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Subject

Review of Genetic Connectivity in Six Species in the Small Pelagic Fishery

Author

Dr Jennifer Ovenden

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UniQuest Pty Limited

UniQuest Pty Limited
Expertise Commercialisation, Research
ABN 19 010 529 898

Level 7, Building 78
Staff House Road
The University of Queensland
St Lucia Q 4072

Postal Address:
PO Box 6069
St Lucia Q 4067

Tel: (61-7) 3365 4037
Fax: (61-7) 3365 4433

Title

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Disclaimer

TABLE OF CONTENTS

1. BACKGROUND

1.1 Overview

On 19 November 2012, the then Minister for Sustainability, Environment, Water, Population and Communities made the Final (Small Pelagic Fishery) Declaration 2012, which came into force on 20 November 2012.

The Final Declaration provides that a commercial fishing activity which:

a. is in the area of the Small Pelagic Fishery (SPF);
b. uses the mid-water trawl method; and
c. uses a vessel which is greater than 130 metres in length, has an on-board fish processing facility and has storage capacity for fish or fish products in excess of 2000 tonnes

is a Declared Commercial Fishing Activity for the purposes of Part 15B of the *Environment Protection and Biodiversity Conservation Act 1999* (the EPBC Act).

The Declared Commercial Fishing Activity (DCFA) is prohibited for up to two years while an expert panel conducts an assessment and reports to the Minister on the activity. The Expert Panel commenced its assessment in February 2013 and will report in October 2014. The Panel's terms of reference can be found at http://www.environment.gov.au/node/16953.

1.2 Need

A central source of uncertainty relating to the operation of the DCFA relates to the potential for any localised depletion of target species to result in adverse impacts on the Commonwealth marine environment, including the target species' predators protected under the EPBC Act.

The Expert Panel has considered the available stock structure information on the target species in the SPF and notes that the latest work available was published in 2008 (Bulman *et al.* 2008). The extent of sub-structuring and connectivity levels in the populations of these species throughout the SPF is likely to have a strong bearing on the potential impacts of any localised depletion. Accordingly, the Panel is interested in identifying what, if any, research has been conducted or information has become available to inform the understanding of stock structure of these species since the last stock structure work was completed. In addition, the

Panel is interested in whether recent developments in genetic techniques are likely to be able to provide further insights into stock structure of the target species.

1.3 Scope

The Panel wishes to commission a desktop review of literature, reports and holdings of egg samples and other genetic material to evaluate the usefulness of any genetic connectivity studies that have been applied to the six SPF species and provide opinion on the potential for this knowledge to be improved, quickly and cost-effectively. The SPF species to be considered are:

- Blue mackerel (*Scomber australasicus*)
- Jack mackerels (*Trachurus declivis and T. murphyi)*
- Yellowtail scad (*T. novaezelandiae*)
- Redbait (*Emmelichthys nitidus*)
- Australian sardine (*Sardinops sagax*)

The review should provide an informed position on the historical, current and future genetic population connectivity tools and analysis techniques applicable to SPF species and an assessment of the relevance and cost-effectiveness of the available genetic connectivity techniques that could be applied.

1.4 Response to tender

Dr Jennifer Ovenden (operating as UniQuest Pty Limited) was contracted in February 2014 to perform a study to inform the Expert Panel assessing the likely effect of Declared Commercial Fisheries Activity (being an activity that occurs in target fishery using mid-water trawling from a vessel of certain characteristics and is greater than 130m in length) in the Small Pelagic Fishery (SPF).

A major source of uncertainty that needs to be resolved by the Expert Panel is potential for localised depletion of target species. In March 2014, the Resource Assessment Group of the SPF (SPFRAG) defined localised depletion as

> *"a persistent reduction in fish abundance in a limited area, caused by fishing activity, over spatial and temporal scales that negatively impact on predatory species and/or other fisheries"*

The Expert Panel believes that connectivity levels among populations (and sub-populations) of target species in the SPF is likely to have a strong bearing on the potential impacts of any localised depletion. This was also emphasised by SPFRAG in March 2014:

> *"Risk of localised depletion is highest for target species with low mobility (e.g. abalone) and lowest for highly mobile species (e.g. pelagic fish). Predatory species with limited foraging areas, especially central place foragers, are most likely to be impacted by localised depletion. Localised depletion is less relevant to highly migratory species or species with large foraging areas. Geographical barriers (headlands, straits) can increase the likelihood of localised depletion by limiting movement rates."*

1.5 Objectives

The Terms of Reference specified the task as follows:

1. A review and summary of the available information on genetic studies pertaining to stock structure of the six target species and collections of genetic material, since the most recent study in 2008 (Bulman et al. 2008).

2. Recommendations for cost-effective options for undertaking further genetic surveys of the species that would help to address assessment of the potential impacts of localised exploitation on any of these stocks within the SPF, taking into account the outcomes from the review of historical studies, and in view of recent advances in molecular techniques and analysis. This would probably include identifying existing collections or holdings of suitable material as well as devising sampling strategies for new collections and broad estimates of possible costs.

1.6 The Small Pelagic Fishery

The Small Pelagic Fishery comprises about half of the total area of the Australian Fishing Zone. It encompasses waters adjacent to the states of New South Wales (NSW), Victoria, Tasmania and South Australia, as well as the southern Western Australian coastline, to the 200 nautical mile limit of the Australian Fishing Zone (Figure 1). There are three sub-areas: the Australian Sardine sub-area that is adjacent to the NSW coastline; the Eastern sub-area that is adjacent to the coast of NSW, the south-eastern coast of Victoria and the eastern coast of Tasmania; and the Western sub-area that covers the remaining areas. The history of the SPF

fishery prior to 2007 is outlined in Bulman et al. (2008). Updated information on fisheries sustainability is described by Moore et al. (2012).

The physical and biological oceanographic characteristics of the area are described in detail in Bulman et al. (2008). Generally, the surface waters are low in nutrients and primary productivity and waters are carried southwards by the Leeuwin Current on the Western Australian coast and the East Australian current. There is an overall eastward movement of water mediated by the subtropical front that operates offshore of southern Australia from west to east.

There are no obvious barriers to longshore movement of life-history stages of fish species in the SPF. The exception to this may be the presence of Bass Strait, which was a biogeographic barrier to marine dispersal at lowered sea levels during past glacial periods. Various genetic studies have shown that dispersal through Bass Strait is largely dependent on life history, with sessile species and even some species with sessile adults and planktonic larvae being most affected by the complexity of currents and eddies in this region (Miller et al. 2013).

2. METHODOLOGY

This study determined the extent to which genetic technology has been applied to the SPF species. To achieve this, information was obtained and evaluated from existing literature as well as from individuals, and groups who are currently working with these species and ecosystems.

Literature databases were searched for recent studies on SPF species. As part of a previous study (Pope *et al.* In preparation), a broad literature survey was performed on 11 April 2012. Several publication databases (Web of Science-Thomas Reuters, Zoological Records, Biosis, Scopus, and ASFA1-Proquest) were searched with no restriction on publication year. Three main queries were combined: “genetic”, “marine” and “Australia”. The syntax for the genetic query was “allozyme*”, “gene flow”, microsatellite*, “SSRs”, “STRs”, “mtDNA”, “phylogeograph*” and “SNP” (terms linked with OR). The marine query was “coral*”, “estuar*”, “intertidal”, “marine”, “reef”, “sea”, “subtidal”, “ocean*”, “brackish”, “mangrove*” and wetland*” (terms linked with OR). For this review, literature was selected for examination if populations were sampled from the southern Australian coastline and were published since 2008.

A follow-up survey was performed on 10 April 2014 on the Web of Science-Thomas Reuters database with the same queries as above, but the marine query was modified to “marine”, “reef”, “sea” and “ocean”. The results were filtered as before. In addition to this, a separate literature search was done using the scientific names of the SPF species as search terms using the Web of Science-Thomas Reuters database, and publications focusing on genetics were selected.

A range of species and regional experts were consulted (Table 1). Discussions focussed around a summary table of genetic information on SPF species (a draft, prepared by the author and emailed to expert) and whether other information was available. The history and importance of the fisheries of each species were discussed in the expert’s region (e.g. states), as well as aspects of the biology of species and likely hypotheses of population structure. Questions were asked about availability of tissue samples for potential genetic projects plus the likelihood of obtaining fresh material from fishers or alongside future fisheries projects.

This information, along with an evaluation of the literature, was used to formulate several research projects to examine how existing and new genetic tools could be used to rapidly and

accurately estimate genetic connectivity in the target species to contribute to localised depletion assessments.

3. RESULTS

3.1 Search for recent genetic studies

Numerous new scientific papers were found on the genetic stock structure of species on the southern coastline of Australia (Table 2). None of them included new information on SPF species. However, the efficacy of the search was high; genetic studies on the SPF species cited by Bulman et al. (2008) were uncovered. No new literature was found using the species-specific search.

A species-specific summary of the genetic stock structure of SPF species is presented below and summarised in Table 3. Only genetic studies that relate to stock structure are included. For example, genetic approaches to discriminate between eggs of *Trachurus declivis* (jack mackerel) and *T. novaezelandiae* (yellowtail scad) (Neira *et al.* Submitted) are not specifically reviewed here.

3.2 Population genetics of blue mackerel (*Scomber australasicus*)

3.2.1. Key biological details

Scomber australasicus (blue mackerel) is widely distributed in the Indo-West Pacific region. It is found from the Red Sea and northern Indian Ocean to the western Pacific Ocean including Southeast Asia, Australia and New Zealand. In Australia, they are abundant in temperate and sub-tropical waters. Juveniles and small adults are generally found inshore. Larger adults form schools in depths of 40-200m across the continental shelf (Schmarr et al. 2007).

The age range of *S. australasicus* (blue mackerel) from three Australian studies where samples were taken from the fisheries catch (fisheries dependent) was one to nine years. In contrast, a similar study in New Zealand indicated they attained a greater age (to 23 years) (Bulman et al. 2008).

Spawning occurs in the shelf waters of southern Queensland and northern New South Wales between July and October and in southern Australia from November to July (Neira et al. 2007). Spawning consists of bursts or pulses of gamete release every two to eleven days (serial spawning). Ovaries contain a random mixture of oocytes at every conceivable developmental

stage (asynchronous development). In Australia, female fecundity was around 70,000 gametes per batch (Neira et al. 2007).

3.2.2. Existing genetic studies

A study by Ward et al. (2007) assessed the suitability of three methods (population genetics, parasitology and otolith chemistry) for determining the stock structure of *S. australasicus* across the range of its distribution in Australian and New Zealand waters.

As part of the Ward study, pilot-scale genetic analyses were performed on fish collected from three locations: Western Australia; southern Queensland; and New Zealand (by Schmarr et al. (2007, 2012). Nucleotide sequence data (345 base pairs; bp) from the control region of the mitochondrial genome (mitochondrial DNA, mtDNA) showed a significant difference between these populations overall (F_{ST} [1] = 0.0898, p = 0.00684). Pairwise mtDNA F_{ST} between populations showed that the Western Australian population was significantly different from both the Queensland and New Zealand populations (F_{ST} = 0.0915, p = 0.0198 and F_{ST} = 0.1389, p = 0.003), but the Queensland population was not significantly different from the New Zealand population (F_{ST} = -0.0063, p = 0.3722). Genetic similarity across the Tasman Sea was previously reported for this species from an earlier mtDNA analysis by Scoles et al. (1998).

Around the same time, Tzeng et al. (2009) tested for population genetic differentiation in this species around Taiwan. They sampled four populations within 200km of the Taiwanese coast, to the north and south as well as to the east and west. Using six nuclear DNA markers (microsatellite loci) there was some statistical support (F_{ST} = 0.007, p<0.001) for distinctive stocks to the north and south of Taiwan. They concluded that populations to the north and south of Taiwan should be considered separate fishery stocks and conservation units for management.

3.2.3. Expectations to test in future genetic studies

Following a detailed analysis of biology, fisheries and oceanography, Bulman et al. (2008) recommended further study to clarify the stock structure of *S. australasicus* (blue mackerel) and *T. novaezelandiae* (yellowtail scad, see section 3.4). These species are more commonly caught in the Eastern subarea where eggs and larvae are entrained in southward and eastward currents.

[1] F_{ST} is the proportion of total genetic variation that is due to division into sub-populations. It is bounded by zero and one. Values for marine populations commonly range from 0.01 to 0.10.

Across the entire SPF, however, Bulman et al. (2008) considered that *S. australasicus* (blue mackerel) and *T. novaezelandiae* (yellowtail scad) were most likely subdivided into an eastern and western stock. The separation between the stocks was most likely to coincide with the location of Bass Strait and Tasmania, which bisects the SPF into the Eastern and Western subareas. While this agrees with existing genetic data on *S. australasicus* (blue mackerel) (Schmarr et al. 2007, 2012), there is no information on genetic stock structure to test this proposal for *T. novaezelandiae* (yellowtail scad).

In addition, for *S. australasicus* (blue mackerel) at least, there may be multiple stocks within the SPF. This is feasible as there were genetically separate stocks in the China Sea over distances around 500km (Tzeng et al. 2009), and the southern coastline of Australia is a much larger region (around 5000km).

3.3 Population genetics of Jack mackerel (*Trachurus declivis*)

3.3.1. Key biological details

Trachurus declivis (Jack mackerel) has a relatively restricted distribution in the south-western Pacific Ocean. It occurs from southern Western Australia to New South Wales and eastwards to New Zealand (Figure 2). The species occurs in continental shelf waters often near the bottom or in mid-water, and occasionally on the surface. They feed on krill, other planktonic crustaceans and benthic fish species (Froese & Pauly 2011). In Australia, it is commonly observed around 42cm and the maximum reported age is 25 years. The age range of mature fish in southern Australia was three to six years (Bulman et al. 2008).

Amongst the 14 species of the genus *Trachurus*, *T. declivis* forms an evolutionary lineage (reciprocally monophyletic clade) with *T. novaezelandiae* (yellowtail scad) and one other species (*T. japonicus*). In a phone conversation (27 Feb 2014), the author and the Expert Panel agreed to exclude *T. murphyi* from this report. The species does not breed in Australian waters and is most likely a vagrant. It is evolutionarily distant to *T. declivis* and *T. novaezelandiae* (Cardenas et al. 2005).

Eggs and larvae of *Trachurus* species were encountered during surveys in southern and eastern Australia between 2002 and 2006. They were sampled from July to October at Bundaberg (Queensland) southwards to the Cape Howe (border of New South Wales and Victoria). A distinction was not made between the eggs and larvae of *T. declivis* (Jack mackerel) and *T. novaezelandiae* (yellowtail scad) (Neira et al. 2007). However, pilot studies showed that subsequent estimates of biomass made from daily egg production applied to *T.*

declivis (Jack mackerel). Genetic methods can now distinguish between *T. declivis* (Jack mackerel) and *T. novaezelandiae* (yellowtail scad) (Neira *et al.* Submitted).

3.3.2. Existing genetic studies

There are two genetic analyses of Australian populations of *T. declivis*. The first measured allelic variation in nuclear DNA using allozyme electrophoresis from 22 locations in southern Western Australia and south-eastern Australia (Richardson 1982). The second assayed sequence variation in mitochondrial DNA using restriction enzymes, using samples from two locations (southern New South Wales, and south-eastern Tasmania) (Smolenski *et al.* 1994).

Richardson (1982) presented convincing evidence for two or more genetically separate populations. For instance, mean allele frequencies for the locus glucosephosphate isomerase (*GPI*; 0.20, 0.50, 0.27 and 0.02) in southern Western Australia were significantly different to the frequencies of those alleles in southern New South Wales (0.03, 0.56, 0.40 and 0.01. Three additional loci differed in allele frequencies among these collection locations. From differences such as this, Richardson (1982) concluded there were genetically separate populations in Western Australia and New Zealand, and that in south-eastern Australian there were several genetically distinct, but overlapping, populations.

His hypothesis of overlapping populations was a consequence of a unique feature of the study; there was an excess of homozygotes (or deficit of heterozygotes) at the majority of collection locations for seven of the eight loci. Richardson (1982) evaluated possible causes for this, and concluded that a Wahlund effect was the most likely. This occurs when two or more genetically distinct groups are sampled from the same collection location (see section 3.7.1.3 for more details). To account for the observation of genetic distinction despite mixing, Richardson (1982) proposed that the spatial ranges of the adults contract during breeding season and expand at other times, but that interbreeding generally does not occur between breeding populations. Further research was strongly recommended on breeding structure, schooling behaviour and movement patterns.

The mtDNA study (Smolenski et al. 1994) supported the outcomes of allozyme work for the Eastern subarea that suggested separate populations. As mtDNA characteristics were not temporally stable between collections of fish from the same location, this was interpreted as support for the Wahlund hypothesis.

3.3.3. Expectations to test in future genetic studies

Bulman et al. (2008) considered that *T. declivis* (Jack mackerel) was most likely subdivided into an eastern and western stock. As for other SPF species (*S. australasicus*; blue mackerel and *T. novaezelandiae*; yellowtail scad), the separation between the stocks was most likely to coincide with the separation between the Eastern and Western subareas (Figure 1). This broadly agrees with existing genetic data (Richardson 1982; Smolenski et al. 1994)

Bulman et al. (2008) concluded there was no evidence to suggest that more than one stock of this species occurred in the Eastern sub-area (Figure 1), following an analysis of the biology, habitat and fisheries landings of this species. However, the possible Wahlund effect, discovered by Richardson (1982) and supported by Smolenski et al. (1994), raises doubts about their single stock status. This should be tested with further studies because, if demonstrated, it would be an important risk factor in possible localised depletion. Later sections provide a framework to explore this idea. Section 3.7.1.2 distinguishes between types of stocks based on the extent of movement undertaken during the lifetime of an individual, and section 3.7.1.3 presents the Wahlund effect as a diagnostic indicator of the occurrence of overlap between two or more genetically distinct stocks. Thus, for *T. declivis* (Jack mackerel) on the east coast, the Wahlund effect diagnoses the presence of two or more genetically discrete stocks. Each stock is proposed to aggregate within a small spatial range during spawning. Depletion of the aggregated breeding stock will have a pronounced effect on reproductive success and hence subsequent recruitment. Depletion during other phases, for example during feeding phases, may affect biomass but not subsequent recruitment.

3.4 Population genetics of Yellowtail scad (*Trachurus novaezelandiae*)

3.4.1. Key biological details

Trachurus novaezelandiae (yellowtail scad) is more widespread than the congeneric *T. declivis* (Jack mackerel). The two species co-occur in the waters of southern Australia and New Zealand (Cardenas et al. 2005). Compared to *T. declivis* (Jack mackerel), it is also found in northern Australian waters (Figure 2). *Trachurus novaezelandiae* (yellowtail scad) form large inshore schools and are most abundant in the waters of New South Wales (Stewart & Ferrell 2001). The fishery in New South Wales is based on two and three year old fish with individuals being found from eight to 11 years of age (Stewart & Ferrell 2001).

Spawning of *T. novaezelandiae* (yellowtail scad) is believed to occur on the continental shelf. Eggs and larvae were captured in the study of Neira et al. (2007), but they were not formally distinguished from those of *T. declivis* (Jack mackerel).

3.4.2. Existing genetic studies

There are no genetic studies of *T. novaezelandiae* (yellowtail scad).

3.4.3. Expectations to test in future genetic studies

Trachurus novaezelandiae (yellowtail scad) may be subdivided into two stocks to the east and west of Bass Strait in the same way proposed for other SPF species (*T. declivis*; Jack mackerel, *S. australasicus*; blue mackerel). Within the Eastern subarea, where they are more commonly encountered, there is no knowledge of population structure. Research is required to delineate populations that may be adversely affected by localised depletion.

3.5 Population genetics of Redbait (*Emmelichthys nitidus*)

3.5.1. Key biological details

Emmelichthys nitidus (redbait) is found in the southern Indian Ocean and South Pacific Ocean including waters adjacent to southern Africa, Australia, New Zealand and Chile. It is a small-bodied (to 36cm) mid-water schooling species (Neira et al. 2008a). In Australia, they are caught from northern New South Wales along the southern temperate coast (including Tasmania) to southern Western Australia (Bulman et al. 2008). They are plankton feeders, and an important prey item of tuna, seabirds and marine mammals (Neira et al. 2008a).

Growth appears to be rapid in this species (Bulman et al. 2008). In Tasmania there were significant differences between regions in age and size at maturity. On the southwest coast of Tasmania mature fish were around four years old and 100mm larger than mature fish on the east coast, which were younger (two years old). In both regions spawning occurred over a discrete two to three month period in spring (Neira et al. 2008b).

Like *S. australasicus* (blue mackerel), *E. nitidus* (redbait) is a batch spawner with females releasing eggs once every three days largely before midnight. Eggs to be spawned during the season are not all present in the ovary prior at once (indeterminate fecundity) and oocyte development is asynchronous (Neira et al. 2008b).

Around 2000, the fishing industry began to target *E. nitidus* (redbait) as an alternative to *T. declivis* (Jack mackerel) on the east coast of Tasmania. Catches of the latter species were prone to large inter-annual fluctuations (Neira et al. 2008b). The abundance of *E. nitidus* (redbait) was consistent with regional alteration zooplankton communities and long-term climate change (McLeod et al. 2012).

3.5.2. Existing genetic studies

There are no genetic studies of the population structure of *E. nitidus.* This needs to be rectified in order to assess the potential effect of localised depletion.

3.5.3. Expectations to test in future genetic studies

As they have similar biological characteristics, the stock structure of *E. nitidus* (redbait) may mirror that of *T. novaezelandiae* (yellowtail scad), *T. declivis* (Jack mackerel) and *S. australasicus* (blue mackerel).

3.6 Population genetics Australian sardine (*Sardinops sagax*)

3.6.1. Key biological details

Australian sardines (also called pilchards) belong to a species that is globally distributed. Worldwide disjunct populations were originally described as sister species within the genus *Sardinops*, but are now referred to as *S. sagax* (Parrish et al. 1989; Ward et al. 2012). Their distribution is anti-tropical. Generally, they are found in eastern boundary currents, but in the Indo-Pacific region they also occur in western boundary currents. They exhibit significant fluctuations in population size. When at a peak, they dominate the entire coastal and continental shelf ecosystems, but at other times are totally absent (Parrish et al. 1989).

In Australia, *S. sagax* (Australian sardine) is found in coastal regions along the entire southern half of the continent, including the southern part of the western and eastern coast. The total linear distance of its distribution is around 6700km, making it the largest geographical distribution of all *Sardinops* populations globally (Izzo et al. 2012). The Australian population does not experience the decadal fluctuations in abundance seen in southern Africa, Japan, South America and North America, suggesting that Australia's marine environmental conditions may be relatively stable in comparison to those systems. Certainly, Australia does not experience the massive upwellings that occur in the eastern boundary currents elsewhere. In addition, fishing pressure in Australia has, in most cases, been constrained within sustainable limits and catches have generally been stable (Izzo et al. 2012). The species also occurs in New Zealand (Ward et al. 2012).

In contrast to other SPF species, *S. sagax* has been the focus of numerous, major research projects in Australia and thus is comparatively more well known. *Sardinops sagax* (Australian sardine) releases numerous batches of pelagic eggs that are under continuous development in the ovaries, in the same manner as *S. australasicus* (blue mackerel) and *E. nitidus* (redbait).

Females spawn around once per week. The number of eggs released varies from 10,000 to 30,000 per individual. Spawning normally occurs in open waters between the coast and the edge of the continental shelf. In most locations, there is one spawning period (summer-autumn) but in Western Australia there are two spawning periods (Ward et al. 2012).

The application of standard methods to determine age (such as counting growth increments in scales and otoliths) is problematic in Australia, as it is in the Californian population (Ward et al. 2012). The pelagic larval duration is relatively long. Eggs hatch after approximately two days and larvae metamorphose after one to two months. Recruitment is strongly linked to larval survival, which is determined by spatial and temporally variable factors such as food availability and predation.

Schooling behavior of *S. sagax* (Australian sardine) in Australia appears to be influenced by factors such as reproductive condition, habitat heterogeneity, food availability, predation levels and vessel noise (Ward et al. 2012). In 1995 and again in 1998-99, a virus caused the mortality of up to 60% of the Australian population. There have been no mortality events since then and the populations have recovered to their previous levels. The virus is now regarded as endemic (Whittington et al. 2008).

3.6.2. Existing genetic studies

There are recent three genetic studies on *S. sagax* (Australian sardine) in Australia (Dixon et al. 1993; Okazaki et al. 1996; Yardin et al. 1998). Dixon et al. (1993) and Yardin et al (1998) are final reports of FRDC-funded projects. Using nuclear genetic loci (allozymes), Dixon et al. (1993) found evidence for a series of quasi-independent genetic populations around the southern coast of Australia. They inferred that mixing occurs between the genetic populations during some of their life cycle, but the extent of mixing and how it varied within and between years was unknown. The locations of Dixon's genetic subgroups were summarised by Izzo et al. (2012). These are presented in Figure 3 along with subgroups defined by other stock identification methods. Two genetic stocks from the study by Dixon et al. (1993) in Western Australia coincided with two of the three distinct centres of spawning reported by Gaughan et al. (2002).

The study by Yardin et al. (1998) collected samples from 23 locations along the southern coastline (south-eastern Queensland, New South Wales, Victoria and South Australia). Samples were not collected from Western Australia. One collection was made from New Zealand. Genetic analysis was completed using six nuclear loci (allozymes). In accordance with Dixon et al. (1993), genetic differentiation was found among collection locations. The

large and significant F_{ST}'s (0.066 to 0.162, depending on which groups of collection locations were compared) were not driven by the inclusion of the New Zealand sample in geographic groups. Like blue mackerel (*Scomber australicus*), there appeared to be no genetic difference between populations in eastern Australia and New Zealand. Despite stratifying the sample collection by gonad maturity, genetic analyses were not presented for spawning compared to non-spawning fish. Yardin et al. (1998) came to a very similar conclusion as Dixon et al. (1993); that the *S. sagax* (Australian sardine) populations sampled consisted of three or more major stocks with overlapping boundaries.

The third genetic study on *S. sagax* (Australian sardine) was by a Japanese group *(Okazaki et al.* 1996) and focused on the worldwide genetic relationships of members of this genus. Their method (restriction enzyme analysis of amplified control region of the mitochondrial genome) provided little information about genetic differentiation among the three Australian populations sampled (Table 3).

Like the study by Richardson et al. (1982) on *T. declivis* (Jack mackerel), the studies by Dixon et al. (1993) and Yardin et al. (1998) found evidence of a Wahlund effect for *S. sagax* (Australian sardine) inferred from an excess of heterozygotes. This phenomenon discussed in detail in the next section (3.6.3) and again in section 3.7.1.3.

3.6.3. Expectations to test in future genetic studies

Sardinops sagax (Australian sardine) was newly included in the assemblage of SPF species when Bulman et al. (2008) was produced. A preliminary assessment of stock structure was included, however, highlighting the existence of separate regional stocks within the fishery. The timing of spawning in eastern (NSW), western (WA) and southern (Vic and SA) populations was put forward as evidence for the existence of separate stocks. Some mixing of spawning populations was suggested, as adjacent regions had some overlap in spawning seasons. This pattern of population structure was widely accepted and subsequently used as a sampling template by Whittington et al. (2008) to study the introduced herpesvirus (Figure 3).

Since then, stock structure boundaries were refined by Izzo et al. (2012) using a weight of evidence approach (Welch *et al.* Submitted). Three additional stocks were defined within the eastern subarea (Victorian, southern New South Wales and southern Queensland). A fourth stock was proposed for the eastern coast of Tasmania. The role of the Bass Strait and Tasmania was considered important in the separation of *S. sagax* (Australian sardine) stocks between the eastern and western subarea. Within the western subarea, Izzo et al. (2012)

emphasized that the Leeuwin Current may transport larvae from Western Australia into waters off South Australia possibly leading to connectivity between stocks (Gaughan 2001).

In Western Australian waters, a study of oxygen and carbon stable isotopes of otoliths showed that *S. sagax* adjacent to Fremantle on the western coast were distinguishable from fish from the southern coast of Western Australia (Edmonds & Fletcher 1997). On the southern coast, studies of egg production and timing of spawning suggested there were three assemblages of spawning adults corresponding to three major fisheries operations in the Albany, Bremer Bay and Esperance Zones (Gaughan et al. 2002). Ward et al. (2012) noted there is likely to be a counter-current movement of juveniles westward to maintain population sizes in southern Western Australia given the prevailing transportation eastwards of larvae by the Leeuwin Current.

There was no *a priori* reason that the genetics of *S. sagax* (Australian sardine) should mirror those of *T. declivis* (jack mackerel), yet the results of Dixon et al. (1993) and Yardin (1998) showed striking similarity to those of Richardson (1982). For example, like *T. declivis* (Jack mackerel), the *S. sagax* (Australian sardine) allozyme loci assayed by Dixon et al. (1993) and Yardin (1998) exhibited a pronounced deficit of heterozygotes. Like Richardson (1982), Yardin (1998) evaluated a range of possible explanations for the deficit of heterozygotes, but came to the same conclusion: it was most likely due to a Wahlund effect (section 3.7.1.3). Yardin (1998) performed cluster analysis of samples from different collections based on the genetic distance between them by and showed genetic association by timing of sampling rather than location, which supports the presence of multiple, largely non-interbreeding genetic stocks but with overlapping geographic distributions. Dixon et al. (1993) took a different approach and searched for genetically homogeneous sets of samples from collection locations. The rationale here was that samples were taken from either spawning groups that were likely to be genetically homogeneous, or from non-spawning groups that were genetically heterogeneous. At some locations, Dixon et al. (1993) reported the presence of genetically homogeneous spawning groups.

For a species with small body size and relatively short longevity, it is not unexpected that the *S. sagax* (Australian sardine) is composed of genetically distinct populations within a range that extends over 6000km in Australia. More surprising, however, is the apparent presence of genetically distinct spawner groups, and the consequent reproductive isolation that must occur between them. Dixon et al. (1993) raised the possibility of homing to estuaries for spawning as estuaries may provide enriched waters required for larval nutrition in low productivity waters. This may be the case in Western Australia as the genetic stocks are centred on distinct

spawning areas (Gaughan et al. 2002). It is unknown whether this occurs elsewhere in their distribution. It is also unknown whether the extent of reproductive isolation among spawning groups could lead to localised depletion if they were the focus of mortality events. Both Dixon et al. (1993) and Yardin et al. (1998) believed that mixing of genetically distinct populations occurred, and under these circumstances localised depletion may not persist in time. The depletion event may be smoothed over by the combined effect of movement and schooling behaviour to maximise fitness. Investigation of the interaction between genetically distinct spawner groups and localised depletion events hinges on the re-analysis of old data and the collection and analyses of new types of genetic data. Projects like this are described below.

3.7 Potential genetic projects that may provide new information to address the likely impact of localised depletion on SPF species

3.7.1. Background

3.7.1.1 Genetics and defining fisheries stocks

A fisheries stock is a reproductively isolated population that can be identified by genetic markers of lineage (Cadrin et al. 2014a). Izzo et al. (p. 13, 2012) adopted a similar definition for their large study on *S. sardinops* (Australian sardine). They stated that *"While many definitions for the term 'stock' are available …., we adopt the working definition of a fish stock as "...an intra-specific group of randomly mating individuals with temporal and spatial integrity".* A scan across the literature will come up with a range of alternate definitions; however, genetics will be the central principle in the majority. Avise (2004) summed it up well by stating that genetics has fundamental appeal because all genetic measurements ultimately depend on the transmission of alleles across generations, something that is universally true for all organisms.

Perceptions about how genetics informs current and future fisheries management issues were surveyed by Dichmont et al. (2012). Eighty-eight stakeholders who were interviewed agreed that genetics offers a diverse collection of versatile and useful tools for informing fisheries managers about issues that have a biological basis. However, it is also true that genetics in stock identification has not been the silver bullet that many had hoped for (Mariani & Bekkevold 2014). Fisheries managers often frown upon genetics for the definition of stock boundaries because a single, clear-cut answer cannot always be provided (Mariani & Bekkevold 2014). Dichmont et al. (2012) found other problems, including

- a general lack of understanding of the potential value of genetic information;
- a perception that genetic studies are expensive;

- a perception that genetic results are often “oversold”;
- a lack of consistency in interpretations of genetic results by researchers; and
- that genetic information is far outweighed by practical management decision considerations.

Dichmont et al. (2012) identified that poor communication was limiting the effective use of genetics in fisheries management in Australia, and provided recommendations of how this could be addressed. Another problem is the astounding speed and pervasiveness of the field, which many find threatening.

Beyond this, there is a lack of in-depth analyses of the relative usefulness of a range of stock identification tools, in addition to and combined with genetics. For example, a recently published book on stock identification methods (Cadrin et al. 2014b) is a valuable compendium of diverse methods and contains numerous examples, but is not definitive about the equivalence (or otherwise) of methods. Without this evaluation, effectively combining information across marker types to define stock boundaries is difficult. The ‘weight of evidence’ approach is commonly used (e.g. on *S. sagax*, Izzo et al. 2012), but more could be achieved if the relative contributions made by each type of data was understood. Welch et al. (Submitted) made a start on this by defining the intrinsic time scales of data used by different stock identification methods.

The informed combination of genetics and single-generation markers (such as ototlith chemistry, parasite abundance and tagging and tracking studies) would make significant headway into the science of defining stocks. Alone, all methods, not just genetic methods, are subject to the criticism of Mariani and Bekkevold (2014) that a single, clear-cut answer on stock boundaries has not always been provided. Ovenden (2013) advocated an integrated approach to understand crinkles in connectivity between populations of marine species.

Propelled by the availability of inexpensive whole genome sequencing and bioinformatics capability, genetics in fisheries is on the move. For example, genes can also be used as within-generation markers (Polanowski et al. 2014) in the same way that otoliths that accumulate the signature of the environment or individual fish accumulate a characteristic parasite load. Genetic changes can occur in adaptive genes within a few generations if the selective pressure is high. With open minds, these and other developments may unite stock identification science. This would be a crucial step forward in the improved spatial definition of populations to assess the effects of localized depletion and to establish management regimes for conservation and sustainable exploitation.

3.7.1.2 Types of fisheries stocks and their spatial ranges

By definition, a stock is maintained by reproduction and subsequent recruitment from within and individuals have characteristic age-specific birth, death and growth rates. Death rates are made up of natural mortality and mortality due to fishing (Figure 4). These biological characteristics of a stock allow predictive modelling of the effects of fishing and other environmental perturbations (Cadrin et al. 2014a).

Fisheries management is conducted spatially, so the definition of the spatial extent of a fisheries stock is imperative (e.g. Figure 3). The spatial range of a stock is the distribution in space of its members. Because the majority of marine species have multiple life history stages, often with their own unique environmental requirements, there are different types of stocks according to the extent to which the spatial distribution of life-history stages overlap.

The simplest spatial definition of a stock occurs when the life history stages of the species co-exist in the same place at the same time. These are called sedentary stocks. The use of single-generation (within life-time) markers such as the chemical composition of otoliths, parasite loads or physical tagging and tracking can readily be used to identify sedentary stocks. Members develop characteristics linked to the constrained environmental range occupied during their lifetime.

By definition, sedentary stocks do not overlap spatially with other stocks and there is little (if any) interchange of individuals between them. A sedentary stock definition is the default spatial definition of a stock; it is often assumed in the absence of information about the distribution of life history stages. It is popular as it also defines the spatial extent of adult life history stages, which are commonly the stage that is targeted by fisheries.

In reality, life history stages of most marine species have different spatial ranges. For example, tiger prawns (*Penaeus esculentus*) occupy at least three spatially distinct ranges during their lifetime; adults occupy open water, larvae occupy near-shore waters and post-larvae occupy sea-grass beds (Condie et al. 1999). Furthermore, ranges may be occupied at different times. The spatial ranges of stocks of these species encompass the distribution of all life history stages in time and space. These are called range-sum stocks.

Pacific salmon species are extreme examples of range-sum stocks. The spatial range of a Pacific salmon stock encompasses the northern Atlantic Ocean, where juvenile and sub-adults forage, as well freshwater habitats of the Pacific coast of North America where adults breed. In

the Atlantic Ocean, salmon stocks are mixed (overlapping). In freshwater, stocks are non-overlapping and genetically distinct. Single-generation markers of stock structure (e.g. chemical composition of otoliths, parasite loads) are less successful in defining these stocks due to the heterogeneous environments encountered during the lifetime of an individual. Genetic markers can define these types of stocks regardless of the life history phase sampled (Bekkevold et al. 2011). When fishing occurs on mixed (or overlapping) stocks the relative proportion of stocks caught can be determined by 'mixed-stock analysis' where individuals are assigned to stocks using genetics or other characteristics. This analysis is an essential first step to auditing the effects of fishing on overlapping stocks and preventing the collapse of individual stocks.

SPF species are likely to consist of sedentary stocks as well as range-sum stocks. Localised depletion on sedentary stocks could result in the rapid loss of a proportion of the species' distribution and overall abundance. Genetic studies to date on *Trachurus declivis* (Jack mackerel) and *S. sagax* (Australian sardine) have shown that these species have range-sum stocks, consisting of groups of adults that may be genetically distinct and spatially constrained (i.e. aggregated) during spawning, followed by periods of less spatial constraint. The degree of spatial constraint on non-adults is unknown. If depletion occurred on adults during aggregation, the effect would be more significant compared to depletion on wide-ranging adults. Depletion on spawning adults would affect recruitment, whereas depletion on wide-ranging adults would affect current abundance. More research is recommended to determine the spatial stock status of all SPF species, and genetics is an ideal tool to achieve this.

3.7.1.3 The Wahlund effect

The Wahlund effect was first described in 1928 (Wahlund 1928). It is an explanation for the observation of a deficit of heterozygotes (also expressed as an excess of homozygotes) at the majority of loci in a sample from a naturally occurring population. If heterozygote deficit occurs in the minority of loci, other explanations are more likely (e.g. natural selection, null alleles, inbreeding). Heterozygote deficit occurs at nuclear markers such as allozyme and microsatellite loci. It does not occur for mtDNA, which is a haploid locus.

When there are two alleles at a locus, the robust Hardy-Weinberg principle allows us to calculate expected frequency of heterozygote individuals. It is equal to 2*a*b (where 'a' and 'b' are the frequencies of alleles 'a' and 'b' at one locus). A heterozygote deficit occurs when the observed frequency of heterozygotes in the sample (as directly counted by the researcher) is less than the expected frequency of heterozygotes (as calculated from the Hardy-Weinberg principle).

The Wahlund effect occurs when two or more genetically different populations are inadvertently sampled from the same sampling location. This is normally avoided because genetically different populations do not co-occur, or if they do individuals are physically distinguishable and thus are not co-sampled.

Imagine two populations where alleles ‘a’ have different frequencies, say 0.1 and 0.3. The allele ‘b’ at the same locus would have corresponding frequencies of 0.9 (i.e. 1 - 0.1) and 0.7 (i.e. 1 - 0.3). If we cannot distinguish between the two populations, and assuming that we sample and then genotype equal numbers of members of the two populations, the frequency of allele ‘a’ would be 0.2 (i.e. the mean of 0.1 and 0.3). The expected frequency of heterozygotes in the mixed sample would then be 2*0.2*0.8 = 0.32. But, the observed (actual) frequency of heterozygotes would be [(2*0.1*0.9) + (2*0.3*0.7)]/2 = (0.18 + 0.42)/2 = 0.30 based on the combined frequencies of heterozygotes in each of the cryptic populations. There would be a deficit of heterozygotes in the study because 0.30 (observed frequency of heterozygotes) is less than 0.32 (expected frequency of heterozygotes).

Richardson (1982) observed a deficit of heterozygotes at all six loci in the study on *T. declivis* (Jack mackerel). Likewise, Dixon et al. (1993) and Yardin et al. (1998) observed a deficit of heterozygotes for *S. sagax* (Australian sardine). A Wahlund effect was proposed in both cases, and the implication was that samples had been inadvertently taken from two or more genetically distinct populations at the sampling locations.

However, here is another way to interpret heterozygote deficiency across all nuclear loci within a study. Johnson and Black (1984) took this alternate route when a heterozygote deficiency was observed at all seven loci from samples of *Siphonaria sp.* (an intertidal limpet) from the Western Australian coastline. They were influenced by conclusions from a previous study on mussels (*Mytilus californianus*) that likewise found heterozygote deficits (Tracey et al. 1975). For these populations of sessile invertebrates, it was concluded that population samples consisted of offspring of small, ephemeral groups of spawning individuals. Each spawning group was so small that binomial sampling effects caused significant shifts in gene frequencies in the offspring compared to the gene frequencies of the surrounding population. Sampling these small groups mimicked the sampling of mixed cryptic populations.

A Wahlund effect for the mollusc studies was not proposed due to the lack of evidence for widespread genetically separate populations. Both mollusc studies sampled a range of locations but found no evidence of genetically distinct populations. The studies by Richardson

(1982), Dixon et al. (1993) and Yardin et al. (1998) found heterozygote deficit **and** genetically distinct populations. Breeding populations of *T. declivis* (Jack mackerel) and *S. sagax* (Australian sardine) are unlikely to suffer binomial sampling as their numbers are large and they are not spatially constrained like sessile mollusc species.

It is possible that small contemporary genetic effective population sizes (CN_e) reported for marine finfish (e.g. Hauser et al. 2002) could lead to binomial sampling effects. However, this would be more likely to lead to a low overall population heterozygosity, which is related to, but distinct from, heterozygote deficiency. Furthermore, if this were the case Wahlund effects would be common in marine finfish.

Wahlund effects are relatively rare in the population genetic literature. Amongst the genetic studies of Australian marine species surveyed by Pope et al. (In preparation), a number were formally tested for the presence of a Wahlund effect by authors of the studies (Table 4). Wahlund tests were largely done on sessile species, but tests were also performed on non-sessile species; for example, eight of 41 finfish species were also formally tested. The bias towards sessile invertebrates was expected following on from the studies of Johnstone and Black (1984) and Tracey et al. (1975).

Of 104 species studied by Pope et al. (In preparation), a Wahlund effect was reported in 13 species; four fish (one of which was *T. declivis;* the studies led by Dixon and Yardin are not published), five coral species, two gastropod species (one of which was *Siphonaria*) and two species of algae (Table 4). The frequency of the Wahlund effect (13/104) is considered to be reasonably accurate as all population genetic studies using nuclear loci compare observed and expected heterozygote frequencies, and if found at all loci a deficit of heterozygotes would have been reported.

Apart from *T. declivis* (Jack mackerel), published evidence for a Wahlund effect in finfish is thin [2]. Two Wahlund studies (i.e. where Wahlund effect was reported) were on the same species (*Acanthochromis polyacanthus,* a coral reef species lacking larval dispersal) and were published in the same year (Ayre et al. 1997; Planes & Doherty 1997). The remaining study sampled yellowtail kingfish (*Seriola lalandi*) from Japan, Australia and New Zealand (Nugroho et al. 2001). Three microsatellite loci were genotyped and all were observed to have heterozygote deficits, but examination of the paper suggests this may have been associated with the mis-scoring of genotypes rather than the presence of a Wahlund effect.

[2] The studies led by Dixon and Yardin on *S. sagax* are not published so is excluded.

Of published studies on 41 finfish species in Australia, a Wahlund effect was only found in two (*T. declivis* and *Acanthochromis polyacanthus*). This number rises to three if the studies led by Dixon and Yardin on *S. sagax* are included. Species that were formally tested, but negative for the Wahlund effect include blue grenadier, *Macruronus novaezelandiae* (Milton & Shaklee 1987), orange roughy, *Hoplostethus atlanticus* (Elliott & Ward 1992), jackass morwong, *Nemadactylus macropterus* (Elliott & Ward 1994), atherinid fish *Craterocephalus capreoli*, (Johnson et al. 1994), spikey oreo *Neocyttus rhomboidalis* (Elliott et al. 1998) and tarakihi (from New Zealand) *Nemadactylus macropterus*, (Burridge & Smolenski 2003),

Two of the three species where a Wahlund effect was reported were SPF species. However, a Wahlund effect is not normally associated with pelagic species. For example, no Wahlund effect was reported for the southern Bluefin tuna *Thunnus maccoyii* (Grewe et al. 1997) and black marlin, *Istiompax indica* (Williams *et al.* In preparation), both of which are pelagic in Australian waters. Neither was a Wahlund effect reported in blue grenadier (*Macruronus novaezelandiae*), a species whose life-history is similar to the SPF species (Milton & Shaklee 1987). The presence of the effect in the SPF species may be associated with the presence of genetically distinct spawning groups plus the mixing of genetically distinct offspring, possibly during the feeding (i.e. non-breeding) phase. The Wahlund effect may have been observed because of the life history state of samples that were taken for genetic analyses.

For the Wahlund effect to have occurred, genetically distinct populations must be present within *T. declivis* (Jack mackerel) and *S. sagax* (Australian sardine). The mechanism by which genetically distinct populations are produced and maintained is unknown, but could include aspects of natal homing associated with spawning, schooling behavior and post-mating reproductive barriers. Post-mating reproductive barriers have been shown to control the dynamics of inter-species hybridization in finfish (Immler et al. 2011; Sui et al. 2012). Whatever the mechanism, it is highly likely that it operates between and within genetically distinct spawner groups (GDSG) at and during spawning times and at spawning locations. Localised depletion of a GDSG would reduce recruitment, remove a slice of the resource and lead to an overall reduction in the genetic diversity of the resource. Knowledge about GDSG is essential for the sustainable management of the SPF fishery. The overall aim of the following four projects is 1) to understand the extent and dynamics of GDSG in *T. declivis* (Jack mackerel) and *S. sagax* (Australian sardine), and 2) test the null hypothesis of random interbreeding in the remaining SPF species.

3.7.2. Potential projects

3.7.2.1 Project 1: Genetic stock structure of Blue mackerel (*Scomber australicus*), Yellowtail Scad (*Trachurus noaezelandiae*) and Redbait (*Emmelichthys nitidus*)

Objective

This project would use standard genetic stock structure analyses to test for the presence of separate stocks (and restrictions to gene flow among populations) throughout the area of the small pelagic fishery. The null hypothesis is random interbreeding.

Rationale

The focus of this project are the three SPF species whose population genetic structure has not been analysed previously (Yellowtail Scad, *Trachurus noaezelandiae* and Redbait, *Emmelichthys nitidus*), or has been analysed at the pilot scale only (Blue mackerel, *Scomber australicus*).

Using a hierarchical sampling design, the expectation of genetic subdivision between the Eastern and Western subareas (Figure 1) would be tested, in addition to the assumption of random interbreeding on smaller spatial scales within each of the subareas.

Methods

Population sampling

Tissue samples could be sourced from the fishery (recreational and commercial). Collaboration with, and assistance from, regional fisheries biologists that have ongoing contact with fishers or surveys is essential for sample collection and successful completion of the work. Sampling would be opportunistic and hence slow. Sampling should be initiated as soon as possible and is assumed to have occurred before the commencement of the project.

The project has been costed for the analysis of forty samples (i.e. individual fish) per location with microsatellite markers and ten per location for mtDNA sequence analysis.

The sampling design should be hierarchical, including closely spaced and distantly spaced locations. A location normally corresponds to one sample site or one fishing event, however several sample sites (for example, from recreational catches) can be combined into one sampling location if separated by less than 10km. The minimum number of sampling locations per species is five. Two regions should have two closely spaced sampling locations separated

by hundreds of kilometres (for example, on the New South Wales Coast and on the Western Australian coast). There would need to be one sampling location in another region (for example, in Victoria or South Australia). Increases to the number of sampling locations should be considered when the extent of resources for the project is known; more sampling locations will result in clearer results.

Genetic markers

The project would use standard population genetic markers, mtDNA and microsatellite markers.

New microsatellite markers will be developed for each species taking advantage of next generation sequencing (NGS) technology (e.g. Williams *et al.* Submitted). Species-specific microsatellite loci will streamline laboratory work and minimise artifacts in the data, some of which can mimic the Wahlund effect. Suitable regions from mtDNA will be chosen following whole mitogenome sequencing that is a by-product of the NGS.

Analyses

Standard genetic analyses (*F*-statistics, isolation by distance, Wahlund effect etc.) would be performed.

Collaboration with fisheries biologists is important to incorporate species-specific and regional information into the genetic analyses.

Costs

The project could be performed in three years by a PhD student, or over one year by employing a full time professional officer. The personnel costs are similar; PhD stipend is $28K per year, so for three years this is $84K, and a professional officer would be around $100K.
No specific allowance is made in the budget for sample collection, except for a small amount for postage and limited local costs.

A breakdown of costs is presented in Table 5.

The estimated cost for this project is $183,800.

Outcome

The occurrence of genetic population structure would have direct implications for the assessment of the effect of localised depletion. The presence of fine-scale structure would be

a significant result, as restrictions to gene flow in the target species would reduce the time to recovery following the depletion event. Broader scale population structure, for example where gene flow was restricted through Bass Strait and around southern coastline of Tasmania, would be a risk factor to population recovery after depletion.

3.7.2.2 Project 2: Adaptive genetic markers for determining the genetic stock structure of Blue mackerel (*Scomber australicus*), Yellowtail Scad (*Trachurus noaezelandiae*) and Redbait (*Emmelichthys nitidus*)

Objective

The objective of this project is the same as project 1 (i.e. to test for the presence of separate stocks and restrictions to gene flow among populations), except that 'adaptive' rather than standard genetic markers will be used. The advantage of adaptive compared to standard markers are explained below.

Rationale

Conventional application of population genetics to define fisheries stocks uses genetic markers (loci) that are 'neutral'. This means alleles (genes) are subject to micro-evolutionary forces such as gene flow and genetic drift, but not to natural selection. They are "neutral" with respect to selection. Patterns of allelic variation at neutral loci can be interpreted in terms of gene flow, as genetic drift is low in marine species with large population sizes. This is the primary data about populations that is used to separate them into genetically distinct stocks.

By using "neutral" markers the complicating effect of natural selection of alleles is removed. However, geneticists are now purposely including loci whose alleles may be under selection for studying genetic differences among populations. "Adaptive" loci are those that play a direct role in survival the natural environment. Heat-shock genes, for example, are activated in response to changes in salinity and temperature and even pollution (Mariani & Bekkevold 2014). Alleles at loci like this can be highly variable among populations that span an environmental range, and hence are extremely valuable tools for genetic stock identification studies. Another similar approach is to harness the power of whole genome sequencing to identify classes of loci (also called candidate loci) that may be important to survival, and assay allelic variation in the same way. This approach was recently used by Hemmer-Hansen et al. (2014) for loci associated with life history traits in Atlantic cod (*Gadus morhua*). They used variation to identify conservation units on finer geographical scales than neutral markers.

Interestingly, the use of adaptive loci moves genetic identification of stocks closer to the current *status quo* where many sources of data are combined together in a weight of evidence approach (Izzo *et al.* 2012; Welch *et al.* Submitted). While also reflecting the recent evolutionary history of a population, adaptive genes can respond to local conditions via natural selection over a small number of generations if the selective pressure is high. Epigenetic modifications to genes (such as methylation) switch genes on or off as an organism grows and develops, and these can be assayed using genetic methods (Polanowski et al. 2014).

In this project, adaptive loci will be used to assay the same samples collected for project 1 (section 3.7.2.1). The expectation would be that more genetic variation would be found with adaptive loci. Stocks defined by adaptive markers (adaptive stocks) may coincide spatially neutral markers (neutral stocks), but the spatial definition would be greater. If adaptive and neutral stocks did not coincide, then the results from both studies would be additive leading to even finer scale spatial definitions.

Methods

Population sampling

Tissues collected for project 1 (section 3.7.2.1) could be re-used for this project.

Genetic markers

The project would use adaptive genetic markers.

In project 1 (section 3.7.2.1) NGS data would have been collected for each species. DNA extraction from samples would also have been completed.

SNP assays will be developed for known adaptive genes (e.g. heat shock proteins, Pan I, MHC genes, Mariani & Bekkevold 2014), as well as candidate genes identified by annotating whole genome data from the target species, or by using candidate genes used in other studies (e.g. Hemmer-Hansen et al. 2014; Smith et al. 2013). Adaptive and candidate genes will be selected based on the likelihood that they may be variable over the environmental gradients occupied by SPF species. Bioinformatics support for this will be needed.

Analyses

Advanced genetic analyses suitable for genes under selection would be performed. Advice will be received here from bioinformatisicts.

Collaboration with fisheries biologists is important to incorporate species-specific and regional information into the genetic analyses.

Costs

The project could be performed in three years by a PhD student, or over one year by employing a full time professional officer.

A breakdown of costs is presented in Table 5.

The estimated cost for this project is $243,200.

Outcome

Stocks defined by adaptive markers (adaptive stocks) would allow assessment of the effects of localised depletion in a slightly different way to stocks defined by neutral markers (neutral stocks). Localised depletion within adaptive stocks would signify the removal of individuals that had been demonstrated to be uniquely adapted to their environment. They may be replaced by immigration of less well-adapted individuals, which may or not may survive in the same way as the removed individuals. Immigrants may or may not respond quickly to the selective pressure of the unique environment.

3.7.2.3 Project 3: Re-analysis of data from *T. declivis* (Jack mackerel) by Richardson (1982) and from *S. sagax* (Australian sardine) by Yardin et al (1998)

Objective

This project would use the latest genetic analysis methods to determine the number and genetic composition of populations that may have been sampled from *T. declivis* (Jack mackerel) by Richardson (1982) and from *S. sagax* (Australian sardine) by Yardin et al. (1998) in two previous studies.

Rationale

Three workers concluded that selected SPF species consisted of several genetically distinct stocks that were allopatric (overlapping). As discussed in section 3.7.1.3, they arrived at this conclusion because they found evidence for a Wahlund effect plus a high degree of genetic differentiation between populations.

However, they were unable to convincingly test this hypothesis. Now there are a number of simulation-based analysis methods that could be used (TESS Durand et al. 2009) and (STRUCTURE Pritchard et al. 2000). The methods can determine the number of genetically

distinct populations (k) among a set of samples. The methods also assign samples to populations and graphically display the extent of geographically separate and potentially overlapping stocks (Figure 5).

Re-analysis of the data from studies by Richardson (1982) and Yardin et al. (1998) may result in an outcome like Miller et al. (2013). They demonstrated that populations of *Donax deltoides* (a commercially important bivalve mollusc found in high-energy sandy beaches) were genetically distinct but overlapped in the Bass Strait area (Figure 5).

Methods

Data entry and analysis

The raw genetic data from Richardson (1982), Dixon et al. (1993) and Yardin et al. (1998)[3] needs to be located. If not in electronic format, it would need to be entered into a database and proofread. The analyses would then be implemented.

Costs

A breakdown of costs is presented in Table 5.

The estimated cost for this project is $24,200.

Outcome

The re-analysis may result in a clearer picture of the genetic stock structure of *T. declivis* (Jack mackerel) and from *S. sagax* (Australian sardine). It is a cost-effective option, as it involves no new project work. It may assist with the design of the next project (3.7.2.4). It may provide insight into spatial patterns of dispersal and recruitment that can be used to evaluate the effect of localised depletion.

However, the outcomes of this project would be constrained by the sampling regimes of the original studies and may not be suitable for addressing stock structure on a sufficiently fine scale to assess the effect of localised depletion. Ideally, project 3 should be followed by a full-scale study to maximise the opportunity for genetic analyses to fully address the issue.

[3] Sent email to Roseline Yardin, 7th May 2014. Roseline.Yardin@austrade.gov.au. Email returned – user unknown.

3.7.2.4 Project 4: Test for separate genetic stocks of *Trachurus declivis* (Jack mackerel) and *Sardinops sagax* (Australian sardine) using fisheries independent (early life stages) and dependent samples

Objective

Using neutral and adaptive genetic markers, this project will test for genetically distinct spawner groups (GDSG) (see section 3.7.1.3 for explanation) using fisheries independent and fisheries dependent samples of *Trachurus declivis* (Jack mackerel) and *Sardinops sagax* (Australian sardine). The fisheries independent samples will be taken from spawning grounds as early life stages. The fisheries dependent samples will be taken from the fisheries catch.

Rationale

Ideally, this project should follow on from project 3 where allozyme data on the target species was reanalysed to determine the number of genetic populations, and their likely pattern of overlap. If available, the outcome of project 3 will assist with spatial sampling design in this project.

This project will have two sources of samples: from spawning grounds and from fished populations that are hypothesised to consist of a mixture of two or more genetic stocks.

Genetic stock identification studies on highly mobile species have been more successful when early life stages have been sampled. For example, a review of the genetic population structure studies of Atlantic Bluefin tuna (*Thunnus thynnus*) conducted with samples from the Mediterranean Sea showed that only 60% found evidence of genetic differentiation *(Viñas et al.* 2010). However, Carrlson et al. (2007) sampled young-of-the-year (YOY) from the Mediterranean compared to elsewhere and found significant F_{ST}'s using microsatellite loci. Early life stages will have experienced less movement and less mixing than adults, so it is logical to pursue stock structure studies using them.

This project will compare genetic population structure from two types of samples: (1) early life stages (i.e. embryos and larvae) sampled during daily egg production method (DEPM) surveys (e.g. *S. sagax*, Ward et al. 2011); and (2) fisheries dependent sub-adults and adults. The expectation is that a Wahlund effect will be found in fisheries dependent samples as well as partial evidence for genetic subdivision among populations, confirming previous studies. The signal of genetic subdivision may be more pronounced among early life stages as they reflect GDSG (section 3.7.1.3).

Two types of nuclear loci will be used: microsatellites and adaptive markers. Adaptive markers are described in section 0. The use of adaptive markers is expected to increase the degree of genetic differentiation uncovered among sub-populations. The inclusion of neutral markers provides a point of comparison for the adaptive markers. Adaptive markers cannot be analysed for the Wahlund effect; neutral markers are needed for this component.

Methods

Population sampling

Fisheries dependent samples will be obtained through collaboration with regional fisheries biologists and the fishing industry.

Early life stages will be subsampled from material collected during DEPM surveys. Collaboration will need to be established with DEPM experts (e.g. Tim Ward, Jeremy Lyle) to obtain a survey schedule for the target species as well as to establish procedures for genetic sample collection during the surveys [4]. DEPM surveys are performed opportunistically, and early project planning is essential. DEPM surveys have not been performed for *T. declivis* (Jack mackerel), although the groundwork for their use has been laid (Neira *et al.* Submitted). Although it would be good to include all SPF species in this study, circumstances will dictate which species to include in project 4; for example, *S. australasicus* (blue mackerel) or *E. nitidus* (redbait) could be included if DEPM surveys were underway for these species.

Practically, fisher and survey activity will determine the spatial distribution of both types of samples. The aim should be to achieve the widest possible spatial coverage. Temporal replicates are less important than spatial coverage.

As for project 1, the sampling design should be hierarchical including closely spaced and distantly spaced locations, where possible.

Early life stages of *Trachurus* may need to be identified using molecular methods (Neira *et al.* Submitted) to begin with, followed by routine morphological identification for the bulk of the samples.

Genetic markers

The project would use microsatellite loci and adaptive genetic markers.

[4] Following the submission of this report in draft stage and the release of FRDC research priorities for 2014/15, Tim Ward and the author have agreed that genetic sampling will be included in the DEPM bid for funds.

NGS data will be from each species to support development of new microsatellite markers for each species in the same way as project 1 (section 0). Adaptive markers will be developed in the same way as project 3 (section 0).

Analyses

Analyses would be tailored for the identification of GDSG as well as the detection of population mixing. Standard genetic analyses (*F*-statistics, isolation by distance, Wahlund effect, etc.) would be performed. Advanced genetic analyses suitable for genes under selection would also be performed. Advice will be received here from bioinformatisicts.

Collaboration with fisheries biologists is important to incorporate species-specific and regional information into the genetic analyses.

Costs

This is a large project. Part of it would be suitable for a PhD student, however the total project would require the involvement of a professional officer. Personnel costs included here are a PhD stipend ($28K per year) plus a part-time professional officer for three years.

A breakdown of costs is presented in Table 5.

The estimated cost for this project is $468,900.

Outcome

This project has an excellent chance of providing definitive answers about stock structure for two species in the SPF fishery. Although expensive, it has the greatest likelihood of “success”; it takes advantage of a 2x2x2 factorial design: two species, two sample types and two genetic marker types, and has a dedicated sampling regime tailored to address the hypothesis of GDSG.

If GDSG (see section 3.7.1.3) are found, their location and occurrence will be of critical importance in assessing the likely effect of localised depletion. These groups drive biodiversity and resource abundance as they make essential contributions to the next generation of the species. GDSG will need the highest level of protection against depletion (localised or otherwise) to allow a sustainable future for the temperate ecosystem and the fishing industry that may be based on the resource.

3.8 Conclusion and recommendations

The study has shown that there have been few developments in the use of genetics to define stock structure of the five SPF species since the study by Bulman et al. (2008). The main outcome of this review is the exploration of the Wahlund effect and its interpretation for the SPF species. Despite being a rare feature of population genetic studies, it has been reported for two of the five SPF species. SPF species may be unique in this way, although it remains to be seen from a more extensive literature review how common the Wahlund effect is worldwide. The presence of a Wahlund effect in SPF species may reflect (1) the presence of genetic differentiation in the spatially immense range occupied by the species in southern Australian waters, and (2) mixing of members of different genetic groups at some time during their life cycle.

If a Wahlund effect is demonstrated by the projects suggested herein, the implication is that genetically distinct spawning groups (GDSG) are present. If discovered, one challenge is to understand the biological mechanisms responsible for these groups. Their existence is certainly not consistent with the paradigm of separate populations in separate spatial ranges (see section 3.7.1.2). The GDSG must be reproductively isolated, and some mechanisms are known that permit genetic differentiation in the absence of physical barriers to interbreeding (see section 3.7.1.3). Lack of knowledge about these details is a major impediment to assessing the likely effect of localised depletion; the new projects suggested here are the best starting points.

The genetics projects have been suggested that may lead to positive outcomes for assessing the likelihood of localised depletion in SPF species. They are focussed on resolving the Wahlund conundrum by testing for the presence of GDSG. Applying genetic analyses to spawning stocks is most likely to yield relevant results; samples for this type of project could be cost-effectively obtained alongside egg sampling for DEPM surveys that are currently underway for the majority of SPF species. Beyond taking advantage of future DEPM surveys, there are few (if any) existing tissue sample collections that could be included in future genetic projects. Genetic projects need dedicated sampling regimes that have been designed by experts to address the specific issue. Too often, the spatial scale of sample collection has not matched the scale of stock definition expected by authorities, and consequently the genetic project is deemed to have fallen short (e.g. Mariani & Bekkevold 2014). The projects described here address this issue by the tailored, *de novo* collection of new material.

The projects suggested harness the power of new developments in genetic technology. Projects 1, 2 and 4 require partial (or full) genome sequence data for the target species. Projects 2 and 4 use a new class of genetic marker (adaptive) that provides increased resolution of genetic differentiation among subpopulations of naturally occurring animals. The rationale for not including close-kin analysis (M. Bravington et al, CSIRO pers. comm.) is that it focuses on estimating spawning stock size, not defining stock boundaries.

The starting point for new work would be project 3, which is a re-analysis of allozyme data from previous studies on *T. declivis* (Jack mackerel) and *S. sagax* (Australian sardine). However, the probability of recovering the original data is low. It would be a valuable exercise, however, if feasible.

Projects 1 and 2 examine the genetic stock structure of SPF species that are data poor. The projects are designed to run side-by-side, however they could be done as two stages of a larger project, or as single projects. As project 2 uses adaptive markers, this is more likely to uncover useful information for assessing the effect of localised depletion.

Project 4 aims to clarify the confusing results from previous genetic analyses and single-generation markers about the stock structure of *S. sagax* (Australian sardine), and could ideally include another species such as *T. declivis* (Jack mackerel) for comparison. The resources required for this large project need to be weighed against the commercial and conservation value of the resource. *Sardinops sagax* (Australian sardine) is the largest and potentially most valuable of the five species and occupies a critical position in food webs containing species of conservation (e.g. Australian sea-lions, Little Penguins) and recreational (e.g. billfish and game fish) importance. The assessment of the effect of localised depletion on *S. sagax* (Australian sardine) would be significantly less risk adverse given the outcomes of project 4.

Collaboration with species and regional experts is explicitly included in the design of the four projects, as are workshops to evaluate and disseminate the outcomes of genetic projects. The science of stock identification rests on the shoulders of many types of scientists and other stakeholders, in addition to fisheries geneticists.

The projects outlined above will add scope to management options for the SPF species, but there are many caveats for use of the outcomes in determining susceptibility to localised depletion. The most significant caveat is that resources spent on projects may or may not clarify the situation, not necessarily due to the shortcomings of the technology used, but

because hypotheses need further refinement. Even if GDSG were defined, their susceptibility to localised depletion would be determined on criteria such as overlap with possible depletion events as well as the likely magnitude (in time and space) of the localised depletion. The development of these and other susceptibility criteria should go alongside the genetic projects described here.

4. TABLES AND FIGURES

Table 1 Record of consultation with SPF experts.

Expert person or group	Institution	Method	Date
Expert Panel	Various	Phone conference	27th Feb 2014
Expert Panel	Various	Meeting, Hobart	29th April 2014
Andy Moore	ABARES, Canberra	Phone	11th April 2014, 13th May 2014
Tim Ward	SARDI	Phone	11th April 2014
Jeremy Lyle	UTAS	Phone	14th April 2014
James Andrews	Victorian Fisheries	Phone	15th April 2014
John Stewart	NSW Fisheries	Phone	15th April 2014
Dan Gaughan	WA Fisheries	Phone	15th April 2014
Paul Taylor	Statfishtics, 14 De Vere Cres, Hamilton, New Zealand (ex NIWA)	Skype	6th May 2014

Table 2 A selection of papers published since 2008 on the genetic stock structure of marine species whose distributions fall within the SPF fishery.

Published date	Species	Reference
2013	*Octopus pallidus*	Higgins KL, Semmens JM, Doubleday ZA, Burridge CP (2013) Comparison of population structuring in sympatric octopus species with and without a pelagic larval stage. *Marine Ecology Progress Series* **486**, 203-212.
2013	*Eleutheronema tetradactylum*	Horne JB, Momigliano P, van Herwerden L, Newman SJ (2013) Murky waters: Searching for structure in genetically depauperate blue threadfin populations of Western Australia. *Fisheries Research* **146**, 1-6.
2013	*Retropinna spp*	Hughes JM, Schmidt DJ, Macdonald JI, Huey JA, Crook DA (2014) Low interbasin connectivity in a facultatively diadromous fish: evidence from genetics and otolith chemistry. *Molecular Ecology* **23**, 1000-1013.
2013	*Arripis trutta, A. truttaceus* and *A. georgianus*	Moore GI, Chaplin JA (2013) Population genetic structures of three congeneric species of coastal pelagic fishes (*Arripis*: Arripidae) with extensive larval, post-settlement and adult movements. *Environmental Biology of Fishes* **96**, 1087-1099.
2013	*Jasus edwardsii*	Morgan EMJ, Green BS, Murphy NP, Strugnell JM (2013) Investigation of Genetic Structure between Deep and Shallow Populations of the Southern Rock Lobster, *Jasus edwardsii* in Tasmania, Australia. *PLoS ONE* **8**.
2013	*Hoplostethus atlanticus*	Varela AI, Ritchie PA, Smith PJ (2013) Global genetic population structure in the commercially exploited deep-sea teleost orange roughy (*Hoplostethus atlanticus*) based on microsatellite DNA analyses. *Fisheries Research* **140**, 83-90.
2012	*Glaucosoma hebraicum*	Berry O, England P, Fairclough D, Jackson G, Greenwood J (2012) Microsatellite DNA analysis and hydrodynamic modelling reveal the extent of larval transport and gene flow between management zones in an exploited marine fish (*Glaucosoma hebraicum*). *Fisheries Oceanography*, **21**, 243-254.
2012	*Centrophorus harrissoni* and *C. isodon*	Daley RK, Appleyard SA, Koopman M (2012) Genetic catch verification to support recovery plans for deepsea gulper sharks (genus *Centrophorus*, family Centrophoridae) - an Australian example using the 16S gene. *Marine and Freshwater Research* **63**, 708-714.
2011	*Seriola lalandi*	Miller PA, Fitch AJ, Gardner M, Hutson KS, Mair G (2011) Genetic population structure of Yellowtail Kingfish (*Seriola lalandi*) in temperate Australasian waters inferred from microsatellite markers and mitochondrial DNA. *Aquaculture*, **319**, 328-336.

2011	*Mytilus galloprovincialis*	Nguyen TTT, Hayes BJ, Guthridge K, Ab Rahim ES, Ingram BA (2011) Use of a microsatellite-based pedigree in estimation of heritabilities for economic traits in Australian blue mussel, *Mytilus galloprovincialis*. *Journal of Animal Breeding and Genetics*, **128**, 482-490.
2011	*Macquaria colonorum*	Shaddick K, Gilligan DM, Burridge CP, Jerry DR, Truong K, Beheregaray LB (2011) Historic divergence with contemporary connectivity in a catadromous fish, the estuary perch (*Macquaria colonorum*). *Canadian Journal of Fisheries and Aquatic Sciences*, **68**, 304-318.
2011	*Lutjanus carponotatus*	Veilleux HD, van Herwerden L, Evans RD, Travers MJ, Newman S (2011) Strong genetic subdivision generates high genetic variability among eastern and western Australian populations of *Lutjanus carponotatus* (Richardson). *Fisheries Research (Amsterdam)*, **108**, 74-80.
2010	*Scylla serrata*	Fratini S, Ragionieri L, Cannicci S (2010) Stock structure and demographic history of the Indo-West Pacific mud crab *Scylla serrata*. *Estuarine Coastal and Shelf Science*, **86**, 51-61.
2010	*Acanthopagrus australis*	Roberts DG, Ayre DJ (2010) Panmictic population structure in the migratory marine sparid *Acanthopagrus australis* despite its close association with estuaries. *Marine Ecology Progress Series*, **412**, 223-230.
2009	*Haliotis rubra*	Miller KJ, Maynard BT, Mundy CN (2009) Genetic diversity and gene flow in collapsed and healthy abalone fisheries. *Molecular Ecology*, **18**, 200-211.
2009	*Lethrinus miniatus* and *Lutjanus sebae*	van Herwerden L, Aspden WJ, Newman SJ, Pegg GG, Briskey L, Sinclair W (2009) A comparison of the population genetics of *Lethrinus miniatus* and *Lutjanus sebae* from the east and west coasts of Australia: Evidence for panmixia and isolation. *Fisheries Research*, **100**, 148-155.
2008	*Hyperoglyphe antarctica, Seriolella brama* and *Seriolella punctata*	Robinson N, Skinner A, Sethuraman L, McPartlan H, Murray N, Knuckey I, Smith DC, Hindell J, Talman S (2008) Genetic stock structure of blue-eye trevalla (*Hyperoglyphe antarctica*) and warehous (*Seriolella brama* and *Seriolella punctata*) in south-eastern Australian waters. *Marine and Freshwater Research*, **59**, 502-514.

Table 3 Summary of key population genetic studies on SPF species.

Species	Spatial range (and number, N) of sampled populations	Genetic marker	Fst range	Notes	Reference
Blue mackerel (*Scomber australicus*)	Australia; WA, SE Qld, NZ. N=3	mtDNA, control region sequence, 345bp	0.09 – 0.13	SE Qld and NZ similar, but both differ from WA.	Schmarr et al (2007, 2012).
	Taiwan; N=4	Nuclear DNA, microsatellites, six loci.	0.007	Some support for separation of stocks to north and south of Taiwan.	Tzeng et al (2009)
Jack mackerel (*Trachurus declivis*)	Australia; NSW, Vic, Tas, WA. N=22	Nuclear DNA, allozymes, six loci (*GPI, EST, IDH, ACON, SORD, ADA, PEP-A* and *PEP-B*)	NA	Strong evidence for several genetic populations in southern Australia, possibly having overlapping distributions dependent on life-history stage (Wahlund). Loci assayed were *Gpi, Est, Icd, Acon, Sord, Ada, Pep A* and *Pep B*).	Richardson (1982)
	Australia; NSW, Tas; N=2	mtDNA, restriction enzyme analysis of whole genome.	NA	Supported allozyme study of Richardson.	(Smolenski *et al.* 1994)
Yellowtail Scad (*Trachurus noaezelandiae*)	No studies	-	-	-	-
Redbait (*Emmelichthys nitidus*)	No studies	-	-	-	-
Australian sardine (*Sardinops sagax*)	Australia: NSW, N=2; Vic, N=2; SA, N=1; WA, N=8. South Africa, N=1	Nuclear DNA, allozymes, six loci (*AAT, AH, EST-4, MPI, PEP B* and *PGM*)	NA	Found evidence for a series of quasi-independent genetic populations around the southern coast of Australia. Proposed mixing occurs between populations.	(Dixon *et al.* 1993)
	Australia: NSW, SA and SWA; N=3	mtDNA, restriction enzyme analysis of control region.	NA	Focus on worldwide evolutionary relationships. Little data on three Australian populations.	(Okazaki *et al.* 1996)

Species	Spatial range (and number, N) of sampled populations	Genetic marker	Fst range	Notes	Reference
	Australia: SE Qld, NSW, Vic, SA; N=23 New Zealand; N=1	Nuclear DNA, allozymes, six loci (*AAT, AH, EST-4, MPI, PEP B* and *PGM*).	0.066 to 0.162	Strong evidence for several genetic populations in southern Australia, possibly having overlapping distributions dependent on life-history stage (Wahlund effect). MtDNA (amplified control region) was analysed with restriction enzymes, but results were poor and replaced with sequencing, but results are not present in report. Loci assayed were *Aat-1, Ah-1, Est-4, Mpi-1, Pep B* and *Pgm-1.*	(Yardin *et al.* 1998)

Table 4 The number of species that were tested for the presence of a Wahlund effect (Wahlund test) and the number that tested positive (Wahlund Found) among population genetic studies on Australian species (All species) from Pope et al. (In preparation). The taxonomic unit is arbitrary, but generally is Class and in some cases Phylum or Order. Units Asteroidea, Holothuroidea and Echinoidea from Pope et al. (In preparation) are grouped here to Echinoderm, and Phaeophyceae and Rhodophyceae are grouped here to Algae.

Taxonomic Unit	Common Name	All species	Wahlund Test	Wahlund Found
Actinopterygii	Finfish	41	8	4
Anthozoa	Corals	10	6	5
Gastropoda	Snails	6	6	2
Mammalia	Mammals	6	0	-
Echinoderm	Invertebrates with tube feet	9	6	0
Malacostraca	Crabs, Prawns, Lobsters	5	1	0
Chondrichthyes	Sharks, Rays, Chimeras	8	0	-
Reptilia	Turtles, Snakes	2	0	-
Bivalvia	Mussels, Clams	3	3	0
Algae	Red and Brown	3	2	2
Angiosperm	Plants	2	1	0
Scyphozoa	Jellyfish	1	0	-
Granuloreticulosea	Amoebas	1	0	-
Cephalopoda	Octopi, Squids	2	2	0
Aves	Bird	1	1	0
Calcarea	Sponges	2	2	0
Ascidiacea	Tunicates	1	0	-
Brachiopod	Lamp shells	1	0	-
Total		**104**	**38**	**13**

Table 5 Estimated project costs for Projects 1, 2, 3 and 4.

Project 1: Genetic stock structure of Blue mackerel (*Scomber australicus*), Yellowtail Scad (*Trachurus noaezelandiae*) and Redbait (*Emmelichthys nitidus*).

Category	Details	Cost (,000)	Comments
Personnel	PhD stipend (three years)	$84.0	Project is costed with prof officer over one year, but could equally be done over three years by PhD student
	Professional Officer	$96.0	$75K pa, plus 28% on costs.
Equipment	Minor lab equipment	$5.0	Covers items like pipettors, mini-centrifuges, repairs
	Major lab equipment	$0.0	Assumes lab is equipped.
Consumables	Sampling costs	$6.0	Goes to regional collaborator for direct costs of sampling, tubes, regional travel, postage, $2K per species. Aim for five sampling locations per species.
	DNA extraction	$3.0	$5 per sample, number of samples per species 40x5=200, three species, total number of samples = 3x40x5=600
	mtDNA sequencing	$1.5	$10 per sample, number of samples per species 10x5=50, three species, total samples = 3x50=150
	NGS	$12.0	Next generation sequencing (NGS) per species for mitogenome sequence and microsat marker development, $4K per species
	Marker development	$4.5	Lab testing microsat and mtDNA primers, $1.5K per species.
	Microsatellite genotying	$10.8	$18 per sample, total samples = 600
	Lab consumables	$6.0	Covers gloves, pipette tips, tubes, plates, chemicals, $10 per sample, total samples = 600
Travel	Workshops	$15.0	Workshop with collaborators and other stakeholders at end of project to discuss and extend results, one per species, $5K per workshop.
Project cost		$159.8	
Overheads	University	$24.0	Around 15% of project cost, assumes funding comes from agency exempt from University recovery of indirect costs (e.g. FRDC).
Project total		**$183.8**	

Project 2: Adaptive genetic markers for determining the genetic stock structure of Blue mackerel (*Scomber australicus*), Yellowtail Scad (*Trachurus noaezelandiae*) and Redbait (*Emmelichthys nitidus*).

Category	Details	Cost (,000)	Comments
Personnel	PhD stipend	$84.0	Project is costed with prof officer over one year, but could equally be done over three years by PhD student
	Professional Officer	$96.0	$75K pa, plus 28% on costs.
Equipment	Minor lab equipment	$5.0	Covers items like pipettors, mini-centrifuges, repairs
	Major lab equipment	$0.0	Assumes lab is equipped.
Consumables	Sampling costs	$0.0	Sampling completed in project 1
	DNA extraction	$0.0	DNA extraction completed in project 1
	mtDNA sequencing	$0.0	Not used in this project
	NGS	$0.0	Next generation sequencing (NGS) completed for each species in project 1.
	Bioinformatics	$25.0	Outsourced to QFAB (http://www.qfab.org) for example. Guessestimate. Could be less.
	SNP assay development	$4.5	Lab testing of SNP assays, $1.5K per species.
	SNP genotying	$60.0	$100 per sample, total samples = 600. Guessestimate until SNP assay is determined. Could be much less per sample.
	Lab consumables	$6.0	Covers gloves, pipette tips, tubes, plates, chemicals, $10 per sample, total samples = 600
Travel	Workshops	$15.0	Workshop with collaborators and other stakeholders at end of project to discuss and extend results, one per species, $5K per workshop.
Project cost		$211.5	
Overheads	University	31.725	Around 15% of project cost, assumes funding comes from agency exempt from University recovery of indirect costs (e.g. FRDC).
Project total		**$243.2**	

Project 3 Re-analysis of data *T. declivis* (Jack mackerel) by Richardson et al. (1982) and from *S. sagax* (Australian sardine) by Yardin et al. (1998).

Category	Details	Cost (,000)	Comments
Personnel	PhD stipend	$0.0	Project would be short term.
	Professional Officer	$16.0	Costed at 2 mo. of a full time salary. $75K pa, plus 28% on costs.
Equipment	Minor lab equipment	$0.0	Covers items like pipettors, mini-centrifuges, repairs
	Major lab equipment	$0.0	Assumes lab is equipped.
Consumables	Sampling costs	$0.0	Goes to regional collaborator for direct costs of sampling, tubes, regional travel, postage, $2K per species
	DNA extraction	$0.0	$5 per sample, number of samples per species 40x5=200, three species, total number of samples = 3x40x5=600
	mtDNA sequencing	$0.0	$10 per sample, number of samples per species 10x5=50, three species, total samples = 3x50=150
	NGS	$0.0	Next generation sequencing (NGS) per species for mitogenome sequence and microsat marker development, $3K per species
	Marker development	$0.0	Lab testing microsat and mtDNA primers, $1.5K per species.
	Microsatellite genotying	$0.0	$18 per sample, total samples = 600
	Lab consumables	$0.0	Covers gloves, pipette tips, tubes, plates, chemicals, $10 per sample, total samples = 600
Travel	Workshops	$5.0	Workshop with collaborators and other stakeholders at end of project to discuss and extend results. One workshop, $5K per workshop.
Project cost		$21.0	
Overheads	University	$3.2	Around 15% of project cost, assumes funding comes from agency exempt from University recovery of indirect costs (e.g. FRDC).
Project total		**$24.2**	

Project 4 Test for separate genetic stocks of Trachurus declivis *(Jack mackerel) and* Sardinops sagax *(Australian sardine)* using fisheries independent (early life stages) and dependent samples.

Category	Details	Cost (,000)	Comments
Personnel	PhD stipend	$84.0	PhD student to work full time on the project. Stipend is $28K per year.
	Professional Officer	$172.8	Costed at three days per week for three years. Full time rate is $75K pa, plus 28% on costs.
Equipment	Minor lab equipment	$5.0	Covers items like pipettors, mini-centrifuges, repairs
	Major lab equipment	$0.0	Assumes lab is equipped.
Consumables	Fisheries dependent sampling costs	$4.0	Goes to regional collaborators for direct costs of sampling, tubes, regional travel, postage, $2K per species. Aim for five sampling locations per species.
	Fisheries independent sampling costs	$20.0	Goes to DEPM survey leaders for direct costs of sampling, tubes, regional travel, postage, $5K per species. Aim for five sampling locations per species. Includes allowance for molecular identification of Trachurus early life stages ($5K) and labour for egg sorting ($5K).
	DNA extraction	$40.0	$5 per sample, Number of samples per species 40x5=200, for two species = 2x40x5=400. Two types of samples; dependent and independent. Total number of samples = 2x400=800
	mtDNA sequencing	$0.0	Not used in this project
	NGS	$8.0	Next generation sequencing (NGS) per species for microsat and adpative marker development, $4K per species
	Bioinformatics	$30.0	Outsourced to QFAB (http://www.qfab.org) for example. Guessestimate. Could be less.
	SNP assay development	$4.5	Lab testing of SNP assays, $1.5K per species.
	SNP genotying	$80.0	$100 per sample, total samples = 800. Guessestimate until SNP assay is determined. Could be much less per sample.
	Marker development	$3.0	Lab testing microsat primers, $1.5K per species.
	Microsatellite genotying	$14.4	$18 per sample, total samples = 800
	Lab consumables	$16.0	Covers gloves, pipette tips, tubes, plates, chemicals, $10 per sample, total samples = 600
Travel	Workshops	10	Workshop with collaborators and other stakeholders at end of project to discuss and extend results, one per species, $5K per workshop.
Project cost		$407.7	

Category	Details	Cost (,000)	Comments
Overheads	University	$61.2	Around 15% of project cost, assumes funding comes from agency exempt from University recovery of indirect costs (e.g. FRDC).
Project total		**$468.9**	

Figure 1 Map of the Small Pelagic Fishery including Zones, © *Commonwealth of Australia 2005* (http://www.afma.gov.au/managing-our-fisheries/fisheries-a-to-z-index/small-pelagic-fishery/maps/)

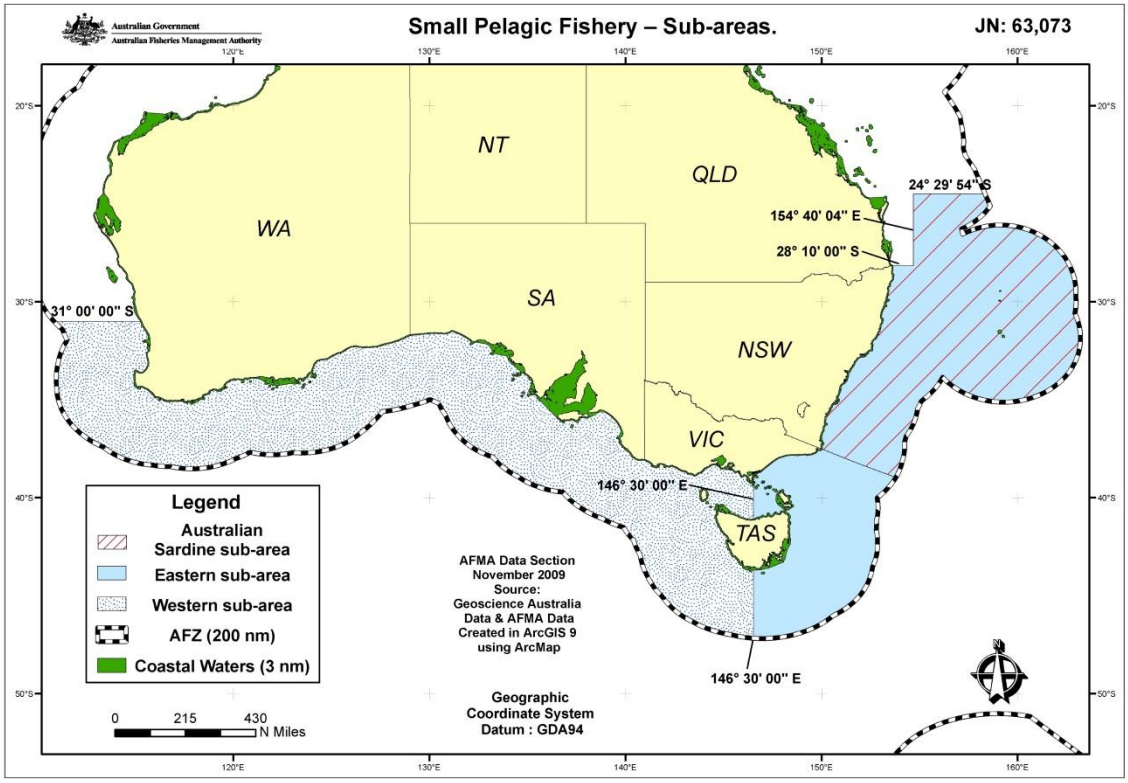

Figure 2 Map of worldwide distribution of *Trachurus* species from (Cardenas et al. 2005) showing overlap in distribution of *T. declivis* and *T. novaezelandiae*, and the likely vagrant status in Australia of *T. murphyi*.

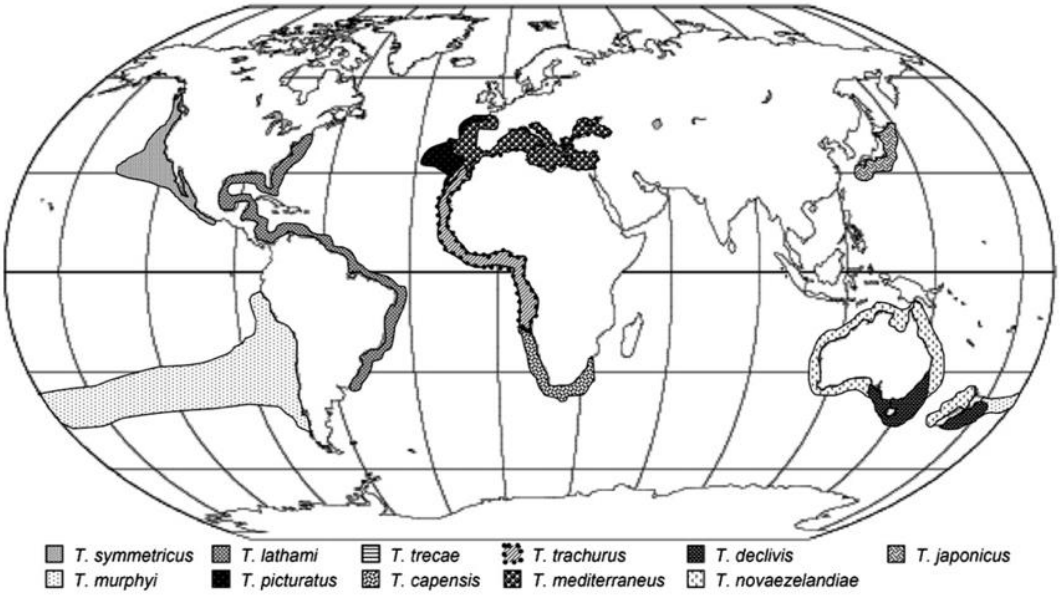

Figure 3 Cut and paste of Figures 3.1 and 7.1 from Izzo et al (2012). Fig 3.1 shows spatial boundaries of *Sardinops sagax* proposed from two genetic studies; green (Dixon et al. 1993) and brown (Yardin et al. 1998). Part B of figure 3.1 shows the consensus stock structure of the species proposed by Whittington et al. (2008) for sampling for disease detection. Fig 7.1 shows stock delineation following integration of available data by Izzo et al. (2012).

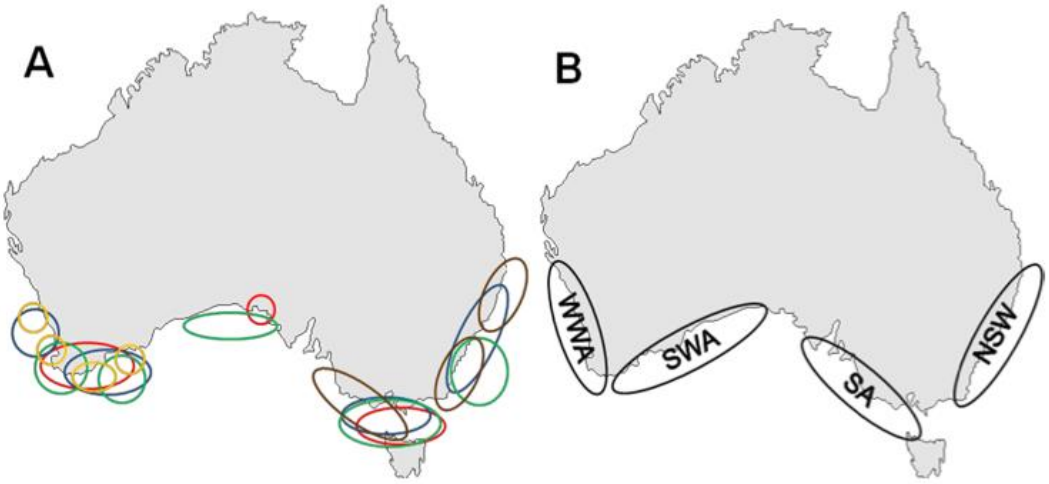

Figure 3.1 Proposed sub-populations of the Australian sardine throughout southern Australia from selected research, including: (A) red = Blackburn (1951); blue = Syahailatua (1992); green = Dixon et al. (1993); orange = Edmonds and Fletcher (1997); and brown = Yardin et al. 1998; and (B) adapted from Whittington et al. (2008) and NSW = south-eastern Queensland/northern New South Wales; SA = Victoria/South Australia; SWA = south coast Western Australia; WWA = west coast Western Australia.

Figure 7.1 Map of Australia showing the approximate boundaries separating the stock units of the sardine. Boundaries are approximate due to limitations of collating literature that varies in both temporal and spatial sampling scales; which may also vary temporally. However, these boundaries conform to the major fishing regions of the species. The dotted line separating Tasmania and main land Australian indicates the uncertainty around the Tasmanian stocks.

Figure 4 A simple biological model of a fisheries stock (after King 2007).

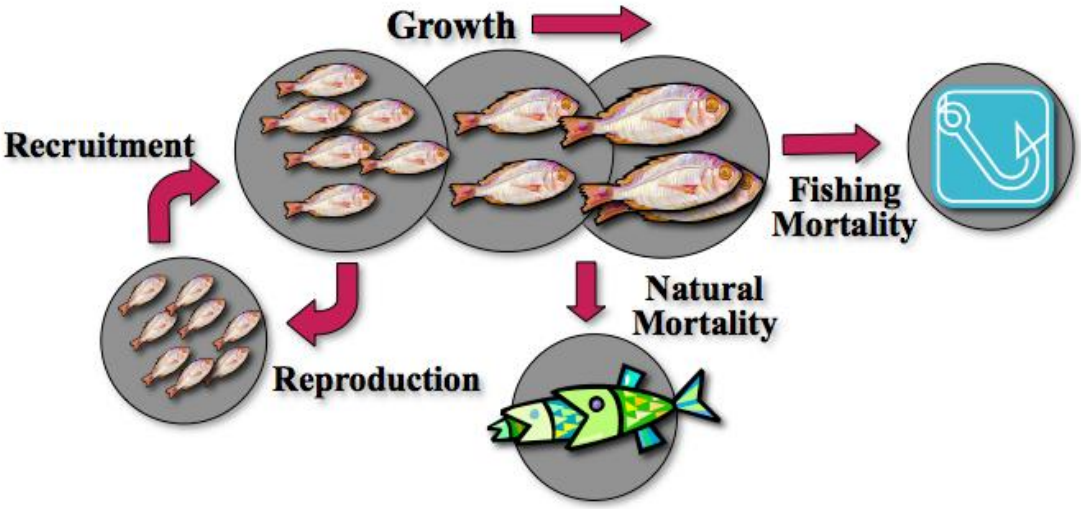

Figure 5 Graphical displays of geographically separate genetically distinct populations in the west (on the left; red above, blue below) and east (on the right; green above, red below), as well as overlapping (admixed) regions (centre; red and green, above; blue and red, below). The figure is taken from the genetic analysis by Miller et al. (2013) of an intertidal mollusc species sampled along the coastline of South Australia, Victoria and New South Wales. The area of where populations are overlapping or admixed is the coastline adjacent to Bass Strait.

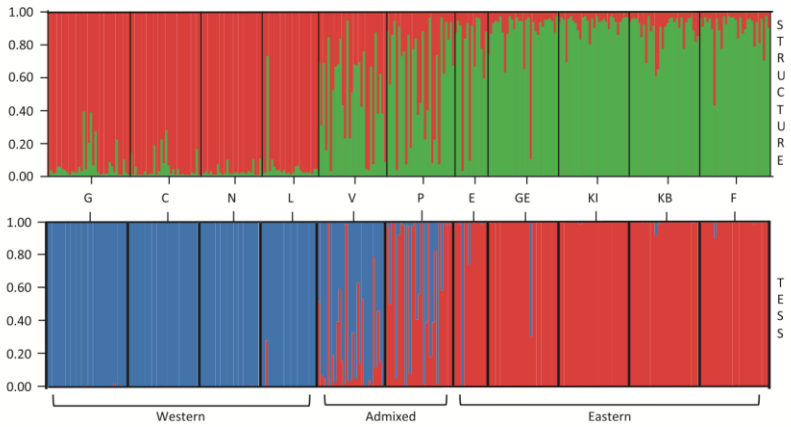

Figure 4. Structure and TESS summary plots of the estimated membership coefficient (*y* axis) for each individual in each two population clusters. Each individual is represented by a single vertical line broken into segments, where segments are proportional to the membership coefficient for each of the population clusters. Individuals are arranged into sites from which they were sampled following the order given in Table 1, and sites are pooled into regions (western, admixed, eastern).

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