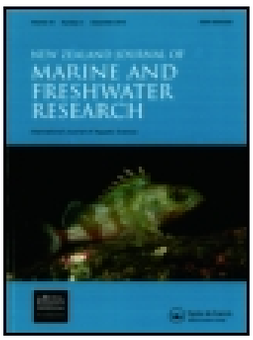

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Genetic variation and population structure in the New Zealand snapper

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ABSTRACT

Fifteen proteins encoded by 23 gene loci were electrophoretically surveyed in two samples of snapper *Chrysophrys auratus* (Forster) from Wellington Harbour and the Hauraki Gulf. Between 17% and 26% of the loci examined were found to be polymorphic and the proportion of heterozygous loci per individual was 7.9% to 8.4%. Using Rogers' genetic distance coefficient an overall similarity of 0.98 was calculated between the two samples.

Three polymorphic loci *Est-4*, *Gpi-1*, and *Idh* were examined in an additional 10 samples from around New Zealand. Two genetically distinct stocks were apparent: one along the west coast, the other along the east coast of the North Island. There was an indication of stock mixing at Ninety Mile Beach and in the Bay of Plenty and East Cape. The distribution of alleles at the *Est-4* locus revealed a third stock in Hawke Bay that is genetically more similar to the west coast than the east coast stock. Hydrological conditions in Hawke Bay are more similar to those of the west coast than to those of the north east coast of the North Island. Thus it is possible that *Est-4* alleles are selectively maintained in response to an environmental factor.

INTRODUCTION

The New Zealand snapper, *Chrysophrys auratus*, is a member of the sea bream family Sparidae and is closely related to the Australian snappers *C. unicolor* and *C. guttulatus* and to the Japanese red sea bream *C. major*. *C. auratus* is a demersal species distributed around the coast of the North Island and along the northwest coast of the South Island of New Zealand, where it is commonly found in shallow water between 20 and 60 m, but ranges down to 200 m. It is the most important teleost in the commercial fisheries of New Zealand, accounting for approximately one-third of the total wetfish landings. The major snapper fishery is centred in the Hauraki Gulf where the abundance of the species is greatest, and where there is a large spawning ground (Paul 1976). Smaller spawning and nursery grounds occur along the east Northland coastline and the outer Coromandel Peninsula, in the eastern Bay of Plenty, and at least one small nursery ground is recorded south of East Cape (Paul & Tarring in press). On the west coast significant spawning and nursery grounds occur in the vicinity of Kaipara and Manukau harbours, in the North Taranaki Bight, and in some years in Tasman Bay.

There is little available evidence as to whether *C. auratus* comprises one large or several small stocks. Tagging studies to date have not ruled out the possibility of larger-scale migrations although most recaptured tagged snapper had moved relatively short distances (Crossland 1976, Paul 1967). Data on size and growth rates have shown that west coast snapper are larger and grow faster than east coast snapper (Longhurst 1958, Paul unpublished data). Longhurst (1958) suggested that these differences may be racial, perhaps related to hydrological conditions. The general trend of increasing size of snapper from north to south might reflect a slow movement of adult fish away from the important northern spawning grounds (Waugh 1973, Paul & Tarring in press).

The applications of protein and enzyme polymorphisms as genetic markers in fisheries population studies have been discussed by Jamieson (1974) and Utter *et al.* (1974), and de Ligny (1969, 1971) has reviewed the proteins studied in commercially important teleosts. It is assumed that differences in allozyme frequencies between stocks of fish indicate that these groups are not freely interbreeding and can be treated as separate units for management purposes. This paper presents data on enzymes studied in *C. auratus* with a view to applying the polymorphic loci as genetic tags in a study of snapper stock structure in New Zealand waters. Electrophoresis, coupled with specific histochemical staining techniques, has enabled estimates to be made of both intra- and inter-specific genetic variation (see Lewontin 1974). Although not the primary aim of this research, the data readily lends itself to an estimate of genetic heterozygosity in *C. auratus*.

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MATERIALS AND METHODS

The location of sampling sites, dates of collection, sample sizes, and tissues collected are given in Table 1. Trawl-caught adult snapper were killed and the tissues dissected out and frozen in liquid nitrogen. For long term storage in the laboratory, samples were maintained at −20°c. Two initial samples of eye, gill, heart, liver, and skeletal muscle tissues were collected from the Hauraki Gulf and from Wellington Harbour and surveyed for the 15 enzymes shown in Table 2, which also shows the buffer systems used and the abbreviations adopted. Additional biological data on sex, length, and age (from otoliths) were collected. All later samples were restricted to liver tissue, plus length and sex data, and analysed for three enzymes: esterase, glucose phosphate isomerase and isocitrate dehydrogenase.

Electrophoretic procedures were carried out at between 2°c and 4°c. Homogenates were prepared by grinding one volume of tissue in two volumes of 0.1 M tris-HCI ph 7.4 and centrifuging at 30 000 *G* for 10 min. The clear supernatants were placed onto 0.3 mm² Whatman No. 4 filter paper wicks and inserted into horizontal starch gels. Electrostarch lot No. 307, at a concentration of 11%, was used for all gels. Five gel-electrode buffer systems described by Selander *et al* (1971) were employed to separate the enzymes: lithium hydroxide, continuous tris-citrate 1, tris-versene-borate, phosphate and phosphate-citrate (Table 2). Staining techniques followed those described by Selander *et al.* (1971) and Shaw & Prasad (1970).

TABLE 1—Location, date of capture, number of fish sampled and tissues collected for snapper *Chrysophrys auratus* samples around New Zealand (e = eye; g = gill; h = heart; l = liver; m = skeletal muscle).

Location	Date	No. of Fish	Tissues
Hauraki Gulf	Nov 1976	88	e, g, h, l, m
Wellington Harbour	Jan 1977	89	e, g, h, l, m
Tasman Bay	May 1977	168	l
Marlborough Sounds	May 1977	103	l
North Taranaki	May 1977	132	l
Kaipara-Manukau	May 1977	115	l
Ninety Mile Beach	May 1977	164	l
Bay of Islands	May 1977	121	l
Bream Bay	May 1977	78	l
Bay of Plenty	May 1977	157	l
East Cape	May 1977	73	l
Hawke Bay	May 1977	111	l

RESULTS

ELECTROPHORETIC PATTERNS OF ENZYMES

Enzymes represented by one electrophoretic zone of activity are assumed to be under the control of a single structural gene. Within this group of enzymes the following stained as a single band and were scored as monomorphic: ADH, GPDH, NDH, and 6PGDH (enzyme abbreviations are listed in Table 2). NDH activity appeared on all dehydrogenase stained gels; it appeared without the addition of substrate but required coenzyme, MTT tetrazolium and phenazine methosulphate. GDH activity was detected as a single band in liver tissue. This enzyme was not included in the estimate of genetic variation in *Chrysophrys auratus* for nearly half the liver tissues analysed exhibited no electrophoretic activity. IDH and XDH appeared as one and three band phenotypes and ME as one and two band phenotypes. The frequencies of the common alleles and numbers of observed and expected heterozygotes for these three loci are presented in Table 3. All three loci showed a good fit to the Hardy-Weinberg equilibrium, with a similar distribution of phenotypes between sexes, and are therefore assumed to represent codominant autosomal genes.

TABLE 2—Enzymes assayed in snapper *Chrysophrys auratus* (e = eye; g = gill; h = heart; l = liver; m = skeletal muscle; PC = phosphate-citrate; LiOH = lithium hydroxide; TC1 = continuous tris-citrate 1; TEB = tris-versene-borate; P = phosphate).

Enzyme	Enzyme Abbreviation	Tissue	Buffer System	No. of loci
Alcohol dehydrogenase	ADH	l	PC	1
Glutamate dehydrogenase	GDH	l	LiOH	1
α-Glycerophosphate dehydrogenase	GPDH	m	LiOH	1
Isocitrate dehydrogenase	IDH	l	PC	1
Lactate dehydrogenase	LDH	e, h, m	TC1	3
Malate dehydrogenase	MDH	m	TEB	2
Malic enzyme	ME	l, m	TEB	1
Nothing dehydrogenase	NDH	l	LiOH	1
6-Phosphogluconate dehydrogenase	6PGDH	m	LiOH	1
Xanthine dehydrogenase	XDH	l	TEB	1
Esterase	EST	e, g, h, l, m	LiOH	4
Peroxidase	PER	h, l	LiOH	2
Glucosephosphate isomerase	GPI	h, l, m	LiOH	2
Phosphoglucomutase	PGM	l, m	P	2
Superoxide dismutase	SOD	h, l, m	LiOH	2

Several enzymes resolved into two or more zones of activity on the starch gels. The genetic criteria for their interpretation are based upon comparison with other species, and for the polymorphic loci on agreement with the Hardy-Weinberg equilibrium. Three monomorphic LDH loci were observed with differing tissue specificities and staining intensities as recorded in other teleosts (e.g., Whitt 1970). Four esterase loci were found in liver tissue. The slowest migrating locus, *Est-4*, was polymorphic for three common alleles, the other loci being essentially monomorphic although rare electrophoretic variants (frequency $P < 0.01$) were observed in some samples. Esterase data for Wellington Harbour and Hauraki Gulf samples are presented in Table 3 and *Est-4* for all samples in Table 4.

TABLE 3—Frequencies of the common alleles of eight variable loci in two samples of snapper *Chrysophrys auratus* (n = number of fish; p = frequency of common allele; obsH = number of observed heterozygotes; expH = expected number of heterozygotes)

Locus		Hauraki Gulf	Wellington Harbour
Idh	*n*	86	82
	p	0.994	0.945
	obsH	1	9
	expH	0.99	8.53
Me	*n*	68	80
	p	0.963	0.981
	obsH	5	3
	expH	4.84	3.04
Xdh	*n*	26	66
	p	0.654	0.591
	obsH	10	32
	expH	11.77	31.9
Est-1	*n*	86	83
	p	1.00	0.994
	obsh	0	1
	expH	0	0.99
Est-3	*n*	86	83
	p	0.994	1.00
	obsH	1	0
	expH	0.99	0
Est-4	*n*	86	83
	p	0.494	0.717
	obsH	46	38
	expH	47.13	34.72
Gpi-1	*n*	84	80
	p	0.691	0.700
	obsH	36	30
	expH	35.87	33.6
Pgm-1	*n*	48	54
	p	0.500	0.435
	obsH	18	25
	expH	28.24	32.64

GPI was seen as three well separated zones which represent two loci and the heteropolymer zone as described in many other teleosts (Dando 1974, Avise & Kitto 1973). The two loci exhibited their strongest activity in different tissues. *Gpi-1* was strongest in liver and *Gpi-2* in skeletal muscle. The heteropolymer zone showed one and two band phenotypes which corresponded to the one and three band phenotypes of *Gpi-1*. Two PGM loci were apparent with the more anodal, and polymorphic, locus staining weakly in liver tissue, and a slower migrating monomorphic locus in skeletal muscle and liver.

The Hauraki Gulf sample showed a significant excess of homozygotes (Table 3; $\chi^2 = 9.69$; d.f. = 5). Activity at this locus was faint and deteriorated with storage time; in both samples approximately 20% of fish could not be genotyped. The two-band heterozygous individuals stained less intensely than the single band homozygotes, suggesting that the apparent homozygous excess could be due to poor electrophoretic resolution of the *Pgm-1* phenotypes. Consequently further samples were not analysed for this enzyme. An alternative explanation could be the presence of a nul-allele in the samples.

A large number of electrophoretic bands were observed for SOD. Two zones of single bands stained at much higher intensity than the others and were treated as two monomorphic loci, one staining more strongly in liver tissue, the other in heart tissue. PER activity was detected as a slow migrating zone of single bands in heart tissue. Liver tissue revealed an additional, faster migrating zone of diffuse activity in which phenotypes could not be determined. Finally, aspartate aminotransferase resolved into two widely separated zones of diffuse activity presumably controlled by two loci.

GENETIC VARIATION IN *C. auratus*

A summary of genetic variation in *C. auratus* is given in Table 5. Calculations are based on the 8 loci in Table 3 and 15 monomorphic loci, *Aat-1, Aat-2, Gdh,* and *Per-1* are not included because of uncertain electrophoretic resolution of phenotypes. An average of 1.39 ± 0.13 (± standard error) alleles per locus was found in the two samples. The number of alleles varies from one for the monomorphic loci to three in *Pgm-1* and *Est-4*. The proportion of loci polymorphic is 26.09% in the Wellington sample and 21.74% in the Hauraki Gulf sample if a locus is considered polymorphic when the frequency of the common allele is no greater than 0.99. The two esterase loci, *Est-1* (P = 0.994 Wellington Harbour) and *Est-3* (P = 0.998 Hauraki Gulf) are monomorphic as is *Idh* in the Hauraki Gulf sample (P = 0.994). If a stricter criterion of polymorphism is employed ($P < 0.95$), then the proportion of loci polymorphic in the Wellington Harbour sample is 21.74% and in the Hauraki Gulf sample 17.39%. Because such estimates are dependent upon sample size, estimates of mean heterozygosity levels are more informative. The observed proportion of heterozygotes per locus is presented and also the expected

under Hardy-Weinberg equilibrium. The large standard errors are produced by the wide variation in frequencies of heterozygotes at individual loci, ranging from 0% for monomorphic loci to over 40% for *Est-4*, *Gpi-1*, *Pgm-1* and *Xdh*. In both the Wellington Harbour and Hauraki Gulf samples the observed heterozygosities are lower than expected due to an excess of homozygotes observed at the *Pgm-1* locus.

To estimate the genetic similarity between the two samples collected in Wellington Harbour and the Hauraki Gulf, the distance coefficient of Rogers (1972) was used. Genetic distance or similarity is the difference between populations expressed as a function of allele frequencies; the formula for the calculation is given in Appendix 1. Based on the 15 monomorphic and 8 variable loci, a genetic similarity of 0.98 was found between the two samples.

POPULATION STRUCTURE IN *C. auratus*

The three polymorphic loci *Est-4*, *Gpi-1*, and *Idh* were analysed in all samples. The numbers of observed phenotypes and allele frequencies are presented in Tables 4, 6, and 7. The expected numbers and fit to Hardy-Weinberg equilibrium are given for *Est-4* and *Gpi-1* but not for *Idh* because of the large number of cells with less than five individuals. At the *Est-4* locus all samples show a good agreement between observed and expected numbers except for the Bay of Plenty sample, which reveals a significant excess of heterozygotes. This could be explained by chance as one sample in twenty would be expected to deviate. However, this is also the only sample out of equilibrium at the *Gpi-1* locus where it shows a significant excess of homozygotes. A test was made for linkage between the phenotypes at these two loci but no disequilibrium was found (χ^2 = 8.4; d.f. = 8). Likewise, similar tests over all samples found no linkage disequilibrium between *Est-4* and *Gpi-1* (χ^2 = 1.3; d.f. = 7), *Est-4* and *Idh* (χ^2 = 2.3; d.f. = 2) and *Gpi-1* and *Idh* (χ^2 = 2.1; d.f. = 1).

It was not possible to determine age classes from the length-frequency data. The snapper is a long-lived fish and there is considerable overlap in length between age classes, above about 30 cm and 5 y (Paul 1976). For *Est-4* and *Gpi-1* the phenotype frequencies were divided into three length classes: 20–29.5 cm, 30–39.5 cm, and 40+ cm if sufficiently large numbers of fish were caught in each class. For *Gpi-1* no differences in frequency were observed between length classes. At the *Est-4* locus two samples Bay of Plenty (χ^2 = 6.16; d.f. = 4) and East Cape (χ^2 = 6.71; d.f. = 3) revealed significant differences in phenotype frequencies between length classes.

To measure the genetic differences between samples the allele frequencies at the three loci have been summed in Rogers' genetic distance coefficient (Table 8). Unlike the values calculated between Wellington Harbour and Hauraki Gulf they canot be interpreted as actual genetic distances as only three loci have been considered. However, they provide a convenient method of summarising the allele frequency differences between samples and also produce a statistic with which to test the hypothesis that two given samples are from genetically isolated stocks. For

TABLE 4—Observed number of *Est-4* phenotypes compared with the expected (in parentheses) assuming Hardy-Weinberg equilibrium in snapper *Chrysophrys auratus* samples from New Zealand waters. Rare phenotypes have been grouped with common phenotypes for χ^2 calculations.

Location	Esterase-4 Phenotypes											*Frequency of* Allele 3	χ^2	*Probability* (4 d.f.)
	12	22	23	33	34	24	13	25	35	44	Total			
Tasman Bay	0	15	54	85	7	5	0	0	0	0	166	0.696		
		(11.92)	(62.08)	(80.34)	(8.32)	(3.32)							3.177	0.70–0.50
Marlborough Sounds	0	6	33	51	4	1	0	0	0	0	95	0.732		
		(5.61)	(33.63)	(50.92)	(3.61)	(1.14)							0.099	> 0.99
North Taranaki	0	7	45	67	4	3	0	1	0	0	127	0.720		
		(7.87)	(45.47)	(65.79)	(5.08)	(1.78)							1.189	0.90–0.80
Manukau-Kaipara	0	5	44	56	2	2	1	0	0	0	110	0.723		
		(7.15)	(40.48)	(57.53)	(2.86)	(1.10)							1.992	0.80–0.70
Ninety Mile Beach	0	18	55	67	8	5	0	0	0	0	153	0.644		
		(15.15)	(61.81)	(63.49)	(8.26)	(3.98)							1.749	0.80–0.70
Bay of Islands	1	33	51	28	2	2	0	0	1	0	118	0.466		
		(30.44)	(55.93)	(25.61)	(1.89)	(2.12)							0.885	0.95–0.90
Bream Bay	0	16	27	21	2	2	0	0	0	0	68	0.522		
		(13.74)	(31.82)	(18.50)	(2.04)	(1.76)							1.474	0.90–0.80
Hauraki Gulf	0	18	37	22	4	5	0	0	0	0	86	0.494		
		(17.72)	(38.53)	(20.98)	(4.47)	(4.13)							0.347	0.99–0.98
Bay of Plenty	0	24	86	32	11	3	0	0	0	0	156	0.516		
		(30.10)	(70.82)	(41.49)	(7.18)	(6.24)							10.375	0.05–0.02
East Cape	0	18	24	21	5	2	0	0	0	0	70	0.507		
		(13.72)	(31.50)	(17.99)	(3.50)	(3.08)							4.637	0.50–0.30
Hawke Bay	1	14	39	45	3	5	0	0	1	1	109	0.610		
		(12.21)	(44.47)	(40.55)	(6.10)	(3.27)							3.908	0.50–0.30
Wellington Harbour	0	4	34	41	3	1	0	0	0	0	83	0.717		
		(4.57)	(30.83)	(42.67)	(2.86)	(1.03)							0.882	0.95–0.90

TABLE 5—Summary of genetic variation in two samples of snapper *Chrysophrys auratus* from New Zealand waters.

	Hauraki Gulf	Wellington Harbour
No. of loci studied	23	23
No. of individuals	88	89
No. of genomes studied per locus	149.7	150.9
No. of alleles per locus (± s.e.)	1.39 ± 0.13	1.39 ± 0.13
Percentage of polymorphic loci	21.74	26.09
Proportion of heterozygotes per locus		
Observed (± s.e.)	0.0843 ± 0.035	0.0791 ± 0.034
Expected (± s.e.)	0.0904 ± 0.038	0.0918 ± 0.041

each pair of samples an approximate 5% critical value is produced (see Appendix 1). If the calculated distance exceeds this value it can be assumed, using a 5% significance level, that the samples have been taken from different stocks. This critical value is dependent upon sample size and allele frequency and for the data presented here lies between 0.045 and 0.065. By the use of this value the samples can be divided into three groups (Table 8):

(a) Tasman Bay, Marlborough Sounds, North Taranaki, Manukau-Kaipara, Ninety Mile Beach, and Wellington Harbour,
(b) Bay of Islands, Bream Bay, Hauraki Gulf, Bay of Plenty, East Cape, and,
(c) Hawke Bay.

Within each group there are no significant differences in allele frequencies while between (a) and (b) and (b) and (c) there are significant differences. The distances between groups (a) and (c) are inconclusive in that some distances exceed and others fall below the critical value.

Contingency tests on *Est-4* alleles between selected samples are presented in Table 9. The west coast samples have been grouped, with the exception of Ninety Mile Beach, and the east coast samples grouped with the exception of Bay of Plenty and East Cape. There are significant differences between west coast samples and Hawke Bay and between east coast and Hawke Bay, thus separating Hawke Bay from the west coast stock. From the allele frequencies (Table 7) it can be seen that the Ninety Mile Beach sample has the lowest *Est-4* allele frequency along the west coast. A comparison of *Est-4* alleles (Table 9) reveals significant differences between Ninety Mile Beach and west coast and between Ninety Mile Beach and east coast samples.

TABLE 6—Observed number of *Gpi-1* phenotypes compared with the expected (in parentheses) assuming Hardy-Weinberg equilibrium in snapper *Chrysophrys auratus* samples from New Zealand waters. The 23 phenotype for North Taranaki has been grouped with the 12 phenotype for χ^2 calculation.

	Glucosephosphate isomerase-1 Phenotypes							
Location	11	12	22	23	Total	Frequency of Allele 2	χ^2	Probability (2 d.f.)
Tasman Bay	10 (10.19)	62 (61.79)	95 (95.02)	0	167	0.754	0.005	> 0.99
Marlborough Sounds	9 (7.31)	37 (40.38)	57 (55.31)	0	103	0.733	0.72	0.70–0.50
North Taranaki	10 (8.58)	46 (49.89)	75 (73.32)	1	132	0.746	0.57	0.80–0.70
Manukau-Kaipara	13 (10.12)	42 (47.84)	60 (57.04)	0	115	0.704	1.68	0.50–0.30
Ninety Mile Beach	14 (10.99)	57 (62.97)	93 (90.04)	0	164	0.741	1.49	0.50–0.30
Bay of Islands	12 (14.28)	59 (54.45)	50 (52.27)	0	121	0.657	0.84	0.70–0.50
Bream Bay	7 (7.72)	35 (33.54)	36 (36.74)	0	78	0.686	0.14	0.95–0.90
Hauraki Gulf	8 (8.02)	36 (35.87)	40 (40.11)	0	84	0.691	0.0008	> 0.99
Bay of Plenty	18 (11.87)	45 (57.13)	75 (69.00)	0	138	0.707	6.81	0.05–0.02
East Cape	7 (7.59)	33 (31.83)	33 (33.58)	0	73	0.678	0.10	0.98–0.95
Hawke Bay	8 (6.38)	37 (40.26)	65 (63.36)	0	110	0.759	0.71	0.80–0.70
Wellington Harbour	9 (7.21)	30 (33.60)	41 (39.20)	0	80	0.700	0.91	0.70–0.50

DISCUSSION

The levels of genetic variation observed in *Chrysophrys auratus* are similar to those reported for other teleosts. The genetic similarity of 0.98 between the two populations is typical of that between local populations of vertebrates (Ayala 1975). Heterozygosity levels are the most reliable estimator of intraspecific genetic variation and for teleosts they range from 0.005 to 0.180 with an average of 0.058 over 31 species from diverse environments (Powell 1975). Valentine (1976) has developed a hypothesis relating genetic variability to trophic resource stability under which a warm temperate species, such as *C. auratus*, might be characterised by a level of genetic variation intermediate between that of cool temperate/polar species and that of subtropical/tropical species. Thus the estimates of 0.084 and 0.079 obtained for snapper are not unusual.

The genetic distance summation over the three polymorphic loci, *Est-4, Gpi-1,* and *Idh,* reveals two genetically distinct populations of snapper around the North Island of New Zealand, one along the west coast, the other along the east coast. Samples collected from Wellington Harbour, Tasman Bay, and Kaipara-Manukau show similar allele frequencies over the three loci. Along the east coast, samples collected between the Bay of Islands and East Cape are similar to each other but dissimilar to those of the west coast. This finding agrees well with the stock separation suggested by Longhurst (1958) based on growth differences.

The *Est-4* locus considered alone represents a more sensitive genetic tag than the summation over three loci. Four of the samples show anomalies at this locus and it is noteworthy that they occur at the geographical limits of the two described stocks. The East Cape and Bay of Plenty samples show a significant difference in *Est-4* phenotypes between length classes, and the Bay of Plenty sample reveals an excess of *Est-4* heterozygotes and an excess of *Gpi-1* homozygotes. These observations indicate stock mixing, particularly as the Hawke Bay sample is genetically distinct from the east coast stock. These samples were collected in May, about 5 months after spawning, when mixing is likely to be at a maximum. Length frequency data obtained by Paul & Tarring (in press) also suggested the possibility of stock mixing at East Cape, resulting from the slow movement of adults southwards, i.e., immigration from the Hauraki Gulf and Bay of Plenty.

The Ninety Mile Beach sample is grouped with the west coast stock when the three loci are taken into account, yet there is evidence that it represents a mixed sample. *Est-4* allele frequencies lie between those of east and west coasts and there is a significant difference in the distribution of *Est-4* alleles between Ninety Mile Beach and the west coast and between Ninety Mile Beach and the east coast. In hydrological terms the west coast shelf can be regarded as one area, influenced by the north flowing Tasman Current (Roberts & Paul 1978). However, there is increasing evidence that Ninety Mile Beach is also influenced by warmer northern water, in common with the northeast coast. It lies in the hydrological transition zone between the west and northeast coasts.

The fourth anomalous sample is Hawke Bay. This sample is genetically distinct from the east coast stock but is not significantly different from the west coast stock when averaged over three loci. The distribution of *Est-4* alleles separates Hawke Bay from the west coast stock. It is not clear why the Hawke Bay sample is genetically closer to the west coast than the east coast samples. Hawke Bay is geographically closer to the latter, and appears to receive immigrants from the north (Paul & Tarring in press); it is separated from the west coast stock by the Wairarapa coastline where snapper are quite uncommon. Hawke Bay seems to be primarily influenced by water coming from the north in the East Cape current, but as a current flowing north from the Cook Strait region reaches almost to this latitude, it does lie close to a hydrological boundary.

Water temperatures might provide some clue to the nature of the Hawke Bay sample. Although the differences are small, surface temperatures in Hawke Bay are closer to those of the west coast than to

TABLE 7—Isocitrate dehydrogenase phenotypes and frequency of the common allele in snapper *Chrysophrys auratus* samples from New Zealand waters

Location	Isocitrate dehydrogenase phenotypes 12	22	23	33	24	Total	Frequency of Allele 2
Tasman Bay	0	150	13	0	0	163	0.960
Marlborough Sounds	0	90	7	0	0	97	0.964
North Taranaki	0	119	8	0	0	127	0.969
Manakau-Kaipara	0	105	6	1	0	112	0.964
Ninety Mile Beach	1	148	10	0	0	159	0.965
Bay of Islands	0	102	3	0	0	105	0.986
Bream Bay	0	49	1	0	0	50	0.990
Hauraki Gulf	0	85	1	0	0	86	0.994
Bay of Plenty	0	152	2	0	0	154	0.975
East Cape	0	69	3	0	0	72	0.979
Hawke Bay	1	105	4	0	1	111	0.969
Wellington Harbour	0	73	9	0	0	82	0.945

TABLE 8—Genetic distance among 12 samples of snapper *Chrysophrys auratus;* distances based on three loci *Est-4, Gpi-1,* and *Idh.*

Location	Genetic distance										
Tasman Bay (TB)											
Marlborough Sounds (MS)	0.02										
North Taranaki (NT)	0.01	0.01									
Manukau-Kaipara (MK)	0.03	0.02	0.02								
Ninety Mile Beach (NM)	0.02	0.03	0.03	0.04							
Wellington Harbour (WH)	0.03	0.02	0.03	0.01	0.04						
Hawke Bay (HB)	0.03	0.05	0.04	0.05	0.02	0.06					
Bay of Islands (BI)	0.12	0.12	0.12	0.11	0.10	0.11	0.09				
Bream Bay (BB)	0.09	0.09	0.09	0.08	0.07	0.08	0.07	0.03			
Hauraki Gulf (HG)	0.10	0.10	0.10	0.09	0.07	0.09	0.07	0.03	0.01		
Bay of Plenty (BP)	0.08	0.08	0.08	0.07	0.06	0.08	0.05	0.04	0.02	0.02	
East Cape (EC)	0.09	0.10	0.10	0.08	0.07	0.09	0.07	0.03	0.01	0.01	0.01
	TB	MS	NT	MK	NM	WH	HB	BI	BB	HG	BP

east Northland, Hauraki Gulf, and even the Bay of Plenty. If the described alleles are adaptive and are selectively maintained through an environmental factor, such as water temperature, then Hawke Bay might be expected to be more similar to the west coast. The same reasoning would explain the apparently mixed stock at Ninety Mile Beach, an area intermediate in temperature between the west and northeast coasts. Certainly there are examples in other teleosts of esterase allozymes correlated with water temperature (Johnson 1977, Koehn 1969, Mitton & Koehn 1975). Ninety Mile Beach, Bay of Plenty, and Hawke Bay are obviously areas requiring further study to clarify the boundaries between stocks. Ideally all samples should be collected during the spawning season when mixing is at a minimum.

Further work is also required to see whether these genetic differences result from the effects of water temperature during a critical (presumably larval) period. From the management viewpoint, the west coast, northeast coast, and Hawke Bay snapper should clearly be treated as separate stocks.

TABLE 9—Contingency tests on *Est-4* alleles between stocks of snapper *Chrysophrys auratus.*

Samples compared	χ^2	d.f.	Probability
Ninety Mile Beach *v.* west coast	6.23	3	0.20–0.10
Ninety Mile Beach *v.* east coast	21.02	3	> 0.001
Hawke Bay *v.* west coast	8.89	3	0.05–0.02
Hawke Bay *v.* east coast	10.94	3	0.02–0.01

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APPENDIX 1—Sampling Error in Rogers' Distance between two populations, x and y, based on gene frequencies at i loci is:

$$D_{xy} = \sum_{i=1}^{L} \left[\frac{A_i}{\frac{1}{2} \sum_{j=1}} (P_{ijx} - P_{ijy})^2 \right]^{\frac{1}{2}} / L$$

where A_i = number of alleles at the ith locus and P_{ijxx} frequency (as a proportion) of jth allele at the ith locus in population x. Sampling error arises in that P_{ijx} is only estimated from a sample. Thus the question arises as to whether a calculated distance is significantly different from zero.

In the special case where all loci are (essentially) biallelic, Rogers' distance takes the simpler form:

$$D_{xy} = \{ \sum_{i=1}^{L} | P_{ix} - P_{iy} | \}/L$$

where P_{ix} is the frequency of one of the alleles at the ith locus in population x. The number of occurrences of an allele in a sample is distributed binomially. If we consider two samples drawn from identical populations in which the frequency of one allele at the ith locus is P_i then, using the normal approximation to the binomial distribution, we find that $| P_{ix} - P_i |$ is distributed approximately seminormally (i.e., like the absolute value of a zero-mean normal variate) with mean:

$$[P_1 \ (1-P_1) \ (1/n_x + 1/n_y)/\pi]^{\frac{1}{2}}$$

and variance

$$(\tfrac{1}{2} - 1/\pi) \ P_1 \ (1-P_1) \ (1/n_x + 1/n_y)$$

where n_x, n_y are the numbers of fish in the two samples. Thus under the hypothesis of no difference between the populations, Rogers' distance has mean,

$$\mu = \left[\{(1/n_x + 1/n_y)/\pi\}^{\frac{1}{2}} \sum_{i=1}^{L} \{P_i \ (1-P_i)\}^{\frac{1}{2}} \right] / L$$

and variance

$$\sigma^2 = \left[(\tfrac{1}{2} - 1/\pi) \ (1/n_x + 1/n_y) \sum_{i=1}^{L} P_i \ (1-P_i) \right] L^2$$

Rogers' distance is not normally distributed, but nevertheless the properties of the seminormal distribution lead to the result that an approximate critical value for testing at the 95% confidence level the hypothesis of no difference in the populations is $\mu + 2\sigma$.

Three alleles were found at the *Est-4* locus but one allele was comparatively rare and most of the variation in frequencies between samples lay in the other two alleles. Some of the frequencies at the IDH locus were too high for our normal approximation to be very accurate, but calculations show that the effect of this is only a very slight underestimation of the critical value.

Almost all of the genetic distances in Table 8 are either much less or much greater than the corresponding critical value, so that its approximate nature is unimportant.