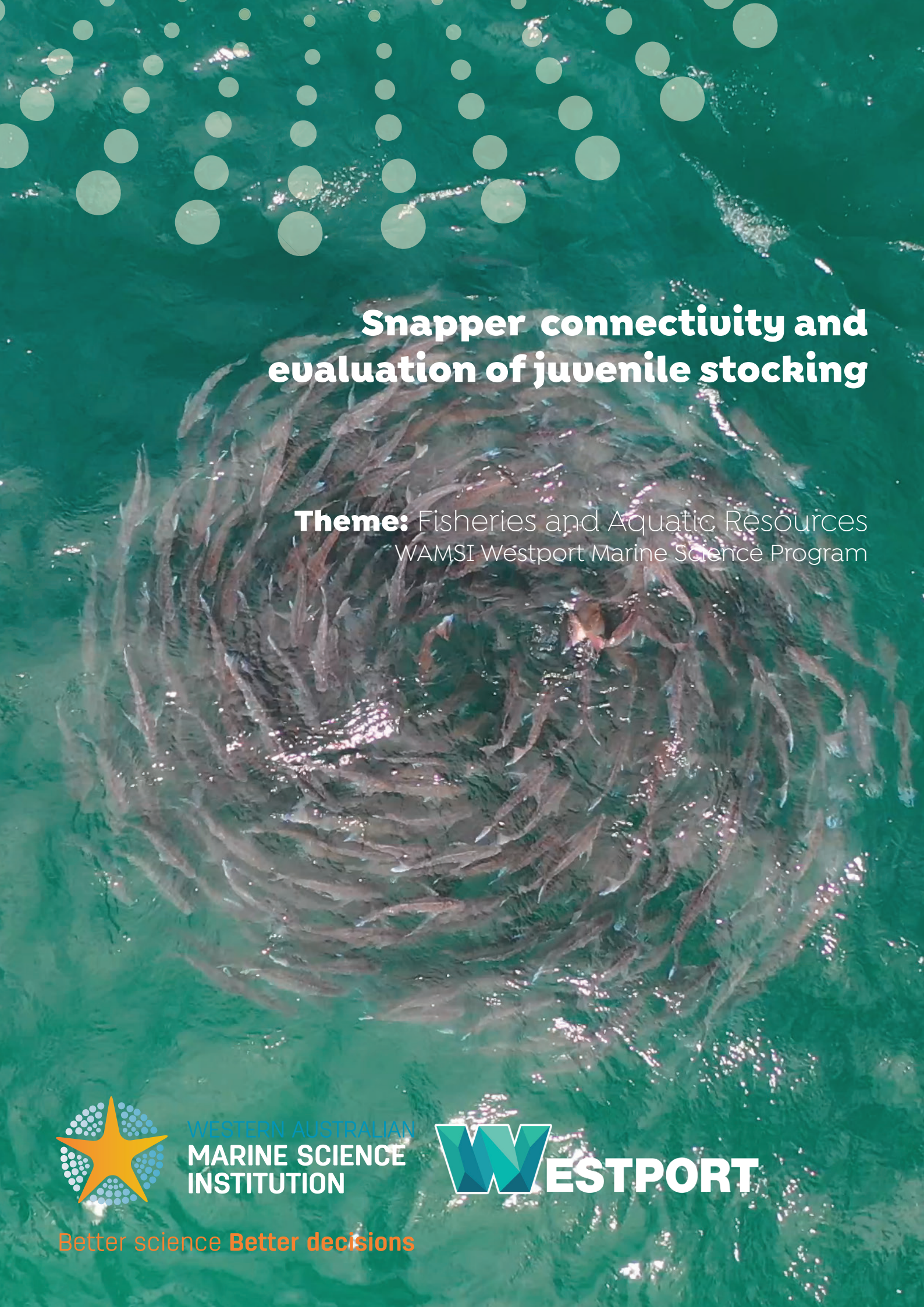

Snapper connectivity and evaluation of juvenile stocking

Theme: Fisheries and Aquatic Resources
WAMSI Westport Marine Science Program

WESTERN AUSTRALIAN MARINE SCIENCE INSTITUTION

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ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a $13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.

FUNDING SOURCES
The $13.5 million WAMSI Westport Marine Science Program was funded by the Western Australian Government, Department of Transport. WAMSI partners provided significant in-kind funding to the program to increase the value to >$22 million.

DATA
Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).

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FRONT COVER IMAGE
Theme: Fisheries and aquatic resources
Front cover image: A school of pink snapper in Cockburn Sound (DPIRD).

Contents

The WAMSI Westport Marine Science Program is a $13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.

Snapper connectivity and evaluation of juvenile stocking

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Project
4.1 Snapper connectivity and evaluation of juvenile stocking.

1 Executive Summary

The Western Australian government has proposed the construction of the Westport container port in Cockburn Sound. Adult snapper (*Chrysophrys auratus*) migrate into this embayment to aggregate to spawn. The resultant juveniles use various habitats in Cockburn Sound as nurseries and subsequently move out to join the broader south-western stock (south of 31°S to Albany), where they later become important in both recreational and commercial fisheries. However, the connectivity and fine scale stock structuring between Cockburn Sound and the lower west coast part of the stock (from 31°S to the Capes region) is less well understood, as is the contribution Cockburn Sound aggregations make to recruitment to the lower west coast.

To be able to evaluate potential effects on snapper from infrastructure development in Cockburn Sound, this study focused on two main objectives:

(1) investigate the genetic relationships and relative contribution of spawning snapper in Cockburn Sound to the broader lower west coast snapper stock (and therefore also its importance to recreational and commercial fisheries), and

(2) evaluate genetic and morphometric methods for monitoring the occurrence of released hatchery-reared juvenile snapper in Cockburn Sound.

<u>Genetic relationships and contribution of Cockburn Sound snapper</u>

This study produced a powerful genomic dataset which demonstrated, for the first time, high connectivity between snapper in Cockburn and Warnbro sounds and adjacent areas along the lower west coast, from the northern metropolitan region (Hillarys to Seabird) to the south-west Capes region (Geographe Bay to Augusta). High genetic migration rates of 86 to 100% were detected between these regions. There was less connectivity (<60% migration rate) with fish from the south coast (Windy Harbour), consistent with a previous broad-scale study that covered much of the Western Australian (WA) coastline (Bertram et al., 2022). The genetic results also demonstrated that recruitment of juveniles in Cockburn Sound and along the lower west coast occurs from local adult spawning events. This finding is consistent with biological studies in Cockburn Sound, indicating that spawning along the whole of the lower west coast would contribute to population replenishment. There was also no unequivocal evidence of localised adaptation, indicating high adaptability to environmental conditions along the lower west coast.

The high connectivity and migration rates demonstrated the strong contribution that snapper in Cockburn/Warnbro sounds make to stocks along the lower west coast and vice versa. In addition, the adult snapper that aggregate to spawn in Cockburn/Warnbro sounds are exclusively large individuals, unlike adults elsewhere along the coast. Estimates of the batch fecundity of such individuals demonstrates that they each contribute relatively more to reproductive potential. This is because in fishes, typically a greater proportion of the larger females, than smaller females, will be in spawning condition during the spawning period. As such, they often have a longer spawning period, produce more eggs and have greater spawning success. The genetic evidence for local recruitment in Cockburn/Warnbro sounds reported in this work, together with the batch fecundity estimates, highlight the important contribution of individuals in spawning aggregations. Other benefits that embayments such as Cockburn Sound confer on early life history stages may include greater protection from predators and food resources than coastal nurseries.

The evidence of equivalent connectivity across the lower west coast indicates that Cockburn and Warnbro sounds could be replenished via natural dispersal and migration if any changes to natural processes occurred because of infrastructure development. However, replenishment would be dependent on the rate of dispersal and migration along the coast at different life stages, any effect of ongoing pressures, and suitable habitat remaining available for spawning, larval settlement and juvenile nurseries. In addition, recruits spawned in Cockburn/Warnbro sounds would subsequently be selected (after reaching the minimum legal length of 500 mm) by recreational and commercial fishers along the lower west coast and therefore any effects of development on recruitment could influence the contribution they make to catches of snapper by these fisheries.

Evaluation of genetic and morphometric methods for monitoring released hatchery-reared snapper

Morphometric and genomic work have demonstrated the potential for these methods to be used in monitoring the hatchery-reared snapper released in Cockburn Sound. Morphometrics of wild and hatchery-reared snapper differed in many aspects, allowing distinction of such individuals from good quality photographs. Further work is required to evaluate whether differences remain between (i) larger hatchery-reared juveniles and fish of the same size collected during trawl surveys of Cockburn Sound, and (ii) between hatchery-reared and wild fish once they are above the minimum legal length for retention of 500 mm on the lower west coast.

The genomic work demonstrated no differences in overall levels of relatedness between hatchery-reared snapper ready for release and wild juvenile snapper. It also demonstrated that the Snapper Guardians program is using an unbiased sample of the wild population, and that hatchery-reared snapper have very low average relatedness (i.e. few pairs of first order kin were identified). These results, in combination with the high genetic diversity found for hatchery-reared snapper, suggest that hatchery-reared fish have high adaptive capacity. This provides strong support for the current method of rearing snapper. It also indicates that the genomics approach used here provides an efficient method to monitor levels of relatedness between samples of hatchery-reared and wild snapper over time. In addition, the current program releases fish at ~3 months old, which minimises the chance of deformities that can occur when fish are reared for long periods prior to release. However, any effects on aggregations and spawning behaviour of snapper as a result of industrial development and operations could influence the efficacy of future hatchery rearing programs using the current methods of wild egg collection rather than maintaining brood stock in tanks.

Since Snapper Guardians commenced in 2014, only four hatchery-reared juvenile snapper have been recaptured and identified from a total of 1,980 juveniles collected by trawl surveys in Cockburn Sound and ~230,000 released hatchery fish across all years. No adult individuals have been identified among 3,952 collected for stock assessment programs in the metropolitan and south-west areas of the lower west coast over that time period. Estimated survival rates of less than 1% could be expected by 5 to 6 years of age (the approximate age at maturity and recruitment to the fishery) and 0.1% by 10 years of

age. Therefore, future stock enhancement programs require consideration of the economic feasibility of producing sufficient fish and whether that will provide observable benefits (either to stocks or to fisheries), the timing and locations of releases, bespoke monitoring programs of released fish and adequate additional funding to conduct such work. Such a program should also focus on (i) the genetic composition of the hatchery fish vs wild fish, adopting the approach used in this study, (ii) initial survival of released fish, as this is when they are most vulnerable to predation and least mobile, and (iii) long-term survival.

2 Introduction

Snapper (*Chrysophrys auratus*) is an important species in both commercial and recreational fisheries in Western Australia (WA; Newman et al., 2023). It is caught by commercial line and gill-net fishers and is a primary target for recreational boat-based fishers in the southern (metropolitan and south-west) management areas of the West Coast Bioregion, an ecological region used in fisheries management in WA, extending from 27°S on the west coast to 115°30'E on the south coast (Newman et al., 2023). This includes the coastal embayments of Cockburn Sound and Warnbro Sound and also Owen Anchorage (Crisafulli et al., 2019; Fairclough et al., 2021; Ryan et al., 2022). Snapper migrate into these coastal habitats each year to aggregate to spawn, with the largest aggregations in these three locations occurring in Cockburn Sound (Wakefield, 2010). These protected habitats are subsequently used as a nursery by snapper juveniles, which remain for 1-2 years, before emigrating to coastal and deeper reef environments (Wakefield et al., 2011). The assumed importance of the aggregations to broader stocks along the lower west coast (south of around Lancelin at 31°S) led to the annual closure to fishing for snapper in Cockburn Sound and Warnbro Sound in the early 2000s, to protect both migrating and aggregating fish (Wakefield, 2010). Following studies of migratory behaviour (Crisafulli et al., 2019), the closure was extended in time and area. It now occurs between 1 August and 31 January and extends westwards beyond Cockburn Sound and Warnbro Sound to protect a greater proportion of migrating and aggregating fish (Figure 2.1; DPIRD, 2023).

Ongoing infrastructure development in Cockburn Sound, including the proposal for a container port (Westport), may influence the behaviour of adult and juvenile snapper in Cockburn Sound during either phases of construction or future operations. For example, dispersal of dredge material from the construction of the port site, deepening of shipping channels or future shipping movements through the sound or while in harbour could elevate levels of suspended solids. If concentrations of suspended solids are high enough, this could result in mortality of snapper larvae (Partridge and Michael, 2010) or smothering of juvenile habitat. In addition, increased shipping activity and associated noise could interrupt migratory and/or aggregation behaviour, potentially affecting reproductive success. Any changes in water flow and circulation within the sound could influence the locations and extent to where snapper larvae settle, which could affect survival. Low water exchange following a period of warm conditions in 2015 resulted in an algal bloom and 'fish-kill' event in Cockburn Sound. Mortalities of adult snapper occurred, which raised the question of the impact of such losses on the contribution of snapper aggregations to stocks on the west coast.

At the time of the fish-kill event, a method had been successfully developed to rear juvenile snapper in hatcheries from wild-captured eggs (Partridge et al., 2017). This allowed the opportunity to release the juveniles into Cockburn Sound (the 'Snapper Guardians' program) as a proposed benefit to stocks and fishing. While this program attracted substantial public interest and has continued, its benefit to the lower west coast stock or to recreational fisheries has never been evaluated.

While understanding potential impacts of the proposed port on snapper in Cockburn Sound is an important question and methods for monitoring them are being evaluated in another WAMSI Westport Marine Science Program (WWMSP) project (Yeoh et al., 2024), understanding the relative importance of snapper in Cockburn Sound to the broader lower west coast stock is also a key question. In addition, evaluation of stock enhancement programs that may contribute to mitigating impacts on snapper in Cockburn Sound requires tools that can be used to monitor the extent of their success.

Therefore, this project has two main components: (1) investigate the genetic relationships and relative contribution of spawning snapper in Cockburn Sound to the broader lower west coast snapper stock (and therefore its importance to recreational and commercial fisheries), and (2) evaluate genomic and morphometric methods for monitoring the occurrence of released hatchery-reared juvenile snapper in Cockburn Sound.

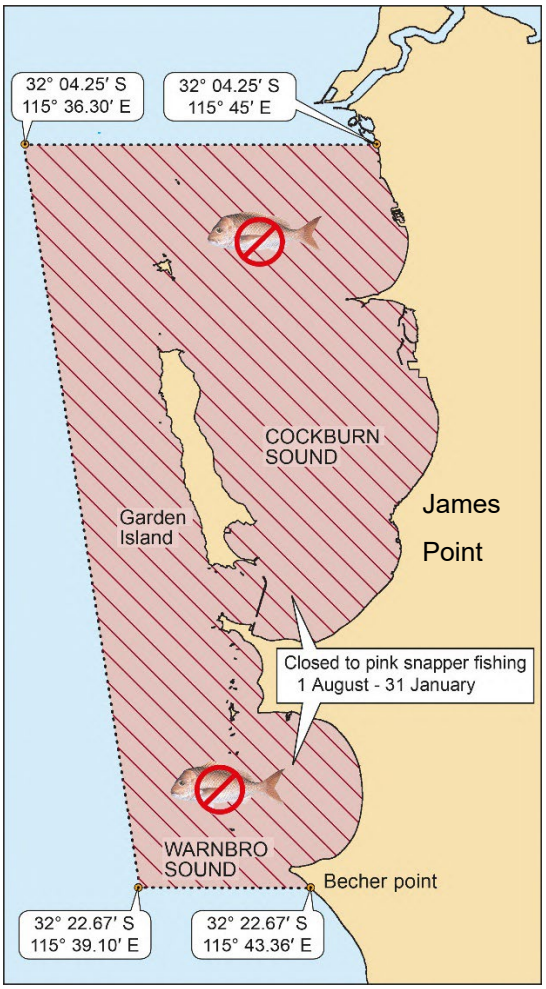


Figure 2.1. Area of annual closure to fishing for Snapper that are migrating to/from or aggregating in Cockburn Sound (DPIRD, 2023).

2.1 Genetic relationships and contributions of Cockburn Sound snapper to west coast stocks

Snapper across the lower west coast (north of Cape Leeuwin) and south coast (east of Cape Leeuwin to Albany) are genetically more closely-related than they are to snapper from Lancelin northwards to the Gascoyne Coast, north of 27°S (Bertram et al., 2022). However, that study demonstrated a degree of isolation by distance, or genetic differences, among snapper at a scale of around 300 km. Thus, this finer-scale study focused on improving the understanding of genetic relatedness between snapper in Cockburn Sound and those along the coast from the northern metropolitan area (Two Rocks) to Windy Harbour on the South Coast.

Adult snapper migrate into Cockburn Sound in late winter/early spring and subsequently leave after spawning in early to mid-summer, with peak spawning generally occurring when water temperatures are between 19 and 21°C (Figure 2.2; Wakefield, 2010; Crisafulli et al., 2019). Adults migrate to and from the sound in multiple years, but not necessarily in consecutive years. When they leave the sound, adults can move 10s to 100s of km, with tagged individuals having been observed as far north as Shark Bay and as far south as Hamelin Bay. However, the vast majority have been recaptured within approximately 20 km (Figure 2.2; Crisafulli et al., 2019). Thus, it is unclear whether individuals that travel long distances from Cockburn Sound after spawning return in subsequent years, whether they spawn in Cockburn Sound for more than just a few years, or would join spawning stocks elsewhere along the coast. Furthermore, it is not completely understood what contribution Cockburn Sound aggregations make to broader west coast stocks.

This work analysed genomic data (i.e. data from thousands of DNA markers) for samples of snapper collected in Cockburn Sound and in adjacent locations of the lower west coast and the south coast. Genomic information provides exceptional power to define the number of demographically distinct management units (i.e. fisheries stocks) and to estimate the amount and direction of connectivity between stocks (Figure 2.3; Bernatchez et al. 2017). When combined with information about habitat mapping and environmental variation, such genomic data can also be used to test if spawning aggregations and their recruits are adapted to particular habitats (Grummer et al. 2019), such as the Cockburn Sound embayment. Specifically, this project will:

(1) clarify patterns of connectivity and the contribution made by Cockburn Sound spawning aggregations of snapper to the broader stock in the lower coast of Western Australia, and

(2) test if aggregating snapper and juvenile recruits in Cockburn Sound exhibit evidence of local adaptation to the Cockburn Sound environment, and how much they differ from snapper in the lower coast of Western Australia.

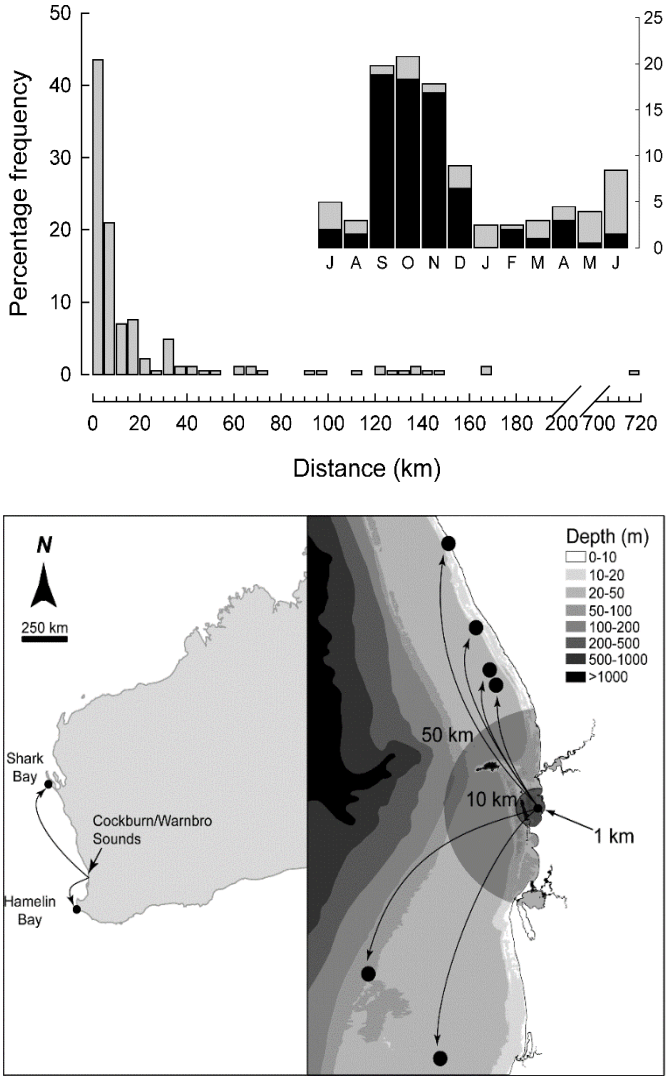


Figure 2.2. (top) Distribution of snapper recapture distances from Cockburn Sound, months in which they were caught and stack bar graph shows whether they were caught in Cockburn and Warnbro Sounds (black) or outside them (grey; with permission from Crisafulli et al., 2019) and (bottom) examples of short and long-distance movements of adult snapper tagged in Cockburn Sound. Black points represent long distances from Cockburn Sound where example recaptures of snapper have occurred and shaded concentric circles on the inset represent area of short distances where snapper have been recaptured, i.e. up to 10 and 50 km from the mid-point of Cockburn Sound.

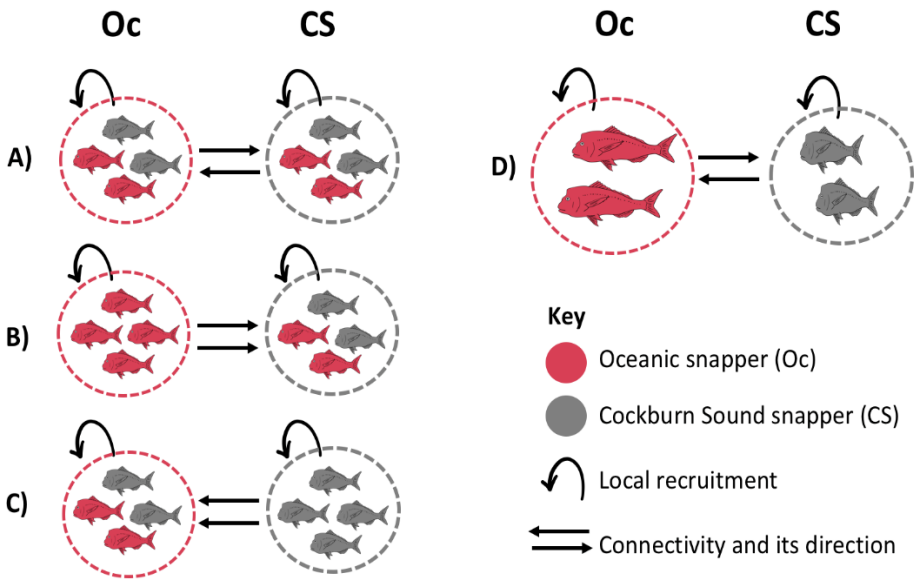


Figure 2.3. Potential scenarios of connectivity between Cockburn Sound (CS) and Oceanic (Oc) snapper to be tested with genomic data. A) high connectivity between both regions results in a single population; B) high connectivity from oceanic snapper results in Cockburn Sound acting as a 'sink'; C) high connectivity from Cockburn Sound snapper results in Cockburn Sound acting a 'source'; D) Local adaptation to environmental conditions in each region results in two distinct snapper populations – this can happen either in the absence, or presence (as shown) of connectivity. All scenarios assume spawning and recruitment within each region.

2.2 Evaluation of methods for monitoring aspects of stock enhancement programs

Stock enhancement involves the release of hatchery-reared individuals into a fishery to improve an already sustainable population (Taylor et al., 2017). Such releases are popular with recreational fishers (Garlock & Lorenzen, 2017; Tweedley et al., 2023a) and have been used globally (e.g. Leber, 2004; Kitada & Kishino, 2006) including in Australia (see review by Loneragan et al. 2013). For example, it has been estimated that > 26 billion juveniles of 180 species are released annually into marine waters of more than 20 countries (Kitada, 2018). However, despite their wide use, many stock enhancement programs are not successful for a number of reasons (Hilborn, 1998; Molony et al., 2003). For example, they may have failed to clearly define the objectives of the program, not conducted hatchery and/or field trials and not developed evaluation methods. To maximise chances of success, releases need to be carefully planned to reduce post-release mortality, including from predation (Poh et al. 2018) and species chosen with an understanding of their movements (Becker et al., 2021, 2023). Given the mixed success of stocking programs, a set of principles was developed to promote responsible stocking which includes "Identify released hatchery fish and assess stocking effects on fishery and on wild stock abundance" (Blankenship & Leber, 1995).

Due to their high economic and recreational value, aquaculture technologies for sparids, in particular gilthead sea bream (*Sparus aurata*) and red sea bream (*Pagrus major*), are well-developed (Basurco et al., 2011). The ability to produce large quantities of juveniles and their value to commercial fisheries and recreational fishers makes species in this family good candidates for stock enhancement (Ryan et al., 2022; Tweedley et al., 2023b; FAO, 2022). Several stocking programs have been successful. In the Bay of Cádiz (Spain), ~18,000 marked gilthead sea bream were released in two size classes, i.e. 15 and 100 g, and recapture rates of 0.03 and 3.52%, respectively, were obtained (Sánchez-Lamadrid, 2004). In the Blackwood Estuary (south-western Australia), 220,000 black bream (*Acanthopagrus butcheri*) were released in 2002-2003, with subsequent monitoring demonstrating that the hatchery-reared fish contributed up to 73% of the commercial catch and up to ~50 % to egg production of one year class (Cottingham et al., 2020). Stocking of snapper into Cockburn Sound and surrounds in Western

Australia, as part of the ongoing Snapper Guardians program (Recfishwest, 2024) was initiated in 2014 and, to date, >230,000 juveniles have been released (Appendix 1). However, no formal evaluation of its success has been conducted or funded as part of this. A similar program was initiated in South Australia in 2021, which has released 530,000 individuals (PIR, 2024). Both these programs expose juvenile snapper to alizarin complexone during the culture and before release, which stains hard body parts, such as otoliths (Appendix 2; Partridge et al., 2017; https://pir.sa.gov.au/research/snapper_recovery/stock_enhancement). This enables those fish to be identified in scientific collections and the effectiveness of stocking programs to be evaluated (Cottingham et al., 2020). However, as the marks are internal, those fish need to be euthanased to remove their otoliths, thus reducing the benefits of the stocking if substantial numbers of fish are collected for identification. As stocking is used to increase numbers of the target species, methods for distinguishing hatchery-reared from wild fish or monitoring the dispersal and abundance of released fish would preferably be non-lethal. While preliminary work by Partridge et al. (2017) suggested that there was no loss of genetic diversity in hatchery-reared snapper versus that of adults in the spawning aggregations, genomic-based analyses offer superior resolution to assess if hatchery-reared juvenile fish used in stocking activities contain levels of genetic diversity and adaptive potential similar to those found in their wild population (Bernatchez et al. 2017).

This project will evaluate the potential for two different non-destructive methods for doing so and address the following objectives:

(1) test if hatchery-reared juvenile snapper used in stocking activities contain levels of genetic diversity and adaptive potential similar to those found in their Cockburn Sound population of origin,

(2) develop a DNA-based method to inexpensively monitor connectivity and the contribution of Cockburn Sound spawning aggregations and hatchery-reared snapper and

(3) determine whether external morphological features collected from photographs of hatchery-reared and wild juvenile snapper (non-lethal methodology) can be used by digital image recognition to distinguish them.

The latter approach has been used to distinguish hatchery-reared from wild individuals of both a sparid and moronid in Europe (Arechavala-Lopez et al. 2012) and preliminary evidence suggests it may work for black bream stocked in the Blackwood River (Appendix 3). If successful, this would negate the need to euthanise snapper to identify whether they are hatchery-reared and help evaluate the success of the Snapper Guardians program. Moreover, future monitoring could include a citizen science component where recreational fishers could provide photographs of their catches for evaluation, i.e. a digital version of the Send Us Your Skeletons Program (Fairclough et al., 2014).

3 Materials and Methods

3.1 Genetic relationships and contributions of Cockburn Sound snapper to west coast stocks

3.1.1 Sample collection

Snapper samples were collected from recreational and commercial landings and fishery-independent sampling by trawling and line fishing by staff of the Department of Primary Industries and Regional Development (DPIRD) in 2021/22 and 2022/23 (see Section 3.2.1 for description of trawling methods). In each year, approximately 40 adult and 40 juvenile snapper were collected in Cockburn Sound (and Warnbro Sound, referred to collectively as Metro) and three broad locations of the lower west coast, one to the north and two to the south of Cockburn Sound, i.e. Metro North (off Hillarys to Seabird), Metro South (off Dawesville to Preston Beach) and South-west (around Geographe Bay) (Figure 3.1). Additional adult samples from the western south coast (Windy Harbour) were also obtained for comparison. A flesh sample from each snapper, to be used for DNA extractions, was preserved in labelled vials in 100% ethanol. Biological data were also obtained from each fish, including its total length (TL to the nearest 1 mm), total weight (where whole fish were obtained; to the nearest 1 g), sex and gonadal development stage. Sagittal otoliths were also removed.

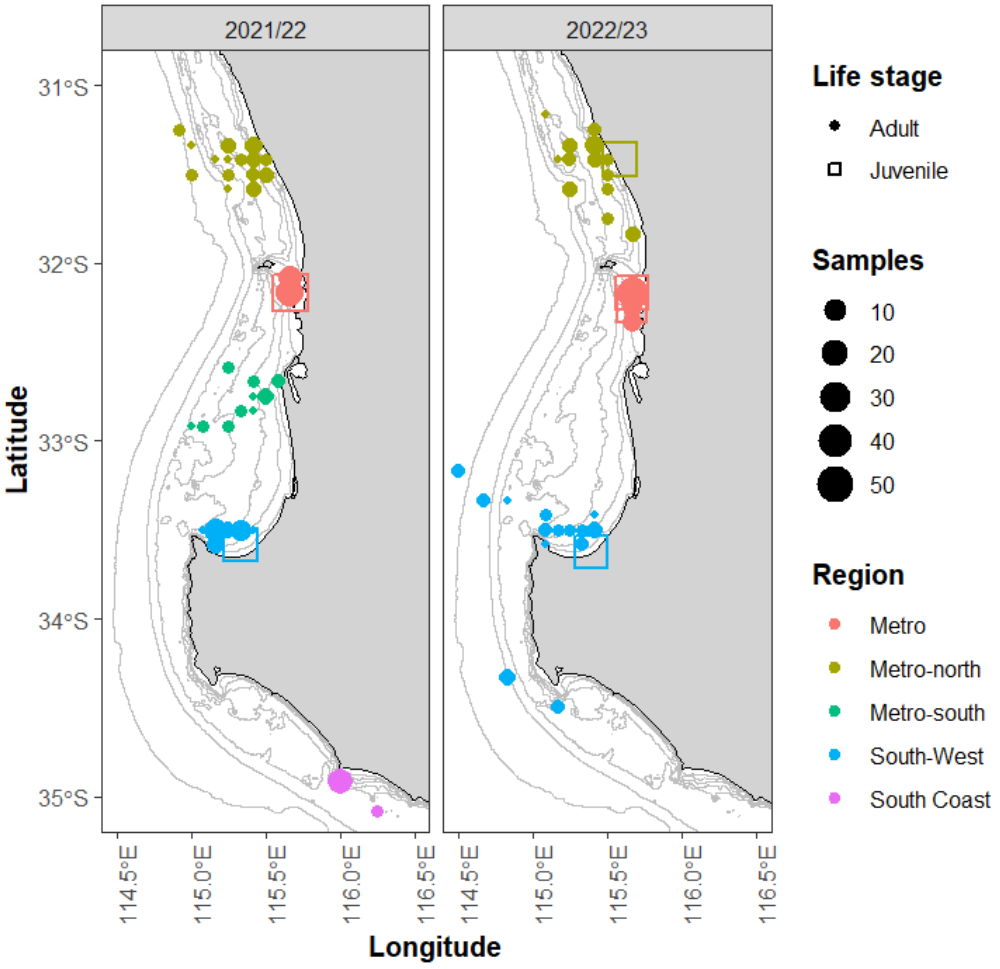


Figure 3.1. Map of locations at which adult and juvenile snapper were collected from recreational and commercial fishers, and fishery-independent trawl and line fishing in 2021/22 and 2022/23, in the Metro (Cockburn and Warnbro Sounds), Metro north (Hillarys to Seabird), Metro south (Dawesville to Binningup), the south-west (Geographe Bay to Augusta) and the western south coast regions (Windy Harbour). Note that fishery reporting blocks (either 5nm×5nm or 10nm×10nm) are sometimes provided by fishers as sample locations. These are converted to latitude and longitude as an approximation of actual sample location.

3.1.2 Laboratory procedures and analysis of genomic data

Genomic sample processing and data analyses were conducted as follows:

- **Tissue dissections and curation**: dissections to obtain subsamples from tissues of all snapper received at Flinders University from the 2021/2022 and the 2022/2023 seasons were conducted. Subsamples were stored in 90% ethanol for curation and subsequent DNA extractions.
- **Extractions of genomic DNA:** a MELFU-modified method of DNA extraction based on a salting-out procedure that has proven successful for Australian snapper (e.g. Bertram et al. 2022) was used for over 1,590 individual extractions including repeat attempts. The concentration, purity, and integrity of each extraction was assessed using Qubit (Life Technologies), NanoDrop (Thermo Scientific), and 2% agarose electrophoresis gels, respectively. Tissue samples preserved in ethanol produced DNA extractions of sufficient quality and quantity for preparation of genomic libraries.
- **Preparation of ddRAD genomic libraries:** A reduced genome representation technique of ddRADseq (double-digest restriction site-associated DNA sequencing) was used in house to trial generating genomic data for 44 individual snappers and 4 replicate samples, which was successful. We then produced genomic libraries for another 816 select individuals representing all the regions (i.e. zone areas) from 2021/2022 and 2022/2023 sampling seasons, as well as from the hatchery program and replicates. We followed the protocol described by Peterson et al. (2012) with a few modifications as described in Sandoval-Castillo et al. (2018). For each sample, ~300 ng of DNA was digested with the restriction enzymes SbfI and MseI, and then ligated with forward and reverse adaptors, with forward adaptors including 1 of 96 individual barcodes designed in-house. Libraries were size selected for 250–800-bp fragments with a Pippin Prep (Sage Science), and then amplified using PCR.
- **Sequencing of ddRAD genomic libraries:** The 2021/2022 genomic libraries were sequenced on an Illumina HiSeq 4000 (150 bp paired end) at Novogene (Hong Kong) in April and in August 2023. The 2022/2023 libraries were sequenced at Novogene (Hong Kong) on an Illumina NextSeq 1000 (150 bp paired end) in November 2023.
- **Bioinformatic analysis of the ddRAD dataset:** Raw sequences from the sequenced genomic libraries were assessed using the software FASTQC (Brown et al 2017). Sequences were demultiplexed using the *process_radtags* module from STACKS (Catchen et al 2013) and quality trimmed (including barcodes and RAD tags) using Trimmomatic v0.39 (Bolger et al 2014). To avoid biases in SNP detection resulting from different sequencing platforms, we also implemented an *in-silico* standardization of the number of reads per sample. The remaining reads were aligned to the MELFU reference snapper genome using Bowtie2 v2.3.5.1 (Langmead and Salzberg 2012) and variants were called using a modification of the GATK pipeline (McKenna et al 2010).
- **Data analysis – genomic diversity, genomic differentiation, connectivity and relatedness:** Observed (Ho) and expected (He) heterozygosity, number of loci (nLoc), number of polymorphic loci (polyLoc), and the population-level inbreeding coefficient (FIS) were estimated using the R package hierfstat (Goudet et al. 2015). Analyses of population differentiation and genetic structure were done both at the level of locality and region (i.e. by pooling localities in their respective region), and with and without including life stage, season and the hatchery sample. Principal Component Analysis (PCA) were carried out using the package vegan (Oksanen et al. 2018) and FST analyses of population differentiation were carried out using hierfstat (Goudet et al. 2015). Analysis of spatial autocorrelation (SAC) were done in GenAlEx (Peakall & Smouse 2012). To understand fine-scale patterns of connectivity (i.e. gene flow), we ran several asymmetric gene flow models using the divMigrate function in the diveRsity R package (Keenan et al. 2013). To test whether hatchery-raised samples represent an unbiased sample from the

highly-connected Metro region, we used the Rpackage related (Pew et al. 2015) to estimate pairwise relatedness for all individuals in each region and compared that with the average pairwise relatedness from the hatchery samples per season, as well as combining the two seasons into a single hatchery sample.

- **Data analysis – seascape genomics:** We used five key oceanographic variables (sea surface temperature (SST), pH, oxygen concentration, salinity, and chlorophyll a) to test if environmental variation in the study region influences genetic diversity and population differentiation. These variables were downloaded from the IMOS (https://imos.org.au/) and Copernicus (https://marine.copernicus.eu/) databases. Variables were parsed based on month of the year, as well as average, minimum and maximum values, resulting in ninety datasets for analysis. After removing correlated variables or those with no sufficient variation in the region, eight variables were selected. All such variables all related to SST. These variables were used to perform RDA and partial RDA controlled by geographic distance using the R package vegan, function rda (Oksanen et al. 2018).

3.1.3 Relative contribution to reproductive potential

Analyses of length frequency distributions and estimated batch fecundity were used to evaluate the relative contribution of Cockburn Sound snapper to the reproductive potential of west coast stocks. Biological data for snapper were obtained from DPIRD data sets derived from fishery-dependent sampling of recreational line fishing catches in the metropolitan and south-west fishery management areas of the west coast between 2000 and 2024 and from research sampling by line fishing over the same time period. In the laboratory, total lengths (TL) or fork lengths (FL) of fish were measured to the nearest 1 mm. Each fish was dissected and its sex and gonadal development stage recorded according to characteristics described in Wakefield et al., (2015). When only FL was measured, it was converted to TL using the equation: $TL = (FL + 23.058)/0.897$ (Wakefield, 2006) for analysis of length frequency distributions.

The length frequency distributions of female snapper collected in Cockburn and Warnbro Sounds were compared with those sampled outside those locations in the metropolitan and south-west areas. Data were limited to fish collected in the main spawning months (October, November, December) (Wakefield et al., 2015), with samples from Cockburn and Warnbro Sounds combined, as adults move between these locations (Crisafulli et al., 2019). Comparisons of length distributions and batch fecundities between locations were made with two subsets of data: (1) for fish assumed to be sexually mature based on whether their TL was ≥ 585 mm, i.e. the estimated TL at which 50% of females reach maturity (Wakefield et al., 2015) and (2) for all female snapper that were in spawning condition (i.e. ovary stage V).

To compare the relative reproductive potential of the females sampled at each location, the batch fecundity of each fish was calculated using the equation: $F = 0.00009436 \times FL^{3.359}$ (Jackson et al., 2012). These were compared using box and whisker plots. In addition, the batch fecundity was estimated for the midpoint of each (50 mm) length class in each data set, and multiplied by the percentage frequency of females in each length class to account for the effect of different sample sizes among sample locations. These values were then summed across length classes to produce a comparable estimate of the overall reproductive potential of fish at each location (based on their total batch fecundity).

3.2 Evaluation of methods for monitoring aspects of stock enhancement programs

3.2.1 Sample collection

A total of 201 and 110 randomly selected hatchery-reared juvenile snapper were collected from the DPIRD fish hatchery in Fremantle in February 2022 and 2023, respectively. No fish were able to be obtained in 2024 (see below). The snapper were grown from fertilised eggs collected by DPIRD staff from Cockburn Sound during the spring spawning aggregation using the methods outlined in Partridge et al. (2017). Juvenile snapper were euthanised in a solution of AQUI-S aquatic anaesthetic by DPIRD staff. The number of samples obtained each year reflects the number of wild snapper egg collection events. In 2022, two egg-collection events occurred, resulting in two aquaculture runs and thus the snapper provided were either 77 or 90 days old, whereas the samples provided in 2023 were produced from a single egg-collection event and the resultant fish were 86 days old. The aim was to obtain morphometric data from ~100 fish from each egg collection event (Table 3.1), which is in line with the sample size used in similar studies (e.g. Arechavala-Lopez et al., 2012). Thus 201 individuals were obtained in 2022 and 110 in 2023.

Morphological aspects of the hatchery-reared snapper were compared to samples of wild juvenile snapper (*n*=328; Table 3.1) caught using small and large trawls conducted by DPIRD in Cockburn Sound over eight seasons between August 2021 and May 2023 (as part of this project and the WWMSP project *Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound;* Yeoh et al. 2024). The large trawl net had an 11 m wide headrope, 1 m opening height and was constructed with 55 mm mesh in the wings and 45 mm mesh in the cod-end. The trawl was twin-rigged (two nets deployed in parallel) and was towed at a speed of ~3.3 knots for 5 minutes (swept area of ~3,750 m^2 per net). The small trawl had a 4.5 m headrope, 0.5 m opening height and was constructed with 51 mm mesh in the wings and 25 mm mesh in the cod end. The trawl was towed at a speed of ~2.7 knots for 9 minutes (swept area of ~2,260 m^2).

The total lengths of individual wild snapper were typically >50 mm standard length (SL), with a mean of 115 mm SL (see Results). In contrast, the snapper obtained from the hatchery in 2022 were much smaller (average = 45 mm SL). Therefore, to facilitate better morphometric comparisons with wild-sourced fish, snapper sampled from the hatchery in 2023 were collected immediately before their release date (Appendix 1), however, these samples were typically only slightly larger (average = 48 mm SL) than those collected from the hatchery in the previous year. To enable more robust morphometric analyses between wild and hatchery-reared snapper, as none of the samples collected by trawl contained previously hatchery-reared and released fish that would have been larger, arrangements were made for an additional sample of juvenile snapper from the hatchery in 2024, which were to be held in the hatchery until they were of a comparable size to wild snapper. However, betanodavirus (a nervous necrosis virus) was detected in the hatchery-reared snapper from this egg collection. As this disease can cause viral encephalopathy and retinopathy and is known to affect over 60 marine finfish species and can cause significant mortality in aquaculture production (Bandín & Souto, 2020) all snapper were euthanised (DPIRD, 2024). As such, no additional snapper were able to be obtained for morphometric analysis in 2024. The next egg collection and aquaculture run is projected to be undertaken in late 2024 for release in early 2025 (DPIRD, 2024).

Table 3.1. Number of wild snapper obtained by trawling in Cockburn Sound and processed for biological data and the number of wild and hatchery-reared snapper used in morphometric analyses. Values outside parentheses indicate samples on which analyses were conducted and values inside parentheses are total samples obtained.

		Field sampling	Morphometric analyses	
Year	**Month**	**Wild snapper caught**	**Wild**	**Hatchery-reared**
2021	April	729 (757)		
	November	183 (186)	182 (182)	
2022	February	357	140(140)	194 (201)
	April	1		
	May	3	3	
	October		11	
	December	10	34	
2023	January		7	
	February	32	32	110 (110)
	April	116		
Total		**1,431 (1,462)**	**322 (409)**	**304 (311)**

3.2.2 Laboratory procedures

In the DPIRD laboratory, a range of biological information, including total length (to the nearest 1 mm) and total weight (to the nearest 0.1 g) were measured and recorded from all snapper. As the otoliths of the hatchery-reared snapper were stained with Alizarin-complexone (Appendix 2), the otoliths of juvenile wild snapper were removed to identify whether they were hatchery-reared fish produced from the Snapper Guardians programs or wild fish. The percentage of fish captured in the wild and processed for biological data that were identified as being hatchery-reared was calculated. For genomic analyses, small tissue samples were taken from the wild-caught fish (see Section 3.1) and triplicate samples of ~80 eggs, 80 early stage larvae (around 10 days post-hatch) and 80 small juveniles just prior to release were collected from the hatchery in early 2022 and 2023. Tissue samples and whole eggs/animals were euthanised as described earlier and then stored in 100% ethanol for genomic analyses. DNA extractions from an individual egg did not provide enough quantity (even after repeat attempts using multiple samples) for ddRAD genomic libraries. Since our data analysis requires individual genotypes to be resolved (i.e. pooling individual egg extractions is not an option), the genomic data for the hatchery snapper sample was based exclusively on the 10 days post-hatch larvae and small juveniles. The digestive tracts of wild-caught juvenile snapper were also removed, preserved in 100% ethanol and transferred for use in the WWMSP project *Trophic pathways and food web structure of Cockburn Sound and Owen Anchorage* (Tweedley et al., 2024).

Prior to dissection, most hatchery-reared and a random subset of the wild juvenile snapper were photographed from the side using a digital camera mounted on a tripod with a light source (Table 3.1; Figure 3.1). The resultant photographs of each snapper were loaded into ImageJ (Abramoff et al., 2004; Schneider et al., 2012) and points representing the 16 morphometric landmarks developed by Arechavala-Lopez et al. (2012) for distinguishing wild and cultured sparids were added manually to each image (Figure 3.2). The *x* and *y* coordinates for each landmark were then uploaded into the software package R and the distance between the 30 different pairs of landmarks (referred to as traits) was calculated in pixels and converted to mm using a ruler or marker of a known length captured in the photograph as a scale (Table 3.2).

3.2.3 Statistical analysis of morphometric data

To assess the appropriateness of using morphometric analyses to distinguish potential differences between wild and hatchery-reared snapper, the standard lengths of individual fish were subjected to one-way Analysis of Variance (ANOVA) in IBM SPSS (Version 28.0.1.0). This test compared three groups (i) wild snapper collected from Cockburn Sound on two sampling occasions in November 2021 and February 2022 and two cohorts of hatchery-reared snapper cultured in (ii) 2021/22 (called 2022) and (iii) 2022/23 (called 2023). As these data were normally distributed, no transformation was required. As a significant difference was detected (see Section 4.3.1), proportional measures between each pair of traits were calculated. This takes into consideration all potential morphometric differences that could be used to distinguish wild and hatchery-reared snapper from photographs of fish derived from recreational fishers that would be highly variable in length and help account for variation in total lengths among individuals (see Reist, 1985). A total of 465 possible combinations of traits (i.e. A1/B6; referred to as ratios) were produced and the values for each subjected to Principal Component Analyses (PCA) in SPSS to determine those components accounted for the greatest variation and objectively provided the best measurements to distinguish differences in morphology among wild and hatchery-reared snapper.

The top ten ratios derived from the PCA analyses based on the size of their eigenvalues were then, in turn, each subjected to one-way Multivariate Analyses of Covariance (MANCOVA) in SPSS. These analyses were used to determine, incorporating SL, whether wild snapper differed statistically from the hatchery-reared snapper (2022 and 2023 separately) and thus establish which ratios, if any, would be most appropriate for distinguishing between snapper in a future monitoring program. MANCOVA also computed the means and associated 95% confidence limit for each ratio standardised to the average fish length in the dataset, i.e. 82.5 mm SL.

Figure 3.2. Photographs of wild (top row) and hatchery-reared (middle row) snapper from 2022 and a diagram (bottom left) of the 16 landmarks developed by Arechavala-Lopez et al. (2012) and (bottom right) the superimposition of those marks on a photograph of a wild snapper from Cockburn Sound. Landmarks: 1, tip of the premaxillary; 2, point of maximum curvature in the head profile curve; 3, anterior insertion of dorsal fin; 4, posterior insertion of dorsal fin; 5, dorsal point at least depth of caudal peduncle; 6, posterior extremity of the lateral line; 7, ventral point at least depth of caudal peduncle; 8, posterior insertion of anal fin; 9, anterior insertion of anal fin; 10, anterior insertion of pelvic fin; 11, insertion of the operculum on the profile; 12, dorsal insertion of pectoral fin; 13 most anterior point of the eye; 14, most dorsal point of the eye; 15, most posterior point of the eye; 16, most ventral point of the eye.

Table 3.2. List of traits and their component landmarks calculated in the current study and based on those developed by Arechavala-Lopez et al. (2012).

Trait	Landmark	Trait	Landmark
A1	1-2	C4	3-8
A2	2-10	C5	4-9
A3	10-11	C6	4-10
A4	1-11	D1	4-5
A5	1-10	D2	5-7
A6	2-11	D3	7-8
B1	2-3	D4	4-7
B2	3-9	D5	5-8
B3	9-10	E1	5-6
B4	2-9	E2	6-7
B5	3-10	F1	1-12
B6	3-11	F2	11-12
C1	3-4	F3	6-12
C2	4-8	Eye L	13-15
C3	8-9	Eye H	14-16

The measurements in mm for each of the 30 traits were also subjected to a range of multivariate statistical analyses using PRIMER v7 and the PERMANOVA+ add-on (Anderson et al., 2008; Clarke and Gorley 2015) to determine whether the morphology of the wild and two cohorts of hatchery-reared snapper from the various egg collections and aquaculture runs (2022 and 2023) differed. To account for the differences in SL among individuals, values of each code for an individual snapper were standardised by the SL of that individual (e.g. A1/SL etc). The standardised values for each trait were then normalised, i.e. mean values are subtracted and divided by their standard deviation, this allows each trait to contribute equally when deriving distances between samples, despite values for some traits being larger than others, e.g. F3 length between pectoral girdle and caudal peduncle vs eye height or length (e.g. Tweedley et al., 2015). These pre-treated data were then used to create a Euclidean distance matrix which was then subjected to one-way Analysis of Similarities (ANOSIM) to test for differences between the three groups (P <0.05) and also an index of multivariate dispersion (MVDISP) to determine the dispersion of each group of samples.

Any differences between groups were visualised using traditional non-metric multidimensional scaling (nMDS) and Bootstrapped metric multidimensional scaling (Bootstrapped mMDS) ordination plots and the variables responsible for those differences were shown using a shade plot (Clarke et al., 2014) of the mean standardised and normalised value for each trait in each group. The order of the groups (x-axis) and traits (y-axis) were determined by separate hierarchical cluster analysis of their mutual associations using a Euclidean distance matrix. A type III SIMPROF test was then employed at each node of the trait dendrogram to determine whether the groups of traits being subdivided were significantly different. This test provided an objective method of grouping together traits whose values are similar within a group (red lines), but statistically significant between groups (Clarke et al., 2008).

Canonical Analysis of Principal Coordinates (CAP; Anderson & Robinson 2003; Anderson & Willis, 2003) was used to find axes through the multivariate cloud of points (each representing a napper) that best discriminate among the three *a priori* groups (i.e. discriminant analysis). Superimposed onto the CAP are vectors for traits whose values changed in a linear direction (Pearson correlation ≥ 0.6) relative to

the CAP axes. Having confirmed that the three *a priori* groups are valid using the "trace" test statistic (see Results), cross-validation using a *leave-one-out* procedure was undertaken to calculate the mis-classification rate. Then, assuming the classification rate is sufficient, the CAP routine would be able to predict the group to which a new sample would belong. Thus, measurements from a new snapper could be taken from a photograph and a prediction made as to whether the individual was wild or hatchery-reared. This is a similar approach to that used by Rogdakis et al., (2011) and Arechavala-Lopez et al. (2012) for ilthead sea bream.

As the snapper obtained in 2022 were grown from two egg collection events conducted two weeks apart, resulting in snapper of 77 and 90 days old being analysed, the same suite of multivariate analyses was conducted only using the hatchery-reared fish from 2022. The only exception was that rather than comparing across three groups (one-way analysis), the statistical design employed was two-way nested, i.e. Cohort (2 levels; 77 days and 90 days) and Tank (8 levels, with tanks L1, L2, L3 and L4 containing fish harvested after 77 days and tanks L5, L7, L8 and L10 containing fish harvested after 90 days). The main purpose of this additional analyses was to investigate the effect of (i) the number of days in culture (i.e. ontogeny) and (ii) the extent of variability among individual aquaculture tanks on morphology.

4 Results

4.1 Genetic relationships between Cockburn Sound snapper and west coast stocks

Data analyses were carried out for 765 individual snapper after removing samples with high missing data and removing replicates used for quality control.

Patterns of genomic diversity, connectivity and relatedness based on the entire genomic dataset:

- All samples were genotyped for 10,791 high quality filtered SNPs (average missing data for all snapper is only 0.4%).
- Table 4.1 summarises the results of the genomic diversity for each snapper locality, for each sampling season (2021/2022 and 2022/2023), and for the hatchery sample. All locality samples displayed high levels of genomic diversity. There was no evidence of population inbreeding (most locality FIS were around zero) and there was no difference in diversity between localities (e.g. expected heterozygosity varied from 0.245 to 0.256 for localities with a sample size of 20 or more individuals).
- The results of the PCA (Principal Component Analysis) of all sampled localities are consistent with the hypothesis of only one population (Figure 4.1). Similar results were obtained when only Metro samples (now including larvae and juveniles from the hatchery) were compared (Figure 4.2 A) or when hatchery samples were removed (Figure 4.2 B), and also between adults and juveniles and between sampling seasons (Figure 4.3). These results are consistent with the nil to very low differentiation between localities (Figure 4.4) or between regions (Figure 4.5) in the FST analyses of population differentiation.
- There was no statistical evidence for isolation by spatial distance among localities (P = 0.62). However, the results of the analysis of spatial autocorrelation (Figure 4.6) indicated positive genetic autocorrelation between individuals sampled at the same site, or same locality (Figure 6c). That means that, at the locality level, individuals are more related to each other than expected by chance. This is suggestive of recruitment to the local subpopulation.
- The results of the gene flow models show high connectivity between all pairs of regions (Figures 4.7, 4.8 and 4.9). A clear exception is the South Coast (region 5), which is not connected to other regions when migration rates are equal or greater than 60% per generation (Nm>0.6) (Figures 4.7-4.9). Another potential exception is the Metro South (region 3), but this conclusion is likely influenced by the very small sample size for Metro South localities (ranging from 2 to 8 individuals) as well as for the total Metro South (n = 22).
- The above-described pattern of high connectivity inferred for the Metro region is not influenced by season (Figure 4.7) or life-stage (Figure 4.8), nor by inclusion of hatchery samples (Figure 4.9). In fact, connectivity between hatchery samples and Metro, as well as between hatchery samples and juveniles were also detected at high levels.

The best summary of patterns of connectivity in the study area comes from estimated migration rates per generation (Nm) using only the very-well sampled regions (Figure 4.10). That analysis confirms the patterns of high connectivity described above. The corresponding diagonal-matrix in that same figure shows that inferred migration rates between each pairwise region ranged from 86% to 100%, attesting to the high symmetric connectivity of snapper in the Metro region and its surroundings.

Table 4.1. Summary of genomic diversity for snapper for each locality, for each season (total and per locality) and for the hatchery sample. N=sample size after bioinformatic filtering. nLoc=# of loci. polyLoc=# of polymorphic loci. Ho and He = observed and expected heterozygosity, respectively. FIS= population-level inbreeding coefficient.

Locality	Region	Life stage	Received	Extracted	Sequenced	N	nLoc	polyLoc	Ho	He	FIS
Seabird	MetroN	Adults	10	7	7	6	10791	8305	0.282	0.243	-0.062
Guilderton	MetroN	Adults	24	22	22	22	10791	10110	0.253	0.250	0.008
Two Rocks	MetroN	Adults	57	56	41	41	10791	10525	0.255	0.252	0.000
Yanchep	MetroN	Adults	6	6	5	5	10791	7636	0.263	0.230	-0.028
Hillarys	MetroN	Adults	6	6	5	5	10791	7798	0.266	0.233	-0.028
Cockburn Sound	Metro	Adults	105	102	80	79	10791	10704	0.258	0.255	-0.004
Warnbro Sound	Metro	Adults	19	19	19	19	10791	10066	0.253	0.249	0.013
Dawesville	MetroS	Adults	6	6	6	6	10791	8187	0.264	0.236	-0.024
Bouvard Reef	MetroS	Adults	8	8	8	8	10791	8708	0.253	0.238	0.005
Preston Beach	MetroS	Adults	6	6	6	6	10791	8137	0.258	0.235	-0.007
Binningup	MetroS	Adults	2	2	2	2	10791	5103	0.255	0.193	0.010
Peppermint Beach	SouthWest	Adults	22	13	13	13	10791	9613	0.255	0.247	0.008
Port Geographe	SouthWest	Adults	23	13	12	12	10791	9444	0.253	0.244	0.008
Busselton	SouthWest	Adults	13	13	12	12	10791	9375	0.250	0.242	0.014
Geographe Bay	SouthWest	Adults	26	26	20	20	10791	10063	0.252	0.248	0.008
Dunsborough	SouthWest	Adults	12	10	7	7	10791	8426	0.253	0.236	0.004
South West Bank	SouthWest	Adults	17	12	7	6	10791	8111	0.251	0.233	0.010
Augusta	SouthWest	Adults	45	16	9	9	10791	9042	0.268	0.245	-0.034
Windy Harbour	SouthCoast	Adults	67	55	20	20	10791	9937	0.253	0.245	-0.007
Season 1, 2021/2022		Juveniles	168	144	90	86	10791	10687	0.254	0.254	0.004
Cockburn Sound	Metro	Juveniles	82	58	50	50	10791	10564	0.253	0.252	0.007
Port Geographe	SouthWest	Juveniles	86	86	40	36	10791	10412	0.255	0.252	0.000
Season 2, 2022/2023		Juveniles	226	201	150	149	10791	10771	0.256	0.256	0.004
Two Rocks	MetroN	Juveniles	74	54	40	40	10791	10486	0.254	0.253	0.009
Cockburn Sound	Metro	Juveniles	54	49	30	40	10791	10511	0.256	0.255	0.003
Warnbro Sound	Metro	Juveniles	48	48	40	30	10791	10502	0.253	0.254	0.003
Port Geographe	SouthWest	Juveniles	50	50	40	39	10791	10514	0.256	0.252	-0.001
Hatchery		Larvae & Juveniles	>500	355	240	232	10791	10777	0.257	0.256	-0.004

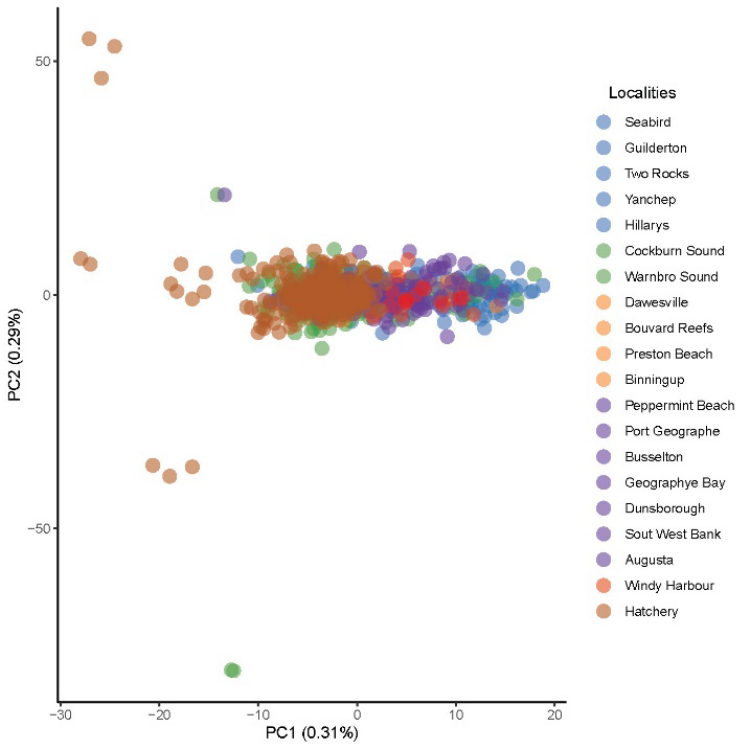


Figure 4.1. PCA based on 10,791 SNPs for snapper collected across all localities and the hatchery sample in 2021/22 and 2022/23 seasons.

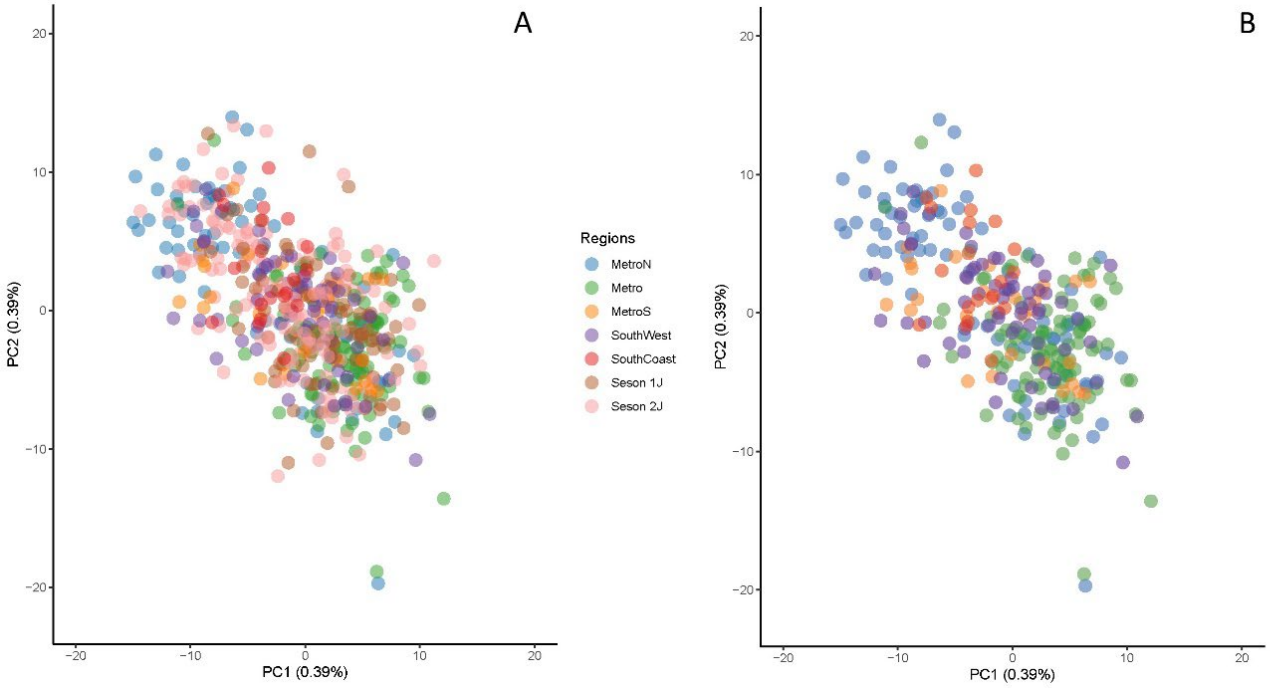


Figure 4.2. PCAs based on 10,791 SNPs for snapper collected in 2021/22 and 2022/23 separated by regions. A) includes both juveniles and adults; B) include only adults. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour), Season 1J and Season 2J are juvenile samples collected in 2021/22 and 2022/23).

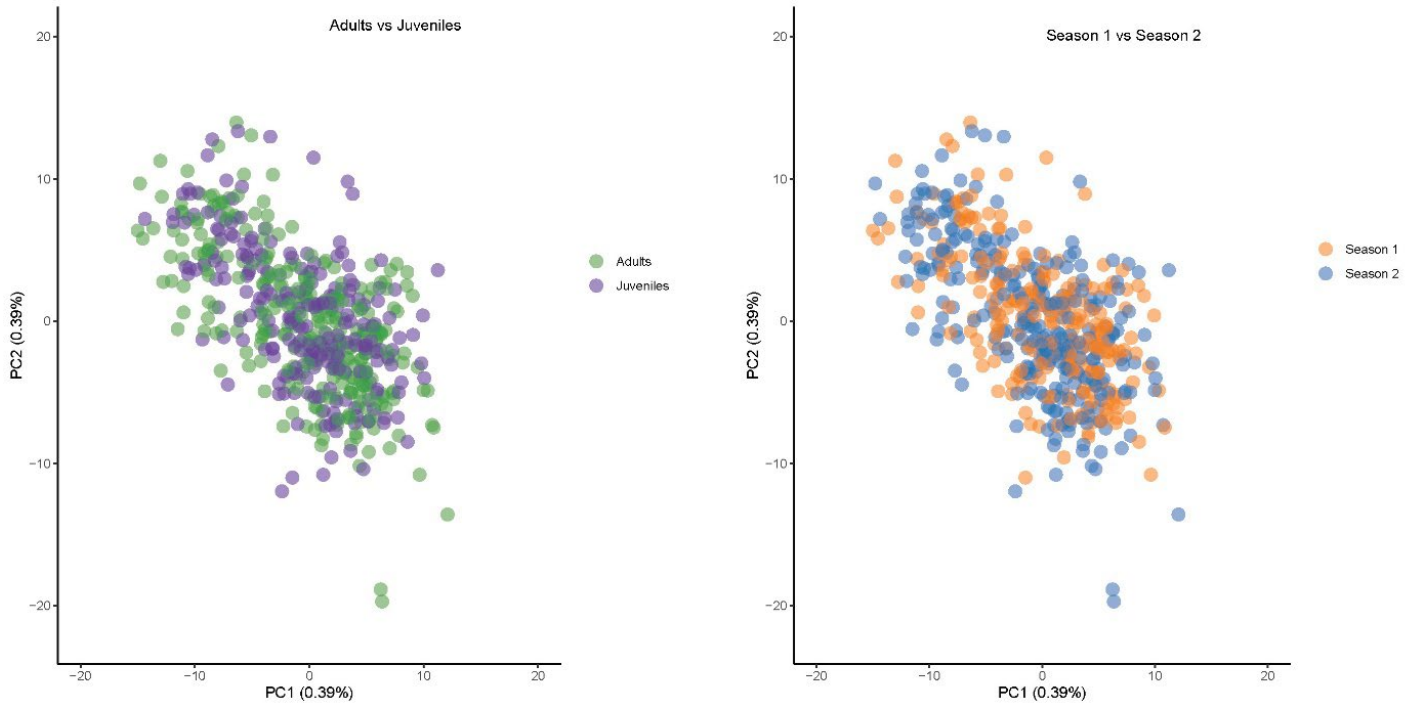


Figure 4.3. Principal components analysis of all SNPs derived from samples of snapper collected in 2021/22 and 2022/23 across all regions A) Comparing adults and juveniles B) Comparing the two different seasons.

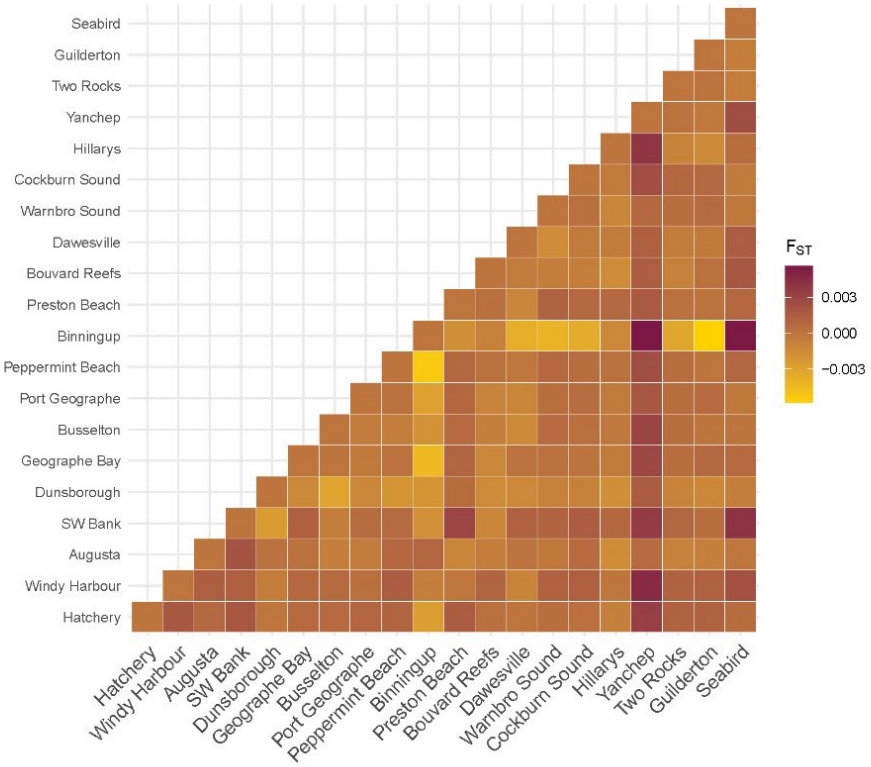


Figure 4.4. Locality-based analysis of population differentiation. Heatmap of pairwise comparisons of FST using 10,791 SNPs for snapper collected in the 2021/22 and 2022/23 seasons and across all localities and from the hatchery.

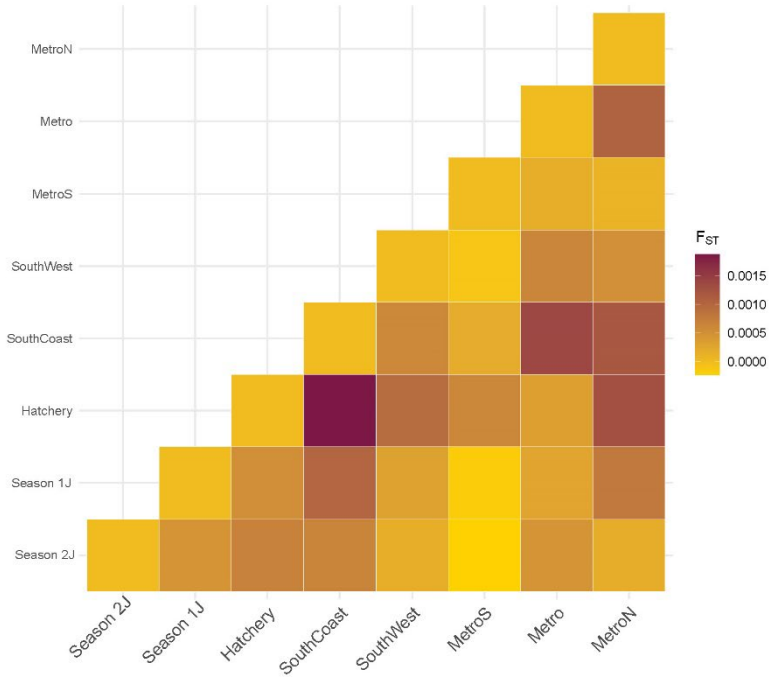


Figure 4.5. Region-based analysis of population differentiation. Heatmap of pairwise comparisons of FST using 10,791 SNPs for snapper collected in the 2021/22 and 2022/2023 seasons and across all regions, from the hatchery and juveniles separated by season. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour), Season 1J, Season 2J and Hatchery are juvenile samples from 2021/22 and 2022/23).

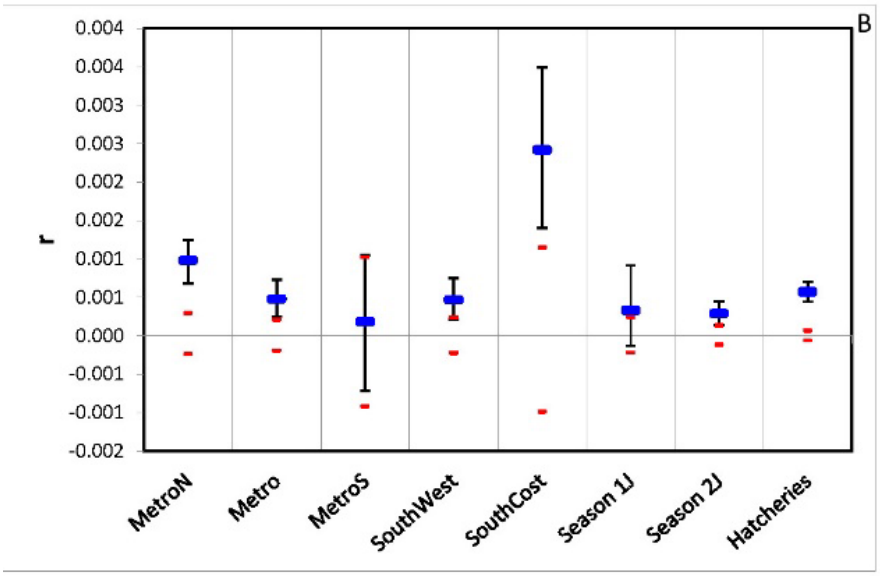


Figure 4.6. Spatial autocorrelation analysis using 10,791 SNPs for snapper collected in the 2021/22 and 2022/23 seasons across all regions, and including the hatchery sample. Red dots indicate the upper and lower bounds of the 95% confidence interval for the null hypothesis of no spatial structure, as determined by permutation. Bars represent the 95% confidence intervals of the error determined by bootstrapping. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour), Season 1J, Season 2J and Hatchery are juvenile samples from 2021/22 and 2022/23).

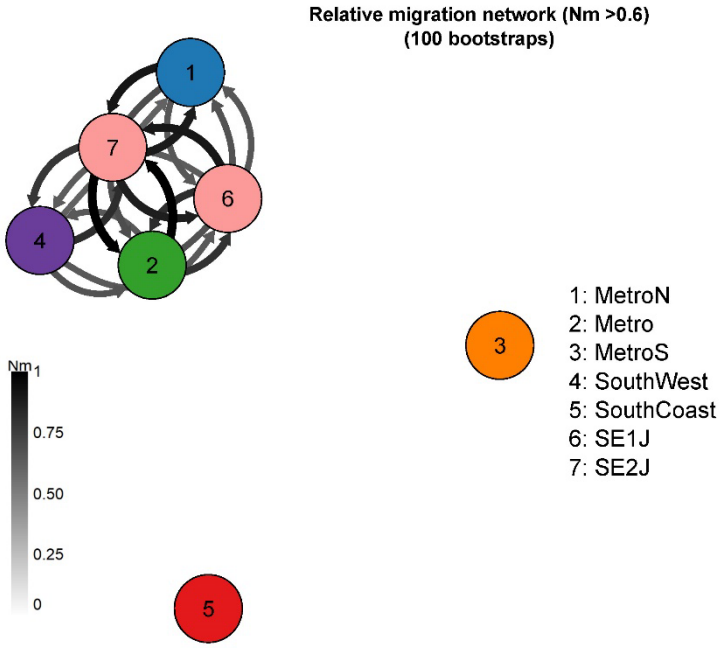


Figure 4.7. Relative migration rates per generation (Nm) among all regions using 10,791 SNPs for adult and juvenile snapper collected in both the 2021/22 and 2022/23 seasons. The thickness and darkness of the arrows are proportional to migration rates. Only migration rates >= 0.6 that were consistently recovered after 100 bootstraps are shown. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour), SE1J are SE2J are juvenile samples from 2021/22 and 2022/23).

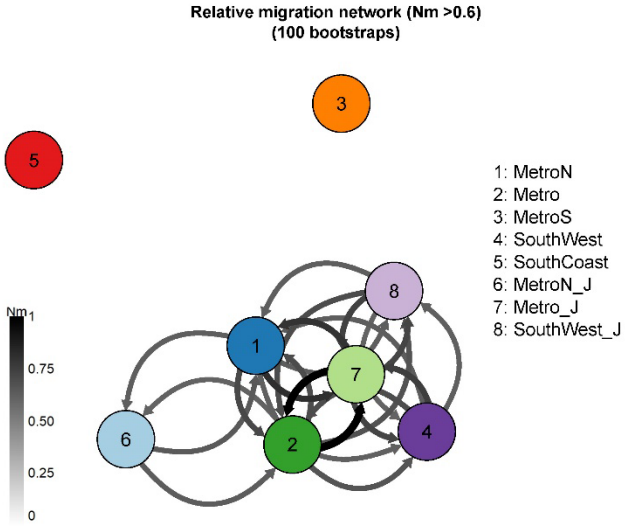


Figure 4.8 Relative migration rates per generation (Nm) among juveniles and adults from each region. The thickness and darkness of the arrows are proportional to migration rates. Only migration rates >= 0.6 that were consistently recovered after 100 bootstraps are shown. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour), MetroN_J – Metro North juveniles (Two Rocks), Metro_J – Metro juveniles (Cockburn/Warnbro Sounds), SouthWest_J – South-west juveniles (Port Geographe).

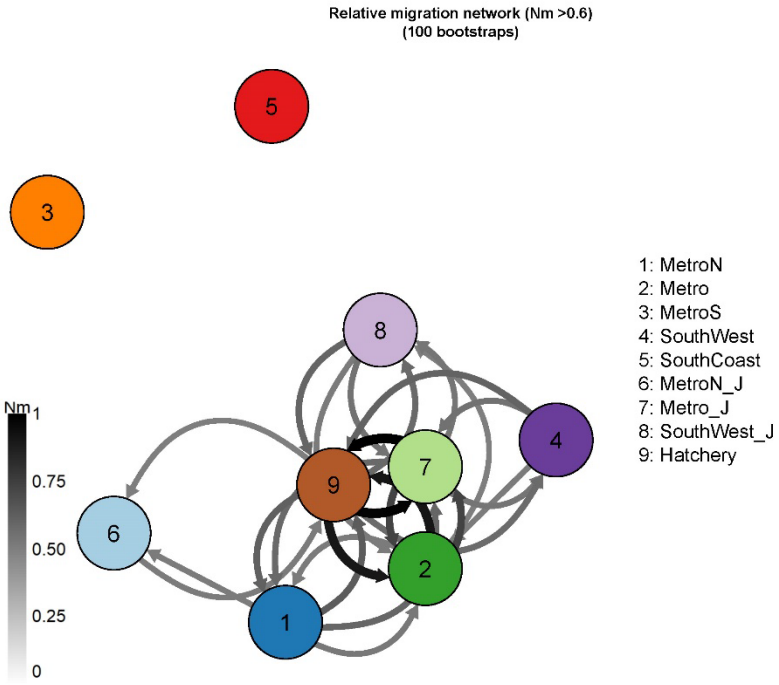
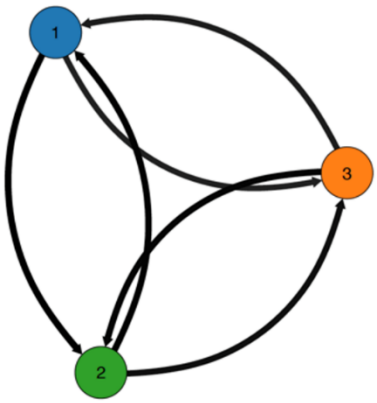


Figure 4.9. Relative migration rates per generation (Nm) among adults and juveniles from each region and hatchery-reared juveniles. The thickness and darkness of the arrows are proportional to migration rates. Only migration rates >= 0.6 that were consistently recovered after 100 bootstraps are shown. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour), MetroN_J – Metro North juveniles (Two Rocks), Metro_J – Metro juveniles (Cockburn/Warnbro Sounds), SouthWest_J – South-west juveniles (Port Geographe), Hatchery – samples obtained from hatchery rearing.



Relative migration network (Nm>0.6)
(100 bootstraps)
1: Metro N
2: Metro
3: SouthWest

Migration	To		
From	**MetroN**	**Metro**	**SouthWest**
MetroN	NA	1.0000	0.8647
Metro	0.9765	NA	0.9341
SouthWest	0.8826	0.9726	NA

Figure 4.10. Relative migration rates per generation (Nm) using 10,791 SNPs for snapper collected in the 2021/22 and 2022/23 seasons across the three well-sampled regions. The thickness and darkness of the arrows are proportional to migration rates (see Figs 4.7-4.9). Only migration rates >= 0.6 that were consistently recovered after 100 bootstraps are shown. The table shows a diagonal-matrix with the inferred migration rates between each pairwise region. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), South-west (Geographe Bay to Augusta).

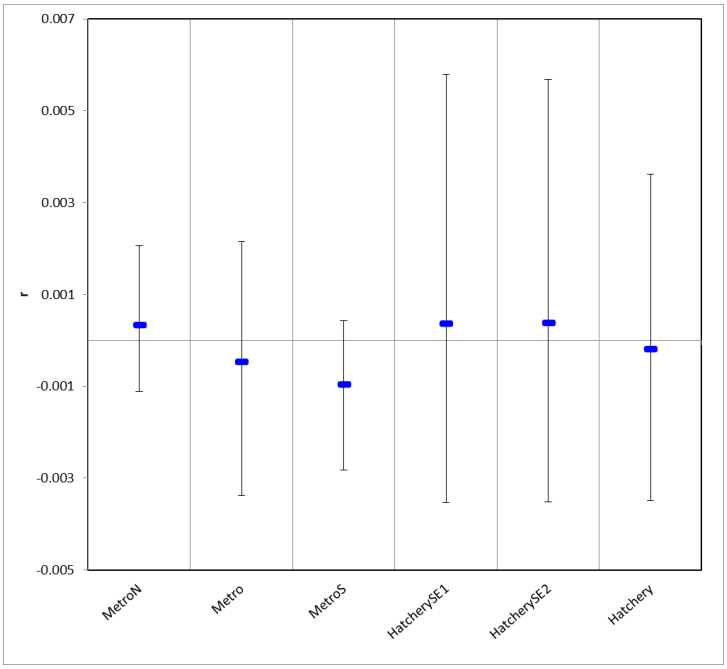


Figure 4.11. Average pairwise relatedness of snapper from the Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sounds) and Metro South regions (Dawesville to Binningup), and from hatchery samples. Hatchery samples are shown per season (SE1 - 2021/22, SE2 - 2022/23), as well as combined seasons. Blue dot represents the average in the sample and bars the 95% confidence intervals..

4.2 Evaluation of localised adaptation using seascape genomics

The RDA shows that 0.87% of the genetic variation in the samples can be explained by the sea surface temperature variables used in the model (Figure 4.12). A significant association was detected between January minimum SST and genetic diversity in our dataset, pointing to a total of 192 candidate adaptive SNPs. However, this association was not statistically significant after controlling for geographic distance. These results indicate that variation in minimum SST is probably shaping adaptive diversity of snapper in the region, but this influence is not impacting on population connectivity after we account for the effects of spatial configuration of the populations.

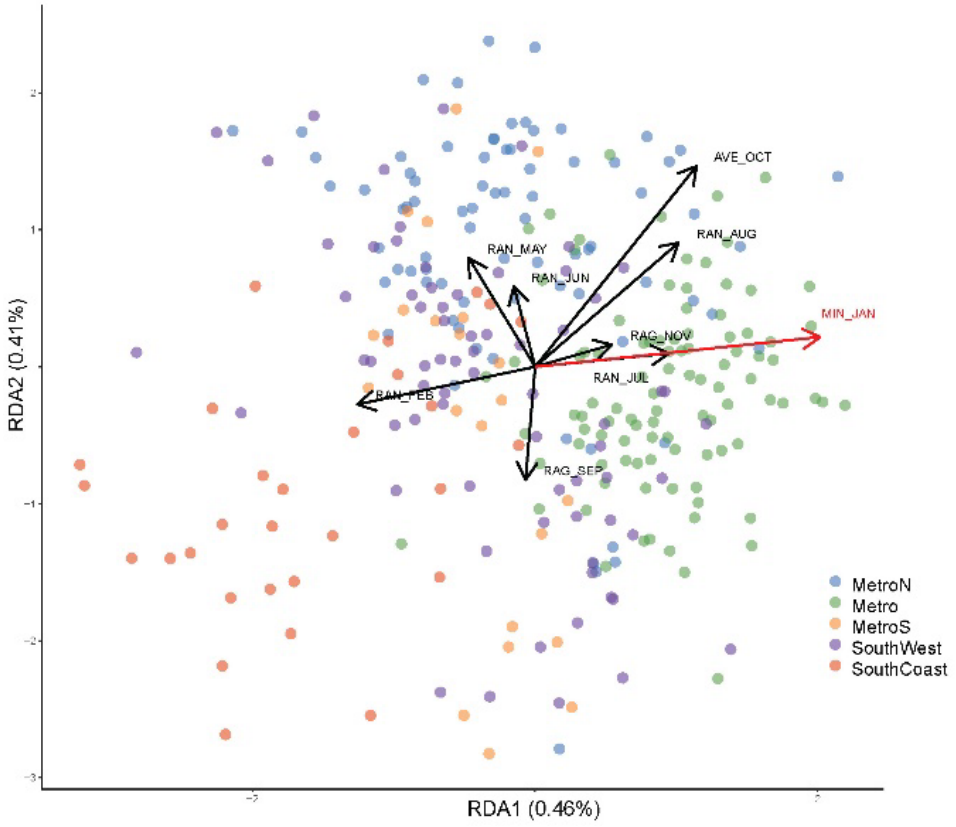


Figure 4.12. Plot of the Redundancy analysis (RDA) conducted on snapper collected during the 2021/22 and 2022/2023 seasons and across all regions. The plot shows the first two RDA axes, which explain 0.87% of the variation in the samples. The sea surface temperature variables retained in the model are shown as arrows. RAN = range, AVE = average and MIN = minimum. Minimum sea surface temperature during January (red arrow) significantly explains the genetic pattern in the region without controlling for the effects of geographic distance. (MetroN – Metro North (Hillarys to Seabird), Metro (Cockburn and Warnbro Sound), MetroS – Metro South (Dawesville to Binningup), South-west (Geographe Bay to Augusta), South Coast (Windy Harbour).

4.3 Contribution of Cockburn Sound snapper to reproductive potential

Female snapper ≥ L_{50} maturity (585 mm) in Cockburn Sound during the main spawning period were mostly much larger than fish sampled during the same months from the metro and south-west oceanic waters, with almost no fish < 700 mm in the embayment (Figure 4.13). Large females were also collected in oceanic waters, but ~50% of fish were < 700 mm. A similar pattern was evident from length distributions of female snapper that were in spawning condition from each location, except that spawning females collected in oceanic waters were as small as 404 mm (Figure 4.14).

As a result, the median batch fecundities of female snapper greater than the L_{50} at maturity and females in spawning condition were greater in Cockburn Sound (681,000 and 648,000 oocytes, respectively) than oceanic waters (340,000 and 374,000 oocytes) (Figures 4.13, 4.14). Based on the percentage frequency of females in each length class, the relative contribution of Cockburn Sound females, in terms of batch fecundity, was ~1.6 times that of snapper in oceanic waters in the metro and south-west (based on the two methods of selecting mature/spawning females from datasets).

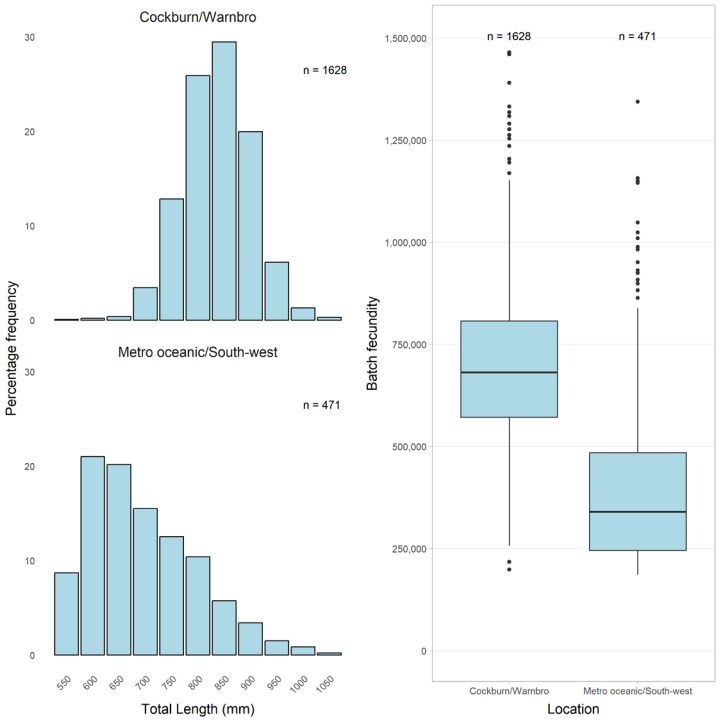


Figure 4.13. Length frequency distributions of female snapper ≥ 585 mm (the estimated length at which 50% of females reach sexual maturity, L_{50}) from Cockburn/Warnbro Sounds and oceanic waters of the metro and south-west regions during the main spawning period (left column) and box and whisker plots of the estimated batch fecundities of all females ≥ L_{50} at maturity (right column).

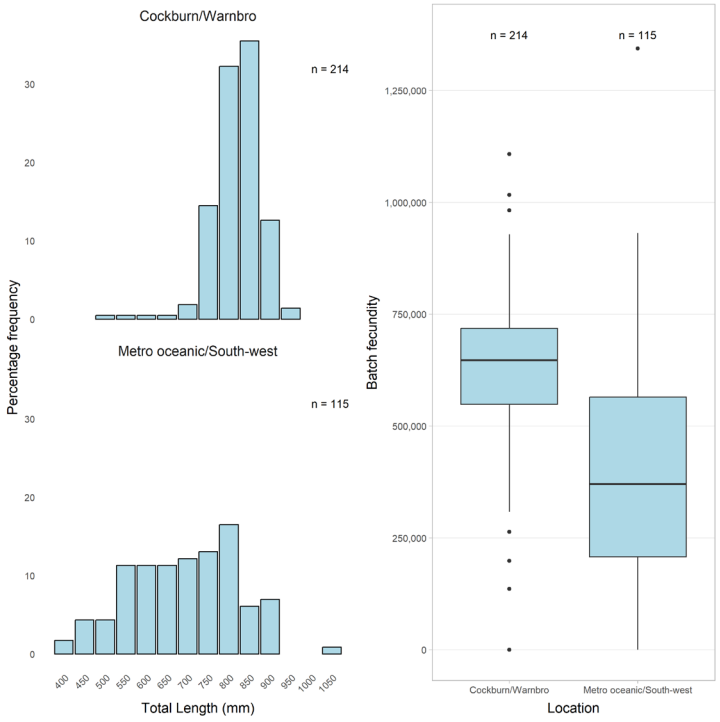


Figure 4.14. Length frequency distributions of female snapper in spawning condition (ovary stage V) from Cockburn/Warnbro Sounds and oceanic waters of the metro and south-west regions (left column) during the main spawning period and box and whisker plots of the estimated batch fecundities of all females in spawning condition (right column).

4.4 Evaluation of methods for monitoring aspects of stock enhancement programs

4.4.1 Genomic analyses for aims 3 and 4

As described in Section 4.1, the comparison between wild caught samples and hatchery snapper showed that genomic diversity of the hatchery sample was high and consistent with that of the wild caught sample. This trend was observed across all localities and there was no evidence of inbreeding in the dataset (Table 4.1; Figures 4.1-4.10).

To test whether hatchery-raised samples represent an unbiased sample from the highly-connected Metro region, we estimated pairwise relatedness for all individuals in each region and compared that with the average pairwise relatedness from the hatchery samples per season, as well as combining the two seasons into a single hatchery sample. The average relatedness from hatchery samples was similar and highly comparable to those found in other Metro wild fish samples (Figure 4.11). A few pairs of individuals from the hatchery showed greater relatedness than any other pair from the Metro samples. Just 16 pairs of full siblings and 18 pairs of second-degree relationships (half siblings, cousins) were found.

4.4.2 Univariate morphometric analyses

ANOVA detected an overall significant difference in the SLs of the snapper from the three groups, i.e. wild and hatchery-reared in 2022 and 2023 (P = 0.001). At a pairwise level, the SL of wild-caught snapper were significantly larger (mean = 114; range = 44-164 mm SL) than those in both hatchery groups (both P = 0.001), but there was no difference between the two hatchery groups (P = 0.180). Hatchery-reared fish had a mean size of 46 mm SL (range = 27-63) in 2022 and a mean of 48 mm SL (range = 36-58 mm) in 2023 (Figure 4.15).

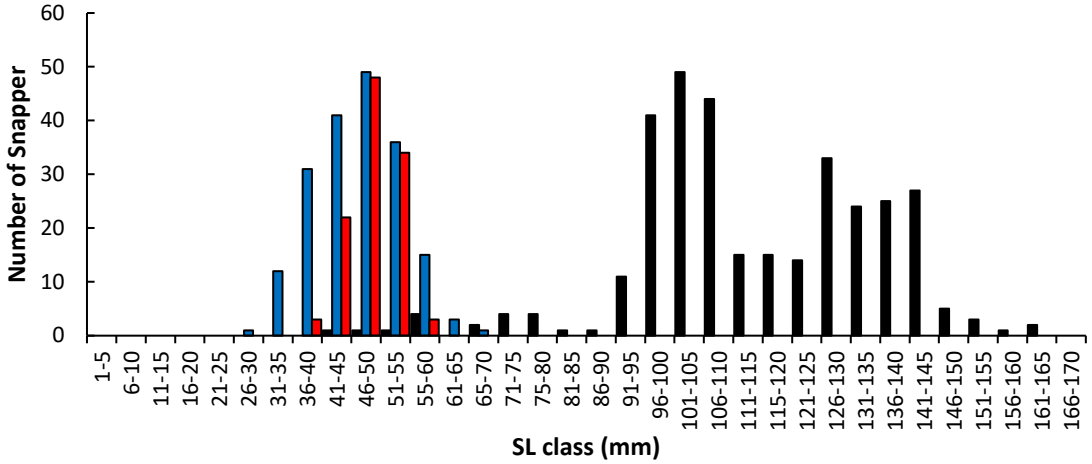


Figure 4.15. Length frequency distributions of the number of wild snapper collected from Cockburn Sound (■) and those reared in the hatchery in 2022 (■) and 2023 (■).

PCA analysis demonstrated that, among the 465 ratios of traits, ten explained 86% of the total variance (Figure 4.16). Of these, A1/B6 and Eye Length/B1 explained 23.4 and 15.0 % of the total variance (Figure 4.17). The relationships between each of those 10 ratios and SL of wild and the two cohorts of hatchery-reared snapper are provided in Figure 4.16. Of those ten, when standardised for SL, significant differences between groups of fish were detected in eight. These were A1/B6, Eye Length/B1, A5/B4, A1/B5, A1/B3, A2/A5, A1/C6 and Eye Height/B1. In four of the eight ratios, significant differences were detected between wild and hatchery-reared snapper (from both cohorts). These included A1/B6, which accounted for 23.4% of the variation and produced the greatest eigenvalue (λ = 109). The mean of A1:B6 for wild snapper (0.52) thus differed significantly from

hatchery-reared snapper in 2022 (0.49, *P* = 0.029) and 2023 (0.40, *P*=0.001; Figure 4.17), noting that differences were also detected between the different cultures (*P* = 0.020). Similar differences were detected between the means of Eye Length/B1, which accounted for 15.0% of the variation (λ = 69.7), with wild snapper (0.55) differing from hatchery-reared snapper in 2022 (0.42, *P* <0.001) and 2023 (0.31, *P* = 0.009; Figure 4.17). However, no differences were detected in this ratio between the two different hatchery cohorts (*P* = 0.248), and this was the only case where a significant difference occurred only between wild and hatchery-reared snapper. Significant differences in means were also detected for A1/B5, with that of wild snapper (0.50) being greater than those of the hatchery-reared snapper in 2022 (0.47, *P* = 0.018) and 2023 (0.37, *P* = 0.001). Similar trends were exhibited by the means of Eye Height/B1 with wild snapper (0.53) being greater than and significantly different to those of hatchery-reared snapper (2022 = 0.46, *P* = 0.021; 2023 = 0.25, *P* = 0.002).

In six of the eight analyses where significant differences were detected, those differences involved the comparison between the two hatchery cohorts, i.e. A1/B6 (*P* = 0.020), A5/B4 (*P* = 0.005), A1/B5 (*P* = 0.016), A1/B3 (*P* <0.001), A1/C6 (*P* <0.001) and Eye Height/B1 (*P* = 0.033) (Figure 4.18). For simplicity, the mean and 95% confidence limit for snapper in each group, standardised for length, are provided in Figure 4.19.

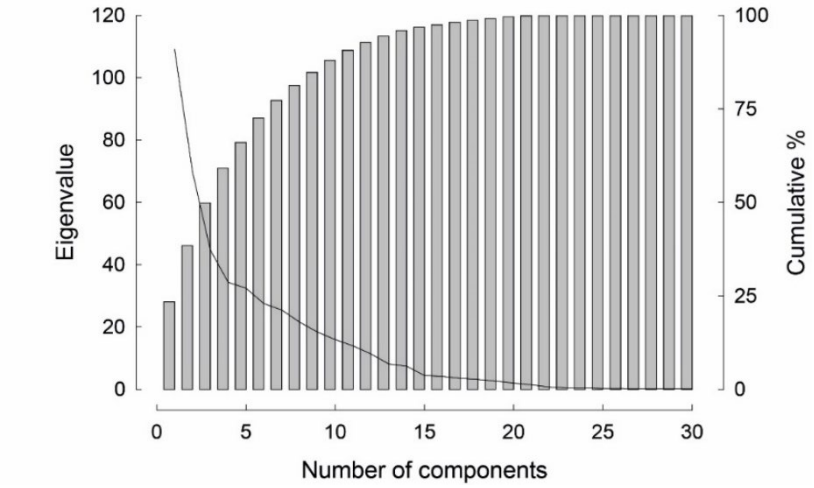


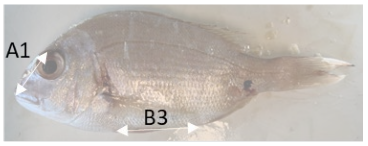
Figure 4.16. Results of Principal Component Analysis showing the Eigenvalue for the 30 most influential component ratios and the cumulative proportion of explained variance explained by increasing numbers of components (ratios).

(a) A1/B6

λ = 109
23.4%
***P* <0.001**

(b) A1/B3

λ = 27.5
5.9%
***P* <0.001**



(c) B1/Eye length

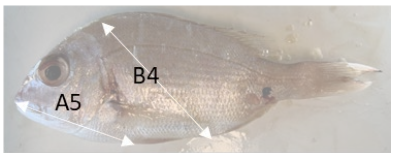
λ = 69.7
15.0%
***P* <0.001**

(d) A2/A5

λ = 25.4
5.5%
***P* =0.004**

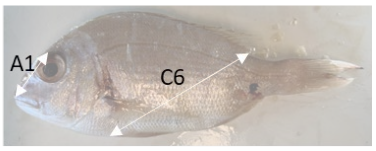
(e) A5/B4

λ = 44.7
9.6%
***P* =0.020**



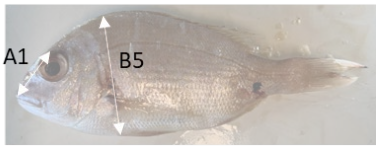
(f) A1/C6

λ = 21.6
4.6%
***P* <0.001**



(g) A1/B5

λ = 34.3
7.4%
***P* <0.001**

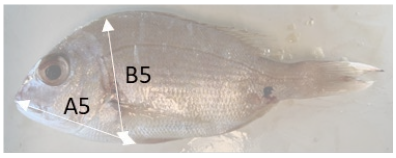


(h) B1/F1

λ = 18.4
3.9%
P =0.813

(i) A5/B5

λ = 32.4
7.0%
P =0.150



(j) B1/Eye height

λ = 15.9
3.4%
***P* <0.001**

Figure 4.17. Top ten ratios identified through Principal Component Analysis (PCA) that provided the greatest degree of explained variance between the wild and two cohorts of hatchery-reared snapper. Eigenvalues (λ) and the proportion of explained variance (%) derived from PCA and *P*-values from MANCOVA. Significant values are highlighted in bold.

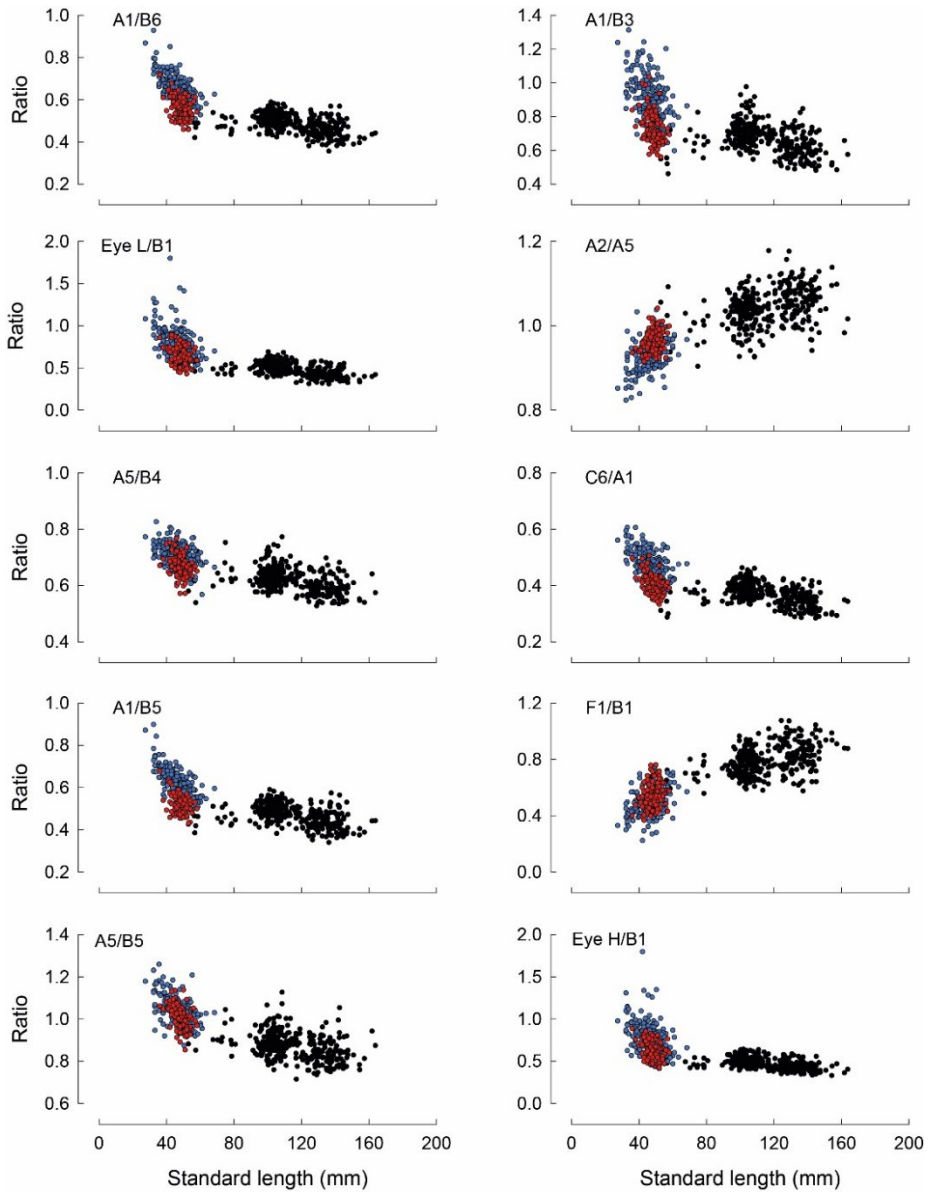


Figure 4.18. Values for the top ten ratios between traits identified through Principal Component Analysis vs standard fish length for wild snapper from Cockburn Sound (■) and those reared in the hatchery in 2022 (■) and 2023 (■).

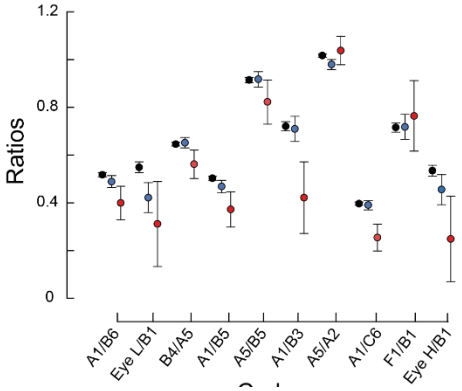


Figure 4.19. Mean value and 95% confidence limits for each of the ten ratios between traits identified through Principal Component Analysis for wild snapper from Cockburn Sound (■) and those reared in the hatchery in 2022 (■) and 2023 (■). Ratios are standardised for an average SL of 82.5 mm and derived from MANCOVA.

4.4.3 Multivariate morphometric analyses

The standardised and normalised lengths of each trait were shown by ANOSIM to differ overall among the three groups, albeit the extent of the difference was relatively low, i.e. $P = 0.001$; Global $R = 0.215$. Differences in morphology were detected in all pairwise comparisons (all $P = 0.001$), being largest between the two hatchery cohorts ($R = 0.301$) and slightly less between the wild and hatchery-reared snapper in 2022 and 2023, i.e. $R = 0.201$ and 0.177, respectively. While the distinction between groups is less clear on the nMDS plot there is clear separation of the mean and associated 95% confidence regions on the bootstrapped mMDS plot (Figure 4.20). The nMDS plot does demonstrate the far larger values of dispersion for the points representing each of the snapper obtained from the hatchery in 2022 and the wild fish (multivariate dispersion = 1.07 and 1.02), compared to hatchery-reared snapper in 2023 (multivariate dispersion = 0.66).

Suites of traits distinguished the various groups of snapper, most notably values were far higher for traits relating to the size of the eye (i.e. eye height and length) and A1 and A6, i.e. distance between the premaxillary and point of maximum curvature in the head and the latter point and the operculum, respectively (Figure 4.21). Thus, these fish have a proportionally deeper head and shorter dorsal fin (C1) than the hatchery-reared fish in both cohorts. Hatchery-reared snapper from 2022 were distinguished by their longer body, i.e. F3 (dorsal insertion of pectoral fin to the posterior extremity of the lateral line) and C6 (posterior insertion of dorsal fin to the anterior insertion of pelvic fin) and their shallower head curve, i.e. B1 (point of maximum curvature in the head profile curve to the anterior insertion of dorsal fin). Finally, hatchery-reared fish in 2023 were distinguished by their longer head, with large values for traits such as F1 (tip of the premaxillary to the dorsal insertion of pectoral fin), F2 (insertion of the operculum to the dorsal insertion of pectoral fin) and A3 (insertion of the operculum to the anterior insertion of pelvic fin).

The same data were subjected to CAP analyses, which used 25 orthonormal PCO axes that incorporated 99.99% of the original variability to produce a constrained CAP plot (Figure 4.22). The canonical correlations for the two axes were relatively large i.e. 0.654 (CAP1) and 0.4912 (CAP2). CAP1 separated both wild snapper and those from the hatchery in 2022 from those hatchery-reared individuals from 2023 based on their low values for F3 and larger values for numerous other traits (Figure 4.22). CAP2 separates the wild snapper (high values for Eye length and width and A1) from hatchery-reared individuals in 2023 (high values for F1, F2 and A3). Significant differences in the three groups in multivariate space were confirmed with a “trace” test statistic, i.e. 1.145; $P = 0.001$. Cross-validation indicated that using the CAP correctly classified 84.15% of juvenile snapper to the group based on their morphology (Table 4.2). Classification rates were best for hatchery-reared snapper in 2023 (>95%) and lowest for hatchery-reared snapper in 2022 (78.24%). In the case where hatchery-reared fish were misclassified, a greater number were misclassified to the wild (Cockburn Sound) group rather than the other hatchery-reared cohort (Table 4.2). For example, of the 32 hatchery-related snapper in 2022 that were incorrectly assigned, 22 were assigned to Cockburn Sound and 10 to the hatchery in 2023.

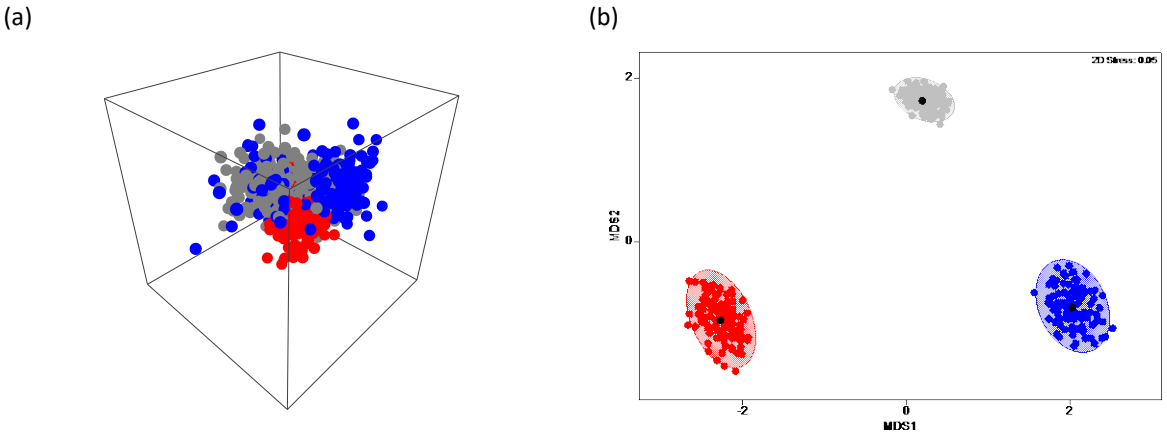


Figure 4.20. (a) Three-dimensional non-metric MDS ordination plot (stress = 0.16) and (b) bootstrapped metric MDS ordination plot constructed using the standardised and normalised value for each trait for each wild snapper from Cockburn Sound (■) and those reared in the hatchery in 2022 (■) and 2023 (■).

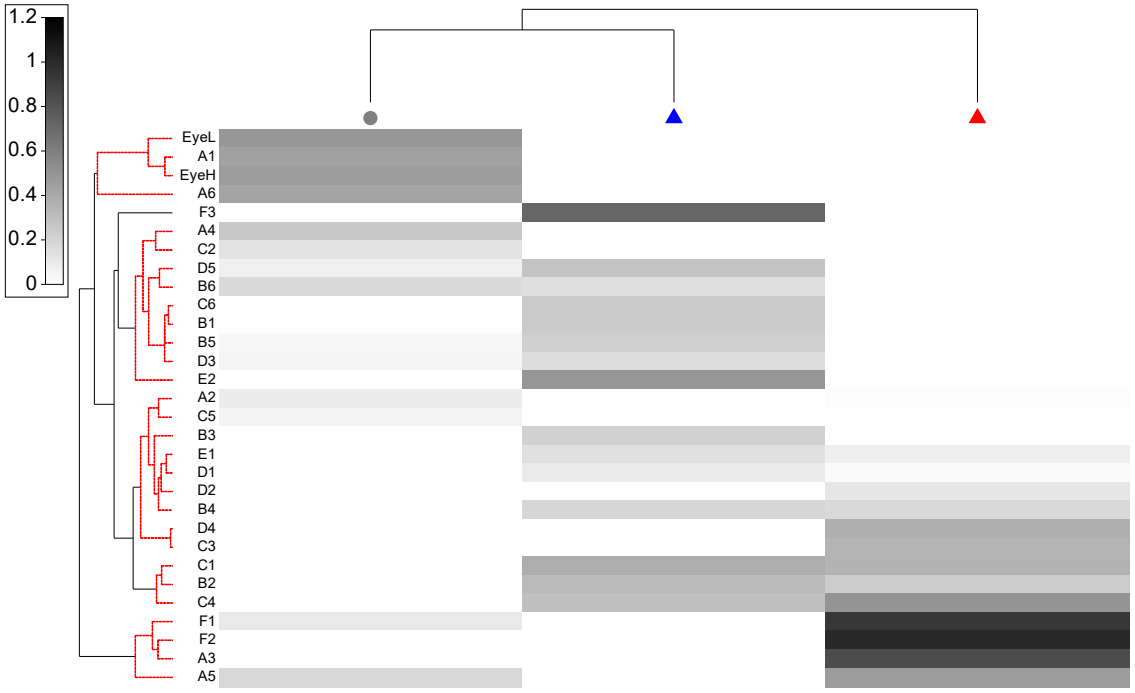


Figure 4.21. Shade plot of the mean standardised and normalised value for each trait for wild snapper from Cockburn Sound (■) and those cultured in 2022 (▲) and 2023 (▲). Traits are ordered by hierarchical cluster analysis, with those joined by red lines having a similar pattern of values across groups. Note only positive values for traits are shown, i.e. highlight which traits were largest.

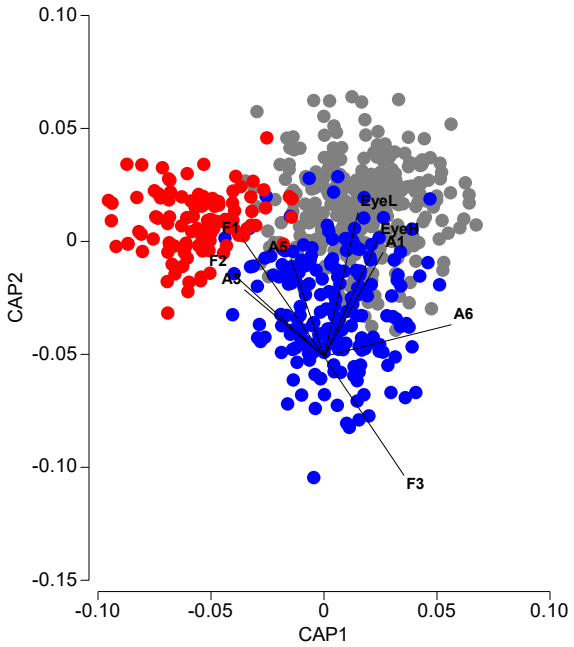


Figure 4.22. Canonical analysis of principal coordinates plot illustrating differences in the morphological traits between wild snapper from Cockburn Sound (■) and those reared in the hatchery in 2022 (■) and 2023 (■). Vectors are provided for traits whose values changes in a linear direction (Pearson correlation ≥ 0.6) relative to the CAP axes.

Table 4.2. Cross-validation results showing the number and percentage of juvenile snapper correctly assigned overall and those for each origin group.

Overall: 84.15% correctly assigned

Original group	Wild	Hatchery 2022	Hatchery 2023	Total	% correct
Wild	275	41	12	328	83.84
Hatchery 2022	32	151	10	193	78.24
Hatchery 2023	4	1	105	110	95.46

As there was considerable variability in the hatchery-reared fish in 2022 compared to those in 2023 (i.e. multivariate dispersion values of 1.07 vs 0.66), the effects of the two egg collections and ages of the resultant snapper when photographed (i.e. 77 vs 90 days) were investigated incorporating the various tanks used in the culture of those fish. While ANOSIM did not detect a difference in the morphology between the two ages of the hatchery-reared snapper from 2022 (Global R = 0.001; P = 0.400), there was a significant tank effect (Global R = 0.268; P = 0.001). This is shown on the associated nMDS plot (Figure 4.23a), where there is considerable overlap of fish of different ages. When the samples in the same plot were coded for tanks some of the tank samples were relatively discrete. Pairwise ANOSIM tests indicate that 24 of the 28 pairwise comparisons were significant (Table 4.3), most notably all those relating to tank L4, which also had greater R-values. The distinctness of the morphology of the snapper in L4 is highlighted on the bootstrapped mMDS plot where the 95% confidence regions for these samples is well separated from those of all other tanks on the left-hand side of the ordination (Figure 4.23c). In fact, excluding L4 led to a reduction in the Global R from 0.268 to 0.148. Among the pairwise comparisons that were not significant were some involving tanks containing fish of different ages, e.g. L2 (77 days) vs L5 (90 days) and L3 (77 days) vs L10 (90 days), with the confidence regions for these tanks overlapping considerably.

Table 4.3. *R*-values derived from post-hoc testing using ANOSIM on the standardised and normalised value for each trait for each hatchery-reared snapper among culture runs and tanks in 2022. Pairwise differences that were not significant, i.e. $P > 0.05$, are shaded in grey.

Culture run		77 day old cohort				90 day old cohort		
	Tank	***L1***	***L2***	***L3***	***L4***	***L5***	***L7***	***L8***
77 day old cohort	***L2***	0.288						
	L3	0.102	0.185					
	L4	0.502	0.803	0.421				
90 day old cohort	***L5***	0.452	0.032	0.268	0.846			
	L7	0.178	0.056	0.096	0.755	0.161		
	L8	0.105	0.096	0.030	0.712	0.207	0.008	
	L10	0.138	0.209	0.042	0.273	0.229	0.213	0.137

The shade plot illustrates that the distinctness of the fish in L4 was mainly due to them possessing a relatively large eye for their size and a longer head (Figure 4.24). There were also several traits that were relatively consistent across fish in all tanks regardless of their age, e.g. C1 and C4. Moreover, fish in L2 were very similar to those in L5 in a number of traits including B1, B5, B6, C6, B3 and B2 despite being in different cohorts. Fish from tanks L3 (77 days) and L10 (90 days) where similar to each other and different from all others due to their lower values for most traits.

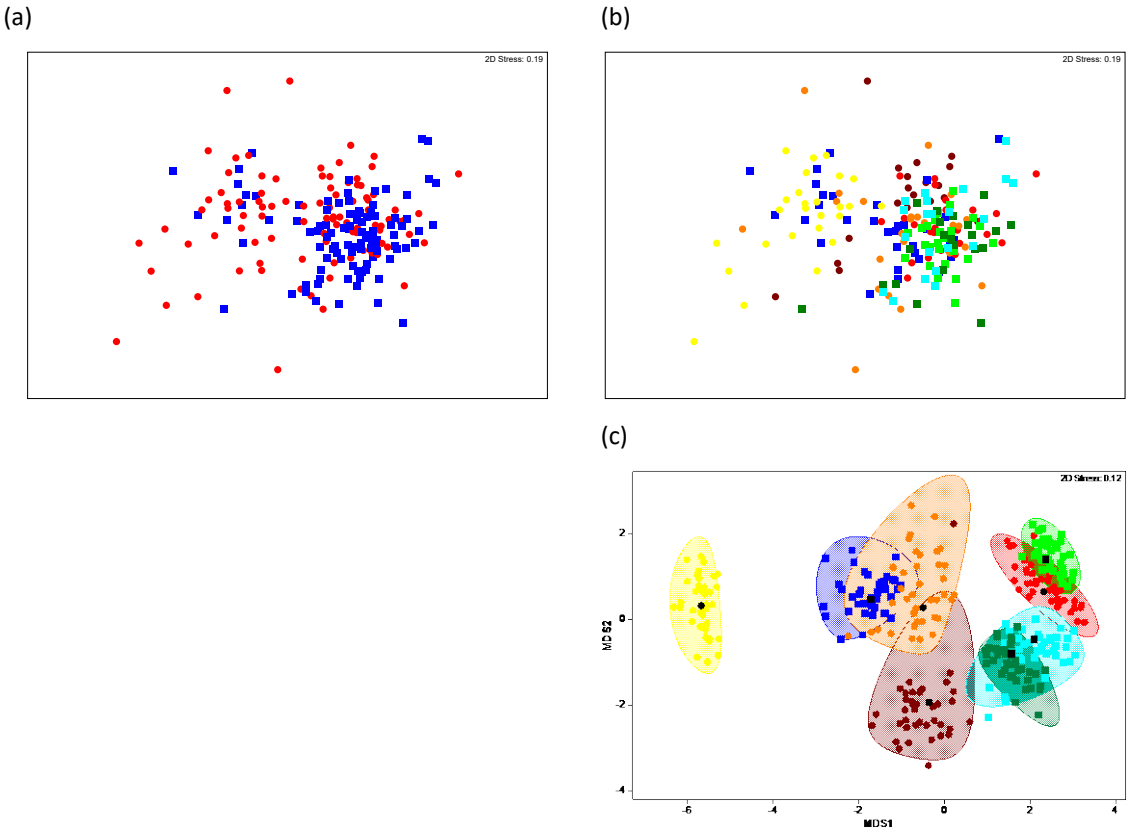


Figure 4.23. (a) Two-dimensional non-metric MDS ordination plot constructed using the standardised and normalised value for each trait for each hatchery-reared snapper in 2022 coded for (a) Cohort (77 days ■; 90 days ■) and (b) Tank (L1 ■; L2 ■; L3 ■; L4 ■; L5 ■; L7 ■; L8 ■; L10 ■. (c) bootstrapped metric MDS ordination plot constructed for each tank.

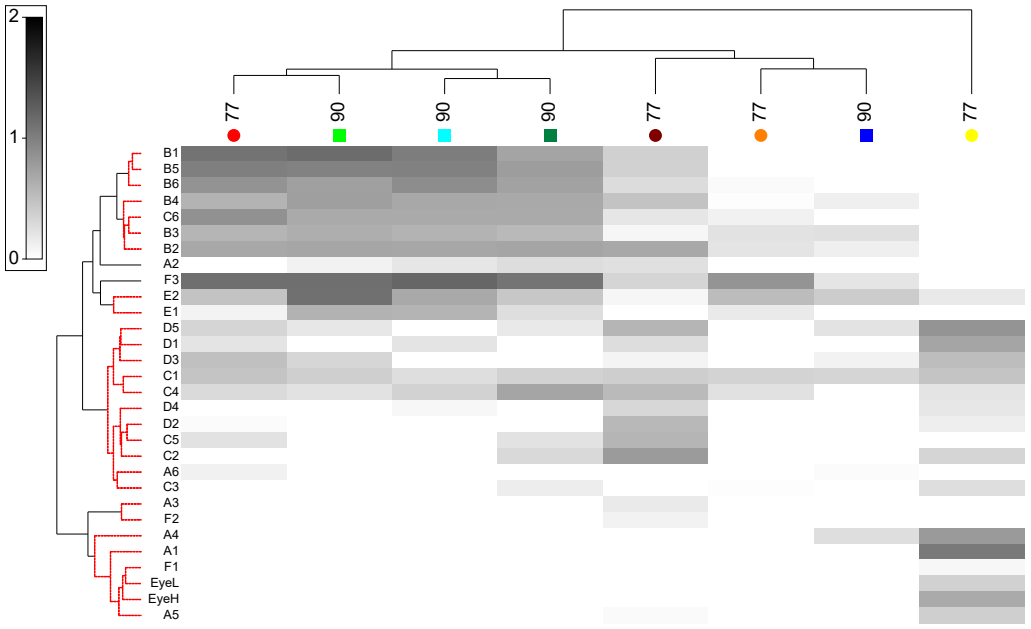


Figure 4.24. Shade plot of the mean standardised and normalised value for each trait for hatchery-reared snapper in each tank in 2022. Snapper in tanks L1 ■; L2 ■; L3 ■; L4 ■ were 77 days old when photographs and those in tanks L5 ■; L7 ■; L8 ■; L10 ■ were 90 days old at the same time. Traits are ordered by hierarchical cluster analysis, with those joined by red lines having a similar pattern of values across groups. Note only positive values for traits are shown, i.e. highlight which traits were largest.

CAP also detected significant differences in the morphology of the hatchery-reared snapper from the different tanks ("trace" test statistic = 2.194; *P* = 0.001). The vectors indicate that the separation of tanks on CAP1 was driven mainly by fish in tanks L4 and L10 having a relatively larger eye whereas those in L2, L5 and L7 possess a longer body (Figure 4.25). Despite using 25 orthonormal PCO axes incorporating 99.00% of the original variability producing the constrained plot with canonical correlations of 0.778 and 0.558, the distinction between the points representing each fish were less clear. This mirrors the reduced differences in morphology observed in the two shade plots (cf. Figures 4.21 and 4.24). Cross-validation indicated that using the CAP correctly classified only 47.93% of juvenile snapper to their correct tank and 59.92% to their correct culture run based on their morphology (Table 4.4). Classification rates varied substantially among tanks, i.e. from as low as 20% for L2 to almost 85% for L4. In fact such was the uniqueness of the morphology of fish in tank L4 that the classification rate declined to 15% when including other tanks in that culture run. Moreover, when fish were misclassified it was rarely to that tank.

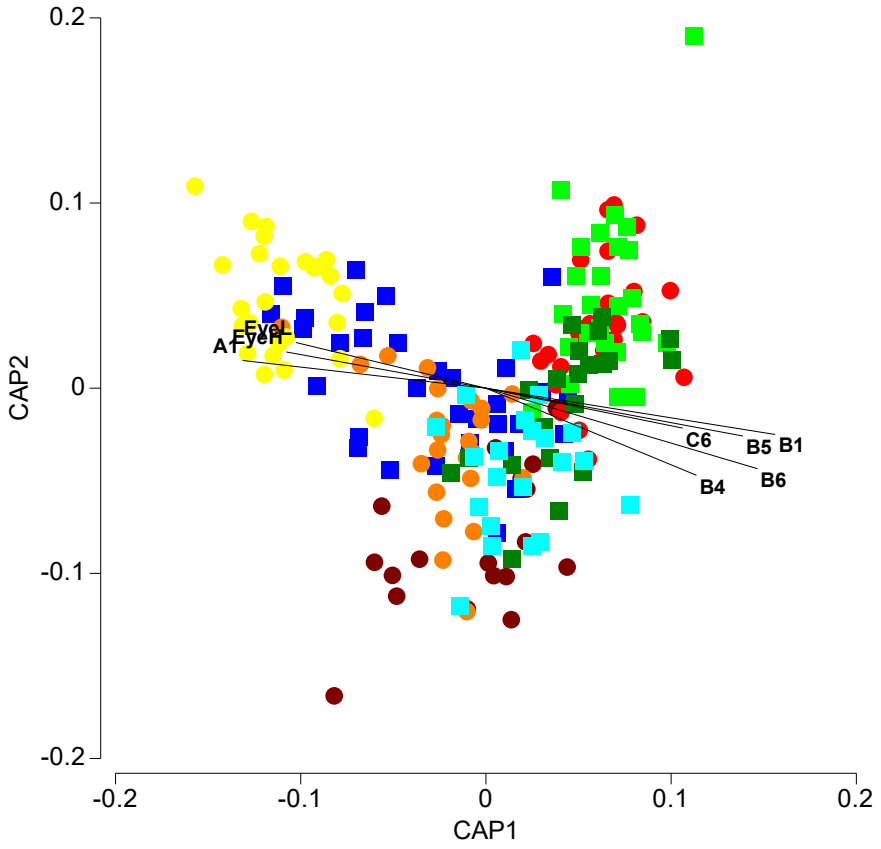


Figure 4.25. Canonical analysis of principal coordinates plot illustrating differences in the morphological traits between hatchery-reared snapper in each tank in 2022. Snapper in tanks L1 ●; L2 ●; L3 ●; L4 ● were 77 days old when photographs and those in tanks L5 ■; L7 ■; L8 ■; L10 ■ were 90 days old at the same time. Vectors are provided for traits whose values changes in a linear direction (Pearson correlation ≥ 0.6) relative to the CAP axes.

Table 4.4. Cross-validation results showing the number and percentage of juvenile snapper correctly assigned to each tank and culture run overall and those for each original group.

Overall: 47.93% (tank level) and 59.92% correctly assigned												
Culture run and tank		**77 day old cohort**				**90 day old cohort**				**Tank level**		**Culture run level**	
	Original group	**L1**	**L2**	**L3**	**L4**	**L5**	**L7**	**L8**	**L10**	**Total**	**% correct**	**Total**	**% correct**
77 day old cohort	*L1*	11	1	2	0	0	1	4	0	19	57.90	8	42.11
	L2	0	5	0	0	10	8	2	0	25	20.00	20	80.00
	L3	2	0	15	3	0	0	3	1	24	62.50	9	37.50
	L4	0	0	0	22	0	0	0	4	26	84.62	4	15.38
90 day old cohort	*L5*	0	9	0	0	15	1	1	0	26	57.69	11	42.31
	L7	1	5	3	0	2	8	1	1	21	38.10	13	61.90
	L8	5	0	3	0	0	5	7	1	21	33.33	14	66.67
	L10	3	0	6	7	2	0	4	10	32	31.25	22	68.75

5 Discussion

5.1 Genetic relationships and contributions of Cockburn Sound snapper to west coast stocks

Bertram et al. (2022) and Gardner et al. (2022) provided insight into patterns of connectivity and other processes at large scales between Cockburn Sound and elsewhere along the west and south coasts, with Bertram et al. (2022) demonstrating delineation of a south-western population from Cockburn Sound to Albany. However, knowledge about fine-scale patterns of connectivity within the south-western Australian 'population' is needed to identify the relative importance of the output of Cockburn Sound spawning aggregations to the broader population with respect to existing and ongoing marine infrastructure development and human use in Cockburn Sound. This study therefore compared individuals from locations within the south-western population, including the northern metropolitan region (Metro North – Hillarys to Seabird), Cockburn/Warnbro sounds (i.e. Metro), southern metropolitan region (Dawesville to Binningup), the south-west region (Geographe Bay to Augusta) and the western south coast (Windy Harbour).

The powerful genomic dataset generated, both in number of DNA markers and in number of individuals genotyped, enabled us to conclusively assess genetic structure within the south-western Australian population and to test several scenarios of connectivity between Cockburn and Warnbro Sounds and oceanic snapper in that south-western population (Fig. 2.3). The results from all analyses of genomic data point to a single and well-connected genetic population within the south-west region. The estimates of relative migration rates per generation varied between 86% and 100% among pairs of locations, except for the south coast, with < 60% migration to regions on the lower west coast. The highest connectivity was estimated between Metro and the adjacent Metro North oceanic snapper (97% and 100%). These results strongly indicate symmetric patterns of connectivity between these two regions, and no evidence of isolation by spatial distance. Importantly, such results remain the same when analyses considered samples from different seasons or life-stages, consistent with the proposal of a single population in south-western Australia, from the south-west region to Metro North. Migration rates between this population and the south coast sites were much lower, indicating they belong to different stocks.

Our results also indicate that snapper are recruiting locally into their subpopulations, including in Cockburn Sound, consistent with the findings of Bertram et al. (2022) that used a much smaller sample across WA and also Jackson et al. (2023) on the mid-west coast. These results also reflect those of previous biological studies that demonstrated that within Cockburn Sound, eggs and larvae produced by spawning aggregations are entrained in wind-driven counter-clockwise currents driving settlement within the sound and the occurrence of juveniles in this nursery environment for around the first 18 months of life (Wakefield, 2010; Wakefield et al., 2011). They also reflect the fact that there is spawning along the open coastline and numerous nursery environments between the northern metropolitan and south-west coasts, such as Geographe Bay, consistent with biological data collections in this and previous studies (see e.g. Fairclough et al., 2013). Therefore, this demonstrates that spawning aggregations in Cockburn and Warnbro sounds and coastal spawning all contribute recruits to the broader, genetically-connected, south-western population. It may also reflect the movement of individuals of this species at both sub-adult and adult stages and possible changes in where individuals spawn during their lifecycle that leads to a more homogenous genomic signature across the lower west coast (see Wakefield et al., 2011; Crisafulli et al., 2019). A different but complementary perspective about stock structure comes from our analyses of adaptation (i.e. seascape genomics). These analyses indicated that variation in sea surface temperature impacts on adaptive diversity of snapper in the south-west region. Although this could potentially lead to adaptive divergence, local adaptation and the evolution of different stocks (e.g. as in Western Australian greenlip abalone; Sandoval-Castillo et al. 2018), it appears that the temperature gradient across the study region is not strong enough to generate population divergence in the presence of high connectivity.

The homogenous genetic signature driven by high connectivity and migration rates demonstrates the equivalent contribution that snapper in Cockburn/Warnbro sounds make to stocks along the lower west coast and vice versa. Furthermore, length distributions of female snapper that are at or above the length at 50% maturity, or contain ovaries that were in spawning condition, and were collected from different locations across the south-western stock, demonstrate that fish that aggregate to spawn in Cockburn (and Warnbro) Sounds are exclusively very large fish. While large fish have been collected at other open coastal spawning locations, they are a relatively small proportion of that part of the south-western stock. Therefore, the large individuals from Cockburn and Warnbro Sounds would contribute disproportionately to reproductive output in terms of the number of eggs that females can produce and that are fertilised by large males. This is consistent with the reproductive output of large females of the West Australian dhufish (Evans-Powell et al., 2024). The relative contribution that snapper in Cockburn/Warnbro sounds make in terms of total batch fecundity was 1.6 times that of mature or spawning fish in oceanic waters of the metro and south-west regions. While the absolute contribution that large Cockburn/Warnbro sounds snapper make to broader stocks cannot be determined without estimates of biomass, it is widely known that aggregating to spawn can confer substantial benefits. Migration and aggregative behaviour is energetically expensive, and there is exposure to predation, therefore the benefit must outweigh the cost. This may come from greater spawning success and improved larval and juvenile survival provided by the greater protection from predation that embayments offer and/or greater food abundance (Molloy et al., 2012).

If Cockburn Sound snapper aggregations and/or their progeny were impacted by existing or future marine infrastructure development or operation, e.g. disruption of aggregative spawning or impacts on nursery habitats, this could directly affect the contribution that these snapper make to the broader stock on the lower west coast. However, evidence of equivalent connectivity across the lower west coast would indicate that Cockburn Sound could be replenished via natural dispersal and migration. The rate of such replenishment would be dependent on the rate of dispersal and migration along the coast. In addition, replenishment assumes that any impacts are not ongoing or long-lasting and that suitable habitat remains available for spawning, larval settlement and juvenile nurseries. Recruits spawned in Cockburn Sound would subsequently be selected (after reaching the minimum legal length) by recreational and commercial fishers along the lower west coast and therefore any short or longer-term effects of development on their abundance may have temporary or longer lasting effects on catches.

5.2 Evaluation of methods for monitoring aspects of stock enhancement programs

5.2.1 Genomics

The genomic comparisons between hatchery-born and wild-caught snapper conclusively demonstrated no differences in relatedness between samples. These results indicate that hatchery-based stocking practices in the Snapper Guardians program uses an unbiased sample of the wild population and, as such, is not negatively impacting on the relatedness (and perhaps on the adaptive potential) of the wild stock. This is consistent with the findings of Prokop (2015) who demonstrated using microsatellites that the genetics of the cultured snapper reflected that of the wild adult snapper population in Cockburn Sound (Partridge et al., 2016).The use of wild collected, fertilised eggs provides a much greater opportunity to maximise genetic diversity and minimise inbreeding than programs that maintain broodstock and therefore must manipulate spawning, e.g. by randomising the selection of individuals to spawn in pairs, to achieve those aims (Fisch et al., 2015). The genomic approach in this study provides a powerful method for longitudinal monitoring of stock enhancement practices of snapper based on eggs collected in the wild.

5.2.2 Morphology

The morphology of the wild and hatchery-reared snapper differed in several aspects and thus using photographs to discriminate between them has potential. The univariate morphometric analyses suggested that most of these differences were located in the anterior region of the body and predominantly the head. Specifically, the distance from the edge of the mouth to the hump on the head (A1) was included in four of the top ten components identified by PCA and the distance from the hump to the base of the dorsal fin (B1) was included in three of those top components. Despite the differences in morphological features, caution must be taken when drawing conclusions as the total lengths of individuals from the hatchery were typically less than those collected in the wild. For example, those collected from the hatchery in 2022 and 2023 were 22-66 and 36-58 mm (TL), respectively, whereas those collected in trawls from Cockburn Sound were 42-185 mm. This may have implications when drawing conclusions from morphometric analyses as the body shape of fish is known to change throughout ontogenetic development (Ahnelt et al., 2020). For example, juveniles typically have larger eyes and heads in relation to total length than their adults (Searle et al., 2021). Thus, the differences between the morphology of hatchery-reared and wild snapper in this study are, at least in part, due to differences in body size. This conclusion is consistent with the fact that many of the discriminating traits included measurements from the anterior region. Multivariate analysis also identified differences in the morphology of wild and both cohorts of hatchery-reared snapper, with traits relating to the eye and anterior region being influential. Using CAP, ~85% of snapper were correctly identified as being wild or one of the two hatchery-reared cohorts. This result is lower than the 99 and 98% obtained for gilthead sea bream in Spain and Greece, respectively (Arechavala-Lopez et al. 2012), but is greater than the 62.9% of hatchery-released fish able to be distinguished by Rogdakis et al. (2011) and similar to those produced by Talijančić et al. (2019, 2021). These comparisons indicated that this cost-effective method does have promise.

To confirm the results of the above analyses, further analyses of wild and hatchery-reared samples of similar lengths are required. Arrangements were made for an additional sample of 2023 hatchery-reared snapper to be grown out to sizes similar to the wild fish collected in Cockburn Sound. However, this did not proceed due to a virus outbreak in the hatchery and the need to euthanise those fish before the planned growout could be completed (DPIRD, 2024). While those hatchery-reared snapper would have been sampled at a similar length to those collected in trawls from Cockburn Sound, they may have incurred additional modifications to morphological features during the additional time spent in the hatchery. For example, deformities of the mouth, such as elongation or bending of the lower jaw (Matsuoka 2003; Okamura et al. 2007) or shortening of the snout (Yamauchi et al. 2006), are common in hatcheries and can occur throughout the lifecycle from larvae to adults (Noble et al., 2012). Thus, the additional time spent in the hatchery may result in those individuals being morphologically different to those hatchery-reared snapper released at an earlier age and size. A proportion of the snapper grown in the DPIRD fin-fish hatchery in Fremantle in 2017 for the Snapper Guardian program were held until they reached a larger size, i.e. an average of 182 mm total length and 135 g wet weight. These fish were found to have a different body shape and 49% of the 89 fish were found to have at least one of several deformities (Tweedley, unpublished data). These included the loss of a septum (33%), a misaligned jaw (17%) and a misshapen head (7%; Appendix 4).

Given the potential changes that can occur with the additional time spent in the hatchery, appropriate morphological comparisons would ideally be made between the wild and hatchery-reared snapper recaptured from the wild. However, it is noteworthy that of the ~230,400 hatchery-reared individuals released, only four have been recaptured and none in the current study (see subsection 5.2.3). While the recaptured individuals were of appropriate length for comparison, i.e. 87-143 mm, these fish were recaptured prior to the initiation of the current study and thus not photographed. In any case, a much larger sample (n = 100) would be required for morphological analyses, which may be challenging to obtain without increased sampling effort. Furthermore, to determine whether this method may be suitable for identifying hatchery fish once they reach larger sizes and join the fishery, i.e. reach the

minimum legal length for retention of 500 mm in the metropolitan and south-west areas of the west coast, samples of wild and hatchery-reared fish would also be needed at that stage. This would require the collection of significant numbers of samples from the west coast to obtain enough hatchery-reared fish among the wild population and to be able to conduct rigorous morphometric comparisons. Greater engagement by the recreational sector in donating such fish to DPIRD via its Send Us Your Skeletons program (www.fish.wa.gov.au/frames), for example, is one way that increased numbers of samples can be obtained. Additional collection methods, such as sampling recreational catches at boat ramps and from filleting stations or from commercial fishers operating outside the metropolitan area are already used to increase such sample numbers. However, very large sample numbers would probably be needed to identify the likely small numbers of hatchery fish vs wild fish in the fished population, i.e. ≥ 500 mm, when those fish would typically be at least 4 years old and up to almost 10 years old (Wakefield et al., 2016).

5.2.3 Implications for stock enhancement programs

Since the enhancement of snapper stocks began in Cockburn Sound in 2014 a total of ~230,400 juveniles have been released. While these releases were not accompanied by a bespoke scientific monitoring program, regular fisheries independent monitoring of blue swimmer crab populations in Cockburn Sound has been undertaken two times a year by DPIRD and sometimes catch snapper (see Sampey et al., 2011 for details). When juvenile snapper are caught, their otoliths are examined to determine if any of those fish were hatchery-reared. Prior to this WWMSP project, regular crab surveys resulted in the recapture of four hatchery-reared juvenile snapper among a total sample of 1,980 snapper from 2014 to 2023. These were a single individual in April 2016 (TL:105, FL: 95), two individuals in April 2017 (TL:107, FL:100 and TL:87, FL:81) and a single individual in December 2020 (TL:143, FL:129). Of the 640 juvenile snapper obtained during sampling for another WWMSP Project (Yeoh et al., 2024), none contained the otolith mark to show they were hatchery-reared. Moreover, none of the snapper caught by recreational fishers in the metropolitan and south-west areas of the West Coast Bioregion and returned to DPIRD through the Send Us Your Skeletons program since releases began at the end of 2014 (n = 1,509 and 2,443 in the two areas, respectively) have been identified as hatchery-reared fish. These recapture rates (i.e. 0.002%) are far lower than those reported in previous studies for hatchery-reared sparids (Table 5.1). Low recapture rates in Cockburn Sound may be influenced by the locations of blue swimmer trawl surveys in relation to preferred habitats of juvenile snapper. Using alternative or complementary methods, such as trapping may be beneficial (e.g. Wakefield et al., 2011). However, recapture rates of individuals of sparids in other enhancement programs, via fishery catches and surveys, were highly variable. If it is assumed that released individuals are evenly mixed among a wild population, recapture rates would be influenced by the relative proportion of the population that they represent at the time of recapture. This would be influenced by the abundance of that wild population, with a greater probability of recapture of hatchery fish in depleted populations, e.g. red sea bream (Kitada & Kishino, 2006). In addition, recapture rates of hatchery fish would decline over time as a function of natural mortality and also fishing mortality (if present) once they are selected by fishing gear. In the case of snapper, natural mortality rates from the age of release (~0.25 years) to around the average age (5-6 years) at which individuals reach both the age at maturity and approximately that at which they reach the minimum legal length in the metropolitan and south-west regions (Wakefield et al., 2015), would result in an estimated 6,100 fish surviving from 1,000,000 released (Figure 5.1). An estimated 1,200 would survive to 10 years of age, assuming fishing mortality was at a sustainable level (around the natural mortality rate M). This assumes there is no additional post-release mortality of released fish. However, release rates are high in boat-based recreational fishing in the metropolitan area for example (Ryan et al., 2022), which could add post-release mortality.

The probability of encountering hatchery fish along an expansive coastline would therefore be low at current release rates, as would their contribution likely be to either reproduction or fisheries. This suggests that releases of very large numbers of hatchery-reared individuals would be required to produce measurable effects in terms of social or fish population benefits. However, the potential benefits of this also need to be considered in relation to competition with wild-spawned snapper and other fish and any negative effects of hatchery rearing, such as morphological differences, that may result in maladaptation to natural environments and therefore reduced survival. These include commonly occurring differences in body shape (e.g. head and mouth), swimming speed and biochemical composition (e.g. fat levels), as a result of being reared in benign hatchery conditions (e.g. constant water flow, lack of structured habitat, food types etc) (Swain et al., 1991, Wringe et al., 2016; Guo et al., 2022).

If hatchery-based stock enhancement of snapper continues, a bespoke monitoring program would also be required to evaluate its success. Such a program needs to focus on:

(i) the genetic composition of the hatchery fish vs wild fish, adopting the approach used in this study,

(ii) initial survival of released fish, as this is when species are most vulnerable to predation and least mobile, and

(iii) long-term survival.

Such a program would align with DPIRD's 'Policy on restocking and stock enhancement in Western Australia' (Department of Fisheries, 2013) and global best practices (e.g. Blankenship & Leber, 1995; Lorenzen et al., 2010). Moreover, evaluation of a release program (timing and locations) can result in marked improvements in the survival of hatchery-reared individuals and improved long-term success. For example, post-release predation of western school prawn *Metapenaeus dalli* was 288% greater when juveniles were released at night than during the day (Poh et al., 2018). Investigation of food availability and the movement of acoustically-tagged hatchery-reared mulloway *Argyrosomus japonicus* indicated that for that species, future releases should be conducted directly into deeper water rather than from shore-based sites (Taylor & Suthers, 2008; Becker et al., 2023). While aspects of the program can be addressed, such as timing and location of releases, long-term monitoring programs would require additional financial resources. Furthermore, any effects on aggregative and spawning behaviour of snapper as a result of ongoing marine infrastructure development and operations could impact on the efficacy of any future hatchery rearing programs. This is because the current approach is to collect fertilised eggs directly from Cockburn Sound, in contrast to the maintenance of brood stock in tanks commonly used elsewhere (Partridge et al., 2017). The DPIRD approach maximises the genetic composition of hatchery-reared fish and negates the costs and challenges of maintaining brood stock.

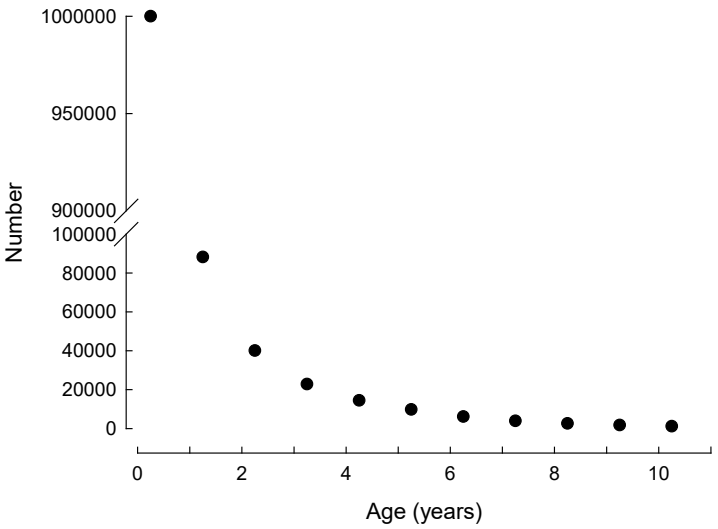


Figure 5.1. Estimated numbers of hatchery-reared snapper surviving each year after release of 1,000,000 individuals at 0.25 years old and a weight of 2.1 g (average weight of snapper at time of release during Snapper Guardians in 2022). Survival determined using Lorenzen (1996) mortality-weight equation. Note there are numerous methods for estimating survival.

Table 5.1. Characteristics of monitoring approaches and recapture rates of sparids from stock enhancement programs.

Species	Location	Releases	Monitoring	Recapture	Study
Sparus aurata	Bay of Cadiz (Spain)	18,253 fish either 15 or 100 g released between 1993 and 1998	Fish marked using tattoo ink, fingerling tags and anchor tags and caught by recreational and commercial fishers	343 recaptures by 2001. 0.03% (±0.05) for 15g and 3.52% (±1.71)	Sánchez-Lamadrid (2004)
Sparus aurata	Algarve (Portugal)	6,102 fish mean fork length 190 (±28) mm released onto an artificial reef	Fish marked with dart style tags (T-bar or Floy tags) and monitored underwater visual census and longer-term mortality via returns from recreational and commercial fishers	378 recaptures (6.2%). Maximum time at sea = 287 days and maximum dispersal 117 km.	Santos et al. (2006)
Diplodus sargus	Algarve (Portugal)	7,520 fish mean fork length 165 (±26) mm released onto artificial reef		337 recaptures (4.5%). Maximum time at sea = 337 days and maximum dispersal 121 km.	
Diplodus sargus	Gulf of Castellam mare (Sicily)	7,284 305 day-old fish mean total length 1,150 (±1) mm and mean weight 32 (±9.9) g released onto an artificial reef in 1999	Fish marked with T-bar tags. Initial mortality was assessed using underwater visual census and longer-term mortality via returns from recreational and commercial fishers	After 15 months 2,100 sightings of tagged fish, last recorded 463 days post-release. 570 fish recaptured (8.2%)	D'Anna et al. (2004)
Diplodus sargus	Gulf of Castellam mare (Sicily)	1,465 fish mean total length 872 (±64) mm. Fish conditioned to i) predators, ii) refuge, iii) predators and refuge or iv) no conditioning (naïve)	Fish tagged with visible implant elastomer tags. Monitored using underwater visual census along transects.	Post-release survival 5.5% for naïve fish and 9.4% for conditioned fish	D'Anna et al. (2012)
Pagrus major	Kagoshim a Bay (Japan)	800,000 to 1,200,000 fish of 60 to 80 mm total length annually	Anchor tag on caudal fin (not always successful) and a formation of a cutaneous bridge on the nostril	9% of fish recaptured. Stocked fish contributed 46 to 74% by number and 39 to 65% by weight to the total catch	Imai (2005)
Pagrus major	Kagoshim a Bay (Japan)	20.8 million fish ~70 mm total length were released between 1974 and 2002 into the bay which is 1,040 km^2	Hatchery-reared fish were identified by their natural marks (i.e. a deformity of the inter-nostril epidermis). Commercial fishing catches examined in fish markets	Recapture rate 8.0% (±0.42). Stocked fish contributed 36% (±16.1) of commercial catches	Kitada & Kishino (2006)
Pagrus major	Sagami and Tokyo bays (Japan)	22.9 million fish ~70 mm total length were released between 1978 and 2000 the bays which cover 1,380 km^2 and reach 700 m deep		Recapture rate 7.1% (±0.29). Stocked fish contributed 40.6% (±19.9) of commercial catches	

Table 5.1 cont. Characteristics of monitoring approaches and recapture rates of sparids from stock enhancement programs.

Species	Location	Releases	Monitoring	Recapture	Study
Acantho pagrus schlegeli i	Hiroshima Bay (Japan)	60,000 fish (20,000 per year) of 40 mm total length between 2000 and 2002	Fish caught by line fishing and identified using the presence of six microsatellite DNA markers	No recapture rate was calculated. Stocked fish represented 12.5 and 13.5% of the total catch in 2003 and 2004, respectively	Gonzalez et al. (2008)
Acantho pagrus schlegeli i	Daya Bay (China)	30,000 thousand fish ~30 mm total length released	Fish caught by trawling and identified using the presence of seven microsatellite loci	169 fish caught of which 2 were assigned to broodstock (1.18%). However, released fish found to have reduced genetic diversity which may have reduced survival	Wang et al. (2021)
Acantho pagrus butcheri	Swan-Canning Estuary (Australia)	775 14-month-old fish ~140 mm fork length	Fish t-bar tagged and recaptures reported by recreational fishers	97 individuals had been recaptured (12.5%) after 31 months	Dibden et al. (2000)
Acantho pagrus butcheri	Blackwoo d Estuary (Australia)	220,000 hatchery-reared juveniles and 102 adult broodstock	Juveniles stained with alizarin complexone and 1217 juveniles and 102 brood were also t-bar tagged. Monitoring conducted using seine and gill nets and tag returns from recreational fishers	Stocked fish contributed 75 and 92% of catches of fishes of the appropriate ages. 57 tag returns (4.3%) were recorded from recreational fishers.	Jenkins et al. (2006)
Acantho pagrus butcheri	Blackwoo d Estuary (Australia)	220,000 hatchery-reared juveniles and 102 adult broodstock	Juveniles stained with alizarin complexone and frames obtained from commercial fishers	Hatchery-reared fish contributed up to 74% of the commercial catch and up to ~50 % to egg production	Cottingha m et al. (2015)
Rhabdos argus sarba	Taiwan	5,769,700 fish between 30 to 100 mm total length released between 2004 and 2018	Five microsatellite loci	An estimated 6-50 of the 459 fish caught were thought to be hatchery-reared (1.3 to 10.9%). Authors stated standards for stock enhancement needed to increase.	Hsu et al. (2020)

6 Conclusions/recommendations

The results of this project demonstrated high connectivity between Snapper in Cockburn Sound and adjacent areas along the lower west coast, from the northern metropolitan region (Hillarys to Seabird) to the south-west region (Geographe Bay to Augusta), with high migration rates between each. However, there was less connectivity with the south coast (Windy Harbour; < 60% migration rate), previously identified as part of a broader south-western population (Bertram et al., 2022). The genetic results also provided evidence of localised recruitment to regions along the coast and in Cockburn Sound, but no strong evidence of localised adaptation. The high genetic connectivity and migration rates between Cockburn Sound and regions of the lower west coast reflects the importance of the contribution of Cockburn Sound snapper to broader stocks and vice versa. In addition, adults that aggregate to spawn in Cockburn Sound are exclusively large fish which would contribute disproportionately to reproductive output, via greater proportions of fish being in spawning condition through the spawning period, longer spawning periods, production of more eggs than small fish and spawning/fertilisation success. This is in addition to the benefits of protection and food conferred to early life stages while in embayments. Therefore, any increased pressures from infrastructure development or operation, such as noise and shipping, could directly affect individual and/or overall contribution to the broader stock on the lower west coast and associated fisheries, whether through effects on adult migration and movement to and from Cockburn Sound, aggregative spawning, larval/juvenile survival or nursery habitats in Cockburn Sound. Such effects may be mitigated by the extensive genetic connectivity across the lower west coast, suggesting that natural dispersal and migration would contribute to replenishment. However, the rate at which this would occur, would be influenced by the rate of dispersal and migration and whether any pressures are ongoing.

Morphometric and genomic work have demonstrated the potential for these methods to be used in monitoring the release of hatchery-reared snapper in Cockburn Sound. Morphometrics of wild and hatchery snapper differed in many aspects allowing potential distinction of such individuals from photographs. However, this would require photographs to be of sufficient quality. In addition, further work is required to evaluate whether differences remain between larger hatchery juveniles and those collected during trawl surveys of Cockburn Sound, and between hatchery and wild fish above the minimum legal length for retention of 500 mm in the metropolitan and south-west areas of the west coast. However, retaining fish for long periods in the hatchery can result in deformities, hence the current practice of releasing fish at ~3 months old. The genomic work demonstrated no differences in average relatedness between hatchery-reared snapper ready for release and wild juvenile snapper. It also indicated that hatchery fish are expected to have high adaptive potential, similar to wild fish. Therefore, there is support for the current methods used to rear snapper for stock enhancement and that this genomic method can be used to monitor for any changes in relatedness between future samples of hatchery-reared and wild snapper over time. However, the currently low recapture rates and estimated survival rates suggest that there is need for consideration of the economic feasibility of producing large numbers of fish to be of measurable benefit either to populations or social and economic objectives, such as fishing. In addition, future monitoring of such stock enhancement programs requires consideration of the timing and locations of releases and bespoke monitoring programs of released fish and adequate funding that would be required to conduct such work. Such a program should also focus on i) the genetic composition of the hatchery fish vs wild fish, adopting the approach used in this study, ii) initial survival of released fish, as this is when they are most vulnerable to predation and least mobile and iii) long-term survival.

7 References

Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics International 11:452: 36–42.

Ahnelt H, Sauberer M, Ramler D, Koch L, Pogoreutz C (2020) Negative allometric growth during ontogeny in the large pelagic filter-feeding basking shark. Zoomorphology 139: 71–83.

Anderson MJ, Robinson J (2003) Generalized discriminant analysis based on distances. AustN Z J Stat 45: 301-318.

Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84: 511-525.

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK.

Arechavala-Lopez P, Sanchez-Jerez P, Bayle-Sempere JT, Sfakianakis DG, Somarakis S (2012) Morphological differences between wild and farmed Mediterranean fish. Hydrobiol 679: 217-231.

Bandín I, Souto S (2020) Betanodavirus and VER Disease: A 30-year Research Review. Pathogens 9: 106.

Basurco B, Lovatelli A, García B (2011) Current Status of Sparidae Aquaculture. In: Pavlidis MA, Mylonas CC (eds) Sparidae. Blackwell Publishing, New Jersey.

Becker A, Lowry MB, Fielder DS, Taylor MD (2021) Dispersal of yellowtail kingfish (*Seriola lalandi*) from a coastal embayment following a recreational fisheries enhancement stocking program: Attempts to integrate aquaculture and habitat-based initiatives. Bull Mar Sci 97: 615-629.

Becker A, Fielder DS, Lowry MB, Taylor MD (2023) Suitability of saltmarsh creeks as release locations for stocked Mulloway (*Argyrosomus japonicus*). Estuar Coast Shelf Sci 292: 108455.

Bernatchez L, Wellenreuther M, Araneda C, Ashton DT, Barth JMI, Beacham TD, Maes GE, Martinsohn JTh, Miller KM, Naish K, Ovenden JR, Primmer CR, Suk HY, Therkildsen NO, Withler RE (2017) Harnessing the power of genomics to secure the future of seafood. Trends Ecol Evol 32: 665-680.

Bertram A, Fairclough, D, Sandoval-Castillo J, Brauer C, Fowler A, Wellenreuther M, Beheregaray LB (2022). Fisheries genomics of snapper (*Chrysophrys auratus*) along the west Australian coast. Evol App 15: 1099-1114.

Blankenship HL, Leber KM (1995) A responsible approach to marine stock enhancement. American Fisheries Society Symposium 15, 167-175.

Bolger, AM, Lohse, M, Usadel, B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120.

Brown J, Pirrung M, McCue LA (2017) FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinformatics 33: 3137-3139.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: An analysis tool set for population genomics. Mol Ecol 22: 3124–3140.

Clarke KR, Somerfield PJ, Gorley RN (2008) Testing of null hypotheses in exploratory community analyses: Similarity profiles and biota-environment linkage. J Exp Mar Biol Ecol 366: 56-69.

Clarke KR, Tweedley JR, Valesini FJ (2014) Simple shade plots aid better long-term choices of data pre-treatment in multivariate assemblage studies. Mar Biol Assoc U K 94: 1-16.

Clarke KR, Gorley RN (2015) PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth.

Cottingham A, Hall NG, Potter IC (2015) Performance and contribution to commercial catches and egg production by restocked *Acanthopagrus butcheri* (Sparidae) in an estuary. Est Coast Shelf Sci 164: 194-203.

Cottingham A, Hall NG, Loneragan NR, Jenkins GI, Potter IC (2020) Efficacy of restocking an estuarine-resident species demonstrated by long-term monitoring of cultured fish with alizarin complexone-stained otoliths. A case study. Fish Res 227: 105556.

Crisafulli BM, Fairclough DV, Keay IS, Lewis P, How JR, Ryan KL, Taylor SM, Wakefield CB (2019) Does a spatiotemporal closure to fishing Chrysophrys auratus (Sparidae) spawning aggregations also protect individuals during migration? Can J Fish Aquat Sci 76: 1171-1185.

D'Anna G, Giacalone VM, Badalamenti F, Pipitone C (2004) Releasing of hatchery-reared juveniles of the white seabream *Diplodus sargus* (L., 1758) in the Gulf of Castellammare artificial reef area (NW Sicily). Aquaculture 233: 251-268.

D'Anna G, Giacalone VM, Vega Fernández T, Vaccaro AM, Pipitone C, Mirto S, Mazzola S, Badalamenti F (2012) Effects of predator and shelter conditioning on hatchery-reared white seabream *Diplodus sargus* (L., 1758) released at sea. Aquaculture 356-357: 91-97.

Department of Fisheries (2013) Policy on Restocking and Stock Enhancement in Western Australia. Fisheries Management Paper No. 261.

Department of Primary Industries and Regional Development (DPIRD) (2023) Recreational fishing guide 2023. Edition 2, March 2023. https://www.fish.wa.gov.au/Documents/recreational_fishing/rec_fishing_guide/recreational_fishing_guide.pdf. Date accessed 30/9/2023.

Dibden CJ, Jenkins G, Sarre GA, Lenanton RCJ, Ayvazian SG (2000) The evaluation of a recreational fishing stock enhancement trial of Black Bream (*Acanthopagrus butcheri*) in the Swan River, Western Australia. Fisheries Research Report 124, 23 pp. Department of Fisheries, Western Australia, Perth, Australia.

DPIRD (2024). Pink snapper stocking on hold for 2024. https://www.wa.gov.au/government/announcements/pink-snapper-stocking-hold-2024. Last accessed. 12/4/2024.

Evans-Powell RT, Hesp SA, Denham A, Beckley LE (2024) Implications of big, old, fat, fecund, female fish (BOFFFFs) for the reproductive potential of a demersal teleost stock. Fisheries Research 272: 106934.

Fairclough DV, Edmonds JS, Jackson G, Lenanton RCJ, Kemp J, Molony BW, Keay IS, Crisafulli BM, Wakefield CB (2013) A comparison of the stock structures of two exploited demersal teleosts, employing complementary methods of otolith element analysis. J. Exp. Mar. Biol. Ecol. 439: 181–195.

Fairclough DV, Brown JI, Carlish BJ, Crisafulli BM, Keay IS (2014) Breathing life into fisheries stock assessments with citizen science. Scientific Reports 4: 7249.

Fairclough DV, Hesp SA, Denham A, Fisher EA, Marks R, Ryan KL, Lek E, Allen R., Crisafulli B.M. (2021) 2021 assessment of the status of the Werst Coast Demersal Scalefish Resource. Fisheries Research Report No. 316. Department of Primary Industries and Regional Development, Western Australia.

Fisch KM, Kozfkay CC, Ivy JA, Ryder OA., Waples RS (2015) Fish Hatchery Genetic Management Techniques: Integrating Theory with Implementation. N Am J Aquac 77: 343–357. FAO (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Gardner MJ, Chaplin JA, Fairclough DV, Potter IC (2022) Microsatellite-based assessment of the genetic structure of snapper, Chrysophrys auratus, in Australasia. Est. Coast. Shelf Sci. 274: 107932

Garlock TM, Lorenzen K (2017). Marine angler characteristics and attitudes toward stock enhancement in Florida. Fish Res 186: 439-445.

Gonzalez EB, Nagasawa K, Umino T (2008). Stock enhancement program for black sea bream (*Acanthopagrus schlegelii*) in Hiroshima Bay: Monitoring the genetic effects. Aquaculture 276: 36-43.

Goudet J, Jombart T, Goudet MJ (2015) Package 'hierfstat'. R package version 0.04-22. Retrieved from http://www.r-project.org, http://github.com/jgx65/hierfstat.

Grummer JA, Beheregaray LB, Bernatchez L, Hand B, Luikart G, Narum SR, Taylor EB (2019) Aquatic landscape genomics and environmental effects on genetic variation. Trends Ecol Evol 34: 641-654.

Guo H, Zhang X, Näslund J, Peng L, Liu C, Tian M, Chai X, Zhang D and Zhang X (2022) Differences in External Morphology, Body Composition and Swimming Performance Between Hatchery- and Wild-Origin Marbled Rockfish (*Sebastiscus marmoratus*). Front. Mar. Sci. 9:912129

Hilborn R (1998) The economic performance of marine stock enhancement projects. Bull Mar Sci 62: 661-674.

Hsu, T-H, Huang C-W, Lee H-T, Kuo Y-H, Liu K-M, Lin C-H, Gong H-Y (2020). Population genetic analysis for stock enhancement of silver sea bream (*Rhabdosargus sarba*) in Taiwan, Fishes: 5, 19.

Imai T (2005). Sea farming of red sea bream *Pagrus major* (Temmick et Schlegel) in waters off Kanagawa Prefecture, Japan with special reference to stock enhancement effect. Bulletin of the Kanagawa Prefecture Fisheries Research Institute 10: 65–71.

Jackson G, Cheng YW, Wakefield CB (2012) An evaluation of the daily egg production method to estimate spawning biomass of snapper (*Pagrus auratus*) in inner Shark Bay, Western Australia, following more than a decade of surveys 1997–2007. Fisheries Research 117-118: 22-34.

Jackson G, Fairclough DV, Fisher EA, Hetzel Y, van der Mheen M, Scoulding B, Gillanders B, Reis-Santos P, Beheregaray LB, Sandoval-Castillo J, Braccin M (2023) Where did the Snapper go? Determining factors influencing the recovery of Snapper stocks on the west coast of Australia. Final Report to Fisheries Research and Development Corporation, FRDC Project 2018-050. Department of Primary Industries and Regional Development, Perth.

Jenkins GI, French DJW, Potter, IC, de Lestang S, Hall NG, Partridge GJ, Hesp SA, Sarre GA (2006) Restocking the Blackwood River Estuary with the black bream *Acanthopagrus butcheri*. Final Report to Fisheries Research and Development Corporation, FRDC 2000/180, Challenger TAFE, Perth, Australia.

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diversity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol 4: 782-788.

Kitada S, Kishino H (2006) Lessons learned from Japanese marine finfish stock enhancement programmes. Fisheries Research 80: 101-112.

Kitada S (2018) Economic, ecological and genetic impacts of marine stock enhancement and sea ranching: A systematic review. Fish and Fish 19: 511-532.

Kitada S (2020) Lessons from Japan marine stock enhancement and sea ranching programmes over 100 years. Rev. Aquac. 12: 1944-1969.

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nature Methods, 9: 357.

Leber KM (2004) Marine stock enhancement in the USA: Status, trends and needs, p11-24. In Leber KM, Kitada S, Blankenship HL, Svåsand (eds). Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Publishing, Oxford.

Loneragan NR, Jenkins GI, Taylor MD (2013) Marine stock enhancement, restocking and sea ranching in Australia: Future directions and a synthesis of two decades of research and development. Rev Fish Sci Aquac 21: 222-236.

Lorenzen K (1996) The relationship between body weight and natural mortality in fish: a comparison of natural ecosystems and aquaculture. Journal of Fish Biology 49: 627-647

Lorenzen K, Leber KM, Blankenship HL (2010) Responsible approach to marine stock enhancement: An update. Rev Fish Sci Aquac 18: 189-210.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297-1303.

Matsuoka M (2003) Comparison of meristic variations and bone abnormalities between wild and laboratory-reared red sea bream. Jpn Agric Res Q 37: 21–30.

Molloy PP, Cote IM and Rynolds JD (2012) Why spawn in aggregations? In Y. Sadovy de Mitcheson, PL Colin (eds) Ref fish spawning aggregations: Biology, Research and Management, Fish and Fisheries Series 35, Springer Science+Business Media BV. pp 57-83.

Molony BW, Lenanton R, Jackson G, Norris J (2003) Stock enhancement as a fisheries management tool. Rev.Fish Biol. Fish 13: 409-432.

Newman SJ, Wise BS, Santoro KG, Gaughan DJ (eds). (2023) Status Reports of the Fisheries and Aquatic Resources of Western Australia 2021/22: The State of the Fisheries. Department of Primary Industries and Regional Development, Western Australia.

Noble C, Cañon Jones HA, Damsgård B, Flood MJ, Midling KØ, Roque A, Bjørn-Steinar S, Cottee SY (2012). Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare. Fish Physiol Biochem 38: 61–83.

Okamura A, Yamada Y, Horie N, Utoh T, Mikawa N, Tanaka S, Tsukamoto K (2007) Effects of water temperature on early development of Japanese eel Anguilla japonica. Fish Sci 73:1241–1248.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'hara R, et al. (2018) Package 'vegan' community ecology package. R package version 2.5-6.

Partridge GJ, Michael RJ (2010) Direct and indirect effects of simulated calcareous dredge material on eggs and larvae of pink snapper *Pagrus auratus*. J Fish Biol 77: 227-240.

Partridge GI, Ginbey BM, Woolley LD, Fairclough DV, Crisafulli B, Chaplin J, Prokop N, Dias J, Bertram A, Jenkins GI (2017) Development of techniques for the collection and culture of wild-caught fertilised Snapper (*Chrysophrys auratus*) eggs for stock enhancement purposes. Fish Res 186: 524-530.

Peakall R, Smouse P (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28: 2537.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7: e37135.

Pew J, Wang J, Muir P, Frasier T (2015) Package 'related: an R package for analyzing pairwise relatedness data based on codominant molecular markers'. R package version, 1.0.

PIR (2024) Stock enhancement. https://pir.sa.gov.au/research/snapper_recovery/stock_enhancement. Last accessed. 12/4/2024.

Poh B, Tweedley JR, Chaplin JA, Trayler KM, Loneragan NR (2018). Estimating predation rates of restocked individuals: The influence of timing-of-release on metapenaeid survival. Fish. Res. 198: 165-179.

Prokop, N.D. (2015) Genetic Implications of Culturing Australasian Snapper(Chrysophrys Auratus) from Wild-caught Eggs for Stock Enhancement. Honours thesis, School of Veterinary and Life Sciences Murdoch University, Perth (pp. 123).

Recfishwest (2024) Snapper guardians. https://recfishwest.org.au/tag/Snapper-guardians/. Last accessed. 12/4/2024.

Reist, J. D., 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Can J Zool 63: 1429–1439.

Rogdakis Y, Koukou K, Ramfos A, Dimitriou E, Katselis G, (2011). Comparative morphology of wild, farmed and hatchery- released gilthead sea bream (*Sparus aurata*) in western Greece. International Journal of Fisheries and Aquaculture 3: 1-9.

Ryan KL, Lai EKM, Smallwood CB (2022) Boat-based recreational fishing in Western Australia 2020/21. Fisheries Research Report No. 327. Department of Primary Industries and Regional Development, Western Australia.

Sampey A, Fromont J, Johnston DJ (2011) Demersal and epibenthic fauna in a temperate marine embayment, Cockburn Sound, Western Australia: determination of key indicator species. J R Soc West Aust 94: 1-18.

Sánchez-Lamadrid A (2004) Effectiveness of releasing gilthead sea bream (*Sparus aurata*, L.) for stock enhancement in the bay of Cádiz. Aquaculture 231: 135-148.

Sandoval-Castillo J, Robinson N, Hart A, Strain L, Beheregaray LB. (2018) Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (*Haliotis laevigata*), along a longitudinal environmental gradient. Mol Ecol 27: 1603-1620.

Santos MN, Lin PG, Pousão-Ferreira P, Monteiro CC. (2006). Preliminary results of hatchery-reared seabreams released at artificial reefs off the Algarve coast (southern Portugal): A pilot study. Bull Mar Sci 78(1): 177-184.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671-675.

Searle PC, Mercer M, Habit E, Belk MC (2021) Ontogenetic shape trajectory of *Trichomycterus areolatus* varies in response to water velocity environment. PLOS ONE 16: e0252780.

Swain DP, Riddell BE, Murray CB (1991) Morphological differences between hatchery and wild populations of Coho salmon (*Oncorhynchus kisutch*): Environmental versus genetic origin. Can. J. Fish. Aquat. Sci. 48: 1783–1791.

Talijančić I, Šegvić-Bubić T, Žužul I, Džoić T, Maršić-Lučić J, Grubišić L (2019) Morphological and ecophysiological adaptations of wild gilthead seabream *Sparus aurata* associated with tuna farms. Aquac Environ Interact 11: 97-110.

Talijančić I, Žužul I, Kiridžija V, Šiljić J, Pleadin J, Grubišić L, Šegvić-Bubić T (2021) Plastic Responses of gilthead seabream *Sparus aurata* to wild and aquaculture pressured Environments. Front Mar Sci 8: 694627.

Taylor MD, Suthers IM (2008) A predatory impact model and targeted stock enhancement approach for optimal release of Mulloway (*Argyrosomus japonicus*). Rev Fish Sci Aquac 16: 125-134.

Taylor MD, Chick RC, Lorenzen K, Agnalt AL, Leber KM, Blankenship HL, Haegen GV, Loneragan NR (2017) Fisheries enhancement and restoration in a changing world. Fish Res 186: 407-412.

Tweedley JR, Warwick RM, Potter IC (2015) Can biotic indicators distinguish between natural and anthropogenic environmental stress in estuaries? J Sea Res 102: 10-21.

Tweedley JR, Obregón C, Beukes, SJ, Loneragan NR, Hughes M (2023a) Selecting from the fisheries managers' tool-box: recreational fishers' views of stock enhancement and other management options. Fishes 8: 460.

Tweedley JR, Obregón C, Beukes, SJ, Loneragan NR, Hughes M (2023b) Differences in recreational fishers' motivations for utilising two estuarine fisheries. Fishes 8: 292.

Tweedley JR, Hyndes GA, McCallum, R, Cox D, Beltran L, Billinghurt J, Haywood M, Krispyn KN, Johnston D (2024). Trophic pathways and food web structure of Cockburn Sound and Owen Anchorage. Draft final report to Western Australian Marine Science Association Westport Marine Science Program, p. 176.

Wakefield CB (2006) Latitudinal and temporal comparisons of the reproductive biology and growth of snapper, *Pagrus auratus* (Sparidae), in Western Australia. PhD thesis, Murdoch University.

Wakefield CB (2010) Annual, lunar and diel reproductive periodicity of a spawning aggregation of snapper *Pagrus auratus* (Sparidae) in a marine embayment on the lower west coast of Australia. J Fish Biol 77: 1359–1378.

Wakefield CB, Fairclough DV, Lenanton RCJ, Potter IC (2011) Spawning and nursery habitat partitioning and movement patterns of Pagrus auratus (Sparidae) on the lower west coast of Australia. Fish. Res. 109: 243–251.

Wakefield CB, Potter IC, Hall NG, Lenanton RCJ, Hesp SA (2015) Marked variations in reproductive characteristics of snapper (*Chrysophrys auratus*, Sparidae) and their relationship with temperature over a wide latitudinal range, ICES J Mar Sci 72: 2341–2349

Wakefield CB, Potter IC, Hall NG, Lenanton R, Hesp SA (2016) Timing of growth zone formations in otoliths of the snapper, *Chrysophrys auratus*, in subtropical and temperate waters differ and growth follows a parabolic relationship with latitude. ICES J Mar Sci 74: 180-192.

Wang X, Weng Z, Yang Y, Hua S, Zhang H, Meng Z (2021) Genetic evaluation of black sea bream (*Acanthopagrus schlegelii*) stock enhancement in the South China Sea based on microsatellite DNA markers. Fishes 6: 47.

Wringe BF, Purchase CF, Fleming IA (2016) In Search of a “Cultured Fish Phenotype”: A systematic review, meta-analysis and vote-counting analysis. Rev. Fish. Biol. Fish. 26, 351–373.

Yamauchi M, Kim EY, Iwata H, Shima Y, Tanabe S (2006) Toxic effects of 2378—tetrachlorodibenzo-p-dioxin (TCDD) in developing red seabream (Pagrus major) embryo: an association of morphological deformities with AHR1 AHR2 and CYP1A expressions. Aquat Toxicol 80: 166–179.

Yeoh D, Mitchell P, Tweedley J, Johnston D, Fairclough D, West L, Krispyn K, Seymour J, Scoulding B, Nilsen J, Fourie S, Norris J, McIlwain J, Middelfart P, Jackson G (2024). Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound. Draft final report to Western Australian Marine Science Association Westport Marine Science Program, Perth, Australia, p. 343.

8 Appendices

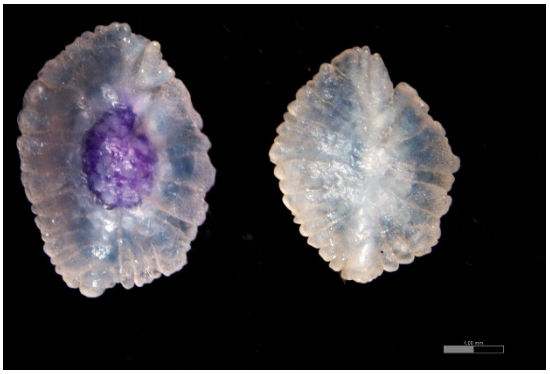
8.1 Appendix 1

Number of snapper released as part of the snapper restocking program. Data provided by DPIRD.

Release	Date	Location	Number	Comment
1	23/12/2014	Boat release Cockburn Sound	1,200	Boat release (Data from RFIF report 2016)
2	23/12/2014	Boat release Cockburn Sound	1,200	Boat release (Data from RFIF report 2016)
3	15/01/2016	Warnbro Beach	15,000	(Data from RFIF report 2016)
4	19/01/2016	Warnbro Beach	10,000	(Data from RFIF report 2016)
5	20/01/2016	Warnbro Beach	10,000	(Data from RFIF report 2016)
6	6/02/2016	Woodman point	5,000	Recfishwest Public Release (Data from RFIF report 2016)
7	17/02/2017	Boat release Cockburn Sound	40,000	Release from Maritime Image
8	18/02/2017	Beach release Woodman Point	3,000	Recfishwest Public Release
9	10/02/2018	Beach release Woodman Point	5,000	Recfishwest Public Release
10	8/02/2020	Beach release Woodman Point	5,000	Recfishwest Public Release
11	12/02/2021	Beach release Woodman Point	6,000	Recfishwest Public Release
12	12/02/2021	Johnston St Boat Ramp Swan River	6,000	
13	10/02/2022	Fremantle Sailing Club Boat Ramp	9,500	
14	10/02/2022	Woodman point	4,000	Recfishwest seagrass release
15	10/02/2022	Cockburn Powerboat Club	8,000	Recfishwest and committee members
16	11/02/2022	Fremantle Sailing Club	16,500	Recfishwest and Club members
17	11/02/2022	Cockburn Powerboat Club	16,500	
18	14/02/2022	Fremantle Sailing Club	11,000	
19	14/02/2022	Cockburn Powerboat Club	11,000	
20	15/02/2022	Cockburn Powerboat Club	8,500	Westport + Minister Saffioti
21	11/02/2023	Woodman's Point Beach	8,000	Recfishwest Public Release
22	13/02/2023	Fremantle sailing club	15,000	
23	13/02/2023	Cockburn Powerboat Club	15,000	

8.2 Appendix 2

Otolith (ear bone) from a (left) hatchery-reared snapper stained with Alizarin-complexone, showing the purple core and a (right) wild-spawned snapper, both caught in Cockburn Sound (Photo: DPIRD).

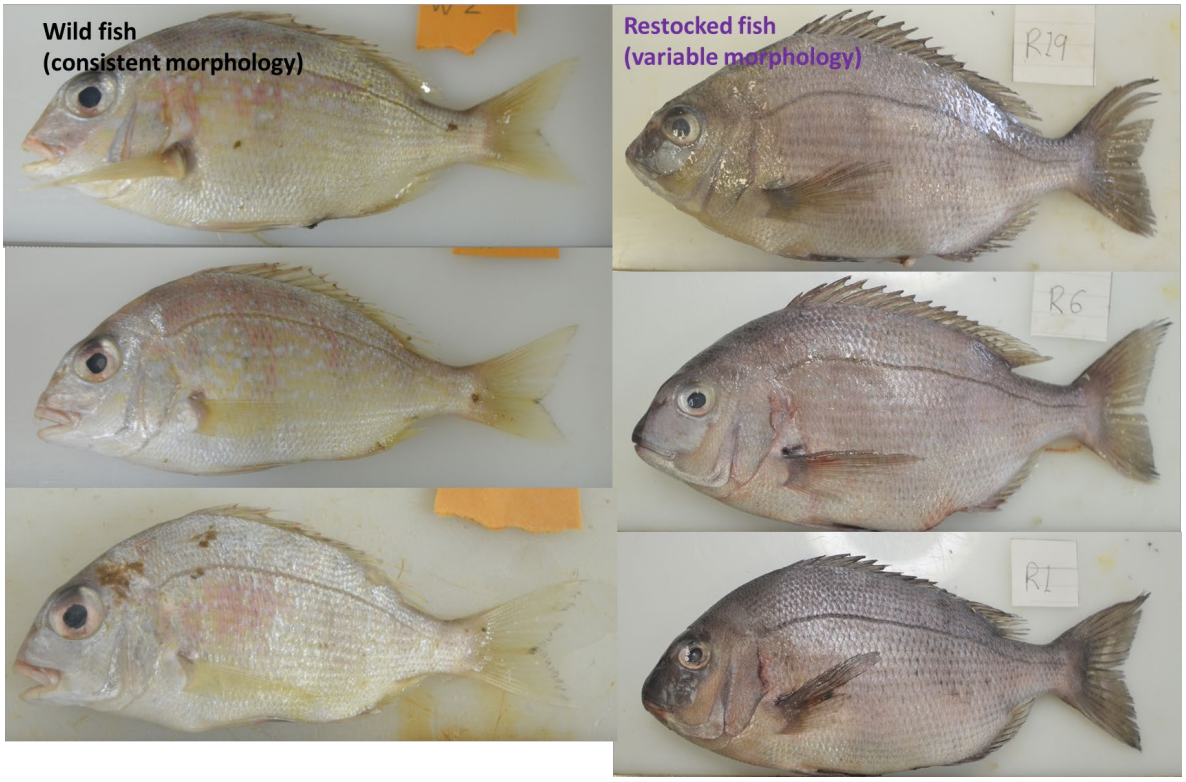


8.3 Appendix 3

Photographs of the body and otoliths from a (top) hatchery-reared black bream stained with Alizarin-complexone, and a (bottom) wild-spawned black bream, both caught in the Blackwood Estuary. Note the differentiation in body shape and scale pattern in the hatchery-reared specimen.

8.4 Appendix 4

Photographs of the body of (left) wild-caught and (right) hatchery-reared snapper from the Snapper Guardian's program in 2017. Note these snapper were held in captivity after the release of juveniles into Cockburn Sound for use in South-Metro TAFE Aquaculture courses.



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Final report	

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