Using population genomics to understand stock structure of Tiger Flathead *(Platycephalus richardsoni)* in southeast Australia

Submitted by

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Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution, and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Amelia Jensen

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Abbreviations

SESSF	Southern and Eastern Scalefish and Shark Fishery
GVP	Gross Value Production
PCR	Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism
NGS	Next-Generation Sequencing
WGS	Whole genome sequencing
AFMA	Australian Fisheries Management Authority
EAC	East Australian Current
PLD	Pelagic Larval Duration
MAF	Minor Allele Frequency
PCA	Principle Component Analysis
DAPC	Discriminant Analysis of Principle Components
AMOVA	Analysis of Molecular Variance

Chapter 1: Literature Review

Introduction

Australia's wild catch fisheries are a key contributor to national and international food security, delivering significant economic and social benefits to the Australian population (Abernethy et al. 2020). There are increasing pressures on Australia's wild catch fisheries with a growing population and climate change presenting significant challenges for the industry (Holbrook & Johnson 2014; Bogard et al. 2019; Smith et al. 2024). Effective fisheries management depends on accurate and up-to-date biological information to ensure the long-term sustainability and health of wild fish populations. (Waples 1998; Benestan 2019).

The Southern and Eastern Scalefish and Shark Fishery (SESSF) is the largest Commonwealth fishery in regards to volume caught. The management area spans almost half of the Australian Fishing Zone, encompassing both Commonwealth and domestic waters (Emery et al. 2024). As of 2023, 38.5% of stocks within the SESSF were either subject to overfishing or uncertain, meaning there is insufficient information to assess whether a stock is sustainably fished accurately (Smith & Dichmont 2017; Butler et al. 2024). Accounting for 24% of the Gross Value of Production (GVP) of Commonwealth fisheries, the SESSF contributes significantly to the Australian economy through the domestic consumption of wild caught fish (Pascoe et al. 2021; Wright et al. 2024). Tiger flathead (*Platycephalus richardsoni*) makes up a substantial component of the wild catch being one of the most valuable species within the fishery (Little et al. 2011; Australian Fisheries Management Authority 2023).

Recent studies have indicated that a portion of the biological parameters used in tiger flathead stock assessments are either out-of-date or unknown (Evans et al. 2022). Additionally, tiger flathead population structure is currently assumed to be panmictic, although no assessment of stock structure has been undertaken for the species (Emery et al. 2023). Poorly understood stock structure for commercially important species can lead to the mischaracterisation of management boundaries which can place populations at risk to overfishing, threatening species longevity and ecosystem function (Taillebois et al. 2021). There are indicators that suggest multiple populations of tiger flathead may exist within the fishery, with differences in growth, appearance and reproductive timings being observed, particularly for fish off the east coast of Tasmania (Liggins 2023).

This literature review aims to examine the current understanding of tiger flathead biology and stock structure in the SESSF. It will synthesise information on the importance of understanding population

198 structure in marine teleosts, particularly from a commercial perspective, and the emergence of
199 population genomics technologies. These findings will provide background context into the analysis
200 of tiger flathead stock structure, contributing to more reliable stock assessments for the species.

201 **Stock structure in fisheries management**

202 Fishery management units are commonly delineated by geographic jurisdictions, assuming a single,
203 panmictic population (Zemeckis et al. 2014; Grummer et al. 2019). However, these conventional
204 stock assessment models can often incorrectly attribute biological stock structure, reducing the
205 accuracy of stock assessments and thus threatening the longevity of a species (Taillebois et al. 2021).
206 Where distinct populations do occur, these may respond differently to fishing pressures impacted by
207 differences in demographic traits such as abundance, mortality, reproduction and growth rates (Cadrin
208 2020). Productivity may vary between populations and if stock assessments do not consider these
209 differences, then less productive populations may be overexploited while more productive
210 populations may be underharvested (Zemeckis et al. 2014). Overharvesting or the unsustainable
211 removal of fish from populations can degrade and reduce the genetic diversity of fish stocks
212 (Andersson et al. 2024). Maintaining genetic diversity enhances a stock's capacity to adapt to
213 environmental changes, supporting the long-term sustainability of fish populations (Allendorf et al.
214 2014). Therefore, effective fisheries management relies on the correct identification of biological and
215 genetic population boundaries (Waples 1998).

216 A biological stock is defined as a group of fish of the same species that inhabit the same area, with
217 similar recruitment patterns (Carvalho & Hauser 1994). Conversely, a genetic population is defined
218 as a geographically and genetically independent group of individuals of the same species, however
219 definitions vary (Waples & Gaggiotti 2006). Population differentiation can vary in scale from
220 complete isolation to a single, homogenous stock (Figure 1). Stock separation may arise due to
221 physical barriers such as sea mounts, deep water and varying oceanographic conditions, such as
222 eddies, fronts, currents and environmental gradients (Grummer et al. 2019; Bertram et al. 2023).
223 Biological barriers also contribute to genetic divergence through differences in vagility, distribution
224 and abundance (Ovenden 2013). Both genetic and genomic techniques can be used to understand
225 stock structure. Genetic studies examine specific genes, or a limited number of loci to understand
226 stock structure, whereas genomic studies look at markers across the entire genome, at a significantly
227 larger scale (Andrews et al. 2016). This provides a more comprehensive analysis of population
228 structure and connectivity (Cuéllar-Pinzón et al. 2016).

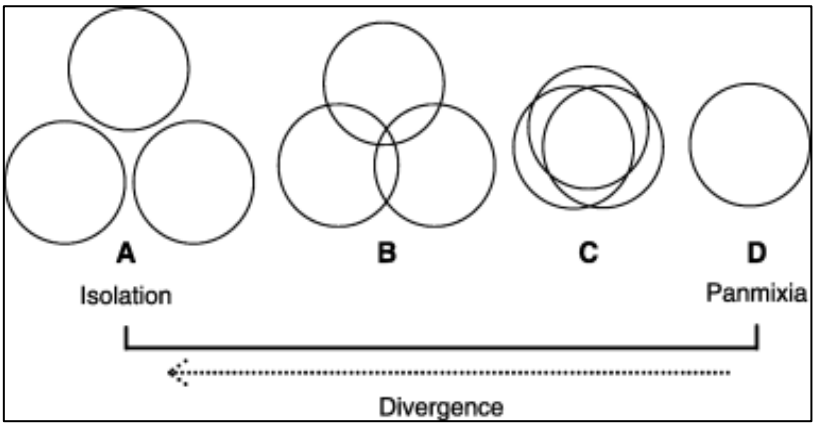

Figure 1: Scale of population differentiation. (A) Complete isolation, (B) Moderate connectivity, (C), Significant connectivity, (D) Panmictic population (Waples & Gaggiotti, 2006).

Misalignment between biological population structure and management areas has been observed for many commercially important species (Reis-Santos et al. 2018; Papa et al. 2022). For example, atlantic cod (*Gadus morhua*) have been managed in the United States' waters as two separate units since 1972. Declared as overfished, a series of management actions were rolled out over decades, such as reducing fishery catch to help rebuild stocks (McBride & Smedbol 2022). Despite this, populations continued to decline, and since then, multiple studies have indicated that a portion of this decline may be due to a misalignment between management units and biological stock structure (Annala 2012; Zemeckis et al. 2014; Northeast Fisheries Science Center (U.S.) 2022). Evidence of genetic variation, movement, dispersal of larvae, spawning locations and timings have all contributed to an updated stock structure of atlantic cod in US waters, highlighting that there are actually five distinct biological populations, with four stocks requiring individual management (McBride & Smedbol 2022). Based on this, the New England Fishery Management Council developed an Atlantic Cod Management Transition Plan which runs from 2025 to 2027 to incorporate these new management units into the fishery management plan (Singer & Macdonald 2024).

Atlantic herring (*Clupea harengus*) is a key ecological and commercial species that has been harvested for centuries (Barrett et al. 2004). The most recent management report of Atlantic Herring indicated that the stock is currently overfished, although not subject to overfishing (National Oceanic and Atmospheric Administration 2024). Early genetic studies undertaken over 40 years ago using a small number of allozyme markers, revealed no genetic differentiation (Andersson et al. 2024). Since then, studies, using whole genome sequencing have found that there are multiple genetically distinct populations of atlantic herring, with genetic differentiation attributed to ecological adaptation such as salinity, water temperature, spawning timelines and light conditions (Han et al. 2020; Bekkevold et al. 2023). This evidence revealed a mismatch between current management units compared to the genetic populations. Therefore, updating stock assessments to reflect these genetically distinct stocks

can improve the accuracy of stock assessments, and thus the quality of management actions (Bekkevold et al. 2023).

Tools and methods for understanding stock structure

A variety of methods can be used to inform stock structure of marine species. Direct measures such as parasitic markers and otolith chemistry can provide insights into a population's broad-scale distributions. Otolith chemistry and morphometrics can provide valuable insights into stock boundaries through identifying different geochemical gradients and regional growth patterns (Nazir & Khan 2021). Additionally, tagging and recapture helps to understand movement patterns and biological boundaries of stocks (Metcalfe et al. 2006; Goddard et al. 2024). Presence and assemblage of parasites associated with different fish can reveal key ecological information on fish stocks and help to identify separate stocks (Poulin & Kamiya 2015). Stable isotopes are another direct marker that can highlight information about the diet and therefore, habitat use of fish (Abrantes et al. 2014). However, these methods only provide information over an individual's lifetime. Comparatively, population genomics provides information over generational timescales (Reis-Santos et al. 2018).

There has been significant development in population genetic approaches and technology. Early molecular techniques include the use of allozyme markers (Davinack 2024; Payet et al. 2024). The first measures of genetic variation were reported in the literature over 50 years ago using protein gel electrophoresis to screen allozyme loci. An allozyme is a form of an enzyme that is encoded by different alleles in the same gene locus. Electrophoresis supports the detection of allozymes, separating the molecules by their charge and size (Davinack 2024). Allozymes supported the detection of distinct populations for a range of marine species (Allegrucci et al. 1997; Bourke et al. 1997; Rossi et al. 1998). For example, a study using allozyme markers found that what was assumed to be two separate species of oysters (*Tiostrea chilensis* and *Teostrea lutaria*) was in fact two genetically distinct populations of the same species (*T. chilensis*) (Buroker et al. 1983; Gosling 2003). However, allozyme markers offer relatively low resolution as they only capture a small number of enzyme loci, resulting in a limited ability to reflect genetic coverage and minimising the ability to accurately assess genetic diversity and population structure (Casillas & Barbadilla 2017). Additionally, studies found that allozyme polymorphisms represented DNA variations that changed amino acid sequencies. Therefore, this only represented 25% of all possible amino acid changes due to its ability to only detect those that change the charge of a protein (Lewontin 1991).

In the 1980's, studies started using methodologies such as PCR (Polymerase Chain Reaction) amplification, microsatellite markers and sequencing of mitochondrial DNA (mtDNA) genes (Cuéllar-Pinzón et al. 2016). These represented significant improvements in screening

methodologies, as the number of variable loci that were able to be analysed increased, thereby providing higher resolution than allozyme markers (Allendorf 2017). However, mtDNA only reflects the maternal line, ignoring paternal gene flow (Cuéllar-Pinzón et al. 2016). This can be limiting when undertaking population genomics studies as it only provides a partial view of ancestry and can underestimate total gene flow within a population (Antoniou & Magoulas 2014).

Recently, further developments in technologies have led to the adoption of genomic tools such as Single Nucleotide Polymorphisms (SNPs) and next-generation sequencing (NGS). SNPs are single base pair changes in the DNA sequence and are spread across the entire genome (Edwards et al. 2007). SNPs can be used to detect genetic differences between regions or stocks, as well as to detect adaptive variation that may be linked to environmental conditions (Wenne 2023; Krustaleva 2024; Lu et al. 2025). SNPs provide a more comprehensive (biparental) genome coverage and higher quality data than other techniques such as microsatellites and mtDNA sequencing (Morin et al. 2004). SNP genotyping has resulted in an increase in accuracy and speed of population genomic analysis at low costs (Cuéllar-Pinzón et al. 2016).

These advances in technology have supported the identification of various fish populations that have previously been unable to be detected. For example, the population structure of highly valuable, migratory species such as yellowfin tuna (*Thunnus albacares*) has previously been studied using methods such as mtDNA and allozyme markers (Appleyard et al. 2001; Díaz-Jaimes & Uribe-Alocer 2006; Grewe et al. 2015). These results considered yellowfin tuna to be a single panmictic population. However, advances in genome-wide SNP genotyping have demonstrated that there are multiple genetically distinct populations (Grewe et al. 2015). This highlights the power of these technological advancements, having the potential to significantly improve management of wild catch fisheries, particularly in relation to stock assessments and reporting (Pecoraro et al. 2018).

Another powerful tool in population genomics is long-read whole genome sequencing (WGS). This provides an even greater power to detect genetic variation within and between populations (Lu et al. 2025). WGS involves the sequencing of larger fragments of the entire genome and the mapping of SNPs onto the genome, supporting the identification of a range of biological processes such as gene flow, adaptation and natural selection (Lu et al. 2025). It also provides access to more SNPs than other methods such as double digest Restriction site Associated DNA sequencing (ddRAD-seq) and DArTseq (Martchenko & Shafer 2023). Figure 2 outlines how long-read WGS can be incorporated into fisheries management, however it is noted that the study outlined in this thesis commenced at step B, rather than step A.

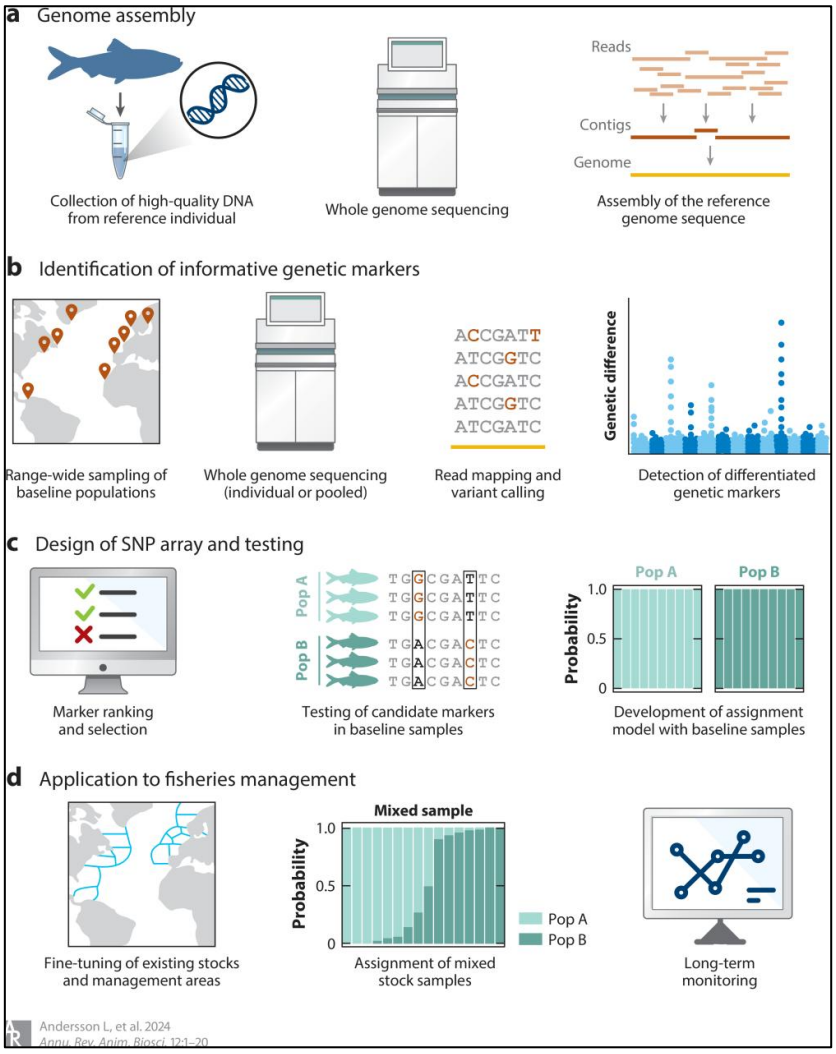

Figure 2: Process for undertaking long read whole genome sequencing for stock structure analysis. (A) Development of a high-quality reference genome sequence for the target species. (B) Collection of samples from target species, followed by whole genome sequencing and detection of genetic differentiation. (C) SNP analysis to inform population structure and (D) Application to management boundaries to support sustainable fisheries management (Andersson et al. 2024).

Southern and Eastern Scalefish and Shark Fishery (SESSF)

The SESSF is a Commonwealth-managed, multi-species and multi-gear fishery that spans nearly half of the Australian Fishing Zone. It operates across both Commonwealth and state waters under Offshore Constitutional Settlement arrangements and encompasses numerous marine parks within its boundaries (Emery et al. 2024). The fishery extends from southern Queensland to Tasmania and across to southern Western Australia, covering depths from 30 to 1200 metres (Figure 3) (Smith & Smith 2001). It includes several sectors: the Commonwealth Southeast Trawl Sector, East Coast Deepwater Trawl Sector, Scalefish Hook Sector, Shark Hook and Shark Gillnet Sector, and the Great Australian Bight Trawl Sector. As the largest Commonwealth fishery, the SESSF accounts for 24%

of the Gross Value of Production (GVP) of all Commonwealth fisheries. In 2022-23, it generated a total GVP of AUD $98.58 million (Wright et al. 2024).

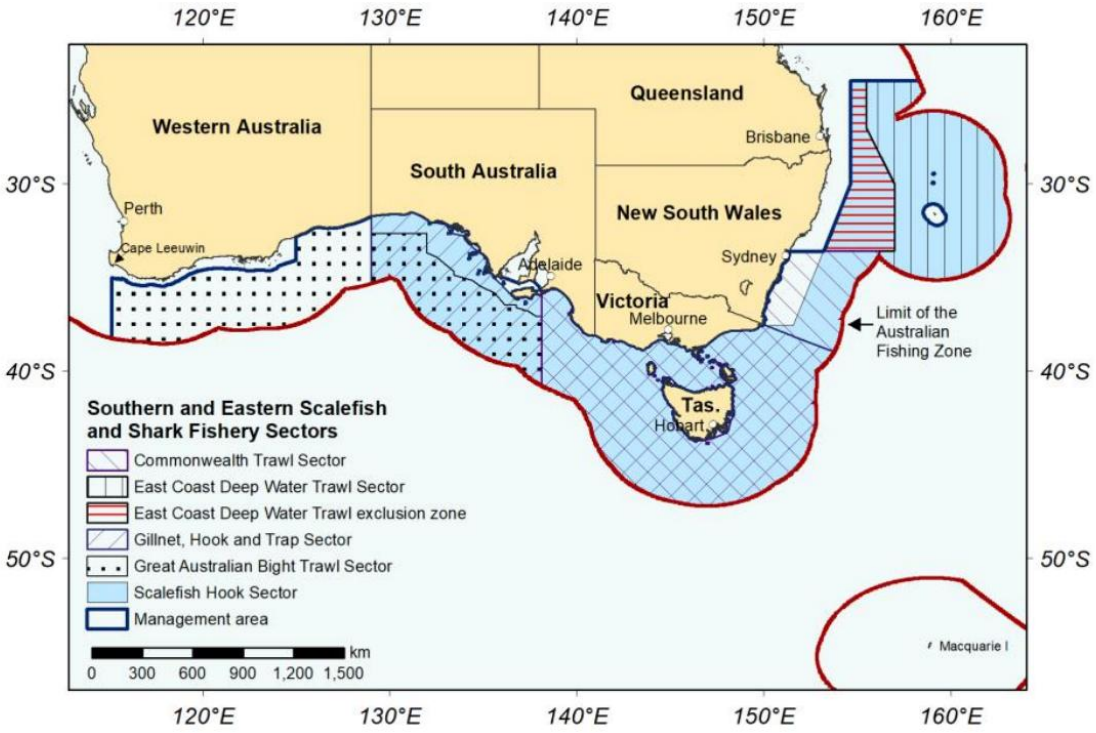

Figure 3: Southern and Eastern Scalefish and Shark Fishery management area (Butler et al. 2024).

The SESSF is managed by the Australian Fisheries Management Authority (AFMA) under a quota-based system, with additional access controls including gear restrictions, electronic monitoring, and spatial closures (Emery et al. 2024). A total of thirty-nine stocks are assessed within the fishery, with the most economically significant species being tiger flathead (*P. richardsonii*), pink ling (*Genypterus blacodes*), blue grenadier (*Macruronus novaezelandiae*), and orange roughy (*Hoplostethus atlanticus*). Together, these four species contribute to 73% of the total value of the fishery (Australian Fisheries Management Authority 2024). In 2023, fishing mortality assessments showed that 62% of stocks were not subject to overfishing, 10% were experiencing overfishing, and 28% had an uncertain status (Wright et al. 2024). Biomass assessments for the same period indicated that 56% of stocks were not overfished, 26% were overfished, and 18% were classified as uncertain (Figures 4 and 5) (Wright et al. 2024).

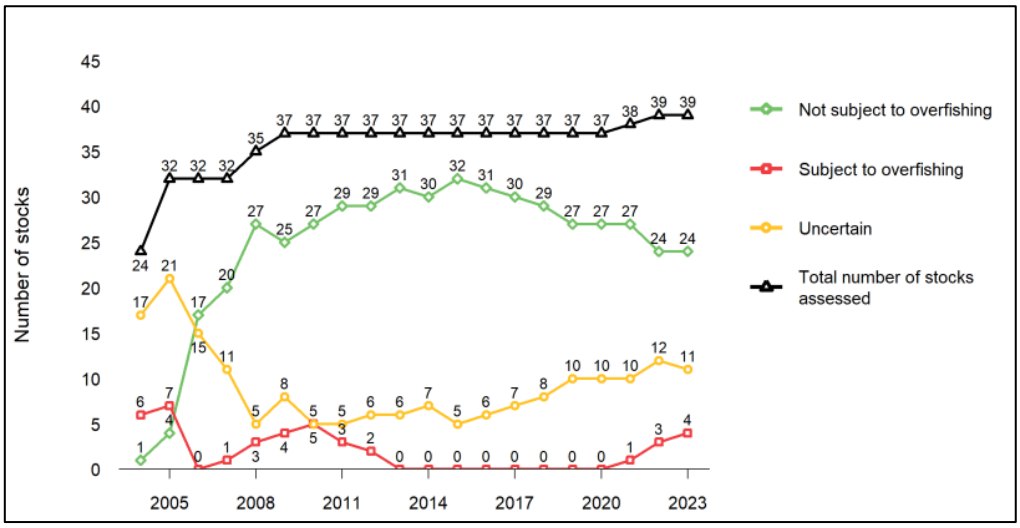

Figure 4: Southern and Eastern Scalefish and Shark Fishery - fishing mortality status for all stocks assessed, 2004 – 2023 (Wright et al. 2024)

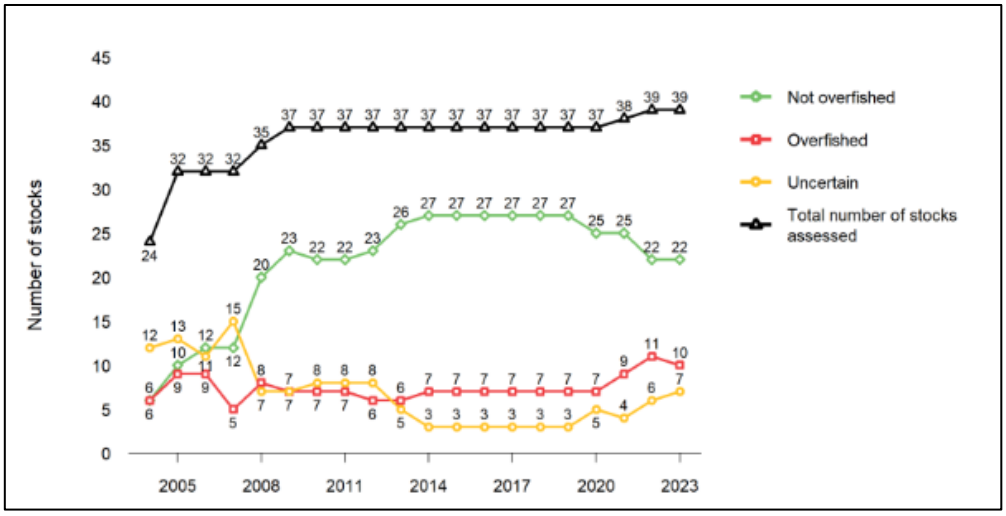

Figure 5: Southern and Eastern Scalefish and Shark Fishery - biomass status for all stocks assessed, 2004 – 2023 (Wright et al. 2024)

The SESSF is also a climate change hotspot, with waters warming at a rate higher than the global average (Hobday & Pecl 2014; DCCEEW 2025). This has caused the East Australian Current (EAC) to intensify, bringing warmer waters poleward (Phillips et al. 2022). Subsequently, there has been an observed shift in species distribution, contributing to the depletion of commercially important species such as the striped trumpeter (*Latris lineata*) and blue warehou (*Seriollela brama*) (Fulton et al. 2024). Conversely, recent modelling by the Australian Fisheries Management Authority indicated that climate change does not have an influence on tiger flathead stock abundance, however, other studies have contradicted this statement, highlighting that changes in temperature, salinity, current direction and UV may impact abundance and survival of the tiger flathead (Pecl et al. 2011; Australian Fisheries Management Authority 2025).

Tiger Flathead

Tiger flathead, also known as King flathead, Trawl flathead and Deepsea flathead, is endemic to Australia. It is a bottom dwelling teleost, inhabiting sandy and muddy substrates across the continental shelf from northern New South Wales, down to Tasmania and across to Southern Australia (Figure 6) (Kailola et al. 1993). Tiger flathead are found in depths ranging from 10 metres to 400 metres, with most commercial catches coming from 50 metres to 200 metres (Tilzey et al. 1990; Edgar 2008; Butler et al. 2023). Tiger flathead is relatively sedentary, spending most of the day resting on the seabed, mud and sand substrate, with no broad-scale geographic movements (Fairbridge 1951; Bruce et al. 2002; Pecl et al. 2011).

Figure 6: Tiger flathead (*Platycephalus richardsoni*) distribution (Australian National Fish Collection et al. 2021)

Tiger flathead are benthopelagic piscivores, with adults feeding on small fish and crustaceans, and cannibalism seen in larger individuals (Bulman et al. 2001; Bruce et al. 2002). They display nocturnal movement patterns, moving into the water column to search for prey (Colefax 1934). Juveniles inhabit shallow waters on the continental shelf and migrate to deeper waters in the outer shelf zone once they mature (Montgomery 1985). Prior to the spawning period, mature fish have been observed migrating back to shallower waters (Kailola et al. 1993). Spawning periods differ between locations; female spawning occurs from October to May in New South Wales, while spawning in the Bass Strait and southern Tasmania occurs from December to February (Fairbridge 1951; Bruce et al. 2002). Tiger

flathead have a life span of around 20 years, reaching sexual maturity at 3 years old (Barnes et al. 2011). Males commonly reach up to lengths of 50cm, while females are larger, growing up to lengths of 60cm (Emery et al. 2023) (Figure 7). They are broadcast spawners with high fecundity; females can produce up to 2.5 million eggs per spawning season (Montgomery 1985). *Platycephalid* eggs and larvae, including those of tiger flathead are pelagic, and are well distributed by the currents and counter currents of the east coast of Australia (Figure 8) (Fairbridge 1951; Taylor et al. 2020). Eggs and larvae are primarily distributed by the EAC and once they hatch, larvae remain in waters with a depth of less than 55 metres while they develop (Montgomery 1985). There is still a significant gap in species specific research regarding pelagic larval duration and distribution. Other *platycephalid* species in southern Australia have pelagic larval durations that range from 20 days to 2 months. For example, the dusky flathead (*P. fuscus*) has a pelagic larval duration around 1-2 months, while the sand flathead (*P. bassensis*) has a pelagic larval duration of approximately 20-40 days (Hamer et al. 2010; Pecl et al. 2011; Hirst et al. 2014).

Figure 7: Tiger flathead (*Platycephalus richardsoni*) caught within the Southern and Eastern Scalefish and Shark Fishery

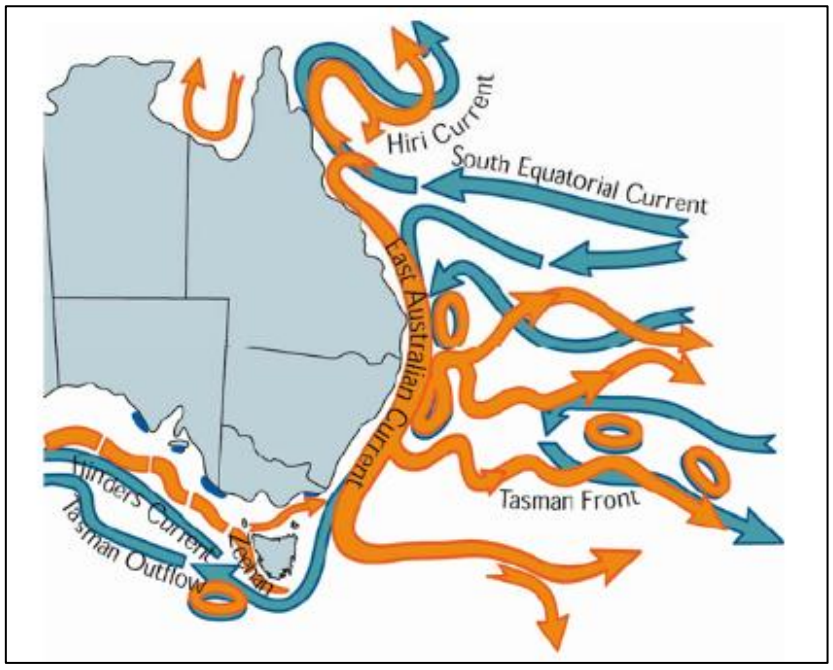

Figure 8: Main ocean surface (orange) and subsurface (blue) currents off eastern Australia (Ridgway & Hill 2009).

Commercial fishing of tiger flathead commenced in 1915 along the east coast of Australia in the steam trawl fishery (Klaer 2004). During the steam trawl period, localised depletion occurred in the New South Wales region, with overfishing causing a stock collapse in the 1940s (Bruce et al. 2002). Heavy fishing combined with fishers moving into deeper waters to maintain catch rates, sustained this collapse, while the type of gear used such mesh net size may have also contributed to declines (Klaer 2001). During World War II, the number of fleets fishing for tiger flathead were significantly reduced, and subsequently, gear restrictions such as minimum mesh size limits were introduced. This reduction in fishing pressure led to the recovery of the stock in the 1960s and 1970s (Novaglio et al. 2018). Danish seine and diesel otter trawlers entered the fishery in the 1930s and 1970s, respectively, with Danish seine trawlers remaining as the primary fishing method for the species to this day (Little et al. 2011; Tuck 2020). Today, tiger flathead remains a commercially important species, with catches primarily managed in the SESSF. The total commercial catch in 2022-23 was 1860 tonnes, making up 21.32% of SESSF Gross Value of Production (GVP) (Australian Fisheries Management Authority 2025).

Tiger flathead is managed as a Tier 1 stock, which is the most robust type of stock assessment undertaken in the SESSF. These assessments incorporate a wide range of high-quality data to make the most informed estimation of recommended biological catch (Dowling et al. 2016). There has been significant development in stock assessment methods for tiger flathead since its inception in 1989. Initially, stock assessments from 1989 to 2001 consisted of catch data, catch rates, age and length.

From 2001 to 2004, additional data and analysis were added to assessments to estimate unfished spawning stock biomass, providing a more dynamic picture of the fishery. In recent years, additional data such as catch per unit effort, ageing errors, discards, fishing mortality rate and conditional age-ate-length data have been comprehensively incorporated into the assessments (Day 2016; Tuck 2020). Currently, the target reference point is to sustain spawning stock biomass at 40% of the unfished level. This target has not been exceeded since the implementation of the reference point in 2010 (Bessell-Browne 2022).

Conclusion

Population genomics provides valuable insights into the evolutionary history of marine teleosts. Understanding population structure is critical in ensuring appropriate management boundaries and catch limits are applied, to support ongoing sustainability of commercially and recreationally exploited important species. Tiger flathead is an important commercial species in Australia, yet its stock assessment is limited by a lack of accurate and up-to-date data. With key biological parameters and stock assessment values used in tiger flathead stock assessments being either out of date or uncertain (e.g. maturity, length-weight relationship), this poses a significant risk to both tiger flathead assessments and the SESSF's long-term sustainability. While tiger flathead is assumed to be a single panmictic population, there have been no studies to date to test this assumption. This literature review highlights the clear need for a comprehensive genomic analysis of tiger flathead population structure in the SESSF and the integration of current and reliable data into management frameworks for the species.

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Chapter 2: Manuscript

Title: Using population genomics to understand stock structure of tiger flathead (*Platycephalus richardsoni)* in the Southern and Eastern Scalefish and Shark Fishery

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Abstract

Effective fisheries management supports the ongoing sustainability and health of wild fish stocks. Appropriate management relies on the accurate identification of populations and stock structure of a species. Tiger flathead (*Platycephalus richardsoni*), a commercially valuable species in the Southern and Eastern Scalefish and Shark Fishery (SESSF), is assumed to consist of a single homogeneous panmictic population; however, no molecular studies have been undertaken to investigate this. Observed differences in growth, appearance, and reproductive timing in tiger flathead across southeast Australia have previously suggested potential stock structure or ecophysiological differences among individuals from different geographic locations. The current study employs a population genomics approach using single nucleotide polymorphisms (SNPs) to assess genetic connectivity and stock structure of the species. DNA was extracted from 188 individuals across eastern Australia from New South Wales to Tasmania, caught and sampled in 2023 to 2024. No fine-scale genetic structuring was found for tiger flathead, suggesting a single panmictic stock across the species' distribution. Results indicate that genomic population structure is congruent with current management strategies, with high gene flow observed, suggesting that large scale

731 movements via ocean currents during the egg and pelagic larval phase likely facilitate genetic mixing.
732 This study represents the first population genomics assessment of tiger flathead, contributing to
733 improved fisheries management knowledge within the SESSF and addresses one of the key gaps in
734 biological parameters for the species.

Introduction

The global population is expected to climb to 10 billion people by 2050, increasing the global demand for food and thus seafood (Guillén et al 2019; Andersson et al. 2024). This is placing increasing pressures on Australia's wild catch fisheries, with climate change contributing to this pressure. (Holbrook & Johnson 2014; Bogard et al. 2019; Smith et al. 2024). Given such challenges, reliable and up-to-date biological data is essential in ensuring appropriate management frameworks are implemented to support sustainability of wild fish stocks (Evans et al. 2022). Population genomics presents a useful opportunity to understand population structuring of marine teleosts, directly contributing to stock assessments for commercially and recreationally important species.

Population genomics in fisheries management

Fishery management units across the globe are commonly delineated by geographical boundaries and anthropogenic considerations rather than biological units. (Zemeckis et al. 2014; Grummer et al. 2019). Additionally, many stock assessments assume panmixia, which occurs when individuals within a population are equally likely to mate with one another with respect to relatedness, geography, sex ratios, genotype and phenotype (Andersson et al. 2024; Walton et al 2025). As genetically distinct populations may respond differently to fishing pressures, this untested misalignment can often reduce the accuracy of biological parameters used in stock assessments, such as productivity and biomass, and can lead to the overexploitation of a species or population (Zemeckis et al. 2014; Cadrin 2020; Andersson et al. 2024). Population genomics is becoming more commonly used in fisheries management frameworks, supporting the identification (or lack thereof) of biological population boundaries (Waples 1998).

A population is defined as a geographically and genetically independent group of individuals of the same species (Waples & Gaggiotti 2006). Genetically distinct populations may arise through a range of physical, oceanographic and biological barriers, such as sea mounts, currents and species vagility which can result in restricted gene flow and reduced mating opportunities (Ovenden 2013; Grummer et al. 2019; Bertram et al. 2023). From allozyme, microsatellite and mitochondrial markers to next-generation sequencing technologies and reduced representation genome scans, there has been an increase in both speed and accuracy of population genomics approaches (Cuéllar-Pinzón et al. 2016). This has led to the increase in the capacity to screen for nuclear markers such as single nucleotide polymorphisms (SNPs). SNPs are abundant and spread across the entire genome, allowing high quality data to be obtained for relatively low costs. SNP analyses provide a unique opportunity to more easily acquire comprehensive genetic data across the genome (hence 'genomic data'), that can be incorporated into fisheries management frameworks (Morin et al. 2004, Payet et al. 2024).

Southern and Eastern Scalefish and Shark Fishery

The Southern and Eastern Scalefish and Shark Fishery (SESSF) is the largest Commonwealth-managed fishery by catch (Wright et al. 2024). Covering depths of 30-1200 metres, the fishery extends the southern end of Australia from Western Australia to Queensland (Smith & Smith 2001). Generating a total GVP of $98.58 million, it accounts for nearly one quarter of the GVP for all Commonwealth fisheries (Wright et al. 2024). Fishing mortality and biomass assessments from 2023 indicate that between 28% to 44% of stocks within the fishery were either subject to overfishing or uncertain (Wright et al. 2024). Additionally, the SESSF is a climate change hotspot, with southeast Australian waters warming at a rate faster than the global average (Hobday & Pecl 2014; DCCEEW 2025). A total of thirty-nine stocks make up the fishery, with one of the most commercially important species being tiger flathead (*Platycephalus richardsoni*). In 2022-23, tiger flathead accounted for over one fifth of the GVP within the fishery (Australian Fisheries Management Authority 2025).

Tiger Flathead

Endemic to southeast Australia, tiger flathead is a demersal marine teleost, inhabiting sandy and muddy substrates (Kailola et al. 1993). They are found in depths ranging from 10 metres to 400 metres, with most commercial catches coming from 50 metres to 200 metres over the continental shelf (Tilzey et al. 1990; Edgar 2008; Butler et al. 2023). Their distribution spans southeast Australia from South Australia to New South Wales, including Tasmania (Australian National Fish Collection et al. 2021). Previous studies have suggested that tiger flathead are a relatively sedentary species with no observed broadscale geographic movements (Fairbridge 1951; Pecl et al. 2011; Bruce et al. 2002). They are benthopelagic piscivores with nocturnal movement patterns, moving into the water column at night to feed primarily on small fish and crustaceans (Colefax 1934; Bulman et al. 2001). Juveniles inhabit shallow waters on the continental shelf and migrate to deeper waters in the outer shelf zone once they mature. Prior to the spawning period, mature fish have been observed migrating back to shallower waters (Montgomery 1985; Kailola et al. 1993). With a lifespan of 20 years, tiger flathead reach sexual maturity around 3 years old and are broadcast spawners, producing up to 2.5 million eggs per spawning season (Montgomery 1985, Barnes et al. 2011). Eggs and larvae of *platycephalids* (Family *Platycephalidae*) are known to be well distributed by the currents of the east coast of Australia (Fairbridge 1951; Gray & Miskiewicz 2000). The pelagic larval duration (PLD) of tiger flathead is unknown, however other *platycephalid* species have PLDs ranging from 20 days to 2 months (Hamer et al. 2010, Pecl et al. 2011, Hirst et al. 2014).

Research gaps

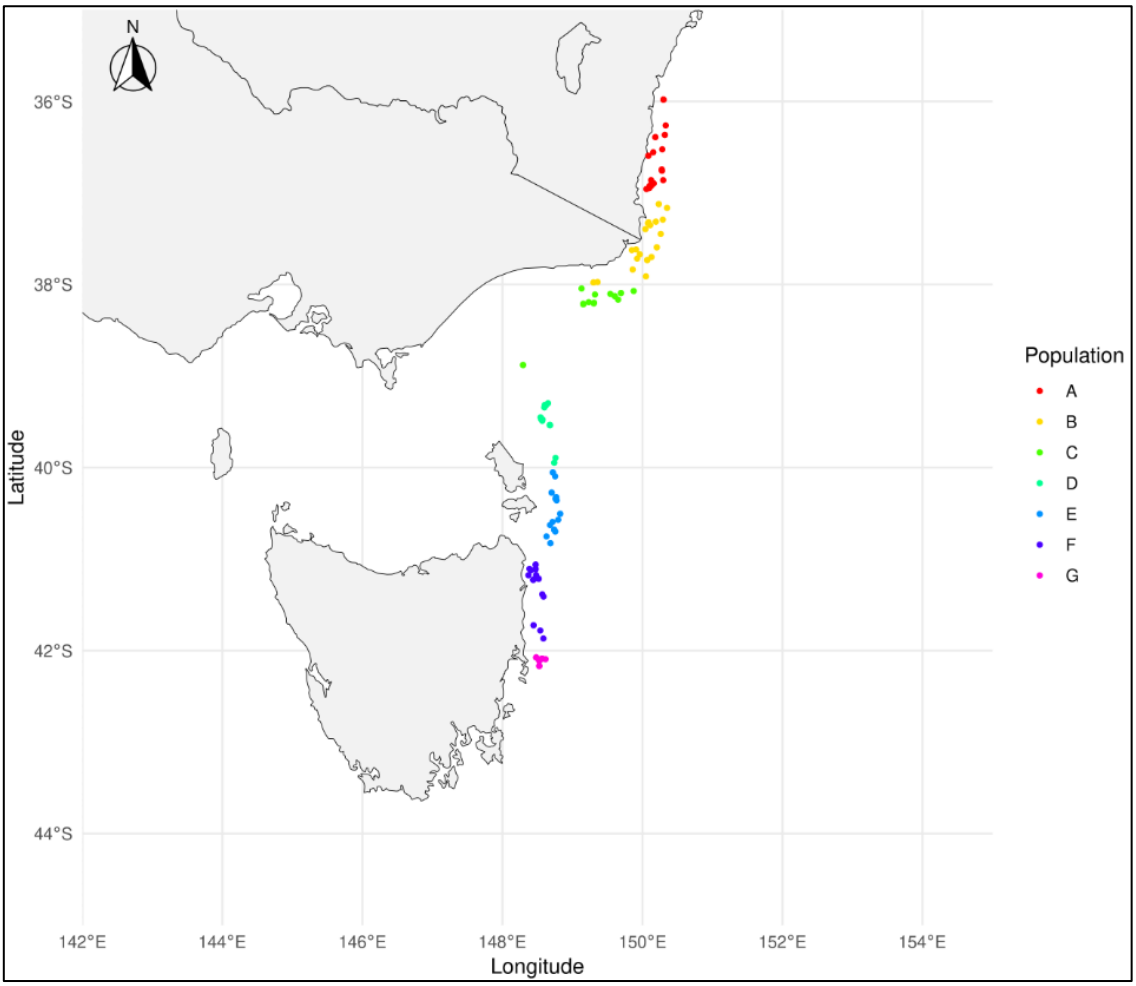
Despite the commercial importance of tiger flathead, much about their ecology and life history remain poorly understood. A study undertaken by Evans et al. 2022 found that many of the parameters used in SESSF stock assessments, including for tiger flathead, are either out of date and / or the reference source of parameters and values used in assessments are unknown. Of the 12 species assessed in the Evans et al. (2022) study, 8 had provenance issues. Among this is tiger flathead, with key parameters used in stock assessments such as maturity and length-weight relationships found to be either older than 10 years, or the biological origin of the parameters and values is unclear or unreliable. Additionally, stock assessments for tiger flathead assume panmictic population structure, despite no previous studies undertaken to investigate this. There has been speculation around the existence of multiple populations of tiger flathead, with observed morphological differences in growth rates, appearance and differences in spawning seasons throughout the geographic range of the species (Kailola et al. 1993). This has been observed in New South Wales with spawning seasons occurring between October and May, whereas individuals off eastern Tasmania and the Bass Straight spawn between December to February (Kailola et al. 1993).

This study represents the first genomic investigation of stock structure for tiger flathead. It looks at the intra-specific genomic connectivity of tiger flathead latitudinally across the SESSF and using this knowledge gained, aims to provide up-to-date data to inform current management strategies, supporting the ongoing sustainability of the species. To test for population structuring for tiger flathead within the SESSF, a null hypothesis of no genetic differentiation across sampling locations (H0) was proposed.

Materials and Methods

Sampling design and collection

Tissue samples of *P. richardsoni* ($n = 188$ individuals) were collected along the southeastern Australia coast between Hobart, Tasmania and Merimbula, New South Wales (Figure 1) in 2023 and 2024. Sampling occurred on the Research Vessel *Investigator,* during three of the CSIRO Southeast Australian Marine Ecosystem Survey (SEA-MES) voyages (Table 1). Individual fish were collected across three voyages to account for temporal and seasonal variability. Individuals were obtained utilising McKenna Trawls and euthanised with AQUI-S. Where possible, sex, weight and length data were also obtained. Muscle samples were collected by dissecting a portion of muscle from above the pectoral fin and storing it at -20°C before returning to Hobart for subsequent sampling and analysis.



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831 **Figure 1:** Sampling location of tiger flathead (*Platycephalus richardsoni*) split by *a priori*
832 populations. A, samples from 35°S-36°S; B, samples from 37°S; C, samples from 38°S; D, samples
833 from 39°S; E, samples from 40°S; F, samples from 41°S; G, samples from 42°S.

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Table 1: Summary of sampling information per voyage, including voyage dates, number of samples per voyage, the minimum length (mm), maximum length (mm) and sex.

Voyage	Voyage start date	Voyage end date	Number of samples	Minimum length (mm)	Maximum length (mm)	Sex
1	1/07/2023	28/07/2023	65	144	582	12 F 3 M 50 unknown
2	05/05/2024	29/05/2024	63	183	577	14 F 8 M 41 unknown
3	14/11/2024	11/12/2024	60	288	595	30 F 30 M

DNA extraction and sequencing

As per Diversity Array Technology's sample preparation guidelines, 10-15mg of muscle tissue was subsampled and submerged in 80% ethanol in Qiagen Collection Micro Tubes (Hilden, Germany) with the associated caps. The scalpel, scissors, tweezers and aluminium foil used on the chopping board were cleaned between each individual sample using 80% ethanol, bleach, water and Kimtech Low Lint Wipers (Georgia, United States of America) to prevent contamination of samples. Samples were posted frozen to Diversity Arrays Technology (Canberra, Australia) for DNA extraction and sequencing. Sequencing was completed using the DArTseq (1.0) - DArTseq Medium density sequencing (1.2 mln reads). This uses the Diversity Arrays Technology proprietary complexity reduction-based sequencing technology. It involves restriction-enzyme digestions, adapter ligation followed by amplification of adapter ligated fragments, targeting the low copy, informative regions of the genome (Kilian et al. 2012; Diversity Arrays Technology n.d.).

Quality control and data filtering

Prior to filtering and analysis, individuals were grouped into arbitrary populations based on their sampling latitude (i.e. 1 degree latitude, split evenly across the region) (Figure 1). As samples were collected at relatively even intervals across the voyage route, this approach was used to explore potential spatial patterns in genetic structure across this geographic extent. This resulted in 7 *a priori* populations ranging from 35°S to 42°S with 26-31 individuals within each population (Table 2).

856 **Table 2:** *A priori* populations, sampling latitudes and number of samples per population.

Population	Latitude	N
A	35°S-36°S	31
B	37°S	26
C	38°S	26
D	39°S	26
E	40°S	27
F	41°S	26
G	42°S	26
Total		**188**

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858 Of the 188 tissues subsampled, the SNP extraction yielded 158,628 SNP markers for 187 individuals.
859 Individual '10080594' was not included due to low-quality DNA and did not pass Diversity Arrays
860 Technology's quality control protocols (Appendix A). Quantitative analyses were undertaken using
861 the statistical package *R* (ver. 4.4.0) The SNP data and metadata produced by DArT Pty Ltd was
862 converted into a genlight object for further filtering using the package *dartR* (ver. 2.9.9.5) (Gruber et
863 al. 2018). Before filtering the dataset, an initial smearplot of the first 20 individuals and 1000 loci
864 was generated using the R package *dartR* (Gruber et al. 2018). This was developed to visualise a

portion of the data and identify any significant missing values, obvious outliers, or patterns (Figure 2).

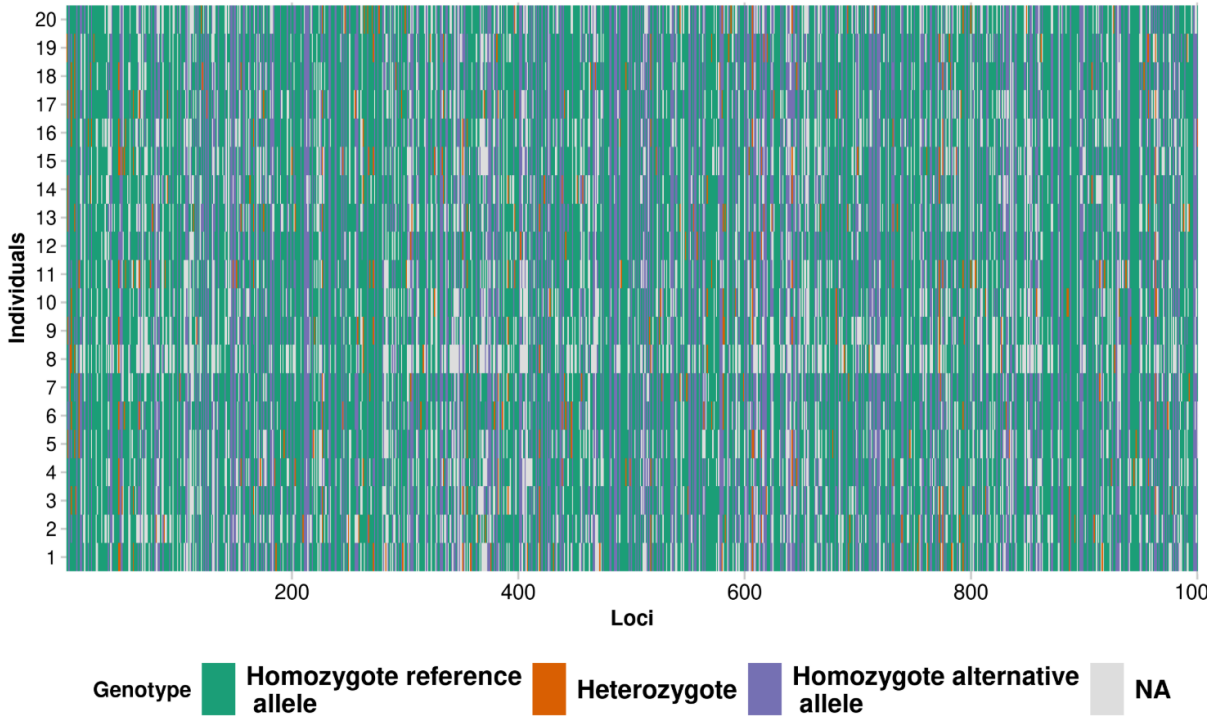


Figure 2: Genotype heatmap across 20 individuals and 1000 SNP loci. Each column represents an individual SNP, while each row represents an individual. Homozygote reference alleles are in green, heterozygotes are in orange, homozygote alternative allele is in purple, and missing values (NA) are in grey.

Various filtering steps were applied to the dataset to improve reliability and quality of the data. A 0.98 reproducibility threshold was applied to the dataset, filtering out 12,118 SNPs (Appendix B). The data was checked for any invariable sites (SNPs that had missing data for all samples), however, no additional loci were removed from the dataset. A Minor Allele Frequency (MAF) filter was applied, with a threshold of 0.02, removing 80,375 loci (Figure 5). The dataset was filtered by read depth, with a lower threshold of 5 and an upper threshold of 100, removing 7,790 loci (Appendix C). The call rate per SNP and individual threshold were set to 0.98 and 0.94 respectively, filtering out 47,271 SNPs and 3 individuals (Appendix D).

The application of these stringent filtering steps ensured SNPs and individuals with low call rates are filtered out, enhancing the quality and reducing bias from missing data. Individuals with unusually low or high heterozygosity were filtered out. The heterozygosity filter was applied with a lower and upper threshold of 0.1 and 0.2, filtering out 2 individuals (Appendix E). SNPs with short distance linkages were also removed to reduce the effect of linkage disequilibrium in the analysis (Appendix F). This removed a total of 1,408 SNPs, creating a final, high-quality dataset of 9,666 SNPs across

182 individuals (Table 3). The data was also quality controlled by assessing the SNP allele frequencies against Hardy-Weinberg Equilibrium to determine if any SNPs significantly deviated from equilibrium. Initially, 3,103 SNPs were identified as outliers from the Hardy-Weinberg Equilibrium, however, p-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure to control for the false discovery rate (Benjamini & Hochberg 1995) (Appendix G). After correction for multiple comparisons, no SNPs significantly deviated from Hardy-Weinberg equilibrium, therefore, no further SNPs were removed from the dataset.

The filtered dataset was checked for any remaining monomorphic loci, which indicated no monomorphic loci needed to be removed. The function *gl.outflank* from the *dartR* package was used to identify and filter for F_{ST}-based outliers (Gruber et al. 2018). No outliers were present in the data. A final smearplot was generated to inspect the data, identify any key areas that may not have been filtered out during the filtering process and identify if any patterns have emerged (Figure 3).

Table 3: Summary of sequential filtering and quality control steps, showing the number of SNPs and individuals removed at each stage.

Filtering step	**SNPs remaining**	**Individuals remaining**
Initial dataset	158,628	187
Reproducibility > 0.98	146,510	187
Minor Allele Frequency < 0.02 removed	66,135	187
Read depth between 5 and 100	58,345	187
Call rate per SNP > 0.98	11,074	187
Call rate per individual > 0.94	11,074	184
Heterozygosity per individual between 0.1 and 0.2	11,074	182
Remove short distance linked SNPs	9,666	182
Final dataset: total polymorphic loci retained	**9,666**	**182**

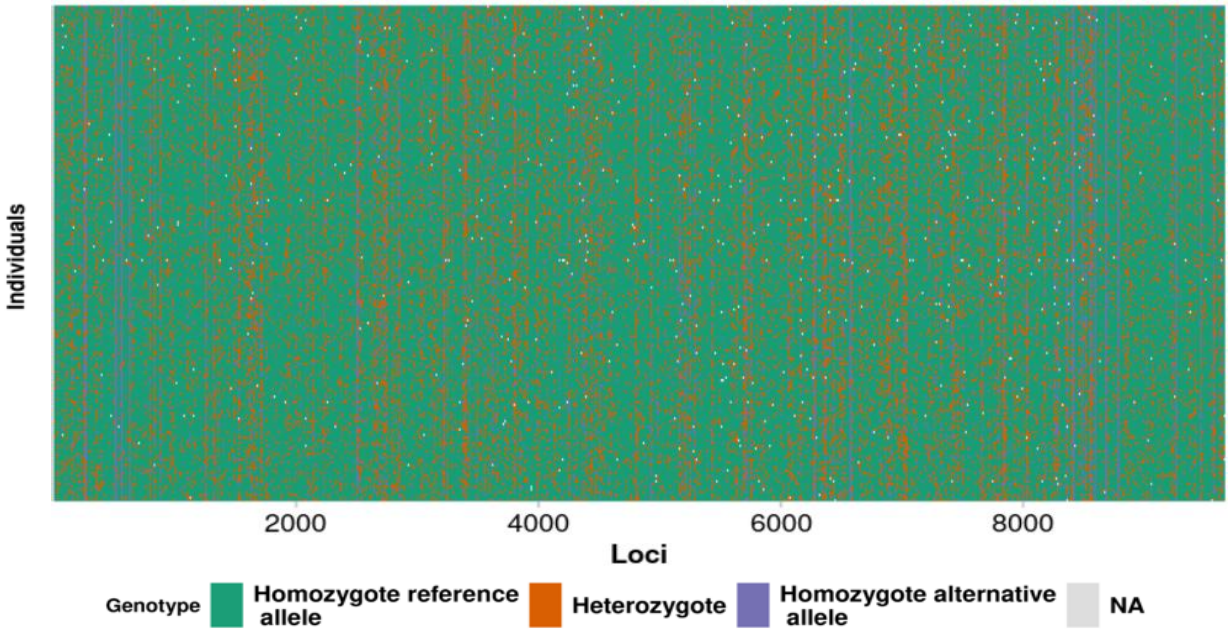


Figure 3: Genotype heatmap across the entire filtered dataset. Each column represents an individual SNP, while each row represents an individual. Homozygote reference alleles are in green, heterozygotes are in orange, homozygote alternative allele is in purple, and missing values (NA) are in grey.

Genetic diversity

The *dartR* package was used to calculate observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), Jost's D and Hedricks G_{ST} (Gruber et al. 2018) (Hedrick 2005; Jost 2008). Genetic differentiation between populations was calculated through fixation indices (F_{ST}), using the R package *dartR* and bootstrapping 100 times (Weir & Cockerham 1984; Gruber et al. 2018). P-values were adjusted using the Benjamini-Hochberg procedure to control for the false discovery rate (Benjamini & Hochberg 1995).

Population structure

Population structure was visualised through a principle component analysis (PCA), discriminant analysis of principle components (DAPC) and a STRUCTURE plot. The PCA was undertaken using the *dartR* package, to detect any genetic similarities and visualise any genetic differentiation (Gruber et al. 2018). A DAPC was executed using the *adegenet* package (ver. 2.1.11) to further visualise any genetic differentiation between pre-defined populations (Jombart 2008). Finally, a STRUCTURE plot was performed using the *LEA* (ver. 3.18.0) package to understand the admixture proportions of individuals across an optimal number of clusters (Frichot & François 2015). An optimal number of ancestral populations (K) was determined using the cross-entropy criterion. Individual admixture coefficients were estimated at K = 1 to K = 8 putative populations with 5 replicates for each value of

K. The optimal K value was determined when the cross-entropy initially decreased (Frichot et al. 2014, Frichot & François 2015). An Analysis of Molecular Variance (AMOVA) was performed using the R package *hierfstat* (Goudet 2005). An AMOVA was performed to further validate the genetic diversity parameters of the study, such as F_{ST} and F_{IS}.

Results

Genetic diversity

All populations had low allelic diversity with mean minor allele frequencies (MAF) near 0 (range 0.112 – 0.121) with an overall mean MAF of 0.056 (Figure 4). Expected heterozygosity was consistent across all locations (H_E = 0.174-0.176, Table 4), with observed heterozygosity slightly lower than expected heterozygosity (H_O = 0.156-0.160, Table 4). The inbreeding coefficients (F_{IS}) were similar across all locations (F_{IS} = 0.070-0.094, Table 4).

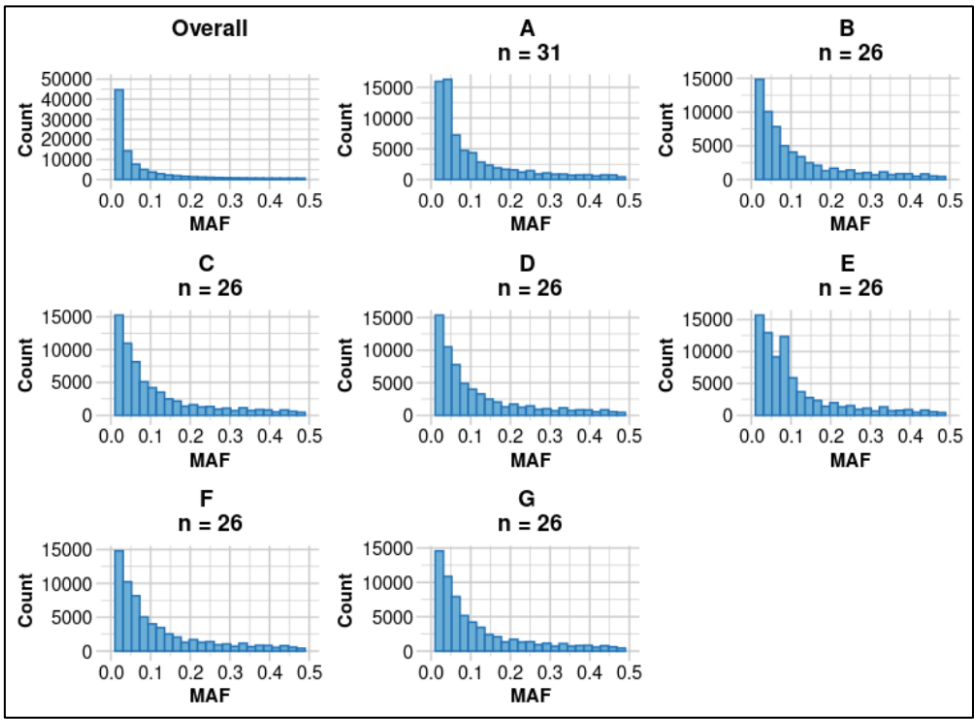


Figure 4: Minor allele frequencies (MAF) for each a priori population of tiger flathead (*Platycephalus richardsoni*) prior to filtering, along with the overall MAF (top left). MAFs >0.02 were removed to improve data quality and enhance statistical power.

Table 4: Population parameters for tiger flathead (*Platycephalus richardsoni*). *Na,* Number of polymorphic SNPs screened; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient.

Population	n	Na	Ho	He	Fis

A	31	9018	0.157	0.175	0.085
B	26	8643	0.156	0.176	0.094
C	26	8657	0.159	0.175	0.074
D	25	8697	0.156	0.174	0.085
E	26	8612	0.157	0.175	0.079
F	24	8707	0.160	0.176	0.074
G	25	8744	0.160	0.176	0.070

Minimal genetic differentiation was detected across all locations, indicated by the overall F_{ST} value (F_{ST} = 0.0002, Table 5) and pairwise F_{ST} values (0.000 – 0.001, Table 4). As per Meirmans & Hedrick 2011, F_{ST} values can be negative, which have been interpreted as 0 for this study. This observation was further supported by an AMOVA, showing an F_{ST} of 0.0003 and an F_{IS} value of 0.0948, with 90.49% of genetic differentiation coming from among individuals, rather than populations, indicating no significant rejection of the null hypothesis (Table 7). Panmixia is further supported by Jost's D and Hedrick's G_{ST} (0.0001 and 0.0003, respectively, Table 5). As the overall and pairwise comparisons indicated no significant genomic differentiation, further testing based on allele frequencies for male and females in each population was not undertaken. When comparing SNP allele frequency differences amongst the arbitrary populations, significant genetic differentiation (F_{ST} = 0.0012) was detected between locations F and B (following Benjamini-Hochberg correction) (P<0.001, Table 6), suggesting evidence to support minimal genetic structuring between the two locations.

Table 5: Overall population parameters and diversity indices. H_O, observed heterozygosity; H_E, expected heterozygosity; F_{ST}, fixation indices; F_{IS}, inbreeding coefficient.

H_O	H_E	F_{IS}	F_{ST}	Jost's D	Hedrick's G_{ST}
0.156	0.172	0.095	0.0002	0.0001	0.0003

Table 6: Pairwise genetic differentiation (F_{ST}) values (below the diagonal) for tiger flathead (*Platycephalus richardsoni*) samples, grouped according to arbitrary populations. *P*-values above the diagonal, significant *P*-values (following Benjamini-Hochberg correction) are shown in bold.

	A	**B**	**C**	**D**	**E**	**F**	**G**
A		0.850	0.740	0.740	0.850	0.630	0.870

B	0.000		0.630	0.630	0.850	**0.000**	0.740
C	0.000	0.000		0.650	0.650	0.420	0.920
D	0.000	0.000	0.000		0.650	0.630	0.850
E	0.000	0.000	0.000	0.000		0.210	0.740
F	0.000	0.001	0.001	0.000	0.001		0.630
G	0.000	0.000	0.000	0.000	0.000	0.000	

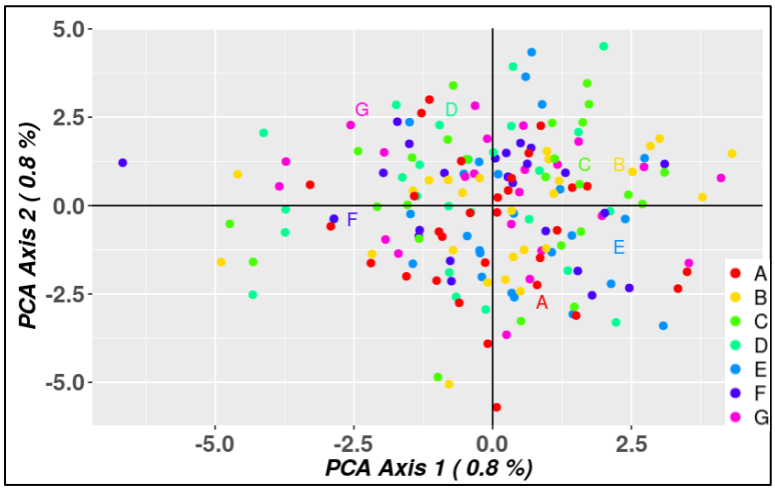
Table 7: Analysis of Molecular Variance (AMOVA) indicating genetic variance among populations, among individuals within populations and within individuals.

Source of Variation	**Sum of squares**	**Variance components**	**% of Variation**
Among populations	0.4320	0.0003(F_{ST})	0.03%
Among individuals within populations	157.2347	0.0948 (F_{IS})	9.48%
Within individuals	1504.6638	-	90.49%
Total	**1662.3310**	**0.0951**	**100.00%**

Population structuring

Population structuring among the seven tiger flathead arbitrary groupings was not evident from a PCA, with no clustering of individuals or populations (Figure 5). In the PCA Axis 1, 2 and 3 accounted for 0.8% of genetic variation, indicating weak structuring. This was further supported with a DAPC, showing significant overlap between locations and individuals in each population were not spatially structured, thereby reinforcing the acceptance of the null hypothesis and assumed panmixia (Figure 6). The optimal number of ancestral populations (K) based on the cross-entropy criterion was two (K = 2). However, the STRUCTURE plot indicated no population structuring between locations as despite the optimal number of ancestral populations being 2, all individuals were assigned with a high probability to a single ancestral population (Figure 7). All analyses support the acceptance of the null hypothesis of no genomic differentiation among tiger flathead samples in the SESSF during 2023 to 2024.

(a)



(b)

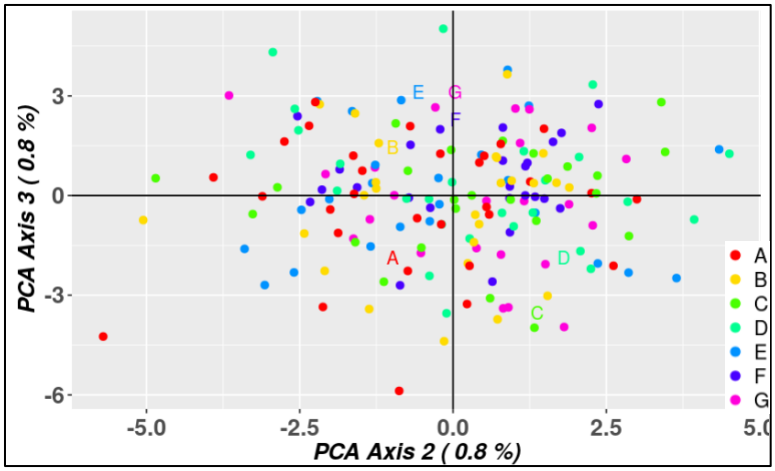


Figure 5: Principle Component Analysis (PCA) of tiger flathead (*Platycephalus richardsoni*)) using neutral loci across axis 1 and 2 (a) and axis 2 and 3 (b). The variance explained by each axis is found in parentheses next to the axis label.

(a)

(b)

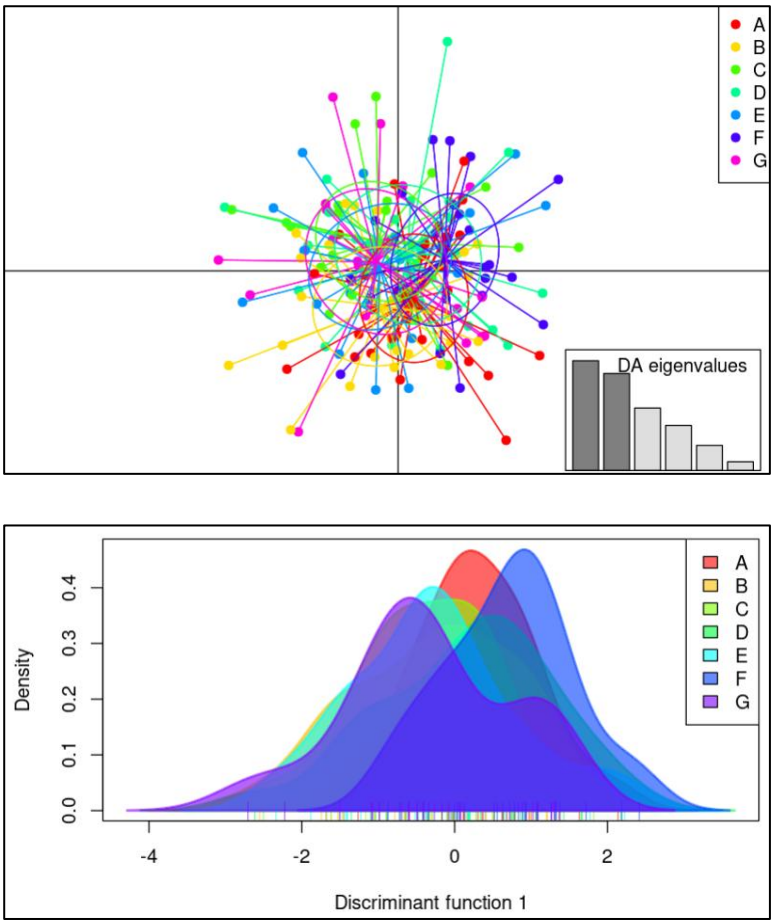
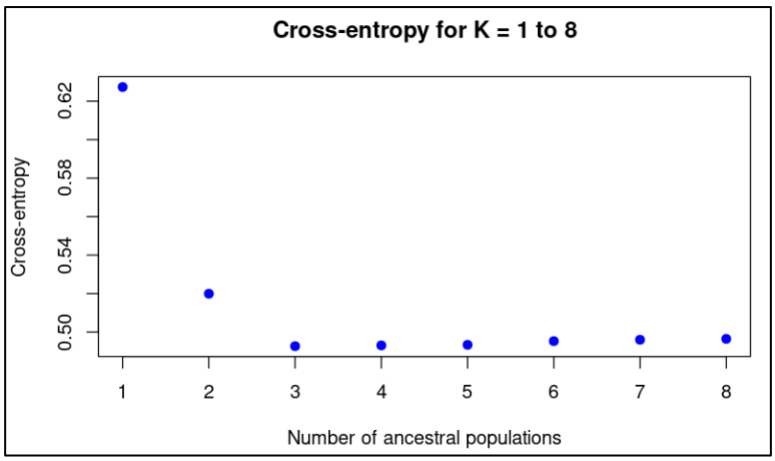


Figure 6: (a) Discriminant Analysis of Principle Components (DAPC) performed with neutral SNP genotypes of tiger flathead (*Platycephalus richardsoni*). Clustering was performed using Bayesian Information, with populations (n = 7) specified as priors. Insets show eigenvalues for the first 6 axes (b) Density distributions of locations along the first discriminant function.

(a)



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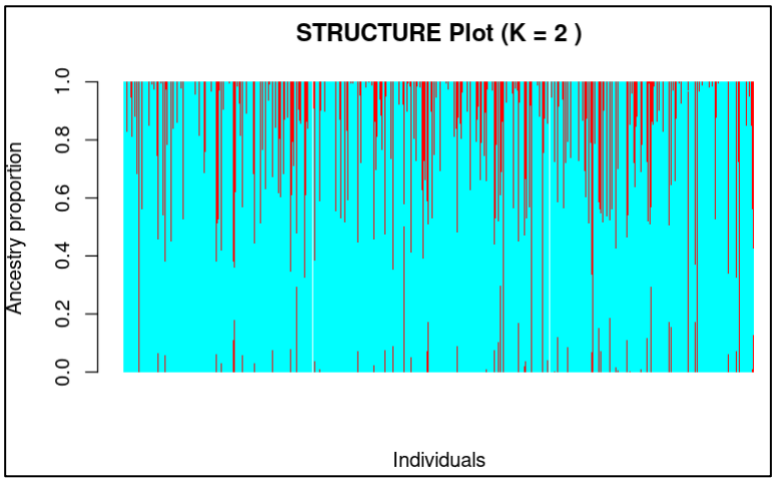

Figure 7: (a) Values of the cross-entropy criterion to determine the optimum number of ancestral clusters (b) Ancestry populations and clustering for tiger flathead (*Platycephalus richardsoni*) based on STRUCTURE outputs for SNPs. Colours represent different ancestry populations based on K values. Each vertical bar represents and individual.

Discussion

Here we present the first comprehensive genomic population analysis of tiger flathead. Results indicate that tiger flathead in southeast Australia consists of a single, panmictic population, supporting the acceptance of the null hypothesis. Based on next-generation sequencing of SNPs, these results contribute to the previously limited knowledge on population structuring for the species and these findings can be used to support the management of tiger flathead, supporting long term sustainability of the species.

Genetic diversity and population structure

Results indicate no evidence of genetic structuring for tiger flathead along the southeast coast of Australia. The F_{ST} values (mean = 0.0002) were low, indicating high gene flow between locations and weak structuring. This is further supported by the Jost's D and Hedrick's G_{ST} values (0.0001 and 0.0003). With values close to zero, this indicates that populations are genetically similar with close to no genetic variation among populations and similar allele frequencies (Hedrick 2005; Jost 2008). Furthermore, the AMOVA shows that over 90% of genetic variation occurs within individuals rather than being at a population level, supporting panmixia. A similar study was conducted by van Herwerden et al 2009 on Red Emperor (*Lutjanus sebae)* across the west and east coast of Australia. Results were comparable, showing a panmictic population, with an overall F_{ST} of -0.006 and majority of genetic variation occurring within individuals rather than populations. Tiger flathead display modest genetic diversity (H_O = 0.156*)*, which is consistent across all arbitrary populations (H_O = 0.156 - *0.160).* Although this is on the lower scale of genetic diversity (0-1), this is consistent with other marine teleosts and there is no sufficient evidence to suggest this is of concern. For example, snapper (*Chrysophrys auratus*) from southeastern Australia displays similar genetic diversity, with observed heterozygosity (H_O) ranging from 0.181 to 0.203 (Bertram et al. 2023).

Although there was one significant pairwise difference between locations B and F (F_{ST} = 0.001, P-value < 0.01), the overall F_{ST} value (0.002) coupled with the other population homogeneity parameters suggest no genetic differentiation. This is important to consider in population genomics as Waples (1998) notes that 'not all statistically significant test results indicate biologically important differences.' If management decisions are based exclusively on statistically significant differences without considering the biological factors, this can result in the loss of economic, social and cultural benefits associated with the harvest and consumption of a stock. Additionally, if we restrict human activities (e.g. harvesting) without considering biological validity, it can increase the difficulty in achieving sustainable resource management in the future (Waples 1998). For example, studies using discrete genetic data assume that sampling is multinominal and a violation of this assumption can result in a significant test result despite there being no differences in populations. When conducting genetic studies such as this, sampling protocols almost always violate this assumption (Waples 1998). Therefore, despite the significant statistical pairwise difference between locations B and F; biologically, it can be assumed that there is minimal to no genetic differentiation between the locations.

These findings are further supported by the STRUCTURE plot, with almost all individuals primarily belonging to one cluster (cyan). Despite the optimal number of clusters from the cross-entropy being

K = 2; looking at these results against the PCA, DAPC and F_{ST} values, it can be concluded that there is minimal to no population structuring for tiger flathead. This is a common limitation of the delta K method for identifying optimal cluster numbers in a STRUCTURE analysis. This method can often result in the over or underestimation of population structure. For example, delta K does not allow the optimal number of clusters to be K = 1, therefore, conducting STRUCTURE analysis alongside other methods such as PCA and DAPC is key in ensuring findings are not misinterpreted (Janes et al. 2017).

These genetic findings suggest that population connectivity is being maintained despite adult tiger flathead being relatively sedentary (Australian Fisheries Management Authority n.d.) This potential for population connectivity aligns with the hypotheses that eggs and larvae are well distributed by the currents and counter currents of the east Australian coast (Fairbridge 1951). Despite limited knowledge on the pelagic larval duration of tiger flathead; other *platycephalid* species such as the dusky flathead (*P. fuscus*) and sand flathead (*P. bassensis*) have pelagic larval durations that range from around 20 days to 2 months (Hamer et al. 2010, Hirst et al. 2014, Pecl et al. 2011). This suggests that the pelagic larval duration of tiger flathead is long enough to support passive movement and mixing along the southeast coast of Australia.

This pattern of dispersal has been widely observed and is the primary dispersal mechanism supporting connectivity of marine populations (Booth et al. 2007; van Herwerden et al 2009). One of the main oceanographic drivers of gene flow for tiger flathead would be the East Australian Current (EAC). The EAC is a western boundary current, running along the east coast of Australia from ~ 22° S to 43°S latitude, reaching velocities up to ~90 cm/s (Ridgway & Dunn 2003). The EAC has powerful dispersal mechanisms for marine fish, transporting tropical larval fish down into temperate waters (Booth et al. 2007). The EAC strengthens in the austral summer (December to February), bringing high eddy kinetic energy and southward extension which in turn, promotes the transportation and connectivity of fish populations (Xu et al. 2022). The strengthening of the EAC over the austral summer also coincides with tiger flathead spawning season, thus promoting gene flow between locations. Other *platycephalid* species also experience larval dispersal and connectivity through ocean currents. For example, a newly recognised *platycephalus* species in China has population connectivity driven by the Taiwan Warm Current and Yellow Sea Warm Current as well as winter monsoon winds. Conversely, a genetically distinct population of the same species in Tokyo Bay is more genetically isolated due to barriers created by the Japanese landmass and Kuroshio and Tsushima Warm Current (Cheng et al. 2019).

Phenotypic plasticity and environmental factors

Given tiger flathead form a single, panmictic population, the observed regional differences, such as differences in growth rates, appearance and spawning seasons, suggest that this is the result of phenotypic plasticity, or epigenetic differences rather than having any underlying population structuring (West-Eberhard 2005). Tiger flathead may adapt to their local environmental conditions such as habitat, temperature and food availability, resulting in varying morphological differences. This has been observed in other marine species, for example, the european anchovy (*Engraulis encrasicolus*) comprises of a single panmictic population in the north-western Mediterranean. However, fish from various locations within this area exhibit morphological differences driven by environmental factors (Tudela 1999). Investigating the epigenetic differences of tiger flathead would provide further understanding behind these observed morphological differences and may provide insight in the species' adaptive capability and resilience. This can be particularly relevant to better understand how the species may adapt to climate change (Mattoo et al. 2025). It is important to note that this type of analysis requires a deeper understanding of the whole genome, however, to support this, a reference genome for the genus *Platycephalus* has recently been sequenced and is currently undergoing assembly (Green, M, personal communication, September 11, 2025).

Differences in size and age of fish observed off Tasmania may also be due to differences in fishing pressure. Through the use of different gear types and more intensive harvesting, fisheries pressure can impact populations by disproportionately removing larger fish from populations. This can result in an evolutionary response, with more heavily fished individuals maturing earlier and at a smaller size (Mishra 2025). Therefore, this would mean that fish off Tasmania are more lightly fished than those off Victoria and New South Wales. Stock assessments for tiger flathead currently account for differences in length compositions in Tasmania through the use of an areas-as-fleets approach (Bessell-Browne 2022). This involves treating different spatial areas as 'fleets' to account varying fishing intensities across a single stock, rather than conducting separate stock assessments (Cope & Punt 2011).

Limitations

It is important to note that due to time and sampling constraints, this study only focusses on eastern individuals across the distribution of the species. Future research should incorporate samples from western Tasmania and the western Bass Strait to strengthen the understanding of tiger flathead population structure across the entire range. Additionally, only five samples were able to be collected from 35° S, therefore these were incorporated into population alongside samples from 36° S. More

samples from 35° S and further north should be collected and incorporated into the analysis in future studies.

Cross-contamination between samples is a risk when conducting bottom trawling and processing. Although all efforts to avoid cross contamination were implemented, this remains a limitation to conducting studies on this scale. Furthermore, multiple technicians conducted sampling of muscle tissue. A standard protocol was used for subsampling; however, multiple handlers can introduce uncertainty and risk of sampling error. Sex specific data was only obtained for a small portion of samples. Given acceptance of the null hypothesis, further sex specific and temporal analyses were not conducted, however this may limit further analysis should it be undertaken in the future.

Although SNPs are a powerful tool for population genomic studies, they also come with a range of limitations which are important to understand. To generate comprehensive SNP datasets, they require large portions of high-quality DNA. This was able to be obtained in this study as tiger flathead are not a species of concern, allowing invasive techniques to be used to obtain DNA. However, this can be difficult to obtain for other species such as those that are protected due conservation concern (Zimmerman et al. 2020). SNPs are only biallelic, which provide less information than polymorphic markers such as microsatellites. However, SNPs are much more abundant and accessible than their microsatellites counterparts, making them a preferred alternative for many studies (Xiong & Jin 1999). Finally, ascertainment bias is a limitation to using SNPs in population genomic studies. This is the result of non-random sampling of individuals and / or biased SNP discovery methods which can skew results (Lachance & Tishkoff 2013). Ascertainment bias can only be removed if the whole genome is sequenced for every individual within a population, which was not able to be undertaken in this study. However, ascertainment bias is less of a concern in population genomics studies as it still allows sufficient detection of individual clustering (Morin et al. 2004).

Management implications and future research

This study is a positive step towards understanding key knowledge gaps for the sustainable management of tiger flathead, an important commercial species within Australia. The findings are congruent with current management strategies, supporting the assumption used in stock assessments that tiger flathead form a single panmictic population (Bessell-Browne 2022). Further research should focus on updating outdated biological parameters such as maturity and weight-length relationship to increase the robustness and reliability of stock assessments for the species. Epigenetic studies on tiger flathead adaptability and resilience, particularly in a warming climate may also be useful in determining how climate change may impact abundance and distribution of tiger flathead. This will

provide insight into the morphological differences observed across the geographic range and the drivers behind those differences.

Ensuring up to date biological data for wild catch fisheries is essential to ensure sustainable fisheries management. Although population structure for tiger flathead is now better understood, ongoing monitoring and population genomic studies still need to be undertaken in the future to ensure we are capturing any genetic structuring that may arise. Should the status of the fishery change, this may need to be conducted on a more regular basis to understand impacts on the genetic diversity and structural changes of the species.

The datasets and raw data analysed in the study are available in the CSIRO and University of Tasmania SharePoint.

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Appendix

Appendix A: Metadata for 188 tissue samples of tiger flathead (*Platycephalus richardsoni)*.

Voyage	Sample ID	Sampling Date	Latitude	Longitude	Length (mm)	Weight (g)	Sex
IN2024_V05	10098937	14/11/2024	-42.16909	148.525897	370	356	M
IN2024_V05	10098877	14/11/2024	-42.16909	148.525897	418	516	M
IN2024_V05	10098882	14/11/2024	-42.16909	148.525897	411	486	F
IN2024_V05	10098906	14/11/2024	-42.16909	148.525897	442	651	M
IN2024_V05	10098903	14/11/2024	-42.16909	148.525897	472	768	M
IN2023_V05	10084740	28/07/2023	-42.11222	148.526193	411	532	NA
IN2023_V05	10084761	28/07/2023	-42.11222	148.526193	449	650	NA
IN2023_V05	10084770	28/07/2023	-42.11222	148.526193	435	570	NA
IN2023_V05	10084808	28/07/2023	-42.11222	148.526193	379	424	NA
IN2023_V05	10084810	28/07/2023	-42.11222	148.526193	477	720	NA
IN2023_V05	10085824	28/07/2023	-42.11222	148.526193	461	636	NA
IN2023_V05	10085923	28/07/2023	-42.094049	148.615655	414	566	NA
IN2023_V05	10084804	28/07/2023	-42.087384	148.565009	408	483	NA
IN2023_V05	10084837	28/07/2023	-42.087384	148.565009	525	1106	NA
IN2023_V05	10084841	28/07/2023	-42.087384	148.565009	382	378	NA
IN2023_V05	10084881	28/07/2023	-42.087384	148.565009	366	367	NA
IN2023_V05	10084916	28/07/2023	-42.087384	148.565009	367	377	NA
IN2023_V05	10084938	28/07/2023	-42.087384	148.565009	340	297	NA
IN2023_V05	10084940	28/07/2023	-42.087384	148.565009	434	670	NA
IN2023_V05	10084968	28/07/2023	-42.087384	148.565009	502	1015	NA
IN2023_V05	10084970	28/07/2023	-42.087384	148.565009	440	587	NA
IN2024_V05	10099047	14/11/2024	-42.075914	148.482196	362	360	F
IN2024_V05	10098989	14/11/2024	-42.075914	148.482196	317	247	F
IN2024_V05	10099049	14/11/2024	-42.075914	148.482196	274	153	F
IN2024_V05	10098986	14/11/2024	-42.075914	148.482196	396	513	F
IN2024_V05	10099035	14/11/2024	-42.075914	148.482196	301	210	M
IN2024_V05	10099315	15/11/2024	-41.86854	148.584459	391	433	M
IN2024_V05	10098850	15/11/2024	-41.781094	148.540289	495	930	M
IN2024_V05	10109862	11/12/2024	-41.723795	148.444162	353	336	M
IN2024_V05	10109811	11/12/2024	-41.723795	148.444162	310	242	F
IN2024_V05	10106769	8/12/2024	-41.410511	148.586604	300	188	M
IN2024_V05	10106875	8/12/2024	-41.385663	148.568725	427	516	F
IN2024_V03	10086172	5/05/2024	-41.227653	148.439928	183	37	NA
IN2024_V03	10086368	5/05/2024	-41.227653	148.439928	238	83	NA
IN2024_V03	10089455	5/05/2024	-41.227653	148.439928	228	79	NA

IN2024_V03	10089482	5/05/2024	-41.227653	148.439928	240	93	NA
IN2024_V03	10089495	5/05/2024	-41.227653	148.439928	264	136	NA
IN2024_V03	10089543	5/05/2024	-41.227653	148.439928	230	76	NA
IN2024_V03	10089569	5/05/2024	-41.227653	148.439928	403	441	NA
IN2024_V03	10089442	5/05/2024	-41.215342	148.517978	346	322	NA
IN2024_V03	10089475	5/05/2024	-41.215342	148.517978	285	172	NA
IN2024_V05	10098800	16/11/2024	-41.176812	148.371173	274	140	M
IN2024_V03	10089843	5/05/2024	-41.175434	148.481131	233	78	NA
IN2024_V05	10099171	16/11/2024	-41.124056	148.405518	286	152	M
IN2023_V05	10085056	24/07/2023	-41.110295	148.473097	373	403	NA
IN2023_V05	10085132	24/07/2023	-41.110295	148.473097	393	736	NA
IN2023_V05	10085135	24/07/2023	-41.110295	148.473097	330	327	NA
IN2023_V05	10085161	24/07/2023	-41.110295	148.473097	430	726	NA
IN2023_V05	10085197	24/07/2023	-41.110295	148.473097	297	209	NA
IN2024_V05	10101568	16/11/2024	-41.107141	148.385994	378	393	F
IN2023_V05	10085208	24/07/2023	-41.060924	148.474113	271	152	NA
IN2023_V05	10085213	24/07/2023	-41.060924	148.474113	354	311	NA
IN2024_V05	10106441	9/12/2024	-40.827154	148.685474	329	300	M
IN2024_V05	10105369	9/12/2024	-40.827154	148.685474	294	230	M
IN2024_V05	10108664	9/12/2024	-40.753485	148.628509	284	171	M
IN2024_V05	10109504	10/12/2024	-40.699212	148.75471	403	567	F
IN2024_V05	10109480	10/12/2024	-40.699212	148.75471	475	906	F
IN2024_V03	10086129	6/05/2024	-40.680163	148.739391	286	183	M
IN2024_V03	10086150	6/05/2024	-40.680163	148.739391	275	143	M
IN2024_V03	10086189	6/05/2024	-40.680163	148.739391	405	544	M
IN2024_V03	10089144	6/05/2024	-40.628374	148.679936	440	532	NA
IN2024_V03	10090638	7/05/2024	-40.594909	148.714739	329	211	NA
IN2024_V03	10090377	6/05/2024	-40.57104	148.795779	363	336	F
IN2023_V05	10083704	25/07/2023	-40.505644	148.825067	384	401	NA
IN2023_V05	10085206	25/07/2023	-40.505644	148.825067	453	775	NA
IN2024_V05	10107676	7/12/2024	-40.358497	148.774063	330	317	M
IN2024_V05	10108818	7/12/2024	-40.358497	148.774063	377	454	M
IN2024_V05	10107500	7/12/2024	-40.340575	148.756774	367	392	M
IN2024_V03	10091161	8/05/2024	-40.333214	148.761691	372	432	M
IN2024_V03	10091162	8/05/2024	-40.333214	148.761691	469	750	F
IN2023_V05	10080179	1/07/2023	-40.323211	148.768471	492	945	F
IN2024_V05	10108390	7/12/2024	-40.274876	148.70242	439	739	F
IN2024_V03	10091074	8/05/2024	-40.274316	148.702581	229	80	NA
IN2024_V03	10091075	8/05/2024	-40.274316	148.702581	354	400	M
IN2023_V05	10080352	2/07/2023	-40.096587	148.752493	414	622	F

IN2023_V05	10080520	2/07/2023	-40.053854	148.719825	339	266	M
IN2023_V05	10080617	2/07/2023	-40.053854	148.719825	397	445	M
IN2023_V05	10080622	2/07/2023	-40.053854	148.719825	424	563	F
IN2023_V05	10080594	2/07/2023	-40.044098	148.714092	304	184	M
IN2024_V05	10101682	18/11/2024	-39.948054	148.739887	361	334	F
IN2024_V05	10101869	18/11/2024	-39.948054	148.739887	468	788	F
IN2024_V05	10101300	18/11/2024	-39.894756	148.75802	344	284	M
IN2024_V03	10091179	9/05/2024	-39.537952	148.678491	299	190	NA
IN2024_V03	10091182	9/05/2024	-39.537952	148.678491	408	573	NA
IN2024_V03	10091190	9/05/2024	-39.537952	148.678491	328	295	NA
IN2024_V03	10091192	9/05/2024	-39.537952	148.678491	478	904	NA
IN2024_V05	10101303	19/11/2024	-39.537573	148.677762	398	459	M
IN2023_V05	10080845	3/07/2023	-39.535029	148.676298	368	329	F
IN2023_V05	10080905	3/07/2023	-39.535029	148.676298	379	406	F
IN2024_V03	10091236	9/05/2024	-39.487866	148.568563	467	748	NA
IN2024_V03	10091267	9/05/2024	-39.487866	148.568563	368	351	NA
IN2024_V03	10091364	9/05/2024	-39.487866	148.568563	292	168	NA
IN2024_V05	10099932	19/11/2024	-39.475514	148.572277	311	196	F
IN2024_V05	10100053	19/11/2024	-39.475514	148.572277	328	274	M
IN2023_V05	10081271	3/07/2023	-39.471432	148.553257	222	74	F
IN2023_V05	10081305	3/07/2023	-39.471432	148.553257	267	124	F
IN2023_V05	10081306	3/07/2023	-39.471432	148.553257	248	99	F
IN2024_V03	10091165	9/05/2024	-39.469257	148.552751	397	506	F
IN2024_V05	10102073	19/11/2024	-39.452368	148.544117	228	85	F
IN2024_V05	10101916	19/11/2024	-39.452368	148.544117	346	337	F
IN2023_V05	10081089	4/07/2023	-39.342528	148.599113	387	458	F
IN2023_V05	10081804	4/07/2023	-39.342528	148.599113	428	587	F
IN2023_V05	10081819	4/07/2023	-39.31761	148.624692	415	508	F
IN2024_V05	10108274	6/12/2024	-39.315066	148.605753	350	345	F
IN2023_V05	10081718	4/07/2023	-39.29805	148.65133	537	1184	F
IN2024_V03	10092835	11/05/2024	-38.879973	148.294572	365	376	M
IN2024_V03	10092862	11/05/2024	-38.879973	148.294572	399	522	F
IN2024_V03	10092869	11/05/2024	-38.879973	148.294572	312	264	F
IN2023_V05	10084715	23/07/2023	-38.216301	149.154564	442	631	NA
IN2023_V05	10084900	23/07/2023	-38.216301	149.154564	497	878	NA
IN2023_V05	10084920	23/07/2023	-38.216301	149.154564	449	652	NA
IN2023_V05	10084924	23/07/2023	-38.216301	149.154564	428	573	NA
IN2024_V05	10108601	4/12/2024	-38.211337	149.154044	529	1376	F
IN2024_V05	10108617	4/12/2024	-38.211337	149.154044	580	1583	F
IN2023_V05	10084510	22/07/2023	-38.204931	149.304733	550	1326	NA

IN2023_V05	10084592	22/07/2023	-38.204931	149.304733	580	1620	NA
IN2023_V05	10084596	22/07/2023	-38.204931	149.304733	558	1474	NA
IN2023_V05	10084650	22/07/2023	-38.204931	149.304733	513	1030	NA
IN2023_V05	10084706	22/07/2023	-38.204931	149.304733	582	1782	NA
IN2024_V03	10093223	15/05/2024	-38.199809	149.307747	577	1596	F
IN2024_V03	10093303	15/05/2024	-38.199809	149.307747	567	1570	F
IN2024_V05	10107047	3/12/2024	-38.19259	149.232267	595	1908	F
IN2024_V03	10094863	20/05/2024	-38.165202	149.652505	571	1476	NA
IN2024_V05	10108880	2/12/2024	-38.127051	149.604202	436	714	M
IN2024_V05	10108877	2/12/2024	-38.127051	149.604202	395	536	F
IN2024_V03	10092526	15/05/2024	-38.109721	149.321603	415	499	NA
IN2024_V03	10094841	20/05/2024	-38.102355	149.540298	453	846	NA
IN2024_V03	10094496	20/05/2024	-38.093468	149.693281	418	590	NA
IN2024_V03	10094570	20/05/2024	-38.093468	149.693281	392	512	NA
IN2024_V05	10107298	30/11/2024	-38.071416	149.87468	394	498	M
IN2024_V05	10108498	3/12/2024	-38.044246	149.130094	385	463	F
IN2024_V05	10107057	1/12/2024	-37.978227	149.300338	528	1200	F
IN2024_V05	10106921	1/12/2024	-37.978227	149.300338	378	491	M
IN2024_V05	10106422	1/12/2024	-37.97331	149.358791	455	618	F
IN2023_V05	10083806	17/07/2023	-37.911535	150.049456	501	1013	NA
IN2024_V05	10104676	29/11/2024	-37.836893	149.860365	351	406	F
IN2024_V03	10096102	22/05/2024	-37.733376	150.066029	402	513	M
IN2023_V05	10081867	15/07/2023	-37.733183	150.068486	485	800	NA
IN2023_V05	10083386	15/07/2023	-37.733183	150.068486	461	774	NA
IN2024_V05	10103064	22/11/2024	-37.717444	149.924109	363	364	M
IN2023_V05	10082373	15/07/2023	-37.700167	150.128705	478	878	NA
IN2023_V05	10083645	16/07/2023	-37.670804	149.962068	406	516	NA
IN2024_V05	10102772	22/11/2024	-37.624079	149.848454	328	250	F
IN2023_V05	10083522	16/07/2023	-37.617319	149.908929	229	75	NA
IN2024_V03	10096806	23/05/2024	-37.593918	150.204906	488	873	NA
IN2024_V03	10096900	23/05/2024	-37.593918	150.204906	436	667	NA
IN2024_V03	10093262	19/05/2024	-37.447453	150.261019	389	519	F
IN2024_V05	10102171	23/11/2024	-37.395047	150.04169	317	226	M
IN2024_V05	10101935	23/11/2024	-37.351707	150.110871	364	388	M
IN2023_V05	10083276	8/07/2023	-37.329757	150.077422	144	18	NA
IN2024_V05	10101653	23/11/2024	-37.319282	150.086646	321	248	M
IN2024_V03	10093822	17/05/2024	-37.313615	150.19154	328	232	NA
IN2024_V03	10093935	17/05/2024	-37.313615	150.19154	288	146	NA
IN2024_V03	10093541	17/05/2024	-37.291586	150.290646	426	624	NA
IN2024_V03	10097888	24/05/2024	-37.163332	150.351226	470	820	F

IN2024_V03	10093625	16/05/2024	-37.121335	150.233585	369	386	NA
IN2024_V03	10093850	16/05/2024	-37.121335	150.233585	319	234	NA
IN2024_V03	10096368	25/05/2024	-36.95673	150.056586	308	196	NA
IN2023_V05	10082761	13/07/2023	-36.944279	150.098437	285	172	NA
IN2023_V05	10082805	13/07/2023	-36.944279	150.098437	347	314	NA
IN2023_V05	10082906	13/07/2023	-36.944279	150.098437	278	149	NA
IN2023_V05	10082909	13/07/2023	-36.944279	150.098437	299	192	NA
IN2024_V03	10096433	25/05/2024	-36.92105	150.105046	338	331	F
IN2024_V03	10096469	25/05/2024	-36.92105	150.105046	332	271	F
IN2023_V05	10082646	13/07/2023	-36.917546	150.127732	366	424	NA
IN2023_V05	10082780	13/07/2023	-36.917546	150.127732	316	230	NA
IN2023_V05	10082801	13/07/2023	-36.917546	150.127732	354	348	NA
IN2024_V03	10095171	26/05/2024	-36.895624	150.161149	380	354	NA
IN2024_V03	10095219	26/05/2024	-36.860557	150.295874	542	1200	NA
IN2024_V03	10096750	26/05/2024	-36.860351	150.123467	344	300	NA
IN2024_V05	10104319	27/11/2024	-36.756202	150.278648	355	352	M
IN2024_V05	10104317	27/11/2024	-36.756202	150.278648	471	1128	F
IN2024_V05	10104285	27/11/2024	-36.756202	150.278648	426	692	M
IN2024_V05	10104278	27/11/2024	-36.742185	150.276154	471	1098	F
IN2024_V03	10097909	27/05/2024	-36.593615	150.084005	370	363	F
IN2024_V03	10098029	27/05/2024	-36.593615	150.084005	343	312	F
IN2024_V03	10097810	27/05/2024	-36.555778	150.153926	400	579	M
IN2024_V03	10097573	27/05/2024	-36.52339	150.282853	514	1270	F
IN2024_V05	10106030	26/11/2024	-36.38998	150.184347	335	299	M
IN2024_V05	10105988	26/11/2024	-36.38998	150.184347	334	270	F
IN2023_V05	10082957	10/07/2023	-36.365783	150.31969	471	884	NA
IN2023_V05	10083059	10/07/2023	-36.365783	150.31969	272	168	NA
IN2024_V05	10105081	24/11/2024	-36.262097	150.33187	361	360	F
IN2024_V03	10098224	29/05/2024	-35.980148	150.299991	329	244	NA
IN2024_V03	10098518	29/05/2024	-35.980148	150.299991	386	438	NA
IN2024_V03	10098548	29/05/2024	-35.980148	150.299991	305	222	NA
IN2024_V03	10098679	29/05/2024	-35.980148	150.299991	316	228	NA
IN2024_V03	10098778	29/05/2024	-35.980148	150.299991	390	478	NA

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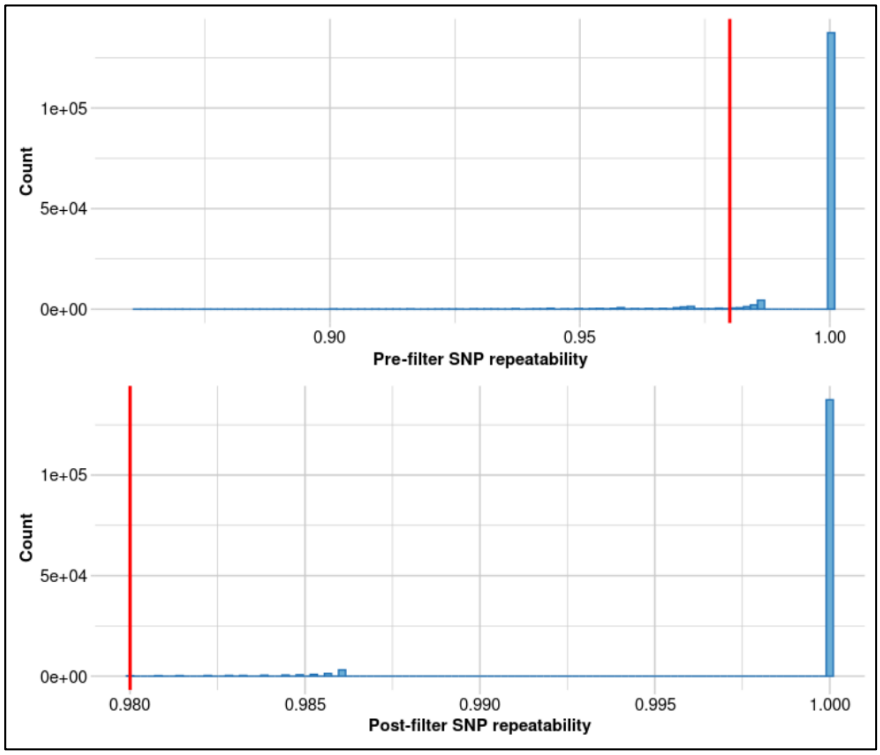
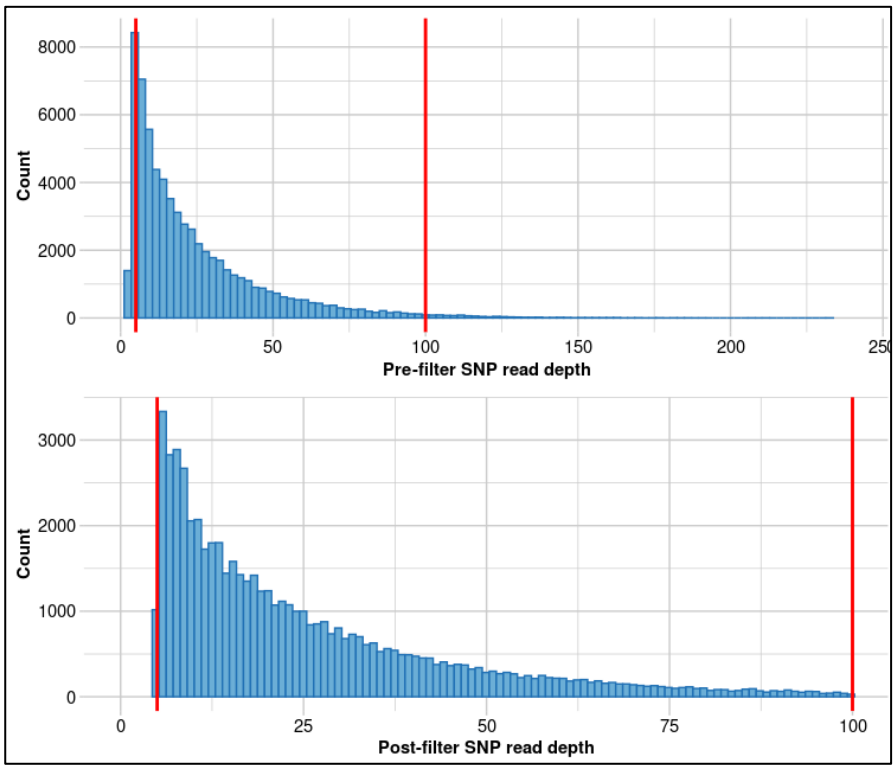
Appendix B: Reproducibility threshold (0.98), filtering out 12,118 SNPs.

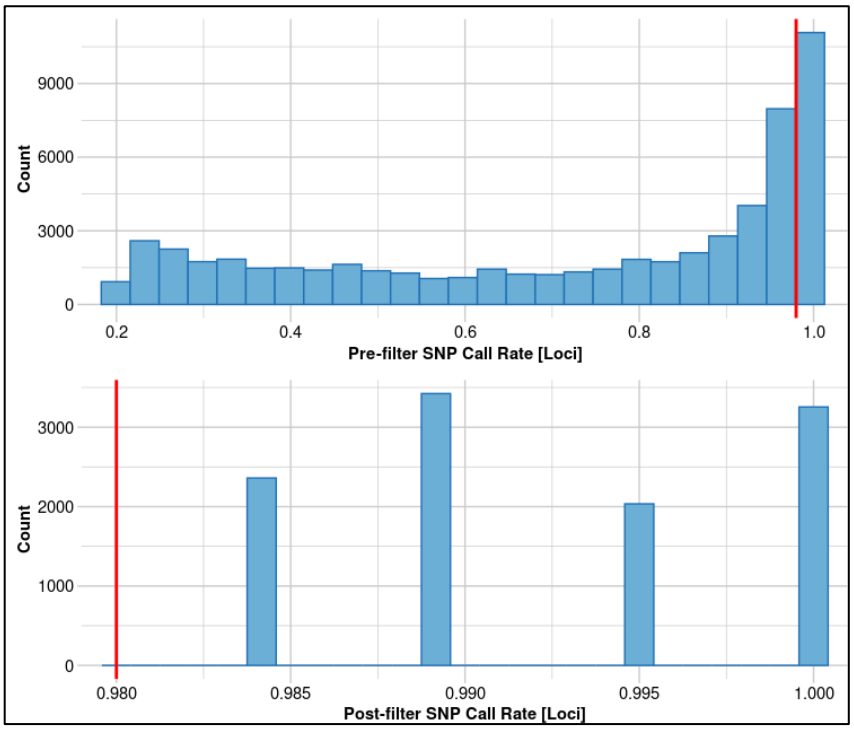


Appendix C: Read depth (lower threshold of 5 and upper threshold of 100) filtering out 7,790 SNPs.

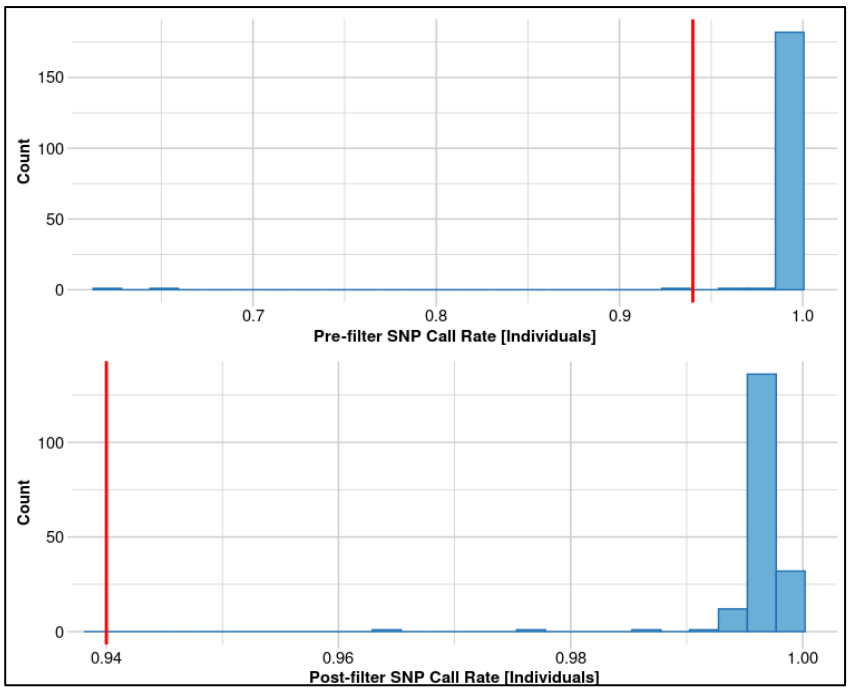


Appendix D: Call rate per SNP (a) and per individual (b) using thresholds of 0.98 and 0.94 respectively, filtering out 47,271 SNPs and 3 individuals.

(a)



(b)



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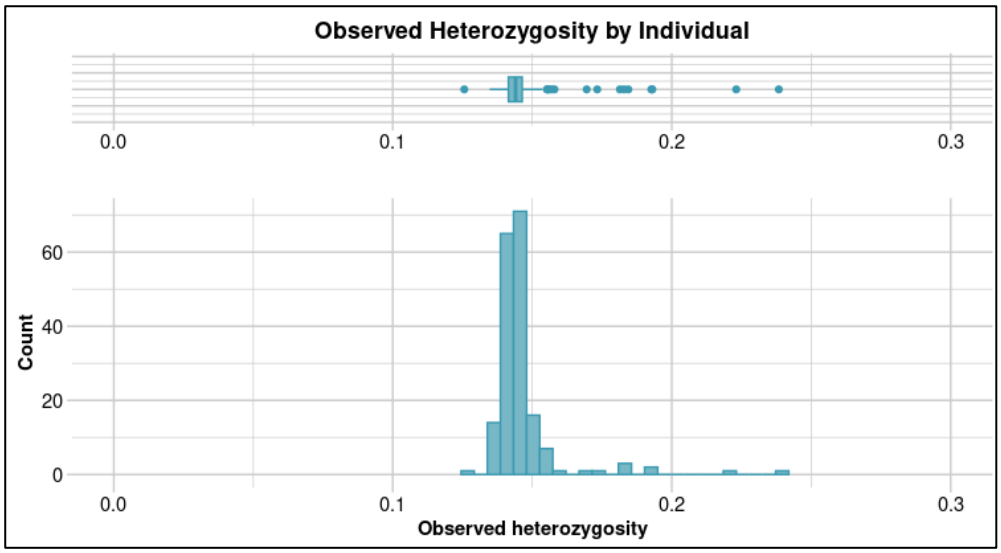
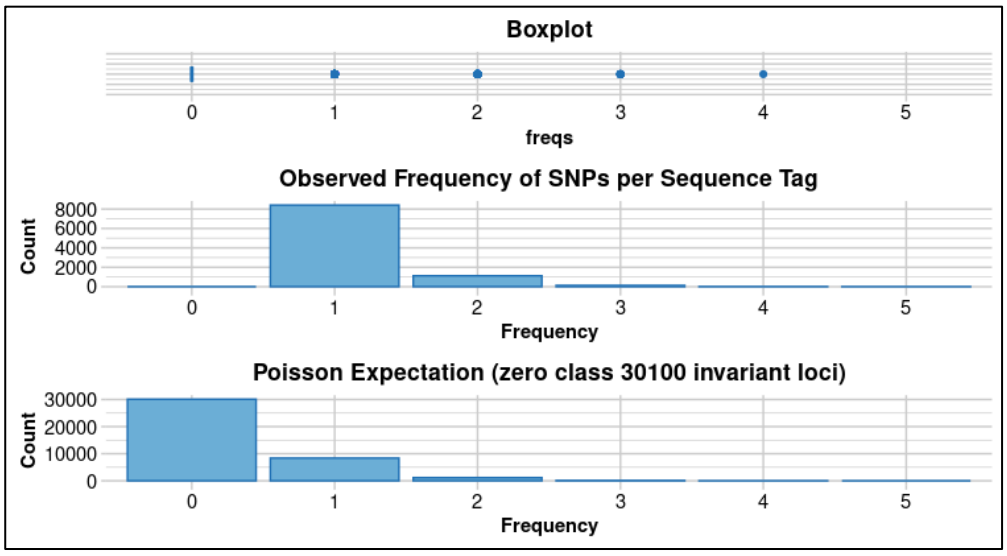
1315 **Appendix E:** Observed heterozygosity (lower threshold 0.1 and upper threshold 0.2) filtering out 2
1316 individuals.

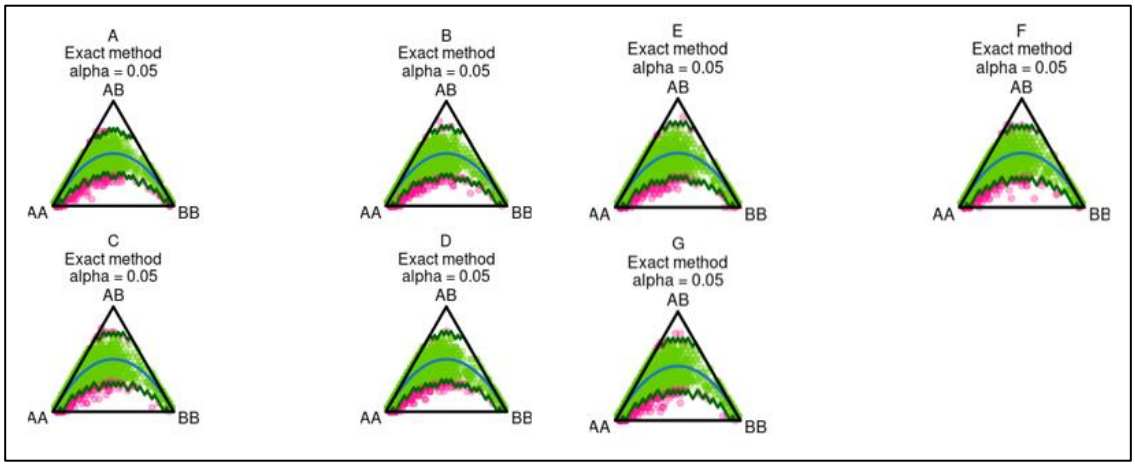


Appendix F: Linked loci removing 1,408 SNPs with short distance linkage

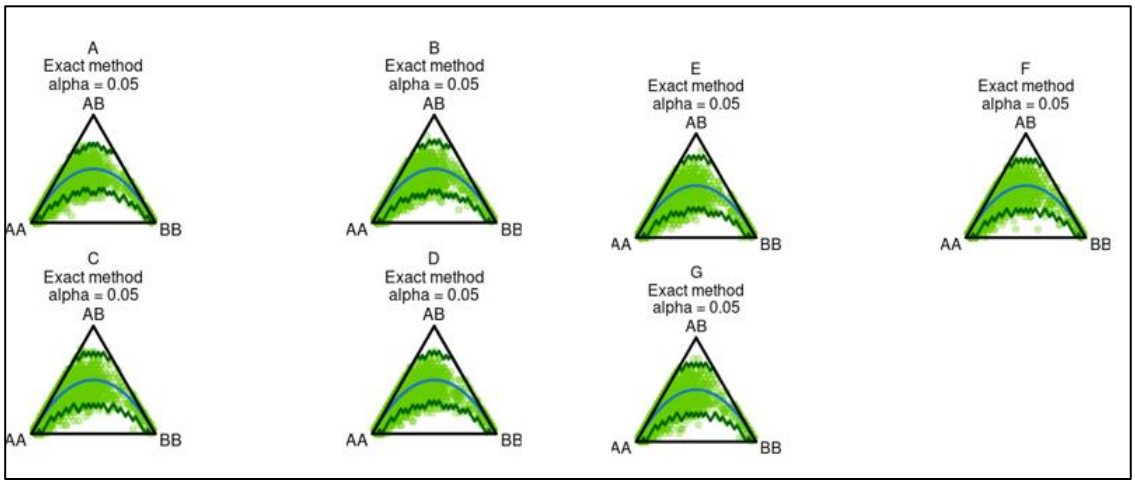


Appendix G: SNPs significantly deviating for Hardy-Weinberg equilibrium (pink) before (a) and after (b) the Benjamini-Hochberg correction.

(a)


A
Exact method
alpha = 0.05
AB
AA
BB
B
Exact method
alpha = 0.05
AB
AA
BB
E
Exact method
alpha = 0.05
AB
AA
BB
F
Exact method
alpha = 0.05
AB
AA
BB
C
Exact method
alpha = 0.05
AB
AA
BB
D
Exact method
alpha = 0.05
AB
AA
BB
G
Exact method
alpha = 0.05
AB
AA
BB

(b)


A
Exact method
alpha = 0.05
AB
AA
BB
B
Exact method
alpha = 0.05
AB
AA
BB
E
Exact method
alpha = 0.05
AB
AA
BB
F
Exact method
alpha = 0.05
AB
AA
BB
C
Exact method
alpha = 0.05
AB
AA
BB
D
Exact method
alpha = 0.05
AB
AA
BB
G
Exact method
alpha = 0.05
AB
AA
BB

1322

1323

Title of the research output: Using population genomics to understand stock structure of tiger flathead (*Platycephalus richardsoni*) in the Southern and Eastern Scalefish and Shark Fishery

Submitted

Declaration of Authorship

All authors agree that they have met the criteria for authorship and are accountable for the content of the research output.

Author order	**Name of author**	**Affiliation(s)**	**Contribution(s)**
Author 1	Amelia Jensen	University of Tasmania	Conducted field work and lab work, conducted analyses, wrote code, wrote manuscript.
Author 2	Karen Evans	UNESCO	Project design, supported lab work, edited manuscript.
Author 3	Sharon Appleyard	CSIRO	Project design, supported lab work, edited manuscript.
Author 4	Madeline Green	University of Tasmania	Project design, wrote code, supported lab work, edited manuscript.

I consent to having my name listed in the acknowledgements section of the above research output:

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Madeline Green	University of Tasmania