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Biochemical Genetics and Stock Assessment of Common Gemfish and Ocean Perch

Final Report, FRDC Project 91/35

John R. Paxton and Donald J. Colgan

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SUMMARY

Recognition of separate breeding stocks and/or similar species is important if commercial fisheries are to be effectively managed. Two targets of the deepwater trawl fishery in southeastern Australia, the common genfish *Rexea solandri* and the ocean perch *Helicolenus percoides* have unanswered questions regarding the number of separate breeding stocks and similar species, respectively. Both species occur around the southern half of Australia and New Zealand. This report describes two years of FRDC funded research on the biochemical genetics of these two fishes in an attempt to resolve these questions.

Fresh or frozen specimens were obtained directly from commercial fishers, from the markets, from fisheries biologists in Australia and New Zealand and on research vessels. Muscle, liver and gonad tissues were removed and kept frozen at -80°C until analysed. A series of counts (fin rays, vertebrae) and measurements of body parts were made on gemfish from five areas to see if different stocks were morphologically identifiable. A similar morphological study of ocean perch has been undertaken by N.S.W. Fisheries.

The biochemical analyses were of three kinds. 1) Enzymes may have a number of distinct forms (called allozymes) which are genetically-determined. Individuals can differ in their allozymic constitution. The enzymes were solubilized by homogenizing the tissue and subjected to electrophoresis, a process which separates allozymes on the basis of their electric charge as shown by differential migration through an inert matrix under an electric field. After electrophoresis, enzyme-specific stains were used to reveal the position of allozymes, enabling the genetic make-up of individual fish to be inferred. 2) The mitochondria of animal cells contain a circular DNA molecule about 15,000 base pairs in length. Restriction endonuclease enzymes were used to make sequence-specific cuts in this DNA. Variation between indviduals or populations in the position of these cuts was assayed by Southern Blotting after agarose gel electrophoresis to separate the mtDNA fragments on the basis of their molecular weight. A cloned fragment of lake trout (*Salvelinus namaycush*) mtDNA was used as a probe in these experiments. 3) The development of the polymerase chain reaction ("PCR") has allowed the production of large amounts of any desired DNA sequence. We applied this technique to a mitochondrial gene of *Rexea solandri* to provide material for determining the sequence of bases in this segment of DNA. The results of all three of these sets of tests were statistically analysed to indicate how much interbreeding there is between fish from different areas.

Gemfish

A total of 288 specimens of common gemfish were analysed for protein electrophoresis (277), restriction fragment length

variation in mtDNA (136), DNA sequencing (14) and/or morphometrics (62). The area totals are: N.S.W. and eastern Victoria (79), Tasmania (26), western Victoria, South Australia and the Great Australian Bight (94) the west coast of Western Australia (47) and New Zealand (42).

No external differences were found in the counts or body measurements from fish from different regions, although a number of measurements were found to vary with age (head length, snout length, eye diameter). However, the biochemical results indicate that two distinct gemfish stocks occur in Australia with almost no interbreeding. All three biochemical analyses gave similar results, although the restriction fragment analysis of mtDNA in which all 11 tested endonucleases revealed fixed (or nearly fixed) differences was more sensitive than protein electrophoresis where only one of 36 enzyme loci showed a nearly fixed difference. The boundary between the stocks is at the western edge of Bass Strait, with the possibility of some limited mixing (and very limited interbreeding) on the west coast of Tasmania. More specimens are needed from this critical area. There is no indication of sub-populations in either the eastern stock (Byron Bay, N.S.W. to southern Tasmania) or the southern/western stock (Westernport, Victoria to Shark Bay, Western Australia.

In contrast, differences between populations from eastern Australia and New Zealand were observed only in small frequency changes in allozymes, restriction site presence or DNA sequences. Our conclusion is that there is some level of mixture of the two populations. In theory, low migration levels (<1%) should result in complete population homogenisation. Our results show that this has not occurred, implying that migration between the areas is not at a high enough level to require fisheries managers to treat them as a single stock.

Ocean Perch

N.S.W. Fisheries biologists have identified a shallow and a deep form of ocean perch. A total of 92 specimens (39 shallow, 53 deep) were obtained. From N.S.W., there were 34 shallow and 15 deep; from Tasmania, five shallow and 16 deep; from Western Australia, 13 deep; and from New Zealand, nine deep (Fig. 2). Protein electrophoresis was conducted on 72 specimens (31 shallow, 41 deep) and mtDNA restriction analysis on 11 specimens (5 shallow, 6 deep). Our results show that the forms do not interbreed along the east coast of Australia, and that intermixing between populations within forms is limited. DNA analyses of many more specimens from throughout the range will be required to obtain accurate estimates of the extent of this limitation.

INTRODUCTION

Recognition of separate breeding stocks and differentiation of similar species are important if commercial fisheries are to be effectively managed. Two targets of the deepwater trawl fishery in southeast Australia, the common gemfish *Rexea solandri* (Cuvier, 1832) and the ocean perch *Helicolenus percoides* (Richardson, 1842), have unanswered questions regarding the number of breeding stocks and the number of similar species, respectively, involved in the commercial fishery. The present study was undertaken, with the financial support of FRDC, to determine if biochemical genetics could be utilised to separate breeding stocks/species in the two fisheries. To simplify presentation, each section of this report is in two parts, A. Gemfish and B. Ocean Perch.

A. Gemfish

The common gemfish is one of six species of the Indo-Pacific genus *Rexea*, four of which are found in Australian waters (Parin, 1989; Parin and Paxton, 1990), and one of 16 Australian species of the family Gempylidae (Nakamura and Parin, 1993). Other gempylids of commercial importance in Australia include the barracouta *Thyrsites atun*, the escolar *Ruvettus pretiosus* and, more rarely, the oilfish *Lepidocybium flavobrunneum*. The common gemfish has been recorded only from the southern half of Australia, from 27°S off Brisbane, Queensland to 31°S off Perth, Western Australia and from New Zealand waters (Parin and Paxton, 1990). This gemfish is caught commercially off N.S.W., eastern Victoria and eastern Tasmania, principally during a winter spawning migration to northern N.S.W. (Graham *et al.*, 1982; Rowling, 1990a,b). The species also is taken commercially in western Tasmania and western Victoria (D. Smith, pers. comm. 11/90), and in increasing numbers in the Great Australian Bight (D. Turner, pers. comm. 10/90); it also has been caught off Perth, W.A. (A. Williams, pers. comm. 8/89). The species is also commercially important in New Zealand. Only one New Zealand species, the common gemfish, is assumed, but other species have not been looked for by the fishery biologists (R. Hurst, pers. comm. 12/89). Two other species of *Rexea* have recently been added to the New Zealand fish fauna (Paulin *et al.*, 1989; Paulin, 1991); one of us (JP) found only one specimen of *R. prometheoides*, from off the west coast of North Island, in the collections of the National Museum of New Zealand in 1989.

The gemfish fishery in Australia developed in the early 1970s to the most important winter-caught species in the southeast trawl fishery, with a maximum of more than 5,000 tonnes caught in 1980. Since 1987 the catch has declined dramatically, with TAC quotas imposed in 1988 (Rowling, 1990a,b; Rowling and Reid, 1992); the quota for 1992 was 200 tonnes (Reichelt and Tilzey, 1992). Concurrently the gemfish fishery in New Zealand expanded from about 3000 tonnes in 1978/9 to a maximum of more than 8000 tonnes in 1985/6, and then declined to 2950 tonnes in 1990/1, less than half the TAC

(Annala, 1992: pp. 79-80).

The question of separate breeding stocks in the common gemfish has obvious and important implications for the management of the fishery. If the species is a single, panmictic population resulting from the winter spawning event off the north coast of N.S.W., eastern Australian and New Zealand quotas should be integrated in a joint management program. The smaller fishery in western Victoria and off South Australia, as well as the potential fishery off Perth, should also be limited in a total management quota. On the other hand, if separate breeding stocks can be identified, separate fishery management plans can be maintained.

In Australia, separate eastern and western gemfish stocks have been assumed for management purposes since 1990 on the basis of "some biological information" [length-frequency distributions of juvenile fish from western Bass Strait appeared six months out of phase with those from eastern Australia (Smith, 1993)]. Fish from western Bass Strait (and the Great Australian Bight) were considered separately as the western stock (Anon., 1990). However, no morphological or biochemical evidence was available to support the assumption. The Australian gemfish has always been considered a separate stock from the New Zealand population, without supporting evidence. In New Zealand, two stocks are also assumed, a southern/west coast stock and a northern/east coast stock (Hurst, 1988; Annala, 1992:81), but these stocks have not been confirmed either biochemically or morphologically. The presumed stock boundaries occur somewhere on the east coast of the South Island and on the west coast, perhaps between North and South Islands (Hurst, in lit., 11/92). Four separate management areas are utilised (Hurst, 1988).

B. Ocean Perch

The ocean perch (also known as the red gurnard perch [Last *et al.*, 1983; Hutchins and Swainston, 1986; May and Maxwell, 1986]) is in the genus *Helicolenus*, with seven species currently recognised from the Atlantic, Indian and Pacific Oceans (Paulin, 1989). It is one (or more, see below) of 80 Australian species in the family Scorpaenidae (Paxton *et al.*, 1989), an important commercial family that includes the rock fishes of the northeast Pacific. The past use of nomenclature for the ocean perch has been confusing, ever since McCulloch (1929-30) incorrectly synonymised *Scorpaena papillosus* (Bloch and Schneider, 1801) with *Helicolenus percoides* (Richardson, 1842). As a result, a number of authors have used *H. papillosus* for this species. Paulin (1982) pointed out McCulloch's (1929-30) error. *H. percoides* was originally described from New Zealand, where it is now known from off North Cape at 34°13'S to the Snares Islands at 48°01'S (Paulin, 1989). In Australia it has been recorded from off Newcastle, N.S.W. (33°S) to off Albany, W. A. (35°S) (Hutchins and Swainston, 1986). It has a broad depth range, from 10 m to more than 750 m (Last *et al.*, 1983).

Ocean perch is caught commercially off N.S.W., Tasmania and western Victoria (Park, 1993). The fishery developed in the mid- to late 1970s, with the species recorded separately at the Sydney Fish Market since 1976. Catches through the Sydney Fish Market have ranged from 200 to 400 tonnes from 1977 to 1989, and 80 to 120 tonnes from 1989 to 1991 (Park, 1993). In 1989 N.S.W. Fisheries scientists K. Rowling and T. Park brought two forms of ocean perch specimens to the Australian Museum, an inshore or shallow form and an offshore or deep form, that differed primarily in colour and capture depth.

Preliminary protein electrophoresis on the tissues of 22 Ocean perch specimens indicated large differences in allozyme frequencies in three enzymes, suggesting the possibility that the forms are distinct species.

Paulin (1982) indicated the New Zealand species represented a species complex that required further study. Paulin (1989) concluded only two species were present, the widespread *H. percoides* and the large-eyed, more southern *H. barathri*; he also recorded this second species from southern Australia, based on figures in Last *et al.* (1983) and May and Maxwell (1986). Preliminary protein electrophoresis failed to reveal species differences in New Zealand material of *H. percoides* (Paulin, in lit., 10/89).

OBJECTIVES

A. Gemfish

The objective of this portion of the study is to use the biochemical techniques of protein electrophoresis and mitochondrial DNA (mtDNA) analysis to ascertain whether separate breeding stocks of the common gemfish can be discriminated.

B. Ocean Perch

The objective here is to use biochemical techniques to determine how many distinct species are present in the ocean perch, genus *Helicolenus*, fishery.

MATERIALS

Muscle, liver and (more rarely) gonad tissues were obtained from fresh or frozen specimens of both gemfish and ocean perch, placed in labelled cryovials and maintained in a -80°C freezer until the biochemical analyses were performed. Muscle tissue, predominantly white, was taken from the right side of each specimen, between the anterior portion of the lateral line and the dorsal fin. Each specimen was sexed macroscopically (if mature enough) and measured, either fork length (fl) or standard length (sl), to the nearest mm; fl was later transformed to sl (to the nearest 5 mm) by the equation:

$$sl = fl - (fl \times 0.07)$$

Most specimens were preserved in 10% formalin, transferred to 75% ethanol, and registered, at least temporarily, in the Australian Museum fish collection for later morphological analysis and, for a more limited number, as permanent voucher specimens.

In order to obtain specimens from as many localities as possible, state fisheries officers were contacted in New South Wales, Victoria, Tasmania and South Australia, as well as CSIRO in Hobart and Perth and New Zealand fisheries personnel in Wellington and Auckland. A special WANTED letter (Appendix 2) was prepared for commercial fishers from Tasmania, as specimens from here proved the hardest to obtain. Eventually individual commercial fishers were contacted from New South Wales, Tasmania and South Australia, with mixed success.

A. Gemfish

A total of 288 specimens of common gemfish were analysed for protein electrophoresis (277), restriction fragment length polymorphism in mtDNA (136), DNA sequencing (14), and/or morphometrics (62). The area totals are New South Wales and eastern Victoria (79), Tasmania (26), western Victoria, South Australia and the Great Australian Bight (94), the west coast of Western Australia (47) and New Zealand (42) (Fig. 1). A list of all specimens utilized is appended (Appendix 3). To test for the effects of seasonality, annual variation, and age variability, the N.S.W. collection included specimens of different sizes from six different months in three different years (Appendix 3). The specimens from other areas are much more limited, sometimes the result of a single collection.

B. Ocean Perch

Only 92 specimens of ocean perch specimens were obtained for study (Appendix 4). Of these 39 were the shallow form and 53 the deep form, from New South Wales (34 shallow, 15 deep), Tasmania (5 shallow, 16 deep), Western Australia (13 deep) and New Zealand (9 deep) (Fig. 2). Of these, protein electrophoresis was conducted on 72 specimens (31 shallow, 41 deep) and preliminary mtDNA restriction enzyme analysis on 11 specimens (5 shallow, 6 deep). Although special WANTED posters and colour photographs of the two forms were sent out in the second year of the project, no specimens from Victoria or South Australia were obtained.

METHODS

The methods utilized for both gemfish and ocean perch are similar, although no morphology, tissue deterioration, or mtDNA sequencing was attempted for ocean perch.

Morphology

A. Gemfish

An initial group of 35 gemfish specimens from N.S.W. (12), S.A. (6), W.A. (8) and New Zealand (9) were examined for any obvious morphological differences (dentition, scalation, coloration), x-rayed for meristic counts of vertebrae, ribs, and fin rays, and measured to the nearest 0.1mm with dial calipers for 30 different measurements. While it was planned to utilise the full range of lengths for each of the four areas, this proved impossible, due to the more limited size range of specimens available from all areas except N.S.W. All of the specimens measured were obtained during this study and initially (sometimes after freezing) fixed in formaldehyde and preserved in alcohol.

The initial analysis of 35 specimens indicated that meristic counts did not differentiate any of the areas and no further specimens were x-rayed. However the data suggested that allometric growth was likely in a number of features including head length, snout length, eye diameter, and snout to pectoral origin. An additional 27 specimens were measured for these four measurements, plus standard length and first and second dorsal spine lengths, to provide the final data set on 62 specimens. Because the few specimens from the west coast of Tasmania appear to be a genetic mixture (see below), they were separated as a distinct group for analysis.

B. Ocean perch

Tim Park of N.S.W. Fisheries is completing a study of the morphology of ocean perch, based on specimens from N.S.W. He has found a number of features that distinguish the shallow and deep forms, including colour and eye diameter (Park, 1992). His work has not been duplicated in this study, and identification of our specimens as shallow or deep forms has been based on colour and capture depth.

Tissue Deterioriation (Gemfish only)

Tissues from eight freshly caught specimens off Sydney (on the commercial vessel Maio I) and seven specimens off Western Australia (on the CSIRO FRV Southern Surveyor) were placed in a liquid nitrogen freezer (Sydney) or -20°C freezer (W.A.) within two and one half hours of reaching the deck. While some of those providing specimens (J. McKenzie, N.Z.) were able to confirm that their fish had tissues frozen within 12 hours of capture, most other specimens had spent an indeterminate length of time on a ship in the sun, under ice

and/or frozen before tissues could be removed.

Shaklee and Keenan (1986:20, fig. 5) demonstrated that, for most enzymes, refrigerated storage of tissues at 5°C for up to 15 days has no detectable effect on the resulting patterns. They also found tissue could be stored frozen at -20°C or -70°C for up to 18 months with little or no qualitative effects on enzyme banding patterns, and that tissue could be thawed and refrozen up to 100 times over a three week period with no detectable effects on banding patterns for most enzymes tested. We are, however, not aware of similar data available for mtDNA analyses. Nor do we know how long gemfish can be kept at ambient temperature before enzymes or mt DNA break down. Therefore the eight specimens caught off Sydney on a commercial vessel were not iced or frozen after the inital tissues were taken and frozen, but placed in a fish box and left out of doors for seven days. Muscle, liver, and gonad (all ovarian) tissues were taken from each of the eight specimens on days 0, 1, 2, 4, and 6 (and from only two specimens on days 3 and 5) around noon, and placed in a -80°C freezer. The fish were in the sun for up to three hours each day. Although it was midwinter (23-29 July 1992), the maximum temperatures ranged from 17.3°C to 21.4°C (and would have been warmer in the sun) and the minimum temperatures ranged from 5.1°C to 12.2°C (Sydney Bureau of Meteorology, pers. comm.). After the seventh day the flesh was so obviously deteriorating that the fish would not have been accepted for tissue samples.

Electrophoresis

Tissue was ground in 1 volume of tissue to 1 volume of homogenising buffer (100ml tris-HCl, pH 7.0, 1mM Na_2 EDTA, 0.5mM NADP and 50µl/100ml β-mercaptoethanol) in hand-held glass homogenisers. The preparation was centrifuged at 13,500 rpm in an MSE Microcentaur centrifuge and the supernatant divided into aliquots held at -80°C awaiting electrophoresis. Electrophoresis was performed on "Titan 111" (Helena, Austin) cellulose acetate gels according to standard procedures (Hebert and Beaton, 1989). Gels were run for 60' (except esterase run for 40', and peptidase-phe-pro 30'), with a constant potential drop of 200V between electrodes. Twenty-two enzyme systems encoded by 36 loci were scored. Staining protocols were adapted from Harris and Hopkinson (1977), Richardson *et al.* (1986), Shaklee and Keenan (1986), and Hebert and Beaton (1989). Fluorescence methods were used for esterase.

The enzymes stained, tissues used, abbreviations following Shaklee *et al.* (1989) used herein, E.C. numbers, running buffer and number of presumptive genetic loci are given in Table 1. Allozymes identified during this study are numerically designated in order of their relative anodal mobility, as were different loci encoding the same enzyme. The following convention for electrophoretic nomenclature is adopted in this report. Protein-encoding loci are italicised,

with letters other than the first in lower case (e.g. *Gpi-2*) whilst the proteins, or allozymes, themselves are in plain capitals (e.g. GPI-2 A).

MtDNA

DNA was extracted from frozen livers, or muscle, if liver was not available from the animal, using the technique of Miller *et al.* (1989). For restriction enzyme digestions, DNA was treated for between 3 and 16 hours with 3 to 5 units of the restriction endonuclease per µg of DNA under the reaction conditions specified by the manufacturer. A panel of restriction endonucleases was initially screened to identify those which cut the mitochondrial DNA ("mtDNA") of both New South Wales and New Zealand specimens. A total of 11 of these endonucleases were routinely applied to DNA samples, although some specimens were examined only for a subset of these. The 11 utilised endonucleases comprised the 6-base recognising enzymes *Hind III* and *Pst 1*, and the 4-base recognising enzymes *Alu 1*, *Ban II*, *Cfo 1*, *Dde 1*, *Hae III*, *Hinf 1*, *Hpa II*, *Mbo 1*, *Sau 3A*. The 6-base cutters *Bam H1*, *Eco R1* and *Xho 1* tested in the initial panel were not used because they cut the mtDNA only once or not at all.

After digestion, samples were run on agarose gels (0.8% for 6-base cutters, 1.5% for 4-base cutters) containing 17µl of 5mg/ml ethidium bromide and 25µl of 10mg/ml RNase A per 250ml gel solution. To enable estimation of fragment sizes, 1µg of *Hind III* - digested lambda DNA was run on 0.8% gels and 1µg of the pGEM™ marker on 1.5% gels. After trimming (including removal of the marker lanes) and following partial de-purination for 10′ in 0.25M HCl (6-base cutters only), alkaline denaturation for 30′ in 0.5M NaOH, 1.5M NaCl and neutralisation for 45′ in 0.025M Na_2HPO_4/NaH_2PO_4, gels were capillary blotted onto Genescreen (Dupont) . After blotting, membranes were baked at 80°C for 3 hours and stored between Whatman 3MM chromatography paper.

Probes were prepared by nick translation (Rigby *et al.*, 1977) of 1/2 µg plasmid DNA. Unincorporated ^{32}P was removed by spun-column chromatography using Bio-Spin columns (Bio-Rad). Nick-translation products were denatured by boiling for 5′ before addition to probe mixtures. Membranes were pre-hybed in 6 x SSC, 0.5 % SDS, 5 x Denhardt's Solution, at 63°C for 2 hours. They were then placed in polyethylene bags with 3 to 5ml of fresh hybridisation solution, bubbles were squeezed out and the denatured probe was added. After sealing, the bags were held at 58°C for 16 hours. The probe was drained from the membranes which were then transferred to trays of 2 x SSC. Washing of the filters followed this protocol:

1 wash for 5′ at room temperature in 2 x SSC
1 wash for 20′ at 37°C in 1 x SSC, 0.5% SDS
1 wash for 20′ at 58°C in 0.5 x SSC, 1% SDS.

After washing, membranes were placed in 2 x SSC awaiting blotting, wrapping in plastic film and autoradiography at -80°C for one to seven days with intensifying screens. After exposure, probe was stripped by shaking the membrane for 3 hours at 75°C in 300ml pre-heated 1mM Tris-HCl (pH 8.0), 0.1 x Denhardt's reagent (Sambrook *et al.*, 1989). Probe removal was confirmed by re-exposure.

The probe used in these experiments was a 9.67kb Bam H1/ Sal 1 fragment of lake trout (*Salvelinus namaycush*) mitochondrial DNA cloned into the Stratagene PBS Bluescript plasmid (Grewe *et al.*, 1993). This clone was kindly provided to us by Dr P. M. Grewe of the CSIRO Division of Fisheries. The fragment includes the D loop region and the 12S rRNA gene. Large-scale DNA preparations from the clone were made following the alkaline lysis procedure detailed in Sambrook *et al.* (1989), following confirmation of plasmid antibiotic resistance and insert size.

DNA Sequencing (Gemfish Only)

The polymerase chain reaction ("PCR") was used to amplify DNA from the mitochondrial 12S rDNA gene according to standard reaction conditions (Sambrook *et al.*, 1989) and a final volume of 100µl. Perkin-Elmer-Cetus PCR Gems™ were used to allow PCR via the "hot-start" modification. The oligonucleotide pair used for amplification was that described by Kocher *et al.* (1989). The Kocher *et al.* (1989) cytochrome B primers were also tested but failed to amplify gemfish DNA in any of a variety of conditions. The step parameters used for the 12S rDNA primers were as follows:

	DENATURATION		ANNEALING		EXTENSION		CYCLES
Step 1	93°	5′	50°	1′	72°	3′	1
Step 2	93°	1′	50°	1′	72°	3′	30
Step 3	93°	1′	50°	1′	72°	10′	1

After PCR was completed, 6 µl of the product was run on an agarose minigel. With our conditions only one band, of the expected size, was detected, so that subsequent gel purification was unnecessary. DNA sequencing was then performed via linear amplification using the Stratagene "Cyclist" ™ kit. Reaction products were run on a 0.4mm x 60cm 6% acrylamide gel at a constant power of 55W for two hours (to reveal small products) or four to five hours (to reveal large products). For 3 New Zealand (NI409, 686, 687) and 3 N.S.W. (NI661-3) specimens, both strands of the DNA were scored by using each oligonucleotide to prime the Cyclist reactions in separate experiments. Short (2 hour) and long (3.5 hour) gel runs were made for each specimen. Only one primer was used in a short gel run for western Tasmanian (NI543, 546), western Victorian (NI529) (both with primer H1478) and other New Zealand (NI685, 690-1, 1035, 1038) (primer L1091) specimens.

Analyses

Electrophoretic results were analysed using the HENNIG 86 package of Farris and Swofford's BIOSYS-1 package (Swofford and Selander, 1981). For cladistic analyses, the locus was treated as the character and allozymes either singly or in combination (for polymorphisms) were used as either ordered or, more generally, unordered states.

The restriction fragment length polymorphism data provided by our experiments were analysed with the assistance of the "REAP" package (McElroy *et al.*, 1992) and DNASIS V (Hitachi Software Engineering Company, Yokohama). Haplotype data were used to discriminate between stocks. No attempt was made to map the actual position of restriction sites in *R. solandri* mtDNA.

DNA sequences were read manually and entered into the DNASIS V program package for analysis.

Two methods were used to estimate the parameter Nm, where N is the (effective) size of each sub-population and m is the probability that a gamete in the offspring generation is an immigrant to the sub-population where it occurs. These are the F-statistics (Wright, 1951, 1978) and conditional allelic frequency (Slatkin, 1981, 1985a, 1985b) approaches.

The overall inbreeding coefficient can be partitioned into components reflecting non-random breeding (F_{IS}) and the effects of between sub-population differentiation (F_{ST}). If the migration rate is small and selection is negligible, then:

$$Nm \approx (1 + (F_{ST})^{-1})/4$$

A number of alternative methods for the calculation of quantities very similar or identical to F_{ST} have been suggested to accommodate complications of the original two-allele per locus situation (Nei and Chesser, 1983; Weir and Cockerham, 1984). The various methods have been reviewed (e.g. Chakraborty and Leimar, 1987; Weir, 1990) and differences between them shown, generally, to be of secondary significance. We have employed the BIOSYS program package (Swofford and Selander, 1981) for the calulation of F_{ST} values. Where estimates of gene flow given below are based on F_{ST}, this will be indicated by Nm-F_{ST}, if it is not clear from the context.

The second main method of estimating gene flow is the conditional allelic frequency approach of Slatkin (1981, 1985a). For this estimation, "private alleles" are identified. These are alleles found in only one population. Slatkin (1985a) found that the average frequency of private alleles ($\bar{p}(1)$) is approximately linearly related to the migration rate by the expression:

$$\log_{10}(\bar{p}(1)) \approx a\log_{10}(Nm) + b$$

where *a* and *b* take values dependent on the number of individuals sampled from each sub-population. Where estimates of gene flow based on the frequency of private alleles are given below they will be designated as $Nm\text{-}\bar{p}(1)$, if this is not clear from the context.

When *Nm* was estimated from the conditional frequencies of private alleles, the parameter values for a sample size of 50 were taken from Barton and Slatkin (1986). The sample sizes in our study do vary between loci and between geographic areas. They are usually of the order of 80 or more for the Eastern Australian area, 120 or more for the Western and 30 or more for New Zealand. Estimates of *Nm* are sensitive to variation around such sample sizes, but not greatly so (Barton and Slatkin, 1986; Slatkin and Barton, 1989). Slatkin (1985a) suggests that an approximation to accommodate sample size variation: estimated *Nm* is divided by the ratio of the actual sample size to 50. The effect of this approach would be reduce the estimates of gene flow - by as much as 50% for the Eastern and Western Australian regions.

RESULTS

Distribution

A. Gemfish

During the course of this study, the range of the common gemfish in Western Australia was extended north some 900 kilometres from off Perth (32°20'S) to north of Shark Bay (23°25'S), based on 25 specimens taken on the FRV Southern Surveyor cruise 1/91 between Geraldton and Exmouth, W.A. and now in the Australian Museum collections (Appendix 3, AMS I.31156-I.31164). This new information was passed on to others and has been utilised in the latest summary publications (Kailola *et al.*, 1993; Nakamura and Parin, 1993).

There was also some question at the beginning of the study whether individuals of the common gemfish were resident year-round in the northernmost portion of their range; all of the Queensland Museum specimens were taken in June and July and could have merely been part of the annual spawning run. However we received specimens from off Coffs Harbout to Byron Bay from May to October (Appendix 2), and presume in the other six months fishers are targetting other species.

B. Ocean Perch

Similarly, the Australian distribution of the ocean perch has been extended on both the east and west coasts of Australia. Previously recorded from Newcastle, N.S.W. (33°S) to Albany, W.A. (35°S) (Hutchins and Swainston, 1986:42), ocean perch on the west coast was taken on the FRV Southern Surveyor

cruise 1/91 as far north as off Shark Bay, W.A. (26°36'S) in 500 m, with 12 specimens in the Australian Museum collections (Appendix 4, AMS I.31171). This new information is now available in the latest publications (Williams, 1992; Park, 1993). More recently a specimen sent by commercial fisher Wayne Piper has extended the eastern distribution north to off Pottsville near Byron Bay, N.S.W. (29°10'S) (Appendix 4, AMS I.34133).

Of interest were the observations of Rodney Trelogin, a lobster trap fisherman from eastern Tasmania. He claimed he could tell whether his traps were over sand or rock because the "dark" (= shallow) forms occur in rocky areas and the "light" (=deep) forms occur over sand, regardless of depth (r. Trelogin, pers. comm. 2/93). However, no sepcimens were made available and his hypothesis could not be tested.

Morphology

A. Gemfish

No significant differences were found in the meristics or morphometrics of gemfish specimens from the four areas of Australia or from New Zealand (Table 2). Nor were differences apparent in other external features such as dentition, fin coloration, extent of head scalation, or position of lateral lines. A number of features were found to undergo allometric growth, with head length, snout length, eye diameter, and snout to pectoral origin all being relatively longer in smaller specimens (Fig. 3). However none of these, or any of the other measurements, distinguished any of the five groups of gemfish from any other.

Biochemical Genetics

A. Gemfish

Allozymic frequencies in *R. solandri* are presented in two tables. In Table 3, frequencies are given for a split of the species into 10 geographic areas. In Table 4, these areas have been lumped into three "regions", these being (A) the east coast of Australia plus the Tasmanian samples (both coasts), (B) Victoria, South Australia and Western Australia and (C) New Zealand. Regions (A) and (B) are distinguished by a very large frequency difference at the *Aat-2* locus. This is not actually a fixed difference between the regions, as allozyme C which is fixed in all areas of region (A), with the exception of two fish in western Tasmania, is present at a frequency of 0.192 in region (B). The most common *Aat-2* allozyme of region (B) is B. This is found in region (A) only in the two exceptional western Tasmanian fish, as a BC heterozygote in NI542 and as a BB homozygote in NI546. It is clear from this distribution that regions (A) and (B) do not constitute a single interbreeding population. The only way in which the absence of the B allozyme from the east coast of Australia can be explained is by highly reduced gene flow from

region (B) to region (A). It appears probable that there is some degree of intermixture of the populations from the two regions in the west coast of Tasmania. The allozymic data do not necessarily indicate that there is interbreeding, as heterozygotes such as NI542 are also found in all areas of region (B).

Other electrophoretically detectable differences between regions (A) and (B) include the finding, at a number of loci, of allozymes which are in low frequency in one region and absent from the other. There are three polymorphic loci (*Adh*, *Est* and *Gpi-1*) with allozymes which differ in frequency by between 10 and 15%. Summary measures of the genetic distance between the regions are given in Table 5, F_{ST} statistics detailing the differentiation for particular loci in Table 6 and estimates of the amount of gene flow in Table 7. The estimates of gene flow (*Nm*) are expressed as the product of the effective population size (*N*) and the migration rate (*m*). The private alleles estimate of gene flow is highly affected by the inclusion of NI542 and NI546. Otherwise, the F_{ST} and $\overline{p(1)}$ approaches give concordant estimates of *Nm* lying between one and two individuals per generation, implying a very low rate of migration between the regions.

There is not a clear distinction in the electrophoretic data between the populations from the east coast of Australia and those from New Zealand. There are fifteen cases where an allozyme is found in low frequency in one region but is absent from the other (*e.g.* EST A, E and F, GPI-1 A, ME-2 A and B). In all but one of these (ADH D) the allozyme is present in eastern Australian waters and absent from New Zealand. This probably reflects the larger sample size from Australian region (A). Only for *Est* and *Gpi-1* are there allozymes which differ in frequency between the regions by more than 10%, and in all cases, these differences are less than 15%. It is notable, moreover, that there are no loci for which an heterogeneity chi-squared analysis suggests that there are significant differences between the regions in allozymic frequency distributions. The high levels of similarity are emphasised by the very low genetic distances (Table 5, Figure 4) and the estimates of gene flow (Table 7) between the regions. These estimates are some six to seven times higher than those for migration between eastern and western Australian regions. The F_{ST} value (0.009 excluding *Est*) is in the range typical of species which show moderate amounts of genetic differentiation (*e.g.* Seeb and Gunderson, 1987; Wehrhahn and Powell, 1987), although they are larger (implying lower migration) than in the other available comparison of New Zealand and Australian fish: in the blue grenadier (*Macroronus novaezelandiae*), F_{ST} is 0.006 (Milton and Shaklee, 1987)

There is little evidence from the electrophoretic data that there are major restrictions on gene flow within the regions described above. This can be seen by inspection of Table 3 and from the inter-area genetic distance matrix of Table 8. Of particular interest here were questions of subdivision in

populations along the southern and western Australian coasts and within New Zealand. In the former case, only *Adh* displays considerable frequency differences between areas. This is due to the absence of allozymes other than B in the Victorian-eastern South Australian area and is probably a chance event, although it may reflect a local selective advantage for the allozyme. There are not enough specimens available from New Zealand for comparisons of regional frequency distributions to be any more than indicative. However, there are no examples of allozymes which differ by more than 10% in the two areas.

Analyses were conducted to ascertain whether there are notable frequency differences between year (1991 *vs* 1992 collections) or size (< 400mm, 400 - 600mm, >600mm) classes in the eastern Australian region (A). The few significant differences were due to the absence of a low frequency variant from one of the classes. There were no significant differences when allelic frequencies were pooled so that all cells for a given locus had expected values greater than five.

Table 9 presents data on the electrophoretic phenotypes of the long-finned gemfish *R. antefurcata* and the small gemfish *R. bengalensis*. Both of these species are clearly electrophoretically distinct from *R. solandri*, the former exhibiting six fixed differences from *R. solandri* and the latter seven among the eighteen loci which were scored. There are eight fixed differences between *R. antefurcata* and *R. bengalensis*.

The mtDNA data are summarised in Table 10, which shows the numbers of each identified haplotype found in each of the three major regions and Table 11 which presents details of the sizes of the scored DNA fragments and their presence or absence in haplotypes. A photograph of a *Hinf 1* Southern blot is shown in Figure 5. The striking differences between the phenotypes of regions (A) and (B) in this figure is typical of the results for all endonucleases. There is a fixed (or nearly fixed) difference between the regions in their haplotypes for every one of the eleven tested enzymes. The only enzyme where a haplotype is found in both regions is *Pst 1* where NI542 from the West Coast of Tasmania has the B haplotype of the western region. Estimates of the degree of mtDNA sequence divergence between the various observed individual phenotypes were generated by the D.exe program in the REAP package and are given in Table 12. The divergence between the eastern and western Australian regions is 0.44, a high value for supposedly conspecific individuals. In contrast to this, there is little evidence of variation between New Zealand and the eastern coast Australian populations. There are haplotypes observed in low frequency in either region (A) or (C) which are absent from the other (*e.g. Cfo 1* C, *Hae III* C, *Hinf 1* C and D and *Mbo 1* C). These haplotypes may, however, be present in both regions, but may not have been detected in one owing to sampling effects. These low frequency variants are independent of each other, implying the scenario that all are of recent origin from the

common haplotypes - and not the alternative of mutation accumulation within single clones permitted by the maternal inheritance of mtDNA. Some note may be taken of the 3 *Hinf 1* D variants, which were all found among the 29 fish scored for this endonuclease from the east coast of the North Island of New Zealand. The absence of this phenotype from other areas is not statistically significant, yet is worth further investigation in relation to stock discrimination within New Zealand.

The mtDNA data do not entirely clarify the affinities of the population on the west coast of Tasmania. The high frequency of the AAT-2 C allozyme suggested that most of the fish from this locality derived from the eastern Australian region, although NI542 and NI546 have the B allozyme. According to its mtDNA phenotype, NI542 is, as suggested by the allozyme data, most likely derived from the western Australian populations. 115 bases of the 12S rDNA sequence of NI546 were sequenced and shown to be identical with that of NI529 from the Western population, in differing from the Eastern Australian form at three sites. The mtDNA of the other fish from this region indicates, however, that their relationships lie in the eastern region. Another complication is the finding of the *Cfo 1* C haplotype in two west Tasmanian fish and nowhere else. The conclusions that can be drawn in this report are limited by the low sample sizes for the region. It is clear, however, that the majority of fish in this area are more closely related to the eastern Australian populations than they are to the western. Whatever, hybridisation occurs between fish from the two regions has a very minor influence on the distribution of genetic variation in the species as a whole.

Apart from the variability in the western Tasmanian area, there is very little intra-regional variation in the mtDNA phenotypes. In particular, there is no indication that there is any biologically significant sub-division in region (B), with the same haplotypes being fixed in all areas of the region for all enzymes.

To test the apparently close relationship between eastern Australian and New Zealand populations further, we undertook the DNA sequencing study of the individuals specified in the 'Methods' section. The listing of the mitochondrial 12S rDNA sequence of NI409 is shown in Table 13. The A at position 35 is replaced by G in all 7 other New Zealand and 3 N.S.W. fish. Otherwise, there is no detectable difference in the sequences of individuals from the two regions. Segments of the mitochondrial DNA, in particular the D loop, do evolve more rapidly than the 12S gene. Sequencing of such segments, using appropriate PCR primer pairs (which are not yet available) might conceivably reveal a major frequency difference between the regions. But such a finding cannot be presumed. Some data were also collected for portion of the 12S segment in western region DNAs. These are shown in Table 14. There are three differences (in 115 bases) between the sequences, which

would be about the level expected from restriction fragment data given that the 12S rDNA gene evolves somewhat slower than other mtDNA.

Tissue Deterioration

The results of the examination of electrophoretic and mtDNA phenotypes in the tissue degradation experiment were somewhat surprising and most reassuring. Samples from three fish from days 1, 3, 5 and 7 were scored for the following enzymes in muscle: AAT, AK, GPD, GPI, IDH, LDH, ME, MPI, PGM and TPI; and for ALD, EST, FBP, GA-3-PDH, G-6-PDH, GPI, LDH, MDH and 6-PGDH in liver. The only changes in phenotype were a reduction in activity for AK and ALD in fish 2, day 7 and in 6-PGDH in fish 3, day 7. In all other cases, there was no diminution in enzyme activity and no generation of breakdown isozymes. These 3 fish were also scored for DNA from liver taken on days 1, 2, 3, 4, 5 and 7. Two were scored for muscle and gonad DNA for the same days. Up to day three there was no apparent degradation of high molecular weight ("HMW") DNA in liver samples. Thereafter, the lengths of DNA on ethidium bromide-stained gels became progressively shorter, until it was mostly less than 300 bp by day 7. However, probing with Lake Trout mtDNA reveals that there is a significant amount of intact gemfish mtDNA in the samples at day 7. A similar pattern was seen for *Hinf 1* digested DNA. There was no reduction at all in the band intensity of any fragment of the mtDNA pattern until after day 4. Day 5 revealed lower intensities, but even at day 7 all fragments of the phenotype were quite clearly scorable. Gonad and muscle tissues were even more resistant to degradation, with significant amounts of HMW DNA remaining at the end of the series, and intact mtDNA being then present in amounts only a little reduced from starting levels.

B. Ocean perch

The allozymic frequencies that were observed in our samples of *Helicolenus percoides* are presented in Table 15. A diagram of the phenetic relationships of the samples is given in Figure 8 which shows a clear separation of the shallow and deep forms. The forms are clearly reproductively isolated to a significant extent, *Est-2* for instance showing a fixed difference between the forms over the whole length of the eastern seaboard, with the exception of the most southerly locality (Sorrel, Tasmania). *6-Pgdh* has very large frequency differences over all of this coastline. Notably, however, the most common allozymes in the forms at Sorrel are the reverse of the most common allozymes in the forms at other localities, implying that whilst there is a general split into two main lineages, there is substantial local genetic differentiation. Other loci which contribute to the genetic distance estimates between sympatric populations are *Fh* and *Sdh-1* at Eden and *Aat-1* and *Pgm* at Sorrel.

The numbers of specimens examined is too small at this stage to make a detailed analysis of stock structure within each of

the forms. At the very least, however, the data suggest that both Sorrel samples are distinct to other populations of their respective forms, as both lie well away from other east coast localities in Figure 8. There is some indication that Western Australian and New Zealand deep forms might also constitute separate stocks.

NOMENCLATURE

A. Gemfish

Rexea solandri was described by Cuvier (1832) on the basis of Solander's manuscript description of New Zealand specimens. No type specimens exist. *Rexea furcifera* Waite, 1911 is a junior synonym (Nakamura and Parin, 1993), with New Zealand also as type locality; the type specimen of this nominal species has not been searched for. As all of the common gemfish populations are considered to belong to the same biological species, there are no nomenclatural problems. If further studies of the western Tasmanian population indicate that the two Australian stocks represent distinct species, a new name would be required.

B. Ocean Perch

Helicolenus percoides was described by Richardson (1842) from New Zealand. Although almost all of the type specimens of species described by Richardson were placed in the British Museum (Natural History), a search by the first author in 1991 failed to find any types of this species.

Paulin (1989) considered *Helicolenus barathri* (Hector, 1875) a valid species, primarily on the basis of a larger eye diameter. This species also has a type locality of New Zealand, off Cape Farewell. Paulin (1989) listed the records and descriptions of *H. percoides* by both Last *et al.* (1983) (as *H. papillosus*) and May and Maxwell (1986), from Tasmania and the southern coast of Australia respectively, as *H. barathri*. The latter decision is presumably based on the eye diameter and colour of the figured specimen. The Tasmanian record (Last *et al.*, 1983: p.317) includes a figure originally published in Scott *et al.* (1973) and presumably from South Australia. While the eye diameter of this figured specimens exceeds 1/3 of the head length, the diagnostic for *H. barathri*, the range of eye diameters given in the description is 1/3 to 1/4, as are those in May and Maxwell (1986), and clearly includes more than one species, presumably also the nominal Australian *Helicolenus percoides*. Paulin (1989) did not examine any Australian specimens. The present study does not have enough specimens from Tasmania, nor any from western Victoria or South Australia, to attempt to solve the problem in southern Australia.

Sebastes alporti was described by Castelnau (1873) from Victorian and Tasmanian waters. While the vast majority of Castelnau's types were deposited in the Museum national

d'Histoire naturelle in Paris, these types were not found, despite a search by the first author in 1991. The present study did not solve the problem of determining how many species of Australian ocean perch exist, or the relationships to the New Zealand species. If a future study determines the Australian ocean perch is one, or more, species distinct from the New Zealand species, Castelnau's name will have validity.

DISCUSSION

A. Gemfish

Our results of the study of gemfish genetics clearly show that there are two populations of common gemfish in Australia with very limited gene flow between the populations. The highly significant difference in the allozymes of *Aat-2*, the fixed or nearly fixed differences in the mtDNA haplotypes of all 11 tested enzymes, and differences in the DNA sequences of the two populations are all indicative of significantly reduced gene flow. Estimates of gene flow (Table 7) between the eastern and southern/western populations in Australia indicate a migration of only 1-2 individuals per year. For fisheries management purposes, these eastern and southern/western populations can be considered completely separate breeding stocks. Virtually none of the specimens of gemfish caught west of Bass Strait will contribute to the spawning run off N.S.W.

Our evidence for two Australian gemfish stocks is supported by the information now known about the breeding of Australian gemfish. The east coast spawning run is well documented (Graham *et al.*, 1982; Rowling, 1990a,b; Kailola *et al.*, 1993), with the vast majority of the east coast gemfish catch coming from the capture of migrating individuals between the months of May and August each year. The commercial fishers are well aware of the annual spawning migration; for instance Fritz Drenkhahn of Eden reported that "Scouts are off Eden this week (15-18/6/92) and the main run should be next week for two weeks" (pers. comm., 6/92). It is clear that prespawning individuals are the main target.

There is still some question about how far north off the N.S.W. coast the actual spawning takes place. Previous indications were that the spawning may take place as far north as off Coffs Harbour, from where gemfish larvae have been identified (Gorman *et al.*, 1987). However in 1992 the gemfish run apparently did not travel as far north as usual, with Laurieton dropline fishers catching few. On 17-18 August 1992, many spent gemfish were caught off Barrenjoey, Sydney that were sent to the markets (Rowling, pers. comm., 8/92). While the timing of the annual spawning run is well known, the precise spawning locality and details of annual variations in spawning locality remain unknown.

Alan Williams of CSIRO Perth (now CSIRO Hobart) recently discovered ripe gemfish off Perth that, when combined with the presence of spent fish, is strongly suggestive of breeding off W.A. (Williams, in litt., 1/92). The presence of both running ripe and spent fish in November indicates at least the western portion of the southern/western gemfish stock is breeding some five-six months out of phase with the eastern stock. This observation correlates well with that of Smith (1993) that the

length-frequency distributions of juvenile gemfish from western Victoria are six months out of phase with those from the east coast (see Introduction above). The lack of reproductively active gemfish from South Australia and the Great Australian Bight may be the result of sampling error with too few samples available, or it may indicate that the entire southern/western stock is also the result of a relatively localised spawning activity off W.A., with larval dispersal facilitated by the Leeuwin Current. We have found no genetic evidence to break this southern/western stock into subpopulations.

As discussed above, there is very low gene flow between the two distinct Australian stocks. In contrast, there is apparently a significant amount of gene flow between the eastern Australian gemfish population and that of New Zealand. In theory, gene flow as small as 1% will prevent local population genetic differentiation (Slatkin, 1985). This presumably explains why Smith *et al.* (1990) found so few commercial fish stocks definable by electrophoretic differences. Our estimates of gene flow are, however, well below this level. From our electrophoretic results, we estimate that the actual numbers of individuals migrating between the regions in any one year and subsequently contributing to the next generation is less than 30. This estimate may be effected by selective differences among allozymes in the two regions. But, without invoking major selective differentials, the estimate would not be increased to such an extent that we should regard the eastern Australian and New Zealand populations as a single stock. The evidence from mtDNA haplotypes and 12S rDNA sequence data also indicates continued gene flow between eastern Australia and New Zealand. Again, however, the finding of variants at low frequency in one region and not in the other is suggestive that local differentiation can occur to some extent. In particular, the presence, albeit in low frequency, of the *Hinf 1* D phenotype may characterise a northern New Zealand stock.

We have not found a fixed genetic difference between the eastern Australian and New Zealand regions. It is true, however, that negative data are never conclusive, as has been shown recently by the successful definition of Canadian salmon stocks (Utter *et al.*, 1992). It is possible that sequencing regions of DNA which evolve more rapidly than the 12S rDNA might show differences between New Zealand and eastern Australian fish. This could be achieved by designing primers specifically for the the D loop region of gemfish mtDNA or by employing the emerging technology of microsatellite DNA analysis. However the case that there is at least some gene flow between the eastern Australian and New Zealand populations will probably remain the most likely scenario even after such further study. It is now certain that trans-Tasman migration occurs for adult fishes, at least for school sharks (Coutin *et al.*, 1992)

Further evidence that we are not dealing with one

homogeneous population originating from the N.S.W. breeding migration is provided by the recent discovery of at least one breeding area of gemfish in New Zealand waters, off Auckland on the northeast of North Island. This is based on only three running ripe individuals, but many fish with highly vascularised gonads were also observed (McKenzie, pers. comm., 7/92). With gemfish from the Wellington area (more than 500 kms south of Auckland) disappearing during the breeding season (Hurst, pers. comm., 6/92), it is possible to infer a breeding migration along the east coast of the North Island of New Zealand similar to that along the east coast of Australia. Very limited data indicate the possibility of another spawning area on the west coast of the South Island of New Zealand, but movement/migrations have not been established (Hurst, in litt., 11/92; Annala, 1992).

A likely scenario for trans-Tasman gene flow is for larval gemfish from N.S.W. to be carried across the Tasman Sea by eddies of the East Australian Current. Such eddies are regularly pinched off the East Australian Current, which moves south from the Coral Sea along the coast towards Tasmania (Nillson and Cresswell, 1980; Cresswell and Legeckis, 1986). Satellite-tracked drifters released on the east coast of Australia have reached New Zealand (Cresswell, pers. comm., 6/93). Francis (1993) discusses trans-Tasman transport of larval fishes in terms of larval longevity and current patterns. All of the trans-Tasman larval dispersal previously studied, of both fishes (Francis, 1993) and echinoderms (Hoggett and Rowe, 1988), have been from west to east. However with a complete lack of knowledge of the length of the larval stage of gemfish, such consideration is largely academic, except to note that larval migration will more likely be from Australia to New Zealand than vice-versa.

B. Ocean perch

Our research on the biochemical genetics of ocean perch has not completely resolved the stocks of Australian and New Zealand ocean perch of the genus *Helicolenus*. Substantial progress has, however, been made in what has been revealed to be a complex taxonomic area. Our electrophoretic analysis of the allozymes of 72 specimens of ocean perch supports the separation of deep *vs* shallow forms over a wide geographic range along the east coast of Australia. The analysis does not, however, fully resolve questions of population structure within ocean perch. For instance, the Sorrel deep sample is genetically more distant to other east coast Australian deep populations than these are to Western Australian samples. The three allozyme differences originally found between the shallow and deep forms off central N.S.W. (see Introduction above) were not found consistently in shallow and deep forms from other areas. Although not originally proposed we were able to perform mtDNA analyses for two restriction endonucleases on 11 specimens (5 shallow and 6 deep). For *Hinf 1*, there was a great deal of variation, with every

individual having at least one unique fragment in its phenotype. There was less variation for *Dde 1*. The 2 shallow form individuals from Eden had a fragment that was absent from all other scored individuals, including two deep form fish from the same locality. However, two Tasmanian shallow form fish had the deep phenotype. The results of our studies suggest that for ocean perch, gene flow can be quite restricted even over relatively small geographic differences. This concords with the studies of Golovan *et al*. (1991) who found statistically significant differences in meristic and morphometric features of three populations of *Helicolenus lengerichi* found on three different seamounts along the Nazca Ridge. More detailed comparisons of many more Australian and New Zealand specimens than were possible in this study will probably reveal a complex of populations/species. Restricted gene flow may be reinforced by the internal fertilization known to occur in at least the Australian specimens of *H. percoides* (eyed larvae have been found in mature females off Sydney), although the length of larval life is unknown. Electrophoresis alone is unlikely to resolve such a potentially complex mixture of populations and/or species. Both mtDNA restriction enzyme analysis and DNA sequencing would probably be required to resolve this situation. Such studies would require additional FRDC funding for completion of the research.

RECOMMENDATIONS

A. Gemfish

Our recommendations for gemfish are in the areas of both research and management.

(1) Our results clearly recognize two separate Australian stocks of gemfish, with a boundary at the western end of Bass Strait, and possibly a low level of mixing in western Tasmania. These results support the continued management of Australian gemfish as two separate eastern (Byron Bay, N.S.W. to southern Tasmania) and southern/western (Westernport, Victoria to Shark Bay, Western Australia) stocks.

(2) While our results indicate mixing of eastern Australian and New Zealand populations, the level is not sufficiently great to indicate that they comprise a single stock. Our recommendation is to continue to manage them as separate stocks. None of our results suggest a scenario that involves a significant number of Australian fish migrating to New Zealand and then returning to Australian waters to spawn.

(3) In the absence of clear-cut evidence for a South Island spawning event, and with the coincidence of concurrent decreases in the gemfish catches of eastern Australia and New Zealand, future management of gemfish should include greater consultation between Australian and New Zealand Fisheries. Certainly New Zealand Fisheries should be supported in their tag and release study of gemfish if this can be shown to be feasible, and commercial fishers in eastern Australia alerted as to the possible existence of tagged gemfish. Also New Zealand Fisheries should be encouraged to make a concerted effort to find the presumed spawning locality off the South Island and to determine its extent. Such a discovery would be additional evidence that the two stocks are distinct and should be managed separately.

(4) The primary area of gemfish research should be to continue genetic investigation of level of discreteness of the east Australian and New Zealand gemfish stocks. One approach would be to obtain DNA sequence information from very rapidly evolving genes, perhaps using DNA microsatellite technology. Also, the frequency differences observed in this study might be investigated in detail by a follow-up survey. Another possible avenue, considering other successes in identifying stocks with parasites, would be to invite a parisitologist like Prof. Klaus Rohde of the New England University to undertake a pilot study to determine if there are any differences in the parasites of gemfish from eastern Australia and New Zealand that could be used as stock markers. The specimens currently preserved in the Australian Museum may be adequate for such a pilot study.

(5) We suggest further researchn into the east Australian spawning event, with the aim of understanding natural

variation in year class success. Currently there is little information available on the environmental parameters (water temperature, salinity, plankton productivity) associated with normal annual variation in the numbers of larvae produced during this spawning. Without this information there cannot be a distinction between a small year class due to natural environmental variation or to increased fishing mortality. Determination of the locality of spawning, and whether this indeed varies annually, might also be addressed in a research cruise in late July- early August. Collection of planktonic larvae may give not only an indication of spawning extent and success, but eventually information about larval life span that will be of value in estimating how easily larvae may reach New Zealand.

(6) An effort could be made to determine if gemfish are spawning anywhere on the south coast of Australia, or whether the southern/western stock is the result of one spawning event. This research will become more relevant if and when this stock develops a commercial fishery.

B. Ocean perch

Considerably more research on the biochemical genetics of ocean perch will be required to distinguish separate stocks/species of this complex, as discussed above. This research should focus on mtDNA or techniques such as "micro-satellite" analysis, which may be even more sensitive in the analysis of population differentiation (Tautz, 1989). The bulk of the fishery is currently in N.S.W., with more than 90% of the marketed catch consisting of the deepwater form (Rowling, pers. comm., 6/90). It must be emphasised that there are at least two species (the shallow and deep forms) in the fishery and its management should recognise this. However, until distinction of the various stocks within the two main species (or species groups) is possible, little advice on strategies for the management of individual stocks can be given.

TECHNICAL SUMMARY

This project used biochemical techniques to investigate the number of separate breeding stocks of the common gemfish *Rexea solandri* and whether more than one species is included in the ocean perch *Helicolenus percoides* fishery. A total of 288 specimens of common gemfish were analysed for protein electrophoresis (277), restriction fragment length polymorphism in mitochondrial DNA (136), sequencing of the 12S mitochondrial rDNA (14), and/or morphometrics (62). For ocean perch, electrophoresis was conducted on 72 specimens (31 shallow, 41 deep) and preliminary mtDNA restriction enzyme analysis on 11 specimens.

Electrophoresis was performed on "Titan 111" (Helena, Austin) cellulose acetate gels according to standard procedures. DNA was extracted from liver and (more rarely) gonad. A total of 11 restriction endonucleases were routinely applied this DNA. Samples were run on agarose gels (0.8% for 6-base cutters, 1.5% for 4-base cutters) and Southern Blotted. Blots were probed with a 9.67kb Bam H1/Sal 1 fragment of lake trout (*Salvelinus namaycush*) mitochondrial DNA cloned into the Stratagene PBS Bluescript plasmid (Grewe *et al.*, 1993). The polymerase chain reaction ("PCR") was used to amplify DNA from the mitochondrial 12S rDNA gene, using the Kocher *et al.* (1989) "universal primer", according to standard procedures (Sambrook *et al.*, 1989) except that PCR Gems™ were used to allow the "hot-start" modification. Sequencing was performed via linear amplification using the Stratagene "Cyclist" ™ kit.

Electrophoretic results were predominantly analysed using Swofford's BIOSYS-1 package and restriction fragment length polymorphism data with the "REAP" package (McElroy *et al.*, 1992) and DNASIS V. Migration rate was estimated using Wright's F-statistics and Slatkin's private alleles approaches.

An initial group of 35 gemfish specimens from N.S.W. (12), S.A. (6), W.A. (8) and New Zealand (9) was examined for obvious morphological differences but meristic counts did not differentiate any of the areas. Nor were differences apparent in other external features such as dentition, fin colouration, extent of head scalation, or position of lateral lines. The possibility of allometric growth was suggested for a number of features including head length, snout length, eye diameter, and snout to pectoral origin. An additional 27 specimens were measured for these four measurements, plus standard length and first and second dorsal spine lengths. In the final data set, all four measurements are relatively longer in smaller specimens. However, none of these distinguished any of the groups of gemfish.

The biochemical data clearly distinguish two Australian stocks of gemfish, these being (A) the east coast of Australia plus the Tasmanian samples (both coasts); and (B) Victoria, South Australia and Western Australia. The regions are

distinguished by a very large frequency difference at the *Aat-2* locus. This is not actually a fixed difference between the regions, as allozyme C which is fixed in all areas of region (A), with the exception of two fish (NI542 and NI546) in western Tasmania, is present at a frequency of 0.192 in region (B).

There is a fixed (or nearly fixed) difference between regions in their mtDNA haplotypes for every one of the eleven tested endonucleases. The only enzyme where a haplotype is found in both regions is *Pst 1* where NI542 from the West Coast of Tasmania has the B haplotype of the western region. Estimated sequence divergence between the regions is 0.44. The mtDNA data suggest the population on the west coast of Tasmania may comprise a mixture of the stocks. According to its mtDNA phenotype, NI542 is, as suggested by the allozyme data, most likely derived from the western Australian populations. 115 bases of the 12S rDNA sequence of NI546 was sequenced and shown to be identical with that of NI529 from the Western population, in differing from the Eastern Australian form at three sites.

Apart from western Tasmania, there is no indication that there is any biologically significant sub-division in either region (A) or (B), with the same mtDNA haplotypes being fixed in all areas of the regions for all enzymes and with no statistically significant electrophoretic divergence.

There is no clear demarcation in the electrophoretic data between the populations from the east coast of Australia (A) and New Zealand (C). There are fifteen allozymes which are found in low frequency in one region but are absent from the other. In all but one of these, the allozyme is present in eastern Australian waters and absent from New Zealand, possibly reflecting the larger sample sizes from the Australian region. Only for *Est* and *Gpi-1* are there allozymes which differ in frequency between the regions by more than 10%. Similarly, there is little evidence of variation between New Zealand and eastern coast Australian populations in mtDNA. There are haplotypes observed in low frequency in either region (A) or (C) which are absent from the other. The three observed *Hinf 1* D variants were all found among the 29 fish from the east coast of the North Island of New Zealand. The absence of this phenotype from other areas is not statistically significant, yet is worth further investigation in relation to stock discrimination within New Zealand.

There is only one variable position in the 12S rDNA sequences of fish from regions (A) and (C). The A at position 35 of NI409 is replaced by G in all 7 other New Zealand and 3 N.S.W. fish. Segments of the mitochondrial DNA, in particular the D loop, do evolve more rapidly than the 12S gene. Sequencing of such segments might reveal a major frequency difference between the regions. The emerging technology of microsatellite DNA analysis might also be employed. However, some gene flow between the eastern Australian and New Zealand

populations will probably remain the most likely scenario.

Deterioration in the quality of DNA preparations and electrophoretic phenotype upon continued exposure was monitored in specimens which were left outdoors for seven days. Muscle, liver and ovarian tissues were taken from specimens on each day. Although the experiment was conducted in midwinter, maximum temperatures ranged from 17.3°C to 21.4°C. Samples from three fish from days 1, 3, 5 and 7 were scored for ten enzymes in muscle and nine in liver. The only changes in phenotype were a reduction in activity for three liver enzymes in one fish each at day 7. Otherwise, there was no diminution in enzyme activity and no generation of breakdown isozymes. These three fish were also scored for DNA from liver taken on days 1, 2, 3, 4, 5 and 7. Two were scored for muscle and gonad DNA for the same days. Up to day three there was no apparent degradation of high molecular weight ("HMW") DNA in liver samples. Thereafter, the lengths of DNA became progressively shorter, until mostly less than 300 bp by day 7. However, probing reveals that there is a significant amount of intact mtDNA in the samples at that time. A similar pattern was seen for *Hinf 1* digested DNA. There was no reduction at all in the band intensity of any fragment of the mtDNA pattern until after day 4. Even at day 7 all fragments of the phenotype were quite clearly scorable. Gonad and muscle tissues were even more resistant to degradation.

The "shallow" and "deep" forms of ocean perch *Helicolenus percoides* are clearly reproductively isolated to a significant extent, *Est-2* for instance showing a fixed difference between the forms over the whole length of the eastern seaboard, with the exception of the most southerly locality (Sorrel, Tasmania). *6-Pgdh*, too, has very large frequency differences over all of this coastline. There are also apparent genetic differences within the forms suggesting that gene flow can be quite restricted even over relatively small geographic distances. For example, the Sorrel deep sample is genetically more distant to other east coast Australian deep populations than these are to Western Australian samples. Intra-form differentiation is marked in the preliminary mtDNA studies. For *Hinf 1*, there was a great deal of variation, with every individual having at least one unique fragment in its phenotype. For *Dde 1*, the two shallow form individuals from Eden had a fragment that was absent from all other scored individuals, including two deep form fish from the same locality. However, two Tasmanian shallow form fish had the deep phenotype.

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Table 1: Summary of Electrophoretic Procedures.

The columns give, in order, the name of the enzyme, its abbreviation used herein, E.C. number, running buffer and number of presumed genetic loci. Running buffer TEM 50 is 50mM tris, 1mM Na_2EDTA, 1mM $MgCl_2$, pH adjusted to 7.8 with maleic acid. Buffer TC 100 is 100mM tris, pH adjusted to 8.2 with citric acid. The tissues used were: muscle M, liver L, or both

ENZYME	ABBREVIATION	E.C. NO.	BUFFER	LOCI	TISSUE
Adenylate kinase	AK	2.7.4.3	TEM 50	2	M
Aspartate aminotransferase	AAT	2.6.1.1	TC 100	3	M,L
Adenosine deaminase	ADA	3.5.4.4	TEM 50	2	L
Alcohol dehydrogenase	ADH	1.1.1.1	TEM 50	1	L
Creatine kinase	CK	2.7.3.2	TEM 50	1	M
Esterase	EST	3.1.1.1	TEM 50	1	L
Fructose-bisphosphate aldolase	FBALD	4.1.2.13	TEM 50	1	M
Fructose-bisphosphatase	FBP	3.1.3.11	TEM 50	1	M
Fumarate hydratase	FH	4.2.1.2	TEM 50	2	M
Glucosephosphate isomerase	GPI	5.3.1.9	TEM 50	2	M
Glucose-6-phosphate dehydrogenase	G-6-PDH	1.1.1.49	TC 100	1	L
Glyceraldehyde phosphate dehydrogenase	GA-3-PDH	1.2.1.12	TEM 50	1	M
α-Glycerophosphate dehydrogenase	GPD	1.1.1.8	TEM 50	1	M
Isocitrate dehydrogenase	IDH	1.1.1.42	TEM 50	2	M
Lactate dehydrogenase	LDH	1.1.1.27	TC 100	1	M
Malate dehydrogenase	MDH	1.1.1.37	TEM 50	2	M
Malic enzyme	ME	1.1.1.40	TEM 50	2	M
Mannosephosphate isomerase	MPI	5.3.1.8	TEM 50	1	L
Peptidase (leu-ala substrate)	PEP-la	3.4.11	TEM 50	1	L
Peptidase (leu-gly-gly substrate)	PEP-lgg	3.4.11	TEM 50	1	L
Peptidase (phe-pro substrate)	PEP-pp	3.4.11	TEM 50	1	L
Phosphoglucomutase	PGM	2.7.5.1	TEM 50	1	M
6-Phosphogluconate dehydrogenase	6-PGDH	1.1.1.44	TEM 50	1	L
Phosphoglyerokinase	PGK	2.7.2.3	TEM 50	1	M
Pyruvate kinase	PK	2.7.1.40	TEM 50	1	M
Sorbitol dehydrogenase	SDH	1.1.1.14	TEM 50	2	L

Table 2: Morphometric and meristic data for individual gemfish *Rexea solandri*

Specimen name Rexea solandri

Reg. no.	I.30909-001	I.30909-002	I.32774-003	I.28998-004	I.28998-003	I.32517-002	I.32517-004	I.32517-003	I.28998-002	I.32517-001	I.28998-001	I.31499-004	I.31500-006	I.31500-002	I.31500-001	I.31500-012	I.31500-007	I.33377-003	I.33377-002	I.33377-0
Locality	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	NSW	ETAS	ETAS	ETAS
Stan Len	214.5	243.4	356.5	367.5	381.9	447.7	485.4	487.6	499.0	582.3	623.0	711.7	715.9	718.6	680.9	683.8	689.5	701.1	656.5	818.0
Fork Len	232.4	265.0		400.2	411.3				538.7		670.8	761.3	770.5	771.8	727.8	737.0	740.2			
Head Len	64.8	71.5	103.6	109.4	112.6	127.8	136.9	142.2	141.1	167.3	172.3	192.5	190.5	187.4	192.0	183.3	188.7	199.7	183.0	223.6
Up Jaw Len	30.7	34.3		51.8	53.1				68.3		83.2	96.3	94.2	92.8	95.0	91.7	89.7			
Snout Len	27.3	30.0	42.3	45.0	46.7	52.7	56.9	57.1	59.3	66.0	71.5	85.0	83.7	81.6	79.2	77.3	79.7	85.7	74.2	93.0
Eye Diam	13.6	14.7		24.2	25.1				27.4		35.0	33.7	35.4	34.5	39.6	33.6	33.3			
Bony Inter	11.6	12.6	19.1	22.7	22.1	25.3	27.4	28.3	30.4	34.8	36.5	39.5	39.3	42.0	37.8	39.0	38.9	40.4	35.7	47.7
Body Depth	33.2	40.0		61.0	60.3				83.6		103.0	112.7	114.0	121.0	119.3	115.7	118.3			
Sn to P	65.4	73.5		110.0	110.5				139.0		172.8	197.3	196.4	195.0	195.9	186.5	189.5			
P Len	29.3	34.7	46.1	51.3	54.5	62.1	67.5	70.1	70.8	76.7	85.0	90.3	91.5	97.0	95.7	91.0	93.0	102.8	92.6	102.8
Sn to V	73.4	82.3	115.9	123.2	125.0	144.7	157.2	150.0	155.7	189.4	192.8	223.8	221.0	213.0	231.5	217.7	211.7	235.1	204.1	261.9
V Len	5.2	6.7		5.7	6.0				8.0		6.7	9.0	12.4	9.1	11.5	6.0	9.3			
Sn to D	61.0	66.7		102.9	104.7				135.8		165.0	177.9	194.7	188.9	177.2	173.7	189.5			
D1 Base	99.8	112.5		177.5	176.7				242.4		330.0	340.0	347.5	357.0	325.3	333.5	347.4			
D2 Base	43.4	46.9		73.0	81.6				107.7		109.0	150.5	156.8	141.1	140.5	139.0	151.5			
Sn to LL2	87.4	95.2		141.3	146.0				193.0		235.1	256.0	261.2	252.4	263.6	254.7	267.6			
Sn to A	163.5	194.7		283.1	298.4				381.8		481.5	552.1	551.1	534.5	540.5	533.6	514.0			
A Base	38.0	44.1		64.0	67.3				91.8		114.9	123.3	119.7	129.9	110.0	117.5	124.5			
LL1 Len	122.0	130.9		213.5	219.1				280.0		391.5	414.3	467.2	396.7	376.6	342.0	455.2			
LL2 Len	170.4	146.7		230.0	238.0				316.0		392.0	457.0	452.1	470.0	432.0	425.9	447.5			
Caud Ped Dep	10.8	11.5		17.1	17.4				24.0		29.3	32.5	33.8	34.0	30.7	33.0	32.3			
Caud Ped Len	14.0	17.2		19.3	24.0				34.2		41.0	48.4	41.6	44.1	46.3	50.0	43.5			
C Len	46.5	47.2		79.2	85.4				100.0		110.0	131.0	136.7	136.6	141.0	136.0	135.5			
D spl Len	15.5	15.6	26.3	26.7	26.2	27.0	29.6	31.2	33.0	31.5	41.5	46.5	41.6	46.4	52.0	47.0	44.7	45.7	40.0	36.1
D ray1 Len	25.2	21.0		38.7	40.9				57.0		60.5	80.4	68.0	62.4	77.5	56.3	62.8			
A ray1 Len	25.2	24.3		35.3	37.5				47.4		56.0	49.5	54.0	59.6	64.0	58.5	58.9			
D sp2 Len			24.8			28.4	29.7	37.8		33.9								52.6	34.5	35.3

Specimen name Rexea solandri

Req. no.	I.32749-004	I.32749-003	I.32749-001	I.32750-001	I.32749-002
Locality	WTAS	WTAS	WTAS	WTAS	WTAS
Stan Len	793.0	740.3	706.5	642.9	587.5
Fork Len	850.0				
Head Len	214.8	200.8	194.9	182.5	161.3
Up Jaw Len	106.5				
Snout Len	91.9	84.0	85.2	74.2	67.2
Eye Diam	38.6				
Bony Inter	43.1	39.2	39.3	36.4	30.1
Body Depth	125.8				
Sn to P					
P Len	115.0	93.9	98.7	89.8	81.3
Sn to V	266.7	238.4	226.2	224.1	176.1
V Len	11.7				
Sn to D	205.5				
D1 Base	331.5				
D2 Base	154.4				
Sn to LL2	288.7				
Sn to A	581.5				
A Base	129.5				
LL1 Len	437.8				
LL2 Len	486.9				
Caud Ped Dep	36.0				
Caud Ped Len	55.6				
C Len	143.7				
D sp1 Len	43.5	29.7	22.3	32.4	33.4
D ray1 Len	65.4				
A ray1 Len	59.0				
D sp2 Len	50.7	35.4	30.2	40.5	33.9

Specimen name Rexea solandri

Reg. no.	I.31504-002	I.31504-001	I.30910-002	I.31504-004	I.31504-003	I.30910-004	I.30910-001	I.30910-003	I.30910-005	I.30910-006	I.31504-011	I.31504-012	I.31504-013	I.32516-001
Locality	SA	-SA	-SA	SA	-SA	SA	-SA	SA	SA	SA	SA	SA	SA	VIC
Stan Len	380.1	330.8	386.8	395.5	396.7	410.0	417.2	467.6	535.0	551.4	771.7	781.7	855.3	428.3
Fork Len	480.4	361.7	420.0			443.5	451.0	511.0	577.2	595.9			920.8	
Head Len	112.1	94.4	117.0	116.7	114.0	119.7	120.0	128.9	143.8	153.0	197.0	206.2	225.5	120.6
Up Jaw Len	52.1	55.0	53.6			58.0	57.1	63.9	71.0	78.3			113.1	
Snout Len	45.4	39.2	46.5	46.6	46.0	49.7	48.2	53.0	60.6	65.3	80.7	90.4	96.3	48.2
Eye Diam	23.4	19.0	27.2			25.6	25.0	26.8	27.6	29.8			37.5	
Bony Inter	22.2	16.3	22.3	23.3	21.8	24.1	24.5	26.6	28.4	32.2	44.9	41.8	43.6	22.8
Body Depth	61.1	53.5	61.9			62.8	65.9	82.8	83.7	90.6			136.3	
Sn to P	111.0	95.8	114.9			118.7	121.0	132.4	147.0	154.2			228.2	
P Len	58.4	45.9	58.5	60.4	54.6	60.3	59.6	66.3	75.0	75.7	101.5	103.6	103.9	63.3
Sn to V	122.8	104.6	123.9	128.4	121.8	132.6	132.5	142.7	162.4	177.6	254.2	235.9	252.0	133.5
V Len	7.6	8.3	4.4			8.6	7.2	11.0	8.6	8.6			12.1	
Sn to D	111.4	95.8	107.8			109.2	112.5	121.5	138.0	143.8			224.4	
D1 Base	17.2	151.6	191.0			196.0	198.9	218.5	256.0	260.7			388.3	
D2 Base	75.1	61.8	72.5			84.2	84.6	98.7	111.6	112.9			176.9	
Sn to LL2	150.0	140.7	155.9			155.5	153.9	179.5	201.0	214.0			326.9	
Sn to A	302.3	257.1	310.0			324.3	329.3	368.7	433.0	441.0			643.3	
A Base	61.6	50.9	64.6			68.0	68.6	85.0	85.0	92.7			135.9	
LL1 Len	196.4	194.5	214.4			246.5	222.2	272.0	327.3	295.0			513.4	
LL2 Len	221.5	199.3	237.2			256.0	262.6	287.9	335.0	335.0			571.9	
Caud Ped Dep	15.8	14.0	17.8			19.4	20.7	24.5	24.7	28.0			35.2	
Caud Ped Len	30.7	20.6	26.2			23.0	25.8	31.7	37.0	40.7			66.6	
C Len	77.9	73.8	76.2			90.0	89.5	83.7	100.0	115.0			163.2	
D sp1 Len	20.6	29.6	23.8	24.6	20.3	28.2	20.0	29.5	35.0	39.2	41.0	44.7	37.7	22.7
D ray1 Len	19.2	22.5	40.3			45.8	49.0	48.2	54.0	56.7			66.5	
A ray1 Len	34.5	26.6	31.6			35.4	38.3	41.8	55.0	54.4			56.1	
D sp2 Len	26.0	26.8		24.7	20.7						40.7	52.3	49.6	29.7

Specimen name Rexea solandri

Reg. no.	I.31284-001	I.31285-001	I.31285-002	I.31156-001	I.31282-004	I.31163-001	I.31282-002	I.32752-004	I.31282-003	I.31284-013	I.31282-001	I.32752-003	I.31284-014	I.32752-002	I.32752-001	I.32748-001	I.32746-001	I.32747
Locality	WA	WA	WA	WA	WA	WA	WA	WA	WA	WA	WA	-WA	-WA	-WA	WA	-WA	-WA	WA
Stan Len	151.0	227.9	231.6	290.0	299.1	303.3	318.8	325.9	336.7	350.8	382.0	385.7	410.0	462.5	609.1	739.0	769.4	779.5
Fork Len				318.0	321.1	324.3	345.5		367.1	378.0	416.1		446.4				834.4	839.3
Head Len	46.5	70.0	70.7	86.0	94.1	89.7	98.9	98.9	106.1	102.2	114.4	110.9	124.6	132.8	174.0	202.9	211.4	209.5
Up Jaw Len				41.3	44.1	44.2	47.0		59.4	50.0	55.6		58.0				101.5	103.8
Snout Len	17.6	27.5	28.8	35.5	37.4	37.4	39.4	39.1	42.3	42.0	46.9	45.1	50.0	53.6	70.7	82.9	86.2	89.4
Eye Diam				18.8	23.7	20.0	23.0		27.0	23.0	26.6		26.5				35.9	32.0
Bony Inter	7.4	12.6	11.6	17.5	19.0	17.5	21.0	18.6	23.0	21.5	24.5	22.2	25.4	25.2	34.5	38.8	44.9	43.2
Body Depth				51.4	50.7	56.3	51.2		58.5	56.5	65.0		70.8				108.2	111.5
Sn to P				85.9	91.7	90.0	94.8		105.2	102.5	114.5		120.6				210.6	210.6
P Len	17.8	29.7	35.7	40.6	47.2	44.0	50.8	48.6	52.8	51.9	58.9	55.8	57.6	66.8	83.9	96.1	108.0	92.2
Sn to V	47.0	68.4	76.7	105.0	97.6	100.0	100.4	103.6	113.6	112.1	d	121.0	134.0	140.4	186.0	216.4	234.2	226.6
V Len				7.7	5.9	8.0	8.1		9.0	8.9			8.2				9.0	7.9
Sn to D				78.0	87.8	81.7	86.4		98.6	98.2	104.6		113.7				199.0	194.9
D1 Base				142.5	140.6	145.5	144.0		154.8	170.0	177.6		209.4				357.8	360.6
D2 Base				56.8	60.2	60.6	64.8		67.1	70.5	76.4		64.0				150.0	155.4
Sn to LL2				115.4	119.2	117.5	127.9		138.4	141.2	154.0		164.5				286.7	288.6
Sn to A				225.2	231.8	229.2	252.6		267.4	267.8	310.0		319.2				618.4	615.4
A Base				49.6	48.5	48.0	54.4		56.7	63.2	63.5		69.8				123.9	125.9
LL1 Len				158.8	150.3	189.0	172.7		149.4	193.0	196.2		255.1				391.5	492.9
LL2 Len				180.0	180.0	185.5	186.7		194.9	212.7	225.0		256.3				497.9	512.1
Caud Ped Dep				13.5	14.1	14.5	14.1		15.0	17.9	17.7		18.4				32.4	34.5
Caud Ped Len				22.6	17.2	17.0	22.0		23.4	24.7	26.1		23.2				47.6	65.6
C Len				59.0	65.7	56.0	62.4		73.0	73.0	76.5		84.4				149.4	123.5
D sp1 Len	7.6	13.5	15.8	19.2	17.4	20.5	18.4		23.7	27.4	20.1	22.4	28.1	26.5	30.9	36.1	34.9	31.8
D ray1 Len				26.6	d	34.0	34.7	16.1	34.3	41.0	38.2		34.7				57.9	38.9
A ray1 Len				26.4	25.8	28.8	29.2		34.2	36.6	35.0		39.4				44.5	55.7
D sp2 Len	12.8	6.8	16.8					21.5	21.7			24.4		33.1	33.4	38.3	38.3	41.0

Specimen name Rexea solandri

Reg. no.	I.30169-001	I.30169-002	I.30169-003	I.31503-001	I.31503-006	I.31503-005	I.31503-003	I.31503-002	I.31503-004
Locality	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Stan Len	480.0	497.0	539.2	660.0	685.7	710.0	732.6	740.0	755.0
Fork Len	525.0	522.0	578.8	712.0	735.0	768.0	780.1	795.0	815.0
Head Len	140.0	141.6	146.9	191.0	196.7	206.6	202.8	208.6	211.0
Up Jaw Len	67.6	70.0	72.9	93.0	94.2	97.8	99.6	101.5	104.0
Snout Len	59.7	60.0	62.8	79.0	82.0	85.6	87.2	90.0	92.7
Eye Diam	28.0	28.6	27.8	39.0	37.9	37.7	37.3	35.5	37.0
Bony Inter	28.0	28.8	30.6	40.0	37.7	42.6	42.7	42.3	46.0
Body Depth	76.2	78.7	85.0	116.7	123.6	131.7	128.0	127.7	131.5
Sn to P	142.6	143.7	153.7	194.6	193.3	205.0	206.7	211.8	217.8
P Len	65.4	74.4	68.8	89.0	90.6	93.7	98.3	93.0	98.9
Sn to V	161.0	162.5	172.3	205.5	225.7	227.0	230.0	234.7	252.4
V Len	5.0	8.0	8.2	6.3	6.2	3.5		6.0	7.2
Sn to D	130.5	137.7	143.2	188.1	182.3	192.8	201.3	194.3	199.4
D1 Base	232.5	228.2	254.5	315.0	322.4	332.2	358.2	346.2	364.3
D2 Base	100.3	103.5	119.2	131.4	143.7	145.0	146.0	129.0	148.2
Sn to LL2	191.0	192.7	209.3	273.0	257.8	274.4	293.0	281.3	301.3
Sn to A	379.3	394.6	419.6	517.2	538.0	561.5	564.0	589.2	594.5
A Base	83.5	80.7	95.3	102.0	113.0	119.4	125.6	121.0	136.7
LL1 Len	269.0	273.4	325.7	305.2	438.9	369.0	480.0	384.0	470.0
LL2 Len	283.5	306.9	334.0	399.0	419.5	438.0	462.0	461.6	481.8
Caud Ped Dep	22.0	23.5	24.2	31.1	33.2	36.0	34.5	32.7	36.3
Caud Ped Len	32.3	35.5	38.6	47.4	49.3	51.7	54.5	50.0	53.6
C Len	95.2	93.8	106.6	137.8	140.0	150.0	145.0	146.5	144.0
D sp1 Len	21.0	30.0	29.2	34.5	38.4	36.2	44.0	42.7	48.0
D ray1 Len	41.0	56.0	56.7	54.2	62.5	73.0	66.0	64.0	77.3
A ray1 Len	40.6	46.3	51.5	63.2	63.7	66.8	68.0		64.3
D sp2 Len									

Table 3: Allozymic frequencies in gemfish *Rexea solandri* samples. Numbers in the row headed (N) indicate sample sizes. The South Australian east sample includes the Westernport, Victoria fish.

	POPULATION									
	New South Wales			Tasmania		South Australia		Western	New Zealand	
ALLOZYME	North	Central	South	East	West	East	Bight	Australia	North	West
AK										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-1										
(N)	17	39	17	12	6	39	38	42	23	10
A	.059									
B	.059	.026					.026		.022	
C	.765	.923	.912	.750	1.000	1.000	.974	1.000	.891	.700
D	.118	.051	.088	.250					.087	.300
AAT-2										
(N)	16	40	22	16	6	44	39	47	23	10
A						.102	.026	.096		
B					.250	.773	.744	.681		
C	1.000	1.000	1.000	.969	.750	.125	.231	.223	1.000	1.000
D				.031						
AAT-3										
(N)	17	37	18	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-1										
(N)	17	34	17	9	6	33	39	45	23	10
A		.015	.029				.026	.022		
B	1.000	.985	.971	1.000	1.000	1.000	.974	.978	1.000	1.000
ADA-2										
(N)	17	34	17	9	6	32	39	47	23	10
A	.971	.956	.971	1.000	.833	.984	.923	.957	.935	1.000
B	.029	.044	.029		.167	.016	.077	.043	.065	
ADH										
(N)	10	26	1			23	27	37	22	
A	.100	.038	.500				.111	.149	.023	
B	.600	.615	.500			1.000	.593	.676	.659	
C	.300	.346					.278	.176	.250	
D							.019		.068	
CK										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	POPULATION									
	New South Wales			Tasmania		South Australia		Western	New Zealand	
ALLOZYME	North	Central	South	East	West	East	Bight	Australia	North	West
EST										
(N)	5	5	13	9	6	37	13	16		7
A	.200						.231			
B	.100	.200	.077	.444		.162	.077	.219		.214
C	.100	.600	.346	.167	.333	.459	.538	.406		.143
D	.600	.200	.346	.333	.667	.311	.154	.313		.643
E			.077	.056		.041				
F			.154					.063		
G						.027				
FBALD										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FBP										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FH-1										
(N)	17	40	20	12	6	40	39	45	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FH-2										
(N)	17	40	20	12	6	40	39	45	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GA-3-PDH										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPD										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-1										
(N)	16	37	19	12	6	40	39	46	23	10
A	.125	.014				.038	.026	.011		
B	.406	.419	.500	.250	.333	.225	.359	.261	.239	.450
C		.054		.333		.100	.064	.033	.087	
D	.469	.514	.500	.417	.667	.625	.526	.674	.674	.550
E						.013	.026	.022		
GPI-2										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G-6-PDH										
(N)	17	40	20	12	6	37	40	47	23	10
A	.029							.011	.022	
B	.971	1.000	1.000	1.000	1.000	1.000	1.000	.989	.978	1.000
IDH-1										
(N)	17	40	20	12	5	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDH-2										
(N)	16	40	20	12	6	37	38	47	23	10
A			.025							
B	1.000	1.000	.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-1										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-2										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	POPULATION									
	New South Wales			Tasmania		South Australia		Western	New Zealand	
ALLOZYME	North	Central	South	East	West	East	Bight	Australia	North	West
ME-1										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ME-2										
(N)	17	40	19	12	6	38	40	47	23	10
A		.013	.026			.026	.013	.021		
B	.971	.975	.868	1.000	1.000	.974	.988	.957	1.000	1.000
C	.029	.013	.105					.021		MPI
MPI										
(N)	17	40	19	12	5	33	40	46	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM										
(N)	17	37	18	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6-PGDH										
(N)	3	8	13	12	5	21	10	17	7	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEP-la										
(N)	17	40	19	12	6	40	40	47	23	10
A	.059									
B	.941	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEP-lgg										
(N)	17	40	19	12	6	40	39	47	23	10
A	.029						.013			
B	.971	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000	1.000
PEP-pp										
(N)	17	40	19	12	6	40	40	47	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGK										
(N)	5	8	13	12	6	40	13	23	23	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.913	1.000	1.000
B								.043		
C								.043		
PK-1										
(N)	17	40	20	12	6	40	40	47	23	10
A	1.000	.925	1.000	1.000	.833	1.000	1.000	1.000	1.000	1.000
B		.075			.167					
PK-2										
(N)	17	40	20	12	6	40	40	47	23	10
A		.025								
B	1.000	.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SDH-1										
(N)	17	40	19	12	2	40	33	36	21	10
A	1.000	1.000	1.000	1.000	1.000	.913	.924	.944	1.000	1.000
B						.013	.015	.014		
C							.061	.042		
D						.075				
SDH-2										
(N)	17	40	19	12	2	40	33	36	21	10
A		.025								
B	1.000	.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 4: Allozymic frequencies in the three major geographic subdivisions of gemfish *Rexea solandri*. Figures in the rows headed (N) indicate sample sizes.

	POPULATION		
	Australia		New
ALLOZYME	East	West	Zealand
AK			
(N)	95	127	33
A	1.000	1.000	1.000
AAT-1			
(N)	91	119	33
A	.011		
B	.022	.008	.015
C	.874	.992	.833
D	.093		.152
AAT-2			
(N)	100	130	33
A		.077	
B	.015	.731	
C	.980	.192	1.000
D	.005		
AAT-3			
(N)	90	127	33
A	1.000	1.000	1.000
ADA-1			
(N)	83	117	33
A	.012	.017	
B	.988	.983	1.000
ADA-2			
(N)	83	118	33
A	.958	.953	.955
B	.042	.047	.045
ADH			
(N)	38	87	22
A	.066	.098	.023
B	.618	.736	.659
C	.316	.161	.250
D		.006	.068
CK			
(N)	95	127	33
A	1.000	1.000	1.000
EST			
(N)	38	66	7
A	.026	.045	
B	.171	.159	.214
C	.303	.462	.143
D	.408	.280	.643
E	.039	.023	
F	.053	.015	
G		.015	
FBALD			
(N)	95	127	33
A	1.000	1.000	1.000

	POPULATION		
	Australia		New
ALLOZYME	East	West	Zealand
FBP			
(N)	95	127	33
A	1.000	1.000	1.000
FB-1			
(N)	95	124	33
A	1.000	1.000	1.000
FB-2			
(N)	95	124	33
A	1.000	1.000	1.000
GA-3-PDH			
(N)	95	127	33
A	1.000	1.000	1.000
GPD			
(N)	95	127	33
A	1.000	1.000	1.000
GPI-1			
(N)	90	125	33
A	.028	.024	
B	.406	.280	.303
C	.067	.064	.061
D	.500	.612	.636
E		.020	
GPI-2			
(N)	95	127	33
A	1.000	1.000	1.000
G-6-PDH			
(N)	95	124	33
A	.005	.004	.015
B	.995	.996	.985
IDH-1			
(N)	94	127	33
A	1.000	1.000	1.000
IDH-2			
(N)	94	122	33
A	.005		
B	.995	1.000	1.000
LDH			
(N)	95	127	33
A	1.000	1.000	1.000
MDH-1			
(N)	95	127	33
A	1.000	1.000	1.000
MDH-2			
(N)	95	127	33
A	1.000	1.000	1.000

	POPULATION		
	Australia		New
ALLOZYME	East	West	Zealand
ME-1			
(N)	95	127	33
A	1.000	1.000	1.000
ME-2			
(N)	94	125	33
A	.011	.020	
B	.957	.972	1.000
C	.032	.008	
MPI			
(N)	93	119	33
A	1.000	1.000	1.000
6-PGDH			
(N)	41	48	16
A	1.000	1.000	1.000
PGM			
(N)	90	127	33
A	1.000	1.000	1.000
PEP-la			
(N)	94	127	33
A	.011		
B	.989	1.000	1.000
PEP-lgg			
(N)	94	126	33
A	.005	.004	
B	.995	.996	1.000
PEP-pp			
(N)	94	127	33
A	1.000	1.000	1.000
PGK			
(N)	44	76	33
A	1.000	.974	1.000
B		.013	
C		.013	
PK-1			
(N)	95	127	33
A	.958	1.000	1.000
B	.042		
PK-2			
(N)	95	127	33
A	.011		
B	.989	1.000	1.000
SDH-1			
(N)	90	109	31
A	1.000	.927	1.000
B		.014	
C		.032	
D		.028	
SDH-2			
(N)	90	109	31
A	.011		
B	.989	1.000	1.000

Table 5: Genetic distances between the three main geographic groupings of gemfish *Rexea solandri*. Wright's Modified Rogers' genetic distances are given below the diagonal and Nei's unbiased distance above the diagonal.

AREA	AUSTRALIA EASTERN	AUSTRALIA WESTERN	NEW ZEALAND
EASTERN AUSTRALIA	**	.019	.001
WESTERN AUSTRALIA	.134	**	.021
NEW ZEALAND	.045	.145	**

Table 6: F_{ST} values for individual loci in the specified pairs of geographic regions of gemfish *Rexea solandri*. Cells without entries represent loci with no variation in either area.

LOCUS	EASTERN-NEW ZEALAND	WESTERN-NEW ZEALAND	EASTERN-WESTERN
Aat-1	.005	.074	.045
Aat-2	.008	.585	.552
Ada-1	.006	.009	.000
Ada-2	.000	.000	.000
Adh	.006	.012	.020
Est	.034	.091	.016
Gpi-1	.015	.001	.013
G-6-pdh	.002	.003	.000
Idh-2	.003		.003
Me-2	.018	.011	.003
Pgk		.010	
Pep-1a	.005		.005
Pep-1gg	.003	.002	.000
Pk-1	.022		.022
Pk-2	.005		.005
Sdh-1		.026	.026
Sdh-2	.006		.006
Mean	.017	.147	.116

Table 7: Estimates of migration rates (*Nm*) between the specified geographical regions of gemfish *Rexea solandri*. Estimates are based on either F_{ST} or $\overline{p}(1)$ statistics. Two sets of estimates are given for comparisons between eastern and western Australian specimens. In the second, NI542 and NI546 from western Tasmania are omitted as they have an *Aat-2* allozyme characteristic of the Western Australian population which is not found elsewhere in the Eastern Australian data set. The *Est* locus is omitted from the eastern Australian-New Zealand estimate as it is based on only seven specimens in the latter country.

COMPARISON	STATISTIC	ESTIMATED *Nm*
Based on F_{ST}		
EASTERN - WESTERN	0.116	1.905
EASTERN - WESTERN No NI542 or NI546	0.128	1.703
EASTERN - NEW ZEALAND	0.009	28.027
WESTERN - NEW ZEALAND	0.147	1.451
Based on $\overline{p}(1)$		
EASTERN - WESTERN	0.025	7.297
EASTERN - WESTERN No NI542 or NI546	0.067	1.271
EASTERN -NEW ZEALAND	0.023	7.577
WESTERN -NEW ZEALAND	0.073	1.148

Table 8: Genetic distances between samples of gemfish *Rexea solandri*. The figures above the diagonal are based on Nei's unbiased distance and the figures below the diagonal on Wright's (1978) modification of Rogers' distance.

	SAMPLE									
	New South Wales			Tasmania		South Aust.		West.	New Zealand	
SAMPLE	North	Central	South	East	West	East	Bight	Aust.	North	West
Northern NSW	**	.005	.005	.007	.006	.029	.022	.020	.003	.002
Central NSW	.087	**	.006	.008	.008	.025	.017	.017	.015	.009
Southern NSW	.087	.085	**	.012	.011	.030	.023	.020	.014	.010
Eastern Tasmania	.098	.102	.115	**	.009	.024	.027	.022	.014	.002
Western Tasmania	.101	.107	.115	.110	**	.012	.015	.011	.007	.004
Western Victoria & Eastern South Australia	.170	.157	.170	.155	.117	**	.005	.002	.033	.026
Great Australian Bight	.151	.133	.150	.165	.131	.076	**	.001	.033	.029
Western Australia	.145	.133	.141	.150	.113	.055	.053	**	.026	.023
Northern New Zealand	.071	.126	.121	.120	.095	.179	.179	.160	**	.006
Western New Zealand	.074	.106	.106	.068	.085	.160	.170	.152	.087	**

Table 9: Allozymes of two specimens of the small gemfish *Rexea bengalensis* and four specimens of the long-finned gemfish *Rexea antefurcata*. The frequency of the observed allozymes was 100%, except for *G-6-pdh* in *R. antefurcata* which had two variants present in equal frequency. Some allozymes have the same mobility (and designation) as those in the common gemfish *R. solandri*. Others (*e.g.* GPD B in *R. bengalensis*) are slower than any seen in *R. solandri* and are given the letter following that of the slowest *R. solandri* form. Allozymes faster than any in *R. solandri* are indicated by A', with A'' being faster again. For comparison, the most common allozymes (>5%) from eastern Australian *R. solandri* are also given.

LOCUS	*R. bengalensis*	*R. antefurcata*	*R. solandri*
Ak	B	A	A
Aat-2	C	C	C
Aat-3	A	A	A
Ada-1	C	B	B
Ada-2	A	A	A
Adh	E	A'	A,B,C
Fbp	A	B	A
Ga-3-pdh	A	B	A
Gpd	B	A'	A
G-6-pdh	B	B: 0.5 A: 0.5	B
Gpi-2	B	A	A
Ldh	A	A	A
Mdh-1	A	A	A
Mdh-2	A	A	A
Mpi	A	A	A
6-Pgdh	A'	A''	A
Pgm	A	A	A
Pk-2	A	A	B

Table 10: Restriction digestion profiles of *Rexea solandri* in the three major geographic regions. The capital letters signify the haplotype for the specified enzyme. Numbers of individuals with a haplotype are written above its designation. Fragments present in the haplotypes are detailed in Table 11. * indicates specimens from western Tasmania.

	AUSTRALIA		NEW ZEALAND
	EASTERN	WESTERN	
ENZYME			
Alu 1	30	33	4
	A	B	A
Ban II	2	2	2
	A	B	A
Cfo 1	25 2*	37	16
	A C	B	A
Dde 1	27	37	17
	A	B	A
Hae III	22 1	16	16
	A C	B	A
Hind III	2	2	2
	A	B	A
Hinf 1	35	35	36 1 3
	A	B	A C D
Hpa II	11 2	13	7 3
	A B	C	A B
Mbo 1	16	9	10 1
	A	B	A C
Pst 1	5 1*	7	9
	A B	B	A
Sau 3A1	3	3	5
	A	B	A

Table 11: Fragments present in restriction digest haplotypes of gemfish *Rexea solandri*. The approximate sizes of the fragments are written (in kb) in the same line as the endonuclease name. Where a fragment is present in a haplotype, "1" is written underneath the relevant fragment size. "0" indicates that the fragment is absent from the specified haplotype.

Alu 1	.85	.83	.70	.56	.47						
A	0	1	1	1	0						
B	1	0	1	1	1						
Ban II	1.60	1.08	.86	.64	.58	.37					
A	1	1	0	0	1	0					
B	1	0	1	1	0	1					
Cfo 1	1.65	1.50	1.40	1.20	1.08	1.03	.99	.90	.80	.61	.54
A	1	0	0	1	1	1	0	0	1	0	1
B	0	1	0	1	1	1	0	1	0	1	1
C	0	0	1	0	0	1	1	0	0	1	1
Dde 1	.73	.58	.49	.47	.39	.34					
A	1	0	1	0	1	1					
B	1	1	0	1	1	0					
Hae III	.94	.68	.66	.58	.49	.37					
A	1	0	0	1	0	1					
B	1	1	0	1	1	1					
C	1	0	1	1	0	1					
Hind III	3.00	1.97	1.65	1.50	1.05	.66					
A	0	1	1	1	1	1					
B	1	1	1	1	0	1					
Hinf 1	4.10	3.50	1.80	1.20	1.07	.94	.60				
A	0	1	0	1	0	1	1				
B	0	1	0	0	1	0	1				
C	0	1	1	0	0	1	1				
D	1	0	0	1	0	1	1				
Hpa II	1.45	1.35	.94	.86	.82	.62	.58	.48			
A	0	1	0	1	1	0	1	0			
B	0	0	1	1	0	1	0	1			
C	1	1	0	1	1	0	1	0			
Mbo 1	2.70	1.80	1.35	1.15	.89	.81	.61	.57			
A	0	1	1	1	0	1	0	1			
B	1	0	0	1	1	1	1	0			
C	0	1	0	1	0	1	0	1			
Pst 1	3.50	1.80	1.50	.88							
A	0	1	1	1							
B	1	1	0	1							
Sau 3A1	2.80	2.50	1.70	1.45	1.25	1.15	1.05	.98			
A	0	1	1	1	0	1	0	1			
B	1	0	0	1	1	1	1	0			

Table 12: Nucleotide diversity (above diagonal) and divergence (below) among populations of gemfish *Rexea solandri* calculated by the REAP DA.exe program (McElroy *et al.*, 1992) according to the formulae of Nei (1987).

	EASTERN	WESTERN	NEW ZEALAND
EASTERN AUSTRALIA		0.043797	0.001544
WESTERN AUSTRALIA	0.043010		0.044249
NEW ZEALAND	0.000007	0.043500	

Table 13: DNA sequence for 12S rDNA of gemfish *Rexea solandri* NI409 from New Zealand

```
  1 AGGAACATAC ATCCCCTATC CGCCCGGGTA CTACAAGCAT TAGCTTAAAA CCCAAAGGAN
 61 TTGGCGGTAC TTTAGATCCC CCTAGAGGAG CCTGTTCTGT AACCGATAAC CCNNTTCAAC
121 CTCACCCTCC CTTGTTTATC CCGCTATATA CCGCCGTCGT CAGCTTACCT GTGAGACTAA
181 TAGTAAGCAA AATTGCATCG CCAGAACGTC AGGTCCAGGT NTAGCGCATG AGAGGGGAAG
241 AAATGGGCTA CATTCGCTAA CGTAGCGAAT ACGAACGATG TACTGAAAAC GTACATCCGA
301 AGGAGGATTT AGCAGTAAGT GGAAAATAGA GTG
```

Table 14: Comparison of (partial) 12S rDNA sequences between eastern and western Australian specimens of gemfish *Rexea solandri*. These sequences have 94.8% identity in an 115 bp overlap. "N" in the NI529 sequence refers to bases that could not be determined. NI661 is from N.S.W and NI529 from western Victoria.

```
                210       220       230       240       250       260
NI661           GTCAGGTCCAGGTNTAGCGCATGAGAGGGGAAGAAATGGGCTACATTCGCTAACGTAGCG
                :::::::::::::.:::::::::::::::::::::::::::::::::::.: :::: :
NI529           GTCAGGTCCAGGTGTAGCGCATGAGAGGGGAAGAAATGGGCTACATTCGCTNATGTAGTG
                        10        20        30        40        50        60
                270       280       290       300       310       320
NI661           AATACGAACGATGTACTGAAAACGTACATCCGAAGGAGGATTTAGCAGTAAGTGG
                :::::::::::::::::::::::::::::.:::::::::::::::::::::::: :
NI529           AATACGAACGATGTACTGAAAACGTACATNCGAAGGAGGATTTAGCAGTAAGTAG
                        70        80        90       100       110
```

Table 15: Allozyme frequencies in ocean perch *Helicolenus percoides*. Numbers in the row headed (N) indicate sample sizes. Note that shallow and deep forms from the same locality are placed side-by-side.

	Coffs Harbour	Wollon-gong	Ulladulla		Eden		Sorrel		Fre-mantle	Geraldton	Shark Bay	New Zealand
	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Deep	Deep	Deep	Deep
AK												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-1												
(N)	6	5	4	5	13	6	3	4	2	6	5	9
A	1.000	1.000	1.000	1.000	.962	1.000	.500	1.000	1.000	1.000	1.000	1.000
B					.038		.500					
AAT-2												
(N)	6	5	6	5	13	6		3	2		5	9
A	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000		1.000	1.000
AAT-3												
(N)	6	5	6	5	13	6	5	2	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-4												
(N)	6	5	5	5	13	6	5	2	2	6	5	9
A	1.000	1.000	1.000	1.000	.962	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B					.038							
ALD												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EST-2												
(N)	6	5	1	5	13	6	5	4	2	6	5	9
A		1.000		1.000		1.000		.750	1.000	1.000	.600	1.000
B	1.000		1.000		1.000		1.000	.125			.400	
C								.125				
FBP												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FH												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	.885	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B					.115							
GA3PD												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.944
B												.056
GPD												
(N)	6	5	6	5	13	6	5	4	2	6	5	8
A										.083		
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.917	1.000	1.000
GPI-1												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-2												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A								.125				
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.875	1.000	1.000	1.000	1.000
IDH												
(N)	6	5	6	5	13	6	5	4	2	6	5	5
A	1.000	1.000	.917	1.000	.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B			.083		.077							
LDH												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-1												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-2												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ME-1												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

	Coffs Harbour	Wollon-gong	Ulladulla		Eden		Sorrel		Fre-mantle	Geraldton	Shark Bay	New Zealand
	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Deep	Deep	Deep	Deep
ME-2												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MPI												
(N)	6	5	6	5	13	6	5	4	2	6	5	9
A		.100					.100					
B	1.000	.900	1.000	1.000	1.000	1.000	.900	1.000	1.000	.917	1.000	1.000
C										.083		
6PGDH												
(N)	6	5	6	4	13	6	5	4		6	5	9
A	1.000	.400	1.000	.375	.769	.333	.200	.625		.500	.600	.111
B		.600		.625	.231	.667	.800	.375		.500	.400	.889
PGM												
(N)	6	5	6	4	13	6	5	4		6	5	9
A	.250	.200	.250	.250	.346	.333	.300			.083	.300	.333
B	.750	.800	.750	.750	.654	.667	.700	1.000		.917	.700	.667
PLGG												
(N)	4	4		2	13	6	3	1	2	6	5	6
A	1.000	1.000		1.000	.923	.917	1.000	1.000	1.000	1.000	1.000	1.000
B					.077	.083						
PK-1												
(N)	6	4	5	3	13	6	3	1	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PK-2												
(N)	6	4	5	3	13	6	3	1	2	6	5	9
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SDH-1												
(N)	6	4	3	4	13	6	5	3	1	6	5	6
A					.154			.167				
B	.250	.625	.500	.750	.500	.583	.300	.167	1.000	.833	.700	
C	.583	.375	.500	.250	.346	.417	.600	.667		.167	.300	1.000
D	.167						.100					
SDH-2												
(N)	6	3	3	3	13	6	5	3	1	6	5	5
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI												
(N)	6				13	6	3	2	2	6	5	9
A	1.000				1.000	1.000	1.000	1.000	1.000	1.000	1.000	.944

Figure 1: Map of gemfish *Rexea solandri* specimens analysed. Numbers between diagonals indicate specimens analysed for: protein electrophoresis/ mtDNA restriction patterns. The letters A, B and C refer to the broad geographic areas.

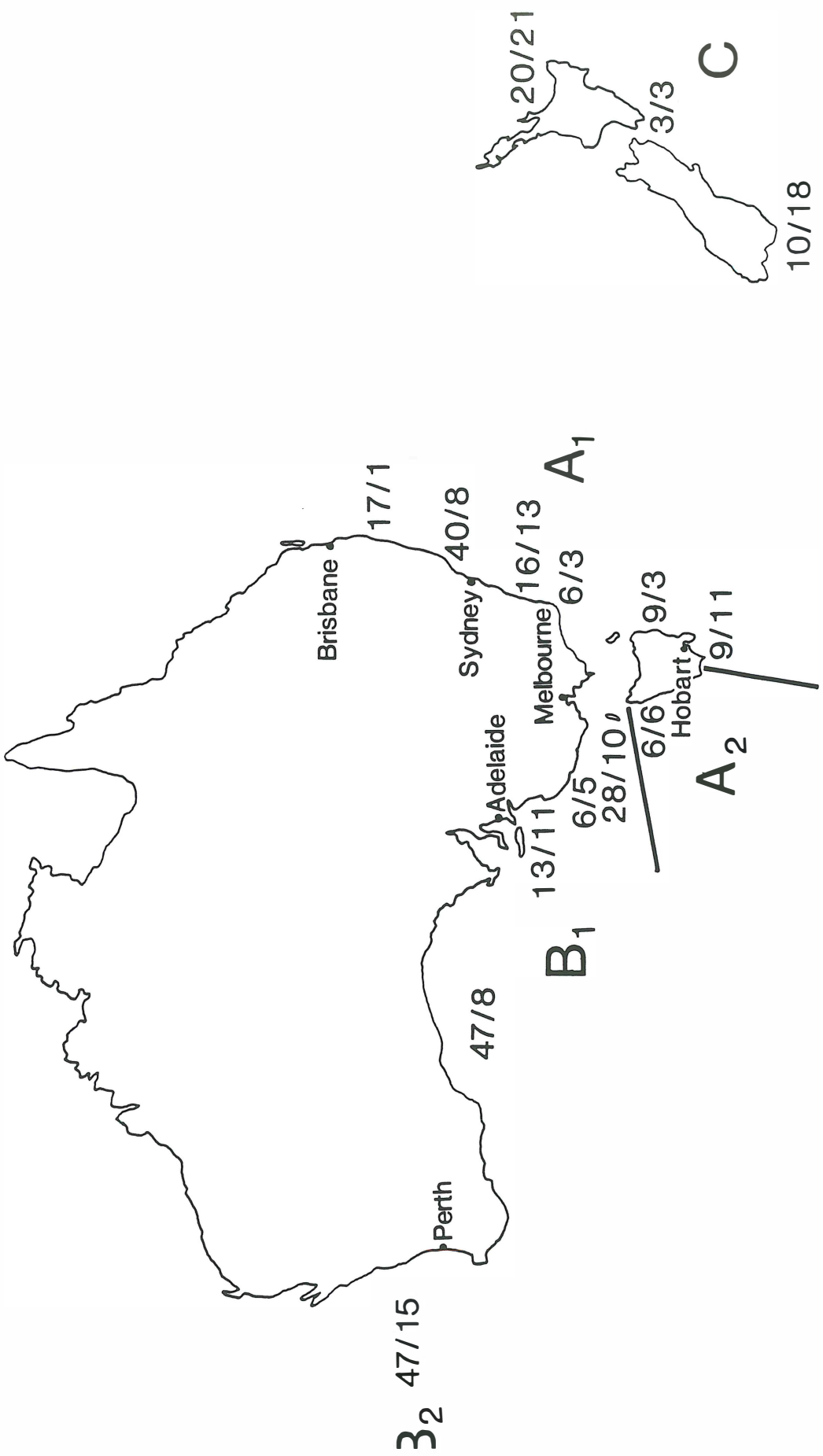


Figure 2: Map of ocean perch *Helicolenus percoides* specimens available for study. Numbers betwen diagonals indicate the specimens available/ analysed for protein electrophoresis/ mtDNA restriction patterns. Filled triangles indicate deep form samples, unfilled triangles indicate shallow form samples.

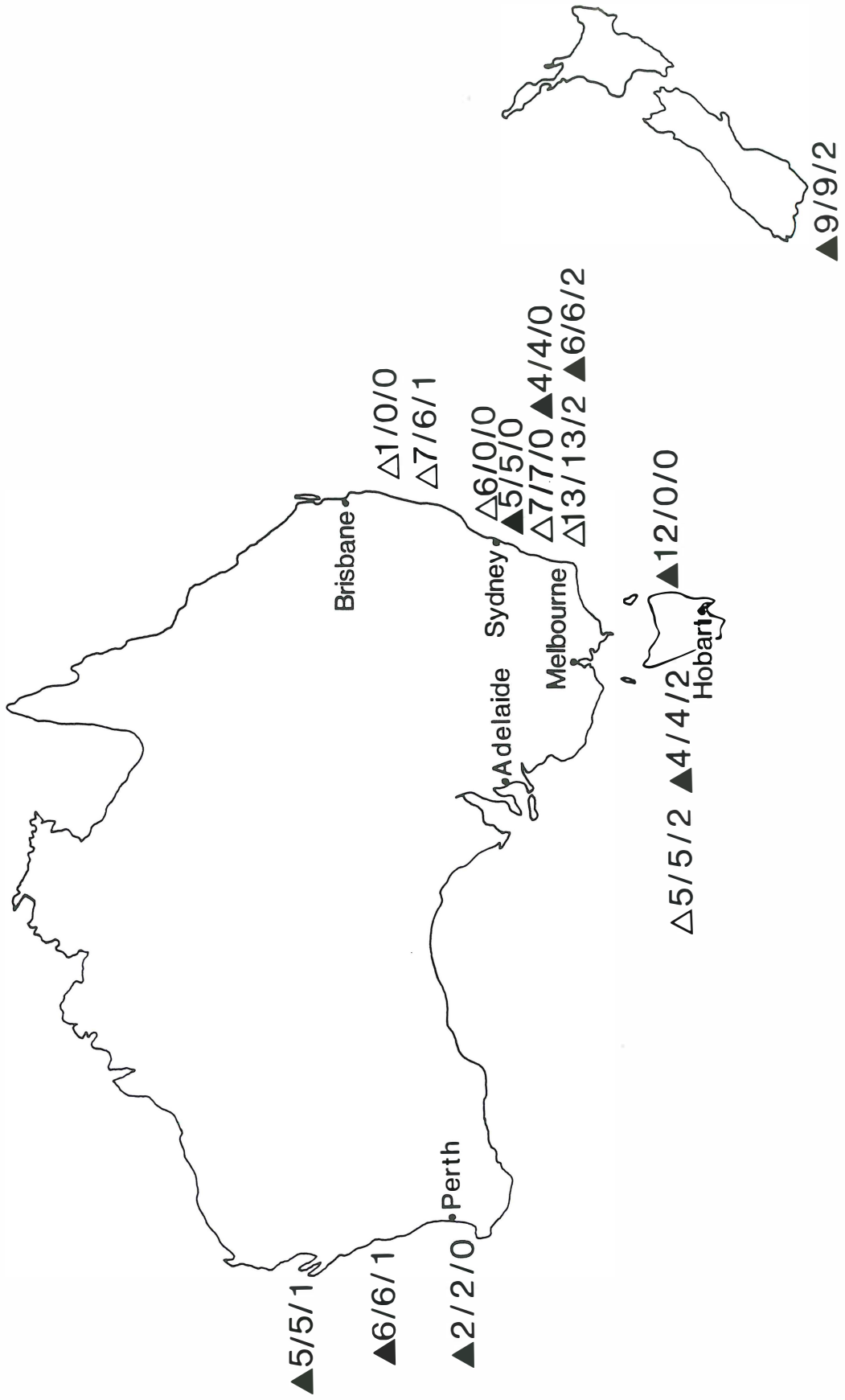


Figure 3: Snout length of gemfish *Rexea solandri* as percent of standard length.

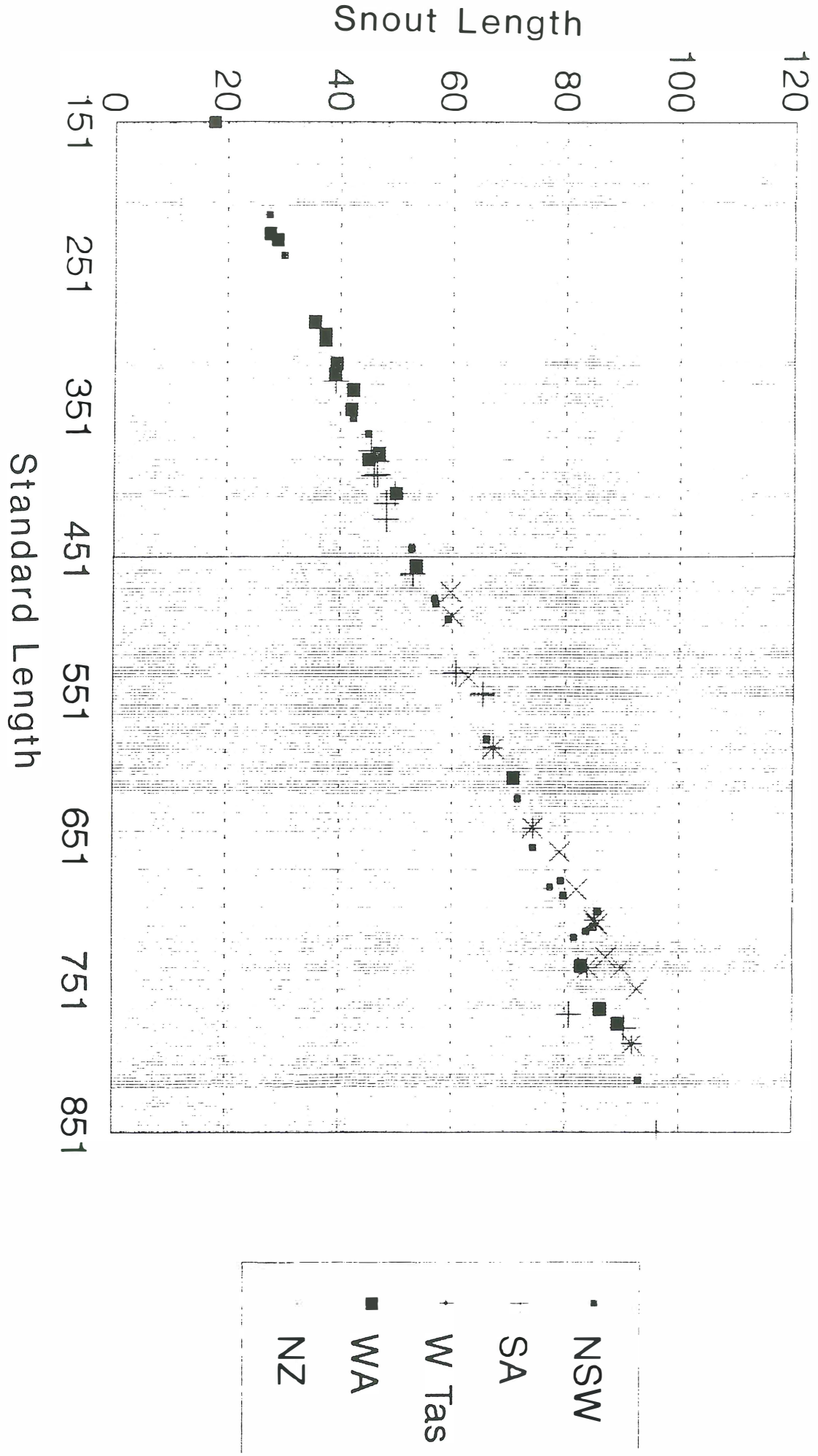


Figure 4: Dendrogram showing the relationships between the three main geographic groupings of gemfish *Rexea solandri* based on Wright's (1978) Modified Rogers' distance.

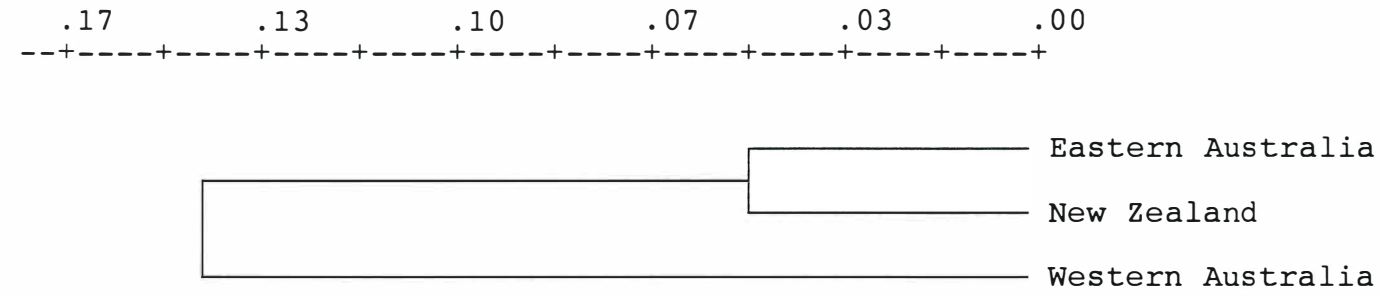


Figure 5: *Hinf 1* phenotypes of gemfish *Rexea solandri* in a Southern Blot probed with lake trout mtDNA. The provenance of the samples is indicated at the top of the photograph. W = Western Australia, S = South Australia, T = Tasmania, N = New Zealand and E = New South Wales.

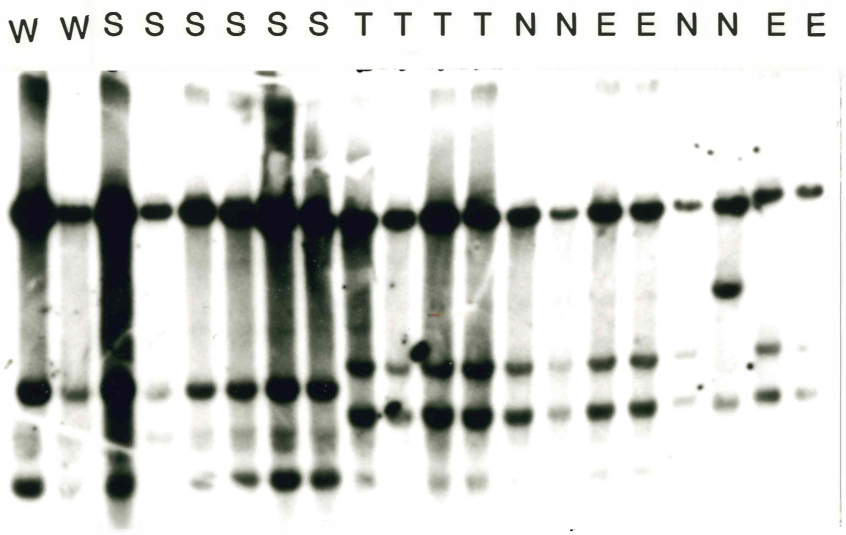


Figure 6: Part of a 12S rDNA sequencing gel comparing gemfish *Rexea solandri* specimens NI662 (N.S.W.) and NI409 (N.Z.). Lanes are identified by the initial letters of the bases at the bottom of the photograph.

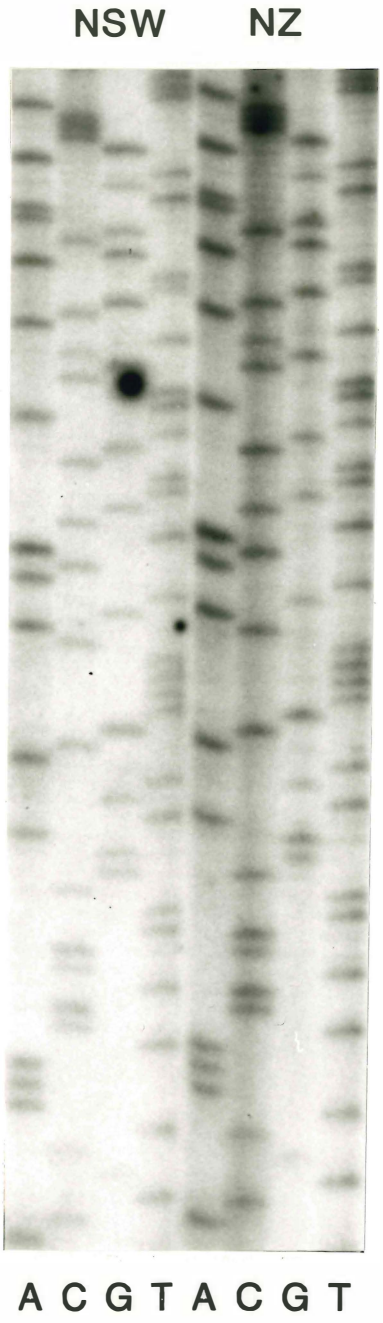


Figure 7: Distribution of known gemfish *Rexea solandri* stocks and breeding areas. Areas are identified by capital letters as in Figure 1.

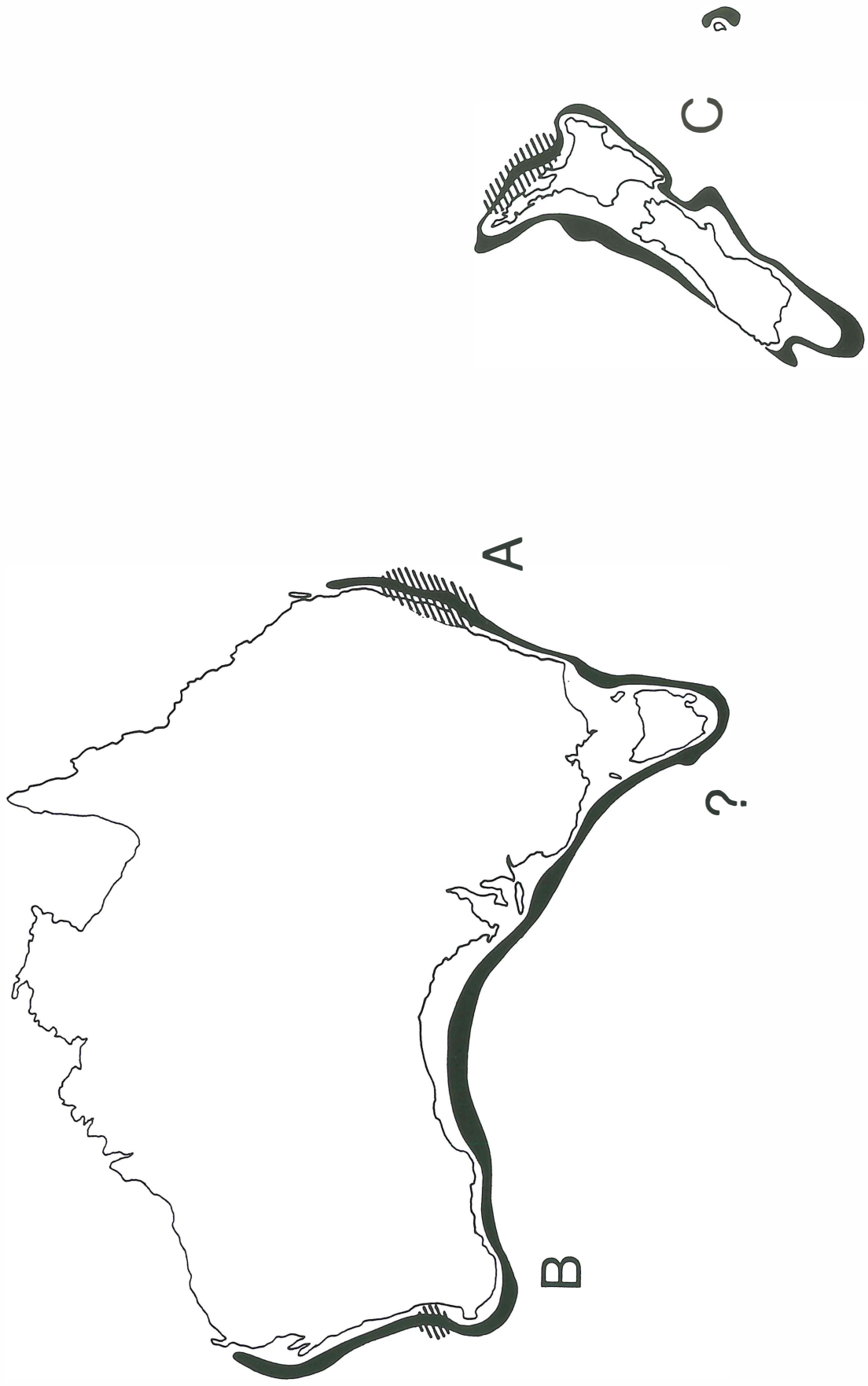
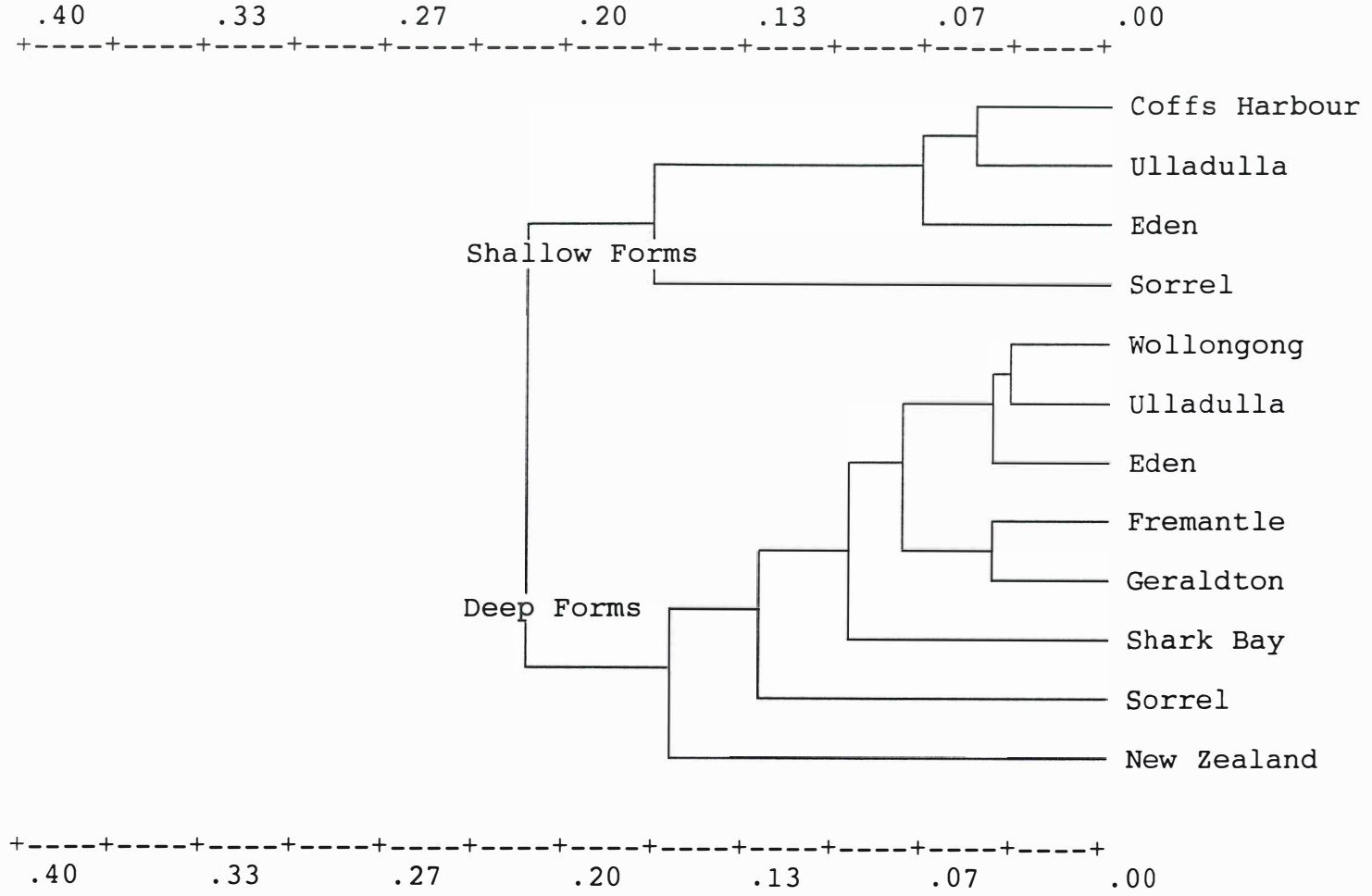


Figure 8: Cluster analysis of ocean perch *Helicolenus percoides* samples using Wright's (1978) Modified Rogers distance.



Appendix 1. Covering Letter Copy

Mr Peter Dundas-Smith,
Executive Director,
Fisheries Research and Development Corporation,
P.O. Box 9025,
Deakin A.C.T. 2600.

Dear Mr Dundas-Smith,

This letter is written, on behalf of Dr John Paxton and myself, to cover our final report on the FRDC funded study (91/35) "Biochemical genetics and stock assessment of the common gemfish and ocean perch". Whilst, this study was carried out under the guidelines in force at the date of contracting to perform it, we have endeavoured as far as practicable to complete it as per the current guidelines.

The investigation has been completed in accordance with the terms of the originally agreed proposal. Broadly stated, the principal conclusions of the study are that: (1) There are two genetically quite distinct stocks of the common gemfish in Australian waters; (2) common gemfish from eastern Australian and New Zealand waters may be distinct stocks but are much closer genetically than are the two Australian stocks; (3) the shallow and deep forms of the ocean perch represent two distinct species.

Two preliminary extensions of the work were considered to be warranted. These were: (1) a DNA sequencing study of one mitochondrial gene region in common gemfish; and (2) a preliminary assessment of mitochondrial DNA fragment length variation in ocean perch. The first of these was conducted to search for a fixed genetic difference between Australian and New Zealand fish. None was found in the selected gene, though it is possible that other genes (as recommended for study, below) will reveal such differences. The second was conducted to begin investigation of what protein electrophoresis has revealed as a complex pattern of population structure in ocean perch.

The total contribution to the project by the FRDC has been $71,038. The contribution by the Australian Museum has been somewhat increased over the initial projections by the time required for the extension work and by its bearing of the costs of DNA sequencing materials. The days spent by Drs Paxton and Colgan on the project have totalled 150 (cost: $28,340) and non-salary expenditures (including consumables and overheads) totalled $11,000, giving a total Museum contribution of $39,340. It is not possible to estimate the contribution of others, particularly those collectors who actively sought specimens for us, but their input has been substantial.

The impact of this study will primarily occur in Australia and New Zealand. It is of particular relevance to fisheries management in the various committees that set quotas

for the Southeast Trawl Fishery and other geographic areas. In Australia, it reinforces the consideration that the two common gemfish stocks must be treated separately. The ocean perch fishery must presently be treated as comprising two species. Stock identification in these species requires further investigation.

We present a series of recommendations for the application of the present results and for further investigation in the body of the report. Among these are identification of the spawning locations of the common gemfish from the southern/western Australian stock. We emphasise the potential of microsatellite DNA analysis to solve the remaining questions of gemfish stock structure and to complement mitochondrial DNA studies of ocean perch.

Copies of this report will be distributed as follows: FRDC 10 bound and 1 unbound hard copy, and 1 copy on diskette.

One copy each to the libraries of:
Bureau of Resource Services, Canberra; N.S.W. Fisheries Research Institute, Cronulla; Victorian Fisheries Research Institute, Queenscliff; Tasmanian Fisheries, Hobart; CSIRO Fisheries, Hobart; South Australia Research and Development Institute, Adelaide; Western Australian Fisheries, Perth; New Zealand Fisheries, Wellington

One copy each to the following individuals:
Kevin Rowling, N.S.W. Fisheries Research Institute; Dave Smith, Victorian Fisheries Research Institute; Jeremy Lyle, Tasmanian Fisheries; Bob Ward, Peter Last and Allan Williams, CSIRO Fisheries; Danny Turner, *Raptis*, Port Lincoln; Jeremy McKenzie, New Zealand Fisheries, Auckland; Rosie Hurst, Don Robertson and Neil Bagley, New Zealand Fisheries, Wellington; Pat Dixon UNSW; Richard Tilzey, Bureau of Resource Services, Canberra.

On behalf of John Paxton and myself, I would like to thank the FRDC for funding this project at the Australian Museum. We have enjoyed the work, and been particularly pleased with the extent of cooperation we have received from fisheries managers, fisheries scientists and the fishers themselves.

Yours faithfully,

Dr Don Colgan

Appendix 2. Wanted Letter Requesting specimens of *Rexea solandri*

GEMFISH WANTED

A study at the Australian Museum in Sydney is trying to find out how many breeding stocks of the common gemfish there are around Australia. Biochemical tests are being conducted on muscle and liver of gemfish from eastern and southern Australia to see if more than one stock is present, and if so, where the dividing line(s) of these stocks are. Results of the study can be used in future management decisions about the fishery.

Because the important biochemical features start to break down once the fish are caught, it is necessary for the fish to be frozen soon after capture; fish covered only with ice for a few days will lose some of their biochemical characters.

The study needs freshly frozen specimens from Tasmania. Ideally we would like 5-6 frozen specimens every 50 kms to 100 kms along both east and west coasts. Each lot should have a label written in pencil giving the depth, date and latitude and longitude of capture. If one lot of 5-6 specimens could be put in the food freezer of each ship only once or twice during the season, we should have enough data to answer the question. If eastern and western gemfish stocks can be confirmed, it will be important for the management of the fisheries to know how the Tasmanian fishes fit into the picture. AND A FEW FROZEN SPECIMENS FROM YOUR CATCH ARE FOR THAT INFORMATION.

To organize collection of your frozen sample(s), please contact Jeremy Lyle in Hobart (002) 278 867. If there are any problems, call me collect in Sydney (02) 339 8139. Your help will be greatly appreciated.

Sincerely,

John Paxton
Senior Research Scientist
Fish Section

P.S. We will also be studying, at the same time as the gemfish research described above, ocean perch. This study will be more complex, as more than one species is involved, and will be of secondary importance in this first year. However if ocean perch could also be frozen, together with a label with their capture data (depth is especially important), it would be of much help to our work. Thanks again.

Appendix 3: Table of gemfish *Rexea solandri* specimens sampled. Areas are as indicated in Figure 1: A1, from northern N.S.W to southeastern Tasmania; A2, western Tasmania; B1, southern Australia from western Victoria to eastern W.A.; B2, from western W.A. from Perth to off Shark Bay; C, New Zealand. NI NUMBERS - identification numbers of frozen tissue samples. AMS REG. I. - all specimen registration numbers in the Australian Museum fish collection begin with I. NO(SL MIN - MAX) - the number of specimens and their range of standard lengths in mm; * indicates converted from fork length to the nearest 5 mm.

AREAS	NI NUMBERS	AMS REGO I	NO.(SL MIN-MAX)	LATITUDE AND LONGITUDE	DEPTH METRES	DATE	COLLECTOR
A1							
	450	31501-001	1(545)	28°33'S,153°50'E	260	27 MAY 1991	W.PIPER
	466-467	31502-001-002	2(650-745)	28°40'S,153°50'E	250	18 JUL 1991	W.PIPER
	611	33286-002	1(348)	29°40'S,150°13'E	366	15 JUN 1992	
	462	32113-001	1(436)	30°12 S,153°28'E	145	?? AUG 1991	
	91-94	28998-001-004	4(370-640)	30°12'S,153°31'E	450	01 AUG 1989	T.NYSSEN,NSWFISH
	463-464	32114-001-002	2(289-296)	30°12'S,153°28'E	145	11 OCT 1991	
	358-359	31280-001-002	2(244-256)	30°20'S,153°30'E		?? JUN 1991	T.NYSSEN,NSWFISH
	182-186	30909-001-005	5(218-255)	30°31'S,153°20'E	139-149	09 MAY 199?	K.GRAHAM,NSWFISH
	437-499,451-460	31500-001-023	23(460-880)	33°50'S,151°45'E	250	22 JUL 1991	A.MAIORANA
	430-436	31499-001-007	7(732-894)	34°00'S,151°50'E	450	20 JUL 1991	A.MAIORANA
	616-617,619-624	33373-001-008	8(765-855)	34°03'S,151°39'E	435-503	23 JUL 1992	J.PAXTON,T.TRNSKI
	651-652	33374-001-002	2(580-760)	34°03'S,151°39'E	435-503	28 JUL 1992	SYD.MARKT.
	95	29302-001	1(247)	34°55'S,115°40'E	390	28 JUL 1989	W.BELLE
	98	29301-002	1(162)	34°55'S,150°40'E		23 FEB 1989	W.BELLE
	664	33357-001	1(410)	35°20'S,149°55'E		28 JUL 1992	
	537-540	32517-001-004	4(455-572)	37°35'S,149°55'E	365-440	?? DEC 1991	F.DRENKHAHN
	589-591	32775-001-003	3(375-427)	37°40'S,150°15'E	448	22 MAY 1992	F.DRENKHAHN
	360-365	31281-001-006	6(155-177)	38°04'S,149°21'E	170-400	04 MAR 1991	C.NEWMAN
	586-588	32774-001-003	3(351-362)	38°07'S,149°59'E	439	21 MAY 1992	F.DRENKHAHN
	614-615	33310-001-002	2(647-650)	38°11'S,149°40'E	274	16 JUN 1992	F.DRENKHAHN
	1070-1075	34158-001-006	6(496-858)	41°52'S,148°45'E	420-470	05 MAY 1993	A.WHITE
	673-675	33377-001-003	3(719-823)	41°53'S,148°08'E	402	29 MAY 1992	TASFISH
	692-699,847-849	33597-001-008	11(383-425)	43°42'S,147°53'E	402	25 AUG 1992	CSIRO
A2							
	545-546	32750-001-002	2(245-616)	43°49'S,146°08'E	450-550	08 DEC 1992	C.SHEARER
	541-544	32749-001-004	4(575-749)	41°58'S,144°35'E	495-585	11 APR 1992	C.SHEARER
B1							
	509-523	31509-001-015	15(401-517)	38°45'S,141°31'E	200-220	22 SEP 1991	T.CRAPPER
	524-536	32516-001-013	13(395-569)	38°16'S,140°28'E	290	30 OCT 1991	T.CRAPPER
	676-681	33518-001-006	6(491-631)	37°50'S,139°40'E	348	28 SEP 1992	D.SMITH,VICFISH
	469-481	31504-001-013	13(335-907)	37°00'S,137°00'E	200	10 NOV 1991	D.VALENTI
	557,563	32762-001-002	2(476-600)	33°45'S,132°12'E	145	07 MAR 1992	N.BRIDGE
	562	32765-001	1(600)	33°21'S,131°42'E	126-130	07 MAR 1992	N.BRIDGE
	176-181	30910-001-006	6(404-533)	33°18'S,130°53'E	170	26 JUN 1990	D.TURNER,SAFISH
	156-158	30437-001-003	3(140-141)	33°25'S,130°23'E	506	26 MAY 1990	M.SCOTT
	558-561	32766-001-004	4(467-535)	33°17'S,130°01'E	150	09 MAR 1992	N.BRIDGE
	488-489	31507-001-002	2(379-417)	33°15'S,127°45'E	135	11 NOV 1991	
	162-175	30911-001-014	14(278-329)	33°12'S,127°02'E	131	25 SEP 1990	D.TURNER,SAFISH
	491-493	31508-002-004	3(316-369)	33°04'S,126°40'E		12 NOV 1991	
	484-487	31506-001-004	4(278-336)	33°18'S,126°15'E	155	12 NOV 1991	
B2							
	494-495,499-502	32743-001-002	6(390-500)	32°29'S,114°54'E	335	29 NOV 1991	A.WILLIAMS,CSIRO
	505	32747-001	1(810)	32°26'S,114°50'E	334	22 NOV 1991	A.WILLIAMS,CSIRO
	496-498	32744-001-003	3(380-470)	32°22'S,114°59'E	342	29 NOV 1991	A.WILLIAMS,CSIRO
	503	32745-001	1(1020)	32°21'S,114°59'E	366	23 NOV 1991	A.WILLIAMS,CSIRO
	506	32748-001	1(770)	32°21'S,114°59'E	366	22 NOV 1991	A.WILLIAMS,CSIRO
	504	32746-001	1(820)	32°12'S,115°05'E	311	22 NOV 1991	A.WILLIAMS,CSIRO
	385-390	31285-001-006	6(225-258)	31°43'S,114°59'E	390-485	10 FEB 1991	CSIRO S.SURV.
	547-550	32752-001-004	4(327-600)	30°00'S,114°27'E	480-490	08 FEB 1991	A.WILLIAMS,CSIRO
	391-396	31286-001-006	6(232-335)	29°58'S,114°27'E	380	08 FEB 1991	CSIRO S.SURV.
	366-368	31282-001-003	3(335-412)	29°19'S,113°57'E	490-505	06 FEB 1991	CSIRO S.SURV.
	371-384	31284-001-014	14(158-440)	29°16'S,113°57'E	320-325	06 FEB 1991	CSIRO S.SURV.
	235	31185-001	1(250)	27°20'S,112°52'E	279-306	03 FEB 1991	J.PAXTON,S.SURV.
	242	31164-001	1(198)	24°54'S,112°11'E	318-344	28 JAN 1991	J.PAXTON,S.SURV.
	220	31163-001	1(320)	24°52'S,112°07'E	444-468	28 JAN 1991	J.PAXTON,S.SURV.
	213	31156-001	1(300)	23°24'S,113°05'E	300-302	26 JAN 1991	J.PAXTON,S.SURV.
C							
	397-416	31290-001-020	20(680-865)*	35°30'S,174°50'E	200	17 JUN 1991	J.McKENZIE,NZFISH
	1035-1038	34149-001-004	4( )	36°30'S,176°12'E		06 MAY 1993	J.McKENZIE,NZFISH
	1046-1048	34151-001-003	3(560-790)	38°04'S,178°46'E	215	15 MAR 1993	N.BAGELY,NZFISH
	1049	34152-001	1(920)	39°43'S,177°40'E	374	19 MAR 1993	N.BAGELY,NZFISH
	137-139	30169-001-003	3(500-525)	41°30'S,175°00'E	110	02 DEC 1989	J.PAXTON
	1056	34155-001	1( )	46°16'S,166°37'E	120	12 FEB 1993	J.McKENZIE,NZFISH
	1055	34154-001	1(870)	46°47'S,168°48'E	116	10 FEB 1993	J.McKENZIE,NZFISH
	1057-1062	34157-001-006	6( )	46°47'S,165°59'E	199	13 FEB 1993	J.McKENZIE,NZFISH
	682-691	33586-001-010	10(550-890)	40°57'S,171°08'E	400	19 AUG 1992	P.McFARLANE,NZFISH
Rexea bengalensis							
	68,73	28932-001,006	167-202	27°05'S,154°08'E	150	12 MAY 1989	
Rexea antefurcata							
	100-1,	29300-002,003	197-304	34°55'S,150°40'E	460	9 MAR 1989	
	109-110	29378,9-001	340-350	34°53'S,150°52'E		19 SEP 1989	

Appendix 4: Table of *Helicolenus percoides* material examined

NI NUMBERS	AMS REGO I	NO.(SL MIN-MAX)	LATITUDE AND LONGITUDE	DEPTH METRES	DATE	COLLECTOR
1063	34133-001	1(207)	29°10'S,153°30'E	220	10 MAY 1993	W.PIPER
103-108	29326-001-006	6(170-222)	30°20'S,153°10'E	(*hallow)	22 AUG 1989	T.NYSSEN,NSWFISH
882	34089-001	1(256)	30°20'S,153°10'E	183	02 OCT 1992	T.NYSSEN,NSWFISH
890-895	34094-001-006	6(140-148)	33°40'S,151°30'E	119	06 JUL 1992	FRV KAPALA
12-16	34191-012-016	5(262-320)	34°55'S,151°00'E	(Deep)	20 OCT 1988	K.ROWLING,NSWFISH
1-11	34085-001-011	11(183-264)	35°55'S,150°00'E	(Shallow)	21 OCT 1988	K.ROWLING,NSWFISH
573-579	32772-001-007	7(200-263)	37°21'S,15O°07'E	92	26 MAY1992	F.DRENKHAHN
580-585	32773-001-006	6(200-253)	37°55'S,149°53'E	137	22 MAY1992	F.DRENKHAHN
567-572	32771-001-006	6(226-243)	38°08'S,149°59'E	439-466	21 MAY 1992	F.DRENKHAHN
1076-1087	34158-007-018	12(277-368)	41°52'S,148°45'E	420-457	06 MAY 1993	A.WHITE
75-79	34086-001-005	5(151-222)	43°20'S,145°30'E	150	21 MAR 1989	CSIRO FRV SOELA
885	34092-001	1(267)	44°00'S,146°23'E	457	18 MAR 1993	G.KRAUS
884	34091-001	1(300)	41°45'S,144°30'E	439	09 MAR 1993	G.KRAUS
886-889	34093-001-004	4(257-321)	42°51'S,144°45'E	439	10 MAR 1993	G.KRAUS
74,80,83	32427-001-003	3( )	40°57'S,143°44'E	775-815	09 MAR 1989	CSIRO FRV SOELA
84-85	32428-001-002	2( )	40°54'S,143°44'E	815-820	09 MAR 1989	CSIRO FRV SOELA
424-425	31288-001-002	2(95-96)	32°08'S,115°09'E	225-230	13 FEB 1991	CSIRO.S.SURV
418-423	31287-001-006	6(170-205)	29°19'S,113°57'E	490-505	06 FEB 1991	CSIRO.S.SURV
241	31184-001	1(210)	27°09'S,112°45'E	370-438	02 FEB 1991	J.PAXTON,S.SURV
227-228	31176-001-002	2(215-320)	27°05'S,112°22'E	713-714	31 JAN 1991	J.PAXTON,S.SURV
224-226	31171-001-003	3(155-190)	26°38'S,112°30'E	500-508	30 JAN 1991	J.PAXTON,S.SURV
134-135	34189-001-002	2(236-363)	47°25'S,179°34'E	374-376	21 NOV 1989	N.BAGELY,NZFISH
136	34190-001-002	1(380)	46°31'S,166°34'E	503-517	11 NOV 1989	N.BAGELY,NZFISH
140-145	30166-001-006	6(254-317)	46°35'S,165°40'E	600-643	11 NOV 1989	N.BAGELY,NZFISH