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Research to underpin better understanding and management of western Gemfish stocks in the Great Australian Bight

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Review

This work was reviewed by Simon Nicol, Bertie Hennecke and Peter Gooday (ABARES), Kevin Rowling (gemfish expert) and Chris Izzo (FRDC). The work was presented to ABARES, the University of Queensland, the Great Australian Bight Resource Assessment Group, the Australian Society for Fish Biology (2015) and the World Fisheries Congress (2016).

Executive Summary

Background

Gemfish (*Rexea solandri*) is a benthopelagic snake mackerel of the Family Gempylidae. Gemfish is found on the continental shelf and slope in southern, southwestern and southeastern Australia and New Zealand. It is found at depths ranging from 100 to 800 metres, but commonly at 300 to 450 metres. Historically Gemfish formed part of a large trawl fishery off the east coast of Australia in the 1970s and 1980s. Catches peaked between 1978 and 1980 at around 5,000 tonnes per year, declining substantially after 1987. The east coast fishery remains in an overfished state with an unavoidable bycatch limit of 100 T. The most recent stock assessment estimated spawning biomass at 15 per cent of the 1968 level.

Fisheries for Gemfish have operated off western Tasmania, south western Victoria and south eastern South Australia as part of the Commonwealth Trawl Sector (CTS) of the Southern and Eastern Scalefish and Shark Fishery (SESSF) and in Great Australian Bight as part of the Great Australian Bight Trawl Sector (GABTS) of the SESSF. These fisheries are managed as a separate stock to the east coast and have a substantially different fishing history and level of exploitation. The most recent stock assessment of the western stock suggested biomass is at 74 per cent of virgin biomass levels.

Great Australian Bight Resources Assessment Group (GABRAG) had sufficient concerns regarding the population structure of the western stock and its impact on the stock assessment (whether western Gemfish constituted a single population and where the boundary between east and west arises) to reject the assessment.

Previous research found genetic subdivision between the eastern stock of Gemfish (eastern Australia including eastern and western Tasmania) and a western stock (South Australia, GAB and Western Australia). Mitochondrial DNA (mtDNA) data showed fixed haplotype differences following the same pattern as the allozyme data, but suggested a much stronger division between the two stocks. Both data sets suggested there were no genetic differences between eastern Australia and New Zealand. However, it was unclear whether these findings were the result of small sample sizes.

This project was initiated through GABRAG to address these stock structure issues and was a collaboration between the Australian Bureau of Agricultural and Resource Economics and Sciences (Andy Moore) and the Molecular Fisheries Laboratory at the University of Queensland (Jenny Ovenden, Carlos Bustamante and Andy Moore). The expectation was that this study would confirm the distinction between the eastern and western stocks, particularly as the classes of genetic markers used for this study mirrored those used previously.

Objectives

The objectives of this project were to:

1) Improve understanding of stock structure for Western Gemfish west of Bass Strait;

2) Improve understanding of spawning locations for western Gemfish west of Bass Strait; and

3) Provide the Australian Fisheries Management Authority with recommendations on stock structure and boundaries on the basis of this evidence.

Methodology

Fish were sampled from the mid-western Great Australian Bight (GAB), from two locations off the southwestern Victorian coastline at Kangaroo Island, (KI) and Portland (Por), from one location in western Bass Strait (WBS) and from one location off the coast of western Tasmania (WT).

To provide genetic comparisons among these target populations, fish were also sampled from the east coast of Tasmania (ET), the eastern coast of New South Wales (NSW) as well as New Zealand (NZ).

Previous research had found large genetic differences between eastern and western populations. However there was large sample size variation between both locations and it was unclear if the genetic differences were the result of biological processes or a sampling artefact. Archived historical samples from this study were obtained from the Australian Museum to confirm the previous results and to test for temporal stability of genetic patterns.

Gemfish from the westesrn part of the species range were sampled during summer and included tissue collection for genetic analysis, gonad staging to gauge likely spawning locations, length data and otoliths for future research. Sampling was also conducted in winter, though few samples were collected at this time and only from a single location (Portland). The western sampling locations were chosen largely on the presence of spawning fish, the occurrence of a fishery in that region and to provide adequate spatial coverage.

Three types of genetic markers were used. MtDNA sequencing of the control region was used to match the restriction digests and hybridization of whole mitochondrial genomes used by previous researchers. The previous study used allozyme markers to study the nuclear genome, while this study used microsatellite loci. In addition, this study used single-nucleotide polymorphism (SNP) loci to allow a direct comparison to standard genetic markers, but with far greater discriminatory power.

Results

This study confirmed that there are two distinct stocks of Gemfish in Australia, with western Bass Strait the boundary between both stocks. The level of differentiation between the stocks for all genetic markers was high. This, along with the largely fixed mitochondrial haplotype differences between populations indicated minimal gene flow between stocks. This level of genetic structuring for a migratory marine finfish species with planktonic larval dispersal and contiguous distribution is very rare. For fisheries management purposes Gemfish to the east and west of Bass Strait can be managed as separate management units.

Eastern gemfish were known to spawn during winter in the north part of their distribution. The data from this study indicated that western Gemfish spawn during summer towards the western end of the Great Australian Bight and potentially off Kangaroo Island. The spatio-temporal differences in spawning behaviour may help to explain the level of genetic isolation reported in this study between stocks.

Immigrant Gemfish were found in both east and west stocks. These immigrants were classified as hybrids as they had the nuclear DNA from one stock and the mitochondrial from the other. However, if full hybridisation is occurring the introgression of genetic material between stocks would lead to the breakdown of stock boundaries. The levels of genetic subdivision between stocks detected in this study indicates that this is not occurring. The most likely explanation for this scenario is that the hybrids are first generation crosses and that backcrossing with either parental stock being prevented. Sterility in first generation hybrids is common for species hybrids, but not typically within a species. It is likely that Gemfish stocks in the east and west have been reproductively isolated for many generations and are either on their own evolutionary trajectory to speciation or speciation has occurred. Both stocks clearly fit the definition of an evolutionarily significant unit and should be managed accordingly.

The study also found evidence for a smaller genetic effective population sizes in eastern Gemfish than western Gemfish. The results are preliminary but warrant further investigation as they may provide insight into why the eastern Gemfish population is not recovering from its overfished state.

Implications

This research has delineated two stocks of Gemfish in Australia and defined the boundary between both stocks. These data are very useful for assigning data for the stock assessment and for changing the management boundary between both stocks.

Recommendations

The report recommends that both eastern and western Gemfish stocks be treated as separate management units and the management boundary between both stocks be moved to a more appropriate location to better reflect both genetically distinct populations. The report also recommends further investigating the small effective population size found for eastern Gemfish.

Keywords

Gemfish, population structure, mtDNA, microsatellites, SNP, migration, admixture

Introduction

Gemfish (*Rexea solandri* Cuvier, 1832) is a benthopelagic snake mackerel of the Family Gempylidae. Snake mackerels are divided into 16 genera and have an elongated body shape including an elongated dorsal fin. There are seven species within the *Rexea* genus distributed throughout the Indian and Pacific Oceans.

Gemfish inhabits the continental shelf and slope in southern, southwestern and southeastern Australia and New Zealand. It is found at depths ranging from 100 to 800 metres, but occurs most commonly at 300 to 450 metres (Rowling 1994). On the eastern Australian coast, spawning occurs on the northern coast of New South Wales in winter. Dense schools of pre-spawners migrate northwards along the continental slope at about 400 metres depth during winter. Larvae are encountered in inshore waters, which may indicate that Gemfish move onto the shelf to spawn, or currents carry larvae inshore from offshore spawning grounds.

A trawl fishery was established in southeastern Australia in the late 1960s when fishers discovered winter aggregations extending from Newcastle (33°S) and Sydney (34°S) on the coast of New South Wales to eastern Bass Strait (39°S) (Rowling 1999). Catches off the east coast peaked in 1978 to 1980 (around 5,000 tonnes per year) and declined substantially after 1987. The most recent stock assessment estimated spawning biomass at 15 per cent of the 1968 level (Little and Rowling 2011). Gemfish in the Commonwealth are managed as separate stocks with the management boundary between eastern and western stocks is delineated at 146°22’E and 42°43’S off the west coast of Tasmania (AFMA 2014).

Fisheries for Gemfish also operate off western Tasmania, southwestern Victoria and southeastern South Australia as part of the Commonwealth Trawl Sector (CTS) of the Southern and Eastern Scalefish and Shark Fishery (SESSF) and in Great Australian Bight as part of the Great Australian Bight Trawl Sector (GABTS) of the SESSF. These fisheries are managed as a separate stock to the eastern stock and have a substantially different fishing history and level of exploitation. The most recent stock assessment (Chambers et al. 2014) suggested biomass is at 74 per cent of virgin biomass levels.

The Great Australian Bight Resources Assessment Group (GABRAG) had sufficient concerns regarding the population structure of the western stock, and its impact on the stock assessment, to reject the most recent assessment. They questioned whether western Gemfish constituted a single population and required more information about the location of the boundary between east and west.

Spawning behaviour

Eastern Gemfish are known to form pre-spawning aggregations off eastern Tasmania each year and migrate to northern NSW to reproduce. Spawning is believed to occur through winter (Rowling 1999). The East Australian Current flows from north to south and has the strongest flow in summer. Eastern Gemfish migrate northward and spawn over winter when the current is weakest, with currents increasing soon after, which would facilitate the transport of larvae back throughout the distribution range of the stock.

The spawning behaviour of western Gemfish is not well understood. Small numbers of gravid western Gemfish have been observed in trawl surveys from the Rottnest Canyon off Perth (Williams 1992) and off southeastern South Australian and southwestern Victoria (DPIFD 1979). Both locations have been postulated as potential spawning location based on these data (Rowling 1999), however these studies only analysed a few gravid females. Commercial

fishers from Beachport and Portland suggest Gemfish migrate to and from the west of Beachport, with returning fish including spent fish and juveniles (Tom Bibby, Terry Moran, Alan Campbell, Michael Burke, and Michael Miriklis pers. comm.).

Comparatively few Gemfish in spawning condition (late stage gonad development) are caught off south east South Australia and south west Victoria (Tom Bibby, Terry Moran pers. comm.) despite several decades of fishing, suggesting this area is not a spawning site. Commercial fishers from the Great Australian Bight report annual aggregations of spawning western Gemfish in the western end of the Great Australian Bight (between longitudes 126° and 128° east) between October and February (J. Raptis pers. comm.). Other fishers (Ben Maas, pers. comm.) suggest Gemfish spawn between Beachport and Kangaroo Island (in the general area of 138° 14' 6" S) and that there are perhaps two spawning seasons per year, in both the Great Australian Bight (GAB) and between Beachport and Kangaroo Island.

Underwater canyons and abysses off Kangaroo Island have previously been postulated as a possible spawning location for western Gemfish (Pattiaratchi 2007), due to the geomorphological similarity to the canyon regions off northern NSW where eastern Gemfish are known to spawn (Rowling 2001).

These reports provided a starting point for the collection of data on spawning Gemfish in the west. For this project, spawning Gemfish were collected as part of AFMA's ongoing observer activities which was requested by the Great Australian Bight Resource Assessment Group (GABRAG) (GABRAG 2011).

Stock subdivision

Parasite analysis (Lester 1990) was used to examine population structuring in Gemfish (Sewell and Lester 1995). They examined parasite species on Gemfish from eastern Australia, eastern Tasmania, eastern South Australia and the GAB. Their results supported the hypothesis that Gemfish from eastern Australia, eastern Tasmania and eastern South Australia constituted a single stock and Gemfish in the GAB were a separate stock.

Colgan and Paxton (1997) used allozyme electrophoresis, mitochondrial DNA and morphological traits to examine population subdivisions in Gemfish within Australia and New Zealand, using fish sampled throughout the species range in Australia and New Zealand. Genetic distance and population pair-wise comparisons of allozyme data indicated genetic subdivision between an eastern stock of Gemfish (eastern Australia including eastern and western Tasmania) and a western stock (South Australia, GAB and Western Australia). Fixed mitochondrial DNA haplotype differences supported this conclusion. Both data sets suggested there are no genetic differences between eastern Australia and New Zealand (Colgan and Paxton 1997).

It is not uncommon to find haplotype frequency differences between populations of marine fish (Ovenden 1990); however, it is rare to find fixed differences in species with such dispersal (adult and larval) potential. The level of distinction observed between these populations is more typical of freshwater fish (Billington and Hebert 1991), or for marine species occurring in different ocean basins (Graves 1998). Such a distinct separation at this smaller geographic scale could be explained by the substantial differences in spawning timing that have been suggested for this species (Tilzey & Chesson 1998, Rowling 1999), which may contribute to possible reproductive isolation of these stocks.

Information for parasite and genetic studies has formed the basis of the current management boundary between eastern and western Gemfish, and was endorsed by GABRAG in 2011. One concern with the genetic study by Colgan and Paxton (1997) was the small sample sizes

used at some locations and the low statistical power of the markers used to delineate stock structure. Small sample sizes can obscure results by not providing sufficient discriminatory power, leading to spurious or ambiguous results (Richardson et al. 1986). Small sample sizes are less likely to be representative of underlying genetic variation, which can accentuate differences between populations (Richardson et al. 1986). This may be an explanation for the differences detected in the mitochondrial data by Colgan and Paxton (1997).

This project was initiated to address these uncertainties in Gemfish stock structure. The expectation was that this study would confirm the distinction between the eastern and western stocks. The classes of genetic markers used for this study mirrored those used previously (Colgan and Paxton 1997). MtDNA sequencing of the control region was used to match the restriction digests and hybridization of whole mitochondrial genomes. The genetic techniques used by Colgan and Paxton (1997) have been improved with methods that have more discriminatory power. They used allozyme markers to study the nuclear genome, while this study used microsatellite loci. In addition, this study used single-nucleotide polymorphism (SNP) loci in the nuclear genome to allow a direct comparison to standard genetic markers.

Objectives

The objectives of this project were to:

1) Improve understanding of stock structure for Western Gemfish west of Bass Strait;

2) Improve understanding of spawning locations for western Gemfish west of Bass Strait; and

3) Provide the Australian Fisheries Management Authority with recommendations on stock structure and boundaries on the basis of this evidence.

Method

Population Sampling

To investigate stock structure within the western stock, fish were sampled from the mid-western Great Australian Bight (GAB), from two locations off the southwestern Victorian coastline (Kangaroo Island, KI and Portland, Por), from one location in western Bass Strait (WBS) and from one location off the coast of western Tasmania (WT).

To provide genetic comparisons among these target populations, fish were also sampled from the east coast of Tasmania (ET), the eastern coast of New South Wales (NSW) and Victoria as well as New Zealand (NZ).

Previous research (Colgan and Paxton, 1997) had found large genetic differences between eastern and western populations. However, there were large sample size differences between both locations and it was unclear if these differences were a result of biological processes or a sampling artefact. Archived historical samples from the Colgan and Paxton (1997) study were obtained from the Australian Museum to include in our study as controls.

The western Gemfish selected for genetic analyses were sampled during summer when the gonad analyses suggested spawning was occurring. Genetic differences among fish from different areas would be most likely to occur during aggregation for spawning. As a contrast, fish sampled during winter at one location (Portland) were also included.

The western sampling locations were chosen largely on the presence of spawning fish and the occurrence of a fishery in that region. The spatial extent of sampling was to provide adequate coverage of possible structuring.

Fin clips were taken from individuals at the wharf within one to six days of capture or one to 10 days of capture for the GAB samples. Samples from fish captured in Western Bass Strait and Western Tasmania were taken at sea, although some were sampled in port.

Where possible, reproductive status was recorded. Female status was collected at all locations except NSW. Male status was recorded only from a few individuals in the west (GAB). Stage 1 was a resting phase with no eggs or sperm present and the gonads were relatively small. Gemfish males and females were difficult to distinguish in stage 1. In stage 2 the gonads begin to develop and fill out and males and females were easier to distinguish. The male gonads typically remain smooth while female gonads will start to colour and develop veins throughout. At stage 3, the development of eggs can be seen in female gonads and in stage 4 the developed eggs are attached together inside gonad. In stage 4 the male gonads were developed but sperm was not free flowing. In stage 5 female gonads, the eggs were fully developed and freely floating inside gonad. This is usually occurs over a very short period. At stage 5 the male gonad was fully developed and sperm flowed freely when touched. For both sexes in stage 6 the gonads were spent and was the period immediately after spawning before returning to resting stage (Figures 1 and 2).

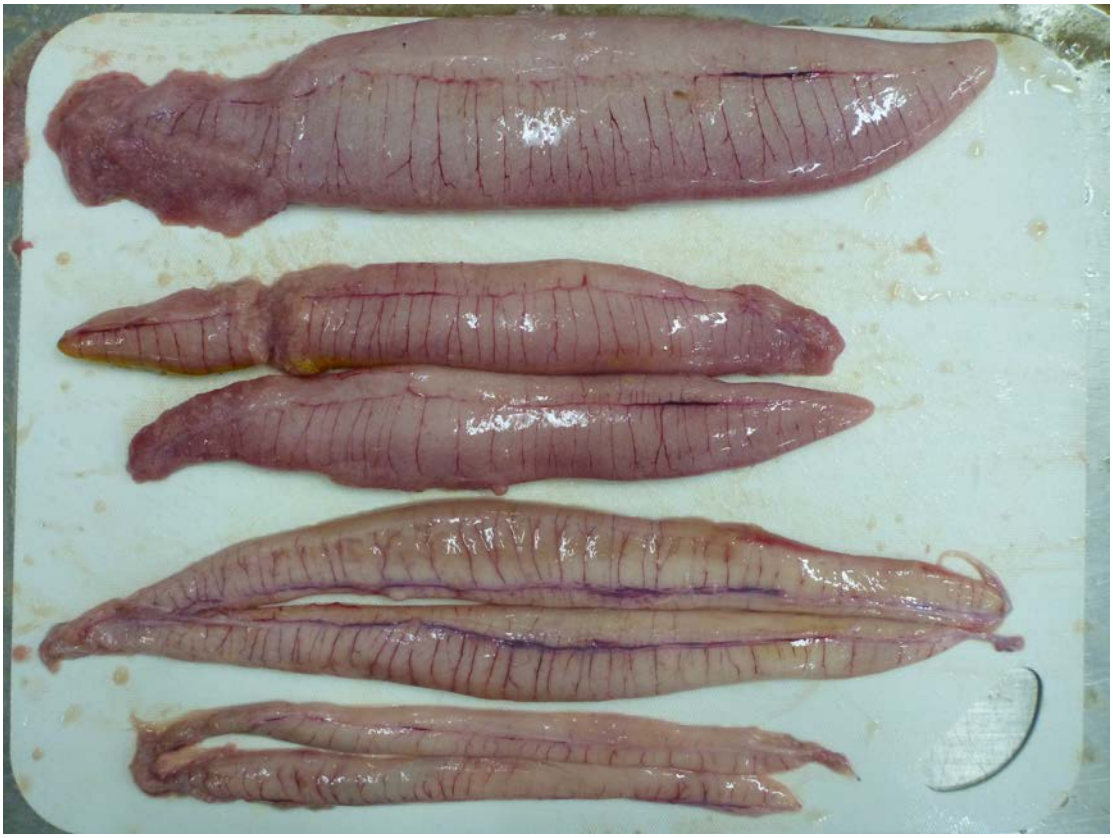

Figure 1. Female Gonad. The pictures below show female stages 2 to 5 (5 at top).

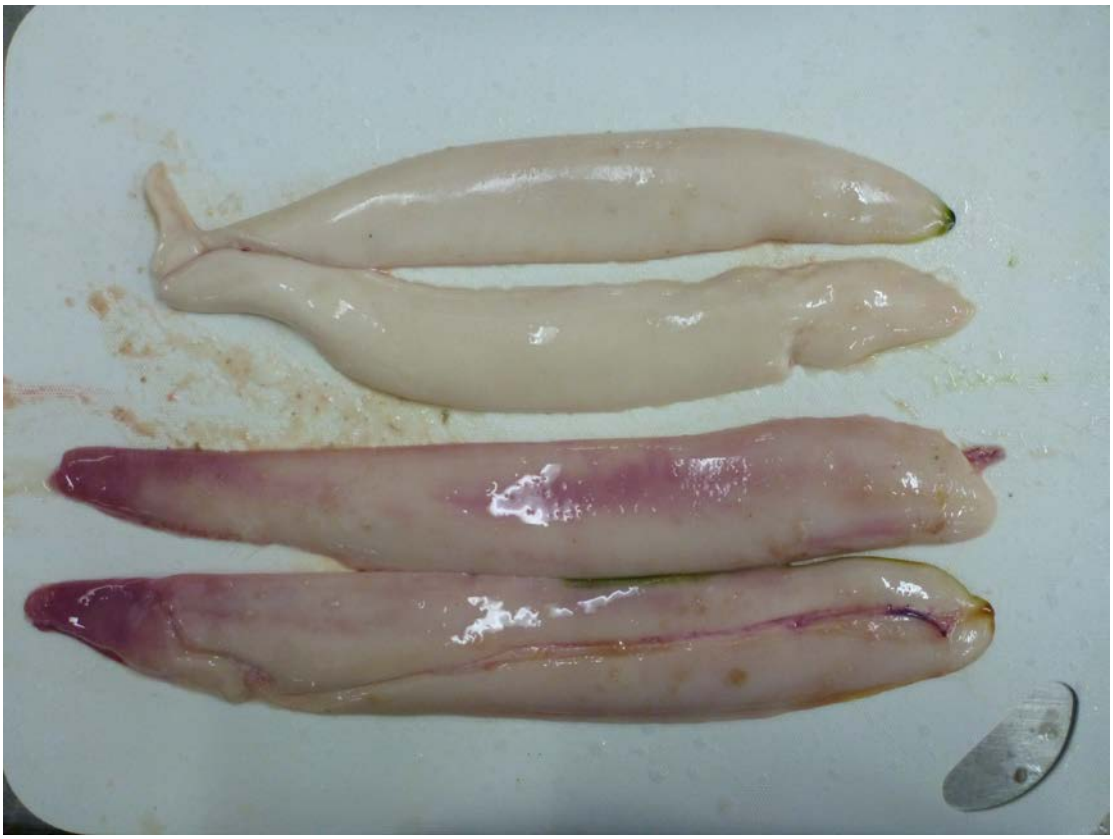

Figure 2. Male gonads stage 4 and 5 (5 on top).

DNA was extracted from fin-clips from individual fish. Fin-clips (approx. 5mm^2) were stored frozen in a 20% salt-saturated dimethyl sulphoxide (DMSO) solution. Sampling locations are described in Figure 3.

Samples from the Colgan and Paxton (1997) study consisted of 90% ethanol-preserved muscle tissues.

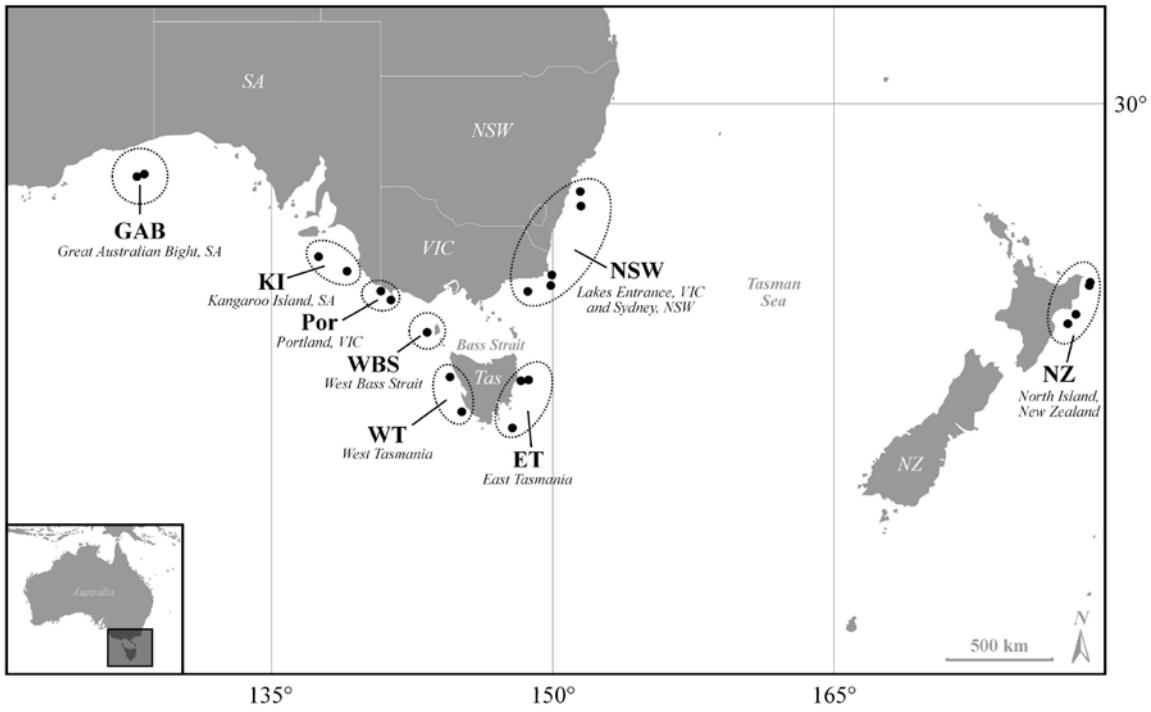

Figure 3. Location of sampling sites representing pooled collection localities for *Rexea solandri* specimens. Localities are New South Wales (NSW), Eastern Tasmania (ET), Western Tasmania (WT), Western Bass Strait (WBS), Portland (Por), Kangaroo Island (KI), Great Australian Bight (GAB) and North Island New Zealand (NZ). See Table 2 for more details.

Genomic DNA extraction

Total genomic DNA was extracted using the salting out procedure of Miller *et al.* (1988). Briefly, a small piece of tissue (approximately 100 mg) was digested overnight at 56°C in a 1.5 ml microcentrifuge tube containing 600 µl of lysis buffer (0.05 M Tris-HCl, pH 8; 0.1 M EDTA; 5 M NaCl and 5 M SDS) and 20 µl of proteinase K (10 mg/ml). Proteins were precipitated using NaCl and discarded. Nucleic acid was precipitated by isopropyl alcohol and ethanol before being dried and resuspended in TE buffer.

One extraction was performed per sample and was used for the analysis of mtDNA, microsatellites and SNP genetic markers.

Next generation sequencing and assembly of the mitochondrial genome of the Gemfish

A single *R. solandri* sample from the GAB (33°24.7'S 128°10.8'W) was selected at random for next generation sequencing. Extracted DNA from this fish was used to generate a library of DNA sequences for the Ion Torrent platform (PGM system, 318 chip, Life Technologies). Genomic DNA was obtained from the single fish using the QIAGEN DNeasy blood and tissue kit and RNA was removed by digestion with RNaseA. The assembly and composition of the mitochondrial genome are described in Bustamante and Ovenden (2014) (Appendix 1).

De novo development of microsatellite markers using next generation sequencing

The Ion Torrent sequencing library was used to identify species-specific microsatellite loci as well as to assemble the mitochondrial genome of Gemfish. A total of 1.616×10^6 sequences were produced. A subset of these (1.395×10^6 sequences), between 150 and 400 base pair (bp) in length, were searched for potential microsatellite loci using the software QDD v.3.1 (Meglecz *et al.* 2014). Sequences were included if the number of repeat units was greater than ten, there was one microsatellite (repeat) region per sequence and the primers designed by QDD were more than 11 bp (5' primer) and 13 bp (3' primer) from the repeat region. Sequences with dinucleotide and imperfect repeat motifs were excluded.

Selected Gemfish sequences were blasted against the Genbank global dataset (Benson *et al.* 2013) to identify and exclude loci that may potentially be located in known coding regions. Finally, Geneious v.7.1.7 (Kearse *et al.* 2012) was used to custom-blast forward and reverse primers back to the NGS library to exclude primers with homology to regions outside the target flanking sequence and to ensure no loci were duplicated.

Forward primers were tailed with a 'CAG-tail' to allow fluorescent labeling (FAM), and reverse primers were tailed with GTTT to ensure complete adenylation (Peters *et al.* 2009). Standard PCR conditions were used to amplify products from 48 putative loci (Williams *et al.* 2014) on DNA extracted from a set of 40 Gemfish samples. The numbers and size of alleles was recorded using Geneious software and observed and expected heterozygosities were estimated from allele frequencies using Genepop v.007 (Rousset 2008).

To optimise the experimental design of population surveys with these loci, a power analysis (POWSIM, Ryman and Palm 2006) was used to simulate the probability of detecting population genetic differentiation across a range of expected F_{ST}'s in three theoretical populations. Hypothetical sample sizes per population (50 and 100) and the numbers of loci (10, 12 and 14) were varied across runs. Loci were excluded if they had 1) a major allele frequency greater than 90% (loci Rsol62 and Rsol70), and 2) potential lower scoring accuracy due to the presence of large numbers of alleles (loci Rsol33 and Rso68).

As recommended by Ryman and Palm (2006), estimates of statistical power for a defined level of divergence was controlled by changing the generations of drift (t) instead of varying the effective population size (Ne), which can cause the loss of low frequency alleles. Values of F_{ST} were set at 0.01, 0.005 and 0.0025, which was equivalent to migration rate of 2.5%, 5% and 10%. The following parameters were used: effective population size (Ne) = 1000; number of simulations = 1000; and generations of drift (t) = 20 (F_{ST} = 0.01), 10 (F_{ST} = 0.005) and 5 (F_{ST} = 0.0025). The degree of significant differentiation (quantified as F_{ST} values) for each replicate run was tested using Chi-square and Fisher's probabilities to test the null hypothesis of genetic homogeneity.

Sanger sequencing of regions of the mitochondrial genome

Laboratory

A section of the highly polymorphic control region of the mitochondrial genome was studied to test for genetic differences among individuals from various collection locations. Primers TDKD_1291L21 (5'-CCT GAA GTA GGA ACC AGA TG-3') and PRO_889U20 (5'-CCW CTA ACT CCC AAA GCT AG-3') from Ovenden *et al.* (2002) were used to amplify the region from 15636 to 16027 (392 base pairs) in the whole mitochondrial genome of the Gemfish (Genbank KJ408216) (Bustamante and Ovenden 2014).

To validate the genetic differences found in the control region between southern and eastern populations, a section of the more conserved ATPase region was amplified and sequenced in a subset of samples. The primers ATP8_7894 and PGSR_8815 were used from Broderick *et al.* (2011) and the region corresponded to 7914 to 8815 (901 bp) (Bustamante and Ovenden 2014).

Polymerase chain reactions (PCR) were performed in a total volume of 10 µl containing 20–50 ng template DNA (1 µl) of template DNA, 1 µl of 10× reaction buffer Taq DNA polymerase buffer (Applied Biosystems, CA), 1 µl of dNTPs (10 mM), 1 µM of each primer, and 0.5 units of Taq DNA polymerase (Applied Biosystems, CA). Negative controls in all PCR runs confirmed the absence of cross-contamination.

Thermal cycling consisted of an initial denaturing step of 3 minutes at 94°C, followed by 35 cycles of denaturing at 94°C for 15 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute; final extension consisted of 72°C for 10 minutes.

Double-stranded DNA products were purified with 1 U of ExoSAP-*it* (GE Healthcare Bio-Sciences, Australia) at 37°C for 45 minutes, followed by an inactivation step at 80°C for 15 minutes.

The cleaned PCR products were sequenced using the BigDye Cycle Sequencing Kit v3.1 (Applied Biosystems, CA). Cleaned products were visualised on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, CA).

Computer analysis

To analyse genetic differences among fish, sequence data were trimmed, assembled and aligned using Geneious software (v8.1.6) with a stringent and conservative pipeline. When all samples were sequenced for a gene region, the raw (.ab1) files were sorted by quality. Those with less than 10% high quality base calls were discarded. Sequence regions with more than a 1% chance of an error per base were trimmed progressively from the sequence ends, and primer sequences were also removed. Sequences were assembled using the medium sensitivity – fast method of the *de-novo* assembly option. The assembly report contained information on contigs consisting of forward and reverse sequences of the same sample. The report also listed singleton sequences (either forward or reverse, but not both). For the control region data, contigs and singletons less than 200 bp in length were discarded. Contigs were visually inspected and mismatches between forward and reverse sequences, if found, were manually edited. Contigs with unresolvable mismatches were removed. Edited contigs and singleton sequences were then aligned to the reference sequence (Bustamante and Ovenden 2014) using five iterations of the medium sensitivity – fast method. Mismatches arising from alignment between contigs and singletons were manually edited. A region of the

alignment that was covered by the majority of the sequences and which was dense in SNPs was manually selected and exported for downstream analysis in .phy format.

DNAsp software (v5) (Librado and Rozas 2009) was used to identify haplotypes. SNPs that contained missing data were excluded.

Arlequin software (v3.5.2.2) (Excoffier and Lischer 2010) was used to find haplotypes from the aligned sequences and the frequency of those haplotypes in groups of samples.

Population pair-wise *phi*-sts were calculated using Arlequin. Genetic distance between haplotypes was estimated using the Tamura-Nei method (Tamura and Nei 1993), with gamma set to 0.25. One hundred permutations were performed to assess the significance of *phi*-sts values.

Pairwise *phi*-sts were calculated between recently sampled collection locations. For this comparison, samples collected at GAB in summer and winter were combined as they were represented by the same control region haplotype. Pairwise *phi*-sts were also calculated between collection locations sampled 20 years ago by Colgan and Paxton (2000).

Microsatellite genotyping

Laboratory

Fourteen microsatellite loci were selected for genotyping from those developed as part of this project (above). Three multiplexed PCRs were performed to genotype each fish at these loci. The first multiplexed PCR contained primer pairs to amplify seven loci, the second two loci and the third five loci (Table 4).

Primer tails used for fluorescent labelling were changed to custom-M13 tails, which allowed the use of any combination of the four fluorescent dyes for with multiplexing. The M13 tail for FAM dye was TTT CCC AGT CAC GAC GTT G, for Vic GCG GAT AAC AAT TTC ACA CAG G, for Ned TAA AAC GAC GGC CAG TGC and for Pet CAC AGG AAA CAG CTA TGA CC. Locus and colour combinations per multiplex PCR were chosen to minimise allele overlap.

One primer stock was set up for each locus consisting of forward and reverse primer pairs, and the corresponding the fluorescently labelled M13 primer (Fam, Vic, Ned or Pet). Primer stocks consisted of 6 µl of 100uM forward primer, 30 µl of 100uM reverse primer and 30 µl of 100uM of M13-fluro labelled primer. For use in PCR, primer stocks were combined into a primer mix in proportions that were determined experimentally to achieve optimal amplification across all loci (Table 1).

Microsatellite PCR amplifications were performed in 96 well plates using BioRad thermocycler (T100). The PCR (14ul) contained 2 µl of template (approximately 20ng genomic DNA), 8 µl of mastermix (2xMyTaq mix, www.bioline.com.au, containing about 0.15 µl of Taq) and 4 µl of primer mix. Primer mix was added to the template, and then the reaction was brought to 95ºC in the PCR machine. The master mix was added and cycling began with denaturing of the template for 3 minutes at 95ºC. This was followed by 37 cycles of 15 seconds at 95ºC, 15 seconds at 57ºC and 45 seconds at 72ºC. This was followed by a final extension step of 30 minutes at 72ºC to ensure complete addition of adenine to the PCR product, which is essential for consistent allele length determination. Positive and negative extraction and PCR controls were used throughout.

PCR products were diluted and amplicon length was determined using capillary electrophoresis on an ABI 3130xl Genetic Analyser (Applied Biosystems, www.applied.biosystems.com). A Liz internal size marker was used.

Genotypes were scored and binned using the microsatellite plugin for the Geneious software v8.1.6 (Kearse *et al.* 2012). Microchecker software (Van Oosterhout *et al.* 2004) was used to determine if heterozygotes were missing across loci. If they were likely to be missing, scoring was checked in Geneious. This continued until the deviation between observed and expected per-heterozygote frequencies was minimised. Genotypes were exported from Geneious into Microsoft Excel format for downstream analyses.

Table 1. Amount of primer stock per microsatellite locus added to primer mix to set up three multiplexed PCRs for genotyping Gemfish at fourteen loci.

Multipexed PCR #	Locus Number (lab no)	Locus Name	Microlitre of primer stock per multiplex per single reaction
1	9	Rsol11	0.250
1	28	Rsol33	0.600
1	19	Rsol22	0.075
1	40	Rsol62	0.060
1	44	Rsol67	0.150
1	45	Rsol68	0.150
1	46	Rsol70	0.150
2	25	Rsol29	0.200
2	27	Rsol31	0.100
3	23	Rsol27	0.350
3	26	Rsol30	0.125
3	29	Rsol54	0.100
3	39	Rsol57	0.150

Computer analysis

Population Structure

GenAlex v 6.501 (Peakall and Smouse 2006) was used to examine per locus and per individual genotyping success as a guide to pruning the dataset of missing allelic determinations. Allelic frequencies per locus by population were calculated in GenAlex. The data conversion and export feature of GenAlex was used to produce input files for downstream analyses in GenePop, Arlequin, STRUCTURE and DAPC.

The Queller and Goodnight (1989) estimator of mean relatedness was calculated between all pairs of individuals represented by their microsatellite genotypes to detect inadvertent duplicates among samples that could arise in the field or laboratory. This was implemented in GenAlex.

GenAlex was also used for an estimate of genetic diversity across population samples. The effective number of alleles per locus was chosen as a measure of genetic diversity as it allows meaningful comparisons of allelic diversity across loci with diverse allele frequency

distributions. The number of effective alleles is a measure of the evenness of the allele frequency distribution, and is equal to [1 / (1-He)] where He is expected heterozygosity. For example, a population with three equally frequent alleles is more diverse than one with a single predominant allele and two rare alleles. It is less affected by sample sizes per population than allelic richness (or number of distinct alleles) per locus (Brown and Weir 1983).

An estimate of genetic effective population size was made using the linkage disequilibrium, single sample method (Waples and Do 2010) using 13-locus microsatellite genotype data. The estimates were made using NeEstimator software V2.01 (Do *et al.* 2014)

Arlequin v3.5.2.2 (Excoffier and Lischer 2010) was used to test for Hardy-Weinberg proportions using exact tests and well as for linkage (gametic) disequilibrium. A large number of Markov chain steps (1 000 000) and dememorisation steps (100 000) were used. *F*STAT (Goudet 1995) was used to estimate global F_{ST}'s per locus to test for a Wahlund effect by comparison with per-locus *F*st's. Population pairwise F_{ST}'s were calculated between populations allowing missing data and with 1000 permutations to estimate p-values using Arlequin.

A Bayesian clustering approach was used on the microsatellite genotype data to test for differential admixtures of groups from collection locations and to determine the immigrant likelihood of individual samples. STRUCTURE v 2.3.4 (Pritchard *et al.* 2000) was used to determine the number of likely groups (k) from two to ten. The method of Evanno *et al.* (2005) was used to display the results. STRUCTURE was run for 20 independent runs for each k with 100 000 MCMC iterations and a burn-in length of 100 000. Allele frequencies were correlated and known population groupings were used to assist the search for likely groups of genotypes. POPHELPER (Francis 2016) was used to display results.

An alternative approach was also used to investigate population structure. Discriminant analysis of principle components (DAPC) focuses on genetic diversity between groups of individuals as a component of the overall diversity of the sample (Jombart *et al.* 2010). It minimizes variation within clusters of individuals while showing differences between groups. It does this by finding synthetic variables or discriminant functions.

DAPC requires prior groups to be defined and it does this using a k-means approach after transforming the data using PCA. In practice, the majority of population genetic data is already clustered. In this project, samples were clustered by collection location.

The number of PC axes retained, and used, in the analysis can be set by a rule-of-thumb (maximum number of PCs divided by three) or by performing cross-validation. For the microsatellite locus dataset, 20 replicates of cross-validation were performed with training set size of 0.9. The optimal number to be retained was 90. However, as the proportion of successful predicted outcomes was largely unchanged from 60 to 90, so 60 PCs were retained. The contribution of alleles to discriminant axis was determined with complete linkage clustering displayed in a loading plot. DAPC was performed in R using package Adegenet (Jombart 2008).

Population Assignment

A pronounced genetic difference between the eastern and western stocks (see below) allowed us to test whether each fish was correctly assigned to the stock from which it was sampled. Fish that were not correctly assigned were regarded as immigrants. Two approaches were used; allele frequency based assignment tests (Paetkau *et al.* 1995) and posterior probabilities (Q-values) using the Bayesian clustering approach STRUCTURE v 2.3.4 (Pritchard *et al.* 2000).

Some fish could not be confidently assigned to one stock or the other. When this occurred, the Q-values suggested that the individual might be a hybrid offspring as a result of interbreeding between fish from different stocks. No attempt was made here to distinguish F1 hybrids from backcrossed hybrids, although this may be possible in future studies. Individuals were designated potential hybrids if their Q-values were greater than 0.3 and less than 0.7. No attempt was made here to validate these cutoffs, but this could be done in future.

Allele frequency based assignment tests (Paetkau *et al.* 1995) were implemented in GenAlex (Peakall and Smouse 2006) on microsatellite genotypes. For each sample, the likelihood of it matching one of two populations was calculated from the per-locus genotype of the sample and the observed frequency of that genotype in either population. The sample was assigned to the population producing the least negative log-likelihood value. If alleles were absent from given populations, then the value of 0.01 was used instead of zero. The 'leave-one-out' option was used that estimates population allele frequencies leaving out the sample under consideration. Populations here were equivalent to the eastern stock or the western stock; the collection locations were grouped into 'east' (NSW, NSW_h, ET_h, NZ_h, WT) and 'west' (Por, Por_w, KI and GAB).

Individuals from the WBS collection location (WBS) were not included in the 'east' or 'west' population grouping. WBS did not have clear links with east or west groups (see below). To investigate whether the WBS location consisted of an admixture of fish from both stocks, individuals from WBS were compared to the 'east' or 'west' groupings.

To confirm the assignments from GenAlex, the posterior probability that the individual in question was correctly assigned (Q-value) was calculated using STRUCTURE where MIGPRIOR was set to 0.1 and k was two.

Genotyping single-nucleotide polymorphisms (SNP)

Laboratory

In addition to mtDNA and microsatellite loci, a third class of genetic markers was used, called single-nucleotide polymorphisms (SNP). Like microsatellite loci, SNP are found in the nuclear genome. SNP loci have only two alleles, unlike microsatellite loci that can have dozens of alleles. However, in studies like this it is feasible to assay thousands of SNP loci compared to an upper limit of around 20 microsatellite loci. SNP loci are simultaneously discovered and assayed whereas microsatellite loci are first discovered then assayed.

The system used for SNP is referred to as DART, or more correctly DArTseq™. It is a combination of DArT complexity reduction methods and next generation sequencing platforms (Donnellan *et al.* 2015; Kilian *et al.* 2012).

To further address the question of multiple stocks within the western region, individual Gemfish were selected from three locations in the western stock; two locations which were close-by (KI and Por) and one distant location (GAB). Individuals were also included from NSW to provide contrast in the data. Individuals were selected when the genomic DNA concentration was high (greater than around 100ng/ul) and the DNA was not degraded (Figure 4).

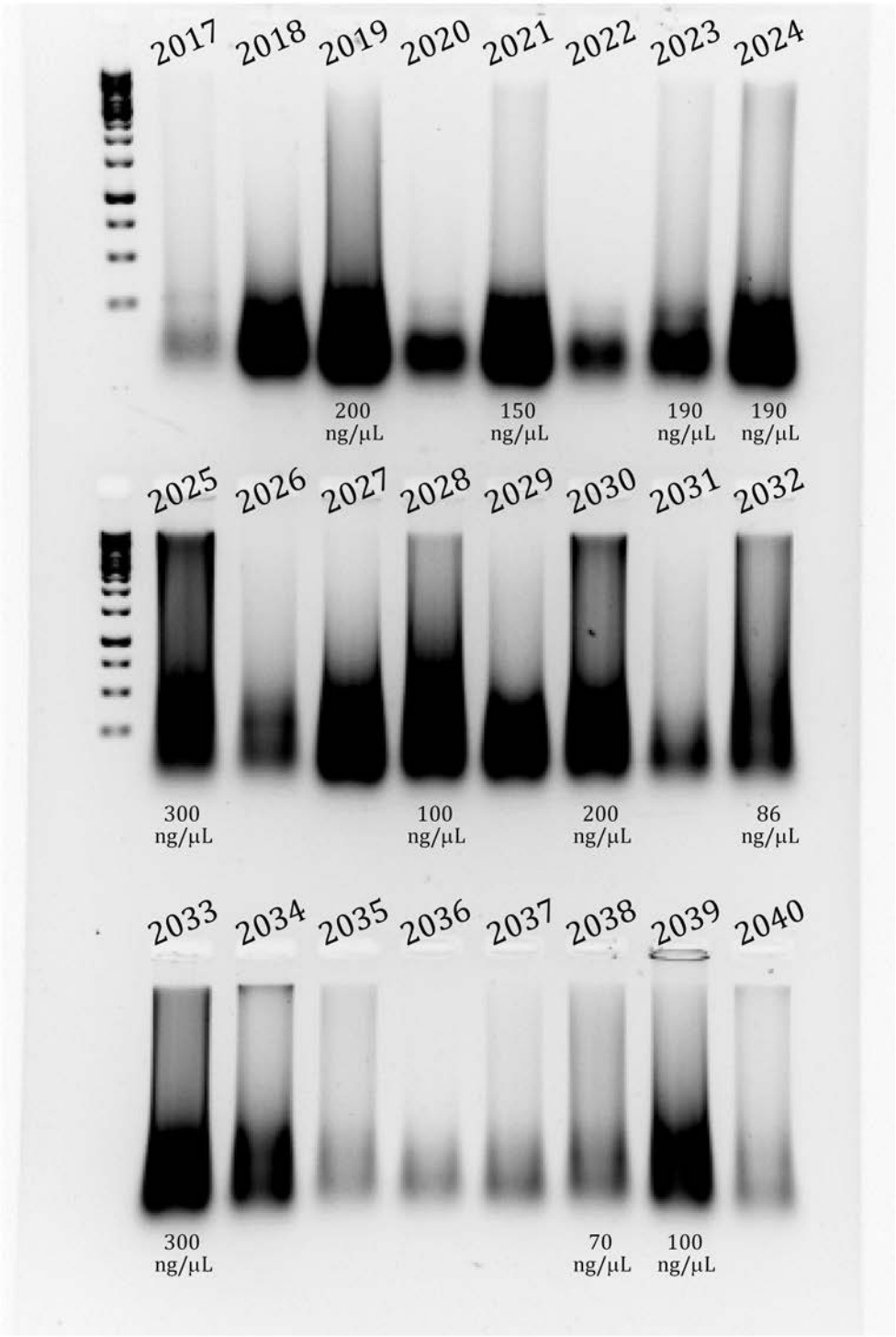

Figure 4. Agarose gel (1%) in Tris/Borate/EDTA buffer solution showing 5 µl of genomic DNA from GAB Gemfish. The DNA concentration (ng/ul) of individuals selected for SNP analysis is shown. Size standards are the 1kb GeneRuler DNA ladder from Thermo Scientific. The smallest bands are 250 and 500bp. The central bright band is 1000bp and the largest band is 10 000bp.

Genomic DNA from each individual was digested with restriction enzymes that recognize a particular sequence of bases. When found, the enzyme cleaves the DNA at that point. For example, the enzyme *Pst*I cleaves DNA at the recognition site 'CTGCAG'. The DNA fragments were then tailed ('barcoded') with (ligated to) a short artificial DNA sequence that identifies the individual. Further tails are added to facilitate the next-generation sequencing *en masse* of pooled, tailed DNA from many individuals simultaneously.

Ligated fragments with both a *Pst*I and *Sph*I adaptor were amplified by PCR using an initial denaturation step of 94°C for 1 minute, followed by 30 cycles with the following temperature profile: denaturation at 94°C for 20 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with an additional final extension at 72°C for 7 minutes. Equimolar amounts of amplification products from each sample were combined before single end sequencing for 77 cycles on an Illumina Hiseq2500. Further details can be found in Donnellan *et al.* (2015).

The DArTseq protocol includes technical replication to assess the quality of the resulting data and 32 of the DNA extractions were divided in two prior to digestion/ligation to act as technical replicates. Two independent libraries were made for those samples and results compared after allele calling to establish calling reproducibility.

Computer analysis

The HiSeq data was formatted as fastq files and processed using proprietary DArT analytical pipelines (DArTsoft14). The steps were: filtering (removing) poor quality sequences, applying stringent selection criteria to the barcode region to ensure the reliable assignment of sequences to specific samples, and collapsing identical sequences into "fastqcall" files.

The SNP data received from Diversity Array Technologies was filtered to remove SNP with a) more than 5% missing allelic determinations across individuals, b) less than a sequencing coverage of 20 times (to minimize sequencing errors in data), c) sequencing coverage more than 80 times (in an attempt to remove paralogs). Minor allele frequencies were calculated for loci remaining after filtration. These tasks were performed with R (R-Development-Core-Team 2010).

Gemfish SNP sequences matching regions of known teleost genomes may be important for survival, and hence under selection (so-called adaptive genes). The filtered SNP data was further screened to identify SNP whose sequence had homology to any of six teleost species for which genomic resources are available. These were the zebra fish, the puffer fish, the Mexican tetra, the Atlantic cod, and the stickleback.

Net genetic diversity between pairs of populations was estimated using F_{ST} (theta; Weir and Cockerham 1984). Data was resampled to produce 95% confidence intervals. Calculations were done using the Diversity package (Keenan *et al.* 2013).

DAPC (Jombart *et al.* 2010) was used to display the genetic diversity of samples between collection locations. Cross-validation was used to determine the optimal number of principal components to retain.

Results

Sampling summary, including biological characteristics

Sampling achieved

In 2013 and 2014, a total of 542 Gemfish individuals were collected from six general locations in Australia (Figure 3, Table 2). Additionally, 59 Australian Museum samples from Australia and New Zealand were obtained (Colgan and Paxton 1997).

Table 2. Numbers of gemfish samples collected during the project in 2013 and 2014 (modern) and 1991 to 1993 (historical, Australian Museum) from locations described in Figure 3. Collections are grouped by month due to the occurrence of seasonal spawning migrations. Month_01 is January and so on. The size, reproductive condition and gender of modern samples was analysed here. Genetic data was collected from a subset of these modern and historical samples (see below).

	Collection location								
	NZ	NSW	ET	WT	WBS	Por	KI	GAB	Total
Modern									
Month_01_2014	-	-	-	-	52	50	50	50	202
Month_02_2014	-	-	-	-	-	50	-	-	50
Month_03_2014	-	-	-	-	-	7	-	-	7
Month_04_2014	-	-	-	44	-	-	-	-	44
Month_07_2014	-	-	-	-	-	45	-	50	95
Month_08_2014	-	100	-	-	-	12	-	-	112
Month_09_2014	-	-	-	-	-	20	-	-	20
Month_11_2013	-	-	-	-	-	-	-	12	12
Total	0	100	0	44	52	184	50	112	542
Australian Museum									
Month_03_1991	-	6	-	-	-	-	-	-	6
Month_03_1993	12	-	-	-	-	-	-	-	12
Month_04_1992	-	-	-	4	-	-	-	-	4
Month_05_1992	-	3	9	-	-	-	-	-	12
Month_08_1992	-	-	10	-	-	-	-	-	10
Month_09_1991	-	-	-	-	-	11	-	-	11
Month_12_1991	-	4	-	-	-	-	-	-	4
Total	12	13	19	4	0	11	0	0	59

Reproductive status and timing of spawning

The ratio between male and females was biased towards females for GAB, KI, WBS, Por and WT locations. In NSW, however, more males than females were collected (Figure 5).

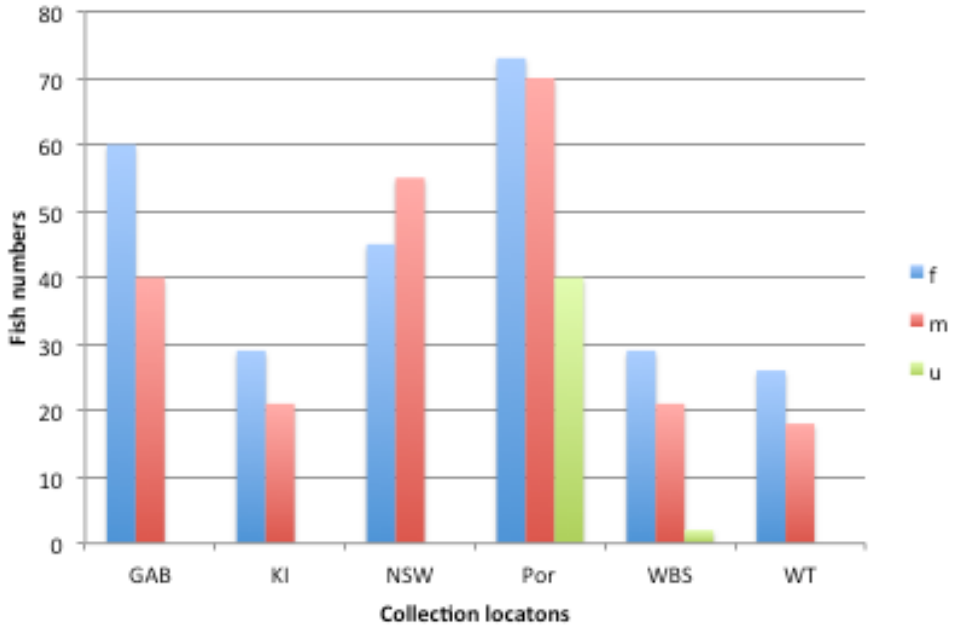

Figure 5. Numbers of male (m) and female (f) Gemfish collected from six locations sampled in 2013 and 2014. The gender of some fish was not determined (u).

Reproductive status was recorded across collection locations and seasons to test the expectation that gemfish in the western stock moved towards the west in summer to spawn. This is in contrast to what is known about the spawning migration in the eastern stock, where fish move northwards in winter to spawn. Thus, in the western stock, fish collected from the most westerly location (GAB) in summer should be in late stages of gonad development or have recently spawned (have spent gonads), and GAB fish collected in winter should be in early stages of gonad development. The average stage of reproductive development for 43 females collected in the GAB in summer (January, 2014) was 3.16 (mode was 3). For winter, the average female stage (17 females collected in July, 2014) was 1.24 (mode was 1). As fish were more advanced in summer compared to winter, these results were consistent with the expectation (Fig. 6).

The expectation of spawning movement in the western stock can also be tested with fish collected from the easterly part of the stock (KI, Por, WBS and WT). In summer, fish from the east should be in earlier reproductive state than fish from the GAB.

There were 29 females collected from KI in summer (January, 2014) and their average reproductive status was 3.41 (mode was 2). In total, 87 females were collected from Portland in summer (January to March 2014) and their average state of reproductive development was 2.08 (mode was 2). The reproductive status of fish collected from Portland in winter (July, August and September 2014) was not recorded. There were 29 females collected from WBS in summer (January, 2014) and their average reproductive status was 2.24 (mode was 2).

There were 25 females collected from WT in late summer (April, 2014) and their average reproductive status was 3.64 (mode was 3). Eight females from WT were recorded as spent (stage 6). In the entire study, only two other females were recorded as spent and these were from KI (Fig. 6).

Generally in summer, fish from the eastern parts of the western stock were less reproductively developed than fish from the western part. This is consistent with the hypothesis that in the western stock, fish move to the west to spawn in summer. The relatively advanced state of females in KI during summer may suggest that the location of spawning in the western stock stretches from there to the GAB.

The genetic analyses presented below suggest that the boundary between the stocks occurs in the WBS and WT location, which affects the interpretation of spawning patterns. This is discussed in detail below.

Season		Female reproductive status				
Austral Summer (November, January, February, March, April)	*Mean*	3.16	3.41	2.08	2.24	3.64
	Mode	3	3	2	2	3
	N	43	29	87	29	25
Austral Winter (July, August, September)	*Mean*	1.24				
	Mode	1	NA	NA	NA	NA
	N	17				
		GAB	KI	Por	WBS	WT
		Far west	*West of centre*	*Centre*	*East of centre*	*Far east*
		Location in western stock				

Figure 6. Measures of female reproductive status (stages 1 to 6) at locations across the western stock in the austral summer and winter. Stage 1 indicates undeveloped ovaries, stage 5 ripe and stage 6 was spent. Fish were collected from November 2013 to April 2014. *N* is the number of females. Some data was not available (NA).

Length frequency

There was variation among the sizes of Gemfish sampled for this project. The largest fish came from the GAB. Fish in the GAB were typically between 70 to 80 cm, with a maximum size of 105 cm (Figure 7).

Fish from KI and Por had similar size ranges from approximately 50 to 80 cm. In Por there were smaller fish (around 45 to 55 cm) compared to KI.

There were differences in sizes between fish collected from the two locations near western Tasmania. At the WBS location, the fish were small, ranging from 43 to 53 cm, with very few above 60 cm. In the WT location, a few hundreds of kilometres to the south, the fish were larger, typically between 65 to 75 cm.

The typical length of gemfish from the NSW population was 35 to 45 cm, with a few measuring from 60 to 70 cm. The fish were sampled in winter (August).

In the NSW and the WBS location, there was evidence of a group of small fish that may represent recent recruits. In NSW these fish ranged from 28 to 34 cm and in WBS they ranged from 35 to 39 cm.

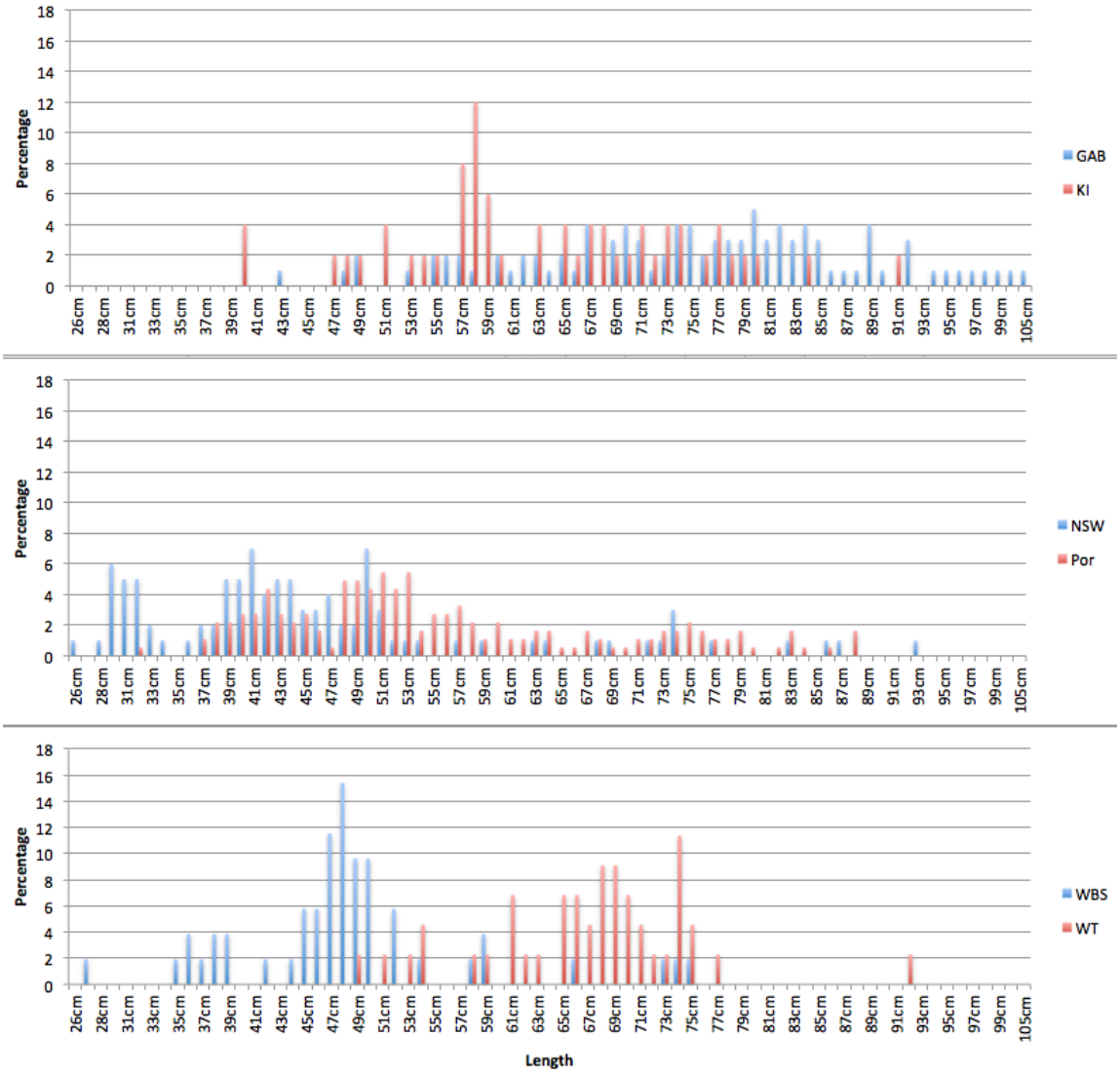

Figure 7. Percentages of Gemfish in length size classes from six collection locations.

Sequence variation in mitochondrial DNA

Summary statistics

The quality of the Sanger control region sequences was low, which was mirrored in the relatively low success rate for microsatellite genotyping (see below). A proportion of sequences (331 out of 452) had more than 10% high quality bases and hence was retained for analyses. Some fish (57) were sequenced in both directions and 207 fish were sequenced in one direction only. Four of the fish sequenced in both directions were discarded because of unresolvable base mismatches.

A 189 bp region was selected from the final control region alignment to maximise coverage and SNP density. Within this region, sequence data was missing from some fish across 92 base positions leaving 97 bases that were present in all fish. Amongst these 92 sites present in all individuals there were 12 polymorphic sites (SNP), which collectively defined seven control region haplotypes. There were six transitions, four transversions and two one-bp insertion/deletions (indels) (Table 3).

Table 3. The character states of 12 SNP of seven Gemfish control region haplotypes, where '.' corresponds to the state in the cell above and '–' is a one bp insertion deletion (indel). Genbank accession numbers are KU64693-9.

SNP position in control region alignment	75	82	83	89	92	94	96	101	116	122	129	135
SNP position in KJ408216	15860	15867	15868	15847	15877	15879	15880	15885	15900	15906	15913	15919
Rsol_CRH01	**C**	**C**	**T**	**A**	**T**	**A**	**T**	**G**	**T**	**G**	**T**	**A**
Rsol_CRH02	.	.	.	.	.	.	**C**	.	.	.	.	.
Rsol_CRH03	**T**	**A**	**C**	**G**	**C**	**–**	.	**A**	**G**	**C**	**G**	**–**
Rsol_CRH04	.	**A**	**C**	**G**	**C**	**–**	.	**A**	**G**	**C**	**G**	**–**
Rsol_CRH05	.	.	.	.	.	.	.	**A**	.	.	.	.
Rsol_CRH06	.	.	.	.	.	.	**C**	**A**	.	.	.	.
Rsol_CRH07	.	.	.	.	.	.	.	.	.	**C**	.	.

Network of mtDNA control region haplotypes

There were two clear groups of haplotypes. The most parsimonious network showed that the most common control region haplotypes (CR01 and CR03) differed by 11 SNP and were related to the less frequent haplotypes by one SNP (Figure 8).

Sanger sequence from the ATPase region was consistent with the large genetic difference between control region haplotypes CR01 and CR03. Among 581 bases, there were 47 SNP, and 44 of them defined ATPase haplotypes from the western compared to the eastern stock (data not shown).

MtDNA haplotype variation among population samples

Haplotype phylogeny was reflected by control region haplotype frequency variation across collection locations. Two collection locations in the southern region (Por, GAB) were represented by 32 and 40 sequences, and of these 97% and 100% were haplotype CRH03. One location in the east (NSW) and on the west coast of Tasmania (WT) also had sufficient sequence sample size (28, 27) and contained 74% and 82% of CRH01. They did not contain haplotype CRH03. The NSW collection also contained CRH02, which was not found in the southern region. The WT collection contained haplotypes CRH05 to 07, also not found in the southern region. The 34 fish sequenced from Western Bass Strait (WBS) contained both CRH01, 02 (58%, 15%) as well as CRH03 (18%), CRH05 (3%) and CRH06 (6%) (Table 4).

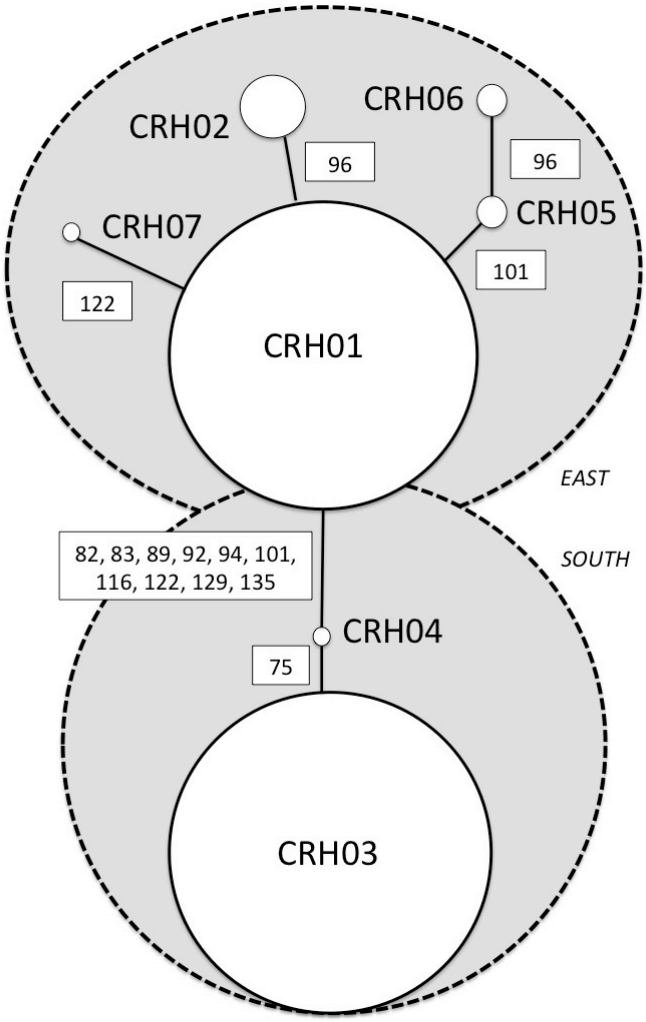

Figure 8. Unrooted network describing the similarity between 12 control region haplotypes from Gemfish. The size of the haplotype symbol is proportional to its overall frequency. The network is shaded to show the geographic distribution of the haplotypes. Branches are labelled with SNP whose character state differs between haplotypes. Homoplasious changes occur for SNP 96, 101, and 122 (Table 3).

The number of different haplotypes (haplotype diversity per location) varied between locations. The two southern locations (Por, GAB) had low diversity (0.061 and 0), while the haplotype diversity of NSW, WT and WBS was higher (0.384, 0.311, 0.597).

The haplotype composition of samples collected 25 years ago from NSW, WT and Por did not significantly differ from those collected during this project. Samples collected in the past from locations not sampled during this project (NZ, ET) showed the most similarity to the eastern region.

Table 4. Frequency of seven mtDNA control region haplotypes in Gemfish sampled from southern and eastern Australia. Groups are labelled as in the map Figure 1, and samples were taken during summer of 2013–14. Locations NSWh, NZh, ETh, WTh and Porh were sampled by Colgan and Paxton (1997) in the early 1990s. Haplotype diversity is equal to 1-sum(freq^2).

		Spatial and temporal sample groups										
Haplotype	All Fish	NSW	NSWh	NZh	ETh	WT	WTh	WBS	Por	Porh	KI	GAB
Rsol_CRH01	82	0.741	1.000	0.429	0.909	0.821	0.500	0.588	0.031	0	0	0
Rsol_CRH02	16	0.259	0	0.429	0.091	0	0	0.147	0	0	0	0
Rsol_CRH03	85	0	0	0	0	0	0.250	0.176	0.969	1.000	0.750	1.000
Rsol_CRH04	1	0	0	0	0	0	0	0	0	0	0.250	0
Rsol_CRH05	5	0	0	0.143	0	0.107	0	0.029	0	0	0	0
Rsol_CRH06	4	0	0	0	0	0.036	0.250	0.059	0	0	0	0
Rsol_CRH07	1	0	0	0	0	0.036	0	0	0	0	0	0
n	194	27	3	7	11	28	4	34	32	4	4	40
Haplotype diversity		0.384	0.000	0.612	0.165	0.311	0.625	0.597	0.061	0.000	0.375	0.000

The amount of population genetic subdivision revealed by *phi*-st analyses reflected the uneven distribution of haplotypes between locations. Samples from NSW, NZ and ET were not significantly different, consistent with the presence of an eastern stock. The western stock consisted of samples from Por, KI and GAB (Table 5).

However, the genetic similarities of fish from WT and WBS was less clear from the *phi*-st analysis. The WT stock was similar to one population in the eastern stock (ET), but distinct from the remainder (NSW, NZ). The WT and WBS stocks were genetically different to the western stock but WBS matched two the eastern stock populations (NZ, ET). The WBS samples were genetically distinct to the WT or Por samples, which is counterintuitive as the collection locations are adjacent on the WBS area (Figure 3, Table 5).

Table 5. *Phi*-st values (below diagonal) for pairwise comparisons among Gemfish samples from locations sampled during this study (1, 4, 5, 6, 7, 8) and from locations sampled by Colgan and Paxton (1997) (2, 3). Location names are the same as Figure 3. Significant p-values (<0.05, above diagonal) and *phi*-st values are in bold.

			1	2	3	4	5	6	7	8
		n	NSW	NZ_h	ET_h	WT	WBS	Por	KI	GAB
1	NSW	27	-	0.250	0.383	**0.007**	**0.008**	**0**	**0**	**0**
2	NZ_h	7	0	-	0.164	**0.022**	0.150	**0**	**0.004**	**0**
3	ET_h	11	0.019	0.159	-	0.338	0.220	**0**	**0**	**0**
4	WT	28	**0.145**	**0.243**	0.022	-	**0.048**	**0**	**0**	**0**
5	WBS	34	**0.121**	0.046	0.055	**0.084**	-	**0**	**0.004**	**0**
6	Por	32	**0.949**	**0.939**	**0.948**	**0.950**	**0.750**	-	0.243	0.446
7	KI	4	**0.962**	**0.944**	**0.981**	**0.970**	**0.658**	0	-	0.081
8	GAB	40	**0.984**	**0.990**	**0.996**	**0.987**	**0.810**	0.007	0.617	-

Microsatellite genotyping

Summary statistics

Gemfish (n=447) were genotyped with microsatellite loci from eight locations in southern and eastern Australia and New Zealand (Figure 1). Portland (Victoria) was represented by samples taken in summer (Por) and winter (Por_w). The GAB was represented by samples taken in summer and winter, but these were pooled due to low sample sizes in the winter sample. Eastern Australian locations were represented by modern samples taken from NSW and 25-year old samples from ET. New Zealand was represented solely by historical samples.

The genotyping success varied significantly between location, sampling time and locus. Interestingly, three of four collection locations that were sampled 25 years previously had similar genotyping success to modern locations. Fish collected 25 years ago from NSW, NZ and ET genotyped at seven to ten loci on average out of 14. Fish from modern locations were genotyped at eight to 12 loci (on average per collection location) out of 14 (Table 6). Samples from Portland collected 25 years ago (Por_h) had poor success with only 2.73 loci on average genotyping per fish. These samples were removed, leaving 432 samples.

Table 6. Numbers of fish genotyped with microsatellite loci per collection location (Figure 1) and per sampling period before and after data pruning. This consisted of the removal of i) samples from two locations (Por_h and WT_h), ii) samples having less than eight loci scored and iii) all data from locus Rsol54 (see text for more detail). The number of samples genotyped per collection location (N) is presented along with the minimum (Min) and maximum (Max) number of loci able to be scored from fish from that location.

	All data				After data pruning			
Collection location	Mean loci per sample	N	Min	Max	Mean loci per sample	N	Min	Max
Modern								
01_GAB	12.35	48	7	14	12.28	40	9	13
02_GAB_w	8.13	16	0	14	12.63	8	11	13
03_KI	12.96	48	8	14	12.17	47	9	13
04_Por	10.91	47	3	14	12.18	33	9	13
05_Por_w	9.93	55	0	13	11.57	44	9	13
06_WBS	9.17	48	1	14	11.85	26	9	13
07_WT	9.57	44	0	14	12.48	27	9	13
10_NSW	10.95	77	2	14	12.05	59	9	13
Historic								
08_WT_h	13.75	4	10	13	Removed	0	0	0
09_ET_h	7.74	19	0	14	12.27	10	10	13
11_NSW_h	10.77	13	2	14	12.33	9	9	13
12_NZ_h	10.62	13	0	14	12.40	11	11	13
13_Por_h	2.73	15	0	14	Removed	0	0	0
Total		447				314		

Of the remaining 432 samples, the genotyping success varied. Some samples yielded data for all 14 loci and some samples had missing data. Three further changes were made to reduce the amount of missing data in the dataset. Another location was removed because of low sample numbers (WT_h, n=4) and one locus was removed (Rsol54), as it could not be scored in samples from Por_w (Table 6). Lastly, individuals were only retained in the final dataset if they had been genotyped at nine or more of the 13 loci.

One locus (Rsol33) had one mutant allele. The sizes of the alleles began at 208 bp and ended at 268 bp in three bp increments, consistent with tri-nucleotide motif of this locus. However, the allele between 229 bp and 235 bp was not 232 bp as expected, but was 233 bp. This is not uncommon for microsatellite loci and the mutant allele was retained.

The number of fish genotyped from GAB in winter was low (8). These samples were added to the GAB sample.

Four pairs of potential duplicate samples were found (Table 7). Two pairs were identified as being similar. Individuals in the pairs differed in allelic composition at one locus. Another similar pair differed by four loci. The fourth pair had identical alleles at 12 loci. One member of this pair (sample 7 002) was removed.

Table 7. Multilocus genotypes of four pairs of samples with similarities determined by the Queller and Goodnight (1989) similarity method. Sample names, population (in brackets) and genotypes are listed. Loci were Rsol11, 19, 22, 27, 29, 30, 31, 33, 57, 62, 67, 68 and 70. Shaded allele pairs differ between samples. Missing alleles are shown as '000'.

First Pair
3160 (WBS) 000 000 154 172 200 203 155 164 197 200 202 208 311 311 000 000 153 153 000 000 166 172 000 000 189 189
3107 (KI) 159 164 000 000 200 203 000 000 000 000 000 000 311 311 223 241 150 153 136 142 166 172 149 149 189 189
Second pair
3483 (NSW) 159 159 000 000 203 203 000 000 194 197 000 000 305 311 214 223 000 000 142 145 166 169 137 146 189 192
6004 (NSW_h)159 159 154 184 203 203 000 000 000 000 208 208 000 000 223 223 153 153 142 142 000 000 137 137 189 189
Third pair
7000 (NZ_h) 159 159 166 172 203 203 152 152 194 200 208 208 311 311 223 233 150 153 142 142 166 166 140 146 189 189
7001 (NZ_h) 159 159 166 172 203 212 000 000 194 200 208 208 311 311 223 233 150 153 142 142 166 166 140 146 189 189
Fourth pair
7002 (NZ) 159 159 172 184 203 206 164 167 194 200 202 211 314 320 211 233 000 000 142 145 166 166 140 146 189 189
7003 (NZ) 159 159 172 184 203 206 164 167 194 200 202 211 314 320 211 233 150 153 142 145 166 166 140 146 189 189

Further information on number of alleles, heterozygosity and fixation indices are provided in Appendix 2.

Tests for conformance to Hardy-Weinberg genotypic proportions and linkage disequilibrium

The null hypothesis of conformance to the Hardy-Weinberg principle (HWP) and linkage disequilibrium (LD) was investigated. There was no evidence of departure from LD at any locus pair across populations (Bonferroni adjusted p-value for an alpha of 0.05 was 0.00064).

Departures from the HWP were detected. After correction for multiple testing, genotypic proportions at some loci were significantly different from expectations in some populations. These were locus Rsol11 in the sample collection from Por, Rsol31 from the GAB, Rsol33 in collections from KI and the GAB, Rsol62 at Por, and Rsol68 in collections from NZ (samples collected 25 years ago), KI and the GAB (Table 8).

In all cases, the departures resulted in a deficit of heterozygotes (Table 8), so explanations such as genotypic frequency differences between the sexes were ruled out as these would result in heterozygote excess (Waples 2014).

The inadvertent scoring of a homozygote instead of a heterozygote can cause heterozygote deficits. This type of scoring can occur when the template quality is poor resulting in low allele peaks that can be overlooked by the software and user. However, there was no discernible relationship between locus-by-population non-conformance to HWP and amount of missing data. The amount of missing data and template quality would be expected to be directly linked, which in turn would drive non-conformance to HWP. For example, for locus Rsol33 there was between 7% and 17% missing data for six population samples, but only two of them were associated with HWP non-conformance. For locus Rsol68, there was no missing data for the two occurrences of non-conformance (population samples NZ_h, KI and GAB), but data was missing from four other population samples (Table 8).

Another explanation for heterozygote deficit is the Wahlund effect, where samples are taken from a population consisting of individuals that are not interbreeding (non-panmictic). Waples (2014) points out that loci with the largest signal of differentiation between populations (F_{ST}) are those where *F*is should be positive, indicating a deficit of heterozygotes. There was no relationship between per-locus *F*is and global F_{ST} for any population (data not shown).

Having ruled out poor quality template and a Wahlund effect, there were few remaining explanations for the observed deficit of heterozygotes. Firstly, there may have been null alleles at one or more loci. If present, however, they would have been at low frequencies or be population-specific to account for their sporadic occurrence across locus-by-population pairs. Also, the expected relationship between the occurrence of null alleles per locus and extent of missing data per locus (as null homozygotes that would be not observed) was not observed.

Another explanation is that sample sizes per locus were insufficient to correctly determine expected genotype frequencies. Even though sample sizes were roughly equal between populations that either conformed or departed from HWP, allelic diversity (a measure of the number of alleles) varied among populations. For example, allele frequencies for loci Rsol33 and Rsol68 differed among populations (Figure 9) and the number of effective alleles for the KI and GAB population samples was larger than the mean (Figure 10). This suggested that higher sample sizes might have been needed for the KI and GAB populations to accurately measure genotypic proportions. The mutant allele (233 bp) in Rsol33 occurred across all populations in similar frequency (approximately 7 to 10%), so it was unlikely to be associated with non-conformance to HWP.

Lastly, the presence of non-conformance in population samples from the southern stock (Por, KI and GAB) could be due to a hidden Wahlund effect associated with unsampled (ghost) populations possibly further to the west of the southern coastline.

As no conclusive explanation could be provided for the non-conformance to HWP for some combinations of loci and populations, genetic tests assuming HWP (*F*-statisitics) were performed with and without loci Rsol33 (two populations out of 10 with departures) and Rsol68 (three population departures).

Table 8. *P*-values less than 0.05 for tests of Hardy-Weinberg proportions in three Gemfish populations sampled 25 years ago (historical) and seven populations sampled for this project (modern). Those tests that were significant after accounting for simultaneous testing (per population) using Bonferroni correction are in bold (alpha=0.05/13=0.0038) and *F*is [(mean expected heterozygosity - mean observed heterozygosity) / mean expected heterozygosity] is given. Where there was missing data, the amount (%) is shown.

	Historical Populations			Modern Populations							
	1	2	3	1	2	3	4	5	6	7	
	NSW_h	NZ_h	ET_h	NSW	WT	WBS	Por	Por_w	KI	GAB+w	
Locus	n=9	n=11	n=10	n=59	n=27	n=26	n=33	n=44	n=47	n=48	Populations with departures
Rsol11	-	-	-	0.0204 (20%)	-	- (7%)	**0.0030 (6%)**	-	- (8%)	0.0326 (12%)	1
Rsol19	-	-	-	- (8%)	-	-	- (9%)	- (9%)	- (8%)	-	0
Rsol22	-	-	-	-	-	-	-	0.0347	-	- (6%)	0
Rsol27	- (11%)	- (20%)	- (18%)	- (11%)	0.0410	- (11%)	- (9%)	- (31%)	- (10%)	0.0118	0
Rsol29	- (11%)	-	- (18%)	-	-	-	-	- (9%)	- (8%)	-	0
Rsol30	-	-	-	- (10%)	-	-	- (6%)	-	- (12%)	-	0
Rsol31	- (11%)	0.0122	- (18%)	-	- (7%)	- (7%)	0.0052	0.0155 (20%)	- (6%)	**0.0030**	1
Rsol33	0.0306 (11%)	-	-	-	- (7%)	- (19%)	- (6%)	0.0137	**0.0001 (8%)**	**0.0007 (18%)**	2
Rsol57	-	- (40%)	-	0.025	- (25%)	- (11%)	-	0.0350 (22%)	-	-	0
Rsol62	-	-	-	-	-	- (11%)	**0.0010 (12%)**	- (15%)	-	-	1
Rsol67	- (11%)	-	-	-	-	-	- (12%)	- (6%)	-	0.0055 (6%)	0
Rsol68	-	**0.0022**	0.0293	- (13%)	-	- (19%)	- (15%)	- (13%)	**0.0001**	**0.0002**	3
Rsol70	- (11%)	-	NA (18%)	- (8%)	-	- (26%)	-	-	- (6%)	- (10%)	0
Loci with departures	0	1	0	0	0	0	2	0	2	3	
% Loci with departures	0.0	7.7	0.0	0.0	0.0	0.0	15.4	0.0	15.4	23.1	
*F*is (Het Deficit)	-	0.1064	-	-	-	-	0.0531, 0.4633	-	0.2761, 0.3645	0.2562, 0.2307, 0.3818	
*F*is (Het Excess)	-	-	-	-	-	-	-	-	-	-	

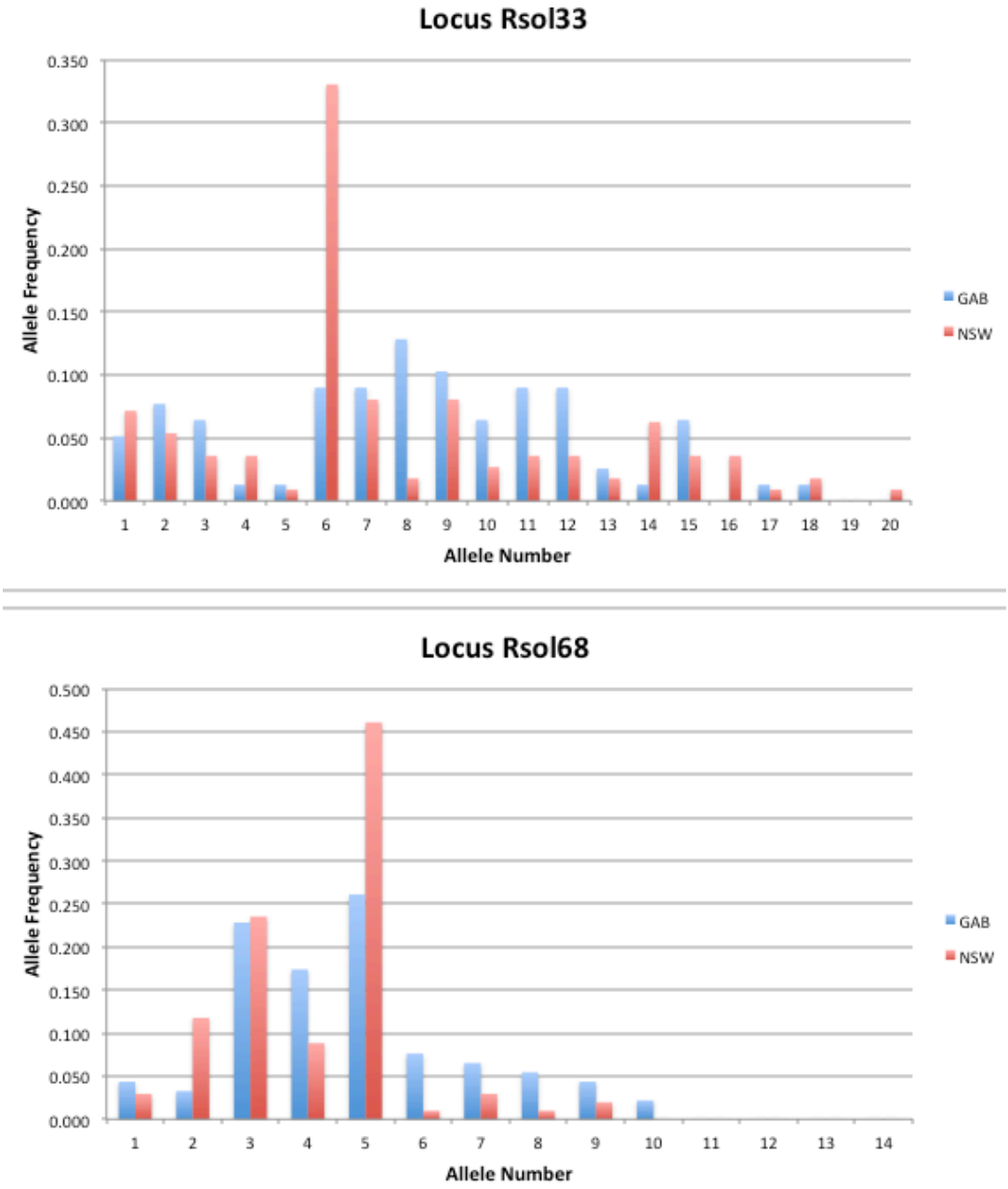

Figure 9. Allele frequency distribution for two loci genotyped in Gemfish sampled from the Great Australian Bight (GAB) and New South Wales (NSW). There were 20 alleles genotyped among all Gemfish sampled during this project for Locus Rsol-33, and 14 alleles among all Gemfish for Locus Rsol-68.

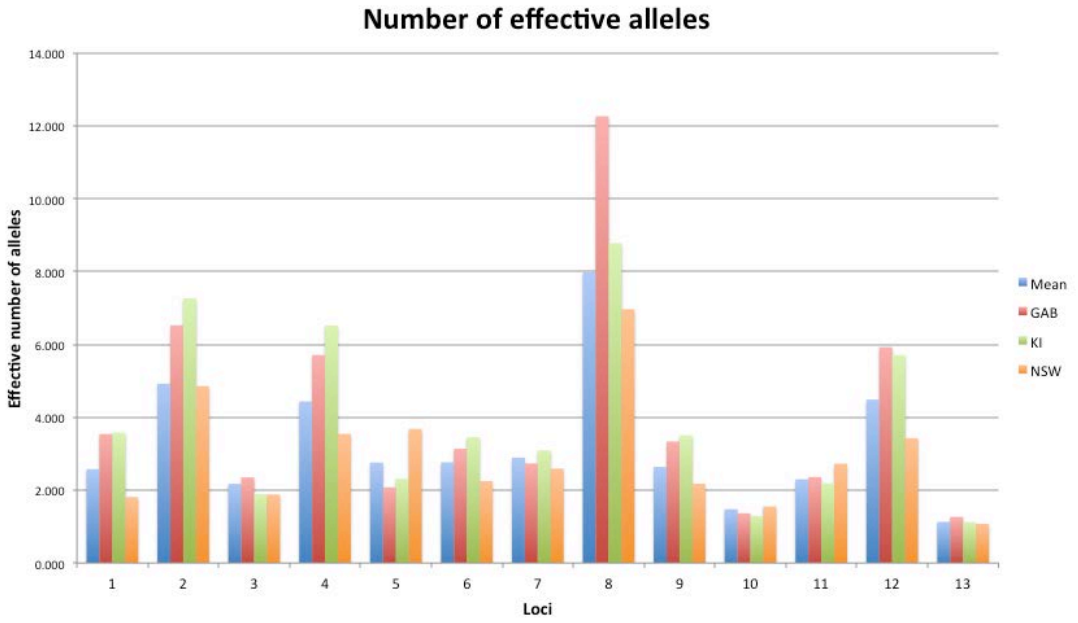

Figure 10. The number of effective alleles at several loci (locus 2, Rsol-19; locus 4, Rsol-27; locus 8, Rsol-33; locus 9, Rsol-57; locus 12, Rsol 68) is higher than the mean for Gemfish sampled from the Great Australian Bight (GAB) and Kangaroo Island (KI), and less than the mean for Gemfish sampled from New South Wales (NSW).

The Gemfish microsatellite genotype dataset is available at UQ eSpace (http://espace.library.uq.edu.au/view/UQ:385440).

Genetic diversity and genetic affective population size from microsatellite genotypes

The genetic diversity per population, as measured by the effective number of alleles, was low in the NSW sample and higher in the samples from the western populations. The number of effective alleles rose for several loci (RSol33, Rsol19, Rsol27, Rsol68, Rsol57 and Rsol31), while the number of effective alleles for other loci was static (Figure 11).

The genetic effective population size (N_e, estimated using the linkage disequilibrium method) of the eastern stock was less than that of the western stock. In this analysis, the eastern population was represented by microsatellite genotypes from NSW and WT, and the western population was represented by genotypes from Por, KI and GAB. The N_e for the eastern population was estimated to be 613; the equivalent estimate for the western population was 6406. These estimates were made using alleles whose frequency exceeded 0.05 (P_{crit} = 0.05). The upper 95% parametric confident limit was unable to be estimated for either population. The lower 95% confidence limit was 166 for the eastern population, and 563 for the western population.

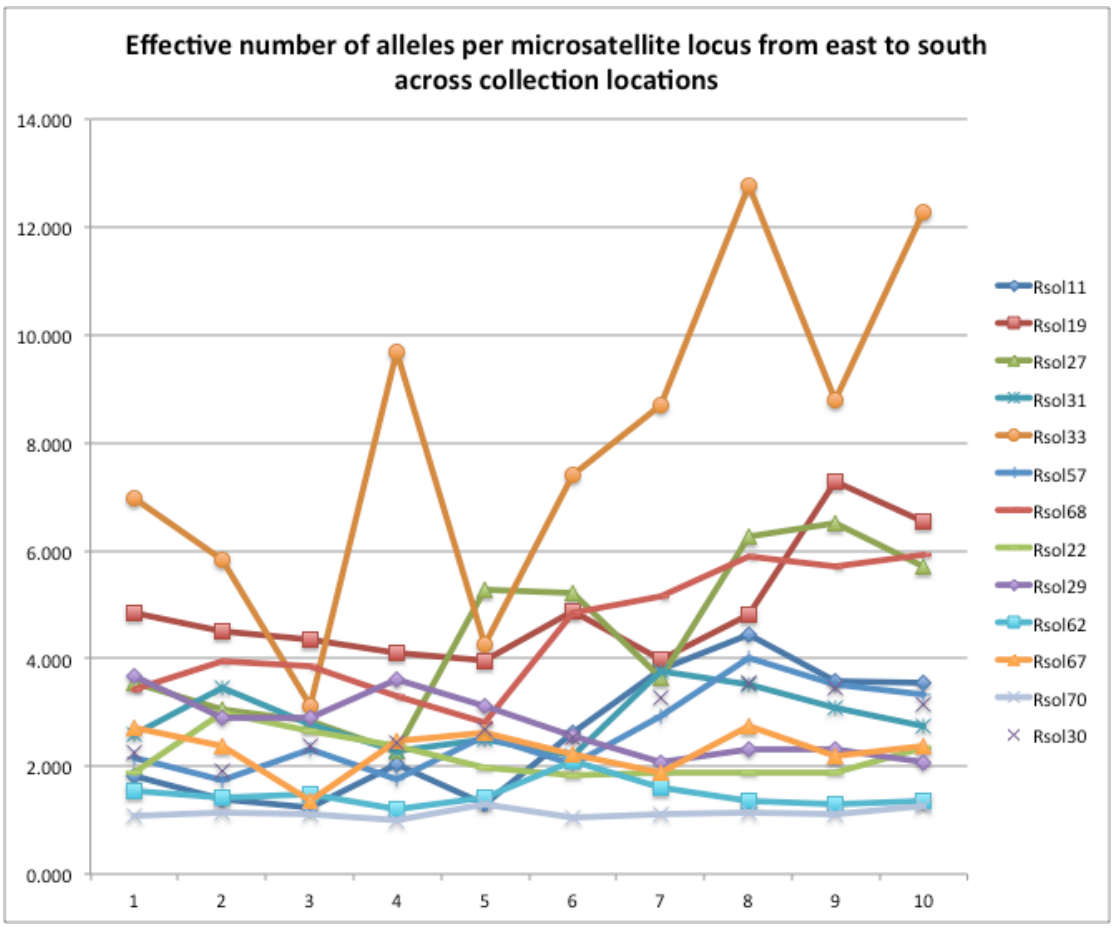

Figure 11. The number of effective alleles increased at some loci from east to south collection locations (1. NSW, 2. NSW_h, 3. NZ_h, 4. ET_h, 5. WT, 6. WBS, 7. Por, 8. Por_w, 9. KI, 10. GAB)

Population genetic structure from microsatellite genotypes

F-statistics

Overall, the microsatellite F_{ST}'s provided evidence of a single western stock from Portland (Vic) to the western Great Australian Bight. The three locations from which samples were analysed within this region (GAB, KI and Por) were not significantly different. There were also no significant differences between samples taken in summer (Por) and winter (Por_w) at Portland (Appendix 3).

Likewise, the existence of an eastern stock of Gemfish was confirmed. The eastern stock encompassed samples from the east coast of NSW (NSW, NSW_h), the east coast of Tasmania (ET_h), the west coast of Tasmania (WT) and New Zealand (NZ_h).

The F_{ST}'s between the eastern and western stocks ranged from 0.03113 to 0.11031 across population pairs from each stock.

The population sampled in Western Bass Strait was significantly distinct from the eastern and the western stocks. It differed from the western stock by F_{ST}'s ranging from 0.01842 to 0.02851. It differed from four of the five populations sampled in the eastern stock (F_{ST} from 0.01046 to 0.0415). It was not significantly different from the NSW population sampled 25 years ago.

The pattern of genetic differentiation among stocks and populations was similar when two loci that showed evidence of non-conformance to HWP (Rsol33 and Rsol68) were removed. The magnitude of F_{ST}'s differed slightly (Appendix 3).

STRUCTURE – A Bayesian approach

The delta-K method (Evanno *et al.* 2005) gave no clear indication of the appropriate number of groups (Appendix 4). STRUCTURE results were visualised with k was set to 2 to correspond to the expected number of major genetic groups (eastern and western stocks). Results for other values of k (3, 4 and 5) are presented in Appendix 5.

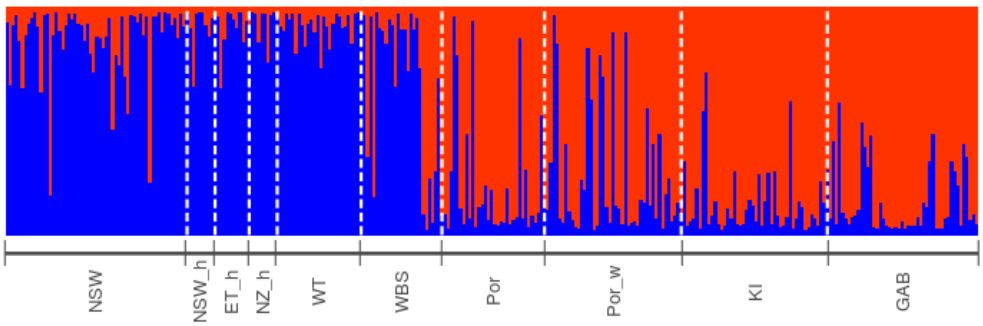

Figure 12. STRUCTURE box-plot showing membership of 313 individuals to two groups (blue, red). Collection locations are labelled and abbreviations are explained in the text.

The membership of individuals to groups (k=2) in the STRUCTURE box plot (Figure 12) matched the likely stock structure of the fishery. Individuals collected from NSW during this study (NSW) and the previous study (NSW_h) as well as individuals from NZ and ET in the previous study (NZ_h , ET_h), plus individuals from WT were largely assigned to the same group (blue, Figure 12). The blue group corresponded to the eastern stock. Likewise, individuals from Portland, collected both in summer (Por) and winter (Por_w), KI and the GAB were largely assigned to the red group, which corresponded to the western stock.

Individuals from one location (WBS) appeared likely to belong either to the red or blue groups. There were individuals that were primarily 'blue' and other individuals that were primarily 'red'. This finding suggested that WBS might be a location where eastern and western stocks mix (see below).

Scattered across the boxplot (Figure 12) there were other examples of outlying individuals. Amongst the blue, or eastern group, there were some individuals that were primarily red. The majority of these appeared in the NSW collection. Likewise, in the red, or western group, some individuals were primarily blue. These outliers were subsequently highlighted by the assignment analyses (see below).

DAPC clustering using microsatellite genotypes

Individuals sampled during 2013 and 2014 from seven collection locations were grouped, based on their 13-locus microsatellite genotypes, into two main clusters by DAPC (Figure 13). One cluster consisted of samples from NSW and WT, while the second cluster consisted of samples from KI, GAB and Por (sampled in winter and summer). The ellipses surrounding the clusters were adjacent, but not overlapping.

Samples from the WBS location overlapped with the two main clusters, possibly reflecting the population's admixed state.

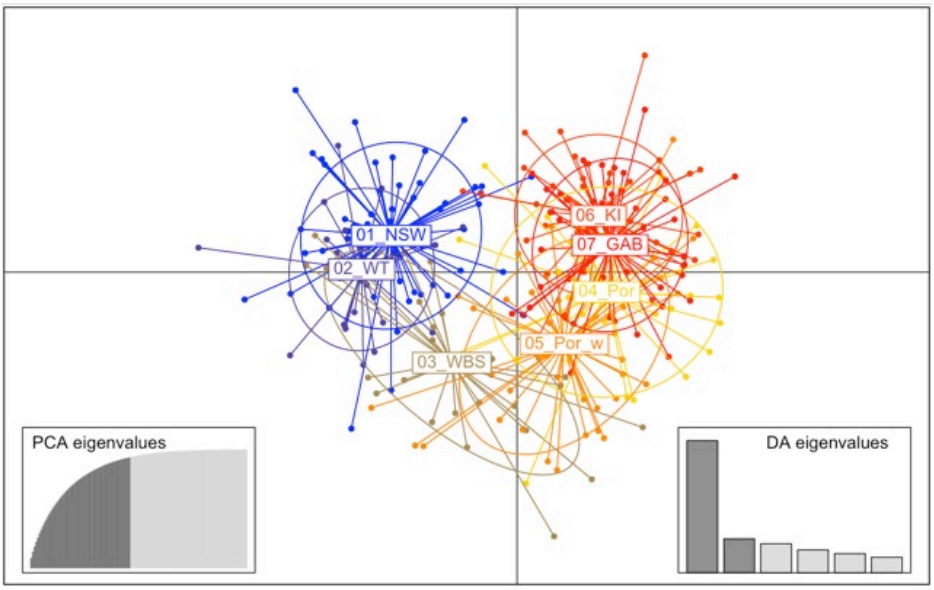

Figure 13. DAPC scatterplot from 13-locus microsatellite genotypes showing samples clustered by collection location (in colour). Location abbreviations are described in Figure 3.

Assignment analysis

As the genetic difference between the eastern and western stocks was pronounced, fish could be tested to determine if it was correctly assigned to the stock from which it was sampled; if not, it was regarded as an immigrant.

Of the 115 fish genotyped from the eastern stock, four fish collected from the eastern stock were genetically assigned to the western stock (Table 9) using allele frequency-based assignment tests (Paetkau *et al.* 1995). Likewise, of the 172 fish genotyped from the west 12 were assigned to the eastern stock. The Q-values (greater than 0.7 or less than 0.3) of the majority of these fish suggested that their nuclear genomes were not admixed, and thus were true (or direct) migrants. All 12 migrants found in the western stock were direct migrants, including fish 3005 and 3006 that were confirmed as migrants based on SNP data (see below, Fig. 16). There were two direct migrants in the eastern stock. The Q-values of the remaining two were low (0.69/0.31, 0.65/0.36), suggesting that they were offspring of migrants (ie. hybrids between eastern and western stock fish).

Table 9. A summary of assignment analyses for the Western and Eastern Gemfish stocks. For collection locations other than WBS, the proportion of migrants is shown (for an explanation of direct and descendant migrant, see text) per stock. The number of fish from each stock in the admixed WBS (Western Bass Strait) collection location is given.

	Western		Eastern	
	N	%	N	%
All collection locations, excluding WBS				
Number of members	148	*86.0*	109	*94.8*
Number of direct migrants	12	*7.0*	2	*1.7*
Number of migrant-descendants	0	*0.0*	2	*1.7*
Number of hybrids	12	*7.0*	2	*1.7*
Total	172		115	
WBS collection location				
Number of fish	7	*87.5*	16	*88.9*
Number of hybrids	1	*12.5*	2	*11.1*
Total	8		18	

The sex ratio of the outliers (direct migrants, migrant descendants and hybrids) in the eastern stock was even (three males, two females and one unknown), and sizes ranged from 41 to 74 cm. They were collected in August 2014 and no reproductive data was collected.

In the western stock, there were 16 outliers at Por (20% of fish sampled), three outliers at KI (6%) and five in the GAB (10%). Of these, 10 were female and nine were male. The gender and reproductive status of three was not recorded. The females ranged from 48 to 100 cm (total length) and their ovaries ranged from undeveloped (stage 2, four females) to developed (stages 3 and 4, six females. The males ranged from 41 to 86 cm (total length).

Admixture analysis

The WBS location was not included in the assignment analyses presented above as this population was hypothesized to be an admixture of eastern and western individuals following the population structure analyses.

Frequency-based assignment tests and Q-value analyses were consistent with this hypothesis. There were 18 (69%) individuals assigned to the eastern stock, and eight (31%) individuals assigned to the western stock (Table 9).

The females of eastern stock origin in WBS ranged from 44 to 66 cm and had stage 2 and 3 ovaries. The western females in WBS were smaller (35 to 39 cm) than eastern females and had stage 1 ovaries, with one fish having stage 3 ovaries. Males of both origins were similar lengths; eastern 45 to 73 cm and western 38 and 52 cm.

Fish collected from WBS were captured in a single trawl shot (R. Wick pers. comm.) ruling out the likelihood that the admixed state of this sample was due to the mixing of eastern and western fish captured at differing locations and times.

SNP genotyping

Summary statistics

Each sample of genomic DNA processed by DArT received on average 1.25 million sequence reads.

After quality control processing by the DArT bioinformatics pipeline, the total number of SNP loci returned was 10 029. The DArT quality control process reduced the number of fish in the dataset from 92 to 76.

About half of the SNP had missing data in more than 5% of 72 fish (5 592 SNP). A conservative approach was taken and these were removed. SNP loci that were sequenced less than 20 times (read depth, coverage) were removed (1 006 SNP). Likewise, SNP loci that were sequenced more than 80 times were removed (894 SNP) as these may have represented loci that were duplicated within the genome (paralogs, pseudogenes). In total, 7 462 SNP were removed. The number retained was 2 567 SNP.

This set of SNP included SNP loci that were conserved across the six teleost species for which genome resources exist (dataset HGLI).

Of these, 47 loci were homologous to regions in known teleost genomes (47 SNP loci only, dataset HGLO).

A third dataset was constructed (HGLE) where these 47 SNP were removed (2,520 SNP).

SNP loci have two alleles (bi-allelic). For example, they could have nucleotides C and T. A homozygote fish can have two copies of C (‘CC’) or T (‘TT’). A heterozygote fish is ‘CT’. The frequencies of least frequent (or minor) allele ranged from 0.01 to 0.5 (Figure 14) in the HGLI dataset.

The frequencies of the 47 SNP with homologies to other teleost genome sequences (HGLE) were similar (Figure 15).

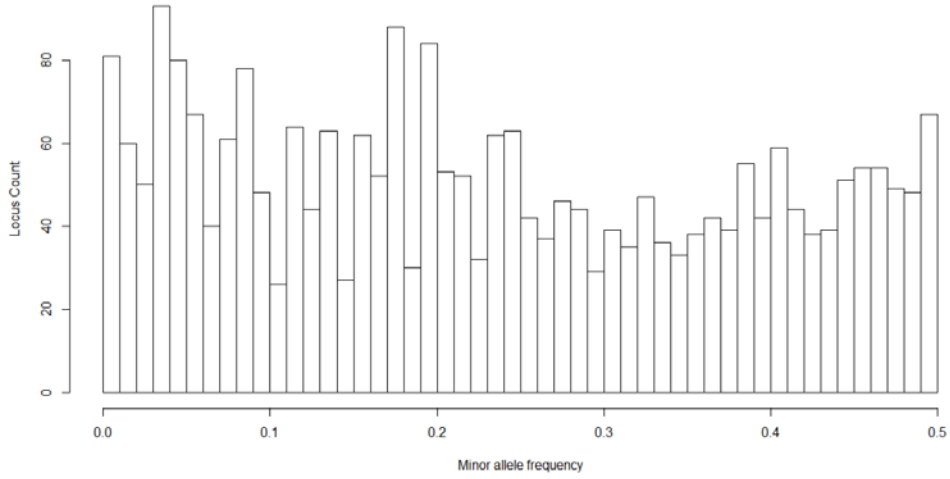

Figure 14. Bar plot of minor allele frequency of the 2,567 SNP in the HGLI dataset.

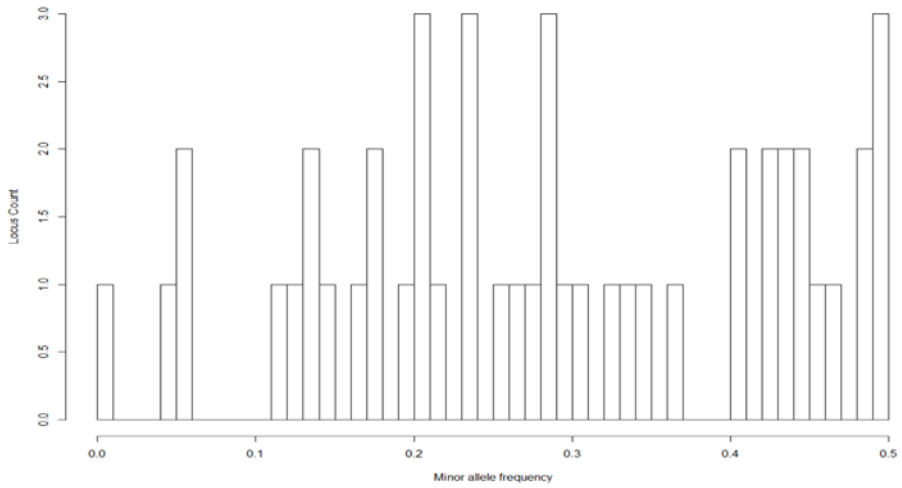

Figure 15. Bar plot of minor allele frequency of the 47 SNP in the HGLE dataset.

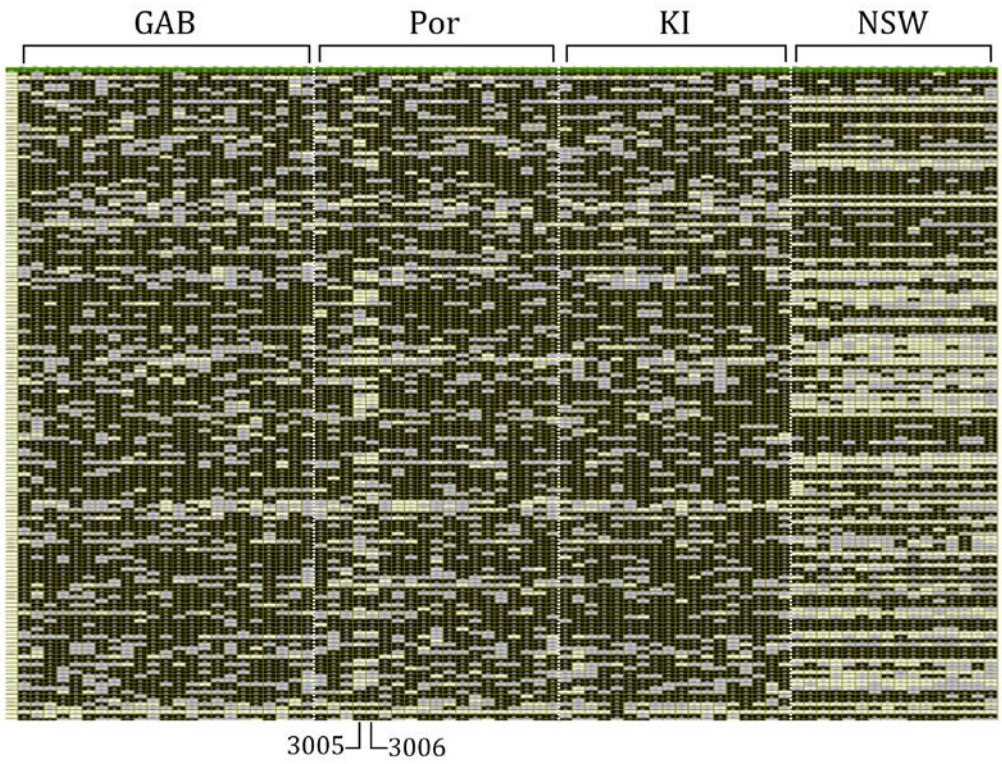

Figure 16. 'Zoomed-out' screen capture image of an excel spreadsheet containing data from an arbitrary 411 SNP loci (horizontal) used to genotype 76 Gemfish from four collection locations (vertical; GAB, n=23; Por, n=15; KI, n=22; NSW, n=16). Black squares represent 0101 homozygotes. White squares represent 0202 homozygotes and grey squares represent heterozygotes (0102). Two individuals (3005, 3006) from Portland (Por) have SNP genotypes matching fish from NSW.

F_{ST} and DAPC analyses of SNP data

A 'zoomed-out' view of the spreadsheet containing the SNP genotypes showed clear differences between fish from NSW compared to other locations (Figure 16).

The SNP genotypes of two fish from Portland matched those of NSW (Figure 16). These fish were identified as migrants from the eastern stock on the basis of their microsatellite genotypes, having Q-values of 0.956 (#3005) and 0.786 (#3006).

The amount of population genetic subdivision among the three western populations was slight for the 2,567 SNP dataset. F_{ST} between samples collected from two closely-spaced locations (Por and KI) was not significant. There was little, if any, evidence for a genetic separation between the most distant location (GAB) and Por and KI (Table 10A).

However, the F_{ST} between the western (GAB, KI and Por) and the eastern (NSW) collection locations was large (0.1878 to 0.2235 depending on populations being compared).

The pattern and magnitude of F_{ST} for the 47 SNP dataset (containing only loci that were flagged as homologous to regions in other teleost genomes) was similar. The 95% confidence intervals were broad as would be expected for a more constrained set of data.

Table 10. Weir and Cockeram's population-pairwise F_{ST} (below diagonal) based on 2 567 SNP in the HGLI dataset (A) and 47 putative 'adaptive' SNP in the HGLO dataset (B). Values above diagonal are 95% confidence limits, and * indicates statistically significant F_{ST}.

A			1	2	3	4
			NSW	Por	KI	GAB
1	NSW	(n=16)	-	0.1774 - 0.1977	0.2122 - 0.2336	0.2133 - 0.2332
2	Por	(n=15)	0.1878*	-	-0.0015 - 0.0046	-0.0005 - 0.0055
3	KI	(n=22)	0.2229*	0.0016	-	0.0012 - 0.0062
4	GAB	(n=23)	0.2235*	0.0025	0.0038*	-

B			1	2	3	4
			NSW	Por	KI	GAB
1	NSW	(n=16)	-	0.0422 - 0.6256	0.0937 - 0.7010	0.1342 - 0.7060
2	Por	(n=15)	0.1759*	-	-0.6931 - 0.0120	-0.1904 - 0.4866
3	KI	(n=22)	0.1951*	0.0013	-	NA
4	GAB	(n=23)	0.2233*	0.0021	0.0019	-

Cluster plots of the discriminant analyses of principal components (DAPC) reinforced the pattern revealed by F_{ST} between populations (Figure 17). For the large SNP dataset (2,657 loci), NSW samples were clustered away from samples from KI, Por and GAB. Two samples from Por (3005 and 3006) had clear affinities with the NSW cluster. These samples had previously been identified from microsatellite genotypes as immigrants into Por from NSW.

The same pattern of clusters was revealed with the 47 SNP locus dataset, but with less distinction between ellipses (Figure 18).

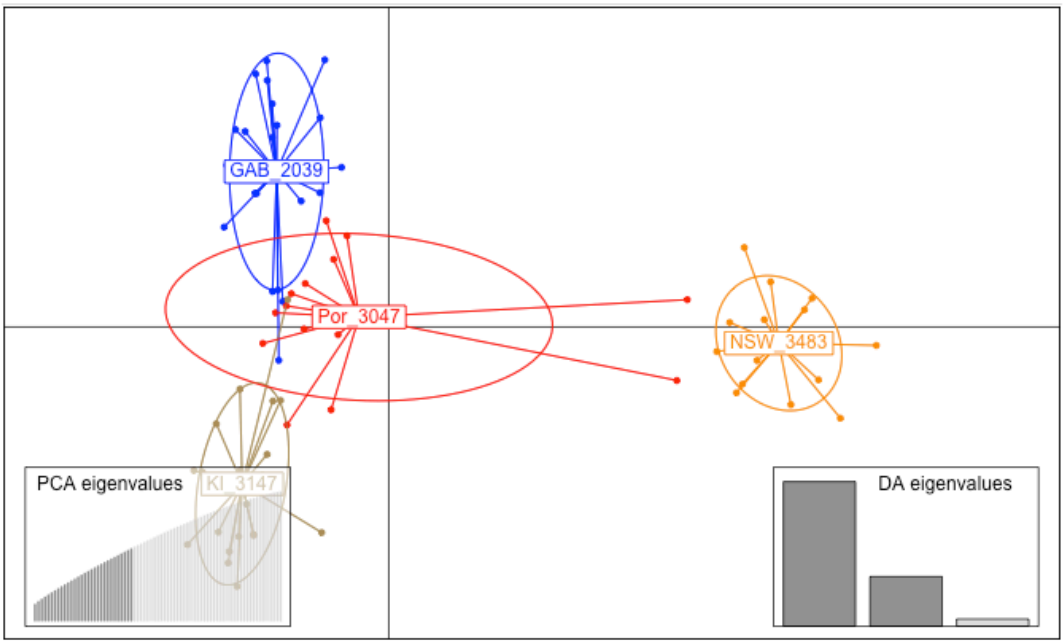

Figure 17. DAPC scatterplot of Gemfish from four locations based on 2 567 SNP loci. Cross validation was used to determine the optimal number of PCs, which in this case was 30.

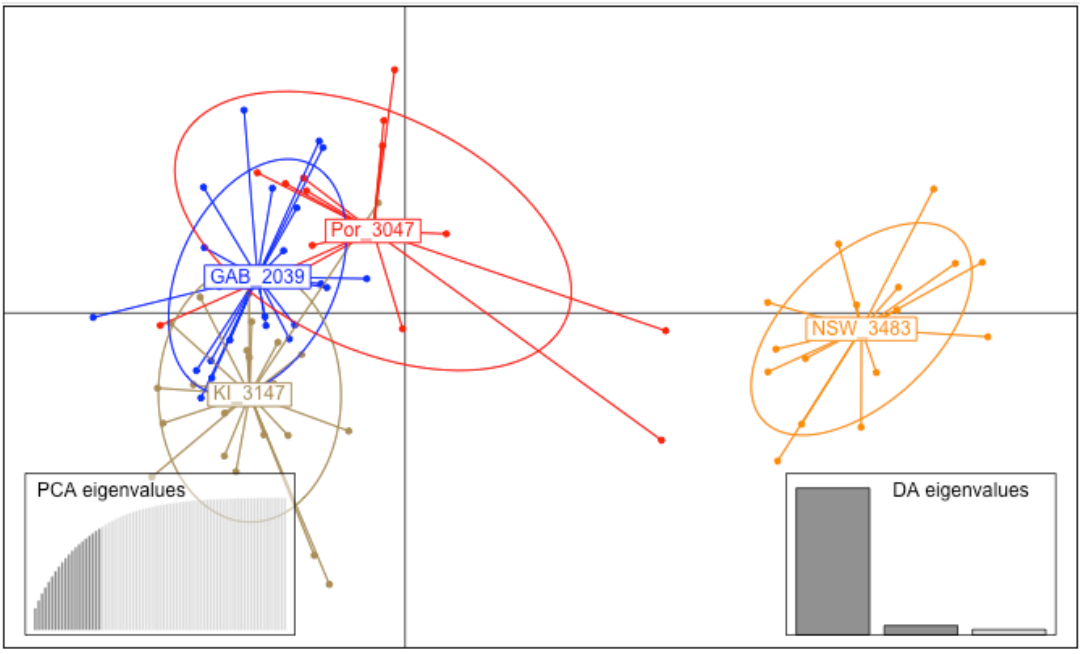

Figure 18. DAPC scatterplot of Gemfish from four locations based on 47 SNP loci. Cross validation was used to determine the optimal number of PCs, which in this case was 20.

Discussion

The objective of this project was to determine the spatial extent and genetic homogeneity of the western stock of Gemfish in southern Australia to assist with the sustainable management of the species. Samples for genetic analysis were taken from five locations in the western zone: from western Great Australian Bight, along the coasts of South Australia and Victoria, western Bass Strait and off the west coast of Tasmania. To confirm separation from the eastern stock, samples were also taken from the NSW coast. Samples from the Australian Museum collected 20 to 23 years prior to this study by Colgan and Paxton (1997) were included to provide extra sampling locations. Nuclear (microsatellite and SNP) and mitochondrial DNA (control region sequence) genetic markers were used to investigate genetic patterns within and between Gemfish population samples.

Despite best efforts in the field and laboratory, the quality of fin clips for genetic marker analysis was poor. Colgan and Paxton (1997) also noted degradation of tissue during their genetic study on Gemfish. In the current project there was no difference between the qualities of samples taken at sea (for example from western Bass Strait and western Tasmania), compared to the remainder that were sampled in ports suggesting that tissue (and the DNA contained within) from this species degrades rapidly. Poor quality genomic DNA reduced the length of the sequence that could be reliably obtained across samples. However, the sequence was derived from the mtDNA control region that was highly variable in Gemfish, as it is in the majority of finfish. The short sequence contained 12 SNP, which defined seven haplotypes that were subsequently shown to discriminate between the western and eastern stocks. A larger number of SNP from a longer region may have defined a large number of haplotypes, but this may have not been as useful because, by chance, some of the haplotypes may be found only once. This may have presented a problem for the sampling regime used here, as singleton haplotypes may have been found exclusively in one population or the other, and thus providing little if any information about genetic relatedness between stocks. Several rounds of pruning was needed for the microsatellite genotype data, and this was a consequence of poor quality DNA. For the SNP analysis, some DNA samples did not pass quality control, but they were replaced. Overall, the poor quality DNA did not affect the usefulness of the genetic data to achieve the project objectives, but in future molecular projects on this species it would be ideal if the problem could be addressed.

Reproductive condition was recorded for the majority of individuals sampled from the western stock to test the hypothesis that spawning occurs in the west, potentially towards the adjacent to offshore canyons off the western end of GAB or off KI in summer as reported by industry. Data collected was consistent with this hypothesis: females from locations in the west (GAB and KI) sampled during summer had more advanced ovarian development than easterly locations. Also, females sampled from the west (GAB) during winter had less ovarian development than summer samples from that location (Fig. 6). However, sample sizes per location for reproductive condition were small and some locations were not sampled during winter due to a lack of fishing activity (example KI, Por, WBS and WT), so the results obtained here are not conclusive. Future studies are needed to investigate the spatio-temporal details of this spawning migration, including the actual spawning locations.

By comparison to samples taken from the eastern Australian seaboard, the presence of a genetically distinct stock in the west was confirmed. Data from mtDNA, microsatellites and SNP supported this conclusion. Two mtDNA haplotypes were diagnostic of the west and east stock and depending on the populations within stocks that were being compared, F_{ST}'s from microsatellite genotypes (0.031 to 0.110) and SNP (0.19 to 0.22) between west and east were statistically significant. Furthermore, there was no evidence of additional stock subdivisions

within the western stock. The genetic methods used had a high sensitivity and were likely to detect stock subdivision if it were present amongst the western samples taken. SNP markers provided information on a large number of loci across the entire nuclear genome and a formal power analysis of the microsatellite loci showed they would have been able to detect very low F_{ST} (around 0.005). In combination with information from the mitochondrial genome, the genetic data presented here is evidence for a single interbreeding western stock of Gemfish.

The study determined the physical location of the boundary between the western and eastern Gemfish stocks. The western stock extends from the western Great Australian Bight eastward through South Australia to southwestern coastline of Victoria. Individuals sampled from the western coast of Tasmania belonged to the eastern stock. When the western coast of Tasmania was sampled in late summer (April), females were in an advanced reproductive state (majority in stage 3) and some were spent (Fig. 6) suggesting reproductive synchrony with the eastern rather than the western stock. Samples from western Bass Strait were an admixture of both eastern and western stocks. Therefore, the boundary between the stocks occurs in the western Bass Strait area.

The broad spatial extent of the eastern stock as proposed by Colgan and Paxton (1997) was confirmed here by *de novo* genetic analysis of museum samples from eastern Tasmania and New Zealand. Within the eastern stock, nine of 13 microsatellite loci showed a general trend of lower numbers of effective alleles compared to the western stock (Figs. 10 and 11). As large populations are more likely to have higher numbers of effective alleles, this genetic contrast between eastern and western stocks suggests that genotypes may hold information about population size. Indeed, the estimates of genetic population size from this study were an order of magnitude larger in the western stock (6 406; lower 95% CI, 563; upper 95% C, unable to be estimated) compared to the eastern stock (613; lower 95% CI, 166; upper 95% C, unable to be estimated). The effective population size for both eastern and western Gemfish is expected to be large. It is unclear why the effective population size is small in the east. There may be factors that are limiting the genetic success of eastern Gemfish, which could be examined in future studies.

Modelling of genetic lineages over generations in iteroparous species is now possible (Blower, Riginos and Ovenden, in prep) for species with tractable life histories. If the modelling was extended to finfish species, this technique could be applied to Gemfish populations. The expectation would be that genetic effective sizes would approximate spawner numbers from conventional fisheries stock assessment models. This method would be independent from fishery specific issues, and may provide insights into why the eastern stock of Gemfish is not recovering.

The genetic difference between the western and eastern Gemfish stock was large, ranging from F_{ST} 0.03 to F_{ST} 0.11 across population pairs. This is counterintuitive considering Gemfish have a contiguous distribution throughout their range with no physical barrier to the movement of life history stages. Paxton and Colgan (1993) found no evidence for morphometric differences between them supporting a single species status for both stocks. Broderick *et al.* (2011) reported a similarly large microsatellite F_{ST} (0.1) between grey mackerel (*Scomberomorus semifasciatus*) stocks in northern Australia, but this coincided with a discontinuity in species distribution around the Kimberley region. The extent of mtDNA difference between Gemfish stocks was significantly higher than for grey mackerel. MtDNA *phi*-sts from the study by Broderick *et al.* (2011) peaked at 0.4, whereas the Gemfish *phi*-sts between stocks were 0.9 and higher with no shared haplotypes between stocks.

Spatio-temporal differences in spawning behaviour could explain the large genetic divergence observed between stocks. Previous studies (Rowling 1999; Prince and Griffin 2001) have shown that adults from the eastern stock move northwards in autumn into the

prevailing southward flowing Eastern Australian Current (Fig. 19). Spawning occurs in aggregations off northern New South Wales in winter. Eastern stock larvae are likely to be advected southwards to adult habitat by the current flow, followed by the return of post-spawning eastern stock adults to feeding grounds in the south. Western stock adults are likely to have similar behaviour but appear to have very different timing. The western stock of Gemfish are thought to move westwards in spring (Chambers et al. 2014) into the eastwardly flowing Leeuwin current (Fig 19) and this hypothesis is supported by data collected in this study. Spawning of western adults appears to occur during summer in the westward extent of the Great Australian Bight and potentially off Kangaroo Island, followed by the presumed advection of larvae and subsequent return of adults to eastern parts of the western stock. In this scenario stocks spawn up to six months apart towards the opposite ends of the species' distribution. The genetic difference between the western and eastern stock may therefore be maintained by a combination of spatial and temporal separation during spawning. Contranatant movement associated with reproduction is common in fish; for example, on the eastern Australian coastline the mullet (*Mugil cephalus*) moves northwards into prevailing currents prior to spawning activity in the surf zone on ocean beaches Kruck et al. (submitted).

Figure 19. Schematic illustration of the larger-scale oceanographic features in the region surrounding southern Australia. Orange arrows indicate surface currents and green arrows indicate subsurface currents. Original figure from Bulman et al (2008).

Western Bass Strait lies within the zone of overlap first proposed for Gemfish by Colgan and Paxton (1997). In the current project, a zone of overlap was confirmed. Individuals from western Bass Strait had the mtDNA haplotype CRH01 that was diagnostic for the eastern stock, combined with fish that contained the mtDNA haplotype CRH03 diagnostic for the western stock (Table 4). The occurrence of overlap or admixture of stocks in Western Bass Strait was strongly supported by frequency-based assignment tests that placed individuals in either the western or eastern stock (Table 9). The zone of admixture was narrow, as it did not extend to the population samples off the western coast of Tasmania, about 200 km to the south, or to the coast off Portland in Victoria, about 200 km to the northwest. This study was not able to test if this overlap zone is stable through time.

The discovery of a genetically admixed population is a first for Australian finfish species. Admixture is a common phenomenon for fisheries species in the northern hemisphere, particularly salmonid species. Pacific salmon in the North Atlantic Ocean consist of numerous genetic stocks, where each stock is associated with recruit production from a spawning population in freshwater habitat on the western coast of the United States. Pacific salmon returning after feeding and growth in the marine habitat to spawn in freshwater are an admixed population. A great deal of effort is expended by fisheries authorities to manage the

harvest of marine admixed stocks to preserve spawning populations, some of which may be less numerous or more under threat than others (Shaklee et al. 1999). The management boundary between the eastern and western stock could be moved north to the west of King Island, which would align with the boundary between zone 50 and 60 of the Southern and Eastern Scalefish and Shark Fishery statistical reporting zones.

The admixture observed in Gemfish is a result of overlapping or abutting stocks at the convergence of both stocks. If this admixture led to full interbreeding between the western and eastern stock, the genetic difference observed here between stocks would not be maintained. Individuals from the admixed zone most likely follow the spatial and temporal pattern of reproduction that is characteristic of the stock to which they belong. Leading up to winter, eastern stock adults from western Bass Strait and western coast of Tasmania are likely migrate with the majority of the eastern stock to the northern coast of New South Wales. As adult Gemfish do not occupy the shallow waters of Bass Strait, the eastern stock adults may undertake an extra leg of the journey; consisting of a movement southwards then eastwards around the southern coast of Tasmania, before turning northwards. Likewise, western stock adults in the admixed zone in western Bass Strait would move towards the west leading up to summer along with the bulk of individuals in the western stock. In this way, reproductive isolation and hence genetic differences between Gemfish stocks are maintained.

Although this seems to be a feasible mechanism for the maintenance of genetic divergence between the eastern and western stocks despite admixture at the stock boundary, Gemfish immigrants were detected suggesting that straying and subsequent interbreeding does occur (Table 9). Locations in the western stock that were further away from the boundary between stocks (Kangaroo Island, 6% of fish; Great Australian Bight, 10%) had fewer migrants than Portland (20% of fish sampled) that was closer to the boundary of Western Bass Strait. It is unusual to be able to detect migrants in natural populations using genetic methods as migration is normally followed by successful participation in spawning in the adopted population. Descendants of immigrants quickly become undetectable as their genotypes become more similar to local individuals. There have been some cases, however, where genetic methods have been used to detect migrants; in bears, for example, where migration did not lead to successful participation in reproduction (Paetkau et al. 1998) and likewise, genetic studies have detected straying of individuals among Pacific salmon spawning populations (Gilk et al. 2004).

A major question remains unanswered; how is the genetic distinctiveness between the eastern and western stock maintained despite migration and interbreeding? Above, it was shown that admixture of the stocks may not lead to homogeneity if interbreeding was prevented by differential spawning times and locations for each stock. But, the amount of immigration reported here between the stocks would rapidly erode genetic distinctiveness. The most number of migrants per generation that could be exchanged per generation while maintaining the Gemfish F_{ST} between stocks (about 0.1 across population pairs) is two to three (see formula in Ovenden, 2013). The number of Gemfish immigrants is an order of magnitude above this.

The answer is that the fitness of the immigrants is most likely lower than local fish. If so, they may not pass their genes into the local stock, and thus genetic distinctiveness would be maintained between stocks. For example, hybrids between eastern and western fish may be unviable or infertile, but this is unlikely as gemfish are a single (albeit genetically diverse) species. Hybridisation between brook trout (*Salvelinus fontinalis*) and bull trout (*S. confluentus*) was found to produce largely F_1 offspring (97 per cent), but F_2's were not detected, suggesting that there was an isolating mechanism preventing backcrosses with either species (Leary et al. 1993). In some cases the introgression of genetic material is unidirectional. Hybridisation between rainbow trout (*Onorhynchus mykiss*) and Apache trout

(*O. gilae apache*) resulted in introgression from rainbow trout to Apache trout but not the reverse (Dowling & Childs 1992). Another explanation is that gemfish immigrants are less able to successfully participate in spawning compared to local fish. This is feasible given the spatio-temporal differences in spawning behaviour of the western and eastern stocks. Migrants from the east that find themselves in the western stock may lack the responses to environmental cues needed to move westwards during summer for spawning. Likewise, migrants from the west that appear in the eastern stock may be less able than local fish to move northwards in the winter to spawn. If migrants lack these behavioural or physiological responses, they would be less able to reproduce and hence their fitness would be lower than local fish. The level of genetic separation between eastern and western stocks indicates that there is very little or no successful backcrossing between first generation (F_1) hybrids and either parental stock, therefore preventing the introgression of genetic material between populations and reinforcing the stock boundary.

Conclusion

The aim of this study was to test for stock structuring between eastern and western populations of Gemfish in Australia, define the boundary between these stocks and test for additional stocks in the west. This study confirmed the presence of non-interbreeding stocks in the east and west of the species' distribution. It clarified that the boundary between the stocks is in western Bass Strait and confirmed the presence of a single stock in the west.

The level of differentiation found between eastern and western stock for all three genetic markers was high, suggesting gene flow is occurring at very low levels. The study found evidence of migrants moving between stocks and hybridisation involving migrants and local fish. However, the hybrids are likely to be first generation crosses and the high genetic separation between populations suggests backcrosses between hybrid progeny and either parental stock are not successful.

The level of genetic subdivision appears rare for a vagile marine fish with planktonic larvae and a contiguous population throughout its distribution. There is no physical barrier separating the stocks such as a break in habitat, absence of requisite biological conditions or land bridge. It appears that gemfish have evolved a spawning behaviour that has them migrating and spawning at different times, which could be maintaining the genetic segregation.

Implications

Results from this work support separate management units for Gemfish east and west of Bass Strait. For the purposes of fisheries management, the western stock, extending from western end of the Great Australian Bight to southern Victoria, can be seen as a single management unit. Gemfish to the south and east of western Bass Straight to the north east extent of the species distribution should be considered a single management unit. The boundary in western Bass Strait coincides with the area adjacent to King Island and the boundary between Southern and Eastern Scalefish and Shark Fishery (SESSF) statistical reporting zones 50 and 60. A realignment of the management boundary to this location would ideally delineate both biological populations.

This research has provided much needed clarity on which statistical reports zone data to include in the stock assessment. The depletion level of both stocks is vastly different and it is important to assign the correct stock to the assessment. These results were used in the 2016 Tier 1 and Tier 4 stock assessments for western Gemfish in 2016, which were used to set a TAC (GABRAG, 2016; Haddon, 2016; Helidoniotis and Moore, 2016). The results of this study have been presented to and discussed at the Great Australian Bight Resource Assessment Group on at least 6 occasions. The implications for the assignment of quota are currently being worked through with the Australian Fisheries Management Authority, but the results suggest that there is no biological reason to manage western Gemfish in the Great Australian Bight Trawl Sector of the Commonwealth Trawls Sector as different stocks. However the is clear evidence break between eastern and western Gemfish.

The implications of the effective population size estimates are that the number of individuals contributing per generation in the eastern stock are far smaller than predicted by the spawning biomass estimates and may mean there is something limiting genetic contributions between generations. This may have implications for why the eastern stock of Gemfish is not recovering.

Recommendations

The results of this project clearly confirm that there two biologically distinct populations of Gemfish in Australia, but clarified the location and extent of this division. The genetic subdivision between both populations is substantial and represent distinct evolutionarily significant units. For the purposes of fisheries management and stock assessment these populations should be treated as separate biological entities. Furthermore, this work has found no evidence for extra stocks within the west. It would be advantageous to move the management boundary between eastern and western stock from its current location to the area to the west of King Island in Bass Strait using the boundary between Southern and Eastern Scalefish and Shark Fishery (SESSF) statistical reporting zones 50 and 60 to better align with the biological separation.

Further development

The project was successful in defining the spatial boundary between the eastern and western stock, and rejecting the hypothesis of multiple stocks within the western stock. As such, no further development is needed on stock structure. However, the study found evidence that the effective population size for eastern gemfish is much smaller than theoretically expected suggesting that there may be factors limiting the reproductive success of spawners. As this information may provide insights into why the eastern stock has not recovered from its overfished state, it is recommended to pursue this line of evidence further in a separate study.

Extension and Adoption

The outcomes of this research have been presented to the Great Australian Bight Resource Assessment Group (GABRAG), the Australian Fisheries Management Authority (AFMA), the Department of Agriculture and Water Resources and the University of Queensland. The results have also been presented at the Australian Society for Fish Biology (ASFB) and the 7th World Fisheries Congress in Busan. ABARES is currently working with AFMA and GABRAG on the implications of this research.

Project coverage

The project has received considerable online coverage through posts on the Molecular Fisheries Laboratory website and Facebook page throughout the course of this project. The results have been presented to industry, government and the wider national and international fisheries community through presentations at the Australian Society for Fish Biology and the 7th World Fisheries Congress in Busan as an invited special presentation.

Project materials developed

One scientific paper has been accepted (Appendix 1.) and another is currently being drafted that will be submitted in 2017.

Appendices

Appendix 1. Gemfish complete mitochondrial genome.

Mitochondrial DNA

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MITOGENOME ANNOUNCEMENT

The complete validated mitochondrial genome of the silver gemfish Rexea solandri (Cuvier, 1832) (Perciformes, Gempylidae)

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Abstract

The silver gemfish Rexea solandri is an important economic resource but vulnerable to overfishing in Australian waters. The complete mitochondrial genome sequence is described from 1.6 million reads obtained via next generation sequencing. The total length of the mitogenome is 16,350 bp comprising 2 rRNA, 13 protein-coding genes, 22 tRNA and 2 non-coding regions. The mitogenome sequence was validated against sequences of PCR fragments and BLAST queries of Genbank. Gene order was equivalent to that found in marine fishes.

Keywords

Eastern gemfish, mitogenome, mtDNA validation, NGS

History

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Rexea solandri is a mesopelagic fish of deep continental shelf and upper slope waters (Pogonoski et al., 2002). Historically overfished in southeastern Australia, it is listed as conservation-dependent species (DAFF, 2007), which restricts its harvest and bycatch. Rexea solandri is distributed from Cape Moreton (QLD) to Shark Bay (WA) and New Zealand waters (Pogonoski et al., 2002). Here we report the sequence of the entire mitochondrial genome of Rexea solandri.

A specimen was collected in South Australian waters (33° 24.7′ S; 128° 10.8′ W) in November 2013, and identified using field guides (Nakamura & Parin, 1993). A fin clip was stored in a 20% salt-saturated dimethyl sulfoxide (DMSO) solution and kept at −20 °C. The experimental protocol followed Blower et al. (2013). Total genomic DNA was extracted using the QIAgen DNeasy kit (Qiagen Inc., Valencia, CA). Mitogenome of R. solandri (GenBank Accession Number: KJ408216) was assembled from one Ion Torrent sequencing (NGS) run on an ION PGM Sequencer (Life Technologies, Carlsbad, CA) using 318v2 chips. About 1,616,041 sequences were produced with a mean read length of 272 bp (mode: 335 bp). Reads were mapped to the complete mitochondrial genome of the Nakamura's escolar Rexea nakamurai (Miya et al., 2013; GenBank Accession Number: NC_022503.1) with the software Geneious Pro v7.0.6 (Biomatters Ltd., Auckland, New Zealand). The 4058 mapped reads were then assembled de novo producing a 16,350 bp mitogenome assembly, similar in size to R. nakamurai mtDNA genome (16,379 bp). Indels (insertion-deletions potentially introduced by homopolymer sequencing errors) were validated based on depth and sequence quality; none were present in coding regions. Annotations were confirmed by comparing MITOchondrial genome annotation Server (MITOS)-generated annotations (Bernt et al., 2013).

The R. solandri mitogenome structure has a gene order typical of fishes and is comprised of 13 protein-coding regions, 22 tRNA genes, 12S and 16S ribosomal RNAs and 2 non-coding areas (origin of replication on the light strand and control region). The mitogenome has an A + T (54.3%) bias as seen in many other marine fishes, and a nucleotide composition of A, 28.5%, T, 25.7%, G, 16.8%, and C, 28.9%.

The proposed mtDNA assembly was validated using ~430 bp length fragments of the control region, amplified from four additional R. solandri specimens following protocol described by Broderick et al. (2011) for PCR (forward primer: TDKD, 5′-CCT GAA GTA GGA ACC AGA TG-3′; reverse primer: PRO, 5′-CCW CTA ACT CCC AAA GCT AG-3′) and sequencing (Applied Biosystems 3130 XL, Carlsbad, CA). Fragments (KJ408217–KJ408220) were 100% identical to the expected gene (control region) without polymorphisms. Additionally, the sequence of each gene was compared using BLAST queries (McGinnis & Madden, 2004). Across genes, the average pairwise identity was 87.9% (Figure 1, min: 62.7%, max: 100%) based on the first ten species hits from the GenBank database. $tRNA^{Glu}$ and $tRNA^{Met}$ had the highest pairwise identity while $tRNA^{His}$ and control region had the lowest values. All coding regions matched the reference genome. A similar analysis for R. nakamurai revealed little (across gene mean: 73.4%) or no match (in four genes) to Genbank sequences.

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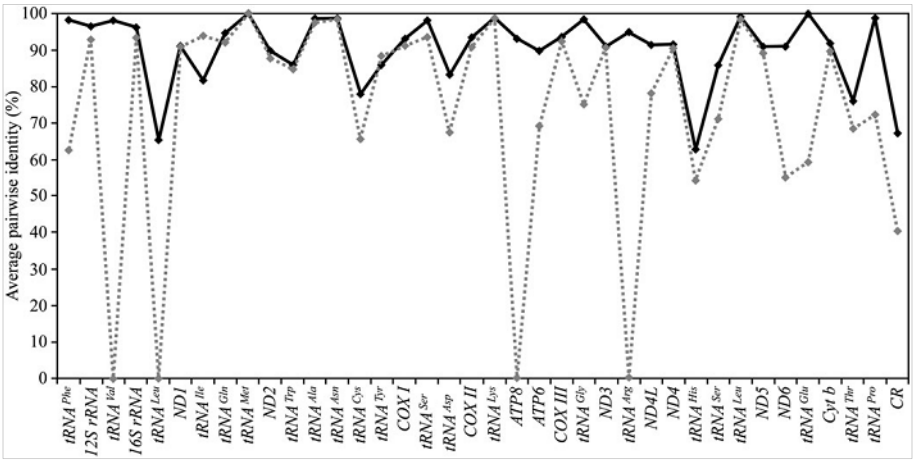

Figure 1. Average pairwise identity (%) of Rexea solandri (solid line) mtDNA genes. Mean values were obtained through BLAST queries for the highest ten species matches obtained. Identity of mtDNA genes from Rexea nakamurai (Miya et al., 2013) is also included (dashed line).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. The authors thank Andy Moore (ABARES, Commonwealth Government) and Raptis P/L for sample collection. CB was supported by was supported by CONICYT–Becas Chile and TUAP–Graduate School of The University of Queensland.

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Appendix 2. Characteristics of Gemfish microsatellite loci

Fourteen out of 48 *de novo* microsatellite loci produced consistent amplicons, with numbers of alleles ranging from three to 13 (Table A2). Loci had tri-repeat motifs, except for locus Rsol11 that was a penta-repeat. Heterozygosity ranged from 0.17 to 0.81. Expected and observed heterozygosity differed for locus Rsol11 and Rsol68.

Table A2.1. Characteristics of 14 polymorphic microsatellite loci designed using Ion Torrent NGS data from *Rexea solandri*. Locus names, forward (F) and reverse (R) primer sequences, annealing temperature (Ta) of PCR, repeat motif, allele size range (bp), total number of alleles (N_A), observed (*Ho*) and expected (*He*) heterozygosity and NCBI accession number are listed. The annealing temperature for all loci PCR's was 57ºC. See text for tailing of primers. (* Major allele frequency greater than 0.90; # Loci with possible heterozygote deficits).

Locus Number (lab no)	Locus Name	Primer sequences (3-5')	Motif	Size range (bp)	*N*	N_A	H_o	H_e	Accession number (bankit 140823)
9	Rsol11#	F: GAACTGCGTTCTGGTCGG	$(AAAGG)_{12}$	155-190	39	7	0.73	0.41	KM406117.1
		R: GGACCTGTCAGCAAGGGC							
16	Rsol19	F: CAGCCAGACAAACGTCCG	$(AAG)_{11}$	159-183	23	9	0.78	0.70	KM406122.1
		R: CTCACATCAGTGTCCTCTGGC							
19	Rsol22	F: GAGGAGACAGAAGAGGCGG	$(AGC)_9$	197-203	33	4	0.48	0.55	KM406111.1
		R: CTGCTGAGTTTGTGCTGGC							
23	Rsol27	F: ATGAGCTGGGTACTAGATGGC	$(AAC)_{10}$	153-186	36	10	0.75	0.86	KM406120.1
		R: TGAACCTGACATGACAACGG							
25	Rsol29	F: AATGATGTAAGTGCCAACGC	$(AAT)_{10}$	194-224	39	5	0.31	0.26	KM406113.1
		R: CAACTTGGTTAAGGCAAGGG							
26	Rsol30	F: TTCTCTACAGCTGACTCGGG	$(AAC)_{10}$	204-216	38	5	0.50	0.58	KM406123.1
		R: GCAGCTGAGTGCAGACGG							

27	Rsol31	F: GGGCATGTAAATTCTCCCG R: AAGAAGCGTAAGAATATCGGC	$(AAG)_9$	305-323	31	7	0.65	0.68	KM406114.1
28	Rsol33	F: GAGAAACGATGCTCCTCGC R: ATTATGATCAGCGTTACGGG	$(AGC)_{12}$	210-261	31	13	0.77	0.87	KM406115.1
29	Rsol54	F: GACTCTAATAACTGGAGTGATGCC R: TGAGCACCACAGAAAGCG	$(AAT)_9$	147-168	35	7	0.63	0.57	KM406110.1
39	Rsol57	F: TACCATGCAAAGATTGAGCC R: GAAAGACTAAAGCATTAGGAGCC	$(ATC)_9$	141-153	27	5	0.52	0.63	KM406112.1
40	Rsol62*	F: GAATGGTAACCGGATGGC R: AACCAGAAACTTGTACCGGC	$(AAC)_{11}$	132-147	23	4	0.17	0.17	KM406118.1
44	Rsol67	F: ATTGGTGTGCTTTCCTCCG R: GCGAATCGTCTCATTTCCG	$(AGC)_9$	161-173	34	5	0.59	0.62	KM406116.1
45	Rsol68#	F: AAGGCCAAACATCCAGCC R: TGCTCGGTTACTGAGAGCG	$(AGC)_8$	139-175	36	11	0.81	0.58	KM406119.1
46	Rsol70*	F: CATGGAGGCAGCTCTGGG R: TTCTCCTGTGAAGAATACAGGC	$(ATC)_9$	192-198	26	3	0.19	0.19	KM406121.1

A power analysis suggested that a small F_{ST} of 0.0025 could be detected with 95% statistical certainty using 12 loci when 100 individuals were genotyped per location (Table A2). With sample sizes per population of 100, the simulation results suggested that using 10, 12 or 14 microsatellite loci were sufficient to recognise population subdivision at greater than 90% across the three levels of differentiation tested (F_{ST} = 0.01; 0.005; 0.0025). Reducing the sample size was found to have a greater effect than reducing the number of loci. When reducing the sample size from 100 to 50, the ability to detect differences at the lowest F_{ST} (0.0025) using 14, 12 and 10 loci decreased to 61%, 59% and 47% respectively. The detection of the highest F_{ST} (0.01) remained robust (>99%) across all samples sizes.

Table A2.2. Percentage of simulations that were able to detect differentiation across a range of F_{ST} values for combinations of numbers of loci (L) and sample size (n). Dark grey shadings present robust combinations (>90%), light grey shading identifies combinations in the mid-range (80%-90%) and non-shaded estimates are non-confident combinations (<80%).

Loci	F_{ST}		
	0.0025	0.005	0.01
14L (n = 100)	97.70%	100%	100%
12L (n = 100)	95.80%	100%	100%
10L (n = 100)	90.20%	99.90%	100%
14L (n = 50)	61.20%	97.00%	100%
12L (n = 50)	59.20%	95.50%	100%
10L (n = 50)	47%	89.10%	99.80%

Table A2.3. Summary statistics of 13 microsatellite loci genotyping of Gemfish (*Rexea solandri*) from ten locations in eastern and southern Australia (see Fig. 3 for location abbreviations). Sample size per locus per population (*N*) is given, as is number of alleles *Na*), number of effective alleles (*Ne*), information index (I), observed heterozygosity (*Ho*), expected (*He*) and unbiased expected heterozygosity (u*He*), and fixation index (*F*)*.

		Rsol11	Rsol19	Rsol22	Rsol27	Rsol29	Rsol30	Rsol31	Rsol33	Rsol57	Rsol62	Rsol67	Rsol68	Rsol70
1. NSW	*N*	47	54	58	52	57	53	58	56	58	56	57	51	54
	Na	5	9	4	7	5	4	6	19	5	7	5	9	3
	Ne	1.812	4.856	1.882	3.546	3.682	2.248	2.592	6.969	2.175	1.554	2.727	3.429	1.077
	I	0.857	1.787	0.865	1.473	1.377	0.970	1.250	2.443	0.910	0.808	1.143	1.539	0.179
	Ho	0.340	0.796	0.431	0.712	0.772	0.604	0.569	0.857	0.724	0.375	0.719	0.706	0.074
	He	0.448	0.794	0.469	0.718	0.728	0.555	0.614	0.857	0.540	0.357	0.633	0.708	0.072
	uHe	0.453	0.801	0.473	0.725	0.735	0.560	0.619	0.864	0.545	0.360	0.639	0.715	0.073
	F	0.240	-0.003	0.080	0.009	-0.060	-0.088	0.074	-0.001	-0.341	-0.052	-0.136	0.004	-0.031
2. NSW_h	*N*	9	9	9	8	8	9	8	8	9	9	8	9	8
	Na	2	7	5	4	3	3	5	9	3	3	4	5	2
	Ne	1.385	4.500	3.000	3.048	2.909	1.906	3.459	5.818	1.742	1.409	2.370	3.951	1.133
	I	0.451	1.692	1.301	1.212	1.082	0.787	1.401	1.977	0.730	0.557	1.034	1.461	0.234
	Ho	0.333	0.778	0.889	0.625	0.875	0.444	0.750	0.625	0.444	0.333	0.625	0.556	0.125
	He	0.278	0.778	0.667	0.672	0.656	0.475	0.711	0.828	0.426	0.290	0.578	0.747	0.117
	uHe	0.294	0.824	0.706	0.717	0.700	0.503	0.758	0.883	0.451	0.307	0.617	0.791	0.125
	F	-0.200	0.000	-0.333	0.070	-0.333	0.065	-0.055	0.245	-0.043	-0.149	-0.081	0.256	-0.067
3. NZ_h	*N*	10	10	10	8	10	10	10	10	6	10	10	10	10
	Na	2	7	4	3	3	3	5	5	3	2	3	6	2
	Ne	1.220	4.348	2.667	2.844	2.899	2.381	2.778	3.125	2.323	1.471	1.361	3.846	1.105
	I	0.325	1.696	1.166	1.072	1.081	0.943	1.278	1.359	0.918	0.500	0.518	1.527	0.199
	Ho	0.200	0.900	0.800	0.750	0.600	0.800	0.500	0.700	1.000	0.400	0.300	0.700	0.100

		Rsol11	Rsol19	Rsol22	Rsol27	Rsol29	Rsol30	Rsol31	Rsol33	Rsol57	Rsol62	Rsol67	Rsol68	Rsol70
	He	0.180	0.770	0.625	0.648	0.655	0.580	0.640	0.680	0.569	0.320	0.265	0.740	0.095
	uHe	0.189	0.811	0.658	0.692	0.689	0.611	0.674	0.716	0.621	0.337	0.279	0.779	0.100
	F	-0.111	-0.169	-0.280	-0.157	0.084	-0.379	0.219	-0.029	-0.756	-0.250	-0.132	0.054	-0.053
4. WT	*N*	27	27	27	27	26	27	25	25	20	27	26	26	27
	Na	3	9	4	8	4	5	6	12	5	5	3	6	3
	Ne	1.298	3.962	1.962	5.283	3.108	2.695	2.490	4.266	2.564	1.418	2.625	2.811	1.303
	I	0.439	1.733	0.929	1.827	1.171	1.188	1.189	1.926	1.140	0.642	1.023	1.272	0.463
	Ho	0.259	0.741	0.481	0.963	0.731	0.593	0.600	0.800	0.700	0.333	0.654	0.500	0.259
	He	0.230	0.748	0.490	0.811	0.678	0.629	0.598	0.766	0.610	0.295	0.619	0.644	0.233
	uHe	0.234	0.762	0.500	0.826	0.692	0.641	0.611	0.781	0.626	0.300	0.631	0.657	0.237
	F	-0.128	0.009	0.018	-0.188	-0.077	0.058	-0.003	-0.045	-0.148	-0.130	-0.056	0.224	-0.115
5. WBS	*N*	24	26	26	23	26	26	24	21	23	23	26	21	19
	Na	5	7	4	9	4	4	6	13	3	4	3	8	2
	Ne	2.636	4.863	1.832	5.212	2.575	2.537	2.190	7.412	2.023	2.112	2.235	4.846	1.054
	I	1.140	1.750	0.847	1.880	1.067	1.140	1.123	2.257	0.862	0.937	0.874	1.772	0.122
	Ho	0.667	0.808	0.462	0.870	0.654	0.538	0.458	0.905	0.609	0.609	0.654	0.810	0.053
	He	0.621	0.794	0.454	0.808	0.612	0.606	0.543	0.865	0.506	0.526	0.553	0.794	0.051
	uHe	0.634	0.810	0.463	0.826	0.624	0.618	0.555	0.886	0.517	0.538	0.563	0.813	0.053
	F	-0.074	-0.017	-0.016	-0.076	-0.069	0.111	0.157	-0.046	-0.204	-0.156	-0.183	-0.020	-0.027
6. Por	*N*	31	30	33	30	33	31	32	31	33	29	29	28	32
	Na	6	10	6	7	4	5	8	18	5	4	4	10	4
	Ne	3.798	3.974	1.894	3.651	2.076	3.280	3.779	8.697	2.943	1.602	1.888	5.141	1.100
	I	1.447	1.706	0.945	1.532	0.952	1.313	1.615	2.499	1.259	0.733	0.861	1.876	0.241
	Ho	0.710	0.767	0.455	0.700	0.424	0.710	0.531	0.774	0.667	0.207	0.586	0.786	0.094
	He	0.737	0.748	0.472	0.726	0.518	0.695	0.735	0.885	0.660	0.376	0.470	0.805	0.091
	uHe	0.749	0.761	0.479	0.738	0.526	0.707	0.747	0.900	0.670	0.382	0.479	0.820	0.092

		Rsol11	Rsol19	Rsol22	Rsol27	Rsol29	Rsol30	Rsol31	Rsol33	Rsol57	Rsol62	Rsol67	Rsol68	Rsol70
	F	0.037	-0.024	0.037	0.036	0.182	-0.021	0.278	0.125	-0.010	0.449	-0.247	0.025	-0.032
7. Por_w	*N*	44	40	44	30	40	42	35	42	34	37	41	38	42
	Na	7	9	5	9	6	7	8	18	10	4	5	10	3
	Ne	4.445	4.827	1.886	6.272	2.327	3.549	3.530	12.783	4.007	1.362	2.756	5.894	1.155
	I	1.637	1.773	0.932	1.988	1.129	1.489	1.617	2.690	1.690	0.557	1.177	1.960	0.290
	Ho	0.773	0.750	0.432	0.767	0.550	0.833	0.600	0.762	0.794	0.297	0.634	0.763	0.143
	He	0.775	0.793	0.470	0.841	0.570	0.718	0.717	0.922	0.750	0.266	0.637	0.830	0.134
	uHe	0.784	0.803	0.475	0.855	0.578	0.727	0.727	0.933	0.762	0.269	0.645	0.841	0.136
	F	0.003	0.054	0.081	0.088	0.036	-0.160	0.163	0.173	-0.058	-0.120	0.005	0.081	-0.066
8. KI	*N*	43	43	46	42	43	41	44	43	45	45	46	47	44
	Na	8	10	6	9	5	7	9	15	5	6	5	12	3
	Ne	3.580	7.265	1.889	6.521	2.316	3.455	3.095	8.784	3.503	1.292	2.185	5.708	1.121
	I	1.527	2.093	0.934	2.016	1.059	1.409	1.502	2.386	1.367	0.557	1.044	1.996	0.247
	Ho	0.628	0.884	0.500	0.929	0.628	0.732	0.614	0.651	0.844	0.200	0.565	0.532	0.114
	He	0.721	0.862	0.471	0.847	0.568	0.711	0.677	0.886	0.715	0.226	0.542	0.825	0.108
	uHe	0.729	0.873	0.476	0.857	0.575	0.719	0.685	0.897	0.723	0.228	0.548	0.834	0.109
	F	0.129	-0.025	-0.062	-0.097	-0.105	-0.030	0.093	0.265	-0.182	0.115	-0.042	0.355	-0.050
9. GAB	*N*	42	48	45	48	47	48	46	39	48	47	45	46	43
	Na	7	12	5	9	5	7	8	17	8	5	4	10	4
	Ne	3.542	6.527	2.353	5.710	2.080	3.137	2.734	12.266	3.337	1.366	2.363	5.927	1.266
	I	1.531	2.072	1.095	1.879	0.999	1.335	1.435	2.613	1.435	0.599	1.023	1.992	0.434
	Ho	0.595	0.875	0.667	0.688	0.468	0.708	0.478	0.718	0.708	0.255	0.422	0.522	0.233
	He	0.718	0.847	0.575	0.825	0.519	0.681	0.634	0.918	0.700	0.268	0.577	0.831	0.210
	uHe	0.726	0.856	0.582	0.834	0.525	0.688	0.641	0.930	0.708	0.271	0.583	0.840	0.213
	F	0.171	-0.033	-0.159	0.167	0.099	-0.040	0.246	0.218	-0.011	0.047	0.268	0.372	-0.107
10. ET_h	*N*	11	11	11	9	9	11	9	11	11	11	11	11	9

	Rsol11	Rsol19	Rsol22	Rsol27	Rsol29	Rsol30	Rsol31	Rsol33	Rsol57	Rsol62	Rsol67	Rsol68	Rsol70
Na	3	5	4	4	4	4	4	12	2	3	3	4	1
Ne	2.051	4.102	2.373	2.282	3.600	2.444	2.282	9.680	1.766	1.204	2.469	3.315	1.000
I	0.860	1.504	1.060	1.040	1.322	1.111	1.040	2.374	0.625	0.368	0.995	1.279	0.000
Ho	0.636	0.818	0.636	0.556	0.889	0.636	0.444	1.000	0.636	0.182	0.727	0.727	0.000
He	0.512	0.756	0.579	0.562	0.722	0.591	0.562	0.897	0.434	0.169	0.595	0.698	0.000
uHe	0.537	0.792	0.606	0.595	0.765	0.619	0.595	0.939	0.455	0.177	0.623	0.732	0.000
F	-0.242	-0.082	-0.100	0.011	-0.231	-0.077	0.209	-0.115	-0.467	-0.073	-0.222	-0.041	#N/A

**Ne* = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2, uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * *He*, *F* = Fixation Index = (*He* - *Ho*) / *He* = 1 - (*Ho / He*) where pi is the frequency of the ith allele for the population & Sum pi^2 is the sum of the squared population allele frequencies.

Appendix 3. Population pairwise F_{ST} values from microsatellite genotypes

Table A3.1. *Rexea solandri* population pairwise F_{ST} values (above) and p-values (below) from 13 microsatellite loci (Rsol-11, Rsol-19, Rsol-22, Rsol-27, Rsol-29, Rsol-30, Rsol-31, Rsol-33, Rsol-57, Rsol-62, Rsol-67, Rsol-68 and Rsol-70) between 10 Gemfish populations. Populations are labelled as in Figure 3. Significant *F*st values are shaded.

*F*st

	1	2	3	4	5	6	7	8	9	10
1. NSW	0									
2. NSW_h	-0.00491	0								
3. ET_h	-0.00459	-0.02345	0							
4. NZ_h	0.00308	-0.0063	0.01133	0						
5. WT	-0.00101	-0.00899	0.00836	-0.00453	0					
6. WBS	0.01046	0.0116	0.01579	0.0415	0.02816	0				
7. Por	0.06758	0.07216	0.06779	0.11222	0.08694	0.02851	0			
8. Por_w	0.04081	0.06055	0.05313	0.09053	0.06132	0.01842	-0.00111	0		
9. KI	0.06318	0.08495	0.07368	0.11031	0.0853	0.03113	0.00483	-0.00203	0	
10. GAB	0.06142	0.07379	0.05539	0.09869	0.0831	0.02819	0.00401	-0.01044	-0.00093	0

Fst p-values

	1	2	3	4	5	6	7	8	9	10
1. NSW	*									
2. NSW_h	0.58789+-0.0138	*								
3. ET_h	0.58008+-0.0173	0.91699+-0.0065	*							
4. NZ_h	0.23242+-0.0134	0.57812+-0.0127	0.11328+-0.0087	*						
5. WT	0.45215+-0.0166	0.73340+-0.0117	0.10742+-0.0079	0.56055+-0.0122	*					
6. WBS	0.00488+-0.0020	0.08594+-0.0090	0.01758+-0.0031	0.00000+-0.0000	0.00000+-0.0000	*				
7. Por	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	*			
8. Por_w	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.56934+-0.0156	*		
9. KI	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.07129+-0.0081	0.69434+-0.0191	*	
10. GAB	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.15820+-0.0121	0.99902+-0.0002	0.66895+-0.0153	*

Table A3.2 *Rexea solandri* population pairwise *F*st values (above) and *p*-values (below) from 11 microsatellite loci (as above, excluding Rsol-33 and Rsol 68) between 10 Gemfish populations. Populations are labelled as in Figure 3. Significant *F*st values are shaded.

*F*st

	1	2	3	4	5	6	7	8	9	10
1. NSW	0									
2. NSW_h	-0.0026	0								
3. ET_h	-0.01551	-0.02669	0							
4. NZ_h	0.00434	-0.00837	-0.00531	0						
5. WT	-0.00205	-0.01095	-0.0088	-0.0064	0					
6. WBS	0.01274	0.01766	0.01712	0.0472	0.02886	0				
7. Por	0.07654	0.08873	0.07074	0.12872	0.0959	0.03524	0			
8. Por_w	0.04485	0.07531	0.05951	0.10053	0.06363	0.02602	-0.00326	0		
9. KI	0.07667	0.1058	0.08426	0.13162	0.09896	0.04309	0.00691	-0.00046	0	
10. GAB	0.06989	0.08492	0.06013	0.10628	0.08787	0.03464	0.00415	-0.00988	-0.00053	0

Fst p-values

	1	2	3	4	5	6	7	8	9	10
1. NSW	*									
2. NSW_h	0.41504+-0.0151	*								
3. ET_h	0.93164+-0.0077	0.88379+-0.0088	*							
4. NZ_h	0.21777+-0.0136	0.47559+-0.0145	0.37598+-0.0161	*						
5. WT	0.51074+-0.0151	0.74219+-0.0122	0.70117+-0.0148	0.54590+-0.0165	*					
6. WBS	0.00879+-0.0035	0.04102+-0.0062	0.03906+-0.0065	0.00000+-0.0000	0.00000+-0.0000	*				
7. Por	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	*			
8. Por_w	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.71289+-0.0152	*		
9. KI	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.04004+-0.0069	0.39746+-0.0142	*	
10. GAB	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.15332+-0.0138	0.99902+-0.0002	0.48730+-0.0139	*

Appendix 4. Delta-k analysis for STRUCTURE runs

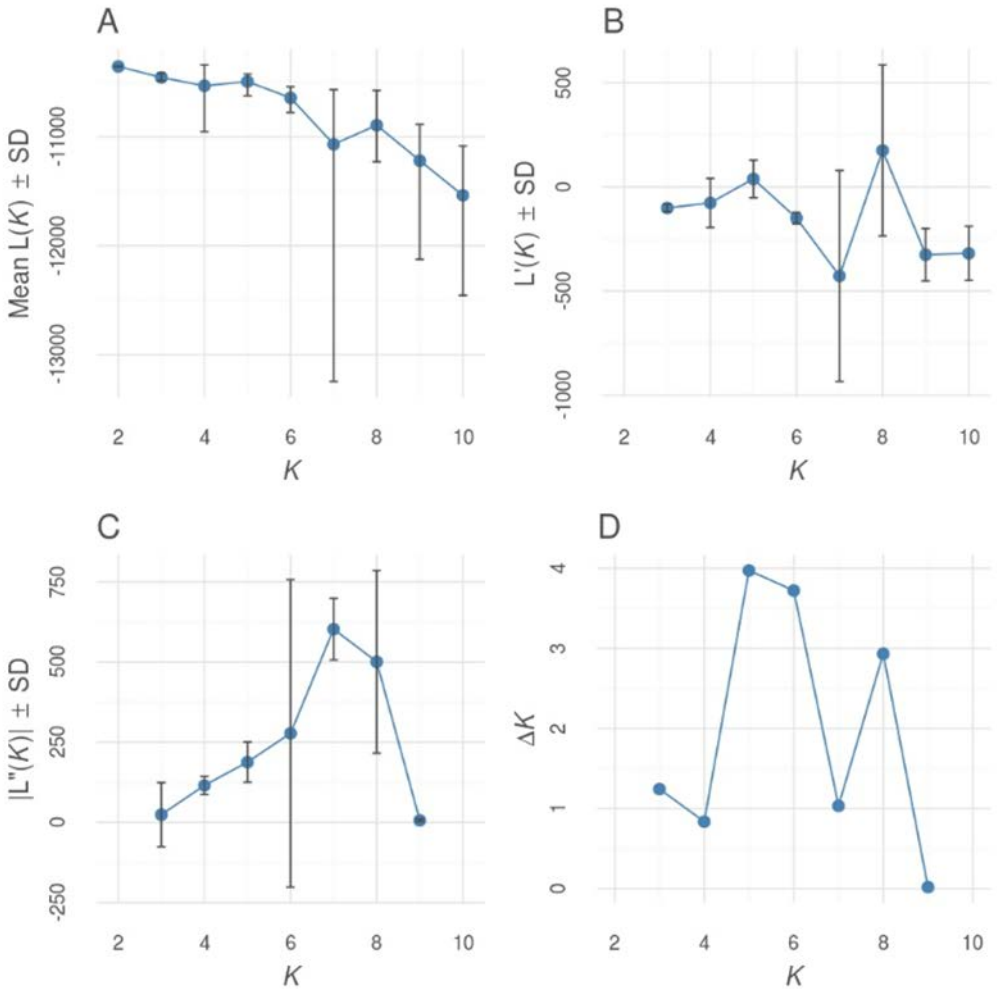

Fig A4. Depiction of various likelihood distributions (A, B, C and D) for the Gemfish microsatellite data when the number of groups (K) is increased from K=2 to K=10 (after Evanno et al, 2005).

Appendix 5

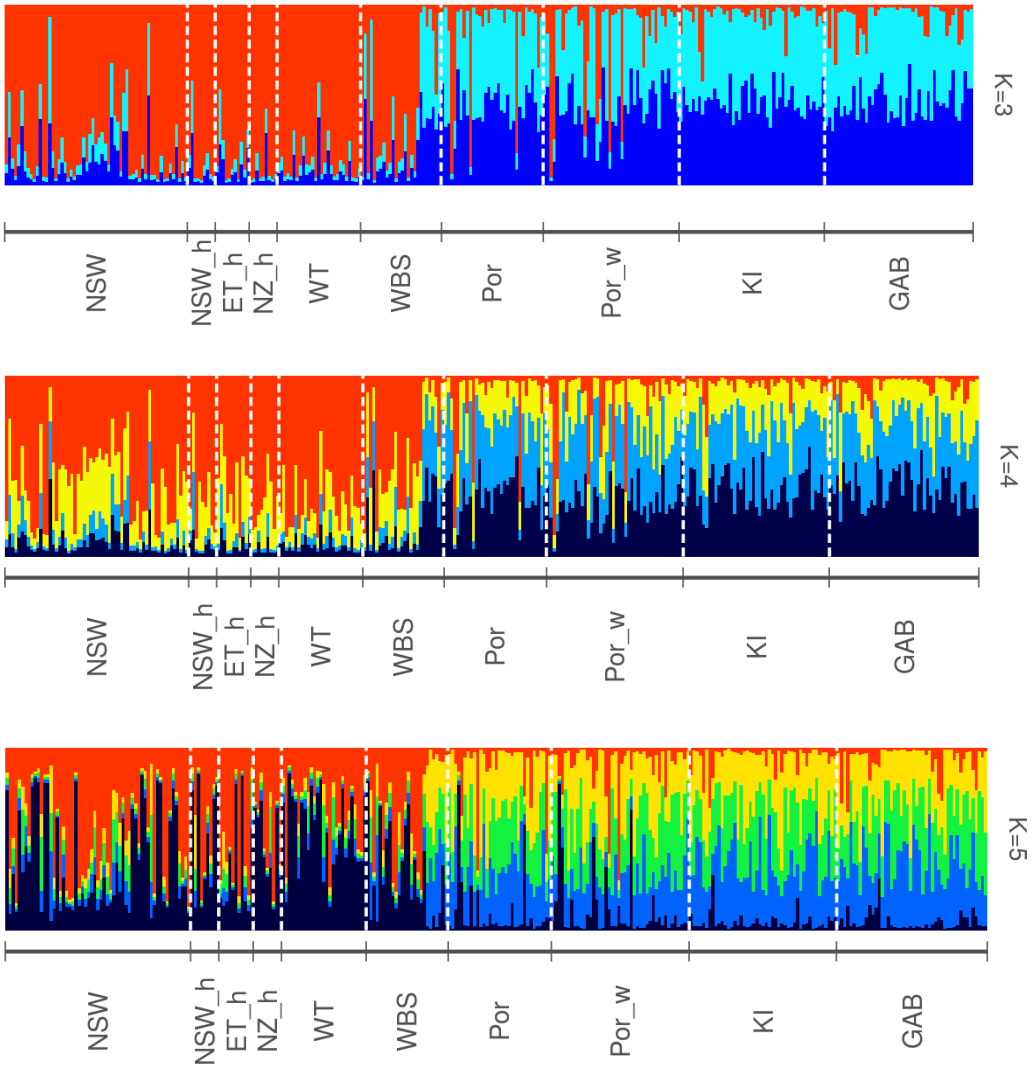

Figure A5. STRUCTURE box-plot showing the membership of 313 individuals to three (k=3), four (k=4) and five (k=5) groups. Collection locations are labelled (abbreviations are explained in the text).

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