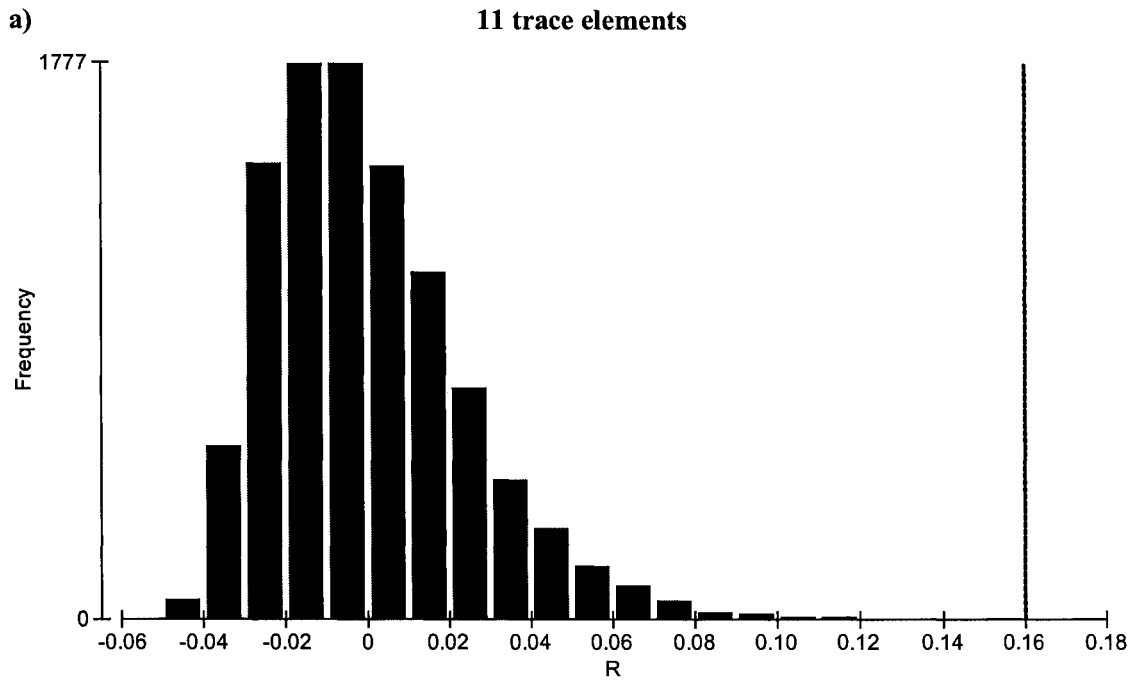


b)

Magnesium and barium



Figures 5a and 5b: 2-dimensional MDS ordinations of the elemental compositions of 40 otolith pairs with clusters from Figures 4a and 4b imposed, respectively. Triangles denote fish from the Waitemata Harbour and squares denote fish from Port Underwood. The upper panel gives the ordination based on all 11 trace elements, and the bottom panel that based on just magnesium and barium. The solid and dotted line ellipses represent the thresholds of similarity in Figures 4a and 4b.



Figures 6a and 6b: Distribution of randomisation based test statistics – R (distances between the observed elemental compositions of 40 fish, which have been randomly assigned to groups of 20 fish per site). The dashed vertical line denotes the observed value of R given the actual origins of each fish. 5a gives the distribution of test statistics based on all 11 trace elements, and 5b those based on just magnesium and barium.

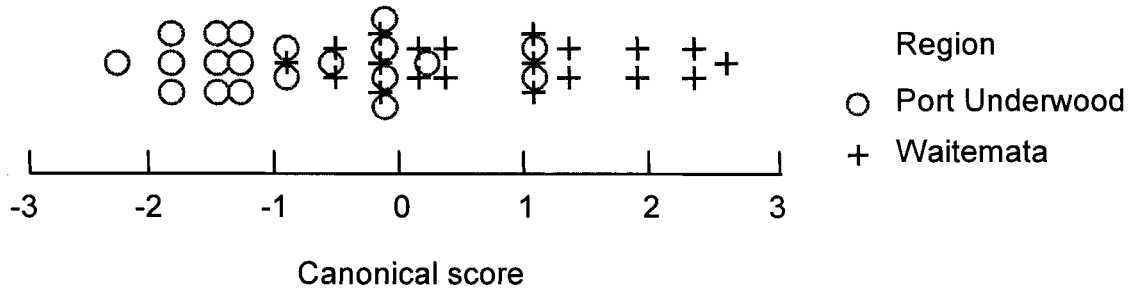


Figure 7: Canonical scores plot from linear discriminant function analysis of kahawai otolith chemistry (Mg, Ba) for samples from Port Underwood and Waitemata.

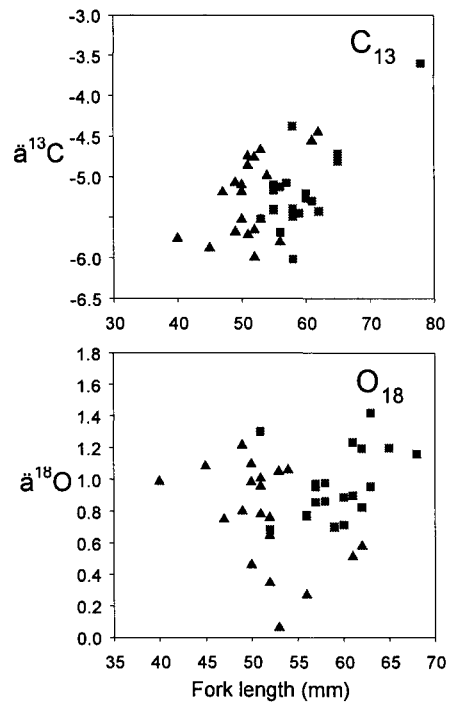


Figure 8: Univariate plots of the isotope ratio values, relative to fish length. Triangles denote fish from the Waitemata Harbour and squares denote fish from Port Underwood.

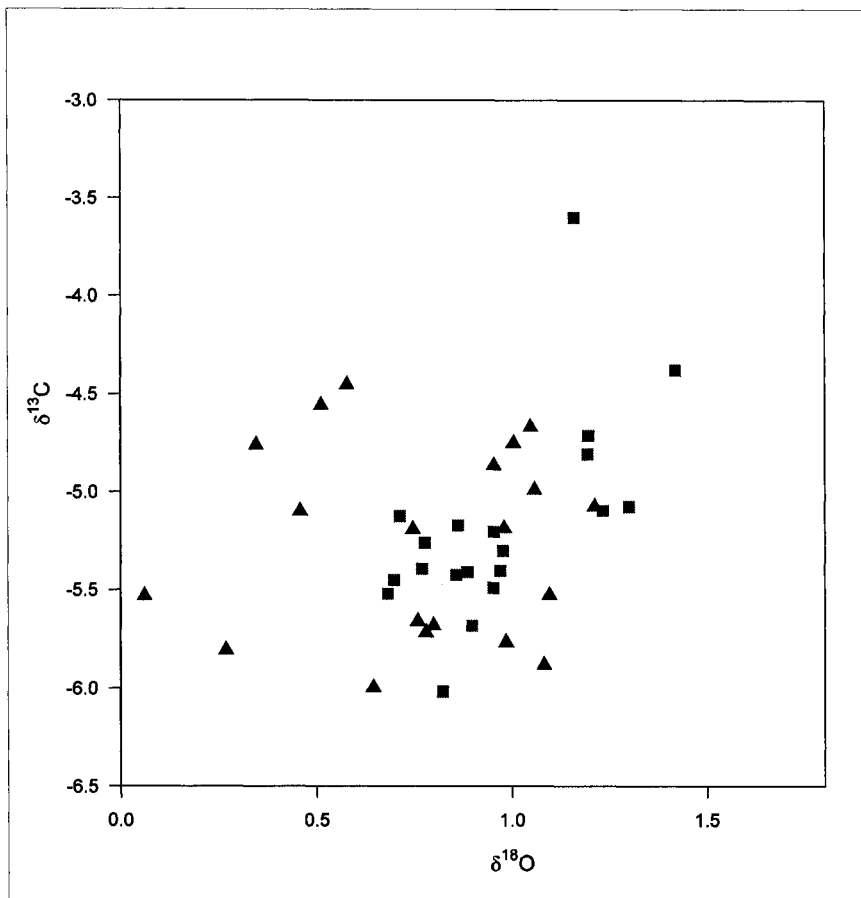


Figure 9: Stable $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotope values from 0-group kahawai otoliths from the Waitemata Harbour (triangles) and Port Underwood (squares).

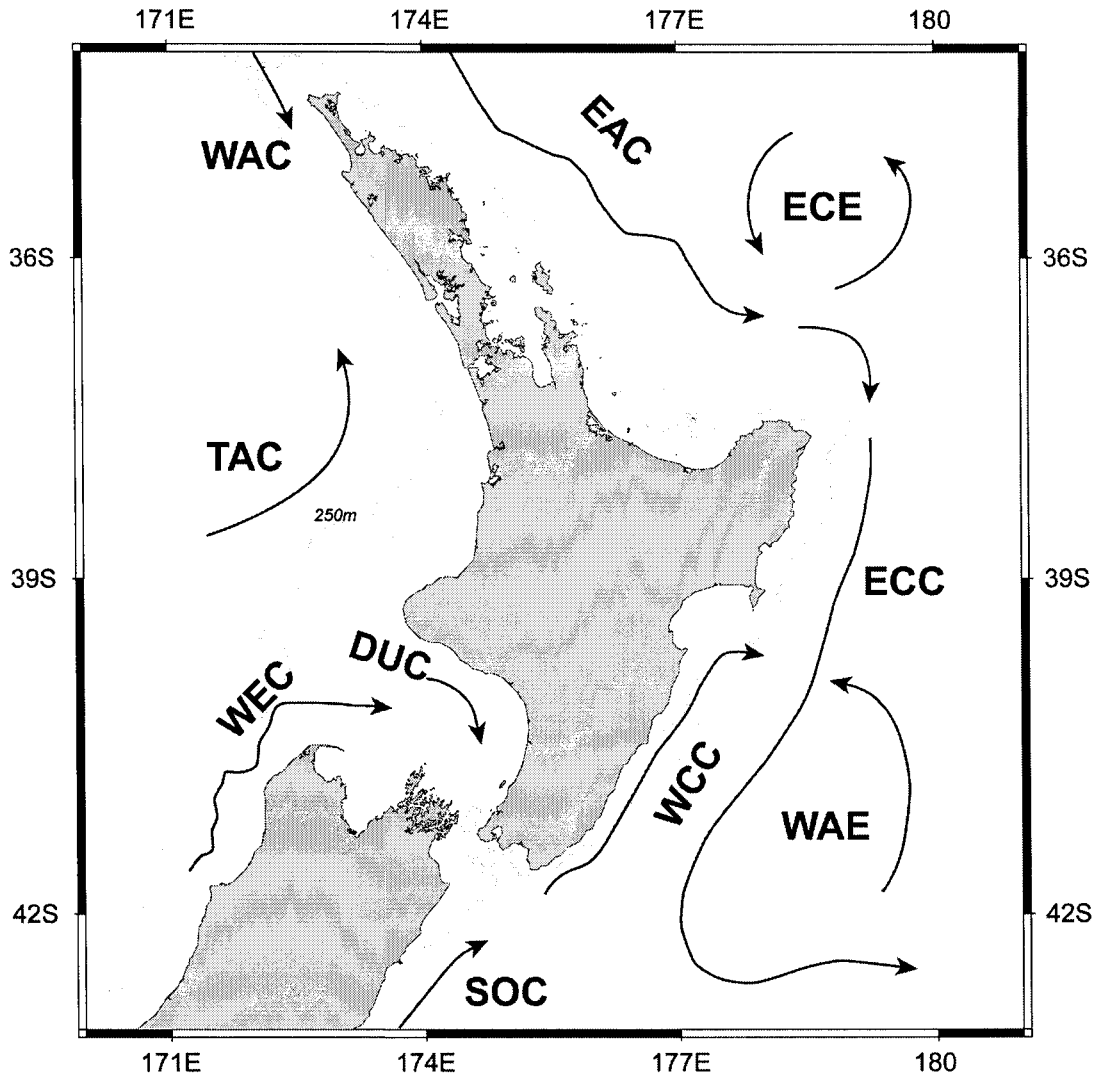


Figure 10: Major current systems around the North Island and northern South Island.
Currents: DUC, D'Urville Current; EAC, East Auckland Current; ECC, East Cape Current; SOC, Southland Current; TAC, Tasman Current; WAC, West Auckland Current; WEC, Westland Current; WCC, Wairarapa Coastal Current. **Eddies:** ECC, East Cape Eddy; WAE, Wairarapa Eddy.

Appendix 1: Trace element analyses of Kahawai otoliths from Waitemata Harbour (w) and Hakahaka Bay (h). Otolith (1) = uncleaned otolith weight; otolith (2) = cleaned otolith weight. LR - low resolution, MR - medium resolution, HR - High resolution.

Fish No	Date sample	Fish size (mm)	Fish weight (g)	Otolith (1) weight (g)		L(LR)	Mg(LR)	Mn(MR)	Fe(MR)	Concentration (ppm = microgram per gram)						
				weight	weight					Cu (MR)	Zn (MR)	Rb(LR)	Ba (LR)	Na(HR)	Ca(HR)	Si(HR)
w2	18.7.06	50	1.56	0.0031	0.0028	0.24842962	21.1445356	0.84326815	2.20146484	0.07001568	0.90539854	0.07062648	1.68988254	3074.94782	327087.688	1170.90646
w4	18.7.06	63	3.14	0.0062	0.0056	0.29166269	23.3798333	1.02165876	0.83387922	0.0739537	0.39784868	0.09112513	2.73082958	3382.46266	397144.238	1648.85209
w6	18.7.06	59	2.42	0.0059	0.0051	0.36058146	26.3791898	1.44005902	1.6510992	0.11811029	0.92739902	0.10224473	2.71698941	3501.69245	406016.587	1594.98268
w8	18.7.06	53	1.9	0.0035	0.0028	0.37121088	24.6674467	1.06224126	3.44569094	0.13165872	1.48175782	0.14349256	2.58431831	4294.46514	485669.893	1794.17163
w16	18.7.06	54	2.04	0.0033	0.0024	0.33536353	27.9195385	1.15566008	4.99817243	0.22847558	1.54416206	0.10418394	2.08357233	4314.60315	441726.952	1498.30241
w17	18.7.06	60	2.72	0.0042	0.0042	0.26624547	19.8633372	1.20658897	2.29568066	0.04233787	1.06399147	0.0760945	3.23189048	3356.96251	395333.738	1425.39451
w18	18.7.06	56	2.17	0.0049	0.0044	0.26653958	21.3634196	1.32944631	1.84748267	0.05385026	0.54275394	0.07329501	2.24630321	3363.37604	371664.434	1390.49459
w21	18.7.06	53	1.96	0.0038	0.0029	0.33618838	22.9855918	1.46388746	2.36769785	0.08610975	1.03700168	0.0962279	1.83576327	3816.6264	408747.791	1376.30014
w22	18.7.06	51	1.77	0.0037	0.0031	0.30715533	21.8365135	1.3574525	7.34006203	0.10067936	1.06011719	0.0830621	1.54373348	3470.83263	366611.737	1278.86971
w23	18.7.06	63	3.1	0.0064	0.0058	0.25003386	19.4722926	1.28939854	0.75034241	0.0598366	1.05169016	0.06869607	2.26122917	3446.31153	385773.266	1398.03743
w24	18.7.06	44	1.05	0.0025	0.0018	0.29592114	21.0937182	1.30332941	3.43596461	0.10775757	1.35467177	0.07383071	1.78000943	3503.70669	372446.327	1355.61773
w25	18.7.06	55	2.19	0.0036	0.0033	0.29680361	20.66609891	1.31330153	2.4280357	0.10857479	1.75817547	0.08418182	1.70820534	3662.85276	406626.456	1538.95374
w27	18.7.06	54	1.91	0.0035	0.0032	0.26950552	18.2732493	0.90117314	1.29929128	0.07877531	1.11806652	0.07602857	1.67728606	3651.37852	390545.159	1371.68311
w29	18.7.06	50	1.49	0.0025	0.0021	0.34939008	23.5384703	1.52280458	3.40468092	0.091110473	1.71673542	0.08492358	2.715325	3847.88908	413967.374	1688.0937
w31	18.7.06	50	1.43	0.0032	0.0028	0.29175809	23.8211941	1.42820629	3.47153995	0.083556265	2.36966196	0.07413882	1.73939525	3485.27919	366637.481	1486.70531
w32	18.7.06	48	1.28	0.0022	0.0018	0.26522308	21.4089264	1.54042206	6.49842798	0.07480475	1.71441927	0.06457634	1.86153033	3156.96464	333758.221	1261.7006
w34	18.7.06	54	2.02	0.0033	0.0026	0.30785962	20.1319365	1.21799912	1.60279063	0.07834056	0.90409982	0.09163884	1.4438604	3479.10646	379357.507	1329.99943
w35	18.7.06	52	1.78	0.0035	0.0031	0.29144889	23.3510297	1.24924703	2.449958	0.09272616	0.78368137	0.09439856	2.13970252	3612.31044	385122.919	1605.55145
w36	18.7.06	52	1.67	0.0028	0.0027	0.31917382	22.4520388	1.02526948	3.05399019	0.06580785	0.88050431	0.09452394	1.91116287	3487.28702	385767.113	1550.1184
w40	18.7.06	47	1.17	0.0028	0.0022	0.32555493	26.6385367	1.42835487	8.43637253	0.13169949	2.92112562	0.07312945	2.10147169	3639.72175	394735.937	1325.46011
h1	1.9.06	61	2.69	0.0054	0.0048	0.25423483	20.833771	1.87073639	1.97759383	0.05523352	0.97655144	0.09294965	1.46947307	3814.91282	422381.882	1382.28409
h4	1.9.06	57	2.47	0.0058	0.0052	0.28727461	22.4595846	1.42951705	8.30847113	0.09333114	0.67027272	0.08202471	1.51275857	3523.53746	396663.564	1380.82978
h5	1.9.06	62	2.6	0.0052	0.0043	0.24006452	20.5066281	1.35304546	1.23431475	0.04313192	0.66766135	0.07806154	1.35941254	3426.00657	400774.694	1325.12428
h6	1.9.06	55	2.03	0.0041	0.0043	0.32302234	18.46529	1.06329403	1.6210583	0.06046421	0.71922559	0.07593209	1.24500113	3389.35612	392875.237	1287.48478

h11	1.9.06	58	2.28	0.0046	0.0046	0.34733729	16.0591431	1.06962054	3.57580268	0.05366861	0.92253377	0.07408166	1.3906776	3717.441	416595.004	1263.82572
h15	1.9.06	58	2.46	0.0052	0.0046	0.3223741	21.1838166	1.41394286	2.34683443	0.05117928	0.68042159	0.07629094	1.61029642	3585.23202	416135.169	1385.48622
h17	1.9.06	58	2.37	0.0053	0.0047	0.27430198	19.7248802	1.08394542	1.84705822	0.09134898	1.02530174	0.0702106	1.22245727	3602.4044	403998.401	1363.24582
h18	1.9.06	55	1.95	0.0036	0.0035	0.24126946	17.2125476	1.15580129	3.13588223	0.21128368	0.92513385	0.06051533	1.224162	3100.59338	355609.76	1207.86337
h19	1.9.06	56	2.37	0.005	0.0045	0.27027964	19.0941179	1.5142986	1.75321646	0.04748652	0.87395156	0.06759029	1.29036784	3234.93948	379336.725	1498.61024
h20	1.9.06	60	2.54	0.0058	0.0058	0.28088519	14.2085311	1.06357664	1.10270277	0.04234971	0.55704271	0.05484292	1.66937357	3020.19176	375707.32	1315.4199
h21	1.9.06	58	2.49	0.0048	0.0041	0.3203362	23.2820726	1.94172447	1.70537065	0.06024872	0.90099025	0.08294807	1.69564876	3858.31162	406592.406	1260.18727
h25	1.9.06	60	2.65	0.0054	0.005	0.24481327	19.2798535	1.62032673	2.43269795	0.03214773	0.81779988	0.08683598	1.05481055	3511.02419	386935.381	1455.07079
h26	1.9.06	53	1.93	0.0045	0.004	0.26180826	19.1727976	1.47465348	4.20499184	0.09162756	1.02716036	0.07621419	1.7998702	3551.5356	390920.265	1277.373
h28	1.9.06	55	2	0.0041	0.0039	0.27108632	17.7005701	1.3780195	4.11418917	0.0423064	0.7037317	0.06880871	1.02087489	3233.59459	352116.641	1470.74756
h29	1.9.06	56	2.35	0.0051	0.0047	0.28631908	18.583943	1.52356746	4.25373001	0.03949324	1.26854444	0.08208895	1.30346865	3747.80986	413560.557	1525.74853
h31	1.9.06	59	2.6	0.0052	0.0047	0.27553946	21.6102672	1.6913414	1.43890424	0.1166746	1.0463099	0.08135862	1.11451912	3530.59785	387781.274	1384.92402
h32	1.9.06	55	2.04	0.0043	0.0042	0.32908606	19.4669976	0.96970566	4.38590338	0.40068857	6.02200639	0.07731411	2.38111847	3607.71558	412615.505	1522.57897
h35	1.9.06	78	6.23	0.0095	0.0088	0.23182329	17.1702665	0.87084168	0.96649476	0.0343002	0.58256427	0.06616974	2.31178567	3324.00973	392872.444	1578.63676
h37	1.9.06	65	3.48	0.0067	0.0061	0.29806666	17.6539112	1.10794984	0.95615705	0.018334	0.61045245	0.08995283	1.85340921	3375.41088	389414.345	1483.48829
h39	1.9.06	65	3.51	0.0059	0.0054	0.28301799	17.9855037	1.56690448	2.08430565	0.05965571	0.63238952	0.08808468	1.27173516	3708.72346	407124.052	1322.58422

Appendix 2: Stable isotope results. w, Waitemata Harbour; h, Hakahaka Bay.

Duplicate analyses were conducted on two separate otoliths for some specimens (Sample Nos and values shown in bold).

Sample No	Date Capture	Fish length	Fish weight ht	Otolith weight (1)	d-13CV-PDB	Mean of 2 d-13CV-PDB	d-18OV-PDB	Mean of 2 d-18OV-PDB
w1	18.7.06	51	1.66	0.00165	-5.75026		0.929001	
w1	18.7.06	51	1.66	0.00165	-5.6822	-5.71623	0.631153	0.780077
w3	18.7.06	47	1.2	0.0012	-5.19198		0.746717	
w5	18.7.06	56	2.17	0.0025	-5.80888		0.253946	
w5	18.7.06	56	2.17	0.0025	-5.80089	-5.80489	0.284642	0.269294
w7	18.7.06	40	0.65	0.00055	-5.76498		0.985342	
w9	18.7.06	45	1.18	0.001	-5.88077		1.08242	
w10	18.7.06	61	2.96	0.0028	-4.60748		0.500061	
w10	18.7.06	61	2.96	0.0028	-4.50655	-4.55702	0.523812	0.511937
w11	18.7.06	51	1.59	0.00155	-4.75031		1.00461	
w12	18.7.06	50	1.53	0.00155	-5.09973		0.458368	
w13	18.7.06	52	1.61	0.00145	-5.66023		0.75846	
w14	18.7.06	52	1.6	0.0014	-5.9967		0.644637	
w15	18.7.06	49	1.41	0.00135	-5.07454		1.21348	
w19	18.7.06	53	1.79	0.00175	-4.66655		1.04834	
w20	18.7.06	53	1.8	0.00185	-5.53094		0.061361	
w26	18.7.06	52	1.84	0.0019	-4.76079		0.346965	
w28	18.7.06	50	1.63	0.00155	-5.18584		0.980302	
w30	18.7.06	54	2	0.00165	-4.98774		1.05814	
w33	18.7.06	50	1.58	0.0016	-5.52709		1.09622	
w37	18.7.06	62	3.23	0.00255	-4.56478		0.564748	
w37	18.7.06	62	3.23	0.00255	-4.34073	-4.45276	0.591496	0.578122
w38	18.7.06	49	1.52	0.00115	-5.68107		0.799144	
w39	18.7.06	51	1.68	0.00145	-4.83626		1.08132	
w39	18.7.06	51	1.68	0.00145	-4.89161	-4.86394	0.826575	0.953948
h2	1.8.06	58	2.34	0.00265	-5.3983		0.807529	
h2	1.8.06	58	2.34	0.00265	-5.20202	-5.30016	1.14682	0.977175
h3	1.8.06	51	1.66	0.00165	-5.07547		1.30099	
h7	1.8.06	57	2.15	0.00265	-5.42473		0.857691	
h8	1.8.06	60	2.55	0.0026	-5.40997		0.886596	
h9	1.8.06	62	2.68	0.00265	-6.01684		0.822718	
h10	1.8.06	63	3.21	0.0037	-4.92229		1.56754	
h10	1.8.06	63	3.21	0.0037	-3.83645	-4.37937	1.27192	1.41973
h12	1.8.06	56	2.14	0.00215	-5.3938		0.770201	
h13	1.8.06	58	2.53	0.00275	-5.17069		0.862614	
h14	1.8.06	61	2.59	0.00245	-5.74652		0.852895	
h14	1.8.06	61	2.59	0.00245	-5.6194	-5.68296	0.941808	0.897352
h16	1.8.06	56	1.98	0.0019	-5.27897		0.687593	
h16	1.8.06	56	1.98	0.0019	-5.24621	-5.26259	0.86508	0.776337
h22	1.8.06	57	2.29	0.00255	-5.49058		0.952434	
h23	1.8.06	63	3.34	0.00235	-5.20492		0.953764	
h24	1.8.06	52	1.84	0.0021	-5.51995		0.683048	

h27	1.8.06	57	2.34	0.0026	-5.40363	0.971057	
h30	1.8.06	60	2.75	0.0028	-5.07276	0.564322	
h30	1.8.06	60	2.75	0.0028	-5.17486	-5.12381	0.862985
h33	1.8.06	59	2.54	0.00285	-5.45092	0.699885	0.713654
h34	1.8.06	61	2.59	0.00255	-5.096	1.23439	
h36	1.8.06	68	3.79	0.00415	-3.60026	1.16167	
h38	1.8.06	62	3.02	0.0036	-4.8059	1.19511	
h40	1.8.06	65	3.4	0.0035	-4.71292	1.19676	

**IA-R022
Calcium
Carbonate**

d-13CV-PDB d-18OV-PDB

	-28.538	-22.51
	-28.633	-22.585
	-28.593	-22.619
	-28.564	-22.593
	-28.637	-22.684
	-28.668	-22.761
	-28.657	-22.792
	-28.694	-22.587
	-28.537	-22.489
	-28.522	-22.539
	-28.572	-22.553
mean	-28.601	-22.61
1 s.d.	0.05923	0.09803
n	11	11
expected	-28.63	-22.69

NBS-18

Calcite

d-13CV-PDB d-18OV-PDB

	-5.14942	-23.0941
	-5.04508	-22.8487
	-5.1185	-23.1115
	-5.17378	-23.1268
	-5.07385	-23.0123
mean	-5.11213	-23.0387
1 s.d.	0.052898	0.11503
n	5	5
expected	-5	-23

**NBS-19
Limestone**

d-13CV-PDB d-18OV-PDB

	1.78208	-2.34078
	1.83793	-2.23346
	1.93268	-2.3985
mean	1.850897	-2.32425
1 s.d.	0.076133	0.083753
n	3	3
expected	1.95	-2.2