# Increasing knowledge of Victoria's growing recreational yellowtail kingfish fishery

Recreational Fishing Grants Program

Image credit: Scott Gray

Your fishing licence fees at work

VICTORIA State Government

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Recreational Fishing Grants Program

**January 2020**

**Recreational Fishing Grants Program Research Report Project No. 14/15/Large/34.**

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# Contents

# Table of Figures

# Table of Tables

# Executive summary

Prior to the early 1990's, yellowtail kingfish (*Seriola lalandi*) were commonly caught by recreational anglers at various offshore locations across Victoria (VIC), primarily in the warmer months. While the historic abundance of yellowtail kingfish in VIC waters was not as high as in New South Wales (NSW) waters, the fish were often very large (up to 30+ kg), particularly around the entrance to Port Phillip Bay ('The Rip'). During the mid-1990's both the number and size of yellowtail kingfish taken by recreational anglers in VIC waters decreased dramatically. This was also observed in the commercial sector where 2–12 tonnes were landed between 1980 and 1993, which then dramatically dropped to <1 tonne per year for almost two decades. Consequently, interest in targeting yellowtail kingfish declined. The low availability of yellowtail kingfish to VIC anglers continued until about 2010 when exceptional yellowtail kingfish catches during summer and autumn were reported. It is clear from the resurgence of targeted recreational fishing that their availability has increased considerably over the last decade.

The reasons for the recent increased availability are unclear, and it is a shared goal for managers and anglers alike, that the current great fishing for yellowtail kingfish in VIC waters is sustained for the long-term. Uncertainties included a limited knowledge of:

1. yellowtail kingfish stock structure around southern and eastern Australia,
2. migratory dynamics and residency behaviour of yellowtail kingfish in VIC waters,
3. length and age composition and basic biological characteristics (growth, reproduction) of yellowtail kingfish occurring in VIC waters.

This project aimed to apply multiple approaches to improve understanding of the yellowtail kingfish fishery in VIC including; genetics, biological sampling, otolith chemistry and a trial of satellite tags. Additional components of the study included a review of historical records of recreationally caught yellowtail kingfish and the potential for marine stocking of the species in VIC waters.

Results from the genetic component of this study confirmed a separate genetic stock of yellowtail kingfish in WA and supported a single stock hypothesis across eastern and south-eastern Australian waters. However, limited sample numbers were available from NSW, and SA was not included in the sampling program. Minor regional genetic variation was detected using the most powerful genetic methods; microsatellites and single nucleotide polymorphisms (SNPs). A properly structured and resourced genetics study using SNPs is recommended to provide definitive conclusions on population structure around southern and eastern Australia, and importantly to identify likely reproductive sources of yellowtail kingfish found in VIC waters.

Growth rates and length/age at maturity for yellowtail kingfish in VIC were generally consistent with studies from NSW. Length/age compositions of recreationally caught fish in VIC varied between the eastern, central and western regions of the State, with greater proportions of larger/older fish present in eastern and western VIC waters. However, across all three regions most fish were less than 100 cm total length, less than 6 years of age, and immature. No evidence of spawning fish was found, although females with developed gonads were observed. The dominance of smaller fish in the contemporary fishery is not consistent with the historical records and anecdotally fish greater than 100 cm and 10+ kg were very common prior to the early 1990's. The current fishery is likely to be in a recovery phase driven by improved management, reduced harvest and higher recruitment over the last decade, and it's therefore likely that high fishing mortality (particularly of juvenile yellowtail kingfish in NSW), and sustained poor recruitment likely influenced the decline of yellowtail kingfish in VIC in the early 1990's rather than an abrupt change in their migratory patterns or habitat preference.

One yellowtail kingfish in this study was successfully tagged with a satellite tag. The fish moved from near Portland in western VIC, east through Bass Strait to near Flinders Island, over a 69-day period from April–June 2017. Unfortunately, two other tag releases failed, due to a combination of equipment failure and possible seal predation. Satellite tags can be deployed successfully on yellowtail kingfish, but they are expensive, and exploration of acoustic tagging is recommended to further explore migration patterns, taking advantage of the Integrated Marine Observing System receiver network around southern Australia.

A trial of otolith stable isotopes in this study, in particular the oxygen isotope ratio ($^{18}O/^{16}O$) supported the application of this technique to expanded studies of thermal history and population structure. The results supported the hypothesis that VIC yellowtail kingfish start their lives in warmer waters outside of VIC, most likely along the eastern seaboard off NSW, but spend more time in cooler waters as they get older. This is consistent with a model of seasonal migration between NSW and VIC and habitat suitability.

Finally, a review of stocking potential indicated that hatchery production of yellowtail kingfish is well established in Australia. However, due to the temperature preferences of the species, and their known summer–autumn occurrence in VIC waters it is likely they would migrate out of VIC waters before they reach the legal minimum length. As such, benefits to VIC anglers would be expected to be limited, and heavily dependent on uncertain return migrations of stocked fish.

# Introduction


Image credit: Scott Gray

Yellowtail kingfish (*Seriola lalandi*) are a relatively large pelagic fish widely distributed throughout the Pacific and Indian Oceans (Nugroho, Ferrell et al. 2001, Sepúlveda and González 2016) and are commonly associated with reefs and near-shore topographic features (Dempster and Kingsford 2003, Hobday and Campbell 2009). In Australia, yellowtail kingfish are found from southern Queensland to the central coast of Western Australia, the east coast of Tasmania, and around Lord Howe and Norfolk Islands (Hughes, Malony et al. 2018). Genetically, kingfish from Western Australia are different to those found around the east coast of Australia and New Zealand (Miller, Fitch et al. 2011). Yellowtail kingfish are a commercially important species with landings from waters of the Commonwealth, Queensland and New South Wales that totalled approximately 120 t in 2017, but have historically exceed 500 t per year during the 1980s (Hughes, Malony et al. 2018). Similarly, culturing of this species is a rapidly growing sector (Tanner and Fernandes 2012, Savage and Hobsbawn 2015), particularly in South Australia where 889 t worth $11.26 million was produced in 2012/13. Although kingfish have a significant commercial value in Australia they are also highly regarded as a recreational species where they are targeted predominantly off South Australia, Victoria, Tasmania and New South Wales.

In Victoria, access to recreational species in marine systems has changed considerably over the past decade. Anglers traditionally targeting species like snapper (*Chrysophrys auratus*) and King George whiting (*Sillaginodes punctatus*) are now also targeting larger gamefish species such as southern bluefin tuna (*Thunnus maccoyii*; Green, Brown et al. 2012), shortfin mako sharks (*Isurus oxyrinchus*) and broadbill swordfish (*Xiphias gladius*). Similarly, targeting of yellowtail kingfish is becoming increasingly popular and it is now a highly sought recreational species, mainly due to their fighting ability and eating quality. Historically (prior to the early 1990's), yellowtail kingfish were caught by recreational anglers at various locations across Victoria including Mallacoota, Wilsons Promontory, Port Phillip Heads and Portland. While the number of yellowtail kingfish taken was not as high as those from other states (particularly NSW), fish were sometimes very large (up to 30+ kg). From the mid 1990's both the number and size of yellowtail kingfish taken decreased dramatically. Consequently, interest in targeting yellowtail kingfish declined with the declining chances of catching one. An estimated 258 tonnes of yellowtail kingfish were caught by recreational fishers in Australia during 2000/01 with less than 1 t estimated to have been taken in Victoria (Henry and Lyle 2003).

Many fishers hypothesised on the cause of decline, but the actual cause is unclear. Since around 2010, reports of recreationally caught yellowtail kingfish from a wide size range have increased greatly with many anglers now targeting yellowtail kingfish at several locations throughout Victoria. Although there are no long-term monitoring data on yellowtail kingfish abundance in Victorian, it is clear from the resurgence of targeted recreational fishing that their availability in Victorian waters has increased considerably over the last decade. The reasons for this increased availability, and whether it will be sustained long-term, are unclear but may relate to broader stock dynamics/productivity and or shifts in spatial distribution or migratory patterns.

In Victoria, commercial take of kingfish is regulated under the Victorian Fisheries Authority Act 2016 and Fisheries Regulations 2009 where they can be taken within the Gippsland Lakes, Corner Inlet, Port Phillip and Westernport, Inshore trawl, and Ocean Fisheries. There are no catch limits that restrict the quantity of yellowtail kingfish that can be landed commercially; however, limited entry and gear restrictions apply for the commercial sector. Yellowtail kingfish are predominantly caught as a bycatch by commercial operators in Victoria. Only 200 kg of catch was reported during 2017 (VFA catch statistics). While there are no recent estimates of recreational catches, it will be a focus of the new Go Fish Vic app and it is reasonable to assume that the current catch and socio-economic value is considerably higher for the recreational sector, especially in Port Phillip Bay.

The objective of fisheries assessment is largely to provide managers and other stakeholders with advice as to the status of exploited fish stocks, the level of fishing pressure on the stocks and to evaluate the consequences of alternative management actions (Punt and Hilborn 1997) with a view to maintaining sufficient biomass that promotes sustainability and meets specified objectives for fishery performance. For the Victorian recreational fishery, anglers are allowed to have a bag/possession limit of 5 yellowtail kingfish greater than or equal to 60 cm

total length; however, the biological basis for such regulation is limited. In Victoria, fundamental stock structure and biological information to support stock assessment is limited for yellowtail kingfish. As increases in numbers caught and targeted recreational effort has become evident, understanding key biological and stock structure information is required to provide choice of alternative management options. Information such as spatial stock structure, temporal (i.e. seasonal) distribution patterns, spawning age, and growth data are basic knowledge requirements to underpin stock assessments and development of ongoing data collection programs. Further, knowledge of stock structure is critical to defining how fishing activities and data sources from other State jurisdictions relate to the dynamics of yellowtail kingfish populations and fisheries in Victorian waters. Greater understanding of the influences on productivity of the fishery will improve the capacity to alter management strategies to ensure yellowtail kingfish are fished sustainably and continue to support a valuable recreational fishery.

# Objectives


Image credit: Jason Taylor

The Victorian Fisheries Authority (VFA) is the State agency responsible for sustainably managing Victoria's fish resources in accordance with the *Victorian Fisheries Authority Act 2016*, *Fisheries Regulations 2009* and relevant policies of the Victorian Government. The VFA has developed goals and objectives for managing wild fisheries (both commercial and recreational) and has implemented scientific programs under which fisheries data are collected and stock condition is assessed. The overarching objective of scientific programs is to provide fisheries managers with evidence needed to determine whether the biomass (or biomass proxy) is at a level sufficient to ensure that, on average, future levels of recruitment are adequate (i.e. not recruitment overfished) and for which fishing pressure is adequately controlled to avoid the stock becoming recruitment overfished[1].

Much research has been conducted on yellowtail kingfish in other states of Australia for assessing biological parameters, biomass and harvest rates (Gillanders, Ferrell et al. 1999, Henry and Lyle 2003, Stewart, Ferrell et al. 2004, Stewart and Hughes 2008) as well as refining techniques for aquaculture of the species (Moran, Gara et al. 2007, Miegel, Pain et al. 2010, Ma 2014, Savage and Hobsbawn 2015). However, for Victorian caught yellowtail kingfish, little is known of their basic biological and population characteristics; therefore, objectives of this project are aligned to meet some of the present knowledge gaps. Objectives of this project are divided between two programs based on their funding source through the Victorian Fisheries Authority's Recreational Fishing Licence Grant Program and the Victorian Governments Target One Million campaign that aims to increase participation in recreational fishing.

**Recreational Fishing Grants Program Objectives**

1. To determine whether yellowtail kingfish caught in Victorian waters are from a single or multiple stock complex using genetic markers.
2. Define population characteristics (age and growth, size structure, spawning characteristics) of Victorian yellowtail kingfish.
3. Determine the future potential of this fishery using historical recreational catch information.
4. To trial the use of satellite tags as one method to understand movement characteristics (spatial, depth, temperature preference) of yellowtail kingfish.
5. To trial otolith chemistry (stable isotope) analyses as a method for investigating yellowtail kingfish temperature preference and population structure.

**Fisheries Victoria Target One Million Objectives**

6. To determine the suitability of yellowtail kingfish for marine stocking.

[1] Recruitment overfished refers to a point where the spawning biomass has been reduced to a level where average recruitment starts to decline.

# Methods and results

## Sample Collection

*The collection of samples is relevant to meeting Objectives 1, 2 & 5*

### Victoria

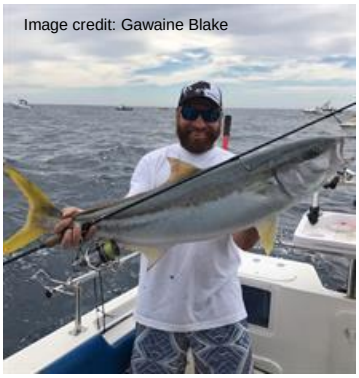
Image credit: Gawaine Blake

Yellowtail kingfish are distributed all along Victoria's coast (Hughes, Malony et al. 2018); however, key regions within the state anecdotally hold greater numbers. Changes in their distribution along Victoria's coast may be a function of habitat suitability, oceanographic variables, food availability and local environmental conditions. Captures of yellowtail kingfish may also be reflective of angler accessibility. In Victoria, key regions including Portland / Port Fairy (Western Victoria), Port Phillip Heads (Central Victoria), and Mallacoota / Marlo (Eastern Victoria) periodically hold large numbers of yellowtail kingfish across a broad size range. Consequently, three 'zones' denoted; 'Western', 'Central' and 'Eastern' were targeted to obtain representative samples across the state (Figure 1). Creel clerks were employed to visit boat ramps and collect yellowtail kingfish frames from recreational anglers and charter boat operators. Local fishing shops were used as 'drop off' stations for anglers to donate frames when creel clerks were not available. A total of 452 yellowtail kingfish frames were donated to the VFA by recreational anglers or caught during targeted research trips between 02 February 2015 – 01 February 2017 (Table 1). As all recreationally caught kingfish frames were above the legal minimum length a Fisheries Research Permit (RP1253 - Appendix) was issued for selected fishers to obtain undersized yellowtail kingfish.

### Interstate

Yellowtail kingfish were also collected as a part of separate monitoring and targeted research projects in New South Wales (NSW Fisheries) and Tasmania (Institute for Marine and Antarctic Studies / University of Tasmania) respectively. Tissue samples for genetic analysis, and otoliths for isotope analysis were kindly donated to the VFA for use within the present project.

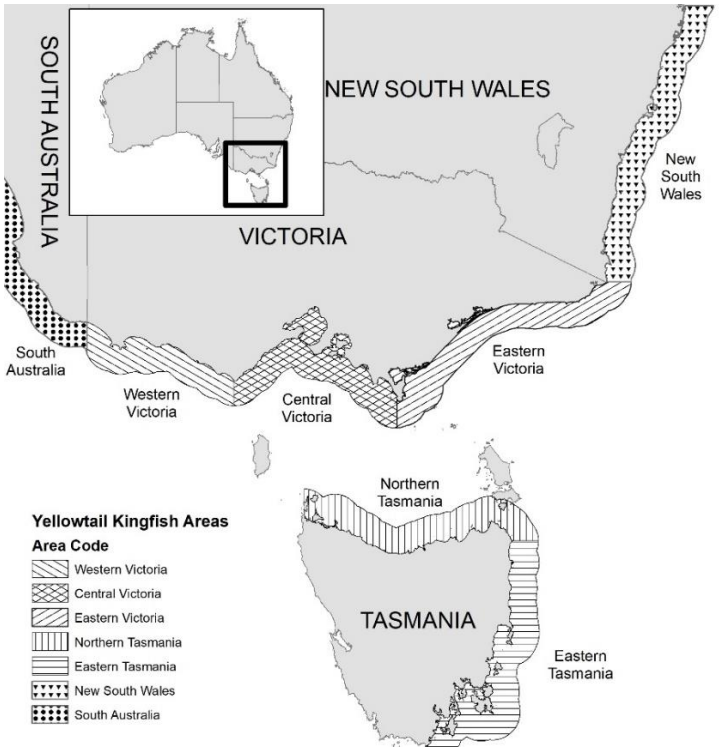


**Figure 1. Regions where yellowtail kingfish were collected for analysis.**

**Table 1. Number of yellowtail kingfish frames obtained from Western, Central and Eastern Victoria above and below the legal minimum limit (LML) from 02/Feb/2015 – 01/Feb/2017.**

| | Western VIC | Central VIC | Eastern VIC | Total |
|---|---|---|---|---|
| **Above LML** | 231 | 79 | 123 | 433 |
| **Below LML** | 3 | 13 | 3 | 19 |

## Genetic stock discrimination

*This section is relevant to meeting Objective 1.*

*Full details of methods and results are presented in Appendix 4.*

Defining the appropriate geographic areas to apply fisheries assessment and management relies on an understanding of spatial stock structure. Genetic techniques are commonly applied to estimate the level of connectivity and interbreeding among fish populations to support the development of geographic biological (i.e. interbreeding) stock structure models. If discrete populations that are targeted by fisheries are indeed separate biological stocks, then alternative management regimes may be needed for each stock and they should be assessed and managed independently.

Image credit: Scott

**Methods**

Genetic diversity and population structure were explored using mitochondrial markers (mtDNA), microsatellites and single nucleotide polymorphisms (SNPs) in order to determine the connectivity of yellowtail kingfish found within Victoria and between Victoria (VIC), New South Wales (NSW) and Tasmania (TAS). Existing publicly available mitochondrial sequence data for individuals from South Australia (SA), Western Australia (WA) and New Zealand (NZ) were included in analyses where possible.

Firstly, mitochondrial markers were used in order to include sequences obtained from yellowtail kingfish caught in south eastern Australian waters with existing archived sequences from fish captured from WA, SA and NZ. Secondly, microsatellite markers were employed to investigate population structure for recently sampled fish across NSW, TAS and three locations in VIC. Thirdly, single nucleotide polymorphisms (SNPs) were used to investigate fine-scale population structure within VIC only.

Tissue samples were collected from yellowtail kingfish caught by recreational fishers in VIC, NSW, TAS and SA between May 2015 and Dec 2018. Victorian samples were collected from the three regions: Western, Central and Eastern (Table 2). Fin clips were taken from VIC, SA and TAS individuals, and muscle tissue was collected for NSW samples only. Fin clips and muscle tissue were preserved in 70% ethanol at -30$^{o}$C for later processing. Size of yellowtail kingfish varied; however, individuals were greater than the legal minimum length for their respective state.

Each of the three types of genetic markers; (1) mtDNA, (2) microsatellites and (3) SNPs were obtained from each individual fish (except for WA - mtDNA only). Genetic diversity (i.e. the total number of different alleles) was estimated for each marker type for each region. In addition, the genetic differences within and between these locations was also assessed in order to determine whether there was any evidence of population structure.

Detailed methods for laboratory analyses are available in appendix 4.

***Data analysis***

mtDNA

Calculations of nucleotide and haplotype diversities were conducted using the program DnaSP v5.10.1(Librado and Rozas 2009). The neutrality tests Fu's Fs and Tajimas D (Tajima 1989) were calculated in Arlequin v3.5 (Excoffier, Laval et al. 2005) for each location separately, for all Victorian populations grouped together and for all Australasian populations separately. Pairwise ΦPT values were also calculated in Arlequin v3.5 for each population separately and for all Victorian populations combined.

Microsatellites

Micro-Checker v2.2.3 (Van Oosterhout, Hutchinson et al. 2004) was used to identify null alleles and errors resulting from stuttering for correction. Corrected data was analysed in Genepop v3.4 (Raymond and Rousset 2003) to identify any deviations from Hardy-Weinberg equilibrium. $F_{ST}$ and $F_{IS}$ were calculated using the program

FSTAT v2.9.3 (Goudet 1995). The structure plot analysis was conducted in STRUCTURE v2.3.2 (Pritchard, Stephens et al. 2000) with the following parameters: burn-in of 100,000 Markov Chain-Monte-Carlo (MCMC) repetitions and five iterations per K (K=1-8). The Evanno method (Evanno, Regnaut et al. 2005) was used to determine the best fit for K.

Single nucleotide polymorphisms

Population structure was assessed using a Discriminant Analysis of Principal Components (DAPC), performed using the R package 'ADEGENET' (Jombart 2008). To determine the optimal value of clusters (K), the Bayesian Information Criterion (BIC) method was implemented without any sampling information, using the function *find.clusters()* and 35 principal components (PCs) were kept.

Diversity indices (number of alleles and inbreeding coefficient - FIS) and population pairwise measures of FST with respective p-values were estimated using the software Genepop (Rousset 2008). Values of observed and expected heterozygosity were estimated using the basicStats() function in the R package "diveRsity" (Keenan *et al.* 2013).

**Table 2. Region, tissue type and number of samples used for mitochondrial (mtDNA), microsatellite and single nucleotide polymorphisms analysis (SNPs) of yellowtail kingfish populations.**

| Region | Tissue type | mtDNA | Microsatellites | SNPs |
|---|---|---|---|---|
| **Western VIC** | Fin | 57 | 56 | 38 |
| **Eastern VIC** | Fin | 57 | 57 | 48 |
| **Central VIC** | Fin | 54 | 53 | 21 |
| **NSW** | Muscle | 6 | 8 | 0 |
| **TAS** | Fin | 60 | 60 | 0 |

## Results

### *mtDNA*

Using mtDNA, there was no evidence for population differentiation between VIC, NSW, SA and TAS, nor between the three Victorian regions. The exception was yellowtail kingfish from WA which was significantly different from all other sampled populations. The haplotype network from the mtDNA analysis exhibits a 'star-like' structure, indicative of a population expansion, with the central and most common haplotype (1) comprising 66.7% of all individuals. Haplotype 1 was present in individuals from all locations with the exception of WA. The second most common haplotype (5) was present in 9.69% of individuals and was only found in all Victorian and Tasmanian locations. Haplotypes 21 (eastern Victoria) and 19 (western Victoria) show the greatest distance from the central haplotype (1) and are separated by four mutational changes. Both of these haplotypes are private, being represented by a single individual. Private haplotypes are evident from five locations; WA (1 private haplotype), eastern VIC (2 private haplotypes), western VIC (2 private haplotypes), TAS (3 private haplotypes) and central VIC (4 private haplotypes) (Figure 2). All pairwise comparisons between VIC locations were low and non-significant suggesting that there was no population differentiation between these locations based on mitochondrial markers.

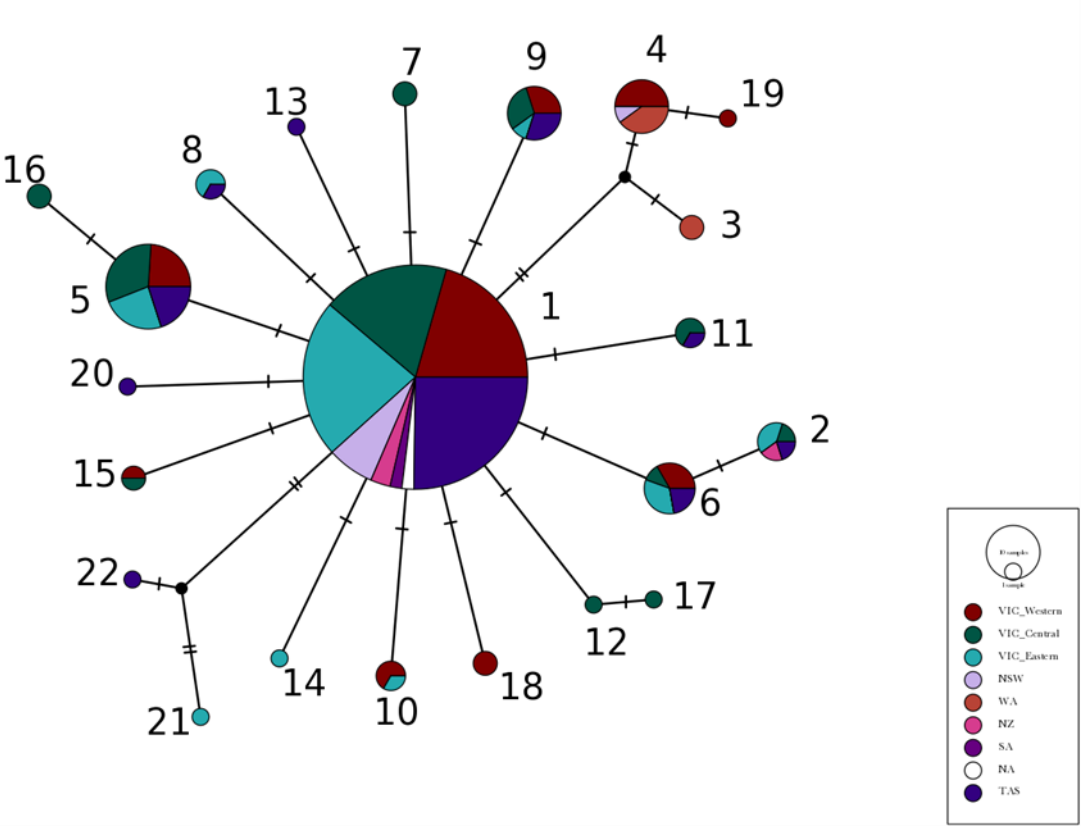


**Figure 2. Median joining haplotype network of yellowtail kingfish from mtDNA. Haplotypes are represented by a single circle proportional in size to the number of individuals, with colours corresponding to sampling regions. Sampling regions are as follows western Victoria (red), central Victoria (green), eastern Victoria (pale blue), New South Wales (lilac), Western Australia (orange), New Zealand (dark blue), South Australia (purple) and Australia (unknown location) (white). Dashes across lines joining haplotypes represent a single nucleotide change and black circles are mutation points.**

***Microsatellites***

*Genetic diversity*

Victoria

No deviations from Hardy-Weinberg expectations were observed for loci pairs from eastern VIC. Central and western VIC populations contained between 1 and two loci pairs, which deviated significantly from expectations respectively. Private alleles were present in all VIC locations with western VIC containing the highest number at 12 and central VIC the lowest with seven. Effective population size was highest for western VIC (9.012) and the lowest effective population size was attributed to central VIC (7.861). The average $F_{IS}$ (inbreeding co-efficient, +ve values indicated higher inbreeding, -ve lower inbreeding than expected from random mating) values for VIC populations were positive with the highest found in western VIC (0.396) and the lowest observed in eastern VIC (0.078). VIC populations showed an observed heterozygosity of <0.5 with the highest recorded from eastern VIC (0.491) (Table 3).

All locations

Deviations from Hardy-Weinberg expectations were found for two TAS loci with all NSW loci pairs congruent with expectations. TAS samples contained nine private alleles which was comparable to VIC, while NSW was the only location to contain no private alleles. NSW contained the lowest effective population size (3.718) and observed heterozygosity (0.362) of all populations but contained the smallest sample numbers. Positive $F_{IS}$ values were found for both NSW and Tasmanian populations (Table 3).

*Pairwise $F_{ST}$*

Victoria

All pairwise $F_{ST}$ values were low, ranging between 0.001 and 0.002, but were statistically significant for all pairs of VIC locations with the exception of the eastern and central pair, which had a pairwise value of 0.55. However, this value was not significant (Table 4).

All locations

$F_{ST}$ comparisons between VIC and TAS regions were low between 0.001 and 0.003 but statistically significant. The only exception was the eastern and central VIC pair, which had a high but non-significant $F_{ST}$ value of 0.477. Due to the small sample sizes from NSW, $F_{ST}$ calculations between this location and VIC or TAS were not possible (Table 4). Non-significant $F_{ST}$ comparisons may largely relate to poor statistical power due to low sample sizes and high variability and should be viewed with caution. $F_{ST}$ comparisons for the VIC regions were significant due to lower within region variation and higher sample sizes, but the differences were very low, and may not be indicative of sustained population structure (see discussion). For example, $F_{ST}$ comparisons that are close to zero indicate no differentiation.

**Table 3. Indices for yellowtail kingfish using nine microsatellite markers across five regions. Sample Size (N), private alleles (NPR), mean number of alleles (Na), effective population size (Ne), observed heterozygosity (Ho), expected heterozygosity (He), allelic richness (AR), loci deviating from Hardy-Weinberg expectations (HW) and FIS.**

| Region | N | NPR | Na | Ne | Ho | He | AR | HW DIS | Fis |
|---|---|---|---|---|---|---|---|---|---|
| **Western VIC** | 56 | 12 | 15.333 | 9.012 | 0.444 | 0.580 | 5.471 | Sdu46 and Sdu16, Sdu29 and Sdu37 | 0.396 |
| **Eastern VIC** | 57 | 9 | 13.889 | 8.637 | 0.491 | 0.546 | 5.025 | | 0.078 |
| **Central VIC** | 53 | 7 | 13.111 | 7.861 | 0.477 | 0.538 | 4.974 | Sdu46 and Sdu32 | 0.084 |
| **NSW** | 8 | 0 | 4.556 | 3.718 | 0.362 | 0.435 | 4.884 | | 0.169 |
| **TAS** | 60 | 9 | 12.444 | 6.400 | 0.372 | 0.542 | 5.025 | Sdu46 and Sdu16, Sdu32 and Sdu10 | 0.419 |

**Table 4. Pairwise $F_{ST}$ values denoting differentiation of nine microsatellites between sampling regions of yellowtail kingfish. Significant values (<0.05) are shown in bold.**

| Population | Western VIC | Eastern VIC | Central VIC | NSW | TAS |
|---|---|---|---|---|---|
| **Western VIC** | | | | | |
| **Eastern VIC** | **0.003** | | | | |
| **Central VIC** | **0.005** | 0.477 | | | |
| **NSW** | 0.73 | 0.352 | 0.239 | | |
| **TAS** | **0.003** | **0.003** | **0.001** | 0.647 | |

*Structure analyses*

Five populations were predicted by the Evanno method (K=5) with a Delta K of 2.807508. However, despite the significant pairwise $F_{ST}$ tests, no clear patterns relating to sampling region were distinguishable from the STRUCTURE analysis (Figure 3).

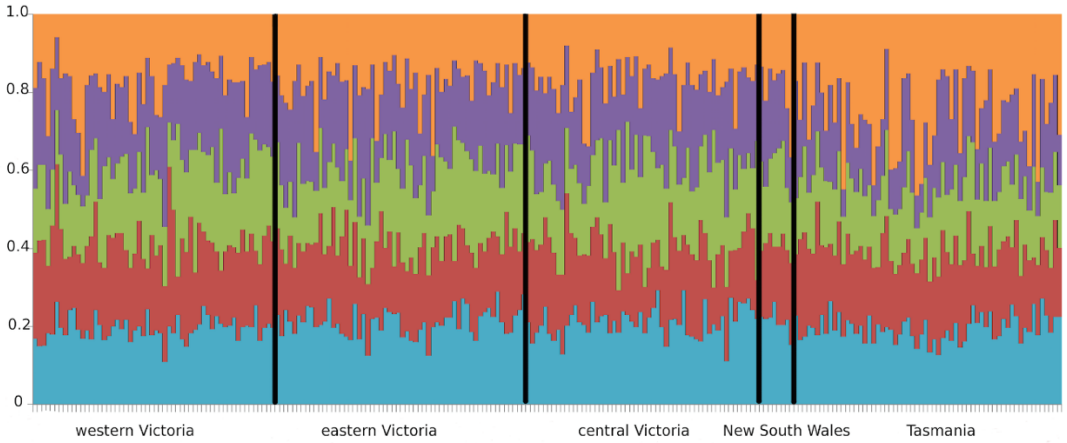


**Figure 3. Structure plot of five populations (K=5) of yellowtail kingfish using nine microsatellite markers. Data are for individual fish samples.**

***Single nucleotide polymorphisms***

Significant genetic structure was detected between Victorian populations when using SNP markers. After filtering, a total of 38,575 SNPs were identified. DAPC (discriminant analysis of principle components) results identified an optimal K value of two, as indicated in the Bayesian Information Criteria (BIC) analysis where the smallest value of BIC is located at two clusters. However, visualisation of the clusters generated by the DAPC revealed three groupings corresponding to the three geographical regions (Figure 4). Additional results are presented in Appendix 4. Pairwise $F_{ST}$ comparisons were statistically significant between central VIC and east and west VIC, but not between east and west VIC. However, similar to the microsatellites, the $F_{ST}$ comparisons were close to zero (Table 5).

**Table 5. a) Pairwise FST values between sampling regions in Victoria based on 38,575 SNPs. Significant values are in bold (p<0.001), and b) indices for yellowtail kingfish 38,575 SNPs. Sample Size (N), number of alleles (Na), FIS (inbreeding co-efficient), observed heterozygosity (Ho) and expected heterozygosity (He).**

| a) | Central VIC | Eastern VIC | Western VIC |
|---|---|---|---|
| **Central VIC** | | **0.000** | **0.000** |
| **Eastern VIC** | **0.0004** | | 1.000 |
| **Western VIC** | **0.0077** | 0.0052 | |

| b) | N | Na | $F_{IS}$ | $H_O$ | $H_E$ |
|---|---|---|---|---|---|
| **Central VIC** | 21 | 60,571 | 0.172 | 0.0996 | 0.120 |
| **Eastern VIC** | 48 | 66,002 | 0.169 | 0.0977 | 0.113 |
| **Western VIC** | 38 | 70,013 | 0.263 | 0.1000 | 0.133 |

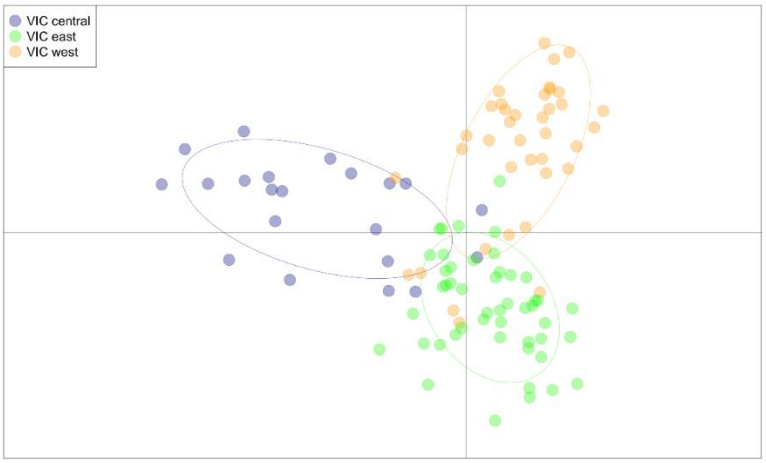


**Figure 4. Discriminate analysis of principal components using 38,575 SNPs. Scatterplot dots represent individuals, while the colours refer to genetic cluster assignment.**

***Genetics results summary***

Overall the results of the genetic analyses confirm a separate genetic stock in WA and support a one stock hypothesis for eastern/south-eastern Australia. While the data from the microsatellites and SNPs analysis, showed some statistically significant comparisons for the VIC and TAS regions, the difference were very minor. The minor genetic variation is possible due to processes operating and smaller spatial and temporal scales creating genetic patchiness and is not indicative of longer-term or broader-scale stock structure that might necessitate a fisheries management response. This is covered in more detail in the discussion section.

## Biological and population characteristics

*This section is relevant to meeting Objective 2.*

### Methods

***Determination of length and weight metrics***


Image credit: Scott Gray

Biological characteristics including, reproduction, age and growth were determined by obtaining meristic data and biological information from yellowtail kingfish frames. For most fish collected, it was not possible to dissect in their fresh state. Consequently, kingfish frames collected throughout the state were frozen and subsequently transported to Queenscliff (VIC), where they were stored frozen for up to one year prior to thawing and dissecting. Such storage techniques are likely to result in body shrinkage (Armstrong and Stewart 1997) that in-turn affects subsequent estimates of biological parameters. For length, shrinkage was quantified by comparing the length of freshly caught yellowtail kingfish with the length of the same individual that was subjected to long-term cold storage (i.e. 1 year at -18ºC). A linear model of Total Length (thawed) and Total Length (fresh) was calculated (Buchheister and Wilson 2005) using the following equation.

$$y = mx + c$$

where $y$ = Total length (thawed), $x$ = Total length (fresh), m and c are model constants

A significant linear relationship was found between fresh and thawed Total Length (y=1.014x - 0.2101, $R^2$ =0.99, F=6851, $df_{1,63}$, $p$<0.01). Where only frozen samples were obtained, the model was used to convert Total Length (thawed) to Total Length (fresh). Length was rounded down to the nearest 0.5 cm. It was assumed that shrinkage occurs at a similar rate irrespective of location of capture and the period over which frames were frozen.

The correlation between total length and fork length was described using the following equation.

$$y = mx + c$$

where $y$ = Fork length, $x$ = Total length, m and c are model constants

The correlation between total weight and total length was described using the following equation.

$$y = mx^c$$

where $y$ = Total weight, $x$= Total length, m and c are model constants

Length frequency distributions were calculated from samples collected to determine if the size differed between each of the three regions; Western, Central and Eastern. Comparisons between distributions were made using a Kolmogorov-Smirnov test. Differences in the average total length of yellowtail kingfish from each zone were determined using an ANOVA.

***Estimates of age***

Various methods have been previously used to estimate the age of yellowtail kingfish such as using otoliths, spines, scales and vertebrae (Gillanders, Andrew et al. 2010). All methods have advantages and disadvantages; however, transversely sectioned otoliths provide the greatest reliability for consistent preparation and increment interpretation (Stewart, Ferrell et al. 2001). Similar methods for preparation and age estimation are described in (Francis, McKenzie et al. 2014) and (McKenzie, Smith et al. 2014). Otoliths from 448 yellowtail kingfish were sent to Fish Ageing Services™ for preparation and analysis (www.fishageingservices.com).

One undamaged otolith from each pair was weighed to the nearest milligram. Otolith mass was recorded for 440 otolith samples. One otolith from each pair was prepared for ageing using a three-stage process of embedding, sectioning and mounting. To embed the otoliths, a thin layer of clear polyester casting resin was poured on to the base of a silicon mould and left to partially cure. Individual otoliths were arranged in two rows of five on the resin. Resin blocks were labelled and coated with another layer of resin and left to cure. Otolith sections were cut using a Gemmasta™ lapidary saw fitted with a diamond-impregnated blade. From each row of otoliths, four sections were taken (approximately 300µm in thickness) to ensure the primordium of each otolith was included in one of the sections. Sections were cleaned and dewatered using alcohol and stored in vials. For identification, each vial contained a sample identification label. A small amount of resin was poured on to a glass slide (50 x 75 mm). Otolith sections were immersed in the resin and the identification label placed at the top of the slide. Once the resin had semi-cured, further resin was applied to the preparations and a cover slip placed on top. Slides were oven cured at 30°C for 3 hours.

Transverse sections were viewed under a compound microscope using various magnifications. Interpretation of presumed annual increments and the location of the first increment was based on previous studies by Francis et al (2014) and McKenzie et al (2014). Increments were counted from the primordium to the otolith edge and a 'readability score' assigned to indicate how decipherable the growth increments were.

1 Has exceptionally clear, unambiguous increments

2 Indicates a clear and confident estimate

3 Indicates that the sample may be subject to an additional interpretation

4 Indicate that the sample is subject to multiple interpretations, and

5 Indicates that the sample is unusable or missing

***Comparison of age estimates***

Repeated readings of the same otoliths provide a measure of intra-reader and inter-reader variability. They do not validate the assigned ages but provide an indication of the size of the error to be expected with a set of age estimates, due to variation in otolith interpretation. Beamish and Fournier (1981) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$IAPE = \frac{100}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right]$$

Where N is the number of fish aged, R is the number of times fish are aged, $X_{ij}$ is the $_i$th determination for the $_j$th fish, and $X_j$ is the average estimated age of the $_j$th fish. The index has the property that differences in age estimates for younger fish will contribute more to the final value than will the same absolute error for older fish (Anderson, Morison et al. 1992).

A total of 25% of yellowtail kingfish were reread by the same person. Precision estimates were compared with the acceptable level of agreement between readings (Morison et al. 1998). The distribution of age estimate differences between first and second readings was calculated, and an ANOVA was used to determine if there was any significant difference between first and second readings.

***Conversion of increment count to age***

Increment counts were converted to age classes based on a theoretical birthday of 1 September which coincides with the approximate time of increment formation that occurs during August / September (Gillanders et al. 2010). As yellowtail kingfish were caught in the summer months, a wide translucent increment was apparent on the otolith edge. Consequently, no adjustment to age was required with increment count equalling age.

***Growth***

Von Bertalanffy growth curves were fitted using sum-of-squares minimisation methods to the male and female length-at-age data separately using R statistics (2016).

$$L_t = L_\infty[1-e^{-k(t-t_0)}]$$

where $L_t$ is the length at age t, $L_\infty$ is the mean asymptotic length, k is the growth coefficient (the rate at which the increase in length decreases) and $t_0$ is the theoretical age at zero length.

***Reproductive characteristics***

Reproductive characteristics of *Seriola spp* (i.e. amberjack, a similar species to yellowtail kingfish) have previously been analysed using histology methods (Micale, Genovese et al. 1993, Marino, Mandich et al. 1995); gonad indices (Smith 1987); and macro gonad staging (Marino, Mandich et al. 1995, McGregor 1995). More recently, Gillanders *et al* (1999) and determined size at maturity and changes in gonad activity of yellowtail kingfish from NSW. Similar methods used in this paper were used to explore the reproductive characteristics for the present research.

Yellowtail kingfish caught in Victorian waters were defrosted and dissected to obtain reproductive characteristics. Sex was determined by visually examining the gonads. Intact gonads of male and female yellowtail kingfish were removed and weighed to the nearest 0.1 g. Gonads were macroscopically staged on a scale of 1 (Immature) – 6 (Spent) for females and 1 (Immature) – 4 (Ripe) for males. Damaged gonads were not weighed; however, were staged where possible. Maturity stage classification was conducted using methods described by Gillanders *et al* (1999) see Appendix 3.

A logistic regression model was used to describe the relationship between total length at maturity was determined. The logistic model was fit in R (Team 2016) with the general linear model procedure. Total length was binned to 5 cm intervals and fish were classified as mature where oocyte stage was =>4 for both sexes.

$$\log\left(\frac{p}{1-p}\right) = \alpha + \beta_1 X$$

Length at 50% ($p$=0.5) mature was determined by

$$x = \frac{\log\left(\frac{p}{1-p}\right) - \alpha}{\beta_1}$$

**Results**

All length measurements were expressed at total length (TL) (cm). The correlation between fork length (FL) and total length was significant ($F_{1,267}$=57370, p<0.001, $R^2$=0.99), where: fork length = 0.896 x total length - 0.985, $R^2$=0.99 (Figure 5). This relationship was used to convert to total length where only fork length was attainable. Approximate weight was determined using the correlation between TL (cm) and weight (kg); where estimated Total weight (kg) = 0.00001 x TL (cm) $^{2.9065}$ (Figure 6).

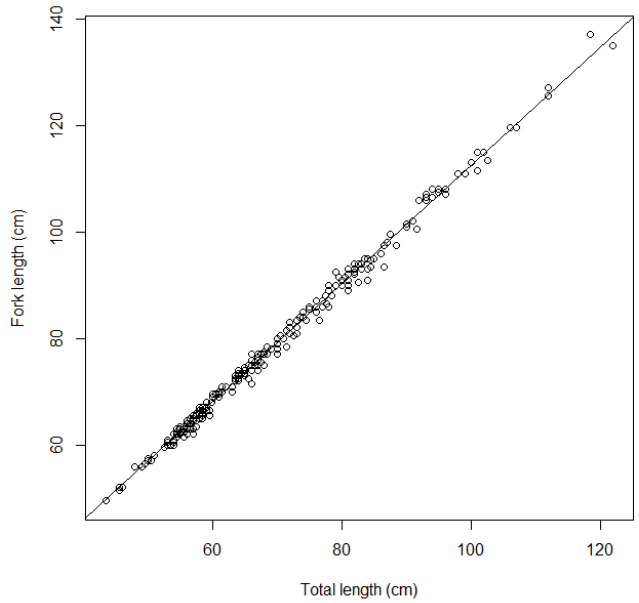


**Figure 5. Relationship between fork length and total length of yellowtail kingfish caught from Victoria.**

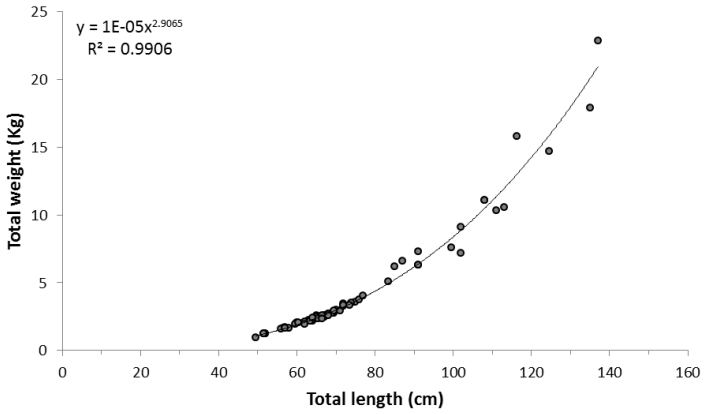


**Figure 6. Length (Total) weight relationship for yellowtail kingfish caught in Victoria (n=72).**

***Size characteristics***

Total length of yellowtail kingfish collected at West, Central and Eastern Victoria ranged from 40 – 130 cm TL (Figure 7). A Kolmogorov-Smirnov test indicated that the size distribution of fish caught was different between each of the three Zones was different (Table 6). Comparing the mean total length of yellowtail kingfish from each Zone using and ANOVA indicated that there was a significant difference between the three groups ($F_{2,527}$=39.4, p<0.001). The average total length of yellowtail kingfish caught was 84.3 cm (±0.79 SE), 70.7 cm (±1.56 SE), and 76.6 cm (±1.11 SE), for Western, Central and Eastern Zones respectively. A Tukey's post hoc test indicated a significant difference in mean total length between Zone. Fish caught in Western Zone were around 13.6 cm (TL) greater than those caught in Central and 7.7 cm (TL) greater that those caught in Eastern Zone.

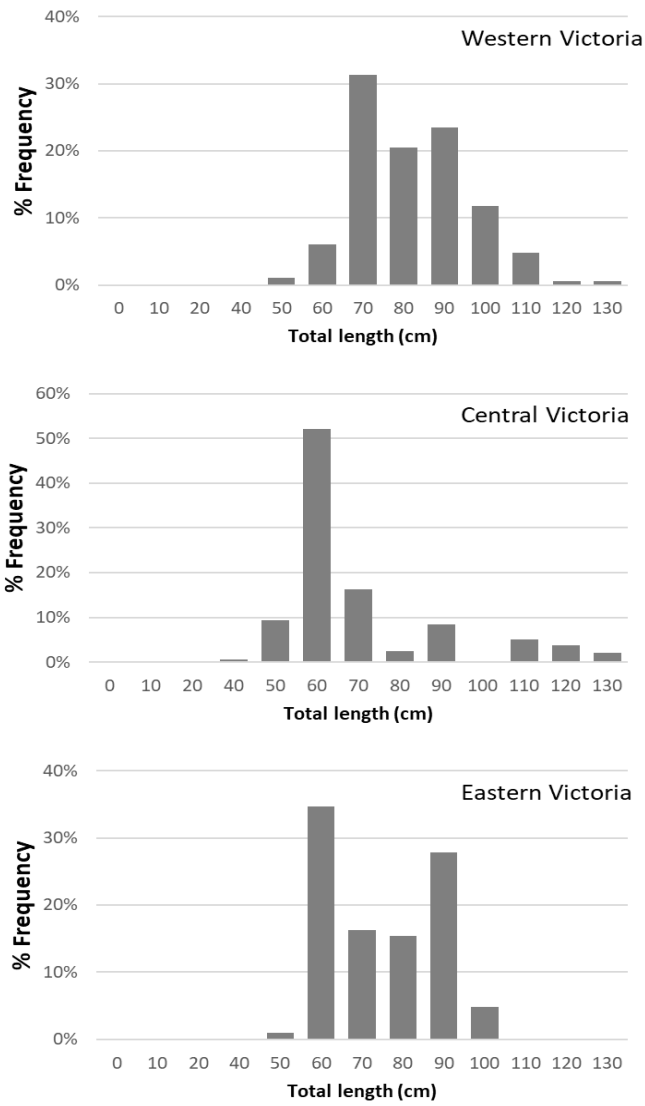


**Figure 7. Percentage total length frequency distribution of yellowtail kingfish caught from Western (n=289), Central (n=98), and Eastern (n=143) Victoria.**

**Table 6. Statistical results from the Kolmogorov-Smirnov test comparing the total length distribution between yellowtail kingfish caught from each Zone.**

| Distribution Comparison (Zone) | D | *P value* |
|---|---|---|
| West – Central | 0.621 | <0.001 |
| West – East | 0.351 | <0.001 |
| Central - East | 0.310 | <0.001 |

### *Age estimates*

Age estimates were determined from 439 of the 448 otolith samples that were collected. Interpretation of incremental structure was relatively difficult with 76% of samples assigned a readability of 4 (i.e. the sample is subject to multiple interpretations), with only 5% of samples possessing relatively clear increments (i.e. readability score of 1 or 2, Figure 9). The index of average percent error for intra-reader variability was 3.4%. An IAPE <5% is considered acceptable repeatability. Age estimates ranged from 1 – 11 years of age with the modal age of 3 years in Western Victoria and 2 years for Central and Eastern Zones (Figure 8). Across all three regions most samples were estimated at ≤ 5 years old. A Kolmogorov-Smirnov test indicated that the age frequency distribution of fish caught was different between each of the three Zones was different (Table 7).

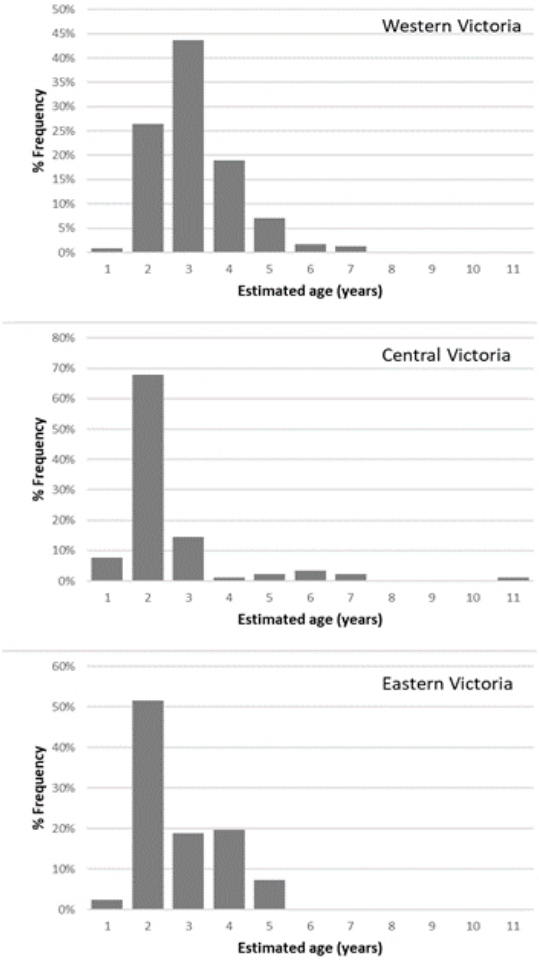


**Figure 8. Percentage age frequency distribution of yellowtail kingfish caught from Western (n=227), Central (n=90), and Eastern (n=122) Victoria.**

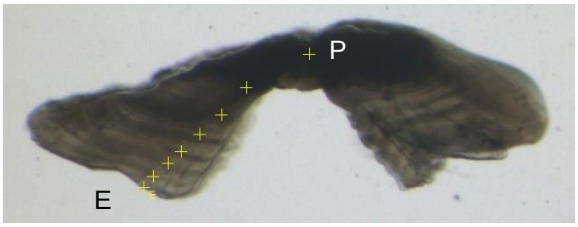


**Figure 9. Transverse section of a yellowtail kingfish otolith. Cross hair indicates position of presumed annual increments and the primordium (P) and edge (E). Estimated age 6 years. Readability score of 1.**

**Table 7. Statistical results from the Kolmogorov-Smirnov test comparing the age frequency distribution between yellowtail kingfish caught from each Zone.**

| Distribution Comparison (Zone) | D | *P value* |
|---|---|---|
| West – Central | 0.482 | <0.001 |
| West – East | 0.267 | <0.001 |
| Central - East | 0.214 | <0.001 |

***Growth***

Growth rates and Von Bertalanffy growth parameters (Table 8) of yellowtail kingfish were similar between Male and Female samples (Figure 10). Growth curves were similar for VIC and NSW yellowtail kingfish (Figure 10), particularly for the comparison with Gillanders et al. (1999). The differences among growth curves and parameters for the Stewart et al. (2004) study is related to their higher $L_{inf}$ which results in lower K.

**Table 8. Von Bertalanffy growth parameters for yellowtail kingfish sampled in Victorian waters, reported for NSW from earlier studies. Note: for comparisons across studies fork length (FL) is used.**

| | **Victoria – this study** | | | **NSW –** Stewart et al. 2004 | **NSW –** Gillanders et al. 1999 |
|---|---|---|---|---|---|
| **Parameter** | Female | Male | All | All | All |
| **$L_{inf}$** | 139.04 | 135.75 | 135.84 | 184 | 125.2 |
| **$T_0$** | -1.29 | -1.08 | -1.23 | -4.4 | -0.7 |
| **K** | 0.146 | 0.156 | 0.149 | 0.054 | 0.189 |

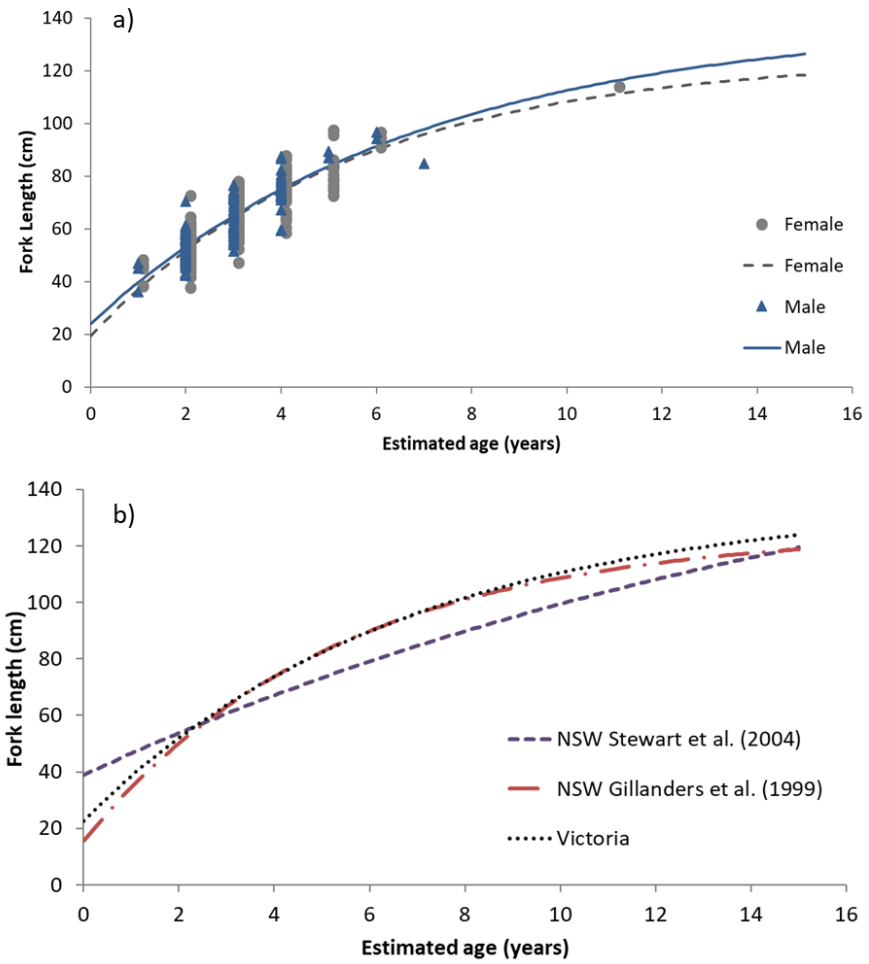


**Figure 10. a) Growth of male and female yellowtail kingfish caught in Victoria (Female n=199; Male n=173). Juveniles or unknown sex is not represented, and, b) comparisons of growth curves between studies in Victoria and NSW pooled across all samples.**

### *Reproductive characteristics*

#### Sex ratio

For all three Victorian regions 25-30% of samples collected could not be sexed confidently due to the fish being immature or gonads were degraded or missing. For samples that could be sexed, males and females occurred in similar proportions for all regions, and there was no bias in the sex ratio (Figure 11a). Gonad stage of yellowtail kingfish caught throughout Victoria varied from stage 1–5 for females and 2–4 for males (Figure 11b) caught between November and April. For males, 41% were mature; whereas 23% of females were mature.

#### Maturity

The total length of yellowtail kingfish where 50% of the samples were mature (length at 50% maturity) was 95.16 cm TL (≈84.3 cm FL) for females and 78.4 cm TL (≈69.3 cm FL) for males (Figure 12). Monthly variation in the gonadosomatic index (GSI) suggests that greatest spawning potential between November and February (Figure 13). Highest GSI was observed during November and December for males and females respectively (Figure 13).

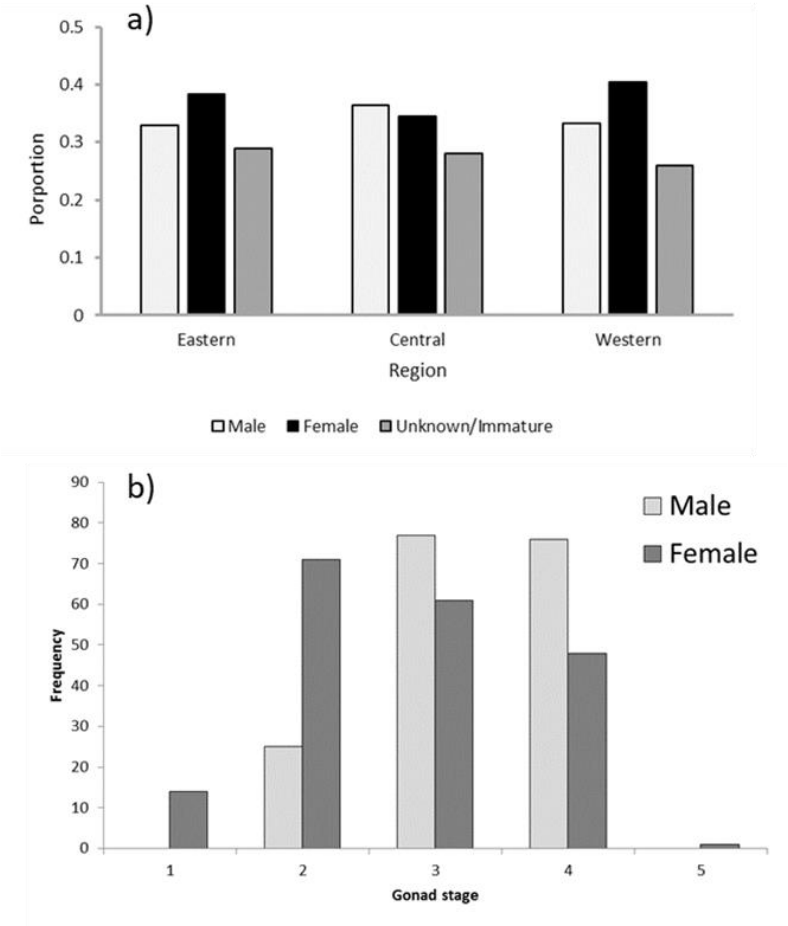


**Figure 11. a) Proportions of female, male and unknown/immature yellowtail kingfish for samples taken in Victorian regions, and, b) distribution of gonad stages for male and female yellowtail kingfish caught in Victoria (Female n=195; Male n=179).**

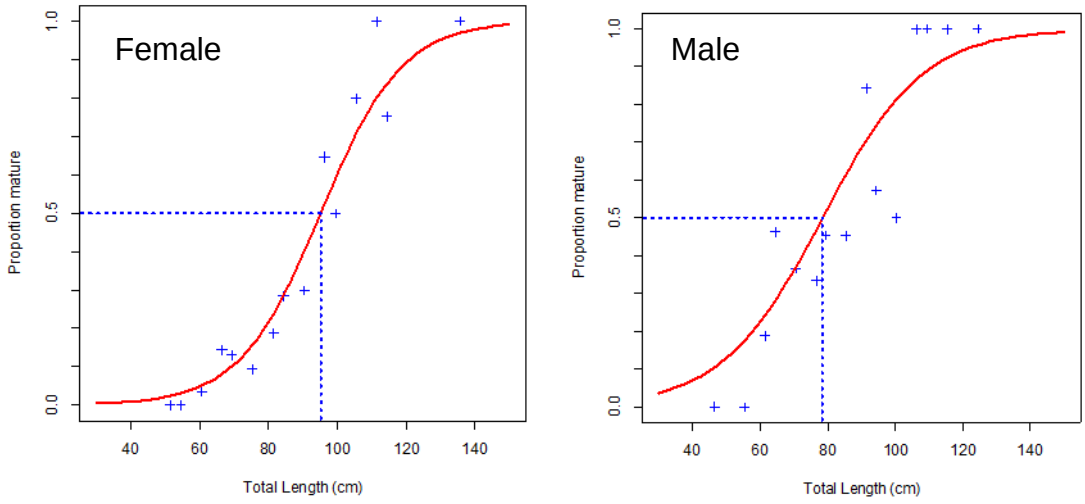


**Figure 12. Size at maturity logistic regressions for female (n=195) and male (n=179) yellowtail kingfish. Blue lines indicate the length of fish where 50% are mature.**

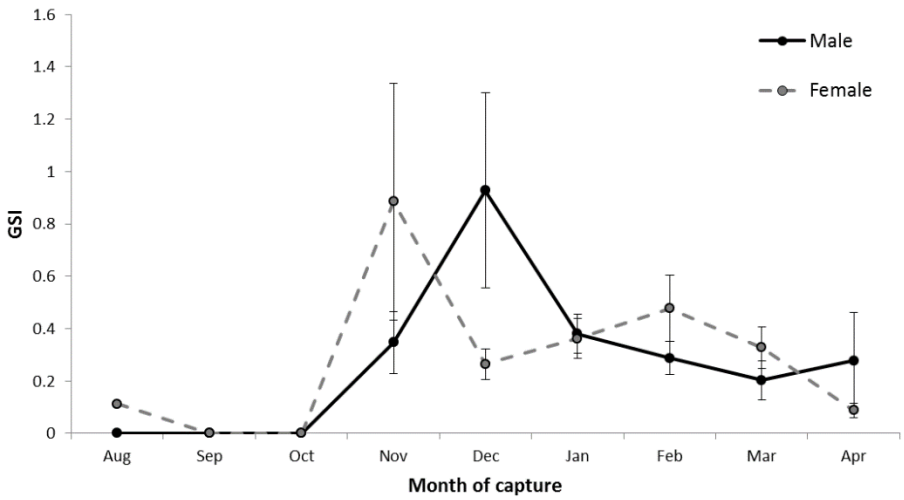


**Figure 13. Monthly variation of mean gonadosomatic index (GSI ±SE) for male and female yellowtail kingfish caught in Victoria from Feb 2015 – Feb 2017.**

## Historical catch records

*This section is relevant to meeting Objective 3.*

Since around 2010, reports of recreationally caught yellowtail kingfish from a wide size range have increased greatly in Victoria after being low from the early 1990's. Many anglers are now targeting yellowtail kingfish at several locations throughout the state. Analysing historical catch records of yellowtail kingfish in Victoria provides a mechanism to infer changes in the population characteristics over time (i.e. spatial and temporal distribution and abundance). A number of information sources (including anecdotal) where used to obtain information such as size (length or weight), location and date of capture of yellowtail kingfish prior to their decline in the early 1990's.

Sources include:

- Game Fishing Association of Victoria and affiliated clubs
- Australian National Sports fishing Association and affiliated clubs
- NSW Fisheries (Fish tagging programme)
- Historical reports, commercial catch information and scientific literature

Information gathered was used to document the change in the fishery over time, infer possible causes of change in numbers and stock structure and to indicate the likelihood of the population and fishery resurgence in Victoria continuing.

### *Commercial fishery catch trends*

Commercial landings of yellowtail kingfish have predominantly occurred on the eastern Australian coast in New South Wales and Queensland, but small catches have been taken in South Australian and Victorian waters at various times. The Commonwealth Southern and Eastern Scalefish and Shark Fishery (SESSF) also takes small harvests in south-east Australian waters (i.e. 9 t in 2017). Commercial catch in Victoria peaked in 1995 (12600 kg) with a dramatic drop in catch from 1994 (Figure 14); which is somewhat consistent with the timing when recreational catch decreased.

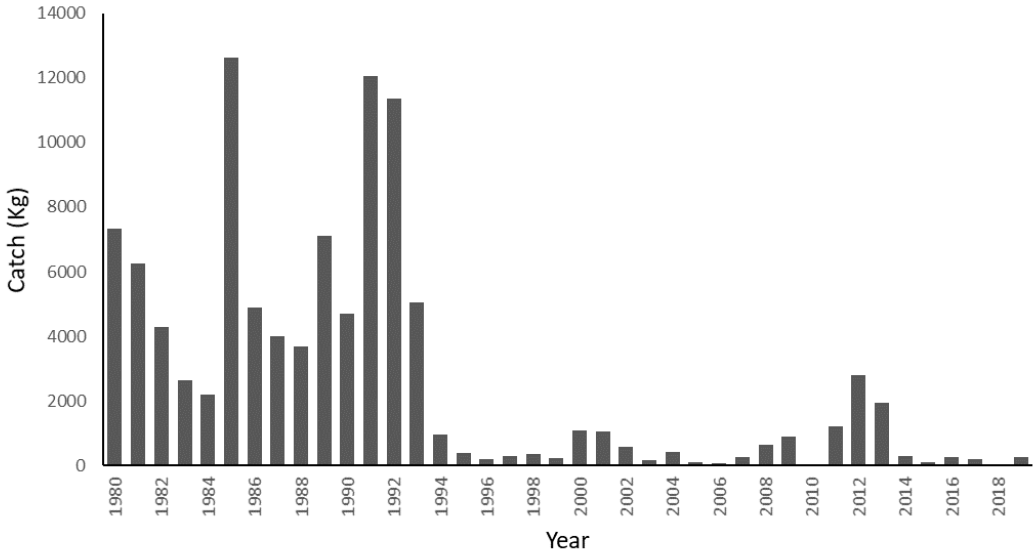


**Figure 14. Commercial catch of yellowtail kingfish landed in Victoria from 1980–2019.**

Commercial landings of yellowtail kingfish in New South Wales have; however, always been the highest of any jurisdiction, but have declined from around 550–600 t per year in the mid-late 1980's, to an average of approximately 150 t per year since the mid-1990's (Stewart, Ferrell et al. 2004). In recent history, a continuous decline in landings has been recorded from 266 t in 2009–10 to 66 t in 2016–17, which is the lowest recorded commercial harvest for the species in New South Wales (Hughs, Molony et al. 2018). Much of the decline in commercial harvest in New South Wales was attributed to the pelagic trap fishery which mostly caught juveniles and was banned in 1996. Since then, yellowtail kingfish in New South Wales have been predominantly been caught by line, which also mostly harvests juveniles. Small commercial catches continue to occur in Queensland, recently ranging 3-14 t per year (Hughs, Molony et al. 2018)

The most recent national reporting (Status of Australian Fish Stocks 2018, SAFS) of stock status for yellowtail kingfish in eastern Australia has classified them as "undefined" (Hughs, Molony et al. 2018), meaning "there is insufficient information available to confidently classify the overall status of the entire eastern Australia biological stock". Importantly, the SAFS status assessment does not include information from Victoria or Tasmania, despite the genetic studies suggesting a single biological stock from South Australia to Queensland. While data is limited outside of New South Wales, the available data from New South Wales suggested the component of the stock in New South Wales waters is currently depleted (Hughs, Molony et al. 2018). The depleted status of yellowtail kingfish in New South Wales is in contrast to the improving status of the fishery in Victoria, but it is noteworthy that the overall stock status is unclear.

### *Recreational fishery catch trend*

Recreational catches of yellowtail kingfish have only been estimated once in Victoria, in 2000/01, when the estimated catch was < 1 tonne (Henry and Lyle 2003). This dated estimate for Victoria is clearly irrelevant now and it will be addressed by the new Go Fish Vic app that was officially launched in December 2019. Recreational catches in other States also continue to be estimated.

The recreational catch for yellowtail kingfish for New South Wales was estimated to be 144 t in 2000–01 (Henry and Lyle 2003) and has declined to 120 t in 2013–14, which was apparently related to a reduction in overall numbers of anglers participating, but no significant change in catch rates (West, Stark et al. 2015).

Recent recreational catch of yellowtail kingfish in Queensland is reported to be less than 10 t (Hughes, Malony et al. 2018).

The State-wide recreational catch of yellowtail kingfish in South Australia has increased in recent times, with the past three phone-diary surveys estimating annual catches of 61 t in 2000–01 (Henry and Lyle 2003), 100 t in 2007–08 (Jones 2009) and 199 t in 2013–14 (Giri and Hall 2015).

### *Interviews with recreational fishery experts*

Expert anglers were interviewed to provide anecdotal opinions and observations of the yellowtail kingfish fishery in Victoria. It is accepted that the last period of good fishing for yellowtail kingfish was prior to 1992. The questioning was aimed to obtain perspectives on changes to the yellowtail kingfish populations in Victoria pre and post early 1990s. Information provided below is a summary of the information received without identifying the information source.

In Victoria during the late 70's, early 80's and up to 1992, yellowtail kingfish caught by recreational fishers on rod and reel mostly ranged in size from 12 – 20 kg. However, during the same period off Port Phillip Heads, bigger fish, some reported being up to 44 kg were caught by both professional fishermen and recreational fishers using lead-lines. Prior to 1992/93 it was very rare to get 'rats' less than 8 kg fish in 'The Rip' (the mouth of Port Phillip Heads). The 'golden years ' seemed to stop in the 92/93 season (November – May). Prior to that, good seasons would produce over a hundred fish for the better crews with fish averaging from 12 – 15 kg. Most fish were caught off Port Phillip Heads with others caught off Portland, Lorne and off Wilsons Promontory.

The exact reason for the decline of yellowtail kingfish catches after 1992 cannot be attributed to a single event but rather a combination of factors. For example, the NSW trap fishery mostly landed immature fish up until 1996 when the practice was banned. Its highly likely that such recruitment overfishing impacted the NSW fishery and subsequently impacted the fishery in Victoria. Similarly, oceanographic conditions may have not been suited for yellowtail kingfish.

Around the time catches declined off Victoria in 1992/93 there were several Taiwanese boats targeting arrow squid in Bass Strait and many people associated that with their disappearance. Noticeably has been the lack of numbers of southern garfish (*Hyporhamphus melanochir*) by comparison to those earlier years. It was a daily occurrence to have them leaping out of the water to avoid boats, something that hasn't been seen in years.

A resurgence of yellowtail kingfish occurred in the summer of 2010/11. Fish typically 5 – 12 kgs were landed predominantly around Port Phillip Heads. Since the resurgence of bigger fish in 2010 there has been a noticeable decrease in numbers of large fish in the rip. Whether that can be attributed to numbers caught effecting stocks or the difficulty in fishing for them effectively due to increased traffic or again an observed fluctuation in lack of preferred bait (ie arrow squid and gars) since then is not clear.

### *Fishing club information*

We contacted 18 fishing clubs in search of historic data on lengths and weights of individual yellowtail kingfish prior to the early 1990's. Only one club, Bellarine Light Game and Sportfishing Club, had suitable historic records dating back to 1973. There were 539 records that had either weight or length records. All records were converted to total length in cm using the weight-length conversion previously described. Of the 539 records, 405 were for fish captured in Victoria between 1974 and 1992. Unfortunately details on specific capture locations were not available for most fish. The length and weight compositions for the 405 records show that the majority of yellowtail kingfish records were >100 cm TL and > 10 kg weight, and the largest fish recorded was approximately 30 kg and 155 cm TL (Figure 15). While it is unclear how biased these club reports are towards larger fish, they do indicate that large yellowtail kingfish were commonly caught in Victorian waters prior to 1993, but that smaller fish < 100 cm were also common. Game fishing records for Victoria also showed the largest yellowtail kingfish that were reported to the Game Fishing Association of Australia were from the 1980's (Table 9).

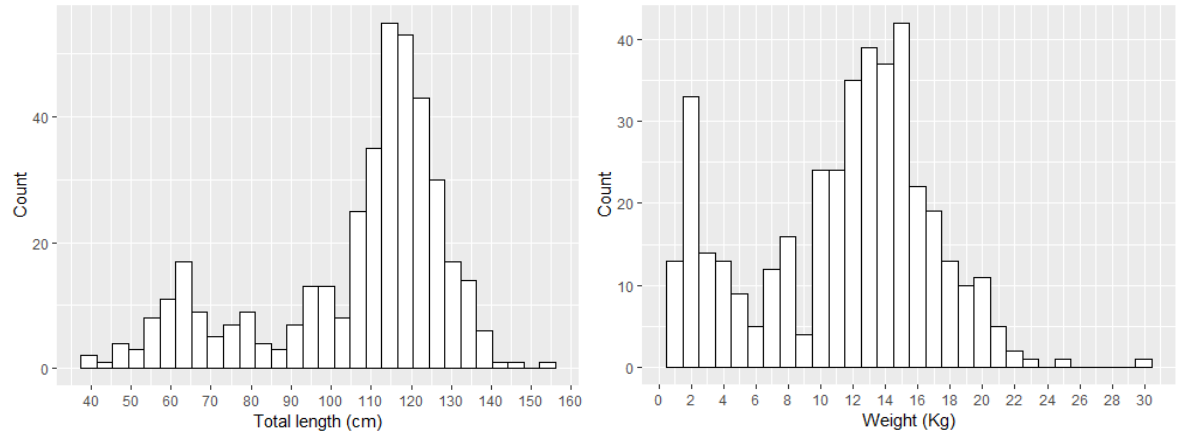


**Figure 15. Length (left) and weight (right) compositions of 405 yellowtail kingfish captured in Victorian waters and recorded by the Bellarine Light Game and Sportfishing Club between 1974 – 1992.**

**Table 9. Victorian saltwater game fishing weight records for yellowtail kingfish with comparison to Australian records (far right column) (courtesy of Game fishing Association of Australia).**

| Line Class | Weight | Capture Date | Angler's Name | Club | Location |
|---|---|---|---|---|---|
| M4 | 4.00 | 15 Feb 1986 | Bill Jenkins | VGFC | Cliffy Island |
| M6 | 14.75 | 22 Mar 1981 | Allan Fisher | VS&GFC | Port Phillip Heads |
| M8 | 12.90 | 2 Feb 2014 | Clinton Adlington | BSGFC | Cape Schanck |
| M10 | 15.50 | 8 Mar 1980 | Brian Higginbotham | VGFC | The Rip |
| M15 | 19.60 | 2 Mar 1982 | Don Di Pietro | VGFC | The Rip |
| F8 | 11.40 | 2 Apr 1987 | Monica Tanian | LVGFC | Cliffy Island |
| F10 | 11.60 | 23 Jan 2016 | Deb Sanders | PMGFC | Portland |

***Tagging data***

There are limited tag/recapture records for yellowtail kingfish either tagged or recaptured in Victoria or tagged in NSW and recapture in Victoria. Table 10 includes seven tag/recaptures involving tagging and or recapture in Victorian waters. Five of these show movement between Victoria and NSW, with movements observed in both directions. Unfortunately, the length or weight data was not reliable and so is not included in this table.

**Table 10. Details of tag/recaptures for yellowtail kingfish tagged and or recaptured in Victorian waters (courtesy of Mick Gamble, NSW Fisheries).**

| Tag No. | Date released | Location released | Date recaptured | Location recaptured | Time at liberty (days) |
|---|---|---|---|---|---|
| **A43203** | 15/02/1986 | Port Welshpool (Vic) | 27/10/1986 | Halibut Oil Field: East Bass Strait (Vic) | 254 |
| **A138215** | 10/05/1987 | Montague Island (NSW) | 28/03/1989 | Port Phillip Bay (The Rip) (Vic) | 688 |
| **A230136** | 14/04/1993 | Portland (Vic) | 14/04/1993 | Portland (Vic) | 0 |
| **A278803** | 27/02/1994 | Woodside (Vic) | 17/12/1994 | The Banks (NSW) | 293 |
| **A278802** | 27/02/1994 | Woodside (Vic) | 18/12/1994 | Moruya (16 Nm Se) (NSW) | 294 |
| **D007764** | 8/04/2007 | Green Cape (NSW) | 26/01/2008 | Port Philip Bay (Ricketts Point, Vic) | 293 |
| **D009885** | 31/03/2007 | Mowarry Point (NSW) | 19/02/2008 | Barwon Heads (Vic) | 325 |

## The use of satellite tags to understand movement characteristics

*This section is relevant to meeting Objective 4.*

Characterising movement patterns and pathways of fish is important for ongoing effective management particularly when the species move between management jurisdictions. Yellowtail kingfish is a fast swimming pelagic species, capable of covering large distances (100 -1000s km) across ocean seascapes (Gillanders, Ferrell et al. 2001). External tagging/recapture studies have shown that yellowtail kingfish can move between SA and NSW, NSW and VIC, and even from NSW to Lord Howe Island and NZ waters (Gillanders, Ferrell et al. 2001). In Australia, tagging is mostly conducted by recreational fishers in NSW, VIC and SA under co-ordination by NSW fisheries. Tagging typically involves limited numbers of avid anglers and sport and game fishing clubs. Typical tag/recapture programs collect data related to locations of release and recapture, and what the fish does is between remains a mystery. Developments in animal tagging technology provide new opportunities to collect detailed information on individual fish movement behaviour. One of these new advances involves satellite tagging. Satellite tags can provide information such as the temperature and depth experienced by a fish at high temporal resolution. Also, models that utilise the temperature and depth records can estimate the geographic locations or regions where a fish was likely to have been at particular times. This project trialled the use of 'miniPAT' pop-up archival tags (PAT) developed by Wildlife Computers (https://wildlifecomputers.com/our-tags/minipat/), to provide information on habitat/depth preference and migration characteristics of individual yellowtail kingfish.

### Methods

Three tags were trialled on yellowtail kingfish were captured using recreational fishing techniques (jigging). Fish were brought onboard where a hose was positioned into the mouth that provide a continue supply of seawater. Each tag was attached to the fish using a tethering approach. A 100 lb plastic coated wire was inserted into one side of the yellowtail kingfish approximately 5 cm below the dorsal spines and passed through to the other side using a needle. This enabled the maximum retention through by utilising the pterygiophore complex. The tag was attached to the wire by crimping the two ends of the wire. Using the same technique, a secondary attachment

point was made toward the tail (Figure 16). Tags were deployed in standby mode which activates the tag to collect data when a depth of 5 metres was attained. Data recorded included, pressure (depth), temperature and light at 600 second intervals. Tags were programmed automatically detach from the fish if the tag is floating on the surface, is at a constant depth ± 2.5m or deeper than 500m for longer than three days, is at a depth below 1400m. Tags were set to detach up to 180 days after fish were tagged and released. Kingfish were tagged towards the end of the kingfish season from February – April. Once tags had detached from the fish they floated to the surface and connected to the Advanced Research and Global Observations Satellite (ARGOS) system where data was uploaded and accessed via Wildlife Computers online portal. Here, an algorithm that uses date/time, light intensity, sea surface temperature and bathymetry were used to determine the geolocation of the fish during liberty.

**Figure 16. Location of wildlife computers miniPAT attached to a yellowtail kingfish prior to release.**

### Results

Three yellowtail kingfish were tagged however only one of the tagged fish (tag 167737, Table 11) provided useful data. It is suspected the other two fish were predated by seals leading to the early tag releases.

**Table 11. Details of satellite tag deployments of three yellowtail kingfish.**

| Tag number | Fish size (TL cm) | Date released | Latitude (released) | Longitude (released) | Programmed release date | Pop-up transmission date | Duration of data collection |
|---|---|---|---|---|---|---|---|
| **167737** | 65 | 05 April 2017 | -38 24.837 | 142 00.967 | 30 Jun 2017 | 13 Jun 2017 | 69 days |
| **167735** | 80 | 19 April 2018 | -38 19.462 | 144 33.220 | 15 Aug 2018 | 30 Apr 2018 | 11 days |
| **167736** | 64 | 27 Feb 2019 | -38 18.930 | 144 27.108 | 31 Jul 2019 | 10 Mar 2019 | 12 days. |

### Tag 167737

This 65 cm yellowtail kingfish was tagged off Lady Julia Percy Island (Western Victoria) on the 5 April 2017, the tag was programmed to release from the fish and transmit data on 30 June 2017, but released and transmitted data slightly earlier on 13 June 2017, resulting in 69 days of data collection. Immediately after tagging, the fish moved out to the continental slope and followed the slope south-east before crossing into Bass Strait and moving east through Bass Strait until the tagged released just north of Flinders Island in mid-June (Figure 17). While the actual locational data is derived by model estimates that have an associated level of uncertainty (displayed in (Figure 18), we can be highly confident that the fish moved east across Bass Strait over the 69 days of tag recording. The estimated central point of the path through Bass Strait is interesting as it included King Island and the string of islands between Wilson Promontory and Flinders Island, consistent with the association of yellowtail kingfish with topographic features such as reefs and oceanic islands (Figure 17).

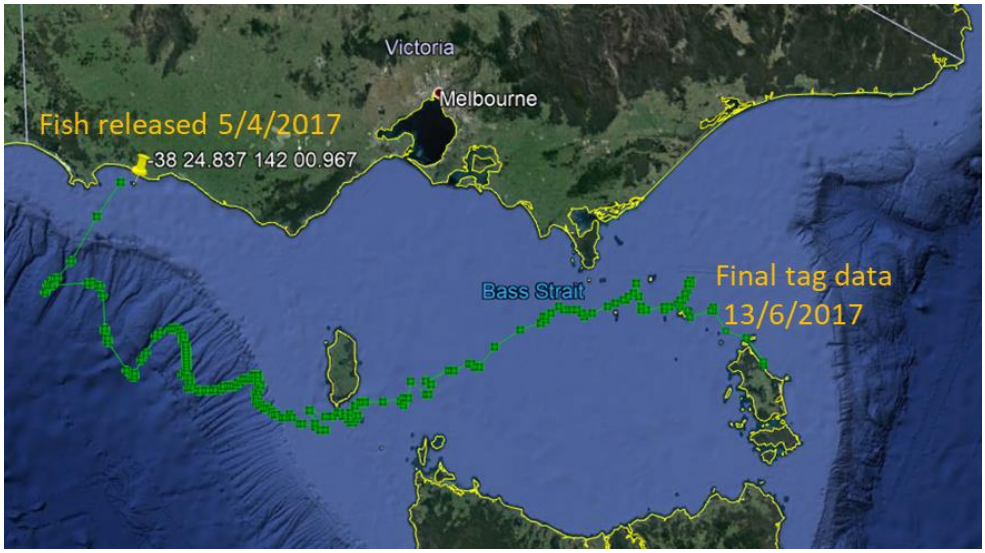


**Figure 17. Modelled projection of yellowtail kingfish (Tag 167737) locations.**

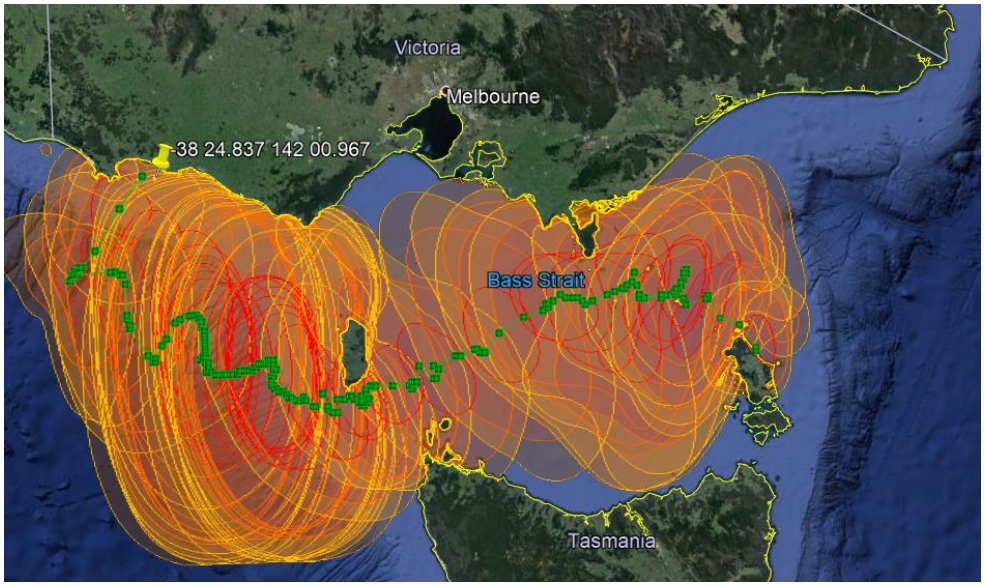


**Figure 18. Modelled projection of yellowtail kingfish (tag 167737) locations with 50%, 95% and 99% confidence intervals.**

The depth profile recorded indicated that the fish moved deeper shortly after release, and as it moved along the continental slope and through into Bass Strait, reaching average daily depths of 70 m. However, it appears that when the fish reached eastern Bass Strait, it mostly resided in near surface waters (i.e. from mid-May onwards) (Figure 19). The average daily temperature records indicated that the fish mostly remained in water temperature >15.5°C (Figure 19). The minimum recorded exposure temperature was 14.5°C and the maximum was 17°C, experienced shortly after release.

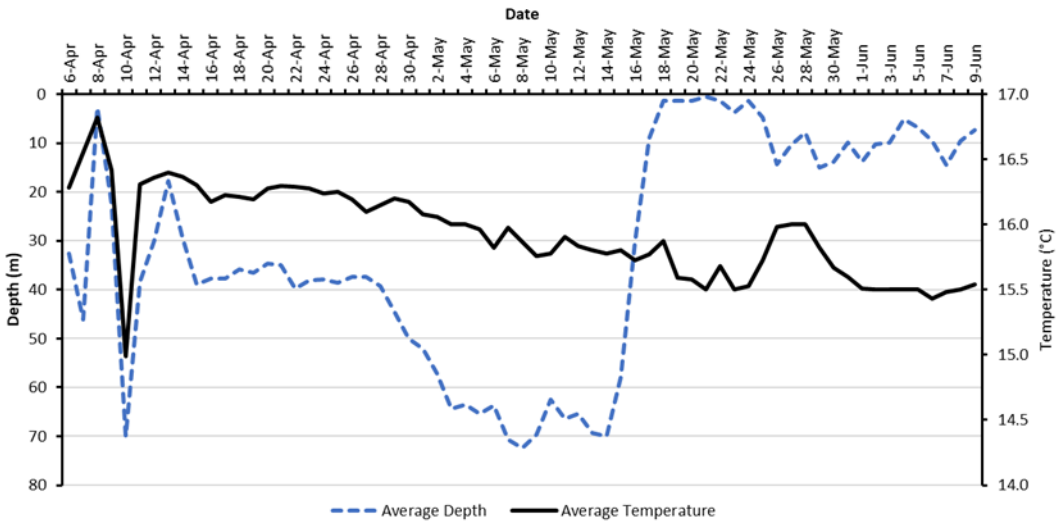


**Figure 19. Average daily depth and temperature of yellowtail kingfish tag 167737 as it moved from Lady Julia Percy Island to eastern Bass Straight from April to May 2017.**

### Tag 167735

After deployed off Barwon heads (Central Victoria), the tag washed up on shore 11 days later. It appears that the first dorsal wire had broken at the crimp. The tag appeared very scratched which was either from abrasion on rocks or teeth marks from a seal. The tag was collected from the beach by a passer-by and it was later sent to the Victorian Fisheries Authority. No data was able to be downloaded from the tag. This event suggested that extreme care needs to be taken when crimping the wire as damage to the wire could have occurred.

### Tag 167736

This fish was tagged in the Port Phillip Heads region, but the tag was found 12 days after the fish was released. Like Tag 167735 it appeared the dorsal wire had broken.

For tags 167735 and 167736 it is unclear whether the wire corroded at the crimp attachment point or if the tagged was pulled from the fish from a predator such as seal. Tag 167737 did remain attached to the kingfish for 69 days suggesting that the attachment method was appropriate. Two attachment points were used to secure the tags on to the fish so it's unlikely that the stainless-steel wire corroded at both.

## Potential of otolith stable isotopes to provide information on temperature exposure and population structure of yellowtail kingfish

*This section is relevant to meeting Objective 5.*

### Background

Stable isotope ratios of carbon ($^{13}C/^{12}C$, i.e. $\delta^{13}C$ ) and oxygen ($^{18}O/^{16}O$, i.e. $\delta^{18}O$) in fish otoliths (earbones) can be used to study fish population structure, metabolic function and temperature exposure histories (Campana 1999, Campana and Thorrold 2001). $\delta^{18}O$ in particular, has been shown to be a useful indicator of temperature experienced by fish due to the fact that the fractionation of the isotopes $^{18}O$ and $^{16}O$ in otolith carbonate is in close equilibrium to that of the surrounding seawater and is strongly influenced by temperature(Iacumin, Bianucci et al. 1992, Thorrold and Campana 1997, Dufour, Pierre et al. 1998, Edmonds, Steckis et al. 1999, Gao and Beamish 1999, Weidman and Millner 2000, Begg and Weidman 2001, Gao, Joner et al. 2001, Stephenson, Edmons et al. 2001). Higher $\delta^{18}O$ values in fish otolith indicate exposure to cooler water.

Unlike $^{18}O/^{16}O$ , the $^{13}C/^{12}C$ ratios in otoliths are not in equilibrium with the surrounding water, and it is thought that much of this disequilibria is related to metabolic effects (Thorrold, Campana et al. 1997). Variation in the

$^{13}C/^{12}C$ isotopic ratio of otolith carbonate may be influenced by variation in both environmental conditions, trophic level of prey and physiology/metabolism (Kalish 1991, Kalish 1991a, Iacumin, Bianucci et al. 1992, Thorrold, Campana et al. 1997, Schwarcz, Gao et al. 1998, Weidman and Millner 2000).

In the case of $\delta^{18}O$, where the influence of temperature has been clearly established, it is possible to use otolith $\delta^{18}O$ to determine if different fish have experienced different temperature regimes during their lives, and therefore infer whether or not they occurred in different ocean regions or perhaps depths. Furthermore, it is possible to convert otolith $\delta^{18}O$ to actual water temperature values which can be related to actual water temperature measurements to provide additional information on likely areas or depths a fish may have used, or perhaps more definitively, to rule out areas or depths.

In this study we conducted a pilot investigation to explore the potential of otolith $\delta^{13}C$ and $\delta^{18}O$ to provide information on yellowtail kingfish population structure and temperature exposure (thermal history). We used samples of yellowtail kingfish otoliths from those collected by the broader project from VIC, NSW and TAS, although there were low sample numbers available for NSW and TAS (Table 12a). For VIC, the samples were haphazardly selected to cover the available size range, males and females, and the Eastern, Central and Western regions of the coast (Table 12b).

**Table 12. Summary of yellowtail kingfish otolith samples used in stable isotope analyses by state (a) and by region of Victoria (b).**

**a) By State and sex (M=Male, F=Female, I=Immature, NA=Unknown)**

| State | N | Sex (M, F, I, NA) | Total Length cm: Mean (range) |
|---|---|---|---|
| **VIC** | 68 | 31xM, 33xF, 4xNA | 82.4 (49.5–137) |
| **TAS** | 10 | 10xNA | 51.4 (42.4–66.4) |
| **NSW** | 15 | 3xM, 6xF, 6xNA | 94.2 (59.5–127.0) |

**b) Victorian samples by region and sex (M=Male, F=Female, I=Immature, NA=Unknown)**

| Region | N | Sex (M, F, I, NA) | Total Length cm: Mean (range) |
|---|---|---|---|
| **Western VIC** | 23 | 12xM, 11xF | 83.8 (57.5–125.5) |
| **Central VIC** | 23 | 8xM, 12xF, 3xNA | 79.8 (49.5–137.0) |
| **Eastern VIC** | 22 | 11xM, 10xF, 1xNA | 83.7 (60.0–106.5) |

Sampling locations within each region: Western=Lady Julia Percy Island, Killarney/Port Fairy, Portland; Central= Barwon Heads/Collendina, The Rip, Cape Woolamai; Eastern=Mallacoota, Marlo

**Methods**

Because yellowtail kingfish otoliths are very small it was not possible to use a micro-sampling approach such as micro-milling i.e. Weidman and Millner (2000) to sample different life-history stages 'within' individual otoliths. In this study one whole sagittal otolith was selected from each fish, weighed to 0.0001 g and then ground to a fine powder using an agate mortar and pestle. The ground otolith powders were transferred to 0.2 ml eppindorf PCR tubes for transfer to the analytical laboratory. All equipment was rinsed in distilled water and dried between each sample being processed. Sample weights ranged from approximately 2–10 mg.

Stable carbon and oxygen isotope ratios were determined using an Analytical Precision AP2003 continuous-flow, isotope-ratio mass spectrometer (School of Geography, Speleothem Research Group Stable Isotope Laboratory, University of Melbourne). Carbon dioxide was evolved from sub-samples of the ground otolith carbonate powder by reaction with 105% orthophosphoric acid in 15mL exetainer vials at 70 °C after atmosphere was replaced with He. Repeat samples of NBS-18 (international standard, known values: -5.01 $\delta^{13}C$, -23.2 $\delta^{18}O$), and the in-house standards NEW1 (known values: +2.40 $\delta^{13}C$, -2.46 $\delta^{18}O$) and NEW12 (known values: -3.80 $\delta^{13}C$, -9.59 $\delta^{18}O$) were run intermittently during analysis runs. NEW1 and NEW12 have been calibrated against international standards NBS-18 and NBS-19. Sample data were normalised to the VPDB (Vienna Pee Dee Belemnite) scale against NEW-1 because it is closest to the otolith values for oxygen. NEW12 and NBS-18 were run as unknowns. Two samples of each otolith powder were analysed. Mean analytical precision for $\delta^{18}O$ and $\delta^{13}C$ were better than 0.05‰.

To indicate water temperature exposure $\delta^{18}O$ values for otolith samples were converted to water temperature estimates using two different fractionation equations from Kalish (1991a) and Høie (2004) (below). The Kalish (1991a) equation is derived from a laboratory rearing study of eastern Australian salmon conducted in Tasmania that covered a temperature range of 13–22°C, similar to that experienced by yellowtail kingfish in south-eastern Australia. The Høie (2004) equation is from a laboratory rearing study of Atlantic cod that covered a temperature range of 6-20°C. The Høie (2004) equation is very similar to various other equations for northern hemisphere species, and inorganic aragonite, in the published literature. However, the Kalish (1991a) equation has different slope and intercept see Dorval (2011) and therefore provides slightly different temperature estimates. Since this has not been done for yellowtail kingfish, we applied these two different conversion equations to provide a likely range of temperature exposure for the yellowtail kingfish sampled in this pilot study.

For the Høie (2004) equations the $\delta^{18}O$ seawater water values were derived from the 'Global Gridded Data Set of Oxygen Isotopic Composition in Seawater' (LeGrande and Schmidt 2006), and we used average values for inshore surface waters across the regions where samples were collected for each State.

Equations for converting $\delta^{18}O$ otoliths to water temperature:

$T(°C) = (\delta^{18}O_{Otolith}(VPDB) - \delta^{18}O_{water}(VPDB) -3.90)/-0.20$ Høie, Otterlei et al. (2004)

$T(°C) = (\delta^{18}O_{Otolith}(VPDB)-6.69)/-0.326$ (Kalish 1991a)

VPDB = Vienna Pee Dee Belemnite

Values of $\delta^{18}O_{water}$ were converted from the VSMOW reference to VPDB using the conversion equation form Freidman and O'Neill (1977)

$\delta^{18}O_{water}(VPDB) = 0.99978* \delta^{18}O_{water}(VSMOW) – 0.22$

VSMOW = Vienna Standard Mean Ocean Water

Sample data for $\delta^{18}O$ and $\delta^{13}C$ were plotted for individual samples by State and collection regions within States, and as means (±SE, standard error) for 10 cm TL bins: 40-49, 50-59, 60-69, 70-79, 80-89, 90-99, 100-109, 110-119, 120-129, 130-139 cm TL.

Mean (±SE) water temperature estimates were plotted for the 10 cm length bins to compare trends with size across States. Note that these trends represented the temperature history integrated across the whole otolith, and therefore the entre life prior to capture.

To estimate temperature exposure for the growth periods associated with each length bin, mass balance relationships were used (Kalish 1991a, Høie, Otterlei et al. 2004). Because some length bins had low sample sizes, including some with only one fish, we used regression estimates of mean otolith weight and mean $\delta^{18}O$ for each length bin rather than the individual data. The regressions of mean otolith weight and $\delta^{18}O$ against the series of 10 cm length bins (i.e. numbered from 1 onwards), were linear and strong for all States and region groups that were used in these analyses ($r^2$ ranged from 0.83-0.99). The mass balance approach estimates the $\delta^{18}O$ for the growth period within an individual length bin (LB) according the following equation:

$((Ot_{wt}\_LB1/ Ot_{wt}\_LB2) * \delta^{18}O\_LB1) + ((Ot_{wt}\_LB2-Ot_{wt}\_LB1)/Ot_{wt}\_LB2)* \delta^{18}O\_GPLB2) = \delta^{18}O\_LB2$

Therefore:

$\delta^{18}O\_GPLB2 = ((\delta^{18}O\_LB2) – ((Ot_{wt}\_LB1/ Ot_{wt}\_LB2)* \delta^{18}O\_LB1)) / ((Ot_{wt}\_LB2- Ot_{wt}\_LB1)/ Ot_{wt}\_LB2)$

$Ot_{wt}$_LB1= mean otolith weight for length bin 1 estimated by regression of otolith weight against length bin,

$Ot_{wt}$_LB2= mean otolith weight for length bin 2 estimated by regression of otolith weight against length bin,

$\delta^{18}O$_LB1 = mean $\delta^{18}O$ for length bin 1 estimated by regression of $\delta^{18}O$ against length bin

$\delta^{18}O$_LB2 = mean $\delta^{18}O$ for length bin 2 estimated by regression of $\delta^{18}O$ against length bin

$\delta^{18}O$_GPLB = $\delta^{18}O$ estimated for the growth period encompassed within the length bin 2

Temperature estimates derived from $\delta^{18}O$ in otoliths were compared with mean annual water temperature estimates obtained for nine ocean regions around south eastern Australia and depths to 100 m. Ocean temperature data were sourced from the CSIRO; CARS - Climatology of Australasian Regional Seas program (http://www.marine.csiro.au/~dunn/cars2009/).

Estimates of temperature exposures for the growth periods within 10 cm length bins are assumed to be representative of the ocean temperatures experience during those particular growth phases. Note that different

fish are used for each length bin. This is different to an individual based analysis of life-history trends from sampling different life-stages within otoliths using micro-mill sampling. The mass balance approach is typically applied to laboratory studies where fish experience the same conditions. For field-based studies it is best applied where samples within each length bin are collected from the same regions/populations and assumes that fish of each larger sizes bins do not have fundamentally different migration/exposure histories to the smaller size bins. This assumption was violated in the case of NSW as discussed above, however, we have included the NSW data in these comparisons, acknowledging this issue. For Victoria, where sufficient samples were available, we also calculated the temperature estimates for each length/growth bin for the Western, Central and Eastern sampling regions.

## Results

The duplicate analyses of each otolith showed that for $\delta^{18}O$, 92% of duplicate samples were within 0.3 ‰, with two samples having differences of between 0.4-0.5 ‰. For $\delta^{13}C$, 98% of the duplicates were within 0.2 ‰, with two samples between 0.21 and 0.26 ‰. For the main analysis we used the averages of the duplicate samples analysed for each otolith.

For the standards run as unknowns, the average accuracy for NEW12 (n=22) was 0.997 ±0.012 SD for $\delta^{13}C$ and 1.001 ±0.010 SD for $\delta^{18}O$. For NBS-18 average (n=9) accuracy was 0.996 ±0.003 SD for $\delta^{13}C$ and 0.976 ±0.014 SD for $\delta^{18}O$.

### *Otolith $\delta^{18}O$ and $\delta^{13}C$*

$\delta^{18}O$ and $\delta^{13}C$ of otoliths increased with length for samples from each State (Figure 20, Figure 21). For $\delta^{18}O$, the samples from VIC generally had higher $\delta^{18}O$ than NSW (Figures 18a, 19). The samples from TAS that overlapped in size with VIC samples had similar $\delta^{18}O$ (Figure 18a). For $\delta^{13}C$ there was strong overlap among States for the comparable length ranges (Figure 20b).

For the VIC samples the relationship between mean $\delta^{18}O$ and length appeared to be non-linear with an increased trend with length until around 100 cm TL before stabilising beyond 100 cm TL (Figure 22). While there was some indication of a similar pattern for NSW, sample size was insufficient, and for TAS there were no samples of larger fish (Figure 20a). For NSW samples there were two outliers for $\delta^{18}O$, these two samples (Figure 20a, samples a and b) had lower $\delta^{18}O$ than for other samples at similar lengths, suggesting they had been in warmer waters. The replicate samples analysed from both these outliers were very similar and their outlying nature did not appear due to analytical errors.

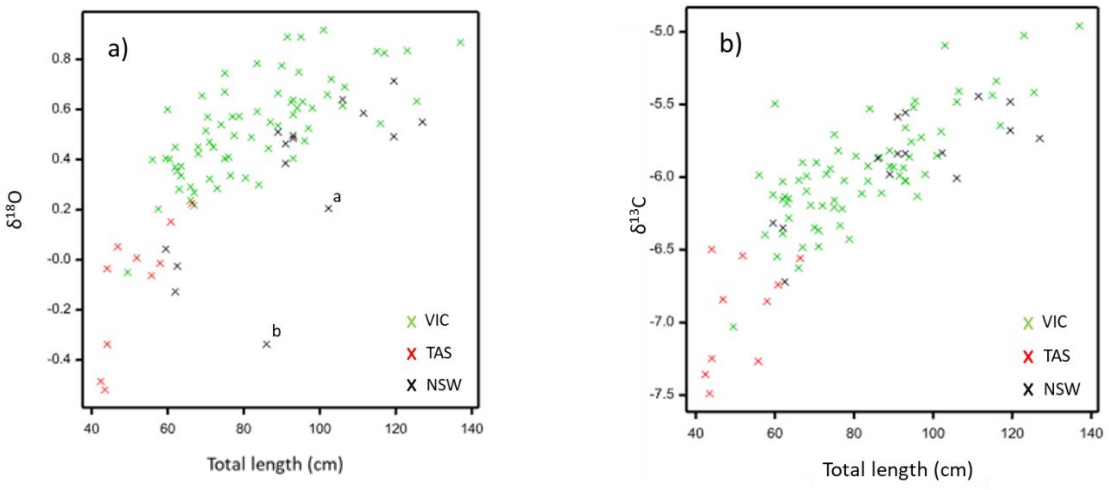


**Figure 20. Scatterplots of a) $\delta^{18}O$ and b) $\delta^{13}C$ versus total length for yellowtail kingfish sampled from Victoria, New South Wales and Tasmania.**

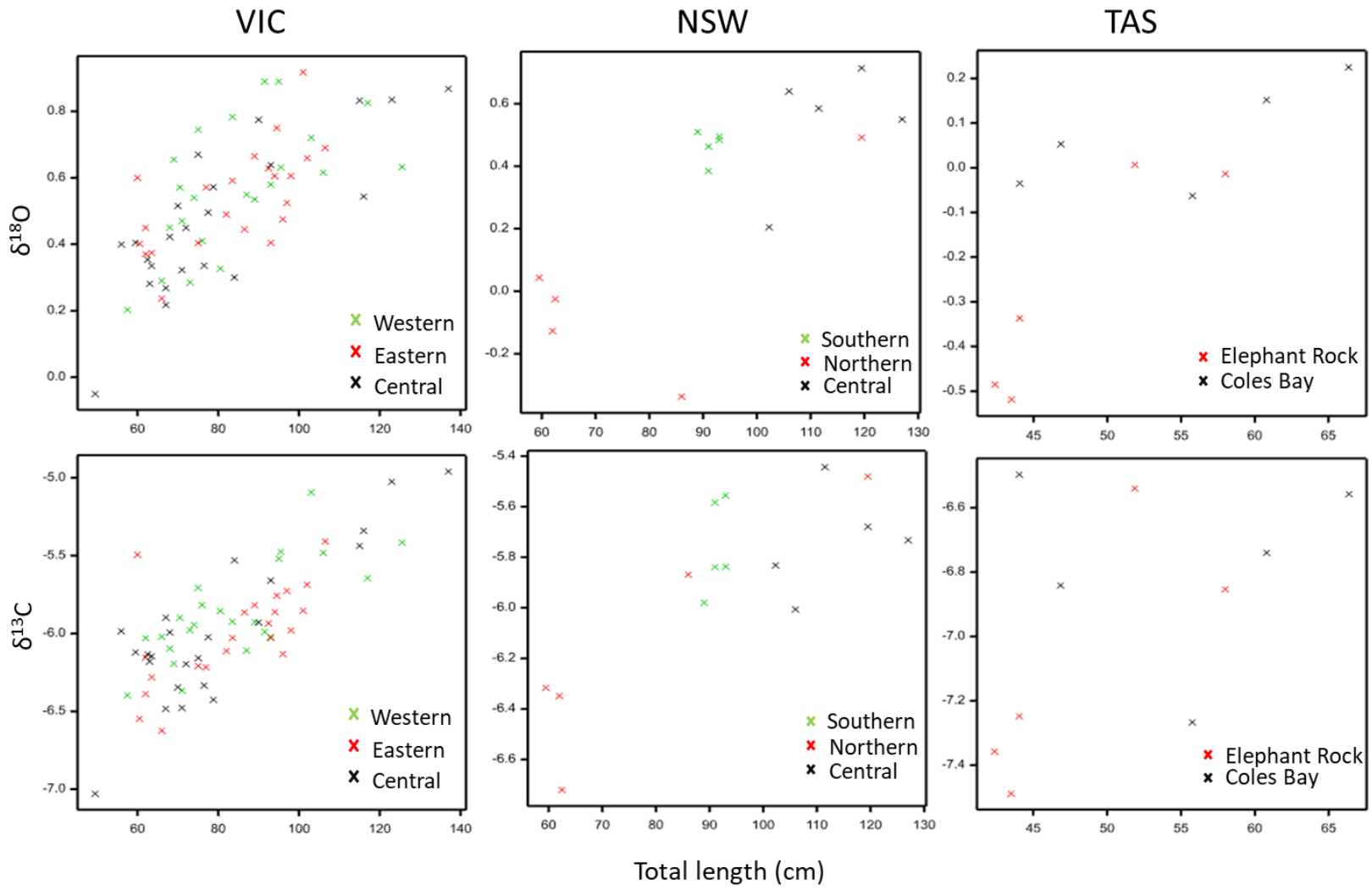


**Figure 21. Scatterplots of (top) $\delta^{18}O$ and (bottom) $\delta^{13}C$ versus total length for yellowtail kingfish sampled from different regions (Victoria, New South Wales) and locations (Tasmania) within Victoria (VIC), New South Wales (NSW) and Tasmania (TAS).**

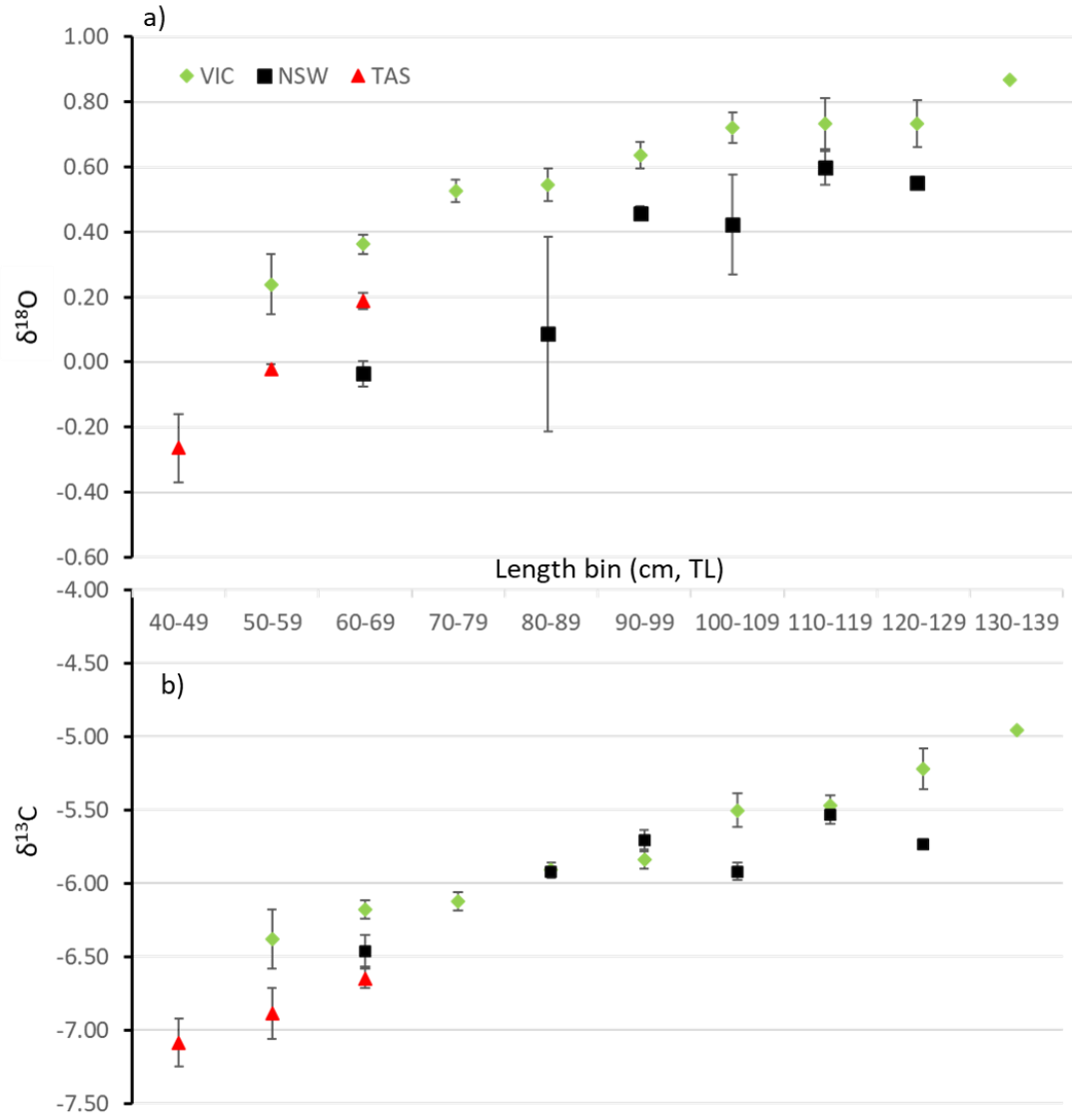


**Figure 22. Plots of mean± SE (standard error); a) $\delta^{18}O$ and b) $\delta^{13}C$ for yellowtail kingfish otoliths sampled from Victoria (VIC), New South Wales (NSW) and Tasmania (TAS) by 10 cm length bins.**

***Temperature exposure estimated from otolith $\delta^{18}O$***

*Integrated life-time temperature exposure*

Temperature estimates derived from otolith $\delta^{18}O$ indicated that juvenile yellowtail kingfish up to 70 cm length sampled in this study mostly resided in water temperatures of 19°C or above irrespective of State or the $\delta^{18}O$-temperature conversion used (Figure 23). The smallest samples from TAS and NSW had average temperature estimates of around 21-22 °C. For the NSW samples, the trend for lower average temperature exposure with increased length bin was likely confounded due to all but one of the larger samples (> 90 cm TL) being from the central and southern regions, and all the smaller samples being from the northern region (Figure 21). Clearly, more samples across the size range and from different latitudinal regions are required to understand temperature exposure trends with age for NSW and TAS. None-the-less, the life-time integrated temperature exposure for the larger NSW samples from the southern and central regions appeared only marginally higher than for the VIC samples across the length bins from 90 cm upwards (Figure 23). For the VIC and NSW samples the life-time temperature exposure estimates appeared to stabilise at between 17-19°C from around 100 cm TL (Figure 23).

*Temperature exposure for specific growth periods/length bins*

There was a consistent trend for decreasing water temperature exposure with growth period as the length bin increased, indicating that yellowtail kingfish progressively spend more time in cooler water with age (Figure 24). For VIC samples the average temperature exposure for the period of life up until 50-59 cm TL (i.e. first 1-2 years of life; this study (Stewart, Ferrell et al. 2004)) was between 19-20°C and decreased linearly to 13°C using Høie et al. (2004) or 15.5°C using Kalish (1991a) for the growth period associated with the 130-139 cm TL bin (Figure 24). For NSW and VIC, temperature estimates for the 120-129 cm TL growth period were very similar at between about 14°C using Høie et al. (2004) and 16°C using (Kalish 1991a). Based on the age length data from this study and previous work by Stewart et al. (2004) and McKenzie et al. (2014) the growth periods above 100 cm TL likely correspond to fish over 6 years age.

For the smallest samples from TAS of 40-49 cm TL, equivalent to about the first 6 months of life (Stewart, Ferrell et al. 2004, McKenzie, Smith et al. 2014), the average temperature exposure was estimated to be 21-22°C, and for the growth period 50-59 cm TL, estimated to be the second six months of life, temperature exposure was lower at 18-19°C (Figure 24). The smaller TAS samples therefore experienced declining temperature exposure over their first year of life. This is consistent with spawning in summer, and the declining temperature exposure over the first year could be due to due lower temperature in winter and or dispersal to more southerly latitudes.

Small juvenile kingfish (<50 cm TL) are often found around FADS in NSW and New Zealand (McKenzie, Smith et al. 2014) and are thought to spend most of their time in the upper 50 m of the water column. If this is the case then the temperature estimates using either the Kalish or Høie methods for fish up to 60 cm TL (i.e. >19°C for whole otoliths (Figure 23), and >18°C for growth periods 40-49, 50-59 cm TL (Figure 24, Figure 25 ) would suggest that the yellowtail kingfish sampled from VIC and TAS waters originated from further north than these two State jurisdictions. Based on the CARS temperature data, for yellowtail kingfish to be exposed to an average water temperature in the upper 50 m of at least 18°C they would need to be at least as far north as south/central NSW (Figure 26). Therefore, it is plausible that yellowtail kingfish from VIC and TAS were derived from spawning along the NSW coast, somewhere from Bermagui to the north (BMG, Figure 26). If they were derived from possible spawning areas in SA, these would have to be in waters warmer than the Port Lincoln region (PTL, Figure 26), which seems unlikely.

Temperature exposure estimates from otolith $\delta^{18}O$ diverged between the Kalish and Høie methods with larger fish size (Figure 24, Figure 25). The regional analysis of VIC samples indicated that, irrespective of sampling region, yellowtail kingfish of the largest sizes (100–140 cm TL) spent the majority of their time in water temperatures from 14-16°C (Høie method) or 15.5–17°C (Kalish method) (Figure 25). It should be noted that these larger fish were mostly less than 8 years age, and most would have been immature. Average annual water temperature data for the CARS data suggest that average exposures of below about 14°C would imply extended residency periods at southern latitudes (i.e. southern TAS region, SET, Figure 26) or at depths below 70 m for areas in western and eastern Bass Strait (i.e. Kngl, EBS, Figure 26). While future application of otolith $\delta^{18}O$ to estimate temperature history for kingfish requires a species-specific validation of the relationship, the temperature estimates using the Kalish (1991a) conversion equation appear more plausible given the predominant summer/autumn occurrence of yellowtail kingfish in VIC and east TAS.

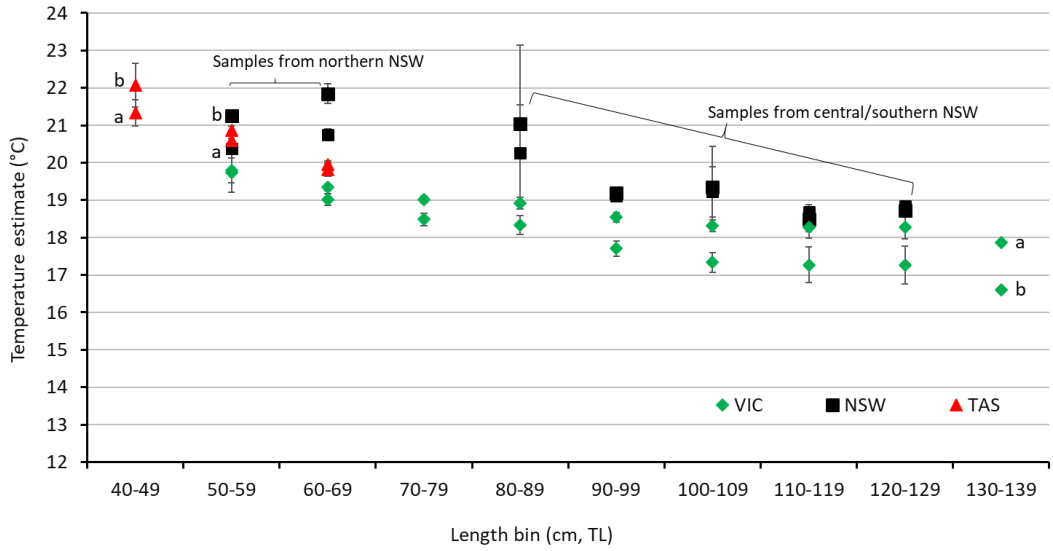


**Figure 23. Plots of mean± SE (standard error) temperature estimates integrated across life derived from $\delta^{18}O$ for yellowtail kingfish otoliths sampled from Victoria (VIC), New South Wales (NSW) and Tasmania (TAS) grouped by 10 cm length bins. a – converted using Kalish (1991b), b – converted using Høie et al. (2004).**

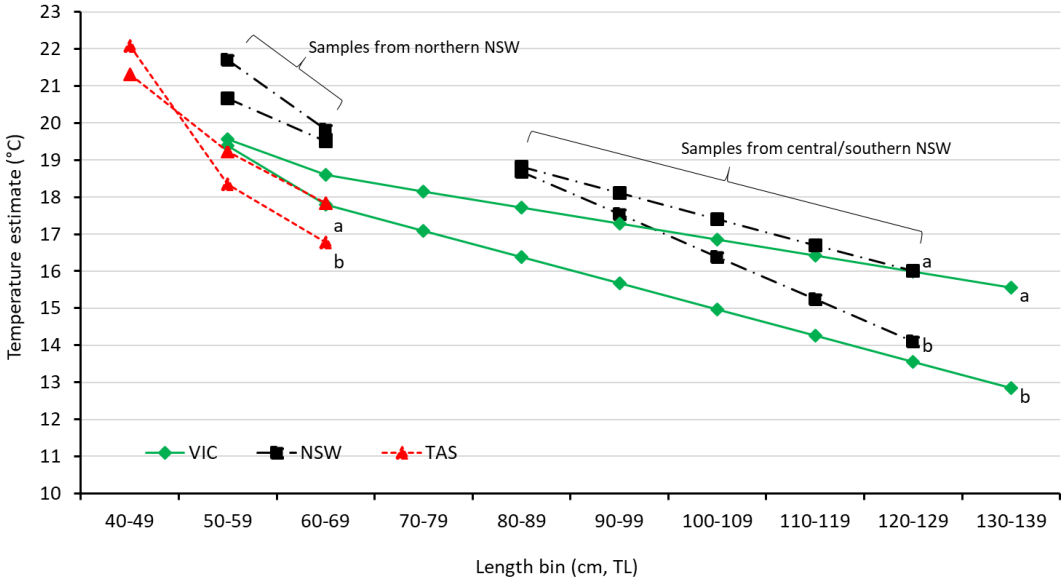


**Figure 24. Plots of estimated mean temperatures (derived from otolith $\delta^{18}O$) experienced by yellowtail kingfish sampled from Victoria (VIC), New South Wales (NSW) and Tasmania (TAS) for the growth periods within 10 cm lengths bins. a – converted using Kalish (1991b), b – converted using Høie et al. (2004).**

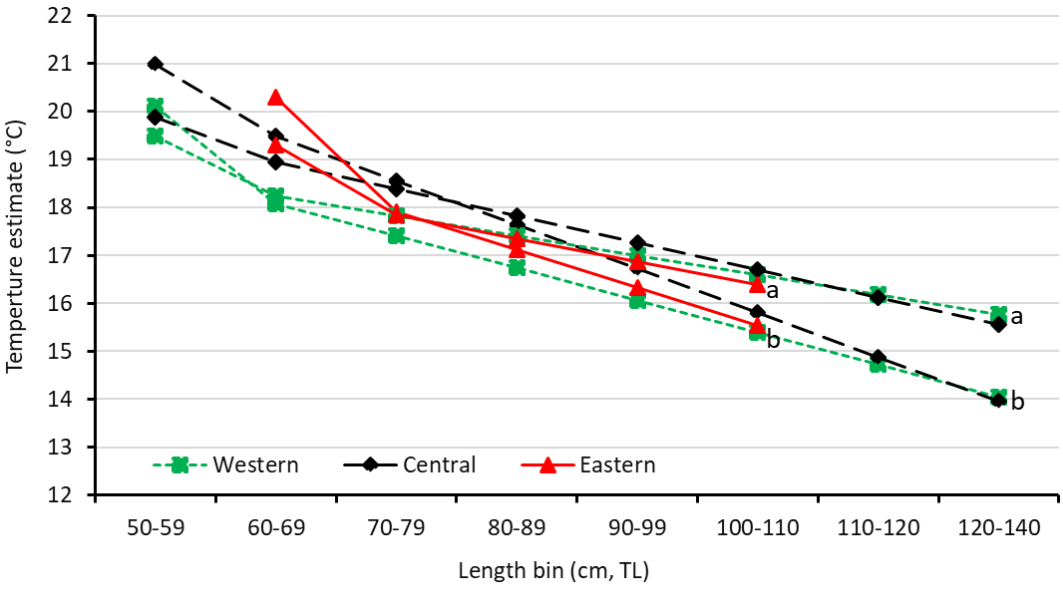


**Figure 25. Plots of estimated mean temperatures (derived from otolith δ18O) experienced by yellowtail kingfish sampled from three Victorian regions for growth periods within 10 cm lengths bins. a – converted using Kalish (1991b), b – converted using Høie et al. (2004).**

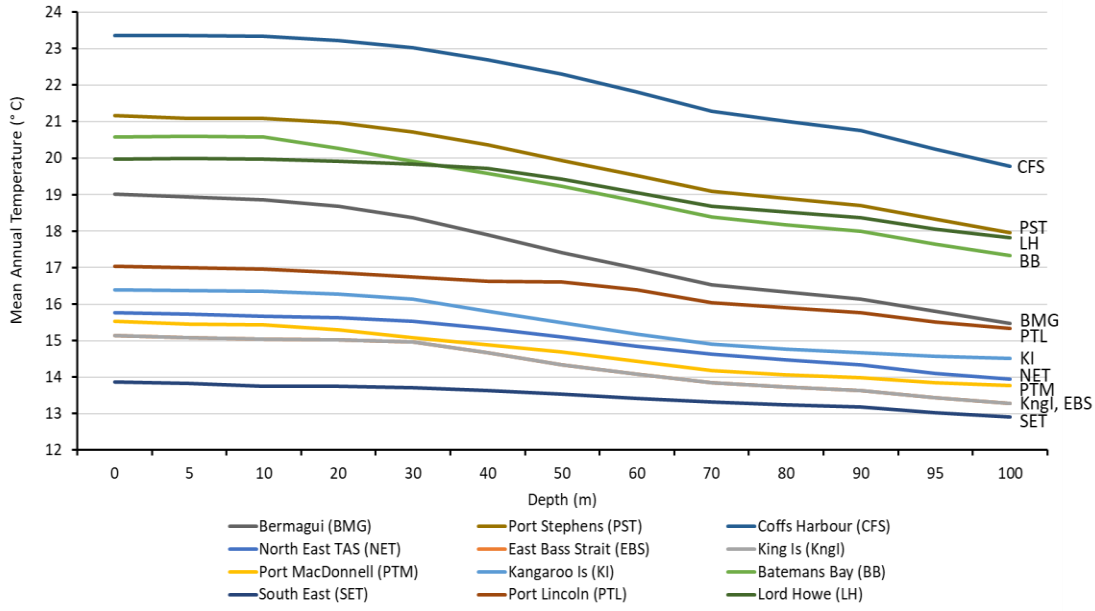


**Figure 26. Comparisons of mean annual water temperatures estimated for nine areas, and depths to 100 m, around south eastern Australia.**

## Suitability of yellowtail kingfish for marine stocking

*This section is relevant to meeting Objective 6.*

### Background

Stocking is a fisheries management tool that has numerous purposes including augmentation and enhancement, creation of new fisheries, conservation, mitigation, community and environmental change (Table 13) (Cowx 1994, Cowx 1998, Cowx 1998, Welcomme and Bartley 1998, De Silva and Funge-Smith 2005, Bell, Bartley et al. 2006, Bartley 2007, Ingram and De Silva 2015, Lorenzen, Cowx et al. 2016, Hunt and Jones 2017). Stocking is one option for managing fish stocks, particularly where recruitment is severely limited. Irrespective of purpose, stocking is intended to rebuild depleted stocks or increase unproductive stocks, usually by releasing large numbers of hatchery-produced juveniles.

Stocking programs therefore require access to sufficient numbers of juveniles of appropriate species and genetic stock that will survive and grow after release and remain in the area to enter the fish population. Stocking hatchery-produced fish is seen as a means of meeting the demands for fish for both sport (recreational angling) and food, and stocking programs are playing an important role in the conservation and recovery of threatened species. Stocking is used by fisheries managers to restore depleted populations of recreationally and commercially significant fish species and may be a potential management option to ameliorate impacts of prolonged recruitment failure, such as experienced by sand flathead in Port Phillip Bay (Hamer, Kemp et al. 2010, Hirst, Rees et al. 2014).

Stocking occurs in freshwater, estuarine and marine environments worldwide. Marine stock enhancement occurs in coastal waters, bays, estuaries and coastal lagoons (Taylor, Palmer et al. 2005, Taylor 2006, Bell, Leber et al. 2008, see also http://www.searanching.org). In developed countries there is a demand for stocking of marine and estuarine environments from the recreational fishing sector (Loneragan 2015), and not surprising this demand is growing in Victoria (Foundation 2009, Taylor 2010). However, not all stocking programs are successful or are likely to be successful. While some have increased yields and generated economic and social benefits, others have either failed to live up to expectations, or their effectiveness have not, or could not be, assessed. Consequently, a responsible approach to stocking, with well-defined management objectives and rationale for stocking is an important first step. However, any stocking program should include plans for monitoring and evaluation, to support continuous learning and understanding of value for money.

Ten principles for responsible marine release programmes have been identified by the *International Symposia on Stock Enhancement and Sea Ranching* (ISSESR) (http://www.searanching.org) (Blankenship and Lee 1995, Blankenship and Leber 1996, Lorenzen, Leber et al. 2010), which are:

1. Prioritize and select target species
2. Develop a management plan
3. Define measures of success
4. Use genetic resources management
5. Use disease and health management
6. Develop restocking objectives and tactics
7. Identify released individuals and assess stocking effects
8. Identify optimum release strategies
9. Identify economic and policy objectives
10. Use adaptive management.

**Stock enhancement in Victoria**

Stocking of fish to establish and enhance recreational fisheries, and to support conservation efforts, has been long practiced for freshwater fish in Victoria (Wharton 1969, Ingram, Barlow et al. 1990, Cadwallader, Barnham et al. 1992, Gooley 1992, Ingram 2013). Stockings have focused predominantly on freshwater salmonids (mainly brown trout, rainbow trout and chinook salmon) and native fish (mainly golden perch, Murray cod, trout cod and Macquarie perch). More recently trial stockings of estuarine and marine species (black bream, estuary perch, mulloway and eastern king prawns) have occurred "The State Government has committed to implementing a marine species stocking program as part of its Target One Million commitment to get more people fishing, more often" (https://vfa.vic.gov.au/recreational-fishing/target-one-million/marine-stocking). The Target One Million Program was launched in 2015 (.

Stocking in Victoria is undertaken in accordance with the "Fish stocking for recreational purposes" Policy (https://vfa.vic.gov.au/operational-policy/strategy-and-policy/policy-statements/fish-stocking-for-recreational-purposes). The Policy outlines the principles for stocking waters to enhance recreational fisheries and applies to stocking all Victorian waters. Although there is no specific policy for marine and estuarine stocking in Victoria, all translocations in the state, including those into marine and estuarine waters are managed in accordance with the Policy. With the exception of recurring stockings, all new stocking proposals are evaluated by a Translocation Evaluation Panel (TEP), which may request that a full impact and risk assessment be submitted for evaluation before permits to stock are granted.

Currently annual stocking plans are developed in consultation with relevant stakeholders, regarding waters to be stocked and species composition number size and timing of stocking.

**Table 13. Stocking purposes, rationale and assumptions.**

| Stocking purpose | Rationale | Key assumptions | Examples |
|---|---|---|---|
| Augmentation and enhancement | Improve production over natural condition | Stocking carried out to supplement an existing fishery where habitat is below carrying capacity or fishery recruitment is limited. | Stocking to enhance recreational and sport fishing opportunities |
| Create new fisheries | Fill a vacant niche | Species performance in new environment acceptable, the environment can support stocking and is below carrying capacity. The resource base will not change substantially. | Creating fisheries in newly created artificial reservoirs, or waters where there is no fishery, or where new species are introduced into existing fisheries. |
| Conservation | Recover threatened species/populations | Stocking within historical range of species. The environment can support stocking and is below carrying capacity. | Re-establishing populations in areas where threatened species have become extinct. |
| Mitigation | Counter disturbance to the environment | Disturbance event has passed. The environment can support stocking and is below carrying capacity. | Recovery of stocks/fisheries affected by flood, drought or fire events. |
| Community change | Improve production over natural condition | Species performance in new environment acceptable, habitat is below carrying capacity and resource base will not change substantially. | Replenishing stocks in culture-based fisheries. |
| Environmental change | Control environmental conditions and aquatic pests | Species stocked will achieve desired outcome. | Examples. Biomanipulation. Control algal blooms in eutrophic ecosystems by enhancing herbivores through a reduction of planktivorous fish and introduction of piscivorous fish. Stocking of selected fish species to control of mosquito larvae. |

**Yellowtail kingfish aquaculture**

*Seriola* species are farmed globally; yellowtail kingfish (*S. lalandi*) in Japan, Australia, New Zealand, USA and Chile, longfin yellowtail (*S. rivoliana*) in the United States, yellowtail (*S. quinqueradiata*) in Japan, greater amberjack (*S. dumerili*) in Japan, the Mediterranean China Korea and Vietnam, and Pacific yellowtail (*S. mazatlana*) in North and Central America (Kolkovski and Sakakura 2004, Sicuro and Luzzana 2016). Japan has been farming *Seriola* since the 1960s, initially relying on harvest of juveniles from the wild (Poortenaar, Hooker et al. 2001, Nakada 2002). Production in Australia and New Zealand commenced in the early 2000s and relies exclusively on production from captive-held broodstock (Fielder and Heasman 2011, Symonds, Walker et al. 2014).

Aquaculture production of *Seriola* species globally over the last decade has ranged from 155,00 to 188,000 tonnes per year (>80% of which is Japanese amberjack) (http://www.fao.org/fishery/statistics/global-aquaculture-production/en). In Australia *S. lalandi* is farmed commercially in NSW (65.7 t produced in 2016/17), SA and WA. In NSW yellowtail kingfish are being farmed as part of a research partnership between Huon Aquaculture and NSW Department of Primary Industries (DPI) (https://www.huonaqua.com.au/about/portstephens/). Yellowtail kingfish are bred at the DPI's Port Stephens Fisheries Institute (PSFI) (Taylors Beach) and then grown-out in pilot scale sea cages offshore in Providence Bay, NSW. The Cleanseas yellowtail kingfish hatchery at Arno Bay (SA) uses selectively bred stock to produce fish that are reared in sea cages in the Spencer Gulf (http://www.cleanseas.com.au/our-kingfish/) (https://www.pir.sa.gov.au/aquaculture/marine_aquaculture/species). Yellowtail kingfish farming in WA occurs off Geraldton in WA (https://www.iofa.com.au/).

**Captive breeding of juveniles**

Captive breeding techniques for yellowtail kingfish are well established with hatcheries producing seedstock specifically for grow-out in aquaculture facilities, mainly sea-cages (e.g. Poortenaar, Hooker et al. 2001, Moran 2007, Fielder and Heasman 2011, Stuart and Drawbridge 2013, Symonds, Walker et al. 2014). There are hatcheries in NSW, SA and WA producing kingfish seedstock.

Yellowtail kingfish are serial spawners with breeding occurring in warmer months of the year (Gillanders, Ferrell et al. 1999, Poortenaar, Hooker et al. 2001). Broodstock are held in large deep tanks (>20,000L, >2m deep). Spawning occurs spontaneously in domesticated broodstock under ambient conditions from late spring to autumn, at temperatures of 17-24$^{o}$C (Fielder and Heasman 2011). Hormone treatments can be used to stimulate maturation and induce spawning. Domestic broodstock can be spawned on-demand year- round (Fielder and Heasman 2011).

Eggs hatch within 2-4 days depending on temperature. Hatched larvae (4.3-4.8 mm TL) commence feeding initially on live food (rotifers, copepods and *Artemia* nauplii) within 3-4 days. After metamorphosis, which occurs at about 3 weeks of age (8-11 mm TL), fry are weaned onto artificial food. Fish take 4-6 weeks to reach 1 g and 15-17 weeks to reach about 20 g (S. Fielder, *pers. comm*). Juvenile yellowtail kingfish are relatively robust (Moran, Smith et al. 2007). Fish are transferred to sea cages from about 5 g in weight (Kolkovski and Sakakura 2007).

**Size, cost and availability of seedstock for release**

There are no fish hatcheries producing yellowtail kingfish in Victoria. In NSW, the PSFI produces yellowtail kingfish for commercial grow-out (https://www.huonaqua.com.au/about/portstephens/) and for stocking (https://www.dpi.nsw.gov.au/fishing/recreational/resources/stocking). Breeding of yellowtail kingfish commences in September and fingerlings are available from November-December. Fish up to 100 g can be supplied by the facility and fish can also be produced out-of-season by environmental manipulation (S. Fielder, *pers. comm*). PSFI maintains populations of both 2$^{nd}$ generation domestic broodstock and wild-caught broodstock, the latter being used for stock enhancement. Seedstock can be purchased from the PSFI with price depending on number, size and time of year requested. For example, during the normal production season price may range from around $2.00/ fish (at 1 g) to over $3.00/ fish (at 20 g), excluding transport costs. In addition, since all broodstock are genotyped, identifying stocked fish post-release can be achieved by generic parentage analysis.

In SA, Cleanseas (Arno Bay) maintains selectively bred domesticated yellowtail kingfish broodstock for production of seedstock, which are grown-out in commercial off-shore aquaculture farms. Since the fish produced at Cleanseas are derived from selectively bred stock, they would not be suitable for stock enhancement purposes for genetic reasons (see below).

**Genetic considerations**

The release or escape of translocated and/or genetically different fish stocks can represent a significant risk to wild populations (Utter 2003, Naylor, Hindar et al. 2005, Kitada, Shishidou et al. 2009, Araki 2010, Satake and Araki 2012). Using broodstock caught from the wild for stock enhance purposes reduces the risks associated with domestication whereas selectively-bred, captive-reared broodstock increase risks and so should be avoided (Rowland and Tully 2004, Araki, Cooper et al. 2007, Ingram, Hayes et al. 2011). Some yellowtail kingfish hatcheries in Australia are using selectively-bred captive bred broodstock, which should not be used for stocking purposes.

In Australia yellowtail kingfish comprise two genetically distinct stocks, one in eastern Australian waters and one in western Australia (Miller, Fitch et al. 2011, this study). Analysis of both mitochondrial DNA and microsatellite data indicated there were no differences between and eastern (New South Wales) and central (South Australia and Victoria) Australian fish (Miller, Fitch et al. 2011, this study). Consequently, releasing yellowtail kingfish into Victorian waters from hatcheries in either NSW or SA (see Section "Captive breeding") poses a reduced genetic risk, providing that broodstock used are 'wild-caught'.

**Disease considerations**

Parasitic infestations are considered a major threat to yellowtail kingfish farmed in sea pens in Australian waters (Sicuro and Luzzana 2016). Common parasites include the myxozoan protozoans *Kudoa* sp. and *Unicapsula seriolae*, the monogenean flatworms *Benedenia seriolae* and *Zeuxapta seriolae* and the digenean *Paradeontacylix* spp. (Beumer, Ashburner et al. 1982, Kolkovski and Sakakura 2004, Hutson, Ernst et al. 2007, Sicuro and Luzzana 2016). However, any translocations of yellowtail kingfish for stocking Victorian waters will require assessment by the Victorian Translocation Evaluation Panel(Victorian Fisheries Authority 2014), which will assess environmental risks, including spread of diseases.

**Previous wild stocking programs**

In Japan, hatchery-bred yellowtail (*S. quinqueradiata*) have been released since 1980 to counter declining commercial catch rates (Mushiake, Yamasaki et al. 2006).

In late 2018, the NSW DPI stocked 7,000 juvenile yellowtail kingfish into Botany Bay and 3,000 into Lake Macquarie to enhance local fishing opportunities. These fish were around 20 cm in length (https://www.dpi.nsw.gov.au/fishing/recreational/resources/stocking). The stockings are being monitored to assess their effectiveness and further small-scale stockings are being planned.

There have been numerous reports of un-intended and accidental escapes of yellowtail kingfish from aquaculture facilities Since 2001 over 65,000 yellowtail kingfish have escaped from aquaculture facilities in SA (https://pir.sa.gov.au/aquaculture/monitoring_and_assessment/register_-_finfish_escape). Most incidents involved escapes of fewer than 1,000 fish but in 2005 there was an event when 30,090 fish (2.38 kg each) escaped. In January 2018, 20,000 yellowtail kingfish escaped from a sea cage in Providence Bay (NSW) that was destroyed in rough seas. https://www.dpi.nsw.gov.au/data/assets/pdf_file/0007/799081/20180209_Huon-Incident-Investigation-Summary.pdf). The impacts of the escapes have not been assessed.

**Post-stocking survival**

The effectiveness of hatchery-bred yellowtail (*S. quinqueradiata*) stockings in Japan depends on timing of releases with improved recovery rates (from 6.4% to 27.7%) occurring when captive spawning is advanced by 2 months (by photothermal manipulation) so that releases of juveniles coincides with the appearance of wild juveniles of the same size (Fushimi 2001, Mushiake, Yamasaki et al. 2006, Mushiake, Yamazaki et al. 2007).

**Post-stocking movement**

Based on tag-recapture studies, yellowtail kingfish are likely to remain within stocking areas following release at lease for a short period of time. Previous tagging studies indicate yellowtail kingfish display both localised and to a lesser extent, larger widespread movement patterns. More commonly, fish exhibit residency across fine-scales with small localised coastal movements (<50 km), but considerably larger (>500 km) and less common movements occur, such as between Australian and New Zealand (Gillanders, Ferrell et al. 2001, Brodie 2016).

However, there is one major caveat, previous tagging studies have involved larger juvenile or mature fish (>40 cm) (Gillanders, Ferrell et al. 1999) that may display very different dispersal/movement patterns compared with small hatchery released fish (1-20 g). Previous tag/recapture summaries have shown that recapture rates also increased with size (Gillanders, Ferrell et al. 1999), which may reflect selectivity of the fishing methods, poor tag retention, and/or wider dispersal of small fish away from the main inshore fishing locations. In any case there is no information on movement of small yellowtail kingfish and the likelihood of achieving local/regional fishery benefits from stocking of yellowtail kingfish to open coastal waters will depend on the level of residency and, if migration occurs post-release, the rate at which stock fish return to release regions over their life.

Along with the uncertainty in post-release movement behaviour of small yellowtail kingfish, seasonal variations in water temperature will also influence residency of yellowtail kingfish in areas stocked within Victorian waters. Yellowtail kingfish are most often found in waters that range in sea surface temperatures of 18-24 °C (Fielder and Heasman 2011, Brodie, Hobday et al. 2015), but can experience temperatures as low as 14 °C (Brodie, Hobday et al. 2015). The optimal temperature window (i.e. >18 °C) typically occurs from late November to April in Port Phillip Bay. For coastal waters the temperature ranges vary depending on the region. For western Victoria (Portland region) the sea surface temperature rarely exceeds 18 °C, for central Victoria temperatures above 18 °C typically occur from January to March, and for eastern Victoria, from January to April. Winter temperatures in Port Phillip Bay can drop to around 10 °C and for coastal waters, typically reach between 13-14 °C depending on the region. The current water temperature regimes in Victoria are clearly not conducive to year-round residency of yellowtail kingfish, and it is likely that fish stocked in open waters would move east and north to find more suitable temperatures in the winter-spring months. Stocked fish in enclosed waters would be expected to experience poor growth under the low winter/spring temperatures, but may still survive, although survival rates are uncertain. Predictions of future increases in sea temperature along the Victorian coast are, however, favourable for yellowtail kingfish, indicating increases in the period of time over which sea surface temperatures will exceed 18 °C each year (Pecl, Ward et al. 2014, Stuart-Smith, Pecl et al. 2016), and the potential for longer residency of yellowtail kingfish along the Victorian coast.

**Supply of hatchery-bred yellowtail kingfish**

Given information on hatchery production of yellowtail kingfish, fish for stocking would be available during summer at a size of 1 – 20 g. Fish would most likely be supplied from the PSFI (NSW), the only hatchery currently producing juveniles from wild-caught broodstock. Prior to stocking an application will need to be submitted to the Translocation Evaluation Panel (TEP) for evaluation.

**Summary of prospects for yellowtail kingfish stocking in Victoria**

Since hatchery-bred juvenile yellowtail kingfish are available, it is feasible to stock this species into Victoria waters. However, considering the temperature preferences of the species, it would be unlikely that fish stocked into Victorian bays and coastal waters, would remain resident until their second year of life when they reach the legal minimum length of 60 cm. It is also highly uncertain as to what proportion of released fish would return to Victorian waters to benefit Victorian anglers. If the objective of stocking is to improve recreational fishing for Victorian anglers, stocking yellowtail kingfish into open waters is unlikely to be beneficial. If stocking is considered as a means to mitigate recruitment failures, it should be considered at a stock wide scale, and therefore including NSW. Stocking of enclosed water bodies, such as estuaries with periodically (multi-year) closed entrances, could be considered in Victoria. The high food resources in such systems would be expected to support stocked fish to reach legal size in 1-2 years. This expected fast growth to legal size would mitigate the risks of entrances opening, and therefore the loss of stocked fish to coastal waters before they become available to anglers. However, stocking of yellowtail kingfish into periodically closed estuaries has ecological risks and potentially could impact on other target species (i.e. predation impacts). Stocking of intermittently closed estuaries would need a thorough ecological risk assessment and be considered in relation to broader objectives for recreational fishing in any candidate estuary. If stocking was to occur monitoring of ecological risks and desired fishery outcomes is essential to inform the implementation of longer-term stocking strategies (Blankenship and Lee 1995, Cowx 1998).

# Discussion

## Genetics

In this study, the mtDNA approach provided confirmation of previous studies (Smith, Pepperell et al. 1991, Miller, Fitch et al. 2011) that yellowtail kingfish in Western Australia are a different genetic stock to eastern Australia and New Zealand, and therefore should be assessed and managed separately. However, the microsatellite and SNPs approaches are recognised as more powerful for resolving population structure and connectivity at finer scales than mtDNA. While overall, the genetic analyses did not provide strong evidence to reject the single stock hypothesis for fisheries management purposes around south-eastern Australia, statistically significant, but very low levels, of regional genetic variation were detected using microsatellites and SNPs (i.e. within Victoria). The high statistical power of microsatellites and SNPs has the implication that statistically significant spatial variation can be detected at scales not indicative of longer-term population mixing and can potentially confound inferences of stock structure for fisheries management purposes.

The variation among regions of the Victoria fishery (SNPs), and between Victorian and Tasmanian samples (microsatellites) was somewhat surprising. More recent SNPs analysis of samples collected in 2018 from South Australia, Tasmania and New South Wales, also showed minor but significant variation between Victoria and the other States (J. Strugnell, pers. comm). Given other information from tag-recapture studies, STRUCTURE analysis of microsatellite data, and the stable isotopes component of this report, it seems highly unlikely that yellowtail kingfish in Victoria would constitute an isolated stock sandwiched between SA and NSW/TAS.

Minor variation in microsatellite and SNP genetic markers at smaller spatial and temporal scales can be explained by the occurrence of fine-scale genetic heterogeneity in time and space (Larson and Julian 1999), referred to as ‘chaotic genetic patchiness’ (Johnson and Black 1982). Chaotic genetic patchiness can be informative of various processors that are influencing population dynamics at local-scales but can confuse inferences of broader scale stock structure for fisheries management. Marine populations often exist as ‘meta-populations’ where reproductive events occur in spatial/temporal clusters, and/or in the case of many fish species, adults aggregate in space and time to spawn, perhaps randomly, or at fixed locations/habitat features. Dispersal of young form these patchy reproductive events can create genetic patchiness in time and space if, for example; juveniles from discrete spawning events tend to disperse/school together, and/or certain reproductive events dominate the successful recruitment of particular cohorts and to particular locations (Larson and Julian 1999, Selkoe, Gaines et al. 2006, Hogan, Thiessen et al. 2010).

It was interesting that for the microsatellite and SNPs that positive $F_{IS}$ values were found for the VIC and TAS sampling regions where sufficient samples were available. This suggested a higher level or relatedness to each other than would be expected from random mating. We suggest the statistically significant variation in microsatellites and SNPs for yellowtail kingfish from the Victorian regions and TAS are due to chaotic genetic patchiness. Further SNPs studies with a more refined sampling design, included nested spatial (regional) sampling within State jurisdictions, sampling across years, and comparisons among and within cohorts/age classes, could provide more definitive information on both broader scale stock structure and finer scale connectivity of yellowtail kingfish around south-eastern Australia.

## Biological and population characteristics

Lack of evidence for genetic stock structure around east and south-eastern Australia is not necessarily indicative of a lack of regional structure in other biological and population parameters important for fisheries management and recreational fishery performance. This study indicated that large/old mature fish are currently rare in Victoria waters, and size compositions vary regionally, with a predominance of smaller and slightly younger fish in central Victorian waters compared to the west coast. While the lack of clear genetic differentiation around south-eastern Australia does suggest a history of broad-scale genetic mixing, the size and age composition data suggest that regional population dynamics in Victoria are currently influenced by variable movement of younger (mostly immature) fish into and out of Victoria from more distant regions. The dominance of the VIC fishery by smaller sub-mature fish is consistent with the fishery being in a recovery phase driven by improved recruitment over the last decade. Furthermore, it supports a theory that overfishing and sustained poor recruitment influenced the decline of yellowtail kingfish, rather than some abrupt change in migratory and residence patterns occurring in the early 1990’s. The lack of larger fish may indicate that the stock is not fully mixed across its range, or that they are just rare in the recovering population and may increase over time.

It was interesting that, although the oldest sample (11 years age) was from central Victoria, the modal ages of the sampled fish were one year older in western Victorian compared to eastern and central Victoria. The reasons for more older samples in western Victorian is unclear, but may relate to the angler(s) involved in sampling being more selective of larger/older fish in western Victoria, and or central Victoria not providing the environmental/feeding conditions preferred by larger fish. Alternatively, catchability of larger fish in central Victoria may also be lower for unknown reasons (i.e. they occur but are harder to catch). However, given the information from historical data where large fish were commonly caught in central Victoria prior to the mid-1990s, and the improved fishing technologies and knowledge, we find the latter theory is unlikely. Because of the high growth rate of yellowtail kingfish the larger fish in western Victoria are only marginally older then the dominant size class in central Victoria. We hypothesise that the larger/older fish in western Victoria relate to a pattern of migration of juvenile fish from waters off NSW, and that the migrants are simply older and larger by the time they reach western Victoria. They may also find better feeding conditions in western Victorian waters and therefore reside there for longer periods than in central Victoria, thus reaching larger sizes.

Migration and regional residency patterns are no doubt a major influence on the dynamics of the Victorian yellowtail kingfish fishery. Such patterns may be influenced by variable process including, water temperature and ocean currents, and prey distributions. To better understand the dynamics of the Victorian yellowtail kingfish fishery, more detailed information on movement patterns of both smaller and larger fish is required. This could involve the use of acoustics tags and the IMOS (Integrated Marine Observing System) network of acoustic receivers around south-eastern Australia (http://imos.org.au/facilities/animaltracking/acoustictelemetry/) that cover the NSW coast, various areas in South Australia, and soon to be enhanced by the installation of a line of receivers off Portland in western Victoria. The larger acoustic tags suitable for yellowtail kingfish can have battery lives of up 10 years, meaning that migration information could be obtained for individual fish from the juvenile through to adult life stage. Movement patterns could then be related to other data on oceanographic conditions to develop models of regional residency patterns and inform predictions of climate change impacts on these patterns (i.e. Brodie, Hobday et al. 2015). Champion (2018) illustrated that between 1995 and 2000 the suitability of habitat (sea surface temperature, sea level anomaly, dissolved oxygen and eddy kinetic energy) for yellowtail kingfish was relatively poor; however, years thereafter were more suitable. A long-term sustained approach to acoustic tagging is required to understand these processes, but this is feasible given the ongoing maintenance of the IMOS acoustic receiver network.

The observation that only 5% of samples collected in Victoria were estimated to be older than 5 years is important in the context of understanding the dynamics in Victoria. Size at 50% maturity for females was estimated at 84 cm FL, similar to that estimated for NSW (83.4 cm FL) (Gillanders, Ferrell et al. 1999). Based on the growth models, most females >84 cm FL would >5 years old. Therefore, there was no clear evidence that Victorian waters are an important area for reproduction and long-term residence of older mature yellowtail kingfish. Furthermore, although well-developed ovaries (stage 4) were observed in some fish during the late spring/summer period, when spawning is also thought to occur in NSW waters (Gillanders, Ferrell et al. 1999) no running ripe fish were sampled.

Despite the wide distribution of this species, little is known about the early life history, and spawning areas are unclear (Gillanders, Ferrell et al. 1999). While females at stage 4 reproductive development have been sampled off NSW (Gillanders, Ferrell et al. 1999) and now in Victoria, there are no confirmed reports of running ripe (stage 5) females from Australian studies. Spawning was earlier thought to occur in July off Coffs Harbour, but February off Narooma, with large fish moving offshore to spawn (Smith 1987). Gillanders et al. (Gillanders, Ferrell et al. 1999) showed that peak gonad development along NSW was in December, and consistent with this timing, larval *Seriola* sp. were captured off the Sydney coast in surface waters during autumn (Gray and Miskiewicz 2000). Small juvenile yellowtail kingfish (i.e. 25-30 cm, < 6 months old) are often found associated with floating objects and Fish Aggregation Devices in NSW and NZ waters(Kailola, Williams et al. 1993, McKenzie, Smith et al. 2014); however, to our knowledge yellowtail kingfish less than 40 cm have not been reported in VIC waters.

While definitive identification of *Seriola lalandi* larvae is not reported in any published Australian studies (genetic techniques may be required to separate *Seriola* larvae to species), the above observations, along with captures or large (i.e. well above the size at maturity) yellowtail kingfish in NSW waters and around Lord Howe Island, support the hypothesis that spawning is occurring off the coast of NSW and or the Tasman Ocean region. However, it is also possible that yellowtail kingfish in east Australian waters may be derived from spawning areas around New Zealand (NZ), as tagging studies have shown movements between NZ and Australian waters, and there is no evidence of genetic differences between yellowtail kingfish in eastern Australia and those around NZ (Smith, Pepperell et al. 1991). The evidence of genetic separation of populations between WA and the south-east and eastern States (SA, VIC, TAS, NSW, QLD) (Miller, Fitch et al. 2011) means that yellowtail kingfish in VIC are not

derived from spawning/juvenile source areas in warmer WA waters. There is also no evidence for spawning of yellowtail kingfish in South Australian waters, although this cannot be ruled out as no detailed reproductive studies have occurred in South Australia.

Growth parameters are important biological parameters for informing other types of fisheries assessment and poorly estimated/biased growth parameters can have major implications for these assessments. Growth parameters should be estimated based on representative sampling of the lengths at age in the populations. However, this is often difficult to achieve with confidence, particularly using recreational fishing based sampling with unknown selectivity patterns. Given this issue it was important to compare growth parameters from this study with those derived from other studies. The growth parameters estimated for yellowtail kingfish sampled in Victoria were very similar to those from an earlier study in NSW by Gillanders et al. (1999) but differed to the estimates from a later study by Stewart et al. (2004). These differences relate to difference in the range of sizes and ages used to estimate the growth parameters and the methods used. For example, the study by Stewart et al. (2004) included fish up to 21 years old, but the current study, and that by Gillanders et al. (1999), only included fish up to 10-11 years of age. The growth parameters for the Gillanders et al. (1999) study were also estimated using a cohort-based approach rather than from otolith aging. The main differences related to the estimates of K (growth co-efficient) being lower for the study by Stewart et al. (2004). This is not surprising as K and $L_{inf}$ are related, and with the higher $L_{inf}$, estimated by Stewart et al. (2004) a lower estimate of K is inevitable. We do not recommend using the K values derived from our study or Gillanders et al. (1999) for further fishery modelling work as they do not represent the full age composition. In terms of comparing age at length, the fitted growth models across a similar age range, however, showed for the three studies that yellowtail kingfish of age 2+ years are on average between 50-54 cm FL (65-70 cm TL) and for age 15+ years, are on average between 118-124 cm FL (141-148 cm TL).

## Historical catch records

The data collected in this study represent a contemporary snapshot of the biological and fishery characteristics of the yellowtail kingfish in Victorian waters. However, given that the contemporary period represents a period of resurgence in yellowtail kingfish in Victoria, we were interested in obtaining an appreciation of whether this new information may be fundamentally different from the earlier period of abundant yellowtail Kingfish, particularly for central Victoria.

Interviews with long-term anglers who were involved in targeting yellowtail kingfish, particularly around Port Phillip Heads, clearly indicated that larger fish from 10-20 kg (i.e. 110-135 cm TL) were common prior to 1993, with fish over 30 kg (150 cm + TL) occasionally caught. Small fish were apparently not that commonly captured. There is little historic information about yellowtail kingfish along most of the Victorian coast, although they have apparently been consistently available in far western Victoria, particularly near Portland. Consistent with the accounts from the long-term recreational anglers, commercial catches in Victorian waters, that were mostly taken in and around Port Phillip Heads, dropped dramatically from approximately 12 tonnes in 1991 and 1992 to less than 1 tonne by 1995 (VFA, unpublished data).

Records from recreational fishery sources (Bellarine Light Game and Sportfishing Club, Game Fishing Association of Australia) confirmed the anecdotal information that larger yellowtail kingfish were common in Victoria prior to the early 1990s. The lack of very large 20kg + yellowtail kingfish in the contemporary Victorian fishery is clearly inconsistent with the available information for the period prior to the early 1990's.

The reason(s) for the dramatic decline in the Victorian yellowtail kingfish fishery in the early 1990s are unclear. However, high fishing pressure on juvenile yellowtail kingfish in NSW waters by the trap fishery during the 1980s may have reduced the potential for juvenile migration south to Victorian waters. The commercial harvest of yellowtail kingfish in NSW decreased from around 600 t per year in the mid-late 1980s to around 100 t per year in the late 1990s, after the use of yellowtail kingfish traps was banned in 1996. Other management measures were also implemented in NSW including a 60 cm LML in 1990, further increased to 65 cm in 2007. These measures have likely contributed to recovery of yellowtail kingfish in south-eastern Australia, however, the status of yellowtail kingfish around south eastern Australia remains uncertain. While the data from Victoria is insufficient to support a comprehensive stock assessment, the available information is consistent with a recovering stock. In contrast, the recent assessments from NSW suggest that the biomass of the stock that occurs in New South Wales waters is likely to be depleted, recruitment is likely to be impaired, and larger fish are still rare in catches (Hughes et al. 2018). The reasons for these contradictory signals are unclear.

The few tag recapture records show movement of individuals between NSW and Victoria in both directions. If the Victorian fishery is largely dependent on migrants derived from reproduction in NSW, the future of the sustained

recovery in the summer/autumn fishery in Victoria is uncertain. The other possibility for the contrasting recent trends in NSW and Victoria may relate to water temperature dynamics. Yellowtail kingfish move their distribution centre southwards in summer to avoid excessively warm waters in the north of their range (Brodie, Hobday et al. 2015). The optimal temperature for yellowtail kingfish in NSW is suggested to be ~22°C (Brodie, Hobday et al. 2015) similar to the recommended aquaculture rearing temperatures of 22°C (Fielder and Heasman 2011), but they can experience temperatures from 14.5 - 25°C (Brodie, Hobday et al. 2015). Increasing southward penetration of the East Australian Current, and a general warming trend for Bass Strait, as a result of climate change may be promoting greater rates of movement of yellowtail kingfish into Victorian waters, which may partly explain the contradicting stock signals in NSW and VIC. Champion *et al* (2018) found a decline in temporal habitat persistence in equatorward bioregions and increases for poleward bioregions (e.g. Eastern Tasmania and Eastern Bass Strait. Understanding the potential for sustained growth and ongoing productivity of the Victorian yellowtail kingfish fishery will depend on a greater understanding of the linkages with NSW and the influence of water temperature on regional movement and residency dynamics.

## The use of satellite tags to understand movement characteristics

The trial of satellite tags resulted in one successful deployment from three attempts, which provided information on a west to east movement through Bass Straight during the autumn. The movement of this fish is consistent with the theory that yellowtail kingfish move to the east from central and western Victoria in search of warmer water in winter. However, clearly no broad conclusions about population level migratory dynamics or pathways can be made from one fish. While the learning from this trial would likely lead to greater success rates with future satellite tag deployment on yellowtail kingfish, the cost of these tags prohibits their use on large enough numbers of fish to make population level inferences. Consideration or future application of satellite tags needs to be evaluated against other tagging approaches, particularly acoustic tags, in relation to key uncertainties around migratory dynamics and pathways.

If satellites tags were to be used in the future it would be suggested that an alternative method of attaching the tags be explored. Observations of the tags when retrieved indicated that the wire used to secure the tag was corrode and broken next to the crimp. High strength monofilament or fluorocarbon may have been a better choice to bridle the tag to the fish.

## Potential of otolith stable isotopes to provide information on temperature exposure and population structure of yellowtail kingfish

This study demonstrated the potential of otolith stable isotope analysis, in particular $\delta^{18}O$, to provide information on environmental temperature history of yellowtail kingfish. Consistent increasing trends in otolith $\delta^{18}O$ with size were indicative of greater exposure to cooler waters as yellowtail kingfish grew and aged. The use of mass balance relationships to estimate temperature exposure for specific growth periods indicated that the first year of life, irrespective of sampling area, was spent in waters of average temperatures above 18-19°C. Based on actual water temperature estimates, this suggested that the yellowtail kingfish sampled in Victorian waters were derived from spawning/juvenile source areas further north and outside of Victoria's jurisdiction. Considering the previous discussion regarding spawning and early life-history we hypothesis that the source area for the yellowtail kingfish that move through Victorian waters is likely to be off central NSW and or the adjacent Tasman Sea. Such results are consistent with modelled habitat suitability of yellowtail kingfish. Here, sea surface temperature, sea level anomaly and eddy kinetic energy were used to determine the relative probability of the presence of yellowtail kingfish (Champion, Hobday et al. 2018). If this is the case the performance of the yellowtail kingfish fishery in Victoria will be highly dependent of the spawning potential and juvenile recruitment in NSW.

The $\delta^{13}C$ of kingfish otoliths showed a commonly observed trend of increasing values (i.e. increased enrichment, less negative values) with size/age. The trend with size was similar for the different sampling areas. Increasing otolith $\delta^{13}C$ with size/age typically relates to a metabolic influence on otolith $\delta^{13}C$, whereby, as metabolic rate reduces with age the contribution of metabolically derived carbon, that is depleted in $^{13}C$ (values of $\delta^{13}C$ are more negative) to the also otolith decreases. There was no clear indication that otolith $\delta^{13}C$ could provide inferences on environmental history or stock structure, although the sample sizes were limited for NSW and TAS. However, we recommend for the minimal additional analytical costs, continuing to measure $\delta^{13}C$ in any future expanded studies to further explore its potential as a metabolic indicator.

The equations used for converting the otolith $\delta^{18}O$ to temperature were derived from literature for other species, and it is likely that the difference among the relationships in the literature is influenced by analytical methods and interspecific differences. Future application of $\delta^{18}O$ as an indicator of thermal history for yellowtail kingfish would

benefit from validation of the relationship between otolith $\delta^{18}O$ and water temperature by controlled studies. Nonetheless in this study the relationships we applied produced similar temperature estimates for the important earlier growth phases, although they diverged for larger sizes/growth phases. The Kalish (1991) equation based on Australian salmon off eastern Australia is perhaps most closely aligned with yellowtail kingfish and the study region, and provided the most plausible temperature estimates for the older growth phases of the Victorian and central NSW samples of 15-17°C. This range also overlapped the average temperature recorded by the satellite tagged yellowtail kingfish that moved between western Victoria, through Bass Strait to eastern Bass Strait from April-June (i.e. 16°C). Overall, the results suggest that for the size/age range sampled in this study, as yellowtail kingfish got larger/older they spent more time cooler waters. However, the average temperatures estimated from otolith growth phases at larger sizes were warmer than would be expected had they remained resident along the coast of central and western Victoria.

An expanded study involving more samples from NSW and TAS, and inclusion of SA samples, would provide a more detailed picture of variation in thermal history for yellowtail kingfish around southern and eastern Australia. Unfortunately, the small size of yellowtail kingfish otolith makes it problematic to use micro-mill approaches to resolve thermal history of individuals necessitating the mass-balance approach applied in this pilot study. This requires more samples to be collected because the data are stratified by size/age categories. A multi-state project would be required to achieve the required sample sizes. To resolve the all-important early life thermal signature, sampling of the smallest yellowtail kingfish possible (i.e. < 30 cm, < 1 year old) would be valuable both to provide a baseline signature for juvenile sources and to estimate the weight/size of otoliths from small juveniles. This could then be used to inform a trial to test whether dissolution of the outer potion of the otoliths from larger fish could isolate the early life period, that could then be analysed for stable isotopes to derive the early life thermal signature of larger fish. Finally, there has been no application of laser ablation ICP-MS (inductively coupled plasma mass spectrometry) for yellowtail kingfish, which can be used to resolve elemental variation (e.g. Mg, Mn, Sr, Ba) at much finer temporal scales within individual otoliths (i.e. Fowler et al. 2017). Comparison of element profiles across otoliths could support information from stable isotopes in inferring population structure of yellowtail kingfish around south-eastern Australia.

### Suitability of yellowtail kingfish for marine stocking

The review of opportunities for yellowtail kingfish stocking in Victoria indicated that a suitable supply of fingerlings was available in NSW at a cost of $2-3 per fish. However, consideration of environmental factors (i.e. water temperature regimes) and uncertainty around movement of small juveniles led to the recommendation that stocking of yellowtail kingfish in Victorian bays and coastal waters would be unlikely to produce clear benefits for Victorian anglers. It is likely that yellowtail kingfish stocked in Victorian open waters would eventually move north into warmers waters along the NSW coast, most likely in the first winter after stocking and before they reach the legal minimum length. Stocking could be considered for intermittently closed estuaries of marine salinities, where entrances are likely to remain closed for at least two – three consecutive years allowing fish to reach the legal minimum legal length for recreational harvest. If entrances are not closed for multiple years it is likely the fish will leave in the cooler months. Stocking of intermittently closed estuaries would have ecological risks, as yellowtail kingfish would predate on other resident species, include potentially some that are important to recreational anglers. If stocking was considered as a management response to recruitment failure and stock/fishery decline it would need to be considered at a stock-wide scale involving all State jurisdictions that have interests in the stock (i.e. QLD, NSW, VIC, TAS, SA).

# Implications

The outcomes of the various sub-components of this project suggest that yellow kingfish in the Victorian fishery are not derived from local spawning and are not a resident stock. We hypothesise that spawning areas off NSW and/or in the Tasman Ocean are the most likely sources of replenishment for the Victorian fishery. If this is true, the Victorian fishery will fluctuate depending on spawning success and migratory dynamics from source populations managed by other jurisdictions. Management changes in NSW may have contributed to the recent recovery in Victoria; however, concerns over stock status in NSW should be addressed in a collaborative manner with NSW managers to ensure the sustained recovery and future performance of the summer-autumn fishery in Victoria.

Approaches to multi-jurisdictional assessment and management collaboration are recommended to ensure the overall management of the biological stock considers the interests of stakeholders in each State jurisdiction. For example the legal minimum limit is 60 cm in VIC, 65 cm in NSW and 45 cm in TAS. In this respect it is helpful that the biological parameters, including size at reproductive maturity and growth are similar across States, but ongoing information to inform stock wide assessments, including fishing mortality rates, representative length and age compositions and catch rate indices are not available for all jurisdictions. The recent edition of the Status of Australian Fish Stocks has defined yellowtail kingfish in south-eastern Australia as of "undefined" status (http://fish.gov.au/report/218-Yellowtail-Kingfish-2018; Hughes et al. 2018). This seems remarkable considering its high commercial, socio-economic and ecological importance, and concerns expressed about the status of the stock component in NSW. Similarly, the SAFS assessment does not include any information from VIC, TAS or SA. Defining and quantifying connectivity and source/sink relationships of yellowtail kingfish around south-east Australia including VIC, TAS and SA is essential for assessment and management to occur at the appropriate scale. Techniques applied in this study such as the SNPs genetic approach, otolith stable isotopes and electronic tagging (including acoustic tags) could be applied in a larger multi-state project on yellowtail kingfish to clarify these uncertainties.

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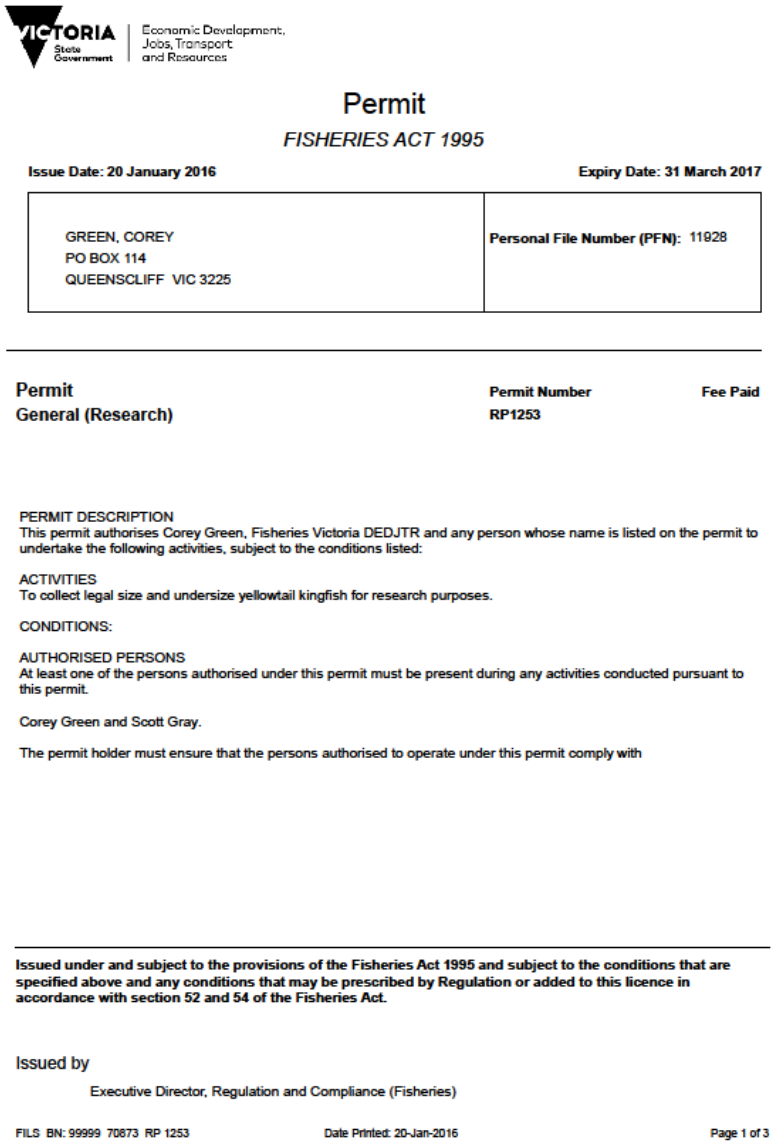
# Appendix 1. Fisheries Research Permit



VICTORIA State Government | Economic Development, Jobs, Transport and Resources

**Permit**

*FISHERIES ACT 1995*

**Issue Date: 20 January 2016** **Expiry Date: 31 March 2017**

| | |
|---|---|
| GREEN, COREY<br>PO BOX 114<br>QUEENSCLIFF VIC 3225 | **Personal File Number (PFN):** 11928 |

| **Permit** | **Permit Number** | **Fee Paid** |
|---|---|---|
| **General (Research)** | **RP1253** | |

PERMIT DESCRIPTION
This permit authorises Corey Green, Fisheries Victoria DEDJTR and any person whose name is listed on the permit to undertake the following activities, subject to the conditions listed:

ACTIVITIES
To collect legal size and undersize yellowtail kingfish for research purposes.

CONDITIONS:

AUTHORISED PERSONS
At least one of the persons authorised under this permit must be present during any activities conducted pursuant to this permit.

Corey Green and Scott Gray.

The permit holder must ensure that the persons authorised to operate under this permit comply with

**Issued under and subject to the provisions of the Fisheries Act 1995 and subject to the conditions that are specified above and any conditions that may be prescribed by Regulation or added to this licence in accordance with section 52 and 54 of the Fisheries Act.**

Issued by

Executive Director, Regulation and Compliance (Fisheries)

FILS BN: 99999 70873 RP 1253 Date Printed: 20-Jan-2016 Page 1 of 3

# Appendix 2. Animal Ethics Permit

**ANIMAL RESEARCH AUTHORITY**
**Department of Primary Industries**
**Fish Animal Ethics Committee**

| Names of Applicants | Address |
|---|---|
| Corey Green | Victorian Fisheries Authority |
| Brent Womersley | P.O.Box 114 |
| Paul Hamer | Queenscliff, Vic. 3225 |
| Scott Gray | |
| Tara Hicks | |

are authorised by

**Department of Primary Industries, Fish Animal Ethics Committee**

**AEC Project Code & Title: DPI Fish AEC Aug15 0098**
Increasing knowledge of Victoria's growing recreational yellowtail kingfish fishery

Subject to the following conditions
Modification granted at Sept 2017 for a project time extension to 30 June 2018

**DPI Fish Animal Ethics Committee**

**This authority remains in force**
**from 24 Aug 2015 to 30 Jun 2018**
Unless suspended, cancelled or surrendered

| | |
|---|---|
| Corey Green | Leanne Gunthorpe |
| Secretary | Chair |

Department of Economic Development, Jobs, Transport & Resources

# Appendix 3. Macroscopic gonad stage descriptors

Macroscopic gonad stage descriptors for yellowtail kingfish (Gillanders, Ferrell et al. 1999).

| | Maturity scale | Macroscopic descriptor |
|---|---|---|
| **Stage** | **Females** | |
| 1 | Immature | Lobes of gonads tend to be oval in shape, generally <60 mm in length. |
| 2 | Mature resting | Lobes of gonads more rounded in cross-section and larger than immature fish. |
| 3 | Developing | Similar to mature resting; lobes usually between 60 and 100 mm. |
| 4 | Late developing | Large eggs, clearly visible. |
| 5 | Ripe mature (running) | |
| 6 | Spent (post spawning) | |
| **Stage** | **Males** | |
| 1 | Immature | Thin, flattened, thread-like lobes of gonads. |
| 2 | Developing | Elongated, ribbon-like testes; ova to triangular in cross-section. |
| 3 | Mature | Similar to developing. |
| 4 | Ripe | Elongated. |

# Appendix 4. Paper Draft: Assessing genetic diversity and population structure in yellowtail kingfish, *Seriola lalandi*, in south eastern Australia

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**Abstract**

Yellowtail kingfish, *Seriola lalandi*, are an increasingly important recreational fish species in south eastern Australia. Scientific knowledge underpins effective management in fisheries worldwide; however, in Victoria little is known of the population structure of yellowtail kingfish. Ascertaining the population structure in Victoria and adjacent states will provide critical information on whether to manage the stock as a single entity or multiple entities. Genetic diversity and population structure was explored using mitochondrial markers, microsatellites and single nucleotide polymorphisms (SNPs) in order to determine the connectivity of yellowtail kingfish found within Victoria and between Victoria, New South Wales (NSW) and Tasmania. Existing publicly available mitochondrial sequence data for individuals from South Australia (SA), Western Australia (WA) and New Zealand (NZ) were included in analyses where possible. Results indicate little evidence for population differentiation between Victoria, NSW, SA and Tasmania, nor between the three Victorian populations, using mitochondrial markers. The exception was WA which was significantly different from all other populations. There was very slight evidence for population differentiation between Tasmania and Victoria and also between Western Victoria and both Eastern and Central Victoria using microsatellite markers. Significant small genetic structure was detected between Victorian populations when using SNP markers. As recreational take of yellowtail kingfish is managed differently in each state, consideration should be made to align management objectives and regulations to ensure their sustainability.

**Introduction**

In Australia, yellowtail kingfish, *Seriola lalandi,* are largely distributed from southern Queensland to the central coast of Western Australia, the east coast of Tasmania, and around Lord Howe and Norfolk Islands (Lowry, Molony et al. 2014). They are a highly sought after recreational species mainly due to their fighting ability and eating qualities. Since around 2010, anecdotal reports of recreationally caught yellowtail kingfish have increased greatly in Victoria and Tasmania with many anglers now targeting yellowtail kingfish at several locations throughout the two states.

In Victoria, fundamental stock structure information is limited on this developing fishery. However, with increases in numbers caught and targeted recreational effort, it is necessary to understand key biological and stock structure information and stock connectivity among the states. Like most developed fisheries, stock discrimination information will be used to help manage this public resource to maintain and potentially improve numbers and size of this iconic species to the recreational community.

The recreational daily take of yellowtail kingfish in Victoria, NSW and Tasmania is five per person; however, the legal minimum length differs between states, being 60 cm, 65 cm and 45 cm respectively (2018 Victorian, Tasmanian and NSW recreational fishing regulation). Determining the genetic connectivity of yellowtail kingfish

among the different states provides a biological context to whether each state should manage their respective fishery independently with alternative regulations or holistically based on the biological stock.

Genetic markers are useful tools for fisheries management as they can detect variation among individuals and populations, improving our knowledge of population structure in marine species. Genetic studies have been previously conducting on yellowtail kingfish (Nugroho, Ferrell et al. 2001, Miller, Fitch et al. 2011). Miller et al. (2011) employed mitochondrial DNA (mtDNA) (ND4) and seven microsatellite loci to assess genetic structure across temperate Australia and NZ. Both marker types supported a genetically distinct Western Australian population with no differences detected between *S. lalandi* sourced from NZ, NSW, SA and Victoria. Recently Premachandra et al. (2017), employed mtDNA (COI), nine microsatellite markes and also Single nucleotide polymorphisms (SNPs) to investigate broad population genetic structure in the Pacific Ocean. They detected population subdivision between the Northeast Pacific, Northwest Pacific and South Pacific using all marker types.

The aim of this study was to determine whether any population genetic structure exists for yellowtail kingfish distributed in South eastern Australian waters (namely Tasmania, New South Wales and three locations in Victoria). Firstly, mitochondrial markers were used in order to include sequences obtained from yellowtail kingfish caught in South eastern Australian waters with existing sequences from fish captured from Western Australia, South Australia and New Zealand. Secondly, microsatellite markers were employed to investigate population structure across NSW, Tasmania and three locations in Victoria and finally single nucleotide polymorphisms (SNPs) were used to investigate fine scale population structure within Victoria only.

**Materials and methods**

Each of the three types of genetic markers; (1) mitochondrial DNA, (2) microsatellites and (3) SNPs were obtained from each individual fish. Genetic diversity (i.e. the total number different alleles) was estimated for each marker type for each location. The locations included Western Victoria, Eastern Victoria, Central Victoria, New South Wales and Tasmania. In addition, the genetic differences within and between these locations was also assessed in order to determine whether there was any evidence of population structure.

*Sample collection*

Tissue samples were collected from yellowtail kingfish caught by recreational fishers in Victoria, New South Wales and Tasmania between May 2015 and April 2016. Victorian samples were collected from three zones: Western (SA/Vic border to Cape Otway lighthouse, Central (Cape Otway lighthouse to Wilsons Promontory and Eastern (Wilsons Promontory to Vic/NSW border, Table 14). Fin clips were taken from Victorian and Tasmanian individuals and muscle tissue was collected for NSW samples only. Fin clips and muscle tissue were preserved in 70% ethanol at -30$^{o}$C for later processing. Size of yellowtail kingfish varied; however, individuals were greater than the legal minimum length for their respective state.

Wherever possible, existing genetic data present on publicly available databases was also included in analyses. This included mitochondrial sequences from individuals from Western Australia, South Australia, New Zealand, New South Wales and Victoria provided by Miller *et al.* (2011).

**Table 14. Location, tissue type and number of samples used for mitochondrial (mtDNA), microsatellite and single nucleotide polymorphisms analysis (SNPs) of yellowtail kingfish (*Seriola lalandi*) populations.**

| Location | Tissue type | mtDNA | Microsatellites | SNPs |
|---|---|---|---|---|
| **Western Victoria** | Fin | 57 | 56 | 38 |
| **Eastern Victoria** | Fin | 57 | 57 | 48 |
| **Central Victoria** | Fin | 54 | 53 | 21 |
| **New South Wales** | Muscle | 6 | 8 | 0 |
| **Tasmania** | Fin | 60 | 60 | 0 |

**DNA extraction**

Complete genomic DNA was extracted from all samples using the DNeasy kit (Qiagen) according to the manufacturer's protocol. Double stranded DNA was quantified using a Qubit fluorometer (Invitrogen).

**Mitochondrial DNA**

A 471 base pair (bp) region of the ND4 gene was sequenced for 234 samples throughout Victoria, NSW and Tasmania. Amplification was achieved using (ND4) forward (Arèvalo, Davis et al. 1994) and (H12293_Leu) reverse (Inoue, Miya et al. 2001) primers. The total volume for each PCR reaction was 25 ul and was composed of 12.5 ul MyTaq$^{TM}$ Red Mix (Bioline), 0.5 ul (10mM) of forward primer, 0.5 ul (10mM) of reverse primer, 10.5 ul of $H_2O$ and 1 ul of genomic DNA (concentration range 0.4ng/ul - 1.8ng/ul). PCR conditions were as follows: Denaturing at 95 $^0$C for 45 sec then 30 cycles of 95$^0$C for 45 sec, annealing at 50$^0$C for 45 sec and 72$^0$C for 1 min. A final extension step at 72$^0$C was carried out for 10 min.

PCR purification and sequencing (in both directions) was conducted by Macrogen Inc. (Korea), and resulting chromatograms were visualized and edited using the program *Geneious* v9.1.0. Additional publicly available sequences from individuals from Western Australia, South Australia, New Zealand, New South Wales and Victoria were included from a previous study by Miller et al. (2011) (Accession numbers JF345183–JF345208). All data was aligned using *MAFFT* in Geneious.

Calculations of nucleotide and haplotype diversities were conducted using the program *DnaSP v5.10.1* (Librado and Rozas 2009). The neutrality tests Fu's Fs and Tajimas D (Tajima 1989) were calculated in *Arlequin v3.5* (Excoffier, Laval et al. 2005) for each location separately, for all Victorian populations grouped together and for all Australasian populations separately. Pairwise $\mathbf{\Phi}_{PT}$ values were also calculated in *Arlequin v3.5* for each population separately and for all Victorian populations combined.

**Microsatellites**

A total of nine previously developed microsatellite loci were used in this study: Sdu01, Sdu03 (Nugroho and Taniguchi 1999), Sdu10, Sdu16, Sdu27 (Renshaw, Patton et al. 2006), Sdu29, Sdu32, Sdu37 and Sdu46 (Renshaw, Patton et al. 2007) for 234 samples across Victoria, NSW and Tasmania. The total volume for each PCR reaction was 12 ul and was composed of 6.25 ul MyTaq$^{TM}$Red Mix (Bioline), 0.075 ul (10mM) of forward primer, 0.25 ul (10mM) of reverse primer, 4.75 ul of $H_2O$, 0.085 µl of 5pmol/ µl fluorophore and 1 ul of genomic DNA (concentration range 0.4ng/ul - 1.8ng/ul). PCR conditions were as follows: Denaturing at 95 $^0$C for 45 sec then 30 cycles of 95$^0$C for 45 sec, annealing for 45 sec (temperatures were dependent on primers and ranged between 50$^0$C and 65$^0$C; Table 15) and 72$^0$C for 1 min, lastly extension at 72$^0$C for 10 min. A total of 234 samples were sent to the Australian Genome Research Facility (AGRF) for fragment separation, and were visualised and scored using the program *Geneious* 5.6.4. Samples were successfully genotyped for between seven and nine loci.

*Micro-Checker* v2.2.3 (Van Oosterhout, Hutchinson et al. 2004) was used to identify null alleles and errors resulting from stuttering for correction. Corrected data was analysed in *Genepop* v3.4 (Raymond and Rousset 2003) to identify any deviations from Hardy-Weinberg equilibrium. FST and FIS were calculated using the program *FSTAT* v2.9.3 (Goudet 1995). The structure plot analysis was conducted in *STRUCTURE* v2.3.2 (Pritchard, Stephens et al. 2000) with the following parameters: burn-in of 100,000 Markov Chain-Monte-Carlo (MCMC) repetitions and five iterations per K (K=1-8). The Evanno method (Evanno, Regnaut et al. 2005) was used to determine the best fit for K.

**Table 15. Annealing temperatures for nine microsatellite loci.**

| Primer | Annealing temperature |
|---|---|
| **Sdu01** | 65$^0$C |
| **Sdu03** | 63$^0$C |
| **Sdu10** | 61$^0$C |
| **Sdu16** | 60$^0$C |

| | |
|---|---|
| **Sdu27** | $60^0$C |
| **Sdu29** | $50^0$C |
| **Sdu32** | $61^0$C |
| **Sdu37** | $60^0$C |
| **Sdu46** | $60^0$C |

**Single nucleotide polymorphisms (Genotyping by Sequencing)**

A total of 108 samples were included in a Genotyping by Sequencing (GBS) analysis in order to generate single nucleotide polymorphisms (SNPs). GBS library preparation and sequencing were conducted by the Australian Genome Research Facility (AGRF). Briefly, three samples were first included in an establishment phase in order to determine the most suitable restriction enzyme combination for genome digestion. The enzymes PstI/MseI were determined to be most suitable.

DNA quality was assessed using a Bioanalyzer (Agilent Technologies) and 108 samples (all from Victoria) were deemed to pass quality control thresholds for GBS. Unfortunately, the NSW and Tasmanian DNA samples were not of high enough quality to enable them to be included. Of these samples, 35 were replicated across library preparation

Library preparation used a ddRAD based (Peterson, Weber et al. 2012) approach. DNA extractions were digested using the restriction enzymes PstI and MseI followed by ligation of using barcoded adapters compatible with the restriction site overhang. Samples were then purified and pooled digested fragments size selected using a Blue Pippin (Sage Science). Amplification of each library was achieved using PCR with indexed primers and single-end sequencing conducted using an Illumina NextSeq500 with 150 cycles in high-output mode.

Sequence data was analysed using the software *Stacks* v1.41 (Catchen, Hohenlohe et al. 2013), demultiplexed and assessed for read quality using FASTQC (Andrew 2010) and trimmed to the size of the shortest read minus two based in order to allow for difference in read length due to variation in barcode sequences. 'Stacks' were then created of similar reads for each sample. The 'stacks' which appear across all samples were collated and then genotypes were determined for the most common polymorphic sites.

The putative markers were filtered to minimize confounding variables. We included only bi-allelic sites and excluded genotypes with more than 50% missing data and with a minor allele frequency (MAF) lower than 0.01 using VCFtools (Danecek et al. 2011). In order to assess batch effect, principal component analysis (PCA) was conducted on the replicates, using the R package 'ADEGENET' v2.0.0 (Jombart 2008). The replicate from each pair with the highest amount of missing data was removed for further analyses.

Population structure was assessed using a Discriminant Analysis of Principal Components (DAPC), performed using the R package 'ADEGENET' (Jombart 2008). To determine the optimal value of clusters (K), the Bayesian Information Criterion (BIC) method was implemented without any sampling information, using the function *find.clusters()* and 35 principal components (PCs) were kept.

Diversity indices (number of alleles and inbreeding coefficient - $F_{IS}$) and population pairwise measures of $F_{ST}$ with respective p-values were estimated using the software Genepop (Rousset 2008). Values of observed and expected heterozygosity were estimated using the *basicStats()* function in the R package ""diveRsity" (Keenan *et al.* 2013).

**Results**

**Mitochondrial DNA**

**Genetic variability**

*Victoria*

Haplotype diversity for all Victorian locations combined was > 0.5 (0.580 +- 0.043). Haplotype diversity was highest in central Victoria (0.630 +- 0.070) and lowest in eastern Victoria (0.499+-0.078) (Table 16). No fixed haplotypes were present in Victorian populations. Nucleotide diversity was relatively low for all populations

ranging between 0.00242 +- 0.00044 and 0.00157 +- 0.00037 for western and eastern Victoria respectively. Tajima's D and Fu's Fs were negative and significant for all Victorian locations combined and for all individual Victorian locations (apart from Tajima's D in western Victoria which was not significant) potentially indicating a population expansion (Table 16).

*All locations*

Haplotype diversity was lowest in NSW (0.00098 +- 0.0008) with the exception of South Australia, which was the only location to contain a fixed haplotype. Nucleotide diversity was relatively low for all locations ranging between 0.00098+-0.0008 and 0.00226 +-0.00073 for NSW and Western Australia respectively. NSW and NZ both possessed negative Tajima's D values and positive Fu's FS with the only significant value detected for Tajima's D in the NSW population (-1.65231). In contrast to all other locations WA had positive Tajimas's D and Fu's Fs values, however they were not significant (Table 16).

**Table 16. Diversity and neutrality indices for Australasian yellow-tailed kingfish (*Seriola lalandi*). $N_{ND4}$ (sample size), h (haplotype diversity), $N_h$ (number of haplotypes), S (number of polymorphic sites), $\pi$ (nucleotide diversity), $P_{TD}$ (probability of Tajima's D) and $P_{Fs}$ (Probability of Fu's Fs). Significant values (<0.05) are shown in bold.**

| | All | All VIC | VIC Western | VIC Central | VIC Eastern | NSW | WA | NZ | TAS | SA |
|---|---|---|---|---|---|---|---|---|---|---|
| $N_{ND4}$ | 258 | 170 | 59 | 54 | 57 | 13 | 6 | 6 | 60 | 3 |
| ***h* (±SD)** | 0.543 (0.00137) | 0.580 (0.043) | 0.613 (0.070) | 0.63 (0.070) | 0.499 (0.078) | 0.154 (0.126) | 0.533 (0.172) | 0.333 (0.215) | 0.458 (0.079) | 0 |
| $N_h$ | 22 | 18 | 9 | 11 | 9 | 2 | 2 | 2 | 10 | 1 |
| ***S*** | 26 | 22 | 10 | 10 | 11 | 3 | 2 | 2 | 11 | 0 |
| **p (±SD)** | 0.00198 (0.00020) | 0.00199 (0.00024) | 0.00242 (0.00044) | 0.00189 (0.00031) | 0.00157 (0.00037) | 0.00098 (0.0008) | 0.00226 (0.00073) | 0.00142 (0.00091) | 0.00131 (0.0003) | 0 |
| **Tajima's *D*** | **-2.11682** | **-2.08877** | -1.30513 | **-1.67792** | **-1.96561** | **-1.65231** | 1.03194 | -1.13197 | **-2.8059** | 0 |
| $P_{TD}$ | 0 | 0.002 | 0.084 | 0.024 | 0.006 | 0.024 | 0.879 | 0.163 | 0.004 | 1 |
| **Fu's *Fs*** | **-19.67617** | **-14.33161** | **-3.20555** | **-7.4073** | **-5.46497** | 0.97596 | 1.7231 | 0.95213 | **-8.02883** | 0 |
| $P_{Fs}$ | 0 | 0 | 0.041 | 0 | 0.007 | 0.586 | 0.766 | 0.595 | 0 | NA |

**Population Structure**

The haplotype network exhibits a 'star-like' structure, indicative of a population expansion, with the central and most common haplotype (1) comprising 66.7% of all individuals. Haplotype 1 was present in individuals from all locations with the exception of Western Australia. The second most common haplotype (5) was present in 9.69% of individuals and was only found in all Victorian and Tasmanian locations. Haplotypes 21 (eastern Victoria) and 19 (western Victoria) show the greatest distance from the central haplotype (1) and are separated by four mutational changes. Both of these haplotypes are private, being represented by a single individual. Private haplotypes are evident from five locations; Western Australia (1 private haplotype), eastern Victoria (2 private haplotypes), western Victoria (2 private haplotypes), Tasmania (3 private haplotypes) and central Victoria (4 private haplotypes) (Figure 27).

All pairwise comparisons between Victorian locations were low and non-significant suggesting that there was no population differentiation between these locations. In addition, the majority of remaining pairwise comparisons also showed non-significant values indicative of panmixia. The exception to this was Western Australia, which was significantly different from all other populations (Table 17). There was significant population differentiation between central Victoria and NSW, yet the low $\Phi_{PT}$ value between these locations suggests very slight differentiation only (Table 17).

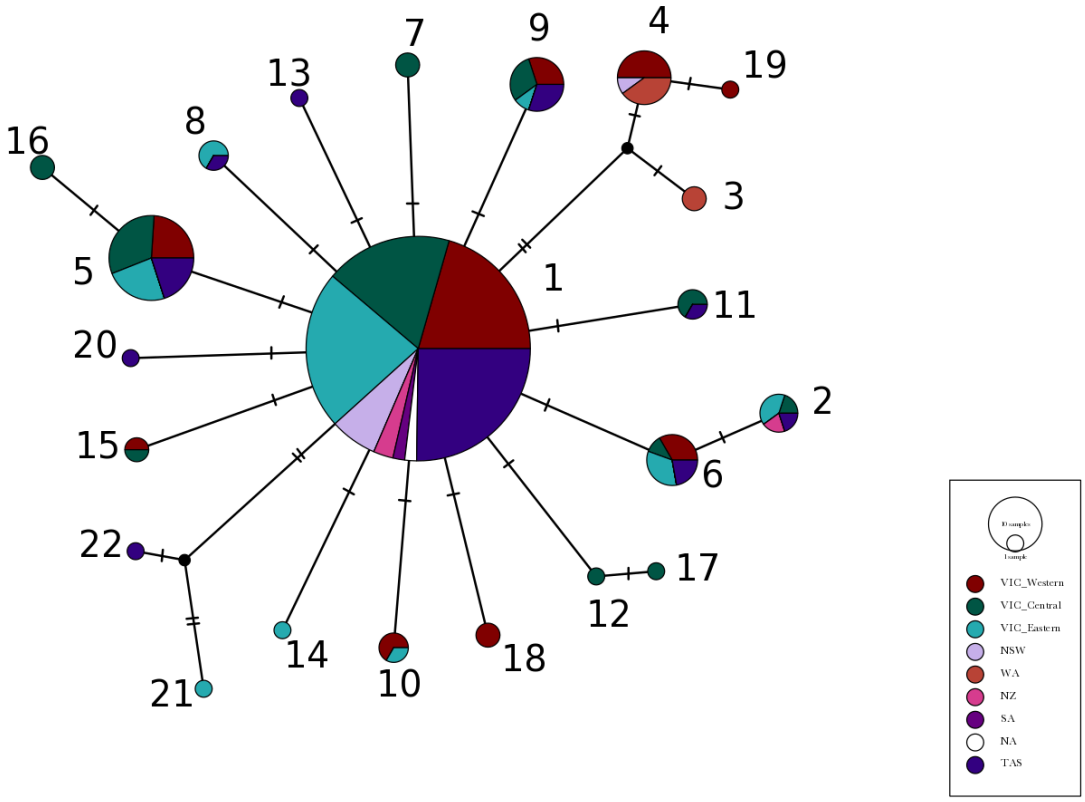


**Figure 27. Median joining haplotype network of Australasian Yellow tailed kingfish (*Seriola lalandi).* Haplotypes are represented by a single circle proportional in size to the number of individuals, with colours corresponding to locations. Locations are as follows western Victoria (red), central Victoria (green), eastern Victoria (pale blue), New South Wales (lilac), Western Australia (orange), New Zealand (dark blue), South Australia (purple) and Australia (unknown location)(white). Dashes across lines joining haplotypes represent a single nucleotide change and black circles are mutation points.**

**Table 17. Pairwise $\Phi_{PT}$ values denoting differentiation of the ND4 gene between populations of Australasian yellow-tailed kingfish (*Seriola lalandi*). Significant values (<0.05) are shown in bold.**

| | All VIC | wVIC | eVIC | cVIC | NSW | WA | NZ | SA | TAS |
|---|---|---|---|---|---|---|---|---|---|
| **All VIC** | | | | | | | | | |
| **wVIC** | -0.00632 | | | | | | | | |
| **eVIC** | -0.00455 | 0.0023 | | | | | | | |
| **cVIC** | -0.00606 | -0.00288 | 0.00324 | | | | | | |
| **NSW** | 0.05012 | 0.05841 | 0.03427 | **0.08005** | | | | | |
| **WA** | **0.42047** | **0.37553** | **0.49241** | **0.39828** | **0.70127** | | | | |
| **NZ** | -0.01599 | -0.00447 | -0.04125 | -0.0012 | -0.02122 | **0.56667** | | | |
| **SA** | -0.03778 | -0.02587 | -0.06936 | -0.0139 | -0.19084 | **0.64179** | -0.15385 | | |
| **TAS** | 0.00123 | 0.00888 | -0.01135 | 0.00973 | 0.01945 | **0.524** | -0.0439 | -0.08798 | |

**Microsatellites**

**Genetic variability**

*Victoria*

No deviations from Hardy-Weinberg expectations were observed for loci pairs from eastern Victoria. Central and western Victorian populations contained between 1 and two loci pairs, which deviated significantly from expectations respectively. Private alleles were present in all Victorian locations with western Victoria containing the highest number at 12 and central Victoria the lowest with seven. Effective population size was highest for western Victoria (9.012) and the lowest effective population size was attributed to central Victoria (7.861). The average FIS values for Victorian populations were positive with the highest found in western Victoria (0.396) and the lowest observed in eastern Victoria (0.078). Victorian populations showed an observed heterozygosity of <0.5 with the highest recorded from the eastern Victorian population (0.491) (Table 18).

*All locations*

Deviations from Hardy-Weinberg expectations were found for two Tasmanian loci with all NSW loci pairs congruent with expectations. Tasmania contained nine private alleles which was comparable to Victorian populations, while NSW was the only location to contain no private alleles. NSW contained the lowest effective population size (3.718) and observed heterozygosity (0.362) of all populations but contained the smallest sample numbers. Positive FIS values were found for both NSW and Tasmanian populations (Table 18).

Pairwise $F_{ST}$

*Victoria*

All pairwise $F_{ST}$ values were low, ranging between 0.001 and 0.002, but significant for all pairs of Victorian locations with the exception of the eastern and central Victoria pair, which had a pairwise value of 0.55. However, this value was not significant.

*All locations*

$F_{ST}$ comparisons between Victorian and Tasmanian locations were low between 0.001 and 0.003 but significant suggesting some population differentiation. The only exception was the eastern and central Victorian pair, which has a high but non-significant value of 0.477. Due to the small sample sizes from NSW, $F_{ST}$ calculations between this location and Victoria were not possible (Table 19).

**Table 18. Indices for Australasian yellow-tailed kingfish (*Seriola lalandi*) using nine microsatellite markers across five populations. Sample Size (N), private alleles (NPR), mean number of alleles (Na), effective population size (Ne), observed heterozygosity (Ho), expected heterozygosity (He), allelic richness (AR) and loci deviating from Hardy-Weinberg expectations (HW).**

| Population | N | NPR | Na | Ne | Ho | He | AR | HW DIS | FIS |
|---|---|---|---|---|---|---|---|---|---|
| **WesternVIC** | 56 | 12 | 15.333 | 9.012 | 0.444 | 0.580 | 5.471 | Sdu46 and Sdu16, Sdu29 and Sdu37 | 0.396 |
| **EasternVIC** | 57 | 9 | 13.889 | 8.637 | 0.491 | 0.546 | 5.025 | | 0.078 |
| **CentralVIC** | 53 | 7 | 13.111 | 7.861 | 0.477 | 0.538 | 4.974 | Sdu46 and Sdu32 | 0.084 |
| **NSW** | 8 | 0 | 4.556 | 3.718 | 0.362 | 0.435 | 4.884 | | 0.169 |
| **TAS** | 60 | 9 | 12.444 | 6.400 | 0.372 | 0.542 | 5.025 | Sdu46 and Sdu16, Sdu32 and Sdu10 | 0.419 |

**Table 19. Pairwise $F_{ST}$ values denoting differentiation of nine microsatellites between populations of Australasian yellow-tailed kingfish (*Seriola lalandi*). Significant values (<0.05) are shown in bold.**

| Population | Western VIC | Eastern VIC | Central VIC | NSW | TAS |
|---|---|---|---|---|---|
| **Western VIC** | | | | | |
| **Eastern VIC** | **0.003** | | | | |
| **Central VIC** | **0.005** | 0.477 | | | |
| **NSW** | 0.73 | 0.352 | 0.239 | | |
| **TAS** | **0.003** | **0.003** | **0.001** | 0.647 | |

*Structure analyses*

Five populations were predicted by the Evanno method (K=5) with a Delta K of 2.807508. However, no clear patterns relating to location are distinguishable (Figure 28).

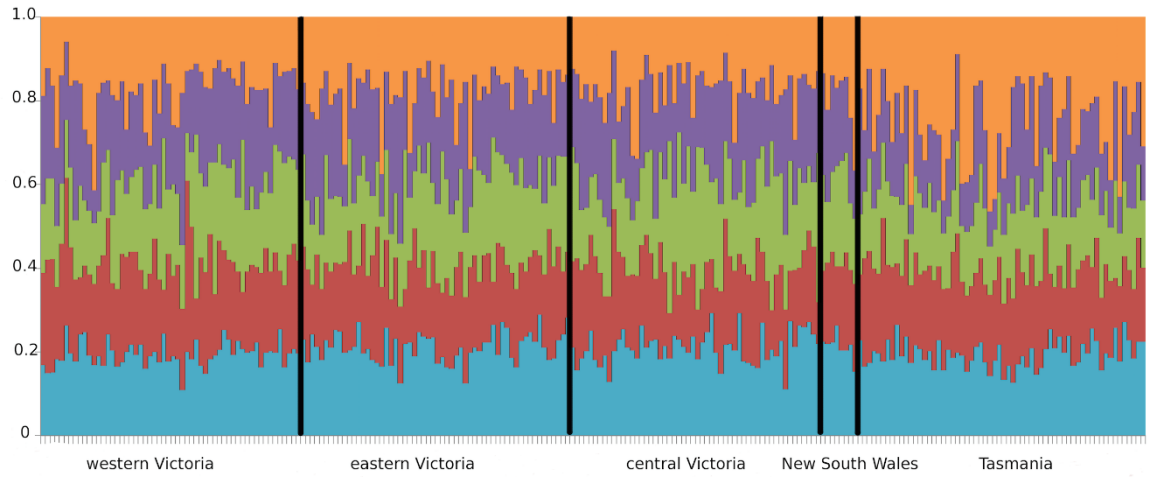


**Figure 28. Structure plot of five populations (K=5) of Australasian yellow-tailed kingfish (*Seriola lalandi*) using nine microsatellite markers.**

**Single nucleotide polymorphisms (Genotyping by Sequencing)**

After filtering, a total of 38,575 SNPs were identified. No clear separation of replicates was evident from the PCA of the replicate samples, hence no batch effect sequencing was detected.

DAPC results identified an optimal K value of two, as indicated in the Bayesian Information Criteria (BIC) analysis where the smallest value of BIC is located at two clusters. Visualisation of the clusters generated by the DAPC revealed three distinct groups corresponding to the three geographical regions (Figure 29).

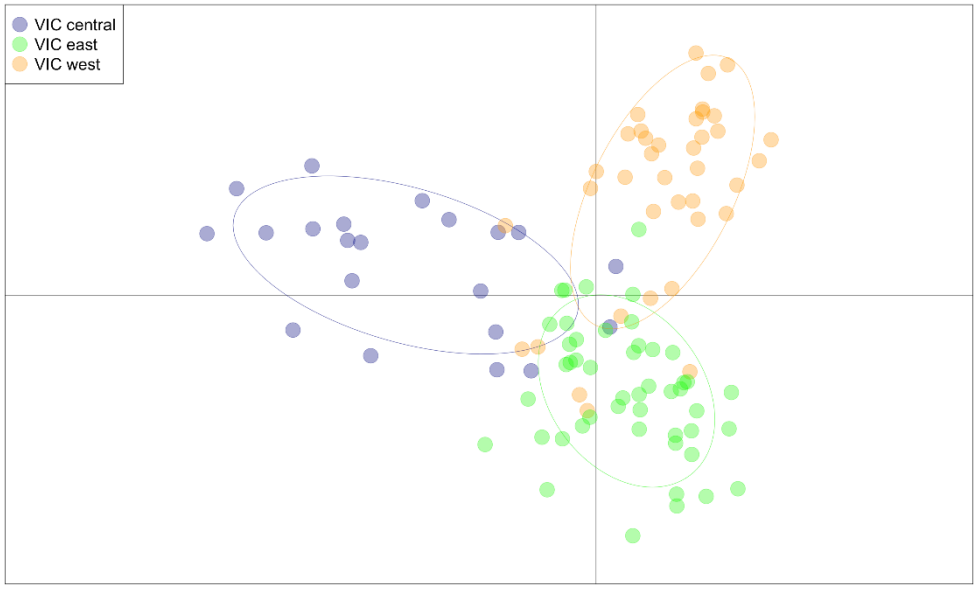


**Figure 29. Discriminate analysis of principal components using 38,575 SNPs. Scatterplot dots represent individuals, while the colours refer to genetic cluster assignment.**

Individual assignment to clusters showed some levels of admixture but stratification of the genetic clusters into regional groups was evident with an assignment proportion (proportion of individuals that were successfully assigned to the correct group) of 87% (Figure 30).

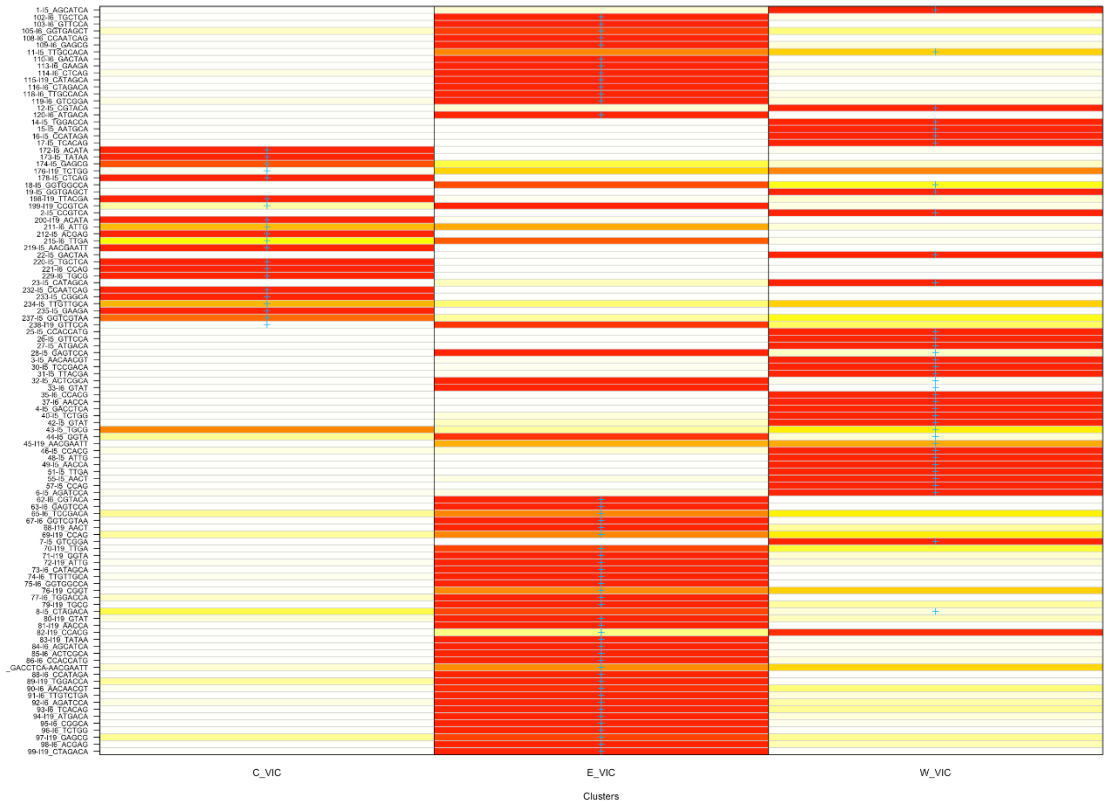


**Figure 30. Genetic group assignment of each individual into the three genetic clusters identified in the DAPC scatterplot (using 38,575 SNPs). Each row represents an individual and the colours represent the posterior probability of assignment to that cluster by the DAPC. Dark red indicates strong probability of assignment to the cluster.**

Pairwise $F_{ST}$ values ranged from 0.0004 to 0.0077. The population Vic Central was significantly different from West and East populations ($p<0.001$, Table 20). $F_{IS}$ values ranged from 0.169 to 0.263 suggesting that individuals in all populations are more related than expected under a model of random mating (Table 21).

**Table 20. Indices for Australasian yellow-tailed kingfish (*Seriola lalandi*) 38,575 SNPs. Sample Size (N), number of alleles (Na), Fixation Index ($F_{IS}$), observed heterozygosity (Ho) and expected heterozygosity (He).**

| | N | Na | $F_{IS}$ | $H_O$ | $H_E$ |
|---|---|---|---|---|---|
| Vic Central | 21 | 60,571 | 0.172 | 0.0996 | 0.120 |
| Vic East | 48 | 66,002 | 0.169 | 0.0977 | 0.113 |
| Vic West | 38 | 70,013 | 0.263 | 0.1000 | 0.133 |

**Table 21. Pairwise $F_{ST}$ values (below diagonal) and p-values (above diagonal) between populations in Victoria based on 38,575 SNPs. Significant values are in bold.**

| | Vic Central | Vic East | Vic West |
|---|---|---|---|
| Vic Central | | **0.000** | **0.000** |
| Vic East | **0.0004** | | 1.000 |
| Vic West | **0.0077** | 0.0052 | |

**Discussion**

Analysis of genetic variability, observed heterozygosity and pairwise measures of yellowtail kingfish caught in south eastern Australia indicted little to no genetic structure in both mitochondrial and microsatellite data. At a broader geographic scale there was little evidence for population differentiation between Victoria, Tasmania and NSW with analyses of mitochondrial data and STRUCTURE analyses detecting no differentiation when using microsatellite markers. Slight, but significant, population differentiation ($F_{ST}$ calculations) was evident between all Victorian locations and Tasmania. Inclusion of previously sequenced ND4 genes from Western Australia did reveal a distinction between western and eastern Australian populations. As such, management of yellowtail kingfish should be considered that incorporates information from Vic, NSW and Tas.

No population differentiation was detected between the three Victorian populations using mitochondrial markers or from STRUCTURE analysis of microsatellite markers. Slight significant population differentiation was detected between Victorian locations using pairwise $F_{ST}$ calculations of microsatellite markers. Significant small genetic structure was detected in Victorian populations when using SNP markers. Despite small differences, population structuring is not likely at a level that warrants investigating differences in population structure that may lead to changes in recreational fishing regulations.

Due to the large expected dispersal ability of many pelagic marine fish species low genetic structure is common (Ward and Elliott 2001). *S. lalandi* have been recorded to move 3000 km along the east coast of Australia in a capture and release study, however the majority of fish were recaptured 50km from their initial site. A trend was present suggesting larger fish tend to move further over time (Gillanders, Ferrell et al. 2001). Similar Victorian large pelagic fish species such as Silver Warehou (*Seriolella punctata*) and the Blue-eye Trevalla (*Hyperoglyphe antarctica*), exhibit a high level of gene flow with no distinct genetic structure found within Victoria when using mtDNA (Robinson, Skinner et al. 2008).

Previous studies have focused on microsatellite loci to examine population structure and as a result few studies using mitochondrial genes, specifically ND4 are currently available for comparison within Victoria. However, Miller et al. (2011) have sequenced three individuals from Victoria, all possessed single haplotype. As a result, no diversity indices are available for these sequences. Other mitochondrial genes such as Cytb have been examined in *S. lalandi* over a larger geographical scale. Haplotype diversity for an Australian population using the Cytb gene revealed a comparable haplotype and nucleotide diversity of 0.463 and 0.001 respectively (Swart 2014).

Private haplotypes were found for all Victorian populations, however pairwise $\Phi_{PT}$ show no significant differentiation between western, central and eastern populations. This pattern is congruent with previous studies, which suggest gene flow between Victorian, NSW and NZ populations (Miller, Fitch et al. 2011).

Low levels of expected heterozygosity were found for all Victorian populations ranging between 0.538 and 0.580 for central and western Victorian populations respectively. These values are slightly higher than previously recorded for

Victorian populations, which averaged 0.45 for seven polymorphic loci (Miller, Fitch et al. 2011). Likewise, heterozygosity values found for the NSW population in this study are also highly similar (0.435) to the previously examined population (Miller, Fitch et al. 2011). No previous studies have examined Tasmanian *S. lalandi*, which, showed similar expected heterozygosity to Victorian populations (0.542). SNPs had a lower average heterozygosity comparing to microsatellites, which is expected when using a bi-allelic rather than multi-allelic marker. Additionally, both markers (SNPs and microsatellites) showed a lower observed heterozygosity ($H_O$) than expected ($H_E$) for all Victorian populations, which can be potentially attributed to inbreeding forces.

Victorian populations all exhibit relatively low effective population size in relation to the number of individuals sampled. Effective population sizes ranged between 9.012 and 7.861 from samples sizes of 56 and 53 for western and central Victorian populations respectively. Low effective population sizes were also recorded for NSW and Tasmanian populations. These relatively low effective population sizes likely indicate historic population bottlenecks. In other words, when a population is substantially reduced in size (bottleneck) its genetic variation can also be reduced. In the case this population recovers after such an event and there is a large number of individuals (census population size), the standing genetic variation may still reflect a much smaller past population size.

Negative Tajima's D and Fu's Fs were found for all Victorian populations due to an excess of low frequency polymorphisms suggestive of a population expansion after a bottleneck. It should be noted that values for the western Victorian population for Tajima's D were not significant however. Recent population fluctuations have been observed in *S. lalandi*, however these results are suggestive of a more historic bottleneck and the subsequent accumulation of haplotypes, which differ by a single nucleotide polymorphism. Historic bottlenecks are believed to be responsible for mild structuring in the blue warehou (*Seriolella brama*) a similar large pelagic fish species within Victoria and between Victorian and Tasmanian populations (Robinson, Skinner et al. 2008).

## Conclusion

Different DNA markers have different characteristics and it is very important to consider applicability to fisheries management and limitations of each type of marker (Waples *et al.* 2008). In this study, while the mtDNA provided insights into the demographic history of Victorian populations (e.g. haplotype diversity was lowest in eastern Victoria), its power to resolve population structure was limited. Microsatellites provide high statistical power in population identification and in this study these markers detected slight significant population differentiation between Victorian locations. However, the SNP markers showed the highest levels of resolution and differences between Victorian populations. SNP markers generally show lower genotyping error rates than microsatellites (Morin & Mccarthy 2007) and occur at a high frequency (one every few hundred base pairs). Therefore using high number of SNPs allows a good coverage of the genome. Additionally, SNPs can also be present in regions of the genome that are under selection and using such markers can add important knowledge for example for identifying locally adapted populations and for studying the effects of natural and anthropogenic pressures on stocks (e.g. environmental change and fisheries exploitation).

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