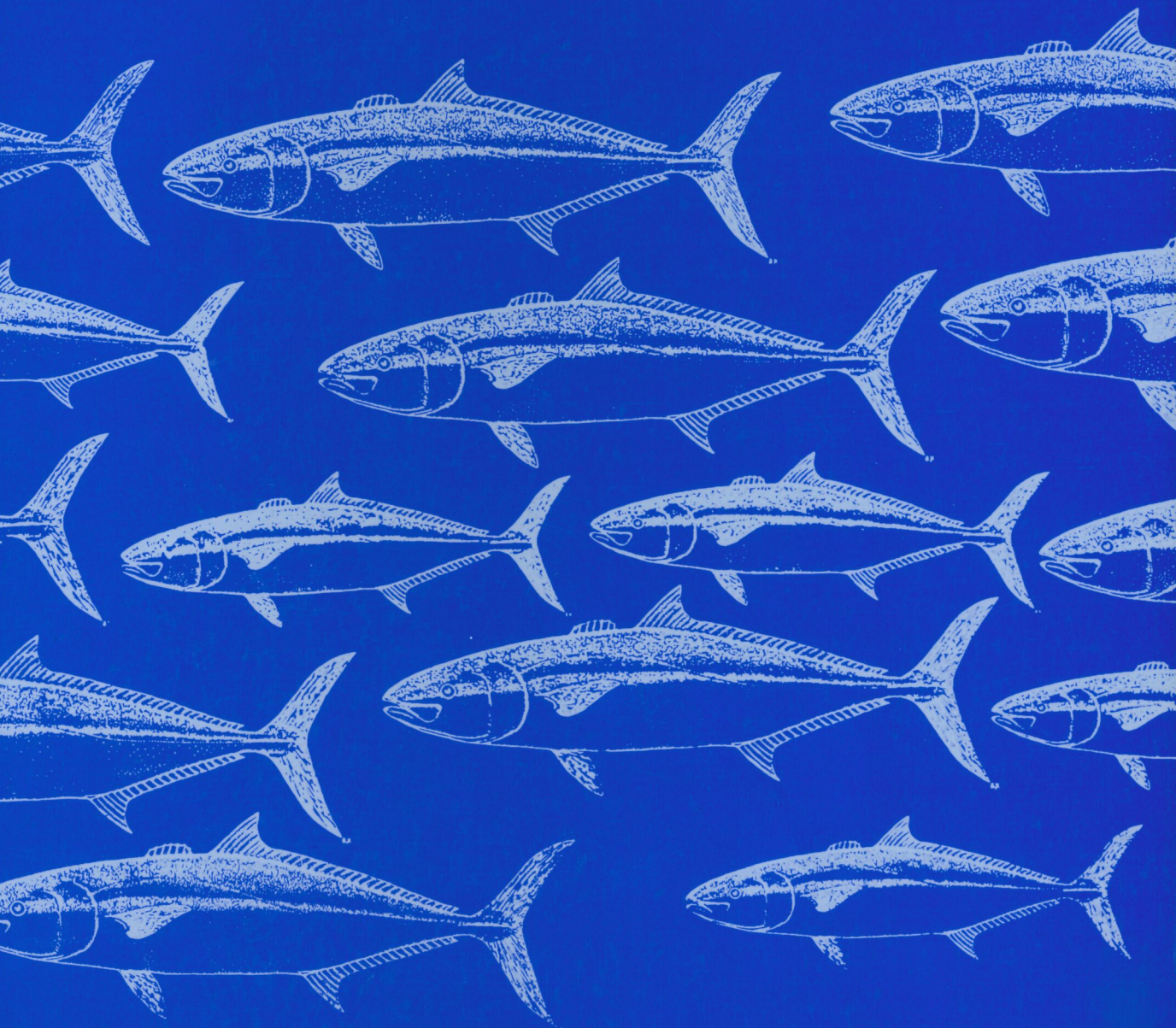
Project FIRTA 86/65
Final Report to FIRC
March 1988



Fisheries Research Institute
NSW Agriculture & Fisheries

Yellowtail Kingfish Stock Identification

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1. INTRODUCTION

The Kingfish Stock Identification Project was instigated in 1985 to provide information on the stock structure of yellowtail kingfish relevant to management. At that time, the N.S.W. catch had more than doubled since 1982 to approximately 360 tonnes per annum and concern was expressed at the possibility of overexploitation of localized stocks.

Of more than 8,000 kingfish tagged by recreational fishermen in New South Wales, some 450 had been recaptured at or near the point of release. Only 17% of fish at liberty for six months or more were recaptured more than twenty kilometres from their release point. Tag returns indicated that juveniles and some adults remain in restricted localities for long periods (more than one year) suggesting the possibility of discrete stocks or resident populations vulnerable to overexploitation.

Since 1985 the total New South Wales catch has continued to increase. Improved market acceptance and higher prices per kilogram contributed to record quantities passing through the Sydney Fish Markets in 1985/86. The introduction of floating kingfish traps has resulted in increased catches of small fish. Floating traps are an effective and economical method of taking kingfish and they have been rapidly adopted in most ports north of Wollongong, and in some southern ports.

The small sizes of fish targetted by trapping has led to renewed conjecture on the possible effects on kingfish stocks, calls for restrictions on gear and the introduction of management strategies. It is therefore timely that this report on kingfish stock identification is available for consideration in future management regimes.

This project has drawn heavily on independent work carried out at the Fisheries Research Institute both before and during the study. The recaptures of large numbers of kingfish tagged by recreational fishermen in or near the location of tagging prompted the initial questions on the possibility of separate kingfish stocks.

The tagging data base has been accumulated since 1974 providing an otherwise prohibitively expensive temporal and geographic distribution of tagged fish. Requests from the Fisheries Research Institute for anglers to tag more kingfish during this project were successful in substantially increasing the numbers tagged. Concentration of effort on kingfish tagging has been maintained throughout the study period and recapture information has proved important in interpretation of electrophoretic and biological data. The ongoing gamefish tagging project continues to provide information on movements and growth.

The Fisheries Research Institute has also provided material assistance in the provision of office and laboratory space, vehicles and vehicle maintainance, computing and secretarial services and library facilities. Assistance in field operations was provided in manpower and logistics and kingfish for sampling were purchased by the Fisheries Research Institute. Successful field sampling relied heavily on the established excellent rapport already existing between research staff and commercial and recreational fishermen. Arrangements for overseas samples were made by Fisheries Research Institute staff who undertook extensive international liaison throughout the study.

A comprehensive report pertaining to this study was submitted as an honours thesis in the Department of Zoology, University of New South Wales, by Mr. Adam Smith, Technical Officer employed on the project. He was awarded First Class Honours, testifying to the quality of the work. The thesis is presented as the major component of this report. A synopsis of results is provided in Segment 4: Summary of Results.

2. DETAILS OF GRANT APPLICATION

Title of Proposal:

Yellowtail Kingfish Stock Identification.

Name of Applicant:

Department of Agriculture, New South Wales.

Division:

Fisheries Research Institute.

Proposal:

To investigate the stock structure of yellowtail kingfish *Seriola lalandi* from the east coast of Australia using electrophoretic and morphometric techniques.

Names of People Responsible for the Programme:

Dr D. Francois, Executive Director (Fisheries.
Dr P. Ayres, Chief, Division of Fisheries.

Qualifications of Staff Employed on the Programme:

J. Diplock, BSc.
Technical Officer, Adam Smith.

Objectives:

To compare kingfish from various localities in eastern Australia in order to identify differences indicative of separate stocks. This information will be used in conjunction with available tagging data to identify stock structure of east coast yellowtail kingfish and clarify their migrations and biology.

Justification:

The yellowtail kingfish is distributed along the eastern and southern shores of Australia. It is abundant in New South Wales and forms an important component of the inshore commercial catch. The catch has fluctuated between 200,000 and 300,000 kg per annum since the mid sixties. The amateur catch is also believed to be considerable, possibly of the same order of magnitude as the commercial catch. Since 1975 approximately 8,000 kingfish have been tagged in New South Wales as part of an ongoing tagging programme. Of these more than 450 have so far been recaptured. Most recaptures have been made at or close to the point of release. Only 17% of fish at liberty for six months or more were recaptured more than twenty kilometres from their release point, and of these only four fish travelled more than 100 kilometres.

The overall recapture rate for yellowtail kingfish is the highest for any species tagged in the programme and in heavily fished areas it may exceed 10%.

Tag returns indicate that juveniles and some adults remain in restricted localities for long periods suggesting the existence of discreet stocks or resident populations which may be prone to overexploitation. A determination of the biological status of the stocks is necessary as effort in the fishery increases.

The high rate of tag returns and the intensity of fishing effort in the major fishing areas of Coffs Harbour, Sir John Young Banks and Montague Island suggest that the populations in these areas may be heavily fished. It is of importance to the management and understanding of the fishery to identify the presence of single or multiple stocks.

Congeneric species in Japanese and in eastern Pacific waters undertake marked migrations, and movement of fish between the major fishing locations on the north and south coasts of New South Wales was therefore expected. No kingfish tagged off New South Wales has been recaptured at Lord Howe Island or vice versa.

The results of this programme will benefit the fishing industry by providing an understanding of the stock structure, an essential aspect of the biological study being undertaken by the Fisheries Research Institute (see supporting data) which will provide the basic information for stock assesment and rational management.

This application seeks funds to purchase suitable equipment and chemicals and employ staff to enable a stock identification study to be undertaken.

Locations of Operation:

The study will be based at the Fisheries Research Institute, Cronulla. Isozyme analyses will be carried out at the School of Zoology, University of New South Wales.

The commercial fishery for yellowtail kingfish in NSW ranges from the Queensland to Victorian borders. Limited commercial fishing takes place on Lord Howe and Norfolk Islands. The majority of the NSW catch comes from Coffs Harbour, Sir John Young Banks and Montague Island. Tissue samples will be obtained and morphometric studies will be carried out both in the field and on fish marketed in Sydney from these locations. Samples will be obtained from Queensland, Victorian, South Australian and Tasmanian waters for isozyme studies and also from Lord Howe Island and Norfolk Islands for comparison.

Proposal in Detail:

(a) Overall Plan.

(1) Sampling: The sampling programme will centre on New South Wales with samples being sought from other states. Two hundred fish, one hundred adult and one hundred juveniles will be collected from each of the following areas:- Coffs Harbour, Sir John Young Banks, Montague Island, Lord Howe Island and interstate sites (and Norfolk Island) as available. Morphometric measurements will be made at the port of landing and a sample of liver and muscle removed from each. The samples would be immediately frozen in liquid nitrogen and later used for isozyme analysis by electrophoresis. This methodology would permit the fish to be marketed in the normal manner.

(2) Isozyme Analysis: A pilot sample of 30 fish will be used for preliminary investigations by electrophoresis. Approximately 40 different enzyme systems will be examined. This should represent between 60 to 70 genetic loci. Those loci which are always expressed in the same form will be discarded as they cannot be used to show differences between stocks. From the remaining polymorphic enzymes 5 or 6 will be selected for detailed study in each population (see sampling above).

(3) Data Analysis: Isozyme data will be analysed using the computer programme PHYLIP version 2.5 (Felsenstein 1981,1982). Differences between populations will be analysed by means of G- tests(Sokal and Rolfe 1973). Morphometric data will be analysed by principle component analysis. Most analyses will be carried out at the Fisheries Research Institute.

Stock analysis using isozymes as genetic markers is a labour intensive procedure. Full time assistance is required for the measurement of fish prior to removal of tissue samples, preparation of tissue samples for electrophoresis, electrophoretic analysis and subsequent data analysis and writing up.

(b) Facilities Available:

Computer facilities, statistical advice, typing and administrative support will be made available by the Fisheries Research Institute. Laboratory space and facilities for electrophoresis will be made available by the School of Zoology, University of New South Wales.

(c) Supporting Data:

The tagging of kingfish will continue through the Institute's Gamefish Tagging Programme and increased effort should ensure approximately 3,000 fish per year being released. A programme on the biology and fishery for kingfish is currently being undertaken by the Fisheries Research Institute. Growth, reproduction and feeding will be examined as well as the importance of the species to the amateur and professional fisheries. Routine sampling at the Sydney Fish Markets will continue.

Proposed Commencement Date: June 1986.

Funds Requested :

	1986/87	1987 (six months)
Salaries	22,768	11,384
Operating Expenses	8,800	1,200
Capital Items	1,200	-
Total	32,768	12,584
Estimated Income	Nil	Nil

Funds Provided by the Applicant:

	1986/87	1987
Biologist (50% of time)	15,000	7,500
Vehicle Running	1,500	500
Gamefish Tags	500	500
Computing Costs	500	500

Co-operating Agencies:

This project will be carried out in co-operation with Dr. P. Dixon of the School of Zoology at the University of New South Wales. Instruction in the techniques of isozyme analysis by electrophoresis and methods of data analysis will be provided if required. Sampling will be undertaken with the co-operation of the Sydney Fish Marketing Authority and the various regional Fishermen's Co-operatives.

Similar Work being Undertaken in Australia:

No similar work is being undertaken on this species in Australia.

Plans for Reporting Results

Results will be published as scientific papers in recognized scientific journals and in Australian Fisheries.

DETAILED STATEMENT OF FUNDS REQUESTED

	1986/87	1987 (six months)
(a) Salaries and Wages		
Technical Officer	19,798	9,899
On Costs (payroll tax, workers' comp., insurance, leave loading at 15%)	2,970	1,485
Total Salaries and Wages	22,768	11,384
(b) Operating Expenses		
(i) Travel Expenses (20 days at $70 for Biologist + T.O)	2,800	
(10 days at $70)		700
(ii) Miscellaneous Expenses	500	500
(iii) Tags	1,500	-
(iv) Chemicals/Glassware	4,000	-
Total Operating Expenses	8,800	1,200
(c) Capital Items		
1 x 35 HC Liquid Nitrogen Freezer	1,200	-
Total Capital Items	1,200	-
Gross Total Cost	32,768	12,584

3. EXPENDITURE REPORT

	Allocation	Expenditure
1986/87		
Salaries	22,768.00	18,076.01
Operating	8,800.00	5,183.95
Capital Items	1,200.00	1,270.00
	32,768.00	24,529.96
1987/88		
Salaries	13,355.00	12,950.73
Operating	1,200.00	4,902.16
	14,555.00	17,852.89
Total	47,323.00	42,382.85

4. SUMMARY OF RESULTS

The yellowtail kingfish Seriola lalandi is a circumglobal species with a disjunct distribution in the northern and southern hemispheres. In Australia it is distributed in coastal waters from Rockhampton in Queensland, around the southern coastline to Shark Bay in Western Australia. S. lalandi is taken by commercial and recreational fishermen throughout the year in New South Wales and at Lord Howe and Norfolk Islands.

More than 660 kingfish have been recaptured from more than 9,000 tagged and released between 1976 and 1987. Most were small with a mean length at recapture of 60 cm l.c.f. (2.02 kg). These fish were recaptured from between 1 to 1205 days after release at distances up to 2,100 km from the tagging locations.

While the majority of fish were recovered near the tag and release site one moved from Sydney to Lord Howe Island and another from Coffs Harbour to New Zealand.

No consistent migration patterns were detected from the tagging data but the large distances travelled by some kingfish illustrate the capability of this long lived, pelagic species to move throughout and beyond NSW coastal waters.

Subpopulation structure of kingfish along the NSW coast was investigated using fish from five locations (Coffs Harbour, Crowdy Head, Greenwell Point, Narooma and Lord Howe Island). Heart and liver tissues were used to screen for polymorphisms.

Seven polymorphic ($p<0.99$) enzymes (ACON, Est-1, Est-2, Gpi-1, Gpi-2, IDH and MPI) were selected for population analysis.

Electrophoretic data were analysed for within population differences and for temporal and spatial variation between populations using G-statistic comparisons and Nei's Genetic Distance (D) and Identity (I). Analyses were performed using a series of computer programs written by Holliday (1987), and Felsenstein's PHYLIP package (1981, 1982).

Statistical analysis using G-statistic comparisons between size groups (small: <70cm l.c.f. large: >80cm l.c.f.) from Lord Howe Island were not significantly different.

Statistical analysis between the five locations were not indicative of spatial structuring. Genetic Distance values averaged 0.0014 which is similar to the expected for a panmictic (randomly mating) population.

Significant temporal allele frequency differences were found between the two populations from Greenwell Point (collected October 1986 and October 1987). These differences may indicate temporally separated subpopulations, genetic drift or patchy larval recruitment.

Samples of kingfish, S. lalandi from New Zealand, Australia and the United States of America; samson fish, S. hippos ; and amberjack, S. dumerili were examined for fixed electrophoretic differences. A survey of 14 enzymes representing 30 presumed loci did not indicate any fixed differences within the S. lalandi samples. A large number of fixed differences were distinguished between the three separate but morphologically similar species of Seriola.

S. lalandi are pelagic spawners. In New South Wales waters spawning occurs in October and November with reproductive activity possibly extending until March. Kingfish are serial spawners and large individuals probably spawn over a period of several months. Juveniles appear off the coast of New South Wales throughout the summer indicating an extended period of recruitment.

5. RECOMMENDATIONS

The commercial and recreational fishery for kingfish in New South Wales should be managed as a single unit. On the basis of electrophoretic and tagging results it appears that kingfish comprise one subpopulation and individuals are capable of dispersal throughout the NSW fishery.

As small taggged kingfish tend to remain at the same locations and show little movement in the first year after tagging they may be prone to overfishing in accessible areas or in areas of local abundance. Fishing methods, such as floating traps, which target on small fish should be restricted until the impact on the stock can be assessed.

No kingfish tagged at either Lord Howe or Norfolk Islands have been recaptured at other locations. Although insufficient numbers may have been tagged at these places to demonstrate large scale movements or exchange, caution should be exercised in the management of kingfish in these geographically isolated areas.

Recommendation for future research

Greenwell Point is the location of the major commercial fishery for kingfish in NSW waters. Replicate electrophoretic samples from this location suggest the possibility of temporal replacement of kingfish subpopulation(s), patchiness of larval recruitment, or genetic drift.

Further examination of kingfish from the Greenwell Point region using electrophoresis, tagging and/or investigation of fish larvae distributions could clarify this problem.

The location of spawning areas and definition of spawning times and duration require further investigation. Field sampling of adult kingfish from the coast of New South Wales and offshore islands for gonadosomatic indices with histological confirmation of reproductive status would elucidate this aspect.

The majority of kingfish tagged so far have been small. Further information is required on the movements and migrations of adult fish. This could be achieved by the increased efforts of recreational fishermen and/or by the charter of a suitable commercial kingfish vessel capable of targetting larger fish. Increased tagging of all size classes at Lord Howe and Norfolk Islands is necessary for a better understanding of the kingfish fisheries in these areas and their relationships with those of coastal New South Wales.

APPENDIX I

Genetic Variation and Dispersal of the Yellowtail Kingfish *Seriola lalandi* from New South Wales waters.

GENETIC VARIATION AND DISPERSAL OF THE YELLOWTAIL KINGFISH, *SERIOLA LALANDI*, FROM NEW SOUTH WALES WATERS

Adam K. Smith

Submitted as partial requirement for an
Honours degree in Biological Oceanography
at the University of New South Wales

November 1987

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ABSTRACT

Subpopulation structure of Seriola lalandi along the New South Wales coast was investigated using electrophoresis and life history information. Seven polymorphic ($p<0.99$) enzymes were selected for population analysis: ACON, Est-1, Est-2, Gpi-1, Gpi-2, IDH and MPI. Heart and liver were used to screen polymorphic loci. S. lalandi were collected from five locations from NSW waters (Coffs Harbour, Lord Howe Island, Crowdy Head, Greenwell Point and Narooma). A replicate sample was collected from Greenwell Point.

Populations of S. lalandi were in agreement with Hardy-Weinberg Equilibrium. G-statistic comparisons between locations were not indicative of spatial structuring of S. lalandi in N.S.W. There are statistically significant allele frequency differences between two populations from Greenwell Point which may indicate temporally separated subpopulations. Genetic distance values average 0.0014 which is similar to the expected for a panmictic population. Tag-recapture results indicated that size is related to dispersal. Spawning period, examined using a Gonad Somatic Index (GSI), did not appear to be syncronised between locations.

Electrophoresis did not differentiate S. lalandi from Australia, New Zealand and California. Three Seriola species which occur sympatrically in NSW waters were readily distinguished by fixed allelic differences.

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1. INTRODUCTION

The yellowtail kingfish, S. lalandi, is an important commercial and recreational species in N.S.W. It is regarded as sashimi quality in Australia and overseas. Recreational fishermen target S. lalandi because it is large, abundant and a strong fighter (Bowerman, 1979; Grant, 1982). Juveniles and some adults have been tagged. A high tag recapture rate (661 recaptures or 10% from 1976-87) and indications of a limited home range have prompted questions concerning the possible over exploitation and stock structure of this species (FRI File 85/2489). Biological information on S. lalandi is mainly anecdotal for Australia. A detailed study of the California fishery revealed two groups of S. lalandi which mix to a limited extent (Baxter, 1960). Australian fishermen often comment on the differences in body shape, average size, colour and variable eating qualities of individuals. A myxosporan parasitic infection appears to be heavy in S. lalandi for south Queensland but rare in NSW fish (Lester, 1982). Because knowledge of S. lalandi was so limited the Fisheries Research Institute, (Cronulla) have begun to investigate the biology of this species. An electrophoretic investigation of S. lalandi throughout NSW waters is one component of this study.

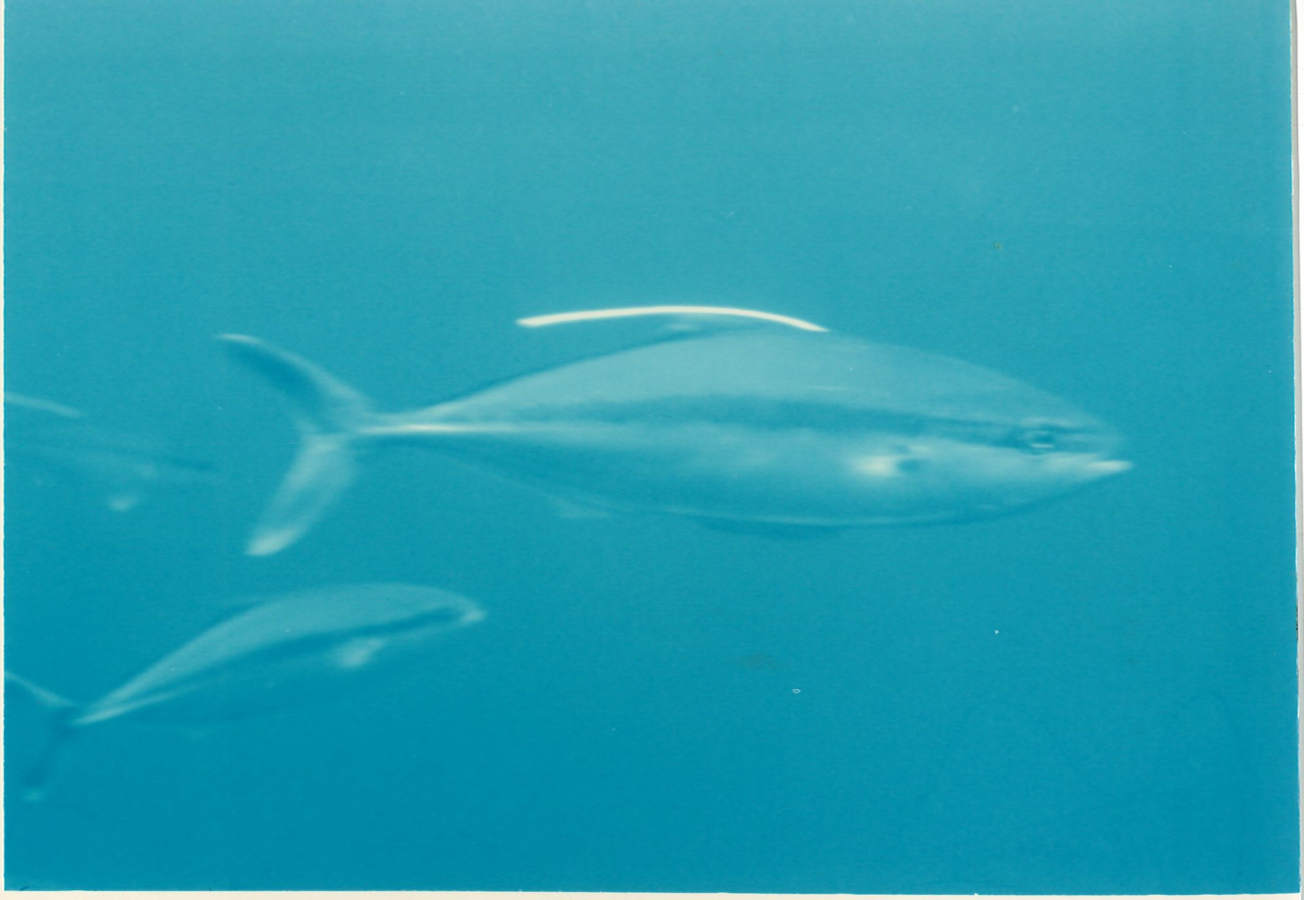
Electrophoresis is a technique which is useful for the delineation of species, species boundaries and population structure. The electrophoretic technique has been widely applied in fisheries investigations because of the importance of defining subpopulations for fishery management. A description of the principles of electrophoresis with examples of investigations of marine teleosts is provided in the accompanying literature review. An understanding of the species life history is important when interpreting electrophoretic results. Therefore, a review of the biology of S. lalandi is presented in Chapter Two.

2. THE YELLOWTAIL KINGFISH Seriola lalandi

2.1 Taxonomic status

The yellowtail kingfish is placed in the family Carangidae, genus Seriola (species name lalandi Note: often misspelt as lalandei or lalandii). It was first described in 1833 by Valenciennes.

S. lalandi has a confusing taxonomic history and numerous synonyms due to few detailed original descriptions (Smith, 1961), variable body shape and colour and disjunct distributions (Laroche, et al ,1983; Smith-Vaniz, 1983). The International Game Fishing Association (IGFA) continues to recognise S. lalandi as a complex comprised of three subspecies: Californian yellowtail, S. lalandi dorsalis; Asian yellowtail, S. lalandi aureovittata; and southern yellowtail, S. lalandi lalandi. This subspecies classification is primarily based on disjunct distribution and lack of evidence of interaction between the subspecies (Anon., 1978, 1985). Mather (1958) examined S. lalandi from the southern hemisphere and east and west coasts of the north Pacific. From counts of dorsal rays, gillrakers and vertebrae he regarded S. lalandi as one species. Gushiken (1983) and Smith-Vaniz (1986) agree with Mather (1958) and concluded that the S. lalandi complex comprises a circumglobal species.



2.2 Description

Body elongate, moderately compressed. Profile from snout to second dorsal fin is gently and evenly convex; the ventral outline is slightly curved from the tip of the lower jaw to the caudal peduncle. The head is rather long, 24.4 to 29.9 percent of standard length. Whole cheek scaly, about 12 series, wide preopercle flange and rest of head naked. Eye smallish. Mouth of moderate size and oblique, extending to below the front third of the eye. Broad bands of small villiform teeth in the jaws, on the vomer, palatines and tongue. Maxilla with round dorso-posterior corner. Maxilla reaches below front margin of pupil (Baxter, 1960; Smith, 1961; Smith and Smith, 1966; Grant, 1982; Gushiken, 1983; Smith-Vaniz, 1986).

D VI-VII + I,30-37. Second dorsal is long and low with an elevated anterior lobe. A II + I,19-23. First two anal spines very small and may be covered by skin in large fish. P i,20. V I,5. The pectorals and ventrals are usually equal in length. Caudal fin is widely forked. Gill raker totals; 27-30 (Mather, 1971, Gushiken, 1983), 14-15 (Smith, 1961), 19-31 (Baxter, 1960; Smith-Vaniz, 1986). Lateral line distinct with pored scale count; 114-162 (Baxter, 1960), 156-203 (Last, et al, 1983, Grant, 1982). Scales are small, generally oblong, cycloid and deeply embedded in the dermis of the skin. Vertebrae total 25 (11 precaudal and 14 caudal).

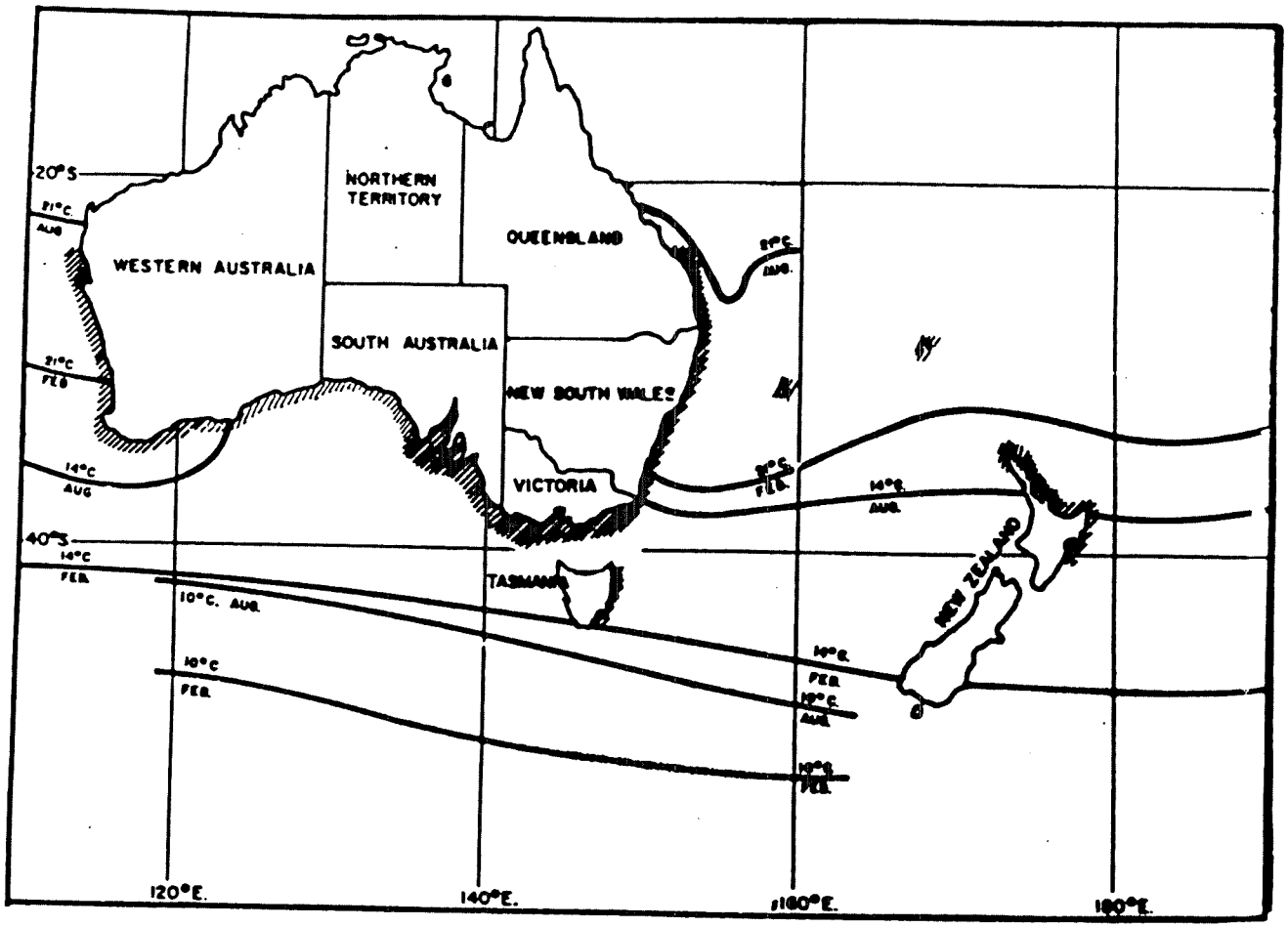


Figure 1. Map to show distribution of Seriola lalandi in Australasia.

2.2.1 Colour

Colour variable. Adult S. lalandi are a steel-blue to purple or green above, silvery white below. Distinct yellow-amber horizontal stripe along the median line of the flanks from the eye to the caudal peduncle. Pelvic fin light yellow, caudal fin bright yellow. Dorsal, anal and pectoral fins are olivaceous to light blue (Thomson, 1977; Coleman, 1983). Large fish sometimes freckled with small black spots. Juveniles are darker than adults and may have several dark vertical stripes depending on the stage of development. Juvenile colouration is described in more detail by Baxter (1960), Brownell (1979) and Sumida, et al,1985.

2.2.2 Size

Maximum recorded length is 2 metres and 55kg from Sydney, Australia. Estimated to reach 2.5 metres and 70kg (Roughley, 1951; Marshall, 1964; Grant, 1982). S. lalandi are common to 1.0 metre and 10-15kg. The size of fish in a school is usually fairly uniform (Roughley, 1951; Whitley, 1962,1980).

2.3 Distribution of adults, juveniles and larvae

S. lalandi are disjunctly distributed in the northern and southern hemispheres. They do not occur in equatorial waters. In the southern hemisphere they are reported from temperate waters of

Australia, New Zealand (North Island), Argentinia, Brazil and South Africa. In the northern hemisphere they are found throughout the Gulf of California, along the Pacific coast of North America, Mexico and British Columbia; Hawaii, and eastern Asia (Southern Japan and Yellow Sea) (Anon, 1978,1985; Gushiken, 1983; Paul, 1986; Uchida and Uchiyama, 1986).

In Australia, S. lalandi are continuously distributed in coastal waters from Rockhampton in central Queensland, around the southern coastline to Shark Bay in Western Australia (Figure 1) (McCulloch, 1927; Ogilby, 1954; Marshall, 1964; Grant, 1982; Hutchins and Thompson, 1983; Russel, 1983). S. lalandi are abundant all year along the New South Wales coast and at Lord Howe and Norfolk Islands (Allen, et al, 1976;) and seasonally common along the remaining states.

Adult S. lalandi prefer rocky shores, reefs and islands. They are occasional visitors to estuaries. Juveniles (<30cm) are generally distributed offshore, near or beyond the continental shelf. They may aggregate beneath floating objects eg. seaweed (Mitchell and Hunter, 1970) or man-made fish attracting devices (FADS) (Matthews, 1973; Deacon, 1984).

In a survey of fish larvae distribution off central Baja California, S. lalandi were collected from nearshore to about 200 miles offshore (Sumida, et al, 1985). In N.S.W., S. lalandi larvae (size <10mm) have been collected from coastal waters of the central coast and Sydney region (A. Miskiewicz pers. comm.)

2.4 Life history information

2.4.1 Reproduction

S. lalandi are pelagic spawners. Adults move 'offshore' to spawn (Walford, 1937; Thompson and Munro, 1974; Anon., 1982; Crooke, 1983). Smith and Paul (1960) examined maturity and fecundity for the Californian yellowtail kingfish. They observed that the state of maturity varied among individuals from the same school of fish. Ova diameter was used to determine that spawning period begins in July and continues until October (northern hemisphere summer and autumn). Ova diameter in ripe females was 1.31-1.80mm. Only small immature ova were present during winter months. The age and size at first maturity was 2 years and 506mm (l.c.f) for some fish, but most spawn at about 3 years and 634mm. Larger individuals apparently spawn a little at a time over at least three months (Smith and Paul, 1960; Kramer and Smith, 1979; Crooke, 1983).

In the southern hemisphere January to March is the spawning period of South African S. lalandi (Smith, 1959). In Australia, Mensforth (1986) recorded the increased juvenile (3-5kg) abundance in January in south Australian waters as evidence of large-scale reproduction in this area. It is also popular opinion that the presence of large kingfish in shallow waters around the south Australian gulfs in October-November is due to spawning behaviour. In N.S.W, spawning of S. lalandi probably occurs from October to November (Anon., 1982). Observations by commercial and recreational fishermen in N.S.W suggests that S. lalandi spawns periodically from February to

November. A GSI calculated from female S. lalandi gonads indicates large ripe gonad condition and an apparent spawning peak during February to March (see Fig 11).

2.4.2 Movements

Berry and Birch (1979) reported that movements and migratory routes of carangids were a complicated pattern of spawning migrations, developmental migrations and temperature induced range extensions. Extensive tagging of Californian yellowtail kingfish indicated that most fish travelled north from central Baja California to California during warmer water (Spring) (Baxter, 1960). Fish from the low peninsula and the Gulf of California did not move large distances and may be a separate group. Dispersal of tagged individuals was related to size. Small S. lalandi (<two years old) were found to have a limited home range. At age three to eight, they school with fish of similar size and travel greater distances. Large fish, eight years and older are apparently sedentary (Baxter, 1960; McClane, 1965; Crooke, 1983).

In Australia, Whitley (1980) stated that S. lalandi probably migrates over great distances. Roughley (1951) reported that in September to October large schools of S. lalandi travel from the north coast of N.S.W in a southerly direction. Seasonal shoaling in Summer was indicated by Blackburn and Tubb (1950). The N.S.W Fisheries Research Institute Gamefish tagging program indicates that juveniles (up to 75 cm) generally remain in the area they were tagged for at least 12 months (Pepperell, 1985). However, one juvenile recently

travelled 800 km from Sydney to Lord Howe Island (K. Deguara, pers comm.). There is limited tag and recapture information for adult fish but it has been inferred that adults travel greater distances than juveniles, which is consistent with the results obtained from California by Baxter (1960). (Pepperell, 1982; 1983; 1984; 1985) (see fig 14).

2.4.3 Feeding

S. lalandi are opportunistic, daytime feeders (Baxter, 1960). They are predominately carnivores, eating fish, squid and crustaceans (Baxter, 1960; Scott, et al, 1974; Bowerman, 1979; Grant, 1982). Small S. lalandi eat mainly small schooling fishes, while large (>70cm) prefer squid (Coleman and Mobley, 1984; Smale, 1985). Smith-Vaniz (1986) reported that S. lalandi follow the pilchard migration from Transkei to Natal (South Africa).

2.4.4 Parasites

A parasite checklist for S. lalandi from Australian waters is presented by Beumer, et al, (1983). Lester (1982) recorded spores of a myxosporan, Unicapsula seriola from skeletal muscle for 16 of 26 fish examined from Brisbane, Queensland. This parasite is responsible for 'milky flesh' upon cooking. One outbreak and four cases of toxic fish poisoning (ciguatera) has been attributed to S. lalandi (Gillespie, et al, 1986).

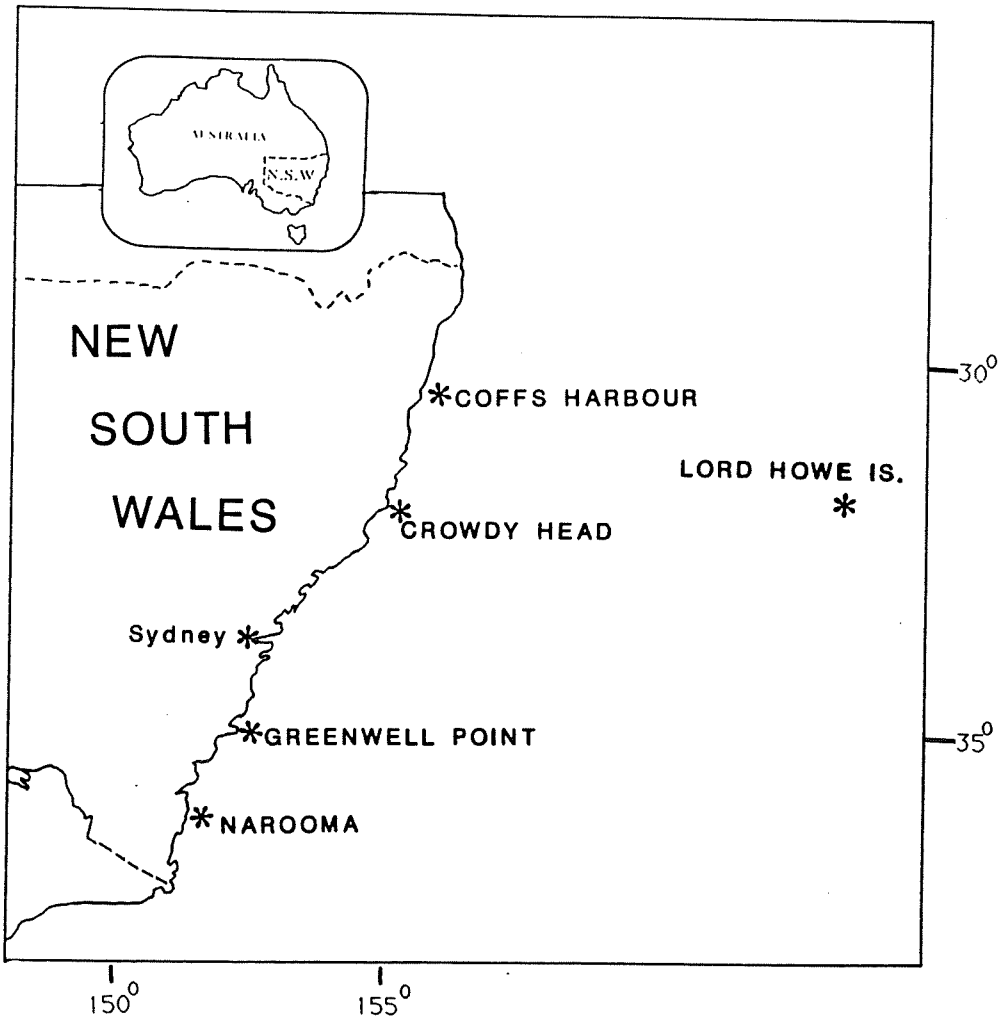


Figure 2. Location map of S. lalandi collections from New South Wales.

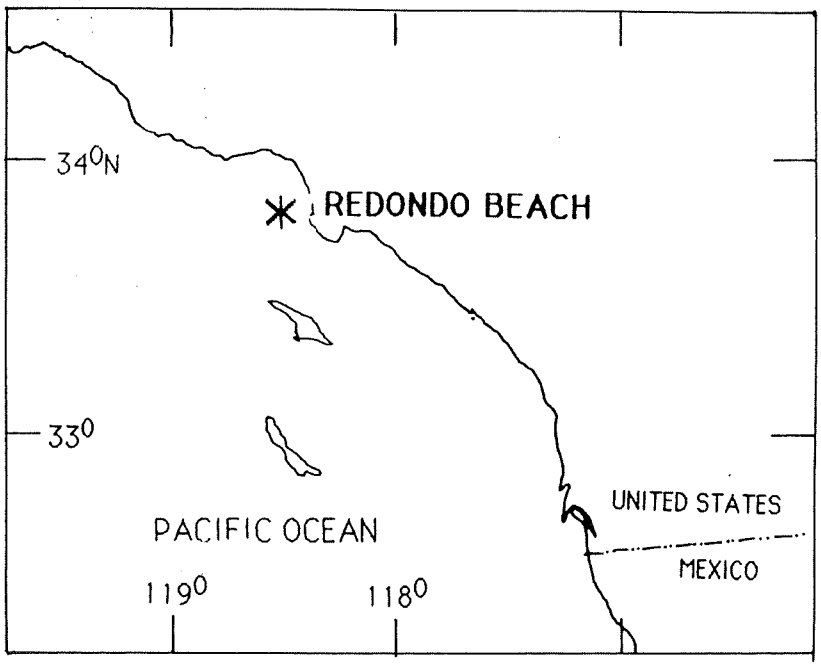


Figure 3. Location map of S. lalandi collections from America.

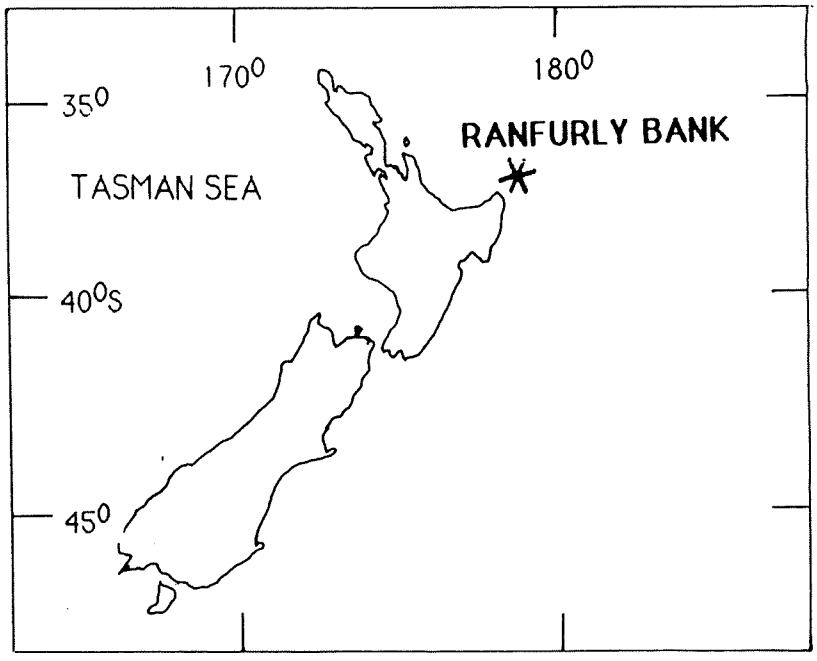


Figure 4. Location map of S. lalandi collections from New Zealand.

3. MATERIALS AND METHODS

3.1 Collection of samples

Tissue and organ samples from S. lalandi from N.S.W waters were obtained with the cooperation of commercial and recreational fishermen. The commercial catch was sampled onboard commercial fishing vessels or by consignment of whole ungutted fish to the Sydney Fish Markets (see Plate 2). The recreational catch was sampled at sportsfishing conventions and from a charter boat operator.

The sampling strategy for a pilot study aimed to collect 10 fish from 2-3 locations, and the investigation of S. lalandi population structure aimed to collect 100 samples from the following locations: Coffs Harbour, Lord Howe Island, Crowdy Head, Greenwell Point and Narooma (see Fig. 2). A total of 553 S. lalandi were collected from N.S.W, between October 1986 and October 1987 (see Table 1). Sample numbers from five locations ranged from 67-109 (mean 94). A replicate data set was sampled from one location (Greenwell Point).

Samples of S. lalandi tissues from Ranfurly Bank (New Zealand) and Redondo Beach (California), and S. hippos and S. dumerili from Coffs Harbour (N.S.W) were obtained with the cooperation of fisheries departments, commercial and recreational fisherman. Table 2 lists the date, location and number of S. lalandi collected from California (see Fig 3) and New Zealand (Fig 4), and collection information for S. hippos and S. dumerili from Australia. Meristic characters used for

TABLE 1. Collection information for S.lalandi from New South Wales.

DATE	NUMBER	SIZE RANGE	COLLECTION SITE	LOCATION DISSECTED	TISSUES SAMPLE
1/9/86	7	79-92	Crowdy Head	SFM	H,L,RM,WM,E
12/9/86	13	60-108	Coffs Harbour	SFM	H,L,RM,WM,K,E
25/10/86	12	57-112	Greenwell Point	Field	H,L
26/10/86	2	82-93	Greenwell Point	Field	H,L
30/10/86	86	77-107	Greenwell Point	Field	H,L
14/11/86	5	93-104	Coffs Harbour	SFM	H,L
10/12/86	102	29-71	Crowdy Head	SFM	H,L
19/1/87	79	45-110	Coffs Harbour	SFM	H,L
22/1/87	23	64-105	Coffs Harbour	SFM	H,L
1/3/87	1	110	Narooma	Field	H,L
7/3/87	39	33-102	Narooma	Field	H,L
8/3/87	23	50-106	Narooma	Field	H,L
21/5/87	14	68-79	LHI-Elizabeth Reef	Consign.	H,L
26/5/87	18	52-72	LHI-Balls Pyramid	Consign.	H,L
5/6/87	10	49-79	Narooma	SFM	H,L
11/8/87	10	99-108	LHI-Balls Pyramid	Consign.	H,L
17/8/87	7	86-105	LHI-Balls Pyramid	Consign.	H,L
19/8/87	15	85-110	LHI-Balls Pyramid	Consign.	H,L
13/10/87	67	69-94	Greenwell Point	Field	H,L
14/10/87	32	75-100	Greenwell Point	Field	H,L

KEY : SFM (Sydney Fish Market), LHI (Lord Howe Island)
Consign. (Consigned tissues airfreighted to Sydney)
H (Heart), L (Liver), RM (Red Muscle), WM (White Muscle)
K (Kidney), E (Eye Lens)

TABLE 2. Collection information for *S. lalandi* from New Zealand and the United States and *S. hippos* and *S. dumerili* from N.S.W.

LOCATION	SPECIES	NUMBER	TISSUES	COMMENT
NEW ZEALAND (RANFURLY BANK,	S. LALANDI NORTH ISLAND)	10	HEART, LIVER, MUSCLE	
AMERICA (REDONDO BEACH,	S. LALANDI CALIFORNIA	2	HEART, LIVER	FISH DEAD 24HRS
AUSTRALIA (COFFS HARBOUR)	S. LALANDI	10	HEART, LIVER, MUSCLE	
	S. HIPPOS	7	HEART, LIVER	
	S. DUMERILI	3	HEART, LIVER, MUSCLE	

identification of S. hippos and S. dumerili are presented in Table 13.

For each sample set details including date, location, length to caudal fork (l.c.f), sex and condition of gonads, and tissue types obtained for electrophoresis were recorded. For 119 S. lalandi, total weight, ungutted weight and gonad weight were measured. Collections of 15 to 40 fish were obtained during February, March, August and October, 1987. The Gonad Somatic Index (G.S.I) was calculated for female fish, as:

G.S.I = (Wg / Wb-Wg) X 100

where Wb is total body weight in grams, and Wg is gonad weight in grams

3.2 Storage of samples

S. lalandi caught by the N.S.W fishery are generally large, valuable fish, so tissue samples were obtained but no whole fish stored. Tissue samples dissected from fresh S. lalandi specimens were stored in 1.8ml Nunc tubes and kept in an insulated foam container (esky) on dry ice (-90°C), wet ice (0°C) or in a freezer and were transferred as soon as possible to liquid nitrogen (-196°C).

Nunc tubes were labelled with details of species, tissue type, individual identification number, date and location of collection. This information was recorded on adhesive tape labels.

Nunc tubes were stored in a 35VHC cryogenic refrigerator. This system allowed 1000 samples to be stored with ready access to individual samples. The two California and ten New Zealand S. lalandi tissues, and six of both S. hippos and S. dumerili which were few in number and needed extensive electrophoretic analysis, were subdivided into two or more different Nunc tubes to create a store of fresh tissue and prevent repeated re-thawing and enzyme denaturation.

Handling of tissues for homogenisation and electrophoretic analysis involved short term storage in small ice-filled eskies. Tissues were returned to liquid nitrogen.

3.3 Preparation of samples

Tissue samples were mixed with an equal volume of cold homogenizing buffer (see Appendix 1) or distilled water, finely chopped with scissors and macerated with a perspex rod. The resulting slurry was centrifuged, using a MISTRAL 6L centrifuge, at 2°C and 2000 rpm for 20 minutes to separated the cellular debris from the supernatant which contained protein in solution. Liver samples often contained a plug of fat after centrifuging. This fat plug was removed with a spatula as it caused a streaky resolution for some enzymes.

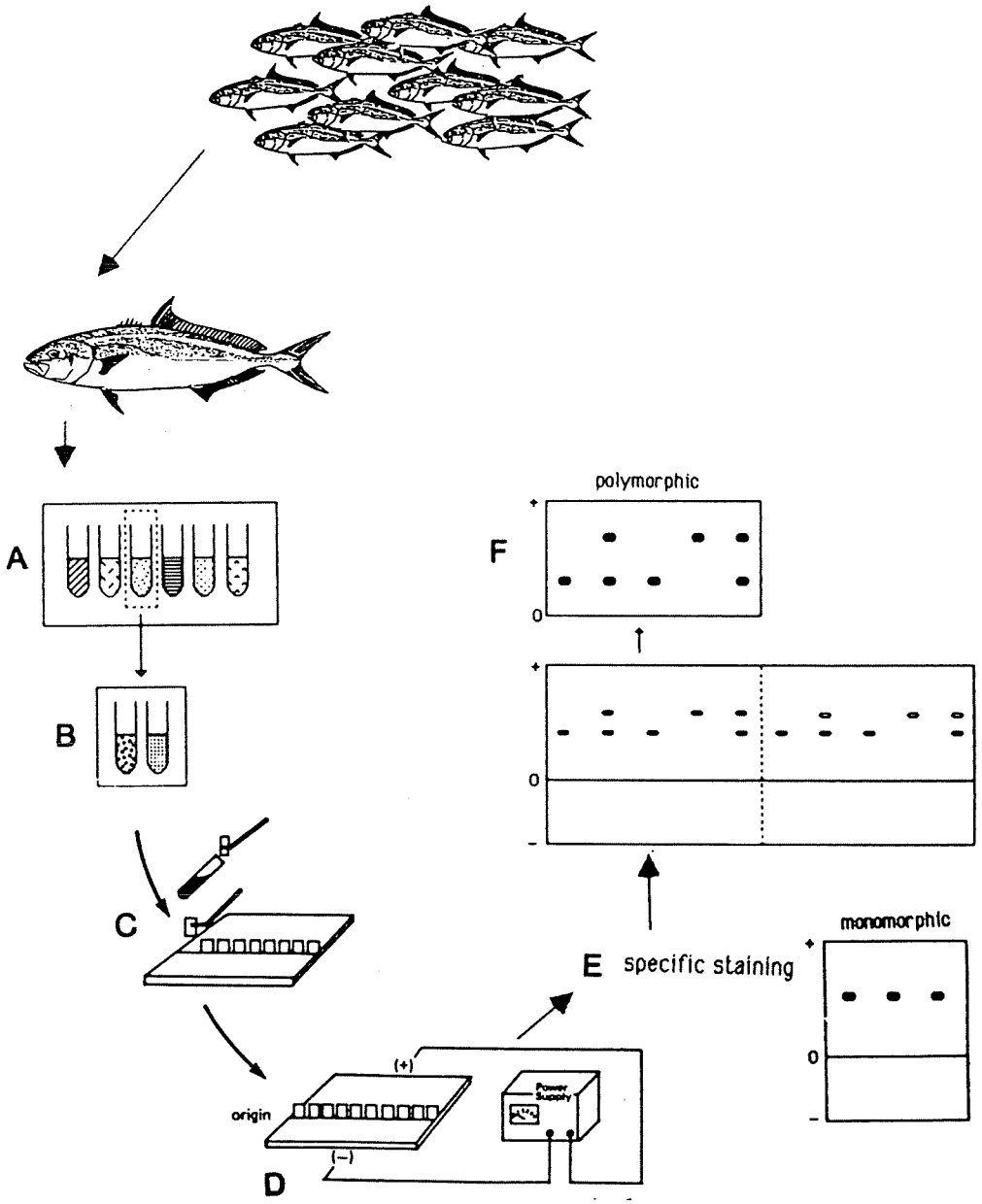


Figure 5. Procedure for starch gel electrophoresis technique.

3.4 Preparation of starch gel

Starch gels were made from 14% SIGMA starch with a range of gel buffers (see Appendix 3). Starch (70gms) was suspended in 500ml of gel buffer (or 42gms in 300ml). This mixture was heated at 250oC at 400rpm on a magnetic stirrer and hotplate, until it reduced in volume and thickened. The hot solution was degassed with a JAVAC vacuum pump and poured into a square perspex mould (dimensions, 500ml: 19.5 x 19.5 x 1cm, 300ml: 19.5 x 19.5 x 0.5cm). The gel was allowed to cool at room temperature and then covered with plastic food wrap to prevent dessication, and stored in a refrigerator overnight.

3.5 Electrophoretic procedure

3.5.1 Pilot study.

Figure 5 illustrates the procedure for the S. lalandi pilot study.

A) Samples from 25 individuals were collected from three locations. A range of tissue samples (liver, heart, white muscle, red muscle, kidney and eye) were dissected (see Figure 6) and placed in 1.8ml Nunc tubes.

B) Tissue samples were divided into two. One was homogenised in an equal volume of homogenising buffer and the other in distilled water.

C) The homogenised protein extract from each fish was absorbed onto a filter paper wick (Whatman #3 filter paper: 10 x 2mm or 5 x 2mm) and placed onto the edge of a cut starch gel using jewellers forceps (see

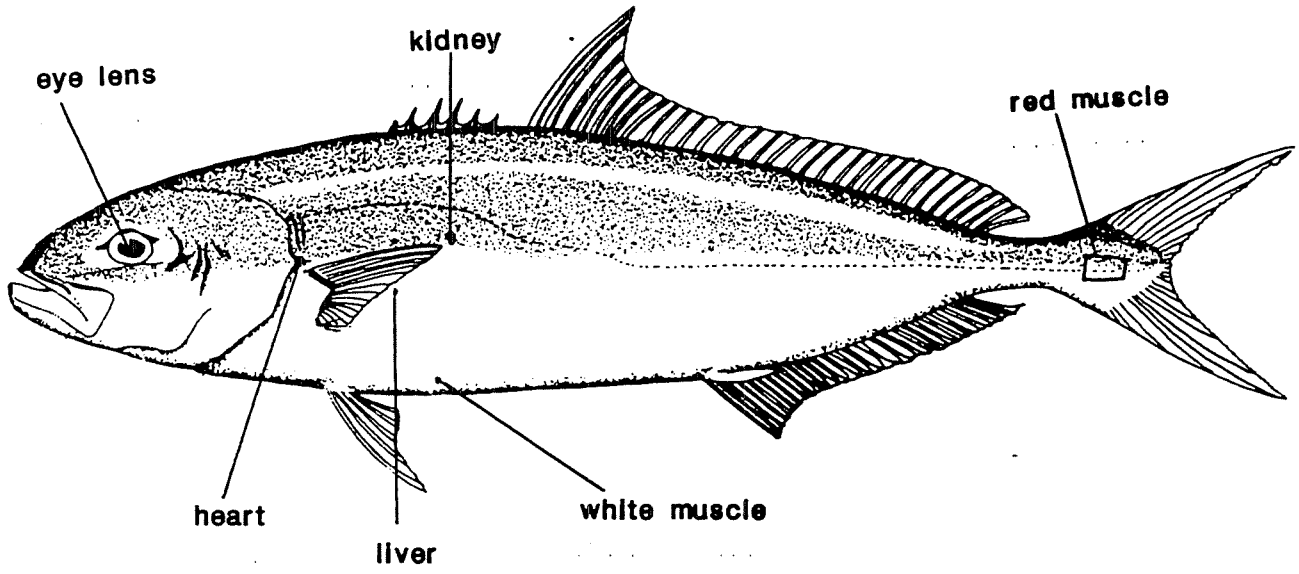


Figure 6. Tissue types sampled for S. lalandi pilot study.

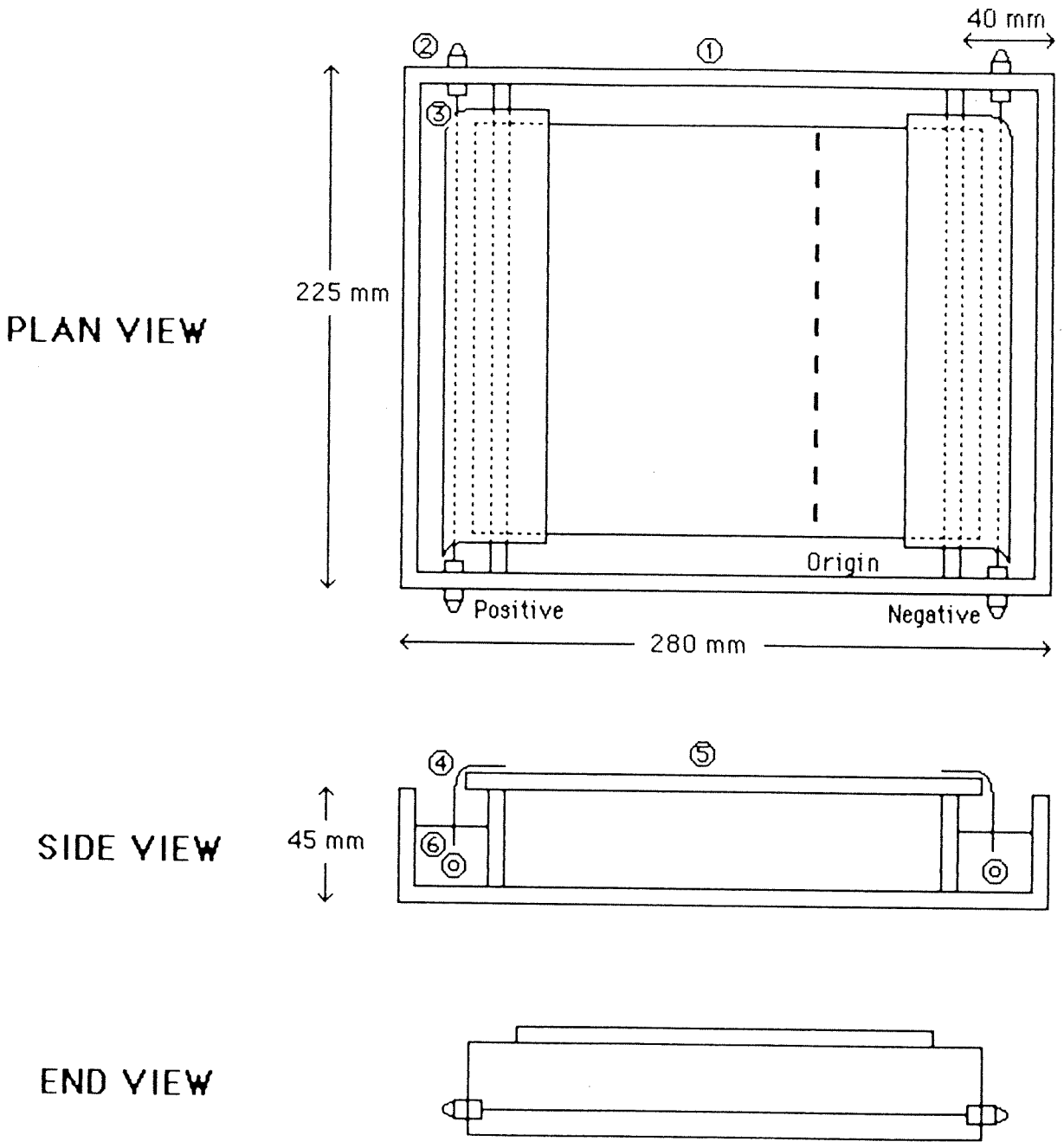


Figure 7. Starch gel electrophoresis apparatus.

PLATE 3 Loading a horizontal starch gel
(Left hand side)

PLATE 4 Connection of loaded gel to power pack.

PLATE 5 Nunc tubes and *S. lalandi* liver and heart tissue.

Plate 3). Samples from 10 fish are shown in fig 5 although typically 25 to 28 samples are loaded on one gel. The pilot study procedure was to load 20 samples of one tissue type (10 in buffer, 10 in water) and a combination of other tissue types. A dye sample was placed in position No. 1 to mark the relative migration of the proteins through the gel.

D) Loaded gels were prepared for electrophoresis as shown in Plate 4. The dimensions of the electrophoresis buffer chamber are given in Figure 7. A direct current was applied across the gel by Heathkit and Pharmacia power packs (see Appendix 2 for conditions). During electrophoresis, protein molecules absorbed on the wick enter and move through the gel.

E) Specific histochemical stains (Recipies in Appendix 3)(modified from Shaw and Prasard, 1970; Harris and Hopkinson, 1976) were prepared while gels were run. A list of enzymes examined in the pilot study is in Table 3. After electrophoresis the gel was sliced (5 slices for 500ml, 3 slices for 300ml). Each slice was placed in a plastic lined tray and covered with a stain and agar overlay. Staining reactions were immediate or required incubation at 37oC in darkness until loci were visualised. Staining reactions were stopped with fixative (Appendix 1) and all gels which showed activity were photographed and scored. The gels were scored by tissue type for amount of activity, resolution (in buffer and water), presumed number of loci and structure (monomorphic or polymorphic).

F) Suspected polymorphic enzymes were further electrophoresed on two to six different buffer systems to obtain the best activity and resolution.

3.5.2 Population study

The laboratory methods for the S. lalandi population study were a similar procedure to Fig 5, however a larger number of individuals were screened and scored for polymorphic loci. The nomenclature of loci follows the recommendations of Richardson, et al, (1986) where multiple loci are numbered sequentially with the most anodal (fastest migrating) loci as 1 eg. Gpi-1 is faster migrating than Gpi-2. Alleles were designated alphabetically, where the fastest migrating allele was labelled A.

3.5.3 Systematic study

A small number of samples of S. lalandi from N.S.W., N.Z and California, and S. hippos and S. dumerili were investigated for 14 enzymes representing 28 presumed loci. The enzymes were selected from the reults of the pilot study.

3.5.4 Electrophoretic data analysis

Genotype numbers for all sample collections were analysed using the G-statistic (ln likelihood method) to determine if the genotype frequencies fitted the Hardy-Weinburg expectation. Smith's H statistic was calculated as an additional test of Hardy Weinburg equilibrium for genotype frequencies when p>Ø.2. Sample collections that had two distinct length classes, and sex ratios for all samples

TABLE 3. Enzymes examined for S. lalandi pilot study

Enzyme	Abbreviation	EC NUMBER
Acid phosphatase	ACPH	3.1.3.2
Aconitase	ACON	4.2.1.3
Adenosine deaminase	ADA	3.5.4.4
Adenylate kinase	AK	2.7.4.3
Alcohol dehydrogenase	ADH	1.1.1.1
Aldolase	ALD	4.1.2.13
Alkaline phosphatase	ALKPH	3.1.3.1
Amino acid oxidase	AO	1.2.3.1
Arginase	ARG	3.5.3.1
Aryl sulphatase	ARS	3.1.6.1
Aspartate aminotransferase	AAT	2.6.1.1
Carbonic anhydrase	CA	4.2.1.1
Catalase	CAT	1.11.1.6
Creatine kinase	CK	2.7.3.2
D-amino oxidase	DAMOX	1.4.3.3
D-aspartate oxidase	DASOX	1.4.3.1
Diaphorase	DIA	1.6.2.2
Enolase	ENOL	4.2.1.11
Esterase	EST	3.1.1.1
Fructose bisphosphate	FDP	3.1.3.11
Fumarase	FUM	4.2.1.2
Gluconate-5-dehydrogenase	GDH	1.1.1.69
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49
Glucose phophate isomerase	GPI	5.3.1.9
Glutamate dehydrogenase	GLUD	1.4.1.3
Glutamate pyruvate transaminase	GPT	2.6.1.2
Glutathione reductase	GSR	1.6.4.2
Glycerol dehydrogenase	GLYDH	1.1.1.6
Glycerol-3-phosphate dehydrogenase	GA3PDH	1.2.1.12
alpha-Glycerophosphate dehydrogenase	GPD	1.1.1.8
Glycolate oxidase	GOX	1.1.3.1
Glyoxylase I	GLO I	4.4.1.5
Glyoxylase II	GLO II	3.1.2.6
Guanine deaminase	GDA	3.5.4.3
Hexokinase	HK	2.7.1.1
Hexosaminidase	HEX	3.2.1.30
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30
Isocitrate dehydrogenase	IDH	1.1.1.42
Lactate dehydrogenase	LDH	1.1.1.27
Leucine amino peptidase	LAP	3.4.11.1
Malate dehydrogenase	MDH	1.1.1.37
Malic enzyme	ME	1.1.1.40
Mannose phosphate isomerase	MPI	5.3.1.8
Peptidase A	PEP A	3.4.11
Peptidase B	PEP B	3.4.11
Peptidase C	PEP C	3.4.11
Peptidase D	PEP D	3.4.13.9
Peptidase S	PEP S	3.4.11
Phosphoglucomutase	PGM	5.4.2.2
Phosphogluconate dehydrogenase	PGD	1.1.1.44
Phosphoglycerate kinase	PGK	2.7.2.3
Pyruvate kinase	PK	2.7.1.40
Sorbitol dehydrogenase	SDH	1.1.1.14
Tetrazolium oxidase	TO	1.15.1.1
Xanthine dehydrogenase	XDH	1.2.1.37

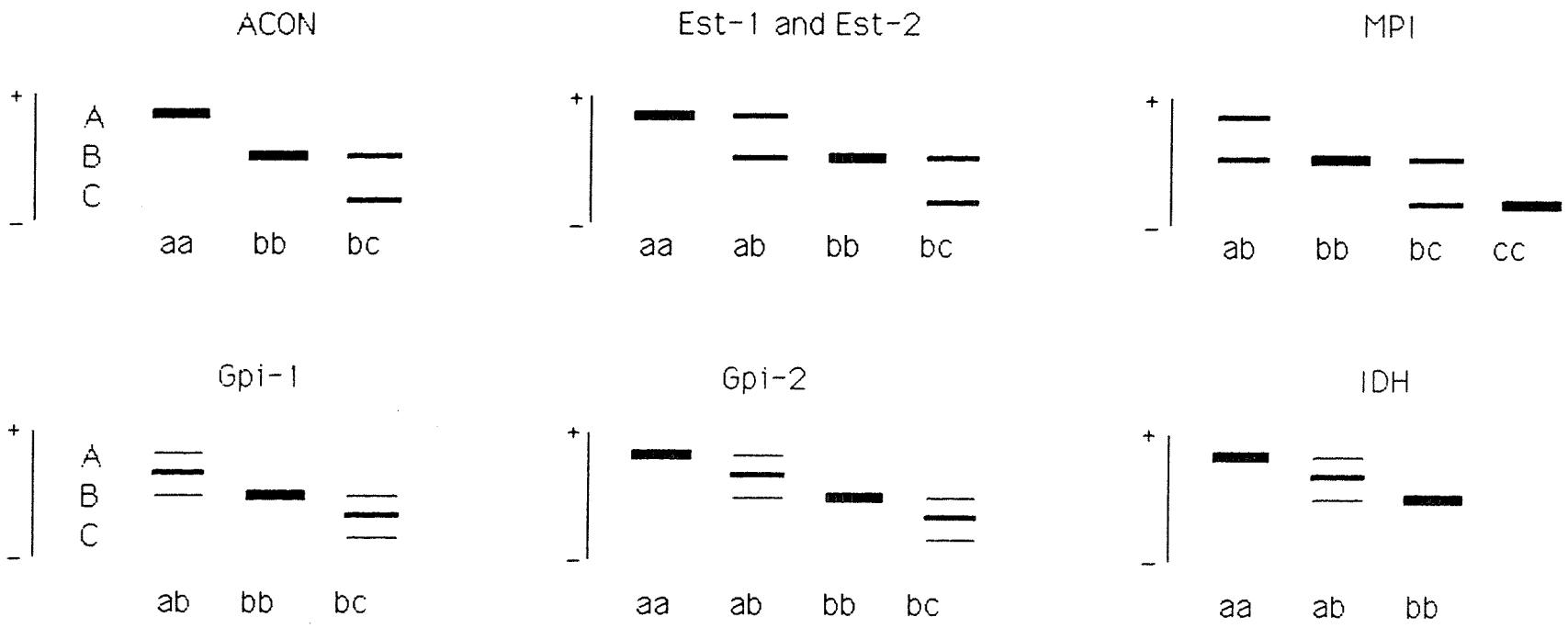


Figure 8. Interpretation of zymogram patterns observed in S. lalandi population study.

TABLE 4. Polymorphic enzymes screened for all samples.

ENZYME	LOCUS	SUBUNIT STRUCTURE	TISSUE	BUFFER
Glucosephosphate isomerase	Gpi-1	dimer	heart	POULIK
Glucosephosphate isomerase	Gpi-2	dimer	heart	POULIK
Isocitrate dehydrogenase	IDH	dimer	liver	TC 7.Ø
Mannosephosphate isomerase	MPI	monomer	liver	TC 7.Ø
Aconitase	ACON	monomer	liver	TC 5.8
Esterase	Est-1	monomer	liver	TC 5.8
Esterase	Est-1	monomer	liver	TC 5.8

TABLE 5. Genotype numbers used for *S. lalandi* population analysis

LOCI	IDH			MPI						Est-1						Est-2					
Location	aa	ab	bb	aa	ab	ac	bb	bc	cc	aa	ab	ac	bb	bc	cc	aa	ab	ac	bb	bc	cc
Coffs Harbour	8	39	53	Ø	2	Ø	85	16	Ø	Ø	5	Ø	98	1	Ø	Ø	4	Ø	1ØØ	Ø	Ø
Crowdy Head	6	41	53	Ø	3	Ø	81	11	Ø	Ø	1Ø	Ø	93	Ø	Ø	Ø	7	Ø	96	Ø	Ø
LHItotal	5	19	3Ø	Ø	Ø	Ø	56	3	Ø	Ø	2	Ø	65	Ø	Ø	1	1	Ø	65	Ø	Ø
GrwellPt1	6	39	52	Ø	Ø	Ø	92	9	Ø	Ø	3	Ø	98	1	Ø	Ø	3	Ø	99	Ø	Ø
GrwellPt2	14	41	43	Ø	2	Ø	85	1Ø	1	Ø	7	Ø	91	Ø	Ø	Ø	2	Ø	96	Ø	Ø
Narooma	7	26	22	Ø	2	Ø	6Ø	8	1	Ø	2	Ø	72	Ø	Ø	Ø	4	Ø	7Ø	Ø	Ø

LOCI	Gpi-1						Gpi-2						ACON					
Location	aa	ab	ac	bb	bc	cc	aa	ab	ac	bb	bc	cc	aa	ab	ac	bb	bc	cc
Coffs Harbour	1	2	Ø	1Ø6	Ø	Ø	1	15	Ø	9Ø	Ø	Ø	Ø	Ø	Ø	1Ø4	5	Ø
Crowdy Head	Ø	2	Ø	97	2	Ø	Ø	12	Ø	89	Ø	Ø	Ø	Ø	Ø	1Ø2	1	Ø
LHItotal	Ø	Ø	Ø	62	Ø	Ø	1	1	Ø	6Ø	Ø	Ø	Ø	1	Ø	57	4	Ø
GrwellPt1	Ø	Ø	Ø	98	1	Ø	1	17	Ø	81	Ø	Ø	Ø	Ø	Ø	75	Ø	Ø
GrwellPt2	Ø	Ø	Ø	98	Ø	Ø	Ø	5	Ø	91	2	Ø	Ø	Ø	Ø	93	5	Ø
Narooma	Ø	Ø	Ø	7Ø	2	Ø	1	14	Ø	66	Ø	Ø	Ø	Ø	Ø	69	2	Ø

KEY: LHI (Lord Howe Island), GrwellPt (Greenwell Point)
Loci as in Table 4.

TABLE 6. Allele frequencies of polymorphic loci investigated for S. lalandi for all sample sites.

ENZYME		LOCATION Coffs	Crowdy	LHI	Grwellptl	Grwellpt2	Narooma
ACON	a	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ
	b	Ø.977	Ø.995	Ø.96Ø	1.ØØØ	Ø.974	Ø.986
	c	Ø.Ø23	Ø.ØØ5	Ø.Ø4Ø	Ø.ØØØ	Ø.ØØØ	Ø.Ø14
EST-1	a	Ø.Ø18	Ø.Ø1Ø	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ
	b	Ø.982	Ø.98Ø	1.ØØØ	Ø.995	1.ØØØ	Ø.986
	c	Ø.ØØØ	Ø.Ø1Ø	Ø.ØØØ	Ø.ØØ5	Ø.ØØØ	Ø.Ø14
EST-2	a	Ø.Ø8Ø	Ø.Ø59	Ø.Ø24	Ø.Ø96	Ø.Ø26	Ø.Ø99
	b	Ø.92	Ø.941	Ø.976	Ø.9Ø4	Ø.964	Ø.9Ø1
	c	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.Ø1Ø	Ø.ØØØ
GPI-1	a	Ø.Ø24	Ø.Ø49	Ø.Ø15	Ø.Ø15	Ø.Ø36	Ø.Ø14
	b	Ø.971	Ø.951	Ø.985	Ø.98Ø	Ø.964	Ø.986
	c	Ø.ØØ5	Ø.ØØØ	Ø.ØØØ	Ø.ØØ5	Ø.ØØØ	Ø.ØØØ
GPI-2	a	Ø.Ø19	Ø.Ø34	Ø.Ø22	Ø.Ø15	Ø.Ø1Ø	Ø.Ø27
	b	Ø.981	Ø.966	Ø.978	Ø.985	Ø.99Ø	Ø.973
	c	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ	Ø.ØØØ
IDH	a	Ø.275	Ø.265	Ø.269	Ø.263	Ø.352	Ø.364
	b	Ø.725	Ø.735	Ø.731	Ø.737	Ø.648	Ø.636
MPI	a	Ø.Ø1Ø	Ø.Ø16	Ø.ØØØ	Ø.ØØØ	Ø.Ø1Ø	Ø.Ø14
	b	Ø.912	Ø.926	Ø.975	Ø.955	Ø.929	Ø.916
	c	Ø.Ø78	Ø.Ø58	Ø.Ø25	Ø.Ø45	Ø.Ø61	Ø.Ø7Ø

KEY: Locations as in Table 5. Enzymes as in Table 4.

TABLE 7. Number of individuals successfully screened for S. lalandi population study.

LOCATION	IDH	MPI	Gpi1	Gpi2	Est1	Est2	Acon
Coffs Harbour	1ØØ	1Ø3	1Ø4	1Ø4	1Ø9	1Ø6	1Ø9
Crowdy Head	1ØØ	95	1Ø3	1Ø3	1Ø1	1Ø1	1Ø3
Lord Howe Island	54	59	67	67	62	62	62
Greenwell Point1	97	1Ø1	1Ø2	1Ø2	99	99	75
Greenwell Point2	98	98	98	98	98	98	98
Narooma	55	71	74	74	72	81	71

were tested by G-statistic for homogeneity of genotype frequencies.

The following list of programs were written by Holliday (1986,1987); Allfreq, Popsep, Popsept, Slpboth, Neistat, Nei4fit, Neiboth, Conplot and Fitplot. These programs form a package (Doc Holliday) which is designed for population analysis. The programs CONTML and FITCH are part of Felsensteins PHYLIP version 2.5 package (Felsenstein, 1981, 1982).

The function of these programs are included in Appendix 4.

4. RESULTS OF ELECTROPHORETIC ANALYSIS OF S. lalandi THROUGHOUT NEW SOUTH WALES

4.1 Pilot study

Eighty presumed gene loci encoding for 55 enzymes (see Table 3 for list of enzymes and abbreviations) were surveyed for genetic variation for six different tissue types of S. lalandi. Appendix 5 summarises the buffer systems, presumed number of loci and observations on tissue activity, resolution and suspected polymorphic loci. There were 11 polymorphic loci ($p<0.99$): ACON, AK, Est-1, Est-2, Gpi-1, Gpi-2, G6PDH, IDH, MPI, SDH and TO.

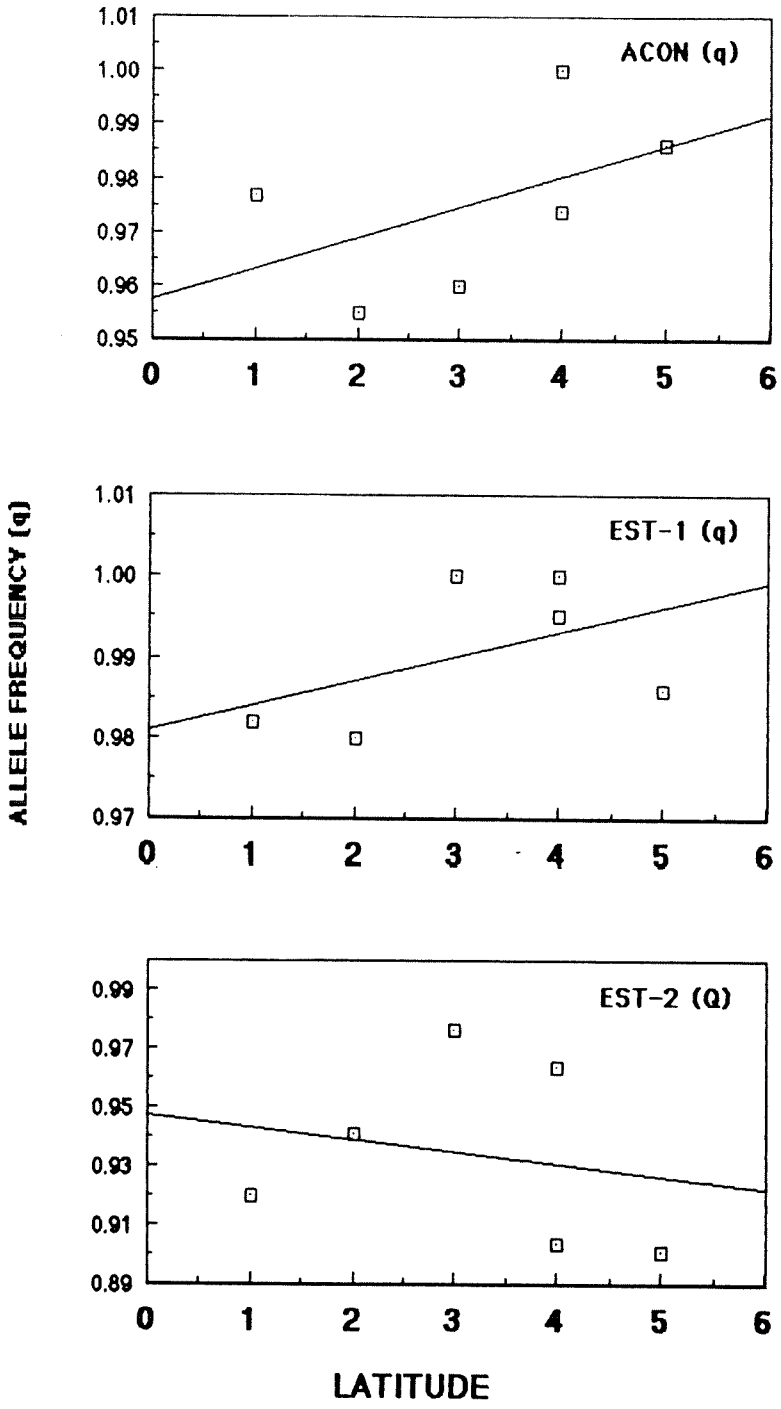


Figure 9 Genotype frequencies of polymorphic loci plotted against latitude.
KEY: 1=Coffs Harbour; 2=Crowdy Head;
3=Lord Howe Island; 4=Greenwell Point
5=Narooma.

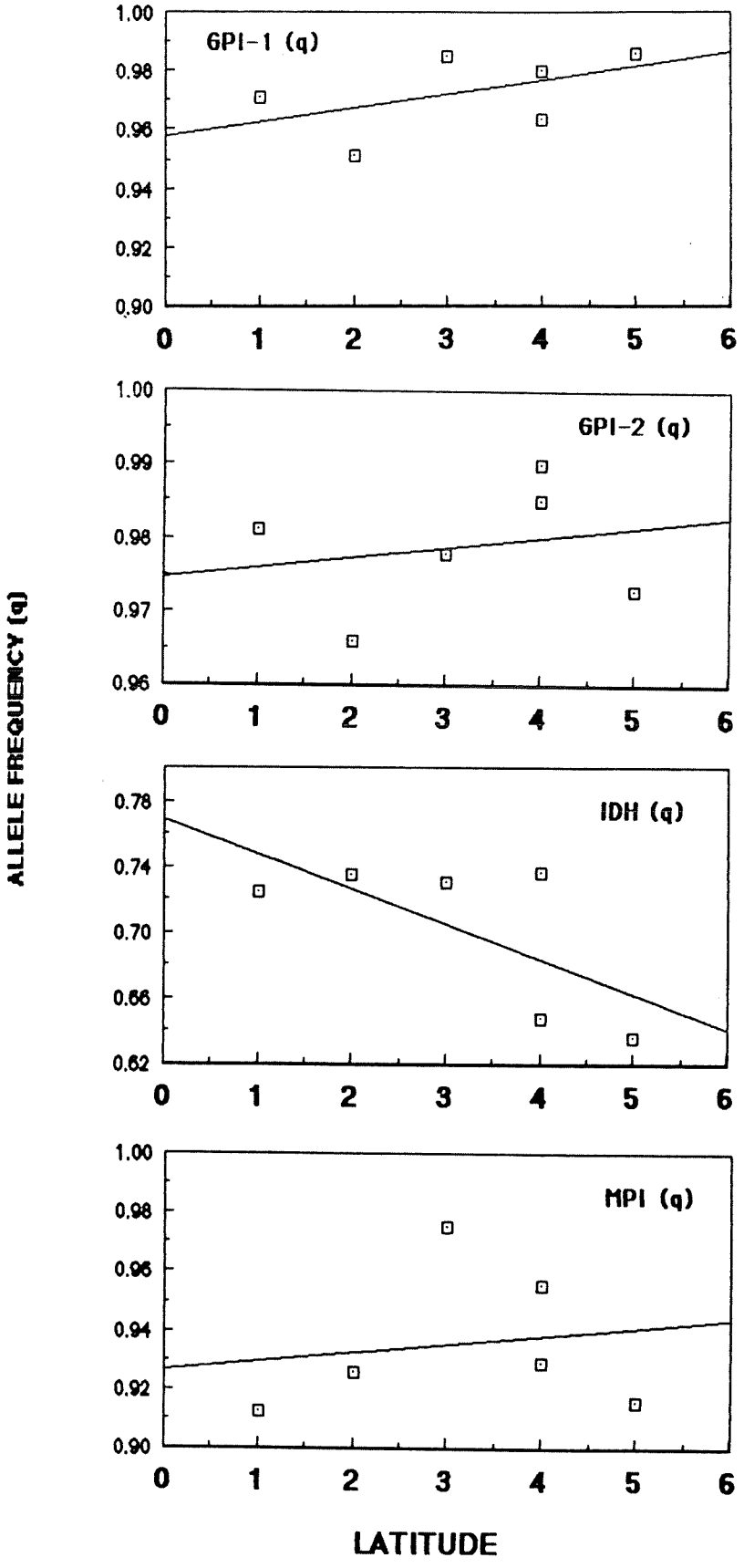


Figure 9. cont.

TABLE 8 Smith's H statistic for IDH genotype frequencies

	p	q	C	N	H x 10^{-3}
Coffs Harbour	0.275	0.725	39	100	5.4 ± 0.78
Crowdy Head	0.265	0.735	41	100	-9.3 ± 0.74
Lord Howe Island	0.269	0.731	19	54	21.0 ± 1.4
Greenwell Point 1	0.263	0.737	39	97	-6.23 ± 0.76
Greenwell Point 2	0.352	0.648	41	98	20.0 ± 1.0
Narooma	0.364	0.636	26	55	-2.76 ± 1.97

KEY: p and q (allele frequency), C (number of heterozygotes)
N (number of individuals), H (Smith's H statistic)

FORMULA: $$H = \frac{4N^2 - (2N - 1)C}{4N(N - 1)}$$

TABLE 9. G-statistic comparison between genotype frequencies of large and small S. lalandi from Lord Howe Island.

otu 1	vs otu 2	g stat	d of f	prob.
lhitotal	lhilarge	4.48485	13	.9849
	lhismall	4.67117	13	.9817
lhilarge	lhismall	11.7249	13	.5503

KEY: lhitotal (Lord Howe Island total), lhi (lord Howe Island) large and small size classes correspond with bimodal length frequency distribution of Fig 12

TABLE 10. G-statistic comparison between genotype frequencies of *S. lalandi* at five locations from New South Wales

otu 1	vs	otu 2	g stat	d of f	prob.
coffs		crowdyhd	12.6435	16	.6986
		lhitotal	20.652	16	.1923
		lhilarge	16.6943	16	.4056
		lhismall	16.3041	15	.3621
		grwellpt1	26.8177	16	.0436 *
		grwellpt2	20.2503	16	.2092
		narooma	18.2223	16	.3110
crowdyhd		lhitotal	22.232	16	.1359
		lhilarge	21.2455	16	.1693
		lhismall	15.2323	15	.4348
		grwellpt1	26.0097	16	.0539
		grwellpt2	21.6948	16	.1533
		narooma	18.5974	15	.2326
lhitotal		lhilarge	4.48485	13	.9849
		lhismall	4.67117	13	.9817
		grwellpt1	24.8061	15	.0526
		grwellpt2	15.0503	15	.4478
		narooma	22.3136	15	.0998
lhilarge		lhismall	11.7249	13	.5503
		grwellpt1	19.6322	15	.1865
		grwellpt2	14.718	15	.4719
		narooma	21.5337	15	.1206
lhismall		grwellpt1	18.9721	14	.1660
		grwellpt2	10.3423	14	.7368
		narooma	14.6676	14	.4012
grwellpt1		grwellpt2	35.4931	16	.0034 *
		narooma	24.0361	15	.0645
grwellpt2		narooma	24.9725	15	.0503

KEY: coffs (Coffs Harbour), crowdyhd (Crowdy Head), lhitotal (Lord Howe Island), grwellpt1 (Greenwell Point 30/10/86) grwellpt2 (Greenwell Point 13/10/87), narooma (Narooma). * indicates a significant difference at the 95% level otu (operational taxonomic unit)

4.2 Population analysis

For the electrophoretic population study of S. lalandi, seven polymorphic loci (ACON, Est-1, Est-2, Gpi-1, Gpi-2, IDH, MPI) were selected on the basis of superior activity, resolution and ease of interpretation. An additional selection factor was that these loci could be screened using only two tissue types, heart and liver. Table 4 details the polymorphic loci, subunit structure, tissue type and running conditions. Interpretations of the zymogram patterns observed in S. lalandi are shown in Figure 8. The genotype numbers are presented in Table 5, and genotype frequencies and number of alleles successfully scored for each polymorphic locus at each locality are presented in Table 6 and 7. Although all seven loci are polymorphic at the Ø.99 level for most of the collection sites, only one locus (IDH) exhibited polymorphism p>Ø.1Ø.

4.2.1 Statistical analysis

Examination of the genotype numbers and frequencies at each polymorphic locus across the five localities revealed that there was little overall variation among populations. The number of observed genotypes were compared with the expected numbers using G-statistics for the seven polymorphic loci, and all samples were in agreement with H-W equilibrium (see Appendix 6). IDH was also tested for H-W equilibrium with Smith's H statistic and no locations were found to be in equilibrium (Table 8). This indicated an excess of IDH heterozygotes for Crowdy, Lord Howe Is. large, Greenwell Pt.1 and an

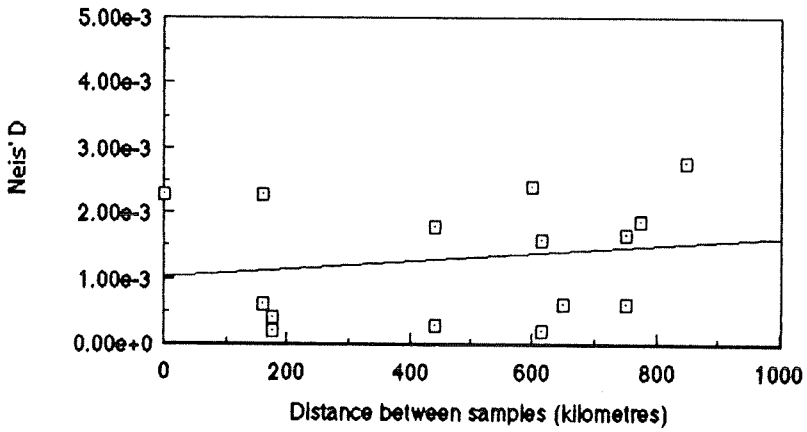


Figure 10. Nei's genetic distance (D) for S. lalandi plotted against geographic distance.

excess of homozygotes for Coffs Harbour, Lord Howe Is. small, and Greenwell Pt-2.

Genotype frequencies for each polymorphic locus were plotted against latitude but no significant pattern of clinal variation was observed (see Figures 9:1-7).

Lord Howe Is. was the only sample site with a bimodal length frequency distribution, ie. large (>85cm) and small (<80cm) S. lalandi (see Figure 12). A G-statistic based on results from seven polymorphic loci showed no significant difference between large and small Lord Howe Is. S. lalandi collections (see Table 9).

Population structure between locations was examined by G-statistic comparisons using the program POPSEP (Appendix 4). Six different structured analyses were run. The input and significant differences are detailed below in A and B respectively (*NB (1)-(4) use genotype frequency information for seven polymorphic loci):

A. (1) Five locations (Coffs Harbour, Crowdy Head, Lord Howe Is., Greenwell Pt-1, Narooma)

(2) Five locations, one replicate and with Lord Howe Is. divided into size classes to give a total of eight otu's.

(3) One location (Greenwell Pt-1) compared to combined north coast (Lord Howe Is., Coffs Harbour, Crowdy Head), south coast (Greenwell Pt-2, Narooma) and NSW total.

(4) One location (Coffs Harbour) compared to combined north coast, south coast, and NSW total. North coast and NSW total excluded Coffs Harbour.

(5) Eight otu's as in (2) using two loci, IDH and MPI.

(6) Eight otu's as in (2) using five loci, Est-1, Est-2, Gpi-1, Gpi-2, ACON.

B. Results of analysis:

(1) significant difference at 95% level between Greenwell Pt-1 and Coffs Harbour (Ø.Ø436).

(2) significant difference between Greenwell Pt-1 and Coffs Harbour and a significant difference at 99% level between Greenwell Pt-1 and Greenwell Pt-2 (Ø.ØØ34) (see Table 1Ø).

(3) significant differences between Greenwell Pt-1 and south coast (Ø.Ø136), north coast (Ø.Ø247) and NSW total (Ø.Ø176) (see Table 11).

(4) no significant difference between Coffs Harbour and other groups.

(5) significant difference between Lord Howe Is. total and Narooma (Ø.Ø486) and Greenwell Pt-1 and Narooma (Ø.Ø456) (see Appendix 7).

(6) significant difference between Greenwell Pt-1 and Coffs Harbour (Ø.Ø346), Crowdy Head (Ø.Ø484), Lord Howe Is. total (Ø.Ø325), Greenwell Pt-2 (Ø.ØØ3Ø) and Narooma (Ø.Ø392) (see Appendix 7).

For analyses (1),(2) and (3), all locations were used alternatively as outgroups to investigate the most likely dendrogram for FITCH and CONTML (see Methods). All CONTML dendrogram branch lengths had large confidence intervals and there was no significant log likelihood difference for CONTML combinations or sum of squares for FITCH combinations (see Appendix 8).

Nei's genetic distance (D) and identity (I) were calculated for seven polymorphic loci for all pairwise sample collections from Groups (1), (2) and (3) above (see Table 12). The largest D value was

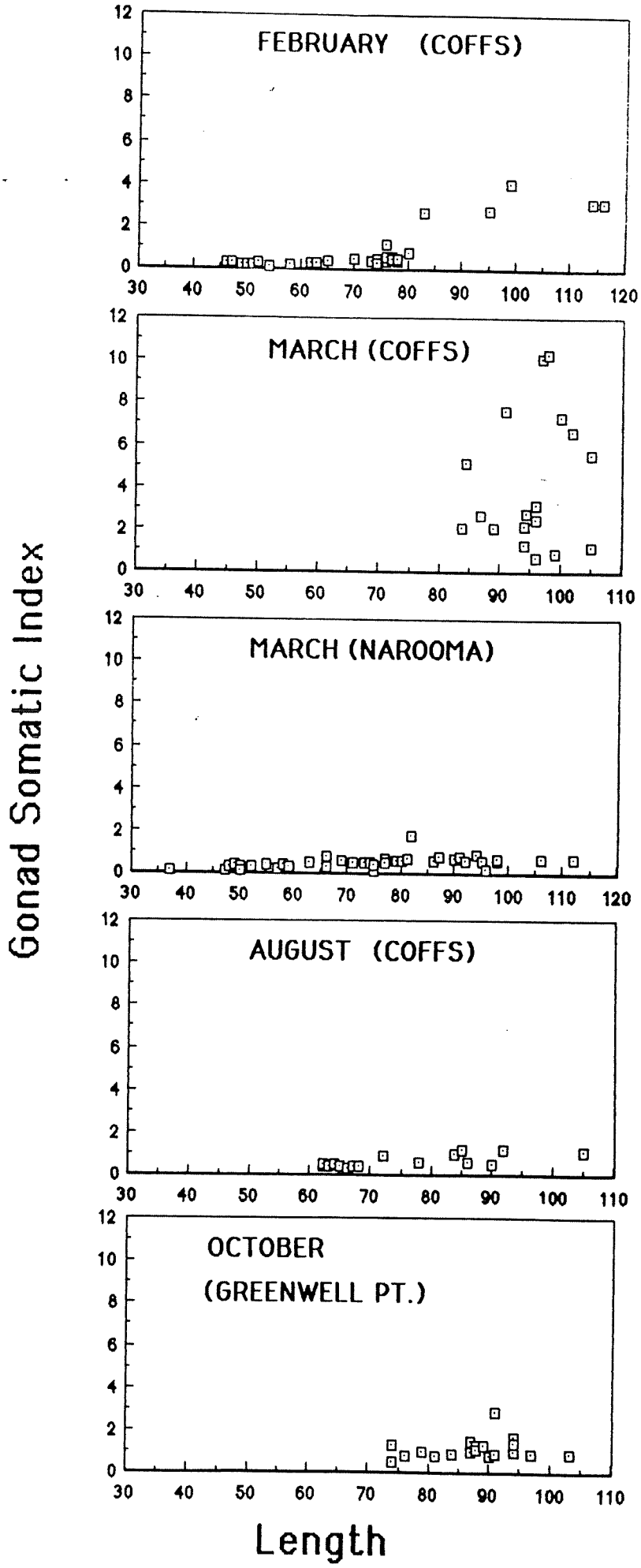


Figure 11. Gonad Somatic Index (G.S.I) plotted against length (l.c.f) for S. lalandi

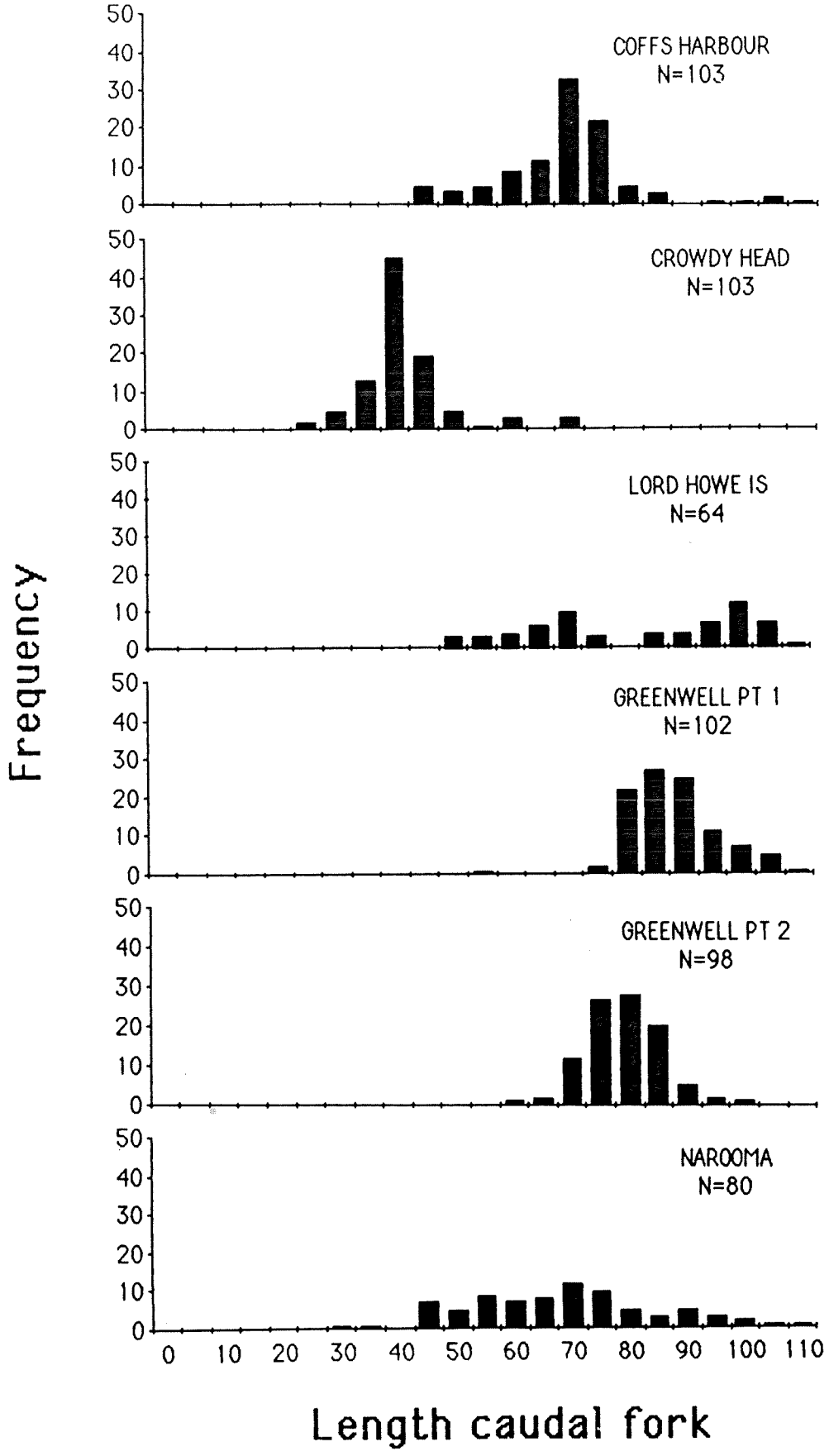


Figure 12. Size frequency distribution of S. lalandi collected from five locations in NSW for electrophoretic analysis.

Ø.ØØ43, between Lord Howe Is. large and Narooma. Greenwell Pt-1 and Greenwell Pt-2 had a D value of Ø.ØØ23. The largest identity was between Coffs Harbour and Crowdy Head (Ø.9998). Nei's D for five locations pairwise comparisons, was plotted against geographic distance and the graph indicated that D was independent of distance (see Figure 1Ø). D was calculated for the 8Ø loci screened for S. lalandi and was close to zero.

4.2.2 Biological information

Biological information on S. lalandi GSI for February, March, August and October is graphed in Figure 11. There is an increased but variable GSI for fish over 8Øcm, for the months of February and March which indicates spawning condition of some individuals. Fish under 75cm have a low GSI which indicates they have not developed. For S. lalandi from Narooma, a low GSI calculated for the same month (March) indicated no spawning fish from a range of length classes. GSI for August and October were small.

Tag-recapture data from 66Ø S. lalandi during 1975-October 1987 indicated a mean recapture size of 6Ø cm (weight 2.Ø2 kg)(see Figure 13). Fish were recaptured from between one and 896 days after release. Distances from tag location ranged to 21ØØkm. The majority of S. lalandi were recaptured near the tag and release location. This is graphed as distance moved from tag location over four time intervals (<6Ø, 6Ø-12Ø, 12Ø-18Ø, >18Ø days) (see Figure 13). Poisson distributions were calculated for distance moved against number of individuals for each of those four periods. The significant result for all tests confirmed that tagged S. lalandi are not randomly

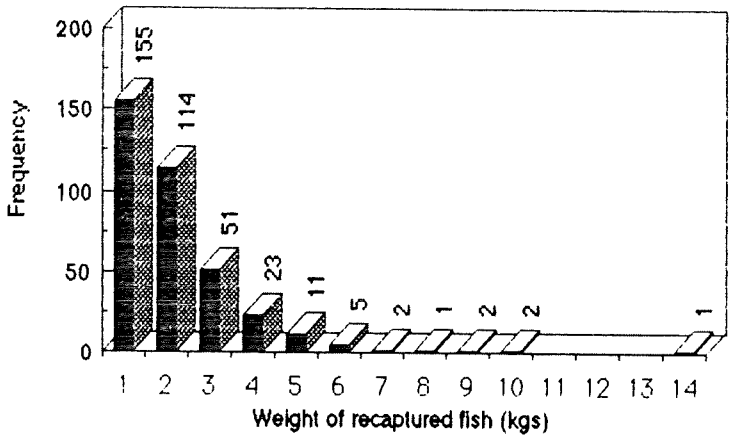


Figure 13. Size frequency of tag recaptured S. lalandi

distributed over distance but are clumped according to a Poisson distribution (see Appendix 9).

Thirty-five S. lalandi were recaptured at distances >50km from the tag and release location. Figure 15: 1,2,3 plots distance and direction, against three variables: length caudal fork, time since release, and month of recapture. Fig 15: 1 illustrates that smaller fish move southward more frequently and greater distances than northward. Fig 15: 2 illustrates that most fish are recaptured soon after release, and that distance travelled is not related to time since release. Fig 15: 3 illustrates a scattered recapture period with no pattern indicative of seasonal movement.

5. DISCUSSION OF POPULATION INVESTIGATION

5.1 Genetic variation of S. lalandi

The proportion of polymorphic loci is a measure of genetic variation. This proportion was 0.137 (11/80) for the pilot study of S. lalandi. This is slightly lower than the average of 0.194 for 41 species of marine fish calculated by Fujio and Kato (1979). A more accurate measure of variation is the average heterozygosity per locus. This was 0.0285 for S. lalandi, lower than the mean of 0.055 ± 0.036 computed by Smith and Fujio (1982) for 106 species of marine teleosts. These measures are not definitive and are influenced by the number and type of enzymes screened. Lewis (1981a; 1981b), Fujino (1976) and Winans (1980) have suggested that large, mobile marine teleosts have low levels of genetic variability, but this was not found by Shaklee, et al, 1983.

TABLE 11. G-statistic comparison of Greenwell Point 1 against grouped populations.

otu 1	vs otu 2	g stat	d of f	prob.
grwellpt1	nthcoast	30.2341	17	.0247 *
	sthcoast	30.9715	16	.0136 *
nthcoast	sthcoast	21.5516	18	.2525

KEY: grwellpt1 (Greenwell Point 1)
nthcoast (Coffs Harbour, Crowdy Head, Lord Howe Island)
sthcoast (Greenwell Point 2, Narooma)
* indicates significant difference at 95% level.

TABLE 12. Genetic distance measures for S. lalandi from five locations within New South Wales.

	LOCATION	1	2	3	4	5	6	7	8	
1	coffs		.0002	.0006	.0009	.0014	.0002	.0016	.0019	
2	crowdyhd	.9998		.0004	.0007	.0014	.0003	.0018	.0024	
3	lhitotal	.9994	.9996		.0003	.001	.0006	.0017	.0028	
4	lhilarge	.9991	.9993	.9997		.0023	.0007	.0032	.0043	(D)
5	lhismall	.9986	.9986	.999	.9977		.0019	.0003	.0012	
6	grwellpt1	.9998	.9997	.9994	.9993	.9981		.0023	.0023	
7	grwellpt2	.9984	.9982	.9983	.9968	.9997	.9977		.0006	
8	narooma	.9981	.9976	.9972	.9957	.9988	.9977	.9994	0	

(I)

KEY: locations as in Table 10. (D) represents Nei's genetic distance
(I) represents Nei's Identity.

Only three of the seven polymorphic enzymes screened for population analysis had a frequency of $p<\emptyset.95$. Richardson, et al (1986) recommends at least six polymorphic loci of $p<\emptyset.9$ were required for population studies. However, this criterion is the ideal case rather than the general procedure and in many population studies, subpopulations have been separated on the basis of frequency differences at one to two polymorphic loci. eg. Genypterus blacodes, K. pelamis, Coryphaena hippurus (Smith, 1979; Lewis, 1981b; Anon, 1986). However, Shaklee, et al, (1982) concluded that population heterogeneity based on a single variable locus (or even two loci) is tentative.

The allele frequency distributions of the seven polymorphic loci all obeyed Hardy-Weinberg expectations when analysed using the G-statistic. This result indicates that random mating occurs between the populations. However, IDH was analysed using Smith's H statistic, and revealed a contradictory result of significant divergence from H-W conditions. Smith's H revealed an excess of homozygotes for Lord Howe Island, Coffs Harbour and Greenwell Point 2. This suggests mixing of two or more subpopulations (Wahlund Effect). Richardson, et al, (1986) proposed that Smith's H is a more sensitive measure of H-W conditions than the Chi-square test, which is similar to the G-statistic used in this analysis. Both analyses are included because it was not expected that there would be a different result for the same data set. The results from Smith's H was suspicious considering the similarity of genotypes for Idh (see Fig 8). This anomaly requires clarification.

5.2 Population structure of S. lalandi

The null hypothesis of electrophoretic population investigations is that all populations have identical allele frequencies. There is a range of statistical methods to test the null hypothesis : G-statistics, Chi-squared test, F-statistics, Anovar, frequency of rare alleles. Many of the methods result in a similar conclusion when applied to the same data (Chakraborty and Leimar, 1987). The G-statistic was used for S. lalandi population structure because it has the advantage over other methods of examining the genetic variation of all polymorphic loci, rather than variation at each individual locus. This provides a more powerful test of population structure. The G-statistic negates the problem associated with Chi-squared tests of arbitrarily pooling rare alleles.

It is important to note that the confidence limits of the statistical tests are related to the population sample size and range of allele frequencies. For samples of 50 - 100 individuals, a 95% confidence interval for individual allele frequency estimates was reported as ±0.10 and ±0.07 respectively (Lewis, 1981b; Richardson, et al, 1986). The sample sizes of S. lalandi populations were in the 50 - 100 category and the range of allele frequency differences was small (see Table 6). IDH allele frequencies between collection sites ranged from 0.263-0.352, a difference of 0.089. The difference was within the 95% confidence limit for allele frequency estimates of ±0.10 to ±0.07. The implications of this confidence limit are that the statistical analysis of S. lalandi allele frequencies using two and five polymorphic loci (Appendix 7) are not as statistically powerful as using the total number of polymorphic loci.

The G-statistic of all pairwise comparisons of S. lalandi collection sites was the initial step of population analysis. The advantage of this approach was that no preconceived assumptions about the samples were made. The disadvantage with this approach is the large number of G-statistics that are required and the choice of an appropriate level of significance. All collection sites were initially analysed by G-statistics at the 95% confidence level. This resulted in a total of 14 G-statistics, only one of which indicated a significant difference between the populations compared. More allele frequency information became available with the collection of a replicate data set (Greenwell Point 2), and Lord Howe Island (LHI) was subdivided into LHI large and LHI small. The allele frequency information was reanalysed and two significant differences resulted from a total of 28 G-statistic comparisons. For the 95% confidence level, approximately one in 20 significant differences would be expected on the basis of chance. Therefore, two significant differences were within the confidence interval for the 28 comparisons. The G-statistic was reanalysed at the 99% confidence level which was more robust (1 in 100 significant differences being due to chance). This resulted in Greenwell Point 1 and Greenwell Point 2 as the only significant difference.

A significant difference between Greenwell Point 1 and Greenwell Point 2 allowed rejection of the null hypothesis of identical allele frequency. Assuming that there were no errors in typing alleles, then several explanations for this genetic difference may be proposed:

(1) that Greenwell Point 1 and Greenwell Point 2 are reproductively isolated and hence are discrete subpopulations;

(2) that Greenwell Point 1 is significantly different from the other

samples due to selection, mutation or random genetic drift at the larval or adult stage;

(3) inbreeding of S. lalandi; or

(4) some combination of these factors.

The significant allele frequency differences observed for Greenwell Point 1 may have resulted from temporal displacement of a subpopulation that reproduces outside NSW (see Fig 18 for a proposed explanation). Samples taken throughout the Australian range would be needed to test this hypothesis. Attempts to obtain population samples from South Australia and Western Australia were unsuccessful. Temporal replacement of subpopulations has been reported for another pelagic species, K. pelamis (Fujino, 1976; Lewis, 1981b; Richardson, 1983; Richardson and Habib, 1987). An assumption of temporal replacement is that S. lalandi would return to particular locations or demonstrate temporal reproductive isolation.

If selection is the suggested cause of S. lalandi genetic differentitation it is difficult to prove. However, changing patterns of allele frequency distribution with increasing age or environmental parameters eg.(temperature) have been reported for a number of marine teleosts eg. Chrysophyrs auratus (Smith, et al, 1979; Johnson, et al,1986). Fishing pressure has also been proposed as a selective force where the fishing technique targets on large size classes which are important for homing of the population eg Salmonids (Maclean and Evans, 1981). The Greenwell Point commercial fishery targets on large individuals but it is not known if the proportion of adults influence the migration and successfully reproducion of the population.

Inbreeding may be an explanation of S. lalandi allele frequency variation, if spawning groups that contribute progeny to the population are small. This may be a possibility for subpopulations from outside N.S.W. which are not large eg. South Australia. Inbreeding is not likely to occur on the east Australian coast because of the abundance of S. lalandi. Preliminary GSI results presented in Figure 11 indicate that only large individuals (>80cm) contribute to reproduction. However, inbreeding is not a likely explanation for a reproductive strategy which is pelagic and prolonged duration.

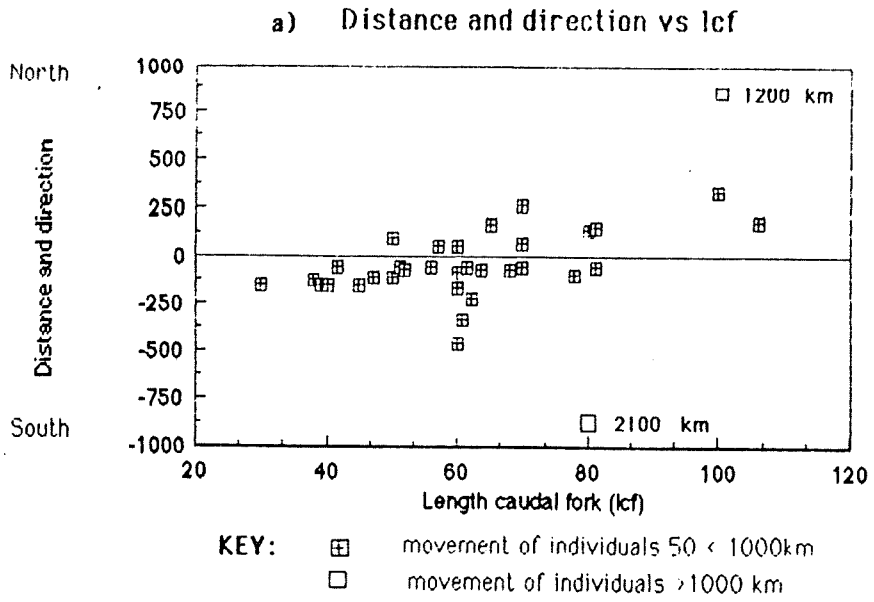
The homogeneity of the populations indicated by the grouped G-statistic between north coast and south coast (Table 11) discounts the possibility of the easterly flowing East Australian Current (EAC) as a barrier to S. lalandi dispersal. The EAC is an episodic, intense southward flowing current which includes a turbulent interactive network of variable currents, eddies and meanders. It separates from the coast in the region between Evans Head and Sugarloaf Point (32° 30'S)(Godfrey, et al, 1980, Pearce, 1980). After separation, the EAC forms a sharp temperature front and moves eastward following the Tasman Front towards New Zealand. The EAC separation point has been proposed as a possible barrier to gene flow for Sillago basensis flindersi (Dixon, et al, 1986) which is less mobile than adult S. lalandi, but both are pelagic spawning species. ~~S. lalandi~~. Because of the prolonged spawning period and pelagic behaviour of S. lalandi the EAC is likely to act as a transport mechanism for adult and larval stages rather than a barrier to gene flow.

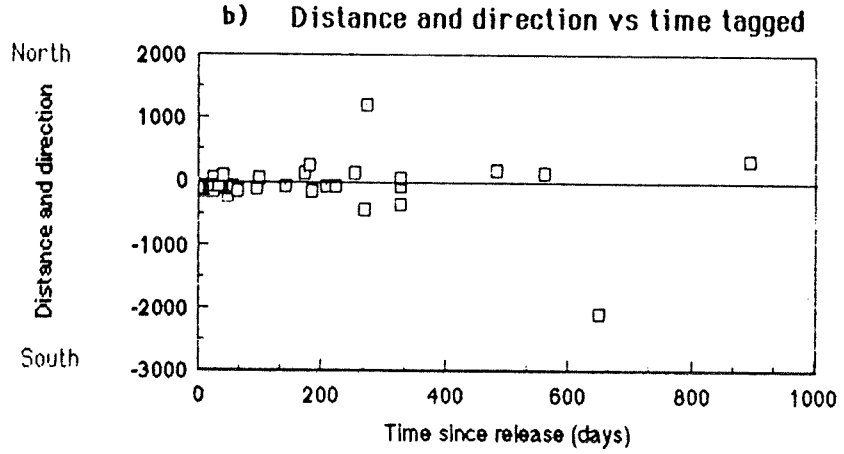
The genetic relationships of S. lalandi from each population, and from grouped populations is presented in dendrograms. These connect the collection sites in a haphazard fashion with no relationship to

geography (Appendix 8). In all cases the standard error of allele frequency and genetic distance is so large that the branch lengths are not realistic. The dendrograms (CONTML and FITCH) do not differentiate any discrete subpopulations or separate Greenwell Point 1 from the other samples.

5.3 Genetic distances and identities

The genetic distances and identities of S. lalandi range from Ø.ØØØ2 to Ø.ØØ43 (mean =Ø.ØØ14). Genetic distance is a measure of the accumulated allele differences per locus (Nei, 1972; 1976). These values have no quantitative criterion for resolving taxonomic questions, but comparison of D values with those published for closely related groups is a useful reference for interpreting results (Shaklee, et al, 1983). Scomber japonicus and S. australasicus, like S. lalandi, are placed in the family Carangidae. They are therefore suitable for comparison of genetic distances. Kijima, et al (1986) reported D values for groups of S. japonicus to range from Ø.ØØØ to Ø.Ø11 (Ø.ØØ5 average) (from 23 loci), and S. australasicus as Ø.ØØ1 to Ø.Ø11 (Ø.ØØ8 average) (from 23 loci). These values were regarded as consistent with intra-group differences (Kijima, et al, 1986). Shaklee, et al (1982) reviewed speciation in marine fishes and compiled genetic distance values for populations with a range of Ø.ØØ2-Ø.Ø65 (average Ø.Ø5) and species from Ø.Ø25-Ø.6Ø9. Winans (198Ø) calculated a D value of Ø.ØØ2 (from 38 loci) among populations of milkfish, Chanos chanos. Grant and Utter (1984) regarded a D value of Ø.ØØ26 $\pm$ Ø.ØØ17 as sufficient to subdivide Atlantic-Bering sea herring Clupea pallasi into an Asian group and an eastern Bering Sea group. If the average D values calculated by Shaklee, et al, (1982) are used as the criterion for distinguishing population structure then





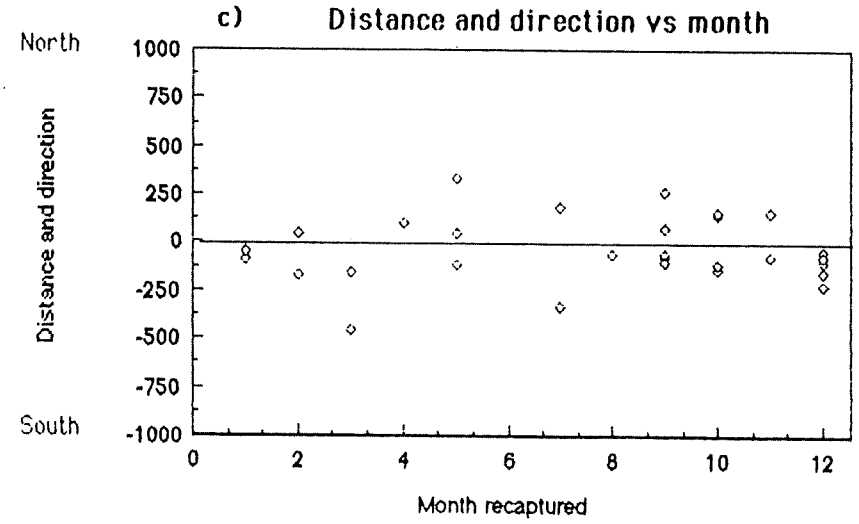


Figure 15. Relationship between distance-direction and a) length caudal fork b) time since release and c) month of recapture

Footnote: individual fish recaptured throughout NSW from 1975-1987

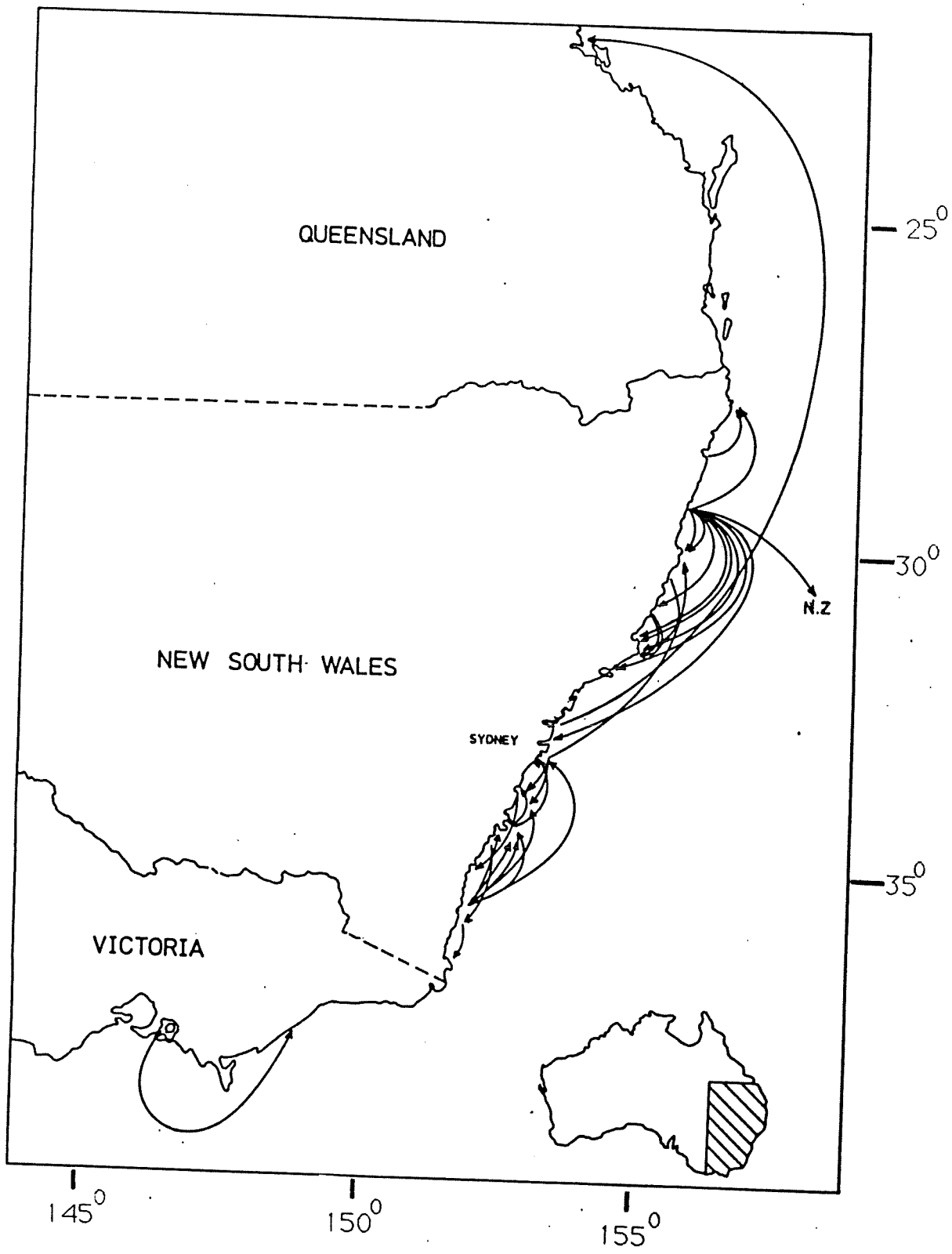


Figure 14. Tag-recapture movements of S. lalandi greater than 50 km along the east coast of Australia.

S. lalandi is one population. However the D value between Greenwell Point 1 and Greenwell Point 2 of Ø.ØØ23 (from seven loci) is similar to the value used by Winans (198Ø) to separate subpopulations. Caution is recommended when inferring population and taxonomic status from genetic distance data because of the large magnitude of the standard error, and the dependence on the number of loci used for calculation.

The level of genetic divergence between S. lalandi populations was plotted against geographic distance (Fig 1Ø). The relationship is linear and similar to the expected relationship for a panmictic population (Richardson, et al, 1986). There is no indication of an increased D value with distance, which is indicative of the isolation by distance model of population structure. An isolation by distance model is not expected since the furthest population samples were separated by approximately 12ØØ km and tag-recapture data have shown individual S. lalandi are capable of moving these distances (Fig 14).

5.4 Independent biological information

Tag recapture results illustrate that one large S. lalandi moved north from Sydney to Rockhampton, Queensland and one from Coffs Harbour south to New Zealand, but the correlation of fish movement with gene flow is unknown (Ihssen, et al, 1981). Tag-recapture data from 66Ø S. lalandi over four time scales conformed with the Poisson distribution. Therefore, movement is clumped, not random. The bias of this data set is the large number of small recaptured fish, and the large number of fish recaptured less the 6Ø days after release. Small fish which are recaptured greater than 5Ø kms from release have moved predominately south, possibly due to the influence of the

southward EAC. Larger fish move greater net distances but are also recaptured after longer time periods (Fig 14, Fig 15). It is not known whether tagging causes stress and behavioural changes to S. lalandi. This is an important consideration in analysing tag data because we assume that the tagged fish are representative of the population. Stanley (1983) and Hampton (1986) indicated that tagging has a significant effect on the condition and behaviour of some pelagic species.

Gyllensten (1985) regarded the amount of variation between populations to be determined by the effective size of populations and the migration rate. A similar theory of migration between spawning stocks was proposed by Grant (1985), using the stepping stone model to account for the genetic similarity of Engraulis capensis. Grant (1985) calculated that as few as 13 migrants may account for the observed genetic divergence between areas. There is no estimate of S. lalandi population size, and the tag data is not sufficient for calculation of migration rate. However, the two individuals which moved large distances (Fig 14) indicate the potential for interaction.

The results from S. lalandi GSI (Fig 11) illustrate that fish from Coffs Harbour are sexually mature in February/March, while those from Narooma are not in spawning condition in March. A different spawning period for north coast and south coast indicates that S. lalandi are not randomly mating throughout their range. However, it is possible that spawning is seasonal and triggered by environmental stimuli eg. temperature, as observations indicate some spawning fish in July and October on the south coast of NSW. The sample from Greenwell Point 1 (collected 31/10/86) contained some spawning fish, and this sample was identified as genetically different from Greenwell

Point 2 fish (14/10/87), which were not in spawning condition. A more complete collection of reproductive samples from north and south coast populations would be needed to verify this apparant separation.

Kornfield, et al, (1982) defined two discrete fall and spring populations of Clupea harengus, but Smith and Jamieson (1986) reanalysed the allozyme data and with life history information they concluded that the discrete subpopulation model did not apply. They regarded the C. harengus stock as a transient sub-division within the species with no taxonomic or evolutionary status. They argue that spawning period is not genetically fixed but environmentally triggered and gene flow occurs between neighbouring spawning aggregations. A similar hypothesis of transient sub-division may explain the apparent genetic variation between Greenwell Point collection sites. There appears to be considerable uncertainty of the genetic structure of marine species (see Shaklee, 1983; Smith and Jamieson, 1986). Electrophoretic data should be substantiated by biological data to infer population structure. S. lalandi has a pelagic adult and larval life history which favours gene flow. In contrast, the barramundi Lates calcarifer has limited dispersal of adult and larval stages which favoured genetic differentiation (Shaklee and Salini, 1983; 1987).

5.5 Proposed population structure models for S. lalandi

The electrophoretic, tag/recapture and reproductive information for S. lalandi are compatible with several population structure models.

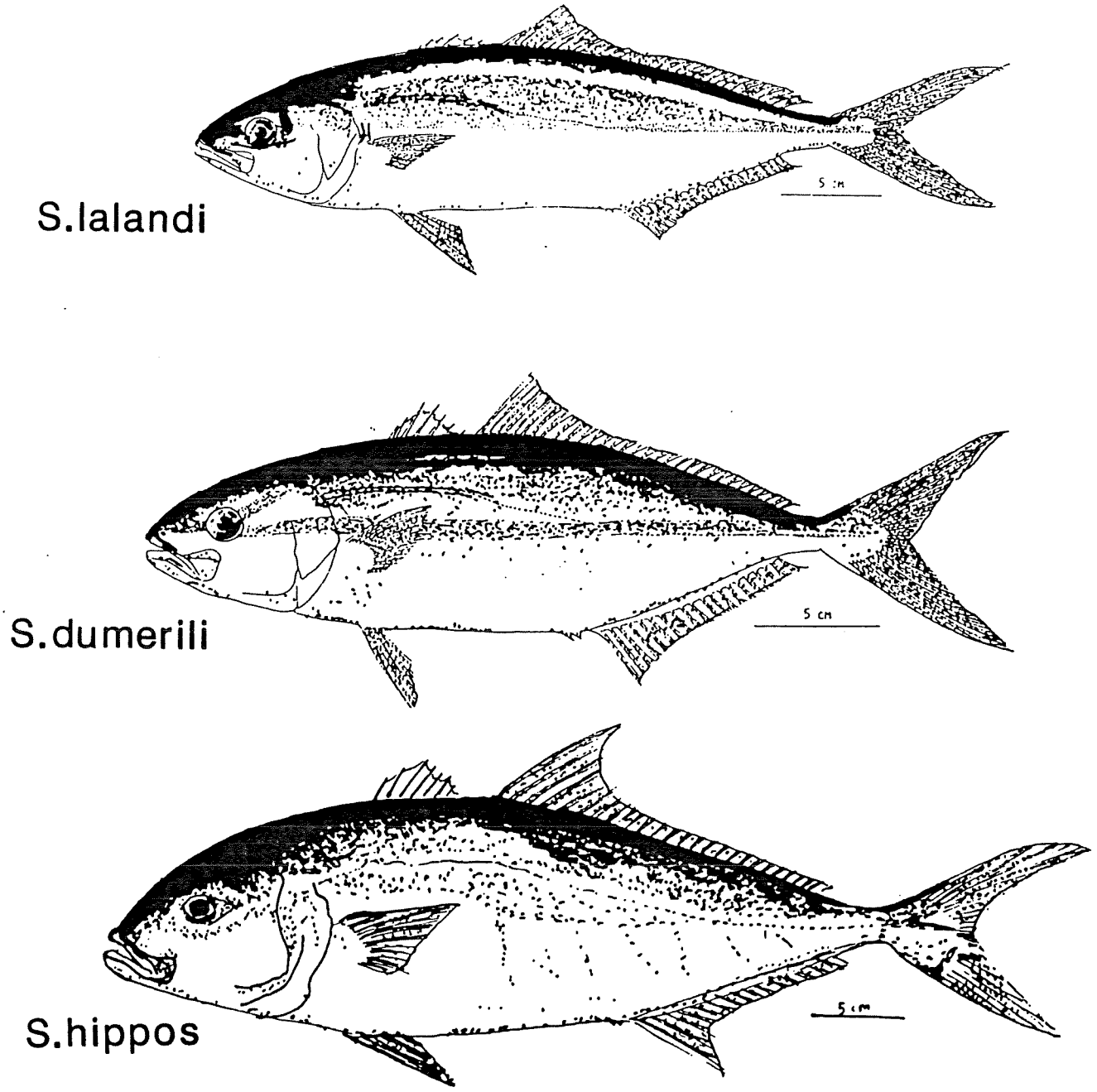


Figure 16. Illustration of S. lalandi, S. dumerili and S. hippos.

1) The distinct subpopulation model, due to significant variation between Greenwell Point 1 and Greenwell Point 2.

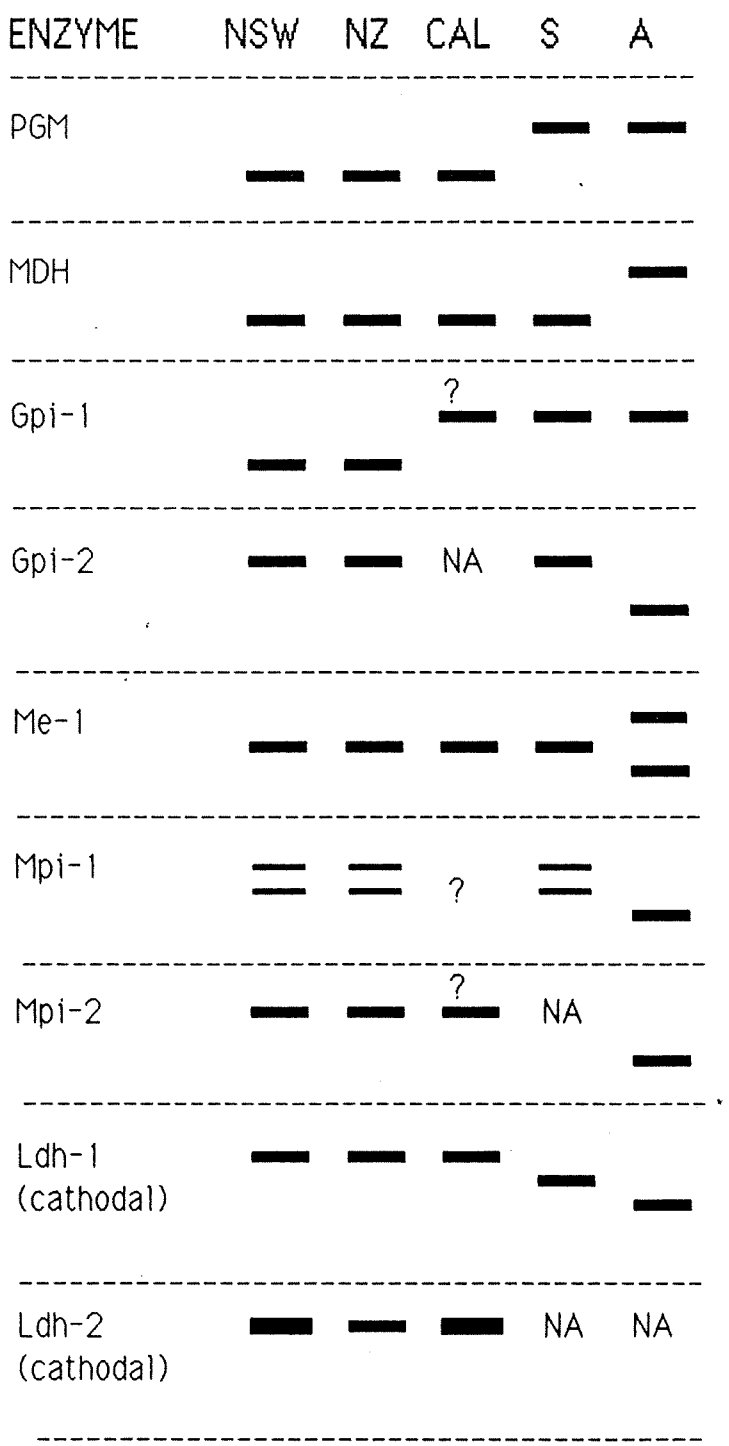
2) The panmictic population model is supported by low genetic distance values, lack of barriers to gene flow, and movement by individuals through the range of the sampling region. The difference between Greenwell Point 1 and Greenwell Point 2 may be explained by differential gene flow.

3) Chance or chaotic model, which proposes that variation may be transient due to a combination of random processes.

Greenwell Point is the most prolific commercial fishery for S. lalandi in NSW. The fishery requires further investigation for temporal replacement of populations. Future study may also be directed towards tagging larger size classes of fish to determine if they move offshore as generally believed, or if they migrate throughout and beyond the N.S.W region. Further electrophoretic investigation on temporal and spatial structure may reveal more differences, as may the more sensitive technique of mitochondrial DNA analysis.

6. RESULTS AND DISCUSSION OF SERIOLA SYSTEMATIC INVESTIGATION

Fourteen enzymes, representing thirty presumed loci were investigated using electrophoresis for three sympatric Seriola species. Samples of S. lalandi from the eastern Pacific (California), were representative of a geographically divergent population, but the poor condition of these samples made interpretation of alleles difficult, or resulted in no activity. These samples were not used for statistical analysis.



KEY:
- NSW - New South Wales S. lalandi
- NZ - New Zealand S. lalandi
- CAL - Californian S. lalandi
- S - S. hippos (samson fish)
- A - S. dumerili (amberjack)
- NA - No activity
- ? - faint

Figure 17a. Relative mobilities of scorable enzymes from heart for Seriola species.

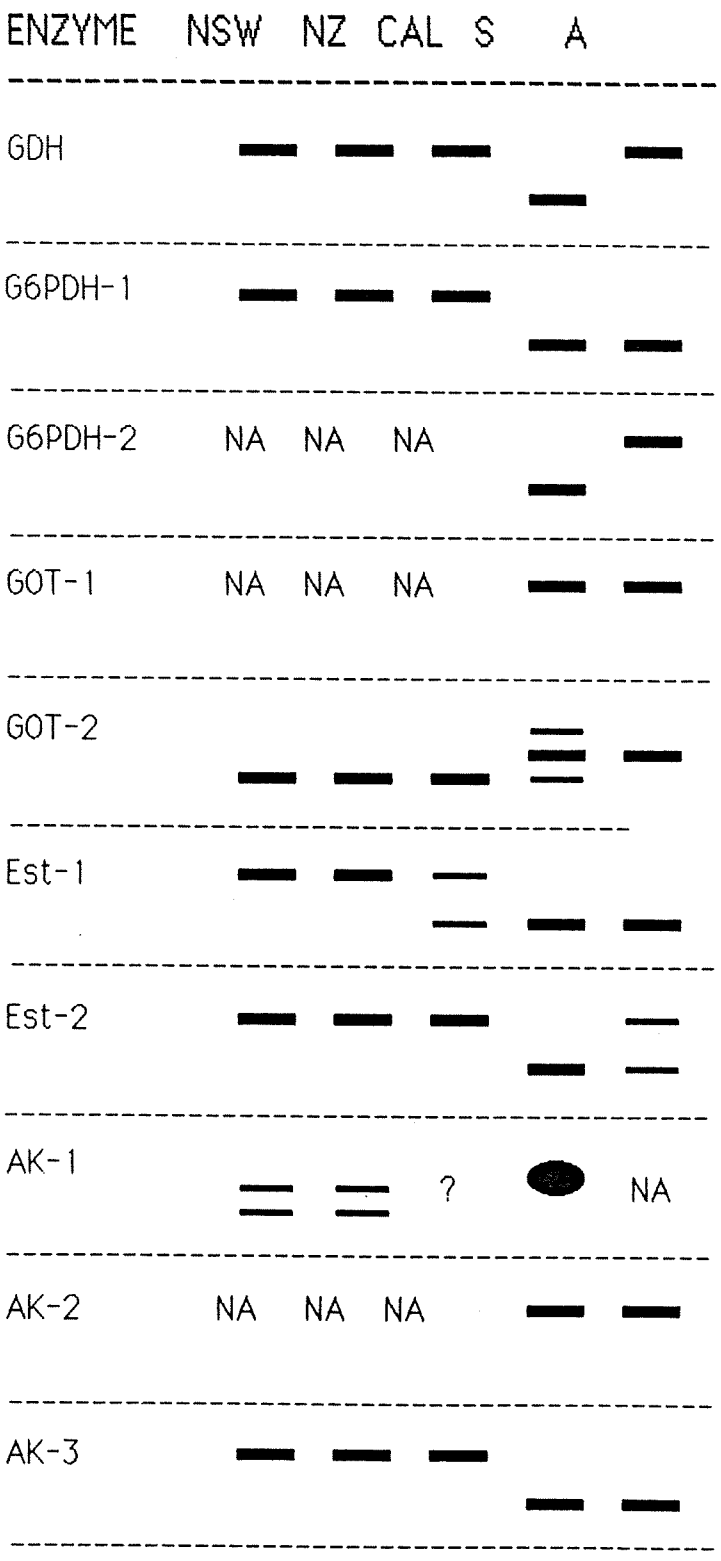


Figure 17b. Relative mobilities of scorable enzymes from liver for Seriola species. See Figure 17a for key.

TABLE 13. Meristic characters of *Seriola* species.

SPECIES	DORSAL	ANAL	OTHER
S. lalandi (10)	D1 V-VI, 33-35	A II-III, 20-21	
S. hippos (7)	D1 VI, 23-25	A III, 16-17	MAXILLA SHAPE
S. dumerili (3)	D VI-VII,30-34	A III, 19-21	GILL RAKER NO.

TABLE 14. *Seriola* species pair comparisons.

Species pairs	Fixed differences
S. lalandi/*S. dumerili*	16/30
S. lalandi/*S. hippos*	14/30
S. hippos/*S. dumerili*	11/30

A large number of fixed differences were observed between the three Seriola species, but no fixed differences were observed within S. lalandi from N.S.W., N.Z. and California (see Figures 17a and 17b). Table 14 shows the number of fixed differences between the three Seriola species. The proportion of fixed differences range from 11/30 to 16/30. This finding is similar to the proportion of allelic differences (20-50%) for closely related species reported by Avise (1975).

The demonstration of fixed allelic differences among samples from sympatric populations is evidence of complete reproductive isolation (Shaklee, et al, 1982). However the test is not reciprocal. A result of no fixed differences cannot be used to establish the conspecific status of samples.

The genetic distance between the three species was calculated using NEISTAT (see Methods). The input file for this program is in Appendix 10. S. lalandi and S. hippos have a D value difference of 0.3295; S. lalandi and S. dumerili of 0.5272, and S. hippos and S. dumerili of .2973 (Table 15). These values are within the range of genetic distances of teleost species (0.025-0.609, average 0.30) calculated by Shaklee, et al 1982. The D value obtained by Kijima, et al, (1986) for S. lalandi and S. dumerili was 0.78. This is higher than the distance of 0.5272 obtained in this study, and is probably due to the smaller number of loci (9) surveyed by Kijima, et al, (1986). The enzymes IDH, MDH and PGM revealed fixed differences in both Kijimas (1986) study and this investigation.

There is no relationship between the genetic distance values and the morphological and meristic differences of Seriola species. S.

TABLE 15. Genetic distances and Identities of three Seriola species.

		1	2	3	4	
1	S.lalandi (NSW)		Ø	.3295	.5272	
2	S.lalandi (NZ)	1		.3295	.5272	(D)
3	S.hippos	.7193	.7193		.2973	
4	S. dumerili	.59Ø2	.59Ø2	.7428	Ø	

(I)

Explanation: Upper right triangle represents Nei's genetic distance (D), lower left represents Identity (I)

lalandi is morphologically and meristically more different to *S. hippos* than to *S. dumerili* but the *S. lalandi* and *S. dumerili* pair comparison has a greater genetic distance. This finding illustrates the application of electrophoresis for separating morphologically similar species.

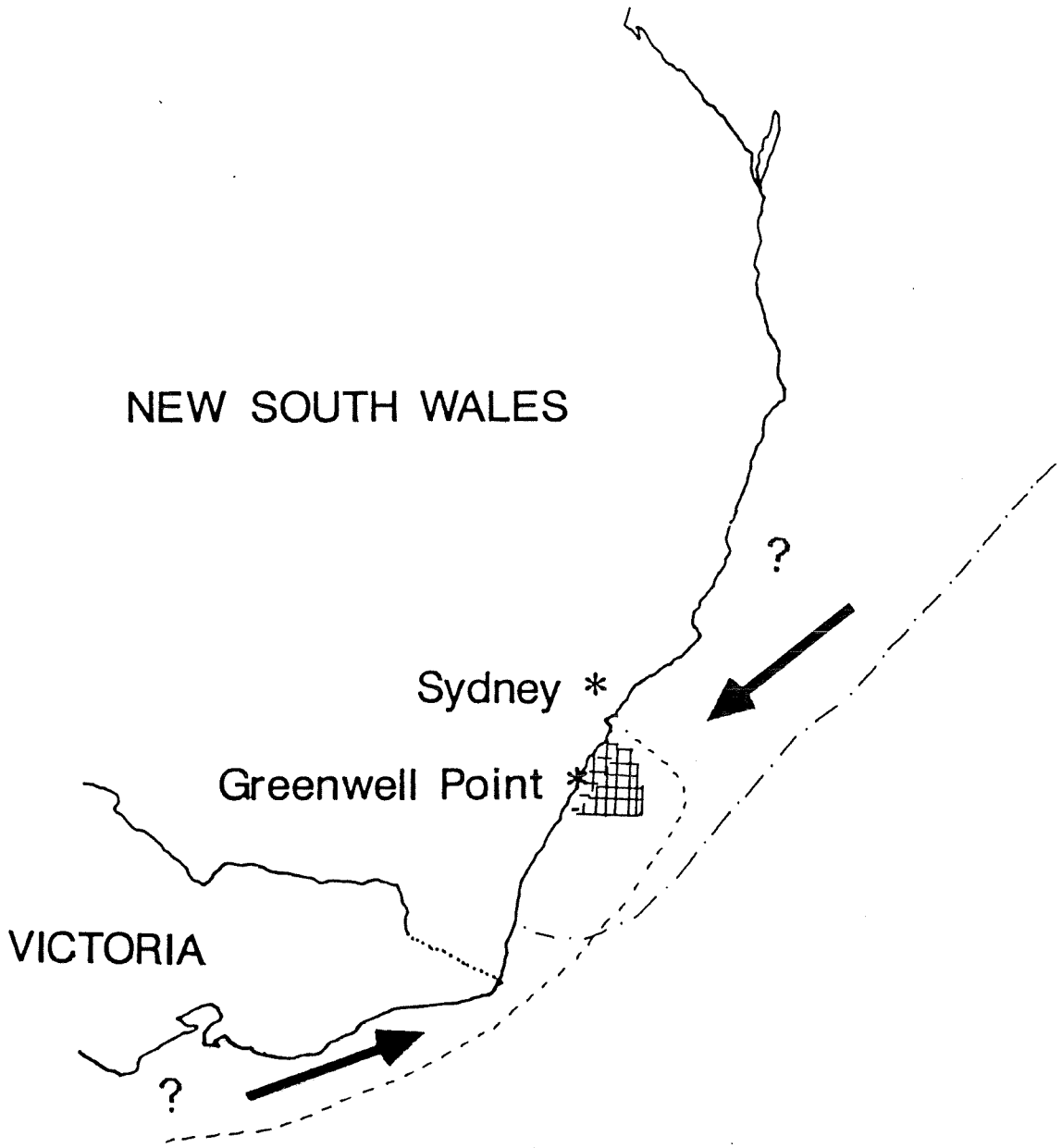


Figure 18 Temporal replacement of subpopulations - Proposed explanation for genetic variation between Greenwell Point 1 (31/ 10/1986) and Greenwell Point 2 (14/10/1987)

CONCLUSION

G-statistic comparisons between populations and genetic distance information suggest that S. lalandi are genetically similar throughout N.S.W with the exception of Greenwell Point 1. Samples from Greenwell Point 1 (30/10/87) and Greenwell Point 2 (14/10/87) differ in allele frequency and reproductive condition. These samples may represent two subpopulations with an overlapping zone of distribution presumably for feeding purposes. Future electrophoretic investigation is necessary to determine the temporal boundaries of these potential subpopulations. Greenwell Point is the major commercial fishery for S. lalandi in N.S.W., and an important consideration for fisheries management is delimiting the number of subpopulations to avoid overexploitation of the fishery.

Electrophoretic investigation of S. lalandi from America, New Zealand and N.S.W did not distinguish fixed genetic differences. Three Seriola species S. lalandi, S. hippos and S. dumerili were readily identified by fixed genetic differences, and genetic distance was calculated between the species.

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Appendix 1.

Laboratory materials

APPENDIX 1.1 Laboratory materials

Starch gel -Lot No. 26F-0215. SIGMA Chemical Company

P.O Box 14508, St. Louis, Mo., 63178 U.S.A

Homogenising Buffer: 0.1 ml Mercaptoethanol

to 100 ml 0.1 M Tris ph 8

Fixing solution: 4 methanol : 5 water : 1 glacial acetic acid.

APPENDIX 1.2 SUBSTRATE SOLUTION RECIPES

Aconitate substrate solution	80 mL 0.2M Tris-HCl pH 8.0; 300 mg Aconitic acid; 1 g Tris.
Esterase substrate solution	500 mg α-naphthyl acetate; 500 mg α-naphthyl acetate; 25 mL acetone; 25 mL water.
GOT substrate solution	292 mg Ketoglutaric acid; 1.064 g L-aspartic acid; 4.0 g polyvinyl pyrrolidone; 400 mg EDTA, Na_2; 11.36 g Na_2MPO_4; water to 400 mL.
Sodium L-Malate substrate solution	24.3 g Na_2CO_3 26.8 g L-Malic acid; water to 200 mL.
Guanine substrate solution	50 mg Guanine dissolved in 10 mL warm 0.1M NaOH; water to 50 mL.

APPENDIX 1.3 STOCK SOLUTIONS USED IN HISTOCHEMICAL STAINING

SOLUTION	CONCENTRATION
o-Dianisidine	10mg/mL
Glucose-6-phosphate Dehydrogenase	10u/mL
MTT	10mg/1.5mL
NAD	10mg/mL
NADP	10mg/mL
Na-Pyruvate	50mg/mL
Phosphoglucoseisomerase	10u/mL
PMS	10mg/mL
Pyrazole	50mg/mL

Appendix 2

Electrophoresis running conditions

Appendix 2. Electrophoresis running conditions and buffer recipes

BUFFER	ELECTRODE BUFFER	GEL BUFFER	CONDITIONS
Tris-borate EDTA pH 9.Ø	42.2g Tris; 2.16g boric acid; 1.64g EDTA Na; to 4 l deionised water.	As for electrode.	3ØØV for 2hrs+ 35ØV for 3hrs
Poulik's	38.2g boric acid; 5.Ø4g NaOH; deionsed water to 2.1 l. pH 8.1	18.6g Tris; 2.1g citric acid; water to 2 l. pH 8.65	5hrs at 35mA
Tris-maleate pH7.8	24.2g Tris; 9.Ø2g maleic acid; deionised water to 2 l.	2ØØml electrode buffer to 2 l water.	4.5hrs at 35mA
Tris-citrate pH 7.Ø	72.29g citric acid; 5ØØml 2M Tris; deionised water to 4 l.	5Øg sucrose; 35ml electrode buffer to 1 l water.	15ØV 4.5hrs
CAM pH 6.1	16.8g citric acid; 19.5ml morpholine; deionised water to 2 l.	1ØØml electrode buffer; water to 2 l.	4.5hrs at 5ØmA
Tris-citrate pH 5.8	42.Ø2g citric acid; 65.6g Tris; deionised water to 2 l.	7Øml electrode buffer; water to 2 l.	15ØV 4.5 hrs

Appendix 4

Description of computer programs used for electrophoretic analysis.

APPENDIX 4: Description of computer programs used for electrophoretic analysis. Programs are in the order they were used for population analysis. Programs 1 to 7 inclusive were written by Holliday (1987). Programs 8 and 9 are part of Felsenstein's (1981; 1982), PHYLIP package.

1) ALLFREQ: The input data is the number of genotypes at each polymorphic locus for each sample collection. This program a) calculates allele frequencies from genotype numbers for each locus, and b) calculates the sample numbers for each locus. The allele frequency file is tabulated in a form suitable for input into CONTML.

2) POPSEP: This program performs G-tests according to Sokal and Rohlf (1983) on all possible OTU (Operational taxonomic units) pairs for the allele frequency of all polymorphic loci. (NB. An OTU may be a sample set, a subdivided sample set or a combined collection).

3) POPSEPT: Prints out an asterisks for significant results at the 95% level of POPSEP in a table form.

4) SIPBOTH: This program does the same as both POPSEP and POPSEPT, but the significance level of the G-test is 1%. ie. asterisks are printed only when $p < 0.01$.

5) NEISTAT: This program calculates Neis genetic distance (D) as revised by Hillis (1984) between each OTU and all other OTUs.

6) NEI4FIT: This program calculates Neis D and tabulates the results in a form suitable for input into FITCH.

7) NEIBOTH: This program produces a table consisting of two triangles, with Neis D in the upper right half and Neis identity (I) in the lower left half.

8)CONTML: (Continuous maximum likelihood) This program uses gene frequencies from ALLFREQ to construct estimates of the maximum evolutionary tree. It assumes that each locus evolves independently by genetic drift. This program was run with all combinations of OTUs as the outgroup to determine the structure of genetic relationships. For a detailed description see Felsenstein (1981, 1982).

9)FITCH: (Fitch-Margoliash and Least Distance Methods) This program uses the output of NEI4FIT. The FITCH program assumes 1) that each distance is measured independently from the others and 2) the distance is, in effect a measure of evolution. These two assumptions are tenuous for D calculated from gene frequency data since additivity and independence are not expected. Therefore CONTML was a more appropriate measure.

10)CONPLOT: This program drives a Graphtec plotter to draw dendrograms of genetic relationships using the input files from CONTML.

11)FITPLOT: This program drives a Graphtec plotter to draw dendrograms of genetic relationships using the input files from FITCH.

Appendix 5

Description of enzymes surveyed, tissues examined, buffer systems and observations from *S. lalandi* pilot study

APPENDIX 5: DESCRIPTION OF ENZYMES STUDIED, TISSUES INVESTIGATED AND PRESUMED NUMBER OF LOCI FOR *Seriola lalandi* ELECTROPHORETIC PILOT STUDY

KEY: TISSUES, L= liver, H= Heart, WM= white muscle, RM= red muscle, K= kidney, E=eye lens
BUFFERS, 1= TBE pH9, 2= Poulik, 3= TM pH7.8 4= TC pH7.Ø, 5= CAM pH6.1, 6= TC pH5.8, *= most suitable buffer system
DESCRIPTION, A= anodal migration, C= cathodal migration P= polymorphic, N/S= no staining

NOTE: Loci are designated by the distance of migration from the most anodal to the most cathodal. e.g. GPI-1 is anodal (faster migrating) than GPI-2

ENZYME	TISSUE	BUFFERS	PRESUMED NO. OF LOCI		COMMENTS
AAT	L	2*,3,6	1 A		POOR RESOLUTION
	RM	2*,3,6	1 A		
	WM	2*,3,6	1 A		
	H	2*,3,6	2 A		
	K	2*,3,6	2 A		
ACON	L	2,3,4,5*,6*	1 ?2 A	P	FAIR RESOLUTION
	RM	2,6*	1 A, 1C		
	WM	2,3*,6	1 A		SLOW TO STAIN
	H	2,3,4,5,6*	1 A, 2 C		
	K	2	1 A		
ACPH	L	1,2*,3,5,6	1 A		POOR ACTIVITY
	WM	2,5*,6	1 A, 1 C		POOR RESOLUTION
	H	2,3*,5,6	1 A		
	K	2	1 A		
ADA	L	2,3,5*	1 A		FAIR RESOLUTION
	RM	2	N/A		
	WM	2	1 A		
	H	2	1 A		
	K	2	1 A		
ADH	L	1,2,3,4,5,6*	1 C		GOOD ACTIVITY
	RM	2	N/A		
	WM	2,3,6	1 A		
	H	2,3*,6	1 A	?P	VARIABLE
	K	2	N/A		
AK	L	2,3*,5,6	2 A		GOOD ACTIVITY
	RM	2*,3,5,6	1 A		VERY STREAKY

	WM	2,3,5,6	1 A		
	H	2,3,5*,6	1 A	?P	CLOSE TO ORIGIN
	K				
ALD	L	1,2*,3,5,6	1 A	?P	POOR RESOLUTION
	RM	2	N/A		
	WM	2,3,5*,6	1 A		
	H	2,3,5*,6	2 C		GOOD ACTIVITY
	K				
ALKPH	L	2,3,6*	1 C		POOR ACTIVITY
	WM	2,3,6	N/A		
	H	2*,3,6	1 A		
	K	2	1 A		
AO	L	2*,3,5,6	1 A		POOR ACTIVITY
	WM	2*,3,6	1 A		
	H	2,3*,5,6	2 A		POOR RESOLUTION
ARG	L	2,6	N/A		
	RM	2,6	N/A		
	WM	2,6	N/A		
	H	2,6	N/A		
	K				
ARS	L	2,6	N/A		
	RM	2,6	N/A		
	WM	2,6	N/A		
	H	2,6	N/A		
	K				
CAT	L	2,3*	2 A		
	RM	2,3*	1 A		POOR ACTIVITY
	WM	2,3*	1 A		
	H	2,3*	1 A		
	K	2*,3	1 A		
CK	L	2*,3,6	1 A		VERY STREAKY
	RM	2,3,6*	1 A, I C		FUZZY
	WM	2,3*,6	1 A		
	H	2,3*,6	1 A		STREAKY
	K	2	N/A		
DAMOX	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	1 A		POOR ACTIVITY
	K	2	1 A		
DASOX	L	2	N/A		POOR ACTIVITY
	RM	2	N/A		SIMILAR RESULT
	WM	2	N/A		TO DAMOX
	H	2	1 A		
	K	2	1 A		

DIA	L	2*,3,6	2 A	?P	DIA-1 STREAKY
	RM	2*,3,6	1 A		LOW ACTIVITY
	WM	2*,3,6	1 A		
	H	2*,3,6	2 A		
	K	2*,3,6	2 A		
ENOL	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
EST	L	2,3,4,5*,6	2 A	BOTH P	SUB BANDING IN
	RM	2*,3,4,5,6	1 A		LIVER QUICKLY
	WM	2,3*,4,5,6	1 A		
	H	2,3,4,5*,6	1 A	?P	FAIR RESOLUTION
	K	2	3 A		GOOD RESOLUTION
FDP	L	2*,3	1 A		POOR RESOLUTION
	RM	2,3	N/A		
	WM	2,3	N/A		
	H	2,3	N/A		
	K	2,3	N/A		
FUM	L	2*,3	1 A		POOR ACTIVITY
	RM	2*,3	1 A		
	WM	2,3*	1 A		
	H	2,3*	1 A		
GA3PDH	L	2,3*,5,6	1 A		VARIABLE
	RM	2,3*,6	1 A		
	WM	2,3,6*	1 C		STREAKY
	H	2,3,5,6*	1 A		VARIABLE
G6PDH	L	1,2,3,4,5*,6	2 A	P	POOR RESOLUTION
	RM	2,3*	1 A		POOR ACTIVITY
	WM	2,3*,5,6	1 A		
	H	2,3,5,6*	2 A		
GDA	L	1,2,3,4,5,6*	1 A		GOOD ACTIVITY
	RM	2	N/A		
	WM	2,3,6	N/A		
	H	2,5,6	N/A		
GLUD	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
GLO I	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		

GLO II	L	2	1 A		POOR RESOLUTION
	RM	2	1 A		
	WM	2	1 A		
	H	2	1 A		POOR ACTIVITY
	K	2	1 A		POOR RESOLUTION
GLYDH	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
GOT	L	2,3*,6	3 A, IC		FAIR ACTIVITY
	RM	2,3*,6	2 A		GOT-2 STREAKY
	WM	2*,3,6	2 A		POOR ACTIVITY
	H	2,3,6*	2 A	? P	POOR ACTIVITY
GOX	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
GPD	L	2,6	N/A		T.O. PROMINENT
	RM	2,6	N/A		
	WM	2,6	N/A		
	H	2,6	N/A		
	K	2,6	N/A		
GPH	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
GPI	L	1,2*,3,4,5,6	1 A		GOOD ACTIVITY
	RM	1,2*,3,4,5,6	2 A	BOTH P	GPI-2 STREAKY
	WM	1,2*,3,4,5,6	2 A	BOTH P	IN MUSCLE
	H	1,2*,3,4,5,6	2 A	BOTH P	INTERACTION BAND
	K	2*,	1 A		GREAT ACTIVITY
GPT	L	2*,3	1 A		LOW ACTIVITY
	RM	2*,3	1 A		LOW ACTIVITY
	WM	2*,3	1 A		SOME SAMPLES N/A
	H	2,3*	1 A		LOW ACTIVITY
	K				
GSR	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
ALPHA HBDH	L	2*,3,6	1 A		LOW ACTIVITY
	RM	2,3*,6	1 A		GOOD ACTIVITY

	WM	2*,3,6	1 A		LOW ACTIVITY
BETA HBDH	L	2*,3,6	1 A		VERY LOW ACTIVITY
	RM	2*,3,6	1 A		LONG TIME TO
	WM	2*,3,6	1 A		STAIN
HEX	L	2*,3,6	1 A		POOR RESOLUTION
	WM	3	N/A		
	H	2,3,6*	1 A		POOR ACTIVITY
	K	2,3	N/A		
HK	L	2*,3	1 A, 1 C		POOR RESOLUTION
	H	2*,3	1 A		LOW ACTIVITY
	K	2*,3	1 A		
IDH	L	1,2,3*,4,5,6	1 A	P	GOOD ACTIVITY
	RM	2,3,6*	1 C		AND RESOLUTION
	WM	2,3,6*	1 C		STREAKY
	H	1,2,3*,4,5,6	1 A		SUB BANDING
	K	2,6	N/A		
LAP	L	2,6*	1 A		FAIR ACTIVITY
	WM	2,6*	1 C		
	H	2*,6	1 A		STREAKY
	K	2	1 A		STREAKY
LDH	L	2,3,4,5,6*	1 A, 3 C		CLEAR
	RM	2,3*,6	1 A, 1 C		GOOD ACTIVITY
	WM	2*,3,6	1 A		STREAKY
	H	2,3,4,5,6*	1 A, 3 C		GOOD ACTIVITY
	K	6	3 C	?P	
	E	2*,3,6	4 A		
MDH	L	2*,3,5,6	1 A		VERY STREAKY
	RM	2*,3,5,6	3 A		STRONG ACTIVITY
	WM	2*,3,5,6	3 A		
	H	2*,3,5,6	1 A		SUB-BANDING
ME	L	2,3,6*	1 A, 1 C		SUB-BANDING
	RM	2*,3,6	1 A, 1		VERY LOW
	WM	2,3,6*	1 A		ACTIVITY IN
	H	2*,3,6	2 A		MUSCLE
	K	6	1 A		
MPI	L	2*,3,5,6*	1 A	P	SUB-BANDING
	RM	2*,3,5	1 A		
	WM	2*,3,5	1 A		SOME VARIATION
	H	2,3,5,6*	1 A	?P	GOOD ACTIVITY
PEP A	L	2*,6	2 A	?P	GOOD ACTIVITY
	RM	2*,6	2 A		ALL TISSUES
	WM	2*,6	2 A		
	H	2*,6	2 A		
	K	2*,6	2 A		

PEP B	L	2*,6	4 A		PEP B 1 VARIABLE
	RM	2*,6	2 A		MUSCLE LOW
	WM	2*,6	2 A		ACTIVITY
	H	2*,6	2 A		POOR RESOLUTION
	K	2*,6	2 A		HIGH ACTIVITY
PEP C	L	2*,6	2 A	?P	GOOD ACTIVITY IN
	RM	2*,6	2 A		LIVER BUT FUZZY
	WM	2*,6	2 A		PEP C 1= PEP A 1
	H	2*,6	2 A		GOOD ACTIVITY
	K	2*,6	2 A		
PEP D	L	2*,6	2 A		FAINT BANDING
	RM	2*,6	2 A		FOR ALL TISSUES
	WM	2*,6	2 A		
	H	2*,6	2 A		
	K	2*,6	2 A		
PEP S	L	2*,6	2 A		GOOD ACTIVITY
	RM	2*,6	2 A		FOR ALL TISSUES
	WM	2*,6	2 A		PEP S 1 SAME
	H	2*,6	2 A		AS PEP A 1 AND
	K	2*,6	2 A		PEP C 1 ?
PGD	L	2,3*,6	1 A		GOOD ACTIVITY
	RM	2,3*,6	1 A		
	WM	2,3*,6	1 A		
	H	2,3*,4,5,6	1 A		T.O PROMINENT
	K	2	1 A		
PGK	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
PGM	L	2*,3,5,6	2 A		PGM-1 POOR, AND
	RM	2*,3,6	1 A		PGM-2 GOOD
	WM	2*,3,6	1 A		ACTIVITY IN H,L
	H	2*,3,6	1 A ?3A		
	K	6	1 A		
PK	L	2	N/A		
	RM	2	N/A		
	WM	2	N/A		
	H	2	N/A		
	K	2	N/A		
SDH	L	2*,3,5,6	1 A	P	NOT CONSISTENT
	RM	2,6*	1 C		PERHAPS NULL
	WM	2,3,6*	1 C		ALLELE IN LIVER
	H	2*,3,6	1 C		
	K	2*	1 A	P	STREAKY

SOD (T.O)	L	2	3 A	P POOR RESOLUTION
	RM	2	3 A	P BEST RESOLUTION
	WM	2	3 A	P IN WM, RM, H
	H	2	3 A	P BUT DIFFICULT
	K	2	3 A	P TO SCORE
XDH	L	2,6	N/A	
	RM	2,6*	1 A	FAIR ACTIVITY
	WM	2,6	N/A	
	H	2*,6	1 A	GOOD RESOLUTION
	K	2*,6	1 A	

SUMMARY: 55 ENZYME SYSTEMS EXAMINED FOR 8Ø PRESUMED LOCI SURVEYED
11 LOCI WERE POLYMORPHIC, OF THESE 7 SHOWED GOOD ACTIVITY
AND SUFFICIENT RESOLUTION TO BE USED FOR POPULATION
ANALYSIS, 5 ADDITIONAL LOCI WERE POSSIBLY POLYMORPHIC
1Ø ENZYMES SHOWED NO ACTIVITY

Appendix 6

Hardy-Weinburg equilibrium tests for 7 polymorphic loci.

APPENDIX 6.1 ACON observed (OBS) and expected genotype frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters.

NULL HYPOTHESIS: ACON gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE						G-STAT	PROB
		AA	AB	AC	BB	BC	CC		
COFFS HARBOUR	OBS	0	0	0	104	5	0	0	NS
	EXP	0	0	0	104	5	0		
CROWDY HEAD	OBS	0	0	0	102	1	0	0	NS
	EXP	0	0	0	102	1	0		
LORD HOWE IS.	OBS	0	1	0	57	4	0	0	NS
	EXP	0	1	0	57.1	3.9	0		
GREENWELL PT1	OBS	0	0	0	75	0	0	0	NS
	EXP	0	0	0	75	0	0		
GREENWELL PT2	OBS	0	0	0	93	5	0	0	NS
	EXP	0	0	0	93	5	0		
NAROOMA	OBS	0	0	0	69	2	0	0	NS
	EXP	0	0	0	69	2	0		
NSW TOTAL	OBS	0	1	0	500	17	0	0.02	NS
	EXP	0	1.2	0	499.5	17.3	0		

NS = not significant. Therefore agrees with Hardy-Weinburg equilibrium.

APPENDIX 6.2. Est-1 observed (OBS) and expected genotype frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters.

NULL HYPOTHESIS: Est-1 gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE						G-STAT	PROB
		AA	AB	AC	BB	BC	CC		
COFFS HARBOUR	OBS	1	2	0	106	0	0	0.8	NS
	EXP	0	3.8	0	105.2	0	0		
CROWDY HEAD	OBS	0	2	0	97	2	0	0	NS
	EXP	0	2	0	97	2	0		
LORD HOWE IS.	OBS	0	0	0	62	0	0	0	NS
	EXP	0	0	0	62	0	0		
GREENWELL PT1	OBS	0	0	0	98	1	0	0	NS
	EXP	0	0	0	98	1	0		
GREENWELL PT2	OBS	0	0	0	98	0	0	0	NS
	EXP	0	0	0	98	0	0		
NAROOMA	OBS	0	0	0	70	2	0	0	NS
	EXP	0	0	0	70	2	0		
NSW TOTAL	OBS	1	4	0	531	5	0	0.36	NS
	EXP	0	7.5	0	530.2	3.3	0		

NS = not significant. Therefore agrees with Hardy-Weinburg Equilibrium.

APPENDIX 6.3. Est-2 observed (OBS) and expected genotype frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters.

NULL HYPOTHESIS: Est-2 gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE						G-STAT	PROB
		AA	AB	AC	BB	BC	CC		
COFFS HARBOUR	OBS	1	15	0	90	0	0	0.396	NS
	EXP	0.7	15.7	0	89.6	0	0		
CROWDY HEAD	OBS	0	12	0	89	0	0	0.858	NS
	EXP	0.4	11.2	0	89.4	0	0		
LORD HOWE IS.	OBS	1	1	0	60	0	0	4.500	NS
	EXP	0.1	2.9	0	59	0	0		
GREENWELL PT1	OBS	1	17	0	81	0	0	0.013	NS
	EXP	0.9	17.2	0	80.9	0	0		
GREENWELL PT2	OBS	0	5	0	91	2	0	0.207	NS
	EXP	0.1	4.9	0	91.1	1.9	0		
NAROOMA	OBS	1	14	0	66	0	0	0.058	NS
	EXP	0.8	14.4	0	65.8	0	0		
NSW TOTAL	OBS	4	64	0	477	2	0	3.905	NS
	EXP	2.5	67.3	0.1	475.1	1	0		

NS = not significant. Therefore agrees with Hardy-Weinburg equilibrium.

APPENDIX 6.4. Gpi-1 observed (OBS) and expected genotype frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters.

NULL HYPOTHESIS: Gpi-1 gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE						G-STAT	PROB
		AA	AB	AC	BB	BC	CC		
COFFS HARBOUR	OBS	0	5	0	98	1	0	0.208	NS
	EXP	0.1	4.8	0	98.1	1	0		
CROWDY HEAD	OBS	0	10	0	93	0	0	0.616	NS
	EXP	0.3	9.6	0	93.1	0	0		
LORD HOWE IS.	OBS	0	2	0	65	0	0	0.000	NS
	EXP	0	2	0	65	0	0		
GREENWELL PT1	OBS	0	3	0	98	1	0	0.000	NS
	EXP	0	3	0	98	1	0		
GREENWELL PT2	OBS	0	7	0	91	0	0	0.206	NS
	EXP	0.1	6.8	0	91.1	0	0		
NAROOMA	OBS	0	2	0	72	0	0	0.000	NS
	EXP	0	2	0	72	0	0		
NSW TOTAL	OBS	0	29	0	517	2	0	0.857	NS

NS = not significant. Therefore agrees with Hardy-WEinburg equilibrium.

APPENDIX 6.5. Gpi-2 observed (OBS) and expected genotype frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters.

NULL HYPOTHESIS: Gpi-2 gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE						G-STAT	PROB
		AA	AB	AC	BB	BC	CC		
COFFS HARBOUR	OBS	0	4	0	100	0	0	0.001	NS
	EXP	0	3.9	0	100.1	0	0		
CROWDY HEAD	OBS	0	7	0	96	0	0	0.102	NS
	EXP	0.1	6.8	0	96.1	0	0		
LORD HOWE IS.	OBS	1	1	0	65	0	0	0.000	NS
	EXP	0	2.9	0	64.1	0	0		
GREENWELL PT1	OBS	0	3	0	99	0	0	0.000	NS
	EXP	0	3	0	99	0	0		
GREENWELL PT2	OBS	0	2	0	96	0	0	0.000	NS
	EXP	0	2	0	96	0	0		
NAROOMA	OBS	0	4	0	70	0	0	0.001	NS
	EXP	0	3.9	0	70.1	0	0		
NSW TOTAL	OBS	0	21	0	526	0	0	0.210	NS
	EXP	0.2	20.4	0	526.4	0	0		

NS = not significant. Therefore agrees with Hardy-Weinburg equilibrium.

APPENDIX 6.6. IDH gene frequencies (p), observed (OBS) and expected genotype
frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters

NULL HYPOTHESIS: Idh gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE			G-STAT	PROB.
		AA	AB	BB		
COFFS HARBOUR	OBS	8	39	53	0.046	NS
	EXP	7.6	39.9	52.5		
CROWDY HEAD	OBS	6	41	53	0.270	NS
	EXP	7	39	54		
LORD HOWE IS.	OBS	5	19	30	0.562	NS
	EXP	3.9	21.2	28.9		
GREENWELL PT1	OBS	6	39	52	0.136	NS
	EXP	6.7	37.6	52.7		
GREENWELL PT2	OBS	14	41	43	0.676	NS
	EXP	12.1	44.7	41.2		
NAROOMA	OBS	7	26	22	0.024	NS
	EXP	7.3	25.5	22.2		
NSW TOTAL	OBS	46	205	253	0.113	NS
	EXP	43.9	209.6	250.5		

NS = not significant. Therefore agrees with Hardy-Weinberg equilibrium.

APPENDIX 6.7. MPI observed (OBS) and expected genotype frequencies and probability of goodness of fit to Hardy-Weinberg (H-W) equilibrium for Seriola lalandi samples from N.S.W. waters.

NULL HYPOTHESIS: MPI gene frequencies conform to H-W equilibrium at each location and for the total N.S.W sample.

LOCATION		GENOTYPE						G-STAT	PROB
		AA	AB	AC	BB	BC	CC		
COFFS HARBOUR	OBS	Ø	2	Ø	85	16	Ø	1.740	NS
	EXP	Ø	1.9	Ø.2	85.7	14.6	Ø.6		
CROWDY HEAD	OBS	Ø	3	Ø	81	11	Ø	2.Ø75	NS
	EXP	Ø.1	2.8	Ø.1	81.5	1Ø.2	Ø.3		
LORD HOWE IS.	OBS	Ø	Ø	Ø	56	3	Ø	Ø.ØØ3	NS
	EXP	Ø	Ø	Ø	56.1	2.9	Ø		
GREENWELL PT1	OBS	Ø	Ø	Ø	92	9	Ø	Ø.41Ø	NS
	EXP	Ø	Ø	Ø	92.1	8.7	Ø.2		
GREENWELL PT2	OBS	Ø	2	Ø	85	1Ø	1	Ø.969	NS
	EXP	Ø	1.8	Ø.1	84.6	11.1	Ø.4		
NAROOMA	OBS	Ø	2	Ø	6Ø	8	1	Ø.995	NS
	EXP	Ø	1.8	Ø.1	59.6	9.1	Ø.4		
NSW TOTAL	OBS	Ø	9	Ø	459	84	Ø	4.38Ø	NS

NS = not significant. Therefore agrees with Hardy-Weinburg equilibrium.

Appendix 7

Results of G-statistic using less than seven polymorphic loci.

APPENDIX 7.1. G-statistic using two polymorphic loci IDH and MPI.

otu 1	vs	otu 2	g stat	d of f	prob.	
coffs		crowdyhd	1.25625	4	.8688	
		lhitotal	6.39582	4	.1715	
		lhilarge	6.19663	4	.1849	
		lhismall	2.88756	4	.5768	
		grwellpt1	4.9223	4	.2954	
		grwellpt2	3.22622	4	.5207	
		narooma	4.76612	4	.3121	
crowdyhd		lhitotal	5.42641	4	.2463	
		lhilarge	5.07579	4	.2796	
		lhismall	3.44649	4	.4861	
		grwellpt1	5.22847	4	.2646	
		grwellpt2	3.93598	4	.4147	
		narooma	7.05537	4	.1330	
lhitotal		lhilarge	.355804	3	.9493	
		lhismall	1.31923	3	.7246	
		grwellpt1	.884062	3	.8293	
		grwellpt2	7.21448	4	.1250	
		narooma	9.55695	4	.0486	*
lhilarge		lhismall	2.12697	3	.5465	
		grwellpt1	1.7482	3	.6263	
		grwellpt2	7.36572	4	.1178	
		narooma	9.14287	4	.0576	
lhismall		grwellpt1	1.18034	3	.7578	
		grwellpt2	1.88772	4	.7564	
		narooma	2.10118	4	.7172	
grwellpt1		grwellpt2	7.04696	4	.1334	
		narooma	9.71042	4	.0456	*
grwellpt2		narooma	2.75661	4	.5993	

KEY: As in Table 10. * indicates significant difference at 95% confidence level

APPENDIX 7.2. G-statistic using five polymorphic loci Acon, Est-1, Est-2, Gpi-1, Gpi-2

otu 1	vs	otu 2	g stat	d of f	prob.	
coffs		crowdyhd	11.3873	11	.4114	
		lhitotal	13.588	11	.2566	
		lhilarge	10.4954	11	.4864	
		lhismall	12.6956	10	.2412	
		grwellpt1	20.8796	11	.0346	*
		grwellpt2	16.7054	11	.1169	
		narooma	11.8195	11	.3774	
crowdyhd		lhitotal	15.868	11	.1461	
		lhilarge	16.1675	11	.1350	
		lhismall	11.0697	10	.3521	
		grwellpt1	19.7868	11	.0484	*
		grwellpt2	17.4472	11	.0953	
		narooma	9.92989	10	.4467	
lhitotal		lhilarge	3.57252	9	.9372	
		lhismall	3.63275	9	.9339	
		grwellpt1	21.0847	11	.0325	*
		grwellpt2	6.68193	10	.7551	
		narooma	13.5976	10	.1922	
lhilarge		lhismall	9.03288	9	.4342	
		grwellpt1	17.4421	11	.0955	
		grwellpt2	7.22907	10	.7037	
		narooma	11.4113	10	.3264	
lhismall		grwellpt1	15.6036	10	.1116	
		grwellpt2	7.01545	9	.6355	
		narooma	12.5646	9	.1833	
grwellpt1		grwellpt2	28.2541	11	.0030	*
		narooma	9.64008	10	.4726	
grwellpt2		narooma	19.0849	10	.0392	*

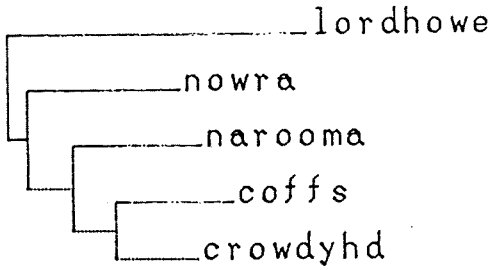
KEY: As in Table 10. * indicates significant difference at 95% confidence interval.

Appendix 8.

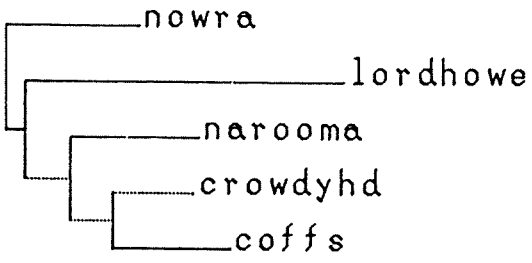
Dendrograms showing the relationships between *S. lalandi* populations from N.S.W.

KEY: grwellpt = Greenwell Point
lordhowe = Lord Howe Island
coffs = Coffs Harbour
crowdyhd = Crowdy Head

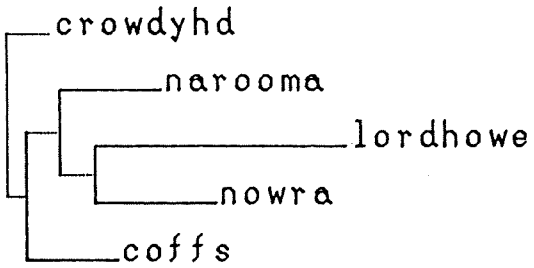
Appendix 8.1a. Dendrograms for five CONTML plots from five populations in NSW. (nowra = Greenwell Point).



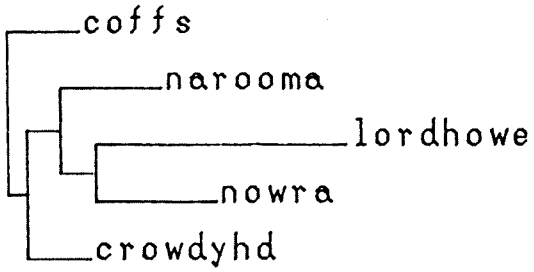
111.15166



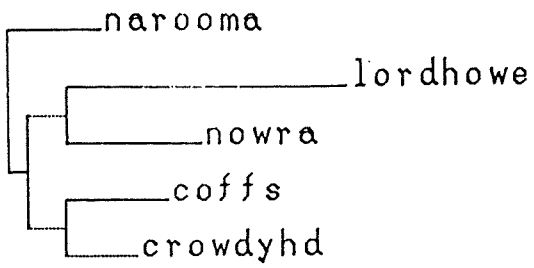
111.15166



111.15166

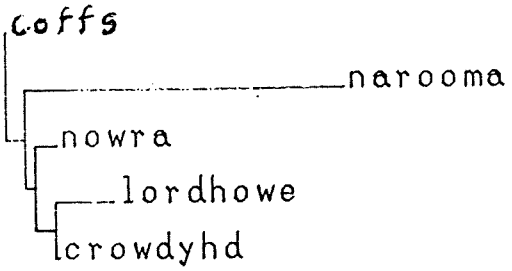


111.15166

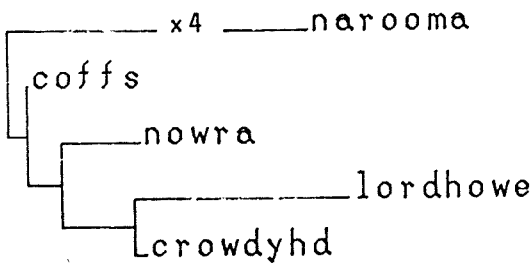


111.15166

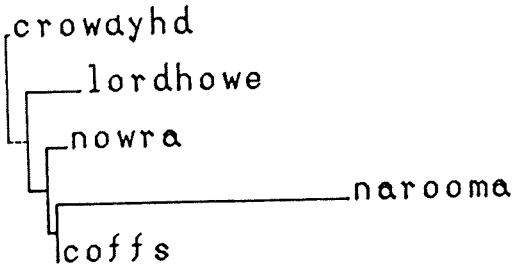
Appendix 8.1b. Dendrograms for five FITCH plots from five populations in NSW. (nowra = Greenwell Point).



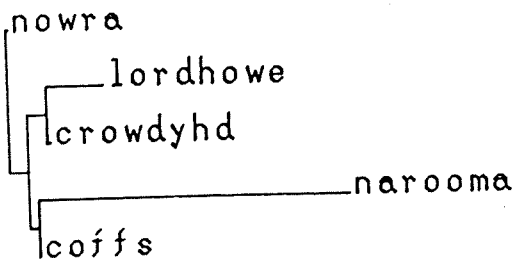
SS = 0.05264 %SD = 5.40776



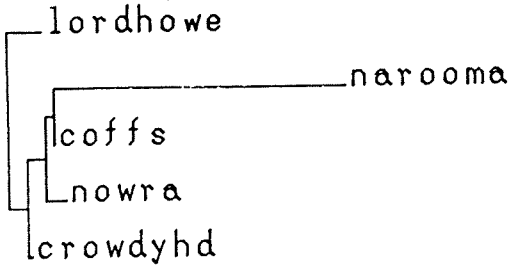
SS = 0.05264 %SD = 5.40776



SS = 0.05264 %SD = 5.40776

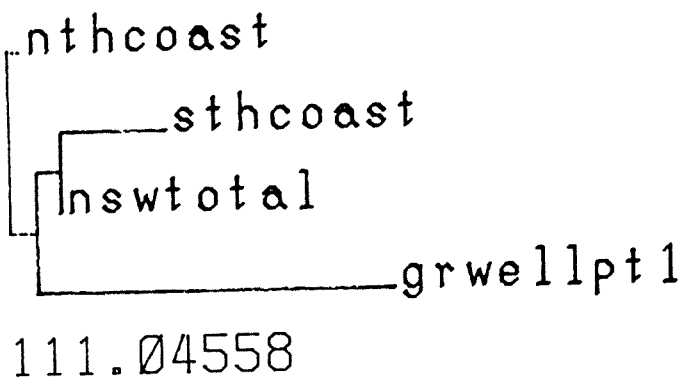


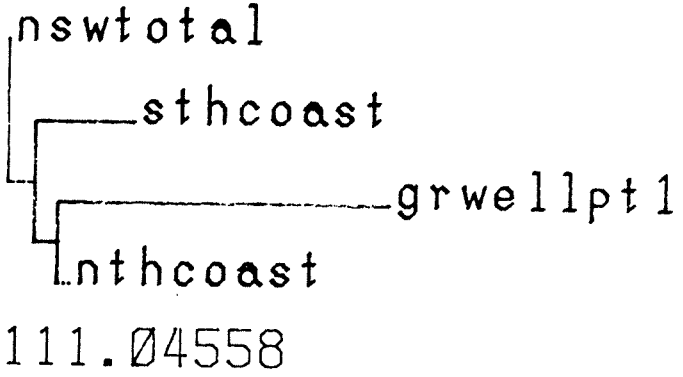
SS = 0.05264 %SD = 5.40776

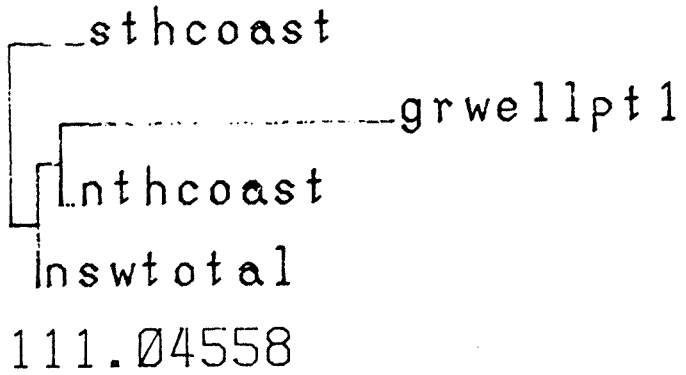


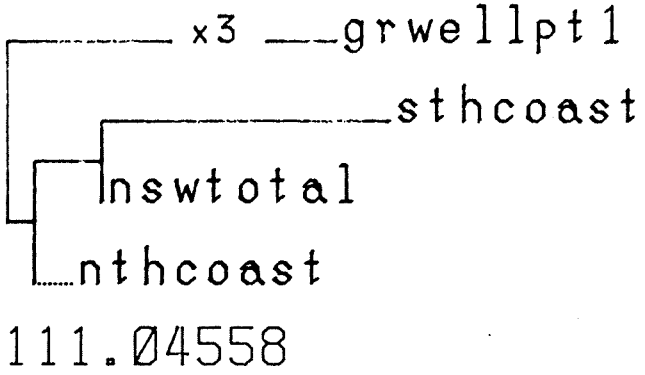
SS = 0.05264 %SD = 5.40776

Appendix 8.3 Dendrograms for four CONTML plots representative of grouped analysis.
(KEY: nthcoast = Lord Howe Island, Coffs Harbour, Crowdy Head; sthcoast = Narooma, Greenwell Point 2; nswtotal = nthcoast + sthcoast.)









Appendix 9.

Poisson distribution tests for tagged/recaptured *S. lalandi*.

Appendix 9.1 Poisson distribution tests for tagged/recaptured S. lalandi over a period of 60days.

Ho: Tagged kingfish are randomly distributed over distance during a 60 day interval between tagging and recapture.

NO. OF KINGFISH PER 25 KM FROM RELEASE	OBS.	f(x)	EXP.	OBS.ln(OBS/EXP)
0	440	0	409.9	31.17
1	7	7	46.3	-13.22
2	5	10	2.57	3.32
3	2	6	0.096	6.07
4	2	8	2.7x10-3	13.20
5	0	0	6.2x10-5	0.00
6	2	12	1.2x10-6	28.70
				69.24

G = 2 x 69.24
= 138.48 (9 D.F.)

P << 0.001 therefore reject Ho. Tagged fish are not randomly distributed with distance for the 60 day period, but clumped.

Appendix 9.2 Poisson distribution tests for tagged/recaptured S. lalandi during a period of 60-120 days.

Ho: Tagged kingfish are randomly distributed over distance during a period of 60-120 days between tagging and recapture.

NO. OF KINGFISH PER 25 KM FROM RELEASE	OBS.	f(x)	EXP.	OBS.ln(OBS/EXP)
0	42	0	30.36	13.63
1	1	1	13.90	-2.63
2	1	2	3.18	-1.16
3	1	3	0.486	0.72
4	1	4	0.055	2.90
5	0	0	5.09x10-3	0.00
6	2	12	3.89x10-4	17.09
				30.55

G = 2 X 30.55
= 61.10 (6 D.F.)

P < 0.001 therefore reject Ho. Tagged and recaptured kingfish are not randomly distributed, but clumped.

Appendix 9.3 Poisson distribution tests for tagged/recaptured S. lalandi over a period of 120-180 days.

Ho: Tagged kingfish are randomly distributed over distance during a 120-180 day interval between tagging and recapture.

NO. OF KINGFISH PER 25 KM FROM RELEASE	OBS.	f(x)	EXP.	OBS.ln(OBS/EXP)
0	11	0	4.23	10.51
1	1	1	5.35	-1.68
2	1	2	3.39	-1.22
3	0	0	1.43	0.00
4	0	0	0.45	0.00
5	0	0	0.11	0.00
6	1	6	0.02	3.73
7	0	0	0.00	0.00
8	0	0	0.00	0.00
9	0	0	0.00	0.00
10	1	10	1.23x10-5	11.30
				22.64

G = 2 x 22.64
= 45.292 (10 D.F.)

P < 0.001 therefore reject Ho. Tagged kingfish are not randomly distributed with distance but clumped.

Appendix 9.4 Poisson distribution for tagged/recaptured S. lalandi for time period >180 days.

Ho: Tagged kingfish are randomly distributed over distance after more than 180 days between tagging and recapture.

NO. OF KINGFISH PER 25 KM FROM RELEASE	OBS.	f(x)	EXP.	OBS.ln(OBS/EXP)
0	50	0	20.18	45.36
1	4	4	24.22	-7.20
2	3	6	14.53	-4.73
3	1	3	5.81	-1.76
4	0	0	1.74	0.00
5	2	10	0.40	3.20
6	1	6	0.08	2.52
7	1	7	0.01	4.60
8	0	0	2.15x10-3	0.00
+9	5	45	2.86x10-4	48.84
				90.83

G = 2 x 90.83
= 181.66 (9 D.F.)

P < 0.001 therefore reject Ho. Tagged kingfish were not randomly distributed over distance but formed a clumped distribution over 25km distances from the tag site for a time period >180 days.

Appendix 10

Neistat input file for three *Seriola* species.

Appendix 10. Neistat input file for three Seriola species

```
     4     30
2 2 3 2 2 2 2 2 2 2 3 3 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2
slalnsw    1.000 1.000 0.000 0.000 1.000 1.000 1.000 0.000
0.000 0.000 1.000 0.000 1.000 0.5 0.5 1.000 1.000 0.000
1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
1.000 1.000 1.000 1.000 1.000 1.000
slalnz     1.000 1.000 0.000 0.000 1.000 1.000 1.000 0.000
0.000 0.000 1.000 0.000 1.000 0.5 0.5 1.000 1.000 0.000
1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
1.000 1.000 1.000 1.000 1.000 1.000
shippos    0.000 0.000 0.333 0.333 0.000 0.000 0.000 1.000
0.000 1.000 1.000 0.000 1.000 0.5 0.5 1.000 0.000 1.000
1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
1.000 1.000 1.000 1.000 1.000 1.000
sdumerili  1.000 0.000 0.000 1.000 0.000 0.5 0.000 1.000
1.000 1.000 0.000 0.5 0.000 0.000 0.000 0.000 0.000 0.000
1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000
1.000 1.000 1.000 1.000 1.000 1.000
```

APPENDIX II

Electrophoretic Investigation of Yellowtail Kingfish.

Between the lines - Fisheries Information Service Newsletter (M.s.)

Electrophoretic Examination of Yellowtail Kingfish

The yellowtail kingfish Seriola lalandi is an important commercial and recreational species in New South Wales coastal waters. Kingfish are keenly sought for their fine sporting and eating qualities. More than 8,000 kingfish have been tagged as part of the Gamefish Tagging Program carried out by the Fisheries Research Institute. Most of the recaptures (more than 450) have been at or near the release site. This prompted the possibility of discrete or local stocks of kingfish existing along the coast of New South Wales with the potential for localised overfishing and important ramifications for management.

To resolve this problem the Fishing Industry Research Trust Account (FIRTA) provided funds for an investigation of the population structure of yellowtail kingfish. The project was initiated by the Fisheries Research Institute and was carried out under the supervision of the University of New South Wales. The principal method used was electrophoresis, a genetic technique which utilises the migration of proteins under the influence of an electric charge. Analysis of kingfish tag/recaptures and reproductive condition facilitated the interpretation of electrophoretic results.

Tissue samples from 553 kingfish were collected with the co-operation of commercial and recreational fishermen. Sampling took place between October 1986 and October 1987 from sites as diverse as Coffs Harbour, Crowdy Head, Lord Howe Island,

Greenwell Point and Narooma.

Statistical analysis of gene frequencies between the five collection sites did not indicate the existence of more than one subpopulation.

The study concluded that kingfish in New South Wales waters consist of one population and should be managed as a single stock.

J Diplock
Biologist