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EXECUTIVE SUMMARY

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Three sets of samples of whole blue mackerel (*Scomber australasicus*) were collected from EMA 1, EMA 2, and EMA 7 during 2003, and a small outgroup sample was collected from New South Wales. Three approaches were used to determine stock relationships among the blue mackerel area samples: meristics, genetics, and parasites. The number of gill rakers, and the number of rays in the first dorsal fin, second dorsal fin, anal fin, pectoral fin and pelvic fin, and the number of anal and dorsal finlets were counted in all specimens. Forty blue mackerel from EMA 1 and EMA 2 were X-rayed whole for vertebral counts. Four characters, gill raker, pectoral fin, first dorsal fin, and second dorsal fin ray counts, showed significant area differences. A fragment of the mitochondrial DNA control region was sequenced in 60 specimens and revealed high haplotype diversity. Two genetic lineages with strong bootstrap support (over 90%) were apparent, but there was no geographical structure, with specimens from EMA 1, EMA 2, and NSW appearing in each lineage. Seven species of parasite were found in blue mackerel: two species of monogeneans (*Kuhnia scombri* and *K. scombercolias*) attached to the gill filaments, two unidentified species of digeneans on the gill filaments, and two species of nematode worms (*Anisakis* sp., *Hysterothylacium* sp.) and one species of acanthocephalan (*Rhadinorhynchus* sp.) in the gut. Three parasite species occurred at low prevalence (the digeneans and the nematode *Hysterothylacium* sp.) and thus are not useful markers. *Kuhnia scombri* was prevalent in EMA 1 and EMA 2, but absent from EMA 7. However, most monogenean parasites in fish are short lived (under 1 year), and so the absence of *K. scombri* in blue mackerel from EMA 7 could be due to seasonal variation. The acanthocephalan *Rhadinorhynchus* sp., although short lived, showed significant differences between blue mackerel from EMA 1 and EMA 2, collected at the same time. Larval *Anisakis* sp. showed significant differences among blue mackerel from EMA 1 and EMA 2, and EMA 2 and EMA 7. Based on the area differences found with meristic characters and parasite markers, blue mackerel are subdivided into at least three stocks in EMA 1, EMA 2, and EMA 7.

1. INTRODUCTION

The blue mackerel (*Scomber australasicus*) is found in coastal waters of the western Pacific around Australia and New Zealand, in the northwest Pacific Ocean and East China Sea, in the north and south Indian Ocean through to the Red Sea, and in the northeast Pacific off Hawaii and Mexico. The equatorial region is a likely barrier to gene flow, with unique DNA haplotypes reported among samples from New Zealand-Australia and Japan-Mexico (Scoles et al. 1998). Blue mackerel are widely distributed in the New Zealand EEZ, but are most abundant around the North Island and northern South Island, to about 44° S off the west coast and 45° S off the east coast. Blue mackerel occur as far south as Stewart Island and east to the Chatham Islands, and are generally caught in water less than 250 m deep (Taylor 2002).

Blue mackerel have recently been introduced into the Quota Management System (QMS), with five Quota Management Areas (QMAs): EMA 1, EMA 2, EMA 3 (combining the southern and southeast FMAs 3, 4, 5, and 6), EMA 7 (combining the west coast FMAs 7, 8, and 9), and EMA 10 (Figure 1). The main fishing areas are around the North Island, with the major catches taken in a targetted purse-seine fishery in EMA 1 and as bycatch in a midwater trawl fishery for jack mackerel in EMA 7. The stock relationships among the main EMA fisheries are unknown.

There is no universal method for determining stock relationships in marine fishes, rather management advice is drawn from different and often independent sources. In recent years stock discrimination studies have moved towards an holistic approach (Begg & Waldman 1999), applying multiple techniques to determine stock relationships (Smith, P. et al. 2002). The common element in applying any discrimination technique, is the null hypothesis of no differentiation. Stocks are identified when the null hypothesis is rejected, but the smaller the spatial scale the less likely that there will be separate genetic or ecological stocks. Here we evaluate three tools that measure different biological characters to assess the stock structure of blue mackerel in New Zealand: 1. phenotypic variation, measured with meristic characters which have a genetic basis but expression of the characters is determined by the biotic and physical environment experienced by individuals during the larval and early juvenile stages; 2. acquired markers, measured with long-lived parasites that accumulate during an individual's life; and 3. genetic variation, measured as mitochondrial DNA haplotypes, inherited characters that are passed down generations and not modified by the environment.

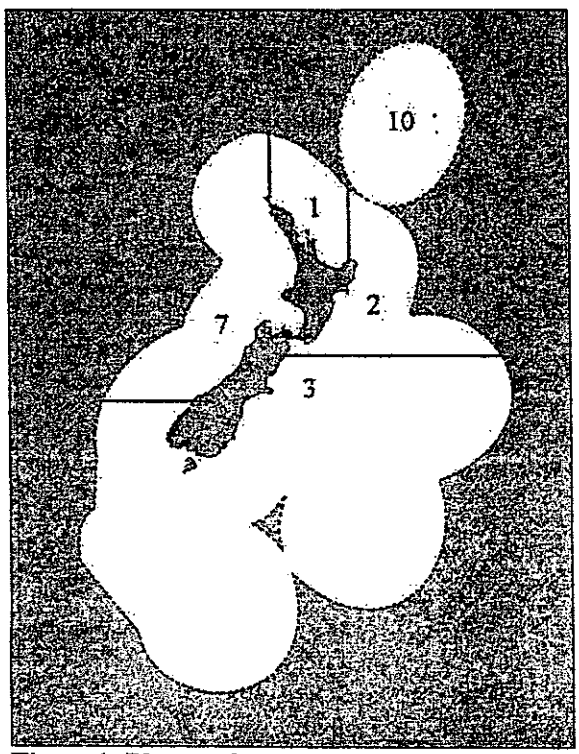

Figure 1: Blue mackerel (EMA) quota management areas.

2. METHODS

2.1 Sample collection

Samples of blue mackerel were collected from three quota management areas: EMA 1, EMA 2, and EMA 7. Two samples, each of about 150 adult whole frozen blue mackerel, were supplied by Sanford Tauranga Ltd. These samples were collected from two widely separated locations at the same time period: North Cape (EMA 1), 31 October 2003, vessel *Tortugas*; and Bare Island, south of Cape Kidnappers (EMA 2), 23 October 2003, vessel *Waihola*. An additional sample of whole frozen blue mackerel, also supplied by Sanford Tauranga Ltd., had been collected in the South Taranaki Bight (EMA 7) during March 2003, aboard *Waihola*. A smaller outgroup sample (n = 20) from New South Wales was supplied by SARDI Aquatic Sciences, South Australia. This geographic outgroup was included to test the sensitivity of the three methods for determining stock relationships between the management areas in the New Zealand EEZ.

2.2 Sample processing

Whole specimens were thawed and fork length, total body weight, sex, gonad stage (visual inspection), and gonad weight recorded. Fin rays were counted in thawed specimens. Otoliths were removed from each specimen for ageing. A small piece of muscle tissue was removed, and stored frozen for genetic analyses. The gut and gills were removed and frozen for parasite analyses. The first left gill arch was stored separately for gill raker counts. A subsample (22 and 20 blue mackerel from EMA 1 and EMA 2, respectively) were X-rayed whole for vertebral counts.

2.3 Meristics

The meristic characters are the serially repeated elements, such as number of vertebrae, fin rays, gill rakers, scales, and pyloric caecae, and were among the first biological markers used to determine stock relationships of marine fishes (Heincke 1898). The meristic characters have a genetic basis (Christiansen et al. 1988,Purdom & Wyatt 1969), but population differences in vertebral numbers and fin rays are modified by environmental factors, such as water temperature, salinity, oxygen, pH, and food availability, experienced during early development, after which the characters are fixed (Lindsey 1988).

Scomber australasicus data taken from the literature show that there is variation in the number of spines in the first dorsal, the number of dorsal and anal finlets, the number of rays in the pectoral fins, and the number of gill rakers on the first arch (Collette & Nauen 1983,Last et al. 1983,May & Maxwell 1980), but no indication is given for numbers or size range of fish analysed.

Fin rays and gill rakers were counted in the east Northland (EMA 1), Wairarapa (EMA 2), South Taranaki Bight (EMA 7), and the New South Wales samples. The number of spines in the first dorsal fin (DI), the number of fin rays in the second dorsal fin (DII), the number of dorsal finlets, the number of fin rays in the anal fin, the number of anal finlets, and the number of fin rays in the pectoral and pelvic fins were counted in the thawed specimens. Paired fins were counted on the left side. The number of gill rakers, on the first left arch, was counted from thawed gill arches under low power magnification. All the gill rakers on the first gill arch were counted without subdividing into upper and lower limbs, as often used for meristic analyses of fishes. Vertebrae were counted from the radiographs.

2.4 Genetics

Total genomic DNA was extracted from 200 to 500 mg of muscle tissue by homogenisation and digestion with proteinase-K, following standard procedures (Taggart et al. 1992). DNA pellets were dried and resuspended in 40 µl sterile water and stored at -20^0C. Several primer pairs were tested to amplify the hypervariable left domain of the control region, from the tRNA to the central conserved region (Table 1); some had been tested previously on *Scomber scombrus* (Nesbo et al. 2000).

Table 1: Control region primers tested in *Scomber australasicus*.

Primer	Primer sequence	Source
L15998	TAC CCC AAA CTC CCA AAG CTA	J. Quintero, pers. com.
CSBDH	TGA ATT AGG AAC CAG ATG CCA G	
L-Pro	TCC CAC CCC TAA CTC CC	Nesbo et al. 2000
12s	CGG TGA CTT GCA TGT GTA AGT TCA	
L-15774	ACA TGA ATT GGA GGA ATA CCA GT	Meyer et al. 1994
H-16498	CCT GAA GTA GGA ACC AGA TG	Meyer et al. 1990

An approximate 600 bp fragment from the $tRNA^{Thr}$ gene to the central part of the control region was amplified with the primer pair L-15774 and H-16498 (Meyer et al. 1990,Meyer et al. 1994). This part of the control region is highly variable in some fishes e.g., eel (Ishikawa et al. 2001). Preliminary screening of genetic markers in 10 specimens of *S. australasicus* from New Zealand had shown a high level of polymorphism in the mtDNA control region (Javier Quinterio, University of Santiago de Compostela, Spain, pers. com. to P.J. Smith, NIWA).

Polymerase chain reactions were performed in 50 µl volumes in a Cetus DNA thermocycler (Perkin-Elmer Corporation, Connecticut). Amplified products were separated in 1.4% agarose gels in a TBE buffer (25 m*M* Tris, 0.5 m*M* EDTA, and 25 m*M* boric acid), stained with ethidium bromide, and viewed under ultraviolet (UV) light. DNA samples were purified using the QIAquick gel extraction kit (Qiagen). Sequences were determined using the ABI Taq DyeDeoxy ™ Terminator Cycle Sequencing Kit according to the manufacturer's directions (Applied Biosystems Inc.) and run on an ABI prism autosequencer.

Sequence data were obtained by sequencing PCR products in both directions on an ABI Prism 377 DNA squencer (PE Applied Biosystems Inc). Sequences were edited in CHROMAS (Technelysium, Queensland, Australia), and aligned in the BIOEDIT programme (Hall 1999). The haplotype diversity (h), the probability that two haplotypes chosen at random from the species are different (Nei 1987), and nucleotide diversity (π), the mean number of nucleotide differences among all haplotypes in a species (Nei 1987), was computed for each area sample. Pairwise F_{ST}'s and exact tests of population differentiation were computed in ARLEQUIN (Schneider et al. 2000). Phylogenies were explored with distance and parsimony methods using PAUP version 4.0 (Swofford 2000). Modeltest version 3.06 (Posada & Crandall 1998) was used to determine the best-fit model using likelihood ratio tests. Support for each internode was evaluated by bootstrap replications (Felsenstein 1985). Conformance of data to a molecular clock was tested with a log-likelihood ratio test (Felsenstein 1981), using the best-fit model with and without the molecular clock restriction, and n-2 degrees of freedom, where n is the number of taxa.

2.5 Parasites

Parasites have been applied as natural tags or markers to disciminate 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 sometimes weak and dependent upon other techniques to confirm stock relationships. There have been several studies of parasites in *S. australasicus* in Australian waters, which simply record the presence of parasite species in samples from one region (Hayward et al. 1998,Perera

1992,Perera 1993). Rohde (1987) showed that the morphology of a mongenean ectoparasite varied between samples of blue mackerel from New South Wales and New Zealand.

The gills and guts of 189 blue mackerel were examined for parasites: 60 blue mackerel from EMA 1, 60 from EMA 2, 49 from EMA 7, and 20 from New South Wales. The parasite study was carried out in two stages. First 70 blue mackerel (25 from EMA 1, 25 from EMA 2, and 20 from New South Wales, were screened for parasites. In the second stage, an additional 119 blue mackerel (35 from EMA 1, 35 from EMA 2, and 49 from EMA 7) were examined for specific parasites identified in stage 1.

Parasites in the gills and guts were located using a dissecting microscope and placed in 10% formalin for identification. The types and numbers of parasites present were recorded for each fish. The ecological terminology used to describe the distribution of parasites amongst fishes followed standard protocols (Bush et al. 1997):

- prevalence = number of infected hosts divided by number of hosts examined;
- intensity = number of parasites found in a sample of infected fish.

The criteria used to determine whether a parasite had potential for use as a stock discriminator followed those established for marine fish (Lester 1990,MacKenzie 1987):

- the parasite should have a lifespan, or remain in identifiable form, in the host long enough to cover the time scale of the investigation,
- the parasite should occur at a reasonably high prevalence, and
- the parasite should be easily detected and identified.

3. RESULTS

3.1 Meristics

Vertebrae were counted in 22 blue mackerel from EMA 1 and 20 from EMA 2. Counts showed little variation in this initial sample (EMA 1: 21 blue mackerel with 32 vertebrae and one with 31; EMA 2: 19 blue mackerel with 32 vertebrae and one with 31), and so no further X-rays were undertaken for vertebral counts.

Merisitic characteristics were measured on 268 mackerel. Several counts were excluded from analyses due to damage to a specific structure. The final counts used for the statistical tests are shown in Table 2. The blue mackerel specimens from New South Wales were in poor condition with damaged fins, and many were excluded from the analyses.

Table 2: Number of gill raker, fin ray, and finlet counts in blue mackerel from three New Zealand areas and New South Wales used in statistical analyses.

Area	Gill raker	Dorsal I	Dorsal II	Dorsal finlets	Anal	Anal finlets	Pectoral	Pelvic
EMA 1	87	95	97	98	97	97	98	98
EMA 2	101	98	101	101	101	101	101	101
EMA 7	15	46	46	46	46	46	45	46
NSW	8	13	13	13	13	13	13	13
Total	211	252	257	258	257	257	257	258

A MANOVA was performed to identify differences between the areas (Table 3). The response variables were numbers of gill rakers, numbers of fin rays in the dorsal I, dorsal II, pectoral, pelvic, and anal fins, and numbers of dorsal and anal finlets. The independent variables were area and sex. Fish length, which acts as a surrogate for age, was included as a covariate.

Table 3: Results of a MANOVA on meristic characters in blue mackerel from four areas; significant values shown in bold.

	Df	Pillai Trace	approx. F	num df	den df	*P*-value
Area	3	0.53	5.14	24.00	573.00	**0.00**
Sex	1	0.03	0.84	8.00	189.00	0.57
Length	1	0.11	2.89	8.00	189.00	**0.00**
Residuals	196					

Univariate ANOVA were tested on individual meristic characters (Table 4). There was a significant difference between areas for four characters: gill rakers, pectoral fin, dorsal I, and dorsal II fin ray counts. For two characters (number of fin rays in the dorsal I and dorsal II fins), area and length had a significant effect (Table 4). These differences are presented in Figures 2–5.

Table 4: Univariate tables from the blue mackerel meristic ANOVA; significant values shown in bold.

Gill raker	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	24.67	8.22	4.86	**0.00**
Sex	1	0.00	0.00	0.00	0.97
Length	1	5.49	5.49	3.25	0.07
Residuals	205	346.97	1.69		

Pectoral fin	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	18.18	6.06	14.46	**0.00**
Sex	1	1.01	1.01	2.40	0.12
Length	1	1.14	1.14	2.71	0.10
Residuals	251	105.21	0.42		

Dorsal I fin	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	26.51	8.84	30.31	**0.00**
Sex	1	0.13	0.13	0.45	0.50
Length	1	1.11	1.11	3.82	**0.05**
Residuals	246	71.72	0.29		

Dorsal II fin	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	5.40	1.80	7.32	**0.00**
Sex	1	0.13	0.13	0.53	0.47
Length	1	1.98	1.98	8.06	**0.00**
Residuals	251	61.72	0.25		

Dorsal finlets	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	0.01	0.00	0.18	0.91
Sex	1	0.00	0.00	0.32	0.57
Length	1	0.01	0.01	1.21	0.27
Residuals	252	2.97	0.01		

Anal fin	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	0.44	0.15	0.65	0.59
Sex	1	0.18	0.18	0.77	0.38
Length	1	0.73	0.73	3.19	0.08
Residuals	251	57.34	0.23		

Anal finlets	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	0.03	0.01	0.47	0.70
Sex	1	0.01	0.01	0.36	0.55
Length	1	0.00	0.00	0.00	0.97
Residuals	251	4.93	0.02		

Pelvic fin	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	3	0.01	0.00	0.51	0.67
Sex	1	0.01	0.01	1.39	0.24
Length	1	0.00	0.00	0.08	0.78
Residuals	252	0.98	0.00		

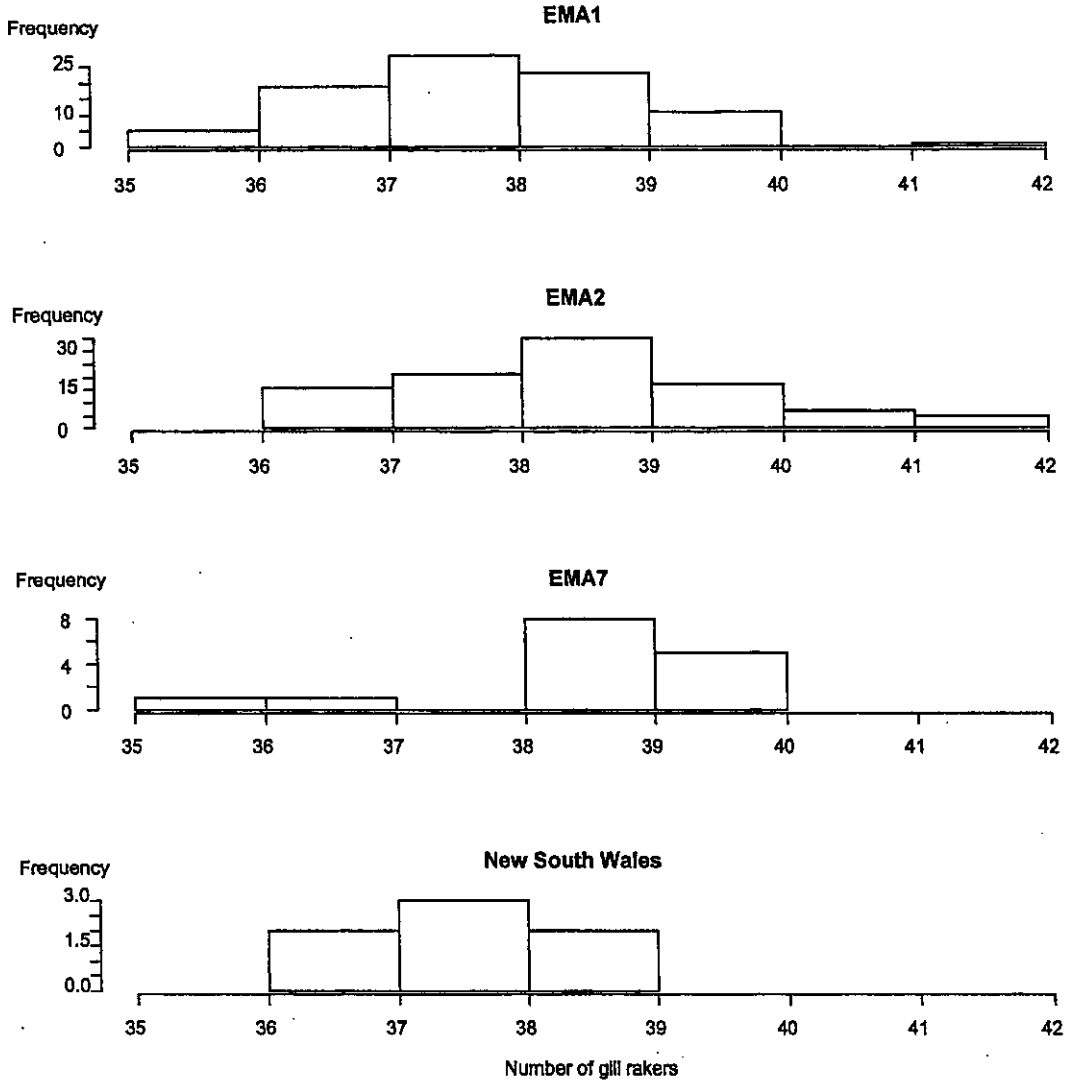

Figure 2: Histograms of gill raker counts in blue mackerel by area.

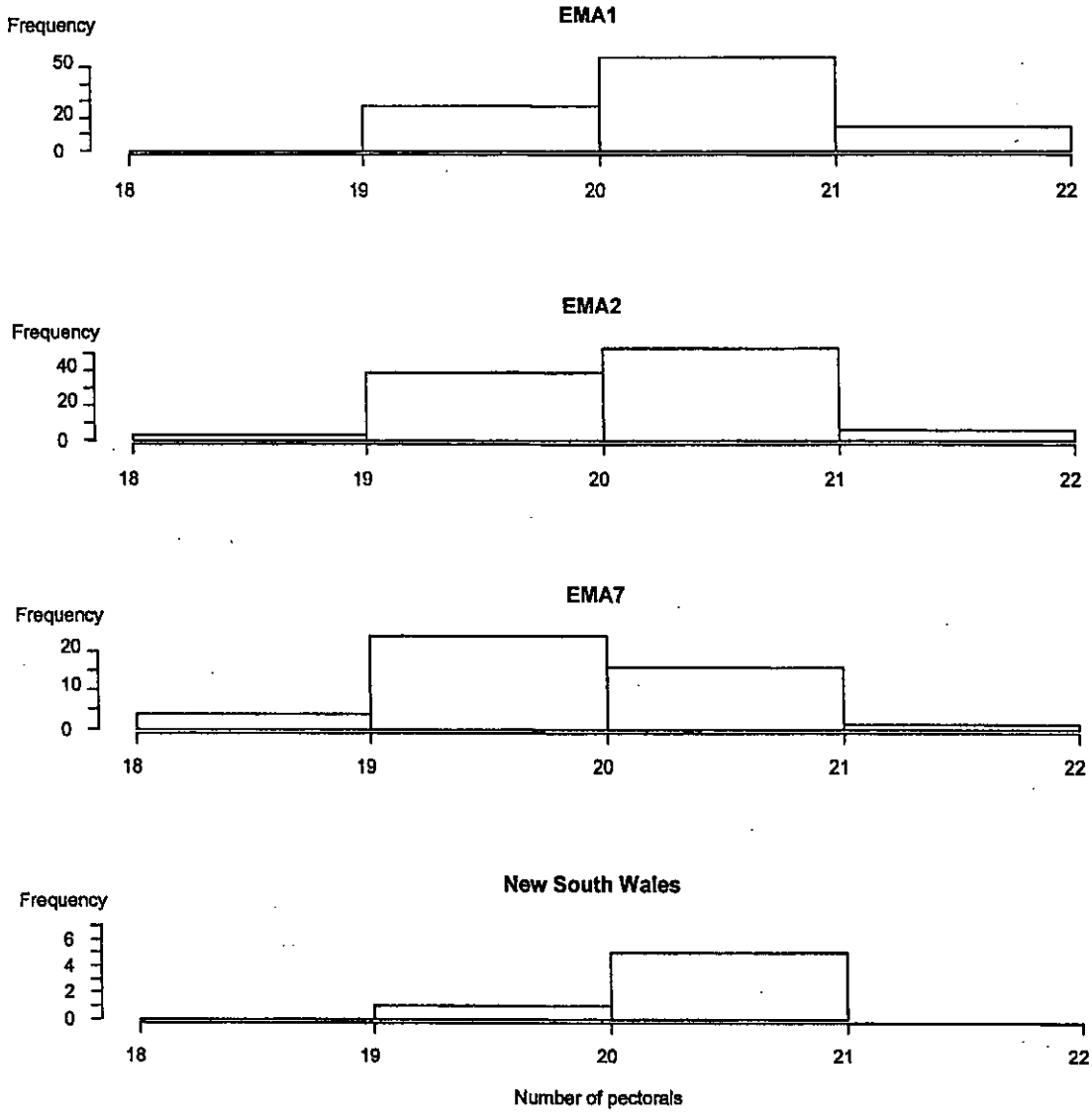

Figure 3: Histograms of pectoral counts in blue mackerel by area.

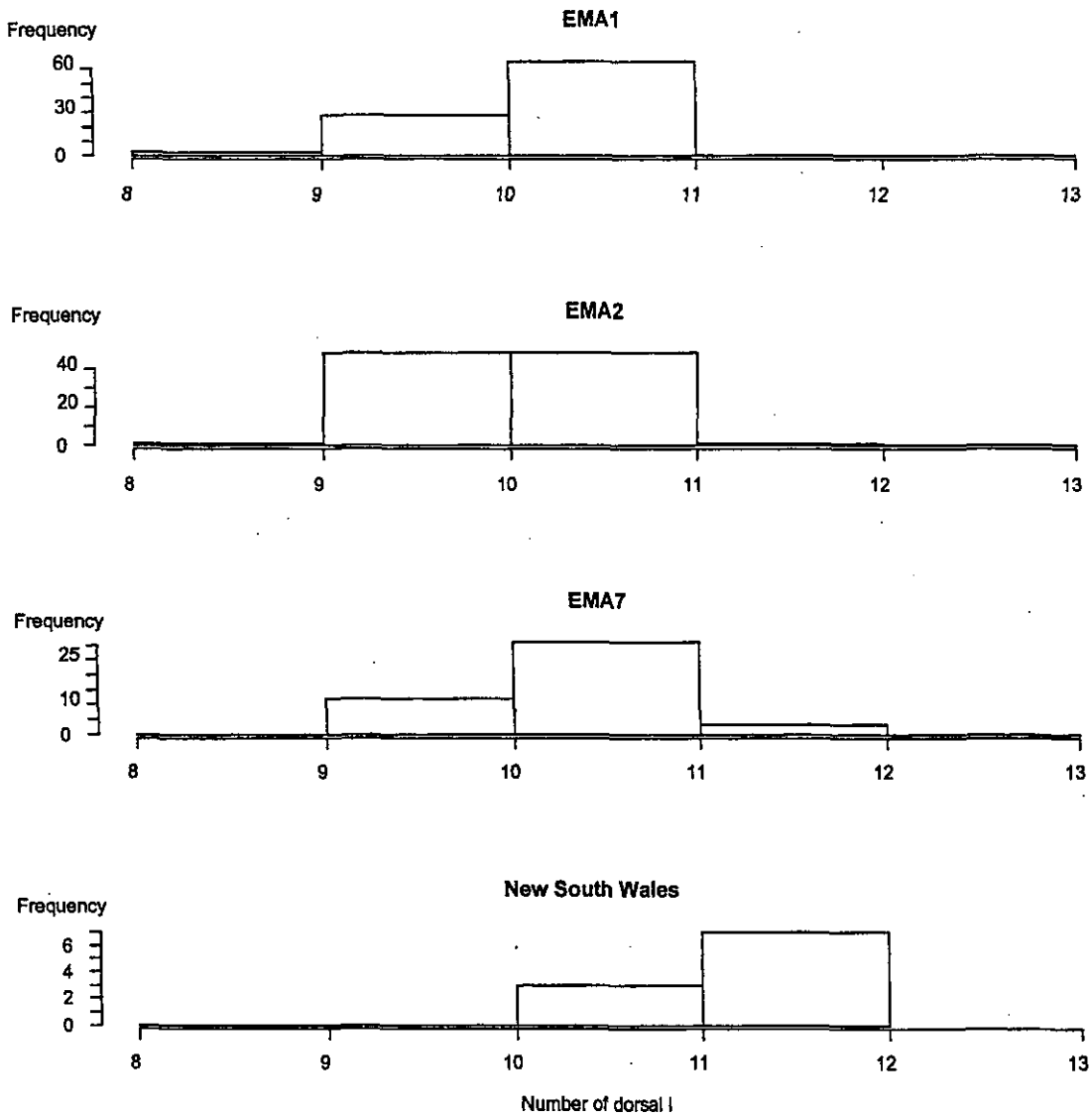

Figure 4: Histograms of dorsal I counts in blue mackerel by area.

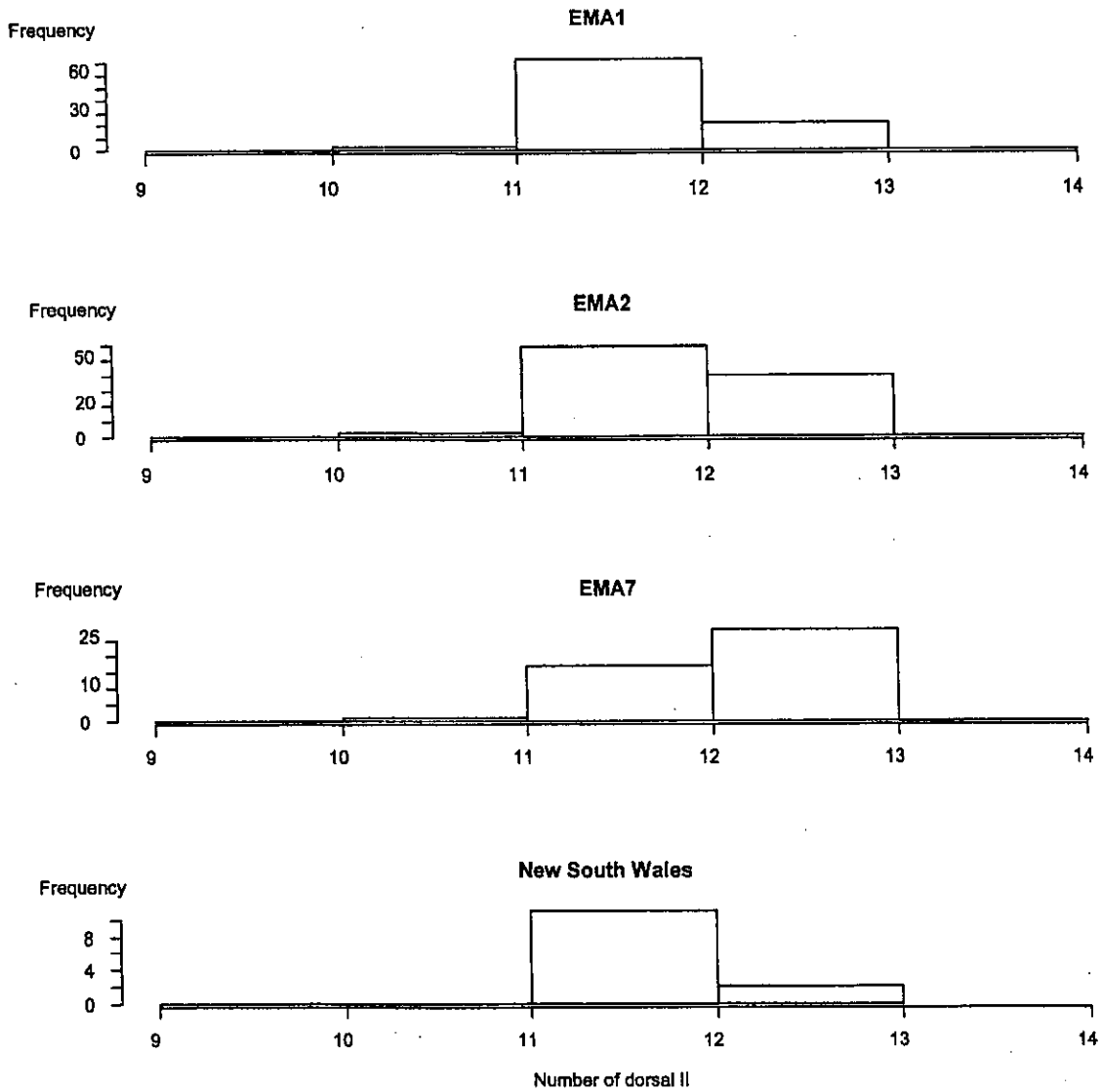

Figure 5: Histograms of dorsal II counts in blue mackerel by area.

MANOVAs and univariate ANOVAs were repeated on samples, firstly excluding EMA 7, with no age data (Table 5). There was a significant difference between areas for four characters: gill rakers, pectoral, dorsal I and dorsal II. In addition, the number of dorsal finlets and anal fin rays were significantly affected by the age of the fish (Table 6).

Table 5: MANOVA table for blue mackerel meristic characters; significant values shown in bold.

	Df	Pillai Trace	approx. F	num df	den df	*P*-value
Area	2	0.47	6.68	16.00	352.00	**0.00**
Sex	1	0.05	1.24	8.00	175.00	0.28
Age	1	0.08	1.83	8.00	175.00	0.08
Residuals	182					

Finally, MANOVAs and univariate ANOVAs were repeated on samples, excluding the sample from New South Wales, using length as a covariate, and then excluding New South Wales and EMA 7, using age as a covariate. There was a significant effect with area in the MANOVAs, and with length but not age (Tables 7 and 8). In the univariate ANOVAs there was a significant difference between areas for four characters: gill rakers, pectoral, dorsal I, and dorsal II fin rays counts (Table 9). In addition, the number of dorsal II and number of anal fin rays were significantly affected by the length of the fish, and the number of anal fin rays and dorsal finlets by age of the fish (Table 10).

Table 6: Univariate tables from the blue mackerel ANOVA (Table 5); *significant values shown in* bold.

Gill raker	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	22.74	11.37	6.64	**0.00**
Sex	1	0.26	0.26	0.15	0.70
Age	1	0.07	0.07	0.04	0.84
Residuals	191	327.20	1.71		

Pectoral	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	9.75	4.87	11.61	**0.00**
Sex	1	0.85	0.85	2.02	0.16
Age	1	0.00	0.00	0.00	0.97
Residuals	207	86.85	0.42		

Dorsal I	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	25.83	12.92	44.59	**0.00**
Sex	1	0.53	0.53	1.83	0.18
Age	1	0.97	0.97	3.33	0.07
Residuals	201	58.23	0.29		

Dorsal II	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	1.83	0.91	3.76	**0.02**
Sex	1	0.15	0.15	0.63	0.43
Age	1	0.43	0.43	1.76	0.19
Residuals	206	50.09	0.24		

Dorsal finlets	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.01	0.00	0.20	0.82
Sex	1	0.00	0.00	0.33	0.57
Age	1	0.08	0.08	5.59	**0.02**
Residuals	207	2.91	0.01		

Anal fin	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.17	0.08	0.35	0.71
Sex	1	0.18	0.18	0.74	0.39
Age	1	1.06	1.06	4.43	**0.04**
Residuals	206	49.19	0.24		

Anal finlet	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.02	0.01	0.59	0.56
Sex	1	0.02	0.02	1.08	0.30
Age	1	0.03	0.03	1.39	0.24
Residuals	206	3.91	0.02		

Pelvic	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.01	0.00	0.55	0.58
Sex	1	0.01	0.01	1.38	0.24
Age	1	0.00	0.00	0.52	0.47
Residuals	207	0.98	0.00		

Table 7: MANOVA table for meristic characteristics.

	Df	Pillai Trace	approx. F	num df	den df	*P*-value
Area	2	0.26	3.40	16.00	366.00	**0.00**
Sex	1	0.03	0.81	8.00	182.00	0.60
Length	1	0.11	2.69	8.00	182.00	**0.01**
Residuals	189					

Table 8: MANOVA table for meristic characteristics.

	Df	Pillai Trace	approx. F	num df	den df	*P*-value
Area	1	0.17	4.40	8.00	168.00	**0.00**
Sex	1	0.05	1.21	8.00	168.00	0.30
Age	1	0.08	1.83	8.00	168.00	0.07
Residuals	175					

Table 9: Univariate tables from the ANOVA.

Response: Gill raker

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	22.89	11.44	6.72	**0.00**
Sex	1	0.02	0.02	0.01	0.91
Length	1	4.51	4.51	2.65	0.11
Residuals	198	337.06	1.70		

Response: Pectoral

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	10.33	5.16	12.40	**0.00**
Sex	1	1.25	1.25	3.00	0.08
Length	1	1.38	1.38	3.31	0.07
Residuals	239	99.49	0.42		

Response: Dorsal 1

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	3.15	1.57	5.60	**0.00**
Sex	1	0.14	0.14	0.49	0.48
Length	1	1.00	1.00	3.57	0.06
Residuals	234	65.82	0.28		

Response: Dorsal 2

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	4.95	2.47	9.84	**0.00**
Sex	1	0.22	0.22	0.88	0.35
Length	1	1.84	1.84	7.33	**0.01**
Residuals	239	60.07	0.25		

Response: Dorsal finlets

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.01	0.00	0.25	0.78
Sex	1	0.00	0.00	0.32	0.57
Length	1	0.01	0.01	1.19	0.28
Residuals	240	2.97	0.01		

Response: Anal

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.32	0.16	0.69	0.50
Sex	1	0.18	0.18	0.78	0.38
Length	1	0.71	0.71	3.11	0.08
Residuals	239	54.29	0.23		

Response: Anal finlet

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.03	0.01	0.63	0.53
Sex	1	0.01	0.01	0.36	0.55
Length	1	0.00	0.00	0.00	0.97
Residuals	239	4.93	0.02		

Response: Pelvic

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	2	0.01	0.00	0.71	0.49
Sex	1	0.01	0.01	1.39	0.24
Length	1	0.00	0.00	0.07	0.78
Residuals	240	0.98	0.00		

Table 10: Univariate tables from the ANOVA.

Response: Gill raker

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	21.15	21.15	12.30	**0.00**
Sex	1	0.10	0.10	0.06	0.81
Age	1	0.02	0.02	0.01	0.91
Residuals	184	316.53	1.72		

Response: Pectoral

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	3.46	3.46	8.29	**0.00**
Sex	1	1.10	1.10	2.63	0.11
Age	1	0.00	0.00	0.00	0.99
Residuals	195	81.37	0.42		

Response: Dorsal 1

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	1.28	1.28	4.66	0.03
Sex	1	0.56	0.56	2.04	0.15
Age	1	1.07	1.07	3.88	0.05
Residuals	189	52.09	0.28		

Response: Dorsal 2

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	1.61	1.61	6.47	**0.01**
Sex	1	0.27	0.27	1.07	0.30
Length	1	0.42	0.42	1.69	0.19
Residuals	194	48.30	0.25		

Response: Dorsal finlets

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	0.01	0.01	0.35	0.56
Sex	1	0.00	0.00	0.33	0.57
Length	1	0.08	0.08	5.34	**0.02**
Residuals	195	2.91	0.01		

Response: Anal

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	0.07	0.07	0.31	0.58
Sex	1	0.18	0.18	0.75	0.39
Length	1	1.07	1.07	4.49	**0.04**
Residuals	194	46.10	0.24		

Response: Anal finlet

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	0.02	0.02	1.04	0.31
Sex	1	0.02	0.02	1.08	0.30
Length	1	0.03	0.03	1.34	0.25
Residuals	194	3.91	0.02		

Response: Pelvic

	Df	Sum of sq	Mean sq	F Value	*P*-value
Area	1	0.00	0.00	0.97	0.33
Sex	1	0.01	0.01	1.38	0.24
Length	1	0.00	0.00	0.50	0.48
Residuals	195	0.98	0.01		

3.2 Genetics

Unambiguously aligned sequences were obtained for 654 bp of control region sequences from 60 specimens. Forty-nine bases were variable and 27 were parsimony informative in the total data set. The base composition was asymmetric and on average A, 32.2%; T, 28.7%; C, 22.8%; and G, 16.3%; variable sites gave a transition to transversion ratio of 1:2.8. Haplotype diversities were high in all samples (h = 1.00 EMA 1, 0.94 EMA 2, and 0.94 NSW). Nucleotide diversities were typical for marine fishes (π = 1.71 ± 0.35% EMA 1, 1.24 ± 0.28% EMA 2, and 1.61 ± 0.34% NSW).

Neighbour-joining trees were constructed based on the HKY +I + G model with unequal base frequencies (Hasegawa, M. et al. 1985). Two lineages with strong bootstrap support (>90%) were apparent in the total data set (Figure 6). There was no geographical basis to these two lineages, with specimens from EMA 1, EMA 2, and NSW appearing in each lineage. Exact tests of population differentiation were non-significant (global test P = 0.14; pairwise tests: EMA1/EMA2 P = 0.12; EMA1/NSW P = 0.51; EMA2/NSW P = 0.36). Given the lack of significant regional differentiation in these preliminary analyses, no further regional samples were tested.

The molecular clock hypothesis was rejected with likelihood-ratio tests, indicating a mutational rate heterogeneity among clades (χ^2 = 81.4, P<0.001, d.f. = 43), and consequently the nucleotide sequence divergences could not be used to estimate the time of phylogenetic events. However, the shallow sequence divergences would indicate a recent evolutionary divergence that corresponds to the late Pleistocene. One scenario is that populations of *S. australasicus* were forced north during the glacial periods and diverged genetically. In subsequent interglacial periods these populations have moved south and are able to interbreed. Alternatively, the control region of *S. australasicus* may have evolved at a more rapid rate than the average rate for teleosts. The northern and southern hemisphere groups of *S. australasicus* were estimated to have diverged 0.48–2.4 million years ago during the mid to early Pleistocene (Burridge 2002).

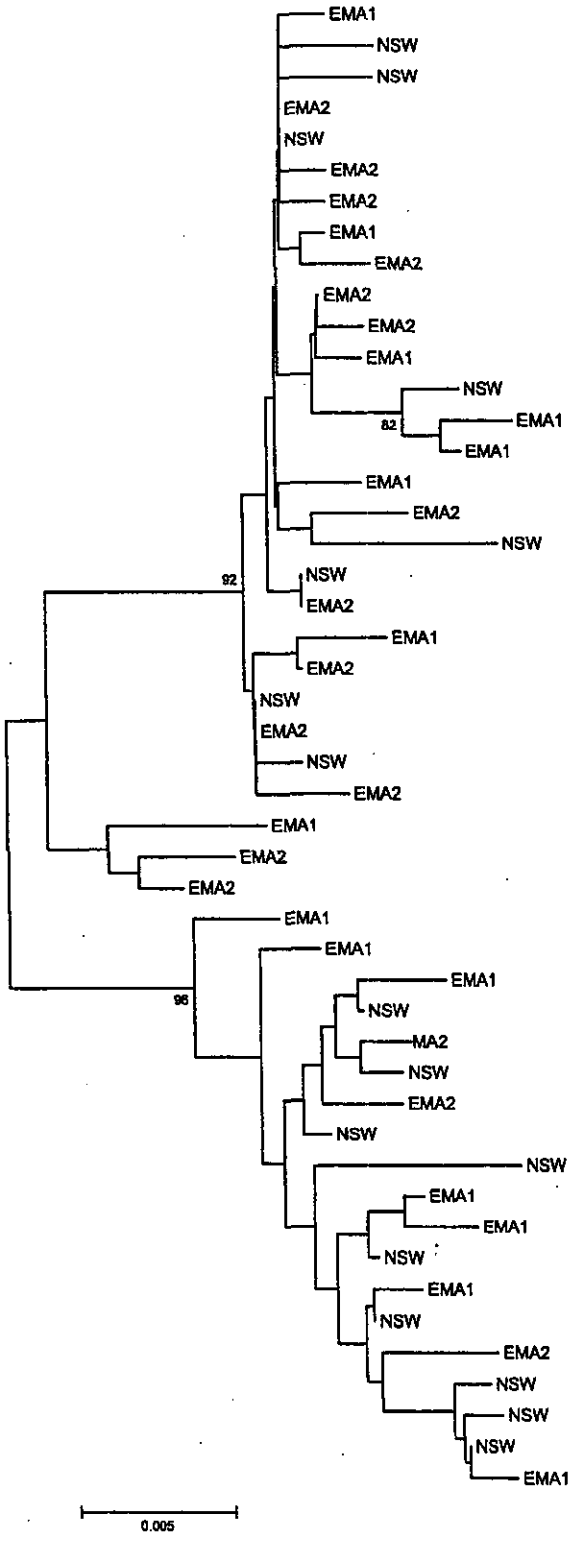

Figure 6: Unrooted neighbour-joining tree for the 667 bp blue mackerel mtDNA fragment including the control region, based on TVM distances. Scale bar represents an interval of nucleotide distance. Numbers at nodes are bootstrap percentages, >75% (1000 replicates). Samples from EMA 1, EMA 2, and NSW.

3.3 Parasites

Seven species of parasite were found in blue mackerel during the stage 1 screening. There were two species of monogeneans (*Kuhnia scombri* and *K. scombercolias*) attached to the gill filaments, two species of digeneans (pedunculate type 1 and didymozoid type 2) also on the gill filaments, and two species of nematode worms (*Anisakis* sp., *Hysterothylacium* sp.) and one species of acanthocephalan (*Rhadinorhynchus* ? sp.) in the gut. The prevalence and mean intensity of parasites found at each site are listed in Tables 11 and 12

Between-area differences in parasite abundance were tested with randomisation tests; firstly employing only positive counts, and secondly employing all observations, including zero counts. A total of 500 bootstrapped datasets generated for each species of parasite. Two-sided *P*-values were calculated by comparing the observed differences in mean abundance to the bootstrap distributions (Tables 13 and 14). To control for the length of the blue mackerel, linear regressions of parasite numbers on length were performed, and the residuals from these regressions used in the randomisation tests (Tables 15 and 16). To control for the age of the blue mackerel, linear regressions of parasite numbers on age were performed, and the residuals from the regressions used in the randomisation tests (Tables 17 and 18).

The two monogeneans on the gills were identified as *Kuhnia scombri*, which occurred only on blue mackerel from EMA 1 and EMA 2, and *K. scombercolias*, which was found only on two blue mackerel from New South Wales (Table 10). The monogeneans were differentiated based on the morphology of their opisthaptor and the size of their hamuli (Figures 7–9) as described by Rohde & Watson (1985) and Rohde (1989). There were significant differences between EMA 2 and EMA 1, and between EMA 2 and EMA 7 (see Table 14), driven by a few heavily infected blue mackerel in EMA 2; host length had little effect (see Tables 14 and 16).

The blue mackerel from EMA 7 were sampled at a different time of year (March) to the two other New Zealand samples (October). The longevity of monogenean parasites in fish is less than 1 year (Trouve et al. 1998,Yamaguti 1963), and so the absence of *K. scombri* in blue mackerel from EMA 7 could be due to seasonal variation in parasite numbers rather than an area effect. Significant differences in monthly abundances of *K. scombri* were reported among samples of blue mackerel collected in Eden, Australia (Perera 1993). The first type of digenean (pedunculate type 1), found at low prevalence (3/60 fish) only in blue mackerel from EMA 1, had a distinctive pedunculate sucker (Figure 10), but could not be identified due to poor fixation. This digenean would have been prone to falling off the gills during the freezing, thawing, and dissection of the samples. The significant between-area differences (see Tables 14, 16, and 18) are statistical artefacts based on a small number of observations in one area, EMA 1 (see Tables 11 and 12).

The second type of digenean occurred in pairs in yellow capsules (Figure 11), and was a member of the Family Didymozoidae (probably subfamily Nematobothriinae). This digenean could not be identified to species level due to inadequate fixation in the frozen and thawed material. The type 2 didymozoid occurred in a few blue mackerel from all areas (see Table 11). The significant between area differences (see Tables 13 and 14) are statistical artefacts based on a small number of observations with one to three blue mackerel infected per area. The didymozoid parasites are generally short lived, less than one year (Jones 1991), and, coupled with the low prevalence in all areas, this suggests that they are not useful markers of blue mackerel stock relationships.

One species of adult nematode, identified as *Hysterothylacium* sp. (based on the presence of an intestinal caecum, ventricular appendix, and excretory pore posterior to the nerve ring), was observed inside the stomach of one blue mackerel from EMA 1, but not in blue mackerel from EMA 2, during the first stage. Subsequently *Hysterothylacium* sp. was detected in all three areas at low intensities (see Table 14), but was absent from the smaller sample from New South Wales. The significant between area differences (see Table 9) are statistical artefacts based on a few observations, and are not apparent in the randomisation tests incorporating all observations (Table 14). *Hysterothylacium* sp. may not be a useful marker for three independent reasons. Firstly, *Hysterothylacium* sp. has a relatively short life span, probably less than 1 year in the definitive host (Iglesias et al. 2002).

Secondly, *Hysterothylacium* sp. has a low prevalence in the New Zealand areas (see Table 11). Thirdly, *Hysterothylacium* sp. was found free on the gills, but is usually found encysted in the mesenteries. Finding *Hysterothylacium* sp. on the gills of blue mackerel was unexpected, and may result from a sampling artefact if some of the stomach fluids were regurgitated by the blue mackerel during capture. Overall, the prevalence of *Hysterothylacium* sp. could not be considered as a biological marker of blue mackerel population structure.

Larval nematodes of the genus *Anisakis*, conforming morphologically to *A. simplex* (Figure 12) were found in the mesenteries of the intestine of blue mackerel from all areas, and at high prevalences in the New Zealand samples from EMA 1, EMA 2, and EMA 7 (see Table 11). There were significant differences between EMA 1 and EMA 2, and between EMA 2 and EMA 7. Larval *Anisakis* sp. encysted in the mesenteries are useful biological tags as they are long lived, and accumulate over the life of the fish (Lester et al. 1988,Sewell & Lester 1995). The presence of apparently dead and degenerating *Anisakis* larvae in some of the blue mackerel (i.e., the worms were dead before capture of the fish) suggests that this species is useful for stock discrimination even after its death, because the parasite remains identifiable. There were fewer *Anisakis* sp. in blue mackerel from New South Wales (see Tables 11 and 12), but length was a contributing factor (see Tables 14 and 16).

Four types of morphologically distinct *Anisakis* larvae have been found in marine fishes (Smith, J. & Wootten 1978). All the *Anisakis* larvae found in the blue mackerel had an oblique ventriculo-intestinal junction and a rounded mucron-bearing tail and conformed to the morphology of *Anisakis* type 1 larvae. However, molecular research indicates the presence of morphologically indistinguishable sibling species of *Anisakis* (Mattiucci et al. 1997). The possibility of cryptic species of *Anisakis* occurring in blue mackerel cannot be discounted without detailed genetic studies of these worms, but the presence of cryptic *Anisakis* species may enhance the usefulness of this marker.

An acanthocephalan, probably a member of the Order Echinorhynchida, and possibly genus *Rhadinorhynchus* based on proboscis hook morphology (Figure 13) and trunk spines (Yamaguti 1963), was found in the intestine of fish from all areas. The acanthocephalan was prevalent in blue mackerel from EMA 1 and EMA 7, but uncommon in blue mackerel from EMA 2 (see Table 11), with significant differences in abundance between areas (see Table 10). Abundance was not influenced by host length (see Tables 14 and 16) and host age (see Tables 14 and 18).

Acanthocephalans have been used as markers to show limited movements between host fish populations on scales as fine as less than 1 km in coral reef environments (Cribb et al. 2000). However, adult acanthocephalans are not long-lived parasites compared to larval stages of nematodes and cestodes, and their life span in the definitive host is usually less than 1 year (Brattey 1988). Hence the utility of the acanthocephalan as a parasite marker may be limited to examination of dynamics of short-term (within-season) migrations, rather than long-term differences between fish populations. The significant differences between blue mackerel from EMA 1 and EMA 2, collected at the same time period, indicate a lack of short-term movement between these areas.

Table 11: Prevalence of parasites (%) found in 189 blue mackerel from four areas.

	EMA 1	EMA 2	EMA 7	NSW
Number of fish examined	60	60	49	20
Monogenea				
Kuhnia scombri	40	43.3	0	0
Kuhnia scombercolias	0	0	0	10
Digenea				
Pedunculate type 1	5	0	0	0
Didymozoid type 2	1.7	3.3	6.1	10
Nematoda				
Anisakis sp.	58.3	81.7	73.5	5
Anisakis sp. (dead)	3.3	16.7	0	0
Hysterothylacium sp.	6.7	3.3	4.1	0
Acanthocephala				
Rhadinorhynchus ? sp.	50	5	34.7	25

Table 12: Mean intensity of parasites found in blue mackerel from four areas.

	EMA 1	EMA 2	EMA 7	NSW
Monogenea				
Kuhnia scombri	2.4	5.4	-	-
Kuhnia scombercolias	-	-	-	1
Digenea				
Pedunculate type 1	1	-	-	-
Didymozoid type 2	2	1	1	2
Nematoda				
Anisakis sp.	9.1	12.4	6.6	4
Anisakis sp. (dead)	6	8.9	-	-
Hysterothylacium sp.	1	1.5	1	-
Acanthocephala				
Rhadinorhynchus ? sp.	4.4	1.33	4.8	1.2

Table 13: *P*-values from the randomisation tests on blue mackerel parasites. Values less than 0.05 (in bold) identify a statistically significant difference between the areas. These results are based on tests of positive (i.e., non- zero) results only. NA, not applicable

Areas	*Kuhnia scombri*	Pedunculate digenean	Didymozoid2	*Anisakis*	*Hysterothylacium*	Dead *Anisakis*	Acanthocephala n
EMA 1-NSW	NA	NA	0.83	0.67	NA	NA	0.10
EMA 2-NSW	NA	NA	**<0.01**	0.32	NA	NA	0.96
EMA 7-NSW	NA	NA	**<0.01**	0.83	NA	NA	0.09
EMA 2-EMA 1	**0.04**	NA	**<0.01**	0.26	**<0.01**	0.57	0.18
EMA 7-EMA 1	NA	NA	**<0.01**	0.44	0.74	NA	0.73
EMA 7-EMA 2	NA	NA	0.98	0.05	**<0.01**	NA	0.15

Table 14: *P*-values from the randomisation tests on blue mackerel parasites. Values less than 0.05 (in bold) identify a statistically significant difference between the areas. These results include zero observations. NA = not applicable.

Areas	*Kuhnia scombri*	Pedunculate digenean	Didymozoid2	*Anisakis*	*Hysterothylacium*	Dead *Anisakis*	Acanthocephalan
EMA 1-NSW	NA	**0.03**	**0.02**	0.09	0.25	0.79	**0.01**
EMA 2-NSW	NA	0.8	**0.03**	**<0.01**	0.33	0.09	0.78
EMA 7-NSW	NA	0.78	0.08	0.12	0.48	0.99	0.07
EMA 2-EMA 1	**0.01**	**<0.01**	0.88	**0.01**	0.78	0.008	**<0.01**
EMA 7-EMA 1	0.11	**<0.01**	0.63	0.86	0.57	0.77	0.37
EMA 7-EMA 2	**<0.01**	0.93	0.62	0.02	0.83	0.006	**<0.01**

Table 15: *P*-values from the randomisation tests on blue mackerel parasites controlling for host length. Values less than 0.05 (in bold) identify a statistically significant difference between the areas. These results are based on tests of positive (i.e., non- zero) results only.

Area	*Kuhnia scombri*	Pedunculate digenean.	Didymozoid2	*Anisakis*	*Hysterothylacium*	Dead *Anisakis*	Acanthocephala n
EMA 1-NSW	NA	NA	0.14	0.20	NA	NA	0.19
EMA 2-NSW	NA	NA	0.60	0.07	NA	NA	0.82
EMA 7-NSW	NA	NA	0.53	0.05	NA	NA	0.22
EMA 2-EMA 1	0.56	NA	**0.044**	0.16	0.05	0.92	0.19
EMA 7-EMA 1	NA	NA	**0.026**	**0.02**	0.92	NA	0.94
EMA 7-EMA 2	NA	NA	0.96	0.47	0.26	NA	0.23

Table 16: *P*-values from the randomisation tests blue mackerel parasites, controlling for host length. Values less than 0.05 (in bold) identify a statistically significant difference between the areas. These results include zero observations.

Area	*Kuhnia scombri*	Pedunculate digenean.	Didymozoid2	*Anisakis*	*Hysterothylacium*	Dead *Anisakis*	Acanthocephala n
EMA 1-NSW	NA	**0.03**	0.13	0.21	0.68	0.25	**0.002**
EMA 2-NSW	NA	0.48	0.26	0.14	0.85	0.96	0.18
EMA 7-NSW	NA	0.62	0.38	**0.006**	0.89	0.13	0.004
EMA 2-EMA 1	0.12	0.07	0.60	0.84	0.33	0.22	**<0.001**
EMA 7-EMA 1	**0.03**	0.05	0.42	0.09	0.39	0.43	0.64
EMA 7-EMA 2	**<0.001**	0.83	0.79	0.12	0.99	**0.03**	**0.004**

Table 17: *P*-values from the randomisation tests blue mackerel parasites controlling for host age. Values less than 0.05 (in bold) identify a statistically significant difference between the areas. The results are based on tests of positive (i.e., non- zero) results only.

Area	*Kuhnia scombri*	Pedunculate digenean.	Didymozoid2	*Anisakis*	*Hysterothylacium*	Dead *Anisakis*	Acanthocephala n
EMA 1-NSW	NA	NA	0.22	0.87	NA	NA	**0.02**
EMA 2-NSW	NA	NA	0.46	0.93	NA	NA	0.32
EMA 2-EMA 1	0.18	NA	**<0.001**	0.76	0.08	0.68	0.33

Table 18: *P*-values from the randomisation tests on blue mackerel parasites controlling for host age. Values less than 0.05 (in bold) identify a statistically significant difference between the areas. The results include zero observations.

Area	*Kuhnia scombri*	Pedunculate digenean.	Didymozoid2	*Anisakis*	*Hysterothylacium*	Dead *Anisakis*	Acanthocephala n
EMA 1-NSW	NA	0.21	0.06	0.53	0.37	0.84	**0.006**
EMA 2-NSW	NA	0.94	0.08	0.51	0.74	0.56	0.07
EMA 2-EMA 1	0.10	**<0.001**	0.80	0.99	0.51	0.43	**0.002**

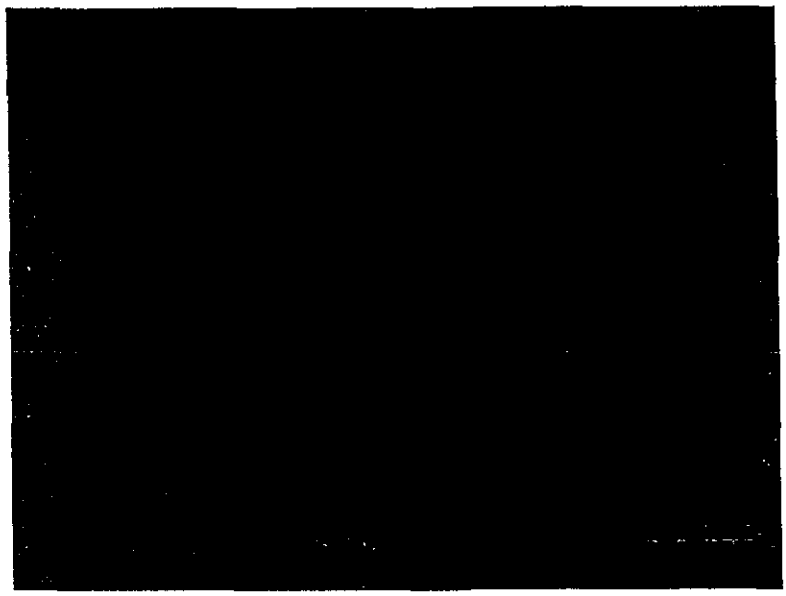

Figure 7: Opisthaptor of *Kuhnia scombercolias* from gills of blue mackerel showing relatively small hamuli (arrows). Scale bar = 40 µm.

Figure 8: Opisthaptor of *Kuhnia scombri* from gills of blue mackerel showing relatively large hamuli (arrows). Scale bar = 100 µm.

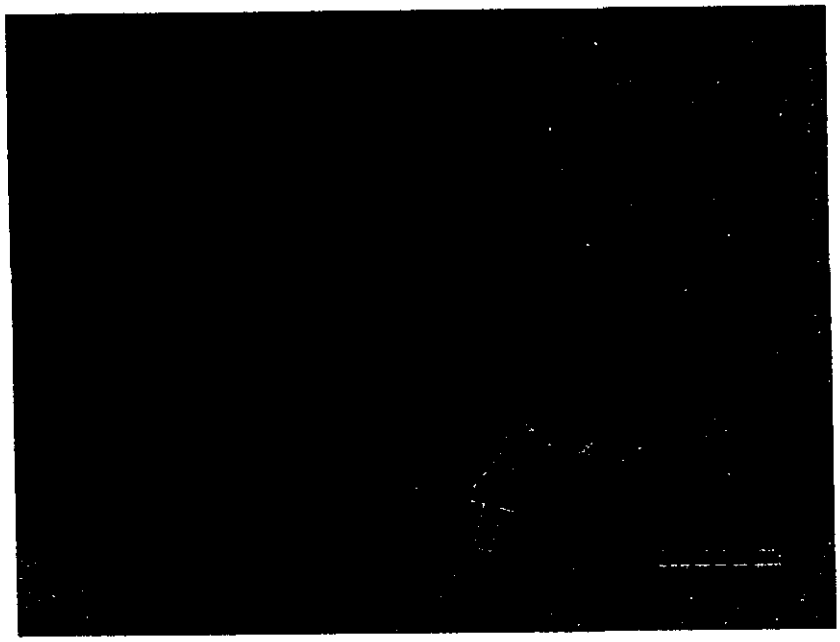

Figure 9: High power view of hamulus (arrow) of *Kuhnia scombri* from gills of blue mackerel. Scale bar = 25 µm.

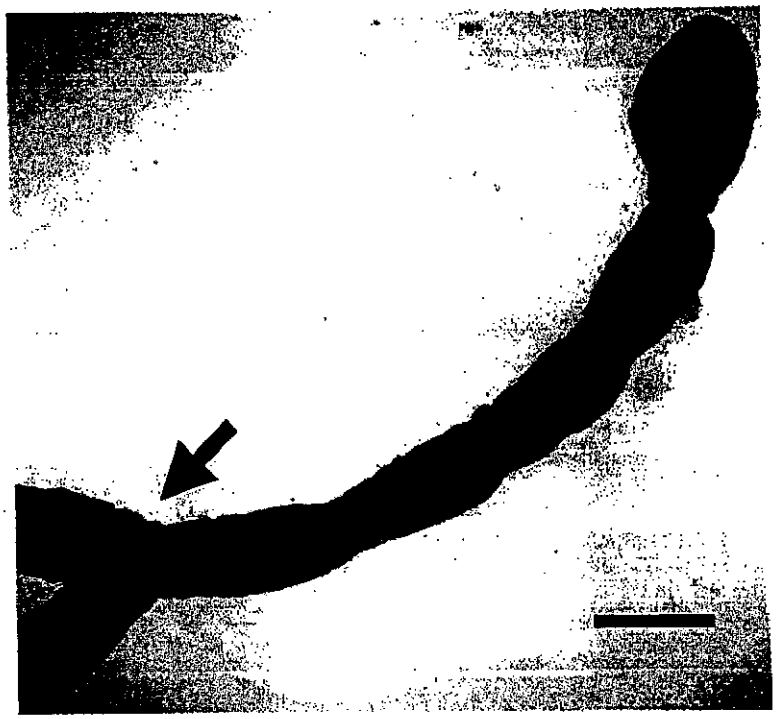

Figure 10: Oral sucker of type 1 digenean with pedunculate ventral sucker (arrow) from gills of blue mackerel. Scale bar = 100 µm.

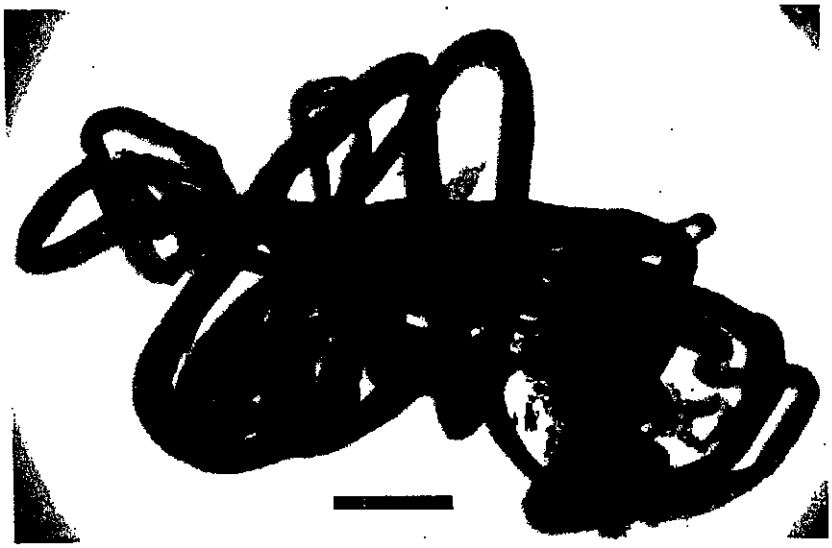

Figure 11: Pair of type 2 didymozoid digenean worms from gills of blue mackerel. Scale bar = 100 µm.

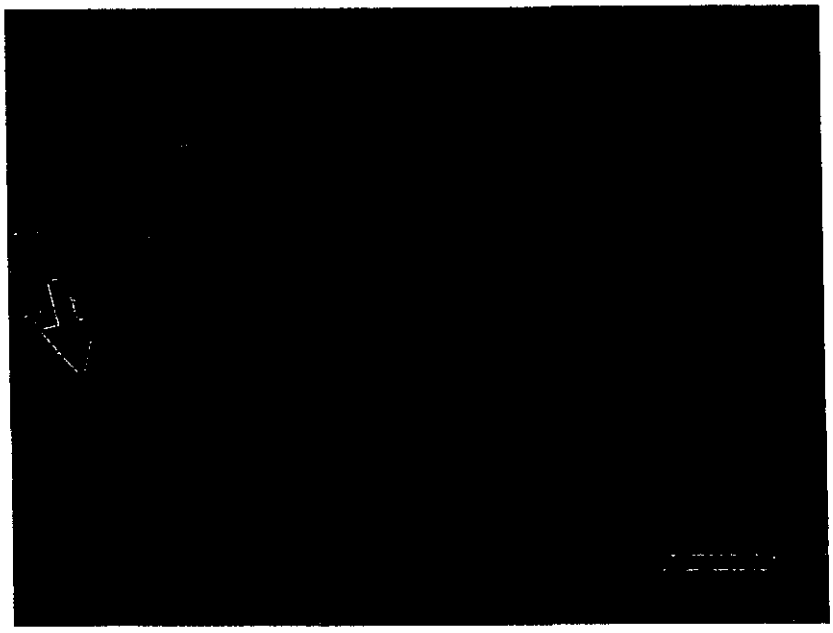

Figure 12: Dorsal lips of *Anisakis* sp. larvae from mesenteries of blue mackerel, showing conspicuous boring tooth (arrow). Scale bar = 50 µm.

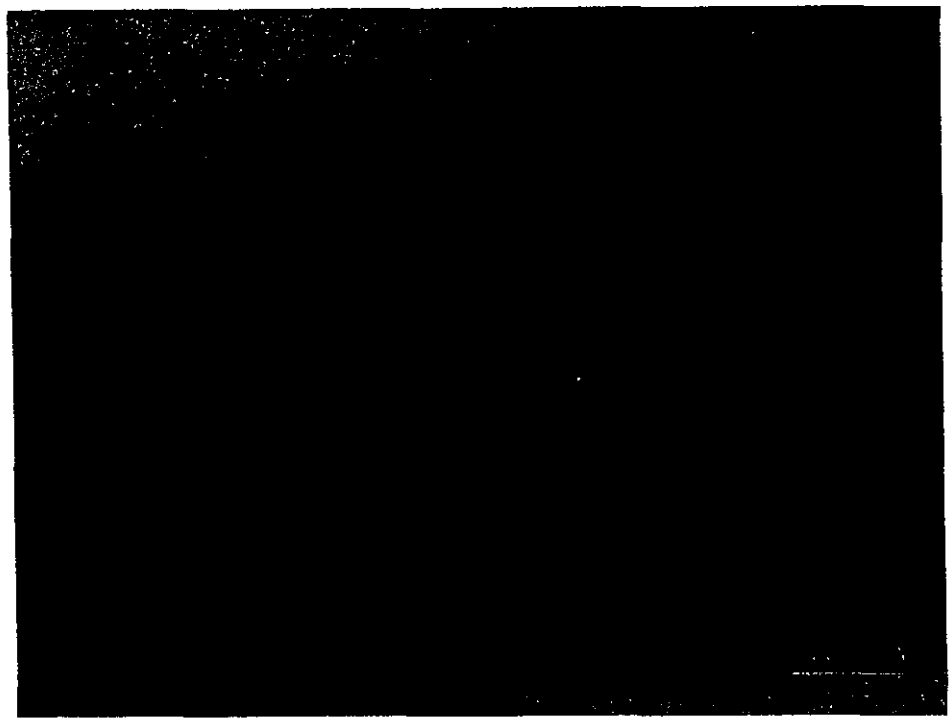

Figure 13: Proboscis hooks of the acanthocephalan from the intestine of blue mackerel. Scale bar = 10 µm

3.3.1 Hamuli (sclerites) in *Kuhina scombri*

The length of the hamuli (see Figures 7 and 8) of *Kuhnia scombri* have been shown to differ between samples of blue mackerel from Australia and New Zealand (Rohde 1987). No *K. scombri* were found in the New South Wales blue mackerel (see Table 7) to test the observations of Rohde (1987). Rohde also reported that the size of hamuli of *K. scombri* did not increase with length of the worm, and found no relationship between hamuli length in *K. scombri* and host size (Rohde 1991,Rohde & Watson 1985).

There was no significant effect of area on the length of the hamuli in *K. scombri* in the current data set. This was determined by the use of a linear model, with hamuli length as the response variable and area and body length as the two predictor variables. Results are given in Table 19.

Table 19: Regression of hamuli length in blue mackerel:

	Value	Std. Error	t value	*P*-value
(Intercept)	84.5076	14.5693	5.8004	0
Area	-1.2517	1.4857	-0.8425	0.4024
Body length	0.0038	0.0035	1.0864	0.281

Residual standard error: 12.67 on 70 degrees of freedom
Multiple R-Squared: 0.02748
F-statistic: 0.9888 on 2 and 70 degrees of freedom, the p-value is 0.3772

4. DISCUSSION

4.1 Hydrology and blue mackerel spawning

The number and distribution of geographically discrete larval-juvenile retention areas can determine the number of stock units in marine fishes, with "stock-rich" species, such as Atlantic herring *Clupea harengus*, characterised by numerous larval retention areas (Sinclair & Iles 1988). The major oceanic feature off the east coast of the North Island is the southward flowing East Auckland Current (EAC), which brings subtropical water down the east coast of the North Island (Figure 14). The EAC diverges near East Cape, with water flowing north and east in a large scale eddy, and the remainder flowing south as the East Cape Current, to the Chatham Rise where it is deflected eastwards, creating a second permanent and large scale eddy off the east coast of the North Island. The Wairarapa Coastal Current (WCC) transports cooler water north along the southeast coast of the North Island (Figure 14). 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 influences Ninety Mile Beach off the northwest coast of the North Island (Roberts & Paul 1978).

Two spawning centres have been reported for blue mackerel: in the Hauraki Gulf, based on egg and larval surveys (Crossland 1981), and in Tasman Bay and South Taranaki, based on the presence of "ripe and running ripe" and "spent" blue mackerel (Hurst et al. 2000). Larval production in the northeast and west coast spawning areas is hydrologically isolated (Figure 14) and may form the basis of separate stocks of blue mackerel. Genetically differentiated stocks of snapper (*Pagrus auratus*) are recognised off the northeast and west coast, contained within the East Auckland Current and Tasman Current respectively (Bernal-Ramirez et al. 2003,Smith, P. et al. 1978). The D'Urville current (Figure 14) also appears to act as a barrier to gene flow isolating snapper in Tasman Bay (Bernal-Ramirez et al. 2003).

Given the potential hydrological barriers and two known spawning populations of blue mackerel, there are three potential stock models for this species in northern New Zealand.

- Two stocks with larvae/juveniles contained in the separate water masses off the northeast coast (EAC, ECE, Figure 14) and west coast (WEC, DUC, TAC, Figure 14), and little adult movement between regions (an island recruitment model).
- Multiple spawning stocks with limited juvenile and adult dispersal (a stepping stone recruitment model).
- A single biological stock with multiple spawning areas and extensive larval/juvenile dispersal from the spawning areas, and extensive adult movement to and from the spawning areas (unit stock model).

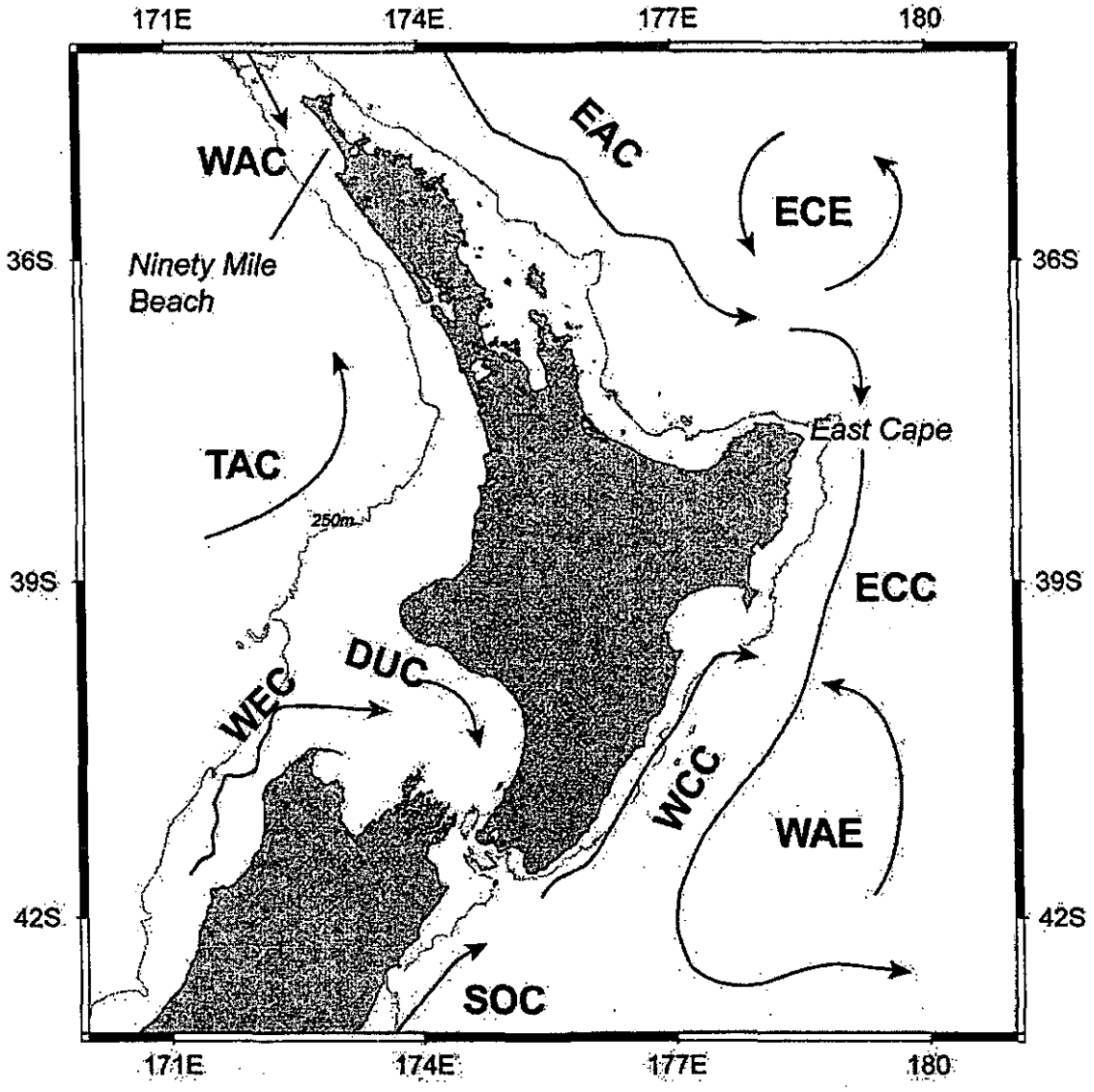

Figure 14: Major current systems around the North Island and northern South Island.

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.

4.2 Phenotypic variation in blue mackerel

The meristic data on New Zealand blue mackerel allow rejection of the single stock hypothesis, and indicate that blue mackerel in EMA 1, EMA 2, and EMA 7 are derived from separate spawning populations, exposed to different environmental conditions during the early larval stages. Counts of pectoral fin rays and gill rakers in *Scomber japonicus* differed between two fishery areas off southern Brazil and the Mar del Plata in the southwest Atlantic, and, coupled with different oceanographic conditions, the meristic data were interpreted as indicating the presence of two stocks (Perrotta et al. 1990).

Average December water temperatures on the northeast coast of New Zealand are about 1 °C warmer than those on the west coast, which in turn are on average about 1 °C warmer than those off the

Wairarapa coast (NIWA data). While laboratory experiments have shown that meristic counts are correlated with water temperature (Fahy 1972,Lindsey 1988,Taning 1946), it is unknown if the observed temperature difference could be sufficient to generate the observed differences in gill raker and fin ray counts in blue mackerel. Ideally, the observed area differences in blue mackerel meristic counts would be tested in additional samples to test for temporal variation, but the preliminary results indicate a difference between EMA 1, EMA 2, and EMA 7, from which we infer that blue mackerel in these areas are derived from different larval production systems.

Morphological characters, which describe shape based on distances between landmarks, such as tip of snout to fin origins, provide another measure of phenotypic variation in fishes. Morphological and meristic data are often collected together as the characters are easy to measure in whole specimens (Junquera & Perez-Gandaras 1993). However, the characters are determined by different biological processes (morphometric characters are determined by the biotic and physical environment experienced by individuals during the post recruitment stages, but meristic characters are determined by the environment during the larval and early juvenile stages) and thus provide different information on phenotypic variation in populations.

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) and Greenland halibut (*Reinhardtius hippoglossoides*) (Bowering 1988). Variation in morphometric characters has been reported in (*Scomber australasicus*) samples collected around Taiwan, with three groups that clustered on sampling period (Chang & Chen 1976). Thus it is likely that morphological variation is non-adaptive and reflects local feeding conditions and spawning times, rather than stock relationships.

Otolith morphology has also been applied as a stock marker for some marine fishes (DeVries et al. 2002). Otolith shape in jack mackerel (*Trachurus trachurus*) showed no significant variation between year classes and sexes, but significant differences between left and right otoliths derived from the same specimens (Murta et al. 1996). In the Atlantic mackerel (*Scomber scombrus*) there are significant age and year class effects in otolith shape, and significant differences among northwest Atlantic and North Sea stocks, but no significant differences among samples from the north and south of the range within the northwest Atlantic (Castonguay et al. 1991).

4.3 Genetics

In general, genetic studies of large pelagic species such as big eye tuna (Appleyard et al. 2002) and yellowfin tuna (Ward et al. 1997) have shown little or no genetic differentiation within ocean basins. In contrast, genetic studies of small pelagics have found genetic differentiation within sea areas. Separate genetic stocks of king mackerel (*Scomberomorus cavalla*) have been reported in the western Atlantic and Gulf of Mexico (Gold et al. 1997,Johnson et al. 1993); separate stocks of school mackerel (*Scombermorus queenslandicus*) are associated with large embayments off the east and north coasts of Australia (Begg et al. 1998); and there are separate stocks of scad mackerel (*Decapterus macrosoma*) between adjoining sea areas in the Sundra Strait and Java Sea (Arnaud et al. 1999). Sequencing of the mtDNA control region in the Atlantic mackerel *Scomber scombrus*, revealed genetic differentiation between three geographically isolated spawning areas off western Europe (Nesbo et al. 2000), whereas early allozyme studies did not (Jamieson & Smith 1987). The eastern Atlantic spawning stocks are genetically differentiated and therefore considered to be reproductively isolated and managed as three independent stocks (Nesbo et al. 2000).

A previous analysis of mtDNA in *Scomber australasicus*, using restriction fragment length polymorphims (RFLP) of the whole mitochondrial genome found no differentiation between Australia (18 fish) and New Zealand (19 fish) (Scoles et al. 1998). The present study found a higher level of genetic variation in the mtDNA control region, but no evidence for regional differentiation in

haplotypes. It is possible that low levels of gene flow are sufficient to inhibit genetic divergence at selectively neutral genetic markers, or that there has been insufficient evolutionary time for genetic divergence to occur amongst large recently isolated populations.

4.4 Parasites

Parasite markers are acquired during the juvenile and adult feeding stages or through inoculation and, depending on the longevity of the parasite, provide a marker of adult feeding behaviour and movement. If the parasites and/or their intermediate hosts have a restricted distribution, then only some groups of fish will be exposed to the parasite. A significant difference in parasite distribution between samples of blue mackerel implies a lack of mixing among those groups. The converse, a lack of differences, indicates that either the parasite and its intermediate hosts are widespread, regardless of the stock structure of blue mackerel, or that blue mackerel move between areas.

Knowledge of the intermediate hosts and their distribution is also critical for interpreting the geographical patterns of parasites in the fish host, for example variations in spatial abundance of the cestode *Grillotia angeli* in *Scomber scombrus* in the northeast Atlantic are limited by the adult worm's definitive host, the monkfish, which is confined to a much smaller geographic range than mackerel (McKenzie & Mehl 1984). The same cestode also showed a dramatic decline in prevalence in *S. scombrus* over time, reducing the value of this parasite as a biological indicator of stock relationships (McKenzie 1990).

Several parasites occurred at low prevalence in the New Zealand blue mackerel samples (the digenaeans, pedunculate type 1, and didymozoid type 2; and the nematode *Hysterothylacium* sp.) and thus are not useful markers of stock relationships. The monogenean *Kuhnia scombri* was prevalent in EMA 1 and EMA 2, but absent from EMA 7. A few heavily infected blue mackerel in EMA 2 led to significant area differences. Most monogenean parasites in fish are short lived (less than 1 year), and so the absence of *K. scombri* in blue mackerel from EMA 7 could be due to seasonal variation in parasite numbers rather than an area effect. Additional samples of blue mackerel would be required to test for temporal variation of *K. scombri*.

The acanthocephalan *Rhadinorhynchus* sp. found in the gut is another short-lived parasite, but the significant differences between blue mackerel from EMA 1 and EMA 2, collected at the same time period, indicate a lack of short-term movement between these areas. Ideally these differences would be tested in additional temporal and spatial samples.

The larval *Anisakis* sp. found encysted in the mesenteries, are possibly the most widely used parasites for biological tags (Lester et al. 1988,Sewell & Lester 1995). The significant differences found among blue mackerel from EMA 1 and EMA 2, and EMA 2 and EMA 7, are indicative of stock differences.

4.5 Tagging

Extensive tagging studies have been carried out on *S. scombrus* in the northeast Atlantic over the past 30 years. In general, tag returns from both external and internal tags are low, but returns demonstrate long-distance movements of mackerel off the west coast of Europe. The assumption of three stocks of *S. scombrus*, based on geographically isolated spawning stocks and early tagging returns, was abandoned by ICES in 1995 after a review of the extensive tagging database (Belikov et al. 1998). However, the single stock interpretation for eastern Atlantic mackerel from tag returns contrasts with the interpretation of recent genetic data which supports three separate stocks (Nesbo et al. 2000).

Tag returns of *S. japonicus* in the Sea of Japan have been low (under 1.5%) (Hasegawa, S. et al. 1998), and follow earlier large-scale experiments between 1950–68 (Usami & Matsushita 1974), and 1967–75 (Ohkawa 1975) which produced low returns (under 1.5%). Early tagging experiments on *S. australasicus* around Taiwan also produced low returns (Chang & Wu 1977). Information on tag

returns can be useful for testing stock relationships (and stock size), but the large scale (10 000+ tags) required and large number of processing sheds and vessels processing blue mackerel suggest that tagging (using either internal or external tags) would not be cost effective in the New Zealand fishery.

5. CONCLUSIONS

1. Four meristic characters, the number of gill rakers, and number of rays in the pectoral, dorsal I, and dorsal II fins, showed significant area differences.
2. A high level of genetic diversity was found in the control region of the mitochondrial DNA, but there was no regional differentiation in distribution of haplotypes.
3. Two parasites, the acanthocephalan *Rhadinorhynchus* sp. and the larval nematode *Anisakis* sp., showed significant area differences. The monogenean *Kuhnia scombri* was prevalent in EMA 1 and EMA 2 but absent from EMA 7; this short-lived parasite should be tested in temporal samples within and between areas.
4. Based on the area differences found with parasite markers and meristic characters, blue mackerel in the EEZ are subdivided into at least three stocks in EMA 1, EMA 2, and EMA 7.

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