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Accelerating Greenlip Abalone stock recovery in South Australia using release of hatchery-reared juveniles

Phase 1 - genetic risk assessment and preliminary cost-benefit analysis

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Abbreviations

ACA – Abalone Council Australia
AIASA – Abalone Industry Association of South Australia
DaRT – Diversity Arrays Technology
F1 – First generation Greenlip Abalone offspring
FX – Greenlip Abalone bred and grown in a hatchery that are either not F1 or are of unknown generation
GVP – Gross Value of Production
M - Mortality
PCA – Principal Component Analysis
PIRSA – The Department for Primary Industries and Regions, South Australia
SA – South Australia
SARDI – South Australian Research and Development Institute (a Division of PIRSA)
SAU – Spatial Assessment Unit
SNP – Single Nucleotide Polymorphism
ToR – Terms of Reference
WZ – Western Zone

Executive Summary

This project was undertaken from 2021 to 2023 by SARDI Aquatic and Livestock Sciences, a division of Primary Industries and Regions SA (PIRSA), to facilitate abalone stock recovery in South Australia, and was deliberately established as the first phase of a multi-phase project. A natural extension, through subsequent phases, would focus on in-water release and monitoring. Three key activities were undertaken. These were (1) a comparison of genetic differences between wild and hatchery-reared Greenlip Abalone; (2) development of a genetic risk-assessment framework as a method for assessing genetic risks associated with release of abalone; and (3) a preliminary cost-benefit analysis of release of juvenile Greenlip Abalone with a user interface to aid use by industry members.

Tissue samples were obtained from wild Greenlip Abalone at eight sites in South Australia (The Gap, Taylor Island, Anxious Bay, Point Westall, Flinders Island, Baird Bay, Tiparra Reef and Avoid Bay; termed 'wild') and from three hatchery cohorts from Yumbah Aquaculture of unknown generation (termed 'FX'). The tissue samples were provided to Diversity Arrays Technology (DaRT), in Canberra. DaRT used single nucleotide polymorphism (SNPs) techniques to analyse the genetic diversity and population structure of the wild and FX Greenlip Abalone. Distance dendrograms, principal component analysis (PCA) plots by site and by wild/hatchery abalone, average allelic richness, expected heterozygosity and pairwise comparisons by sample comprised the primary analyses. The key finding from the genetic analysis was that the genetic diversity and population structure of wild Greenlip Abalone was found to be different from hatchery FX Greenlip Abalone. Notably, samples from wild and FX Greenlip Abalone formed distinct clusters in the PCA plots with no overlap. Similarly, average allelic richness and expected heterozygosity were slightly higher for wild samples indicating potential inbreeding in the FX Greenlip Abalone.

The genetic suitability of releasing FX Greenlip Abalone into wild populations was discussed and assessed using a risk assessment that was developed during an expert-based, risk assessment workshop focusing on genetic risks. The workshop was held across two days and included over 25 participants that spanned expert geneticists, fishery scientists, fishery and policy managers, abalone industry members, and hatchery/farm representatives. The resultant risk assessment, which is objective and follows a consequence-likelihood format, provides a useful method to assess risks (i.e., loss of genetic integrity in wild abalone) from releasing juvenile abalone for stock recovery or subtidal aquaculture. Key likelihood (e.g., release number) and consequence (e.g., genetic base of release juveniles (i.e., broodstock founders)) risk factors are identified. Guidelines for scoring these, along with potential risk scores and potential risk rankings were developed. Utility of the risk assessment was assessed using multiple case studies, with the method being demonstrated to be robust across diverse, hypothetical case study scenarios. As this method is applied and further assessed under real conditions, and with periodic review, it is expected to become a useful tool for assessing risk of future abalone releases for stock recovery and subtidal aquaculture.

A preliminary cost-benefit analysis model for assessing release of juvenile Greenlip Abalone as a strategy to aid recovery of Greenlip Abalone stocks in South Australia was undertaken. This model was built in Excel, with a user interface, to aid ease of use by industry. The model uses inputs on growth and survival following release, costs of release and fishing and harvest value – with many of the model parameters able to be 'user specified'. Costs and benefits are measured in both biomass and dollars and are compared with two alternative investment choices: foregoing catch and financial investment. This cost-benefit analysis model will be a useful tool for industry to evaluate the merits of potential future abalone releases for stock recovery.

Keywords

Greenlip Abalone, genetic risk assessment, cost-benefit analysis, single nucleotide polymorphism, stock recovery, subtidal aquaculture.

Introduction

Wild abalone catches in Australia have declined by 50% nationally and 39% in South Australia over the past 20 years (Status of Australian Fish Stocks Reports). In South Australia, most of this decline was in the Western Zone Fishery, particularly for Blacklip Abalone (*Haliotis rubra*) over the past decade (Stobart and Mayfield 2021). While Greenlip Abalone (*Haliotis laevigata*) catches have declined less than those for Blacklip Abalone and are currently 15% below the long-term catch of 78 t, the relative economic importance of this decline is greater because they have a higher sale value (Blacklip Abalone - ~$95/kg, Greenlip Abalone - ~$120/kg; Stobart et al. 2022). Obtaining information that informs the release of juvenile Greenlip Abalone, including directly into commercial Greenlip Abalone grounds, could facilitate substantial recovery and growth of this sector (see also Hart 2015).

Abalone is a high-value product with small increases in volume potentially translating to large increases in value for the fishery and in turn, support of regional economies and communities. Stock recovery – aimed at recovering stocks classified as depleting or depleted – is therefore one of the highest research priorities for the SA Western Zone Fishery, and abalone fisheries nationally.

There are areas of the Western Zone Abalone Fishery where Greenlip Abalone are depleting, with biomass levels well below those observed historically (Stobart and Mayfield 2021). Despite reduced catches, some areas may not recover quickly without intervention and recovery may be further impeded by climate change that could, for example, lead to a productivity bottleneck in the recruitment or early life history phases. The Western Zone wild-catch abalone industry is therefore considering a stock-release program to accelerate Greenlip Abalone stock recovery in South Australia using release of hatchery-reared juveniles to help 'future-proof' the industry. The Central Zone wild-catch abalone industry is also seeking to establish a stock release program to re-build Greenlip Abalone stocks in depleted areas using hatchery-reared juveniles.

Abalone populations in Australia are assumed to be made up of numerous biologically and genetically discrete stocks with adaptive sub-populations (Mayfield et al. 2014; Miller et al. 2009, 2014; Sandoval-Castillo et al. 2017). Mitigation of genetic (i.e., loss of genetic integrity in wild abalone) and disease risks through stock recovery activities minimises impacts to wild abalone populations. There are key Government and industry needs for commencing a stock recovery program using hatchery-reared juveniles. The key industry need is to test the release of juvenile Greenlip Abalone in the Western and Central Zones to evaluate the long-term economic viability. To support this important industry goal, the key Government need is for data to underpin release policy. There are existing protocols for managing the disease risks, but those for managing potential genetic risks are less advanced. Up-to-date knowledge of the phylogeography and population genetics of Greenlip Abalone (after Miller et al. 2014, Sandoval-Castillo et al. 2017), including those available from hatcheries, is required to enable review, and potential amendment to, *Part A of the Prohibition of Entry into and Movement within South Australia of Aquaculture Stock Notice 2020* issued under section 33 of the *Livestock Act 1997* which states that "Aquaculture stock comprised of abalone that have been hatchery reared in the State or taken from waters of the State must not enter a licence area, unless:

> a) the abalone are moved directly from a licence area in which aquaculture is carried out by means of a semi-closed system to another licence area in which aquaculture is carried out by means of a semi-closed system; or

b) for a licence area that is wholly below the low water mark, if there is a population of wild abalone within an area surrounding the licence area and extending out 1 kilometre (km) from the boundary of the licence area, the abalone are descendants of broodstock abalone collected from within that area surrounding the licence area; and

c) prior written approval of the Chief Inspector of Stock has been obtained and all conditions of the approval are complied with; and

d) all requirements in the Translocation Protocol relating to the movement of abalone are complied with."

The principles of this 'Livestock Notice' have also been applied in assessments for the release of hatchery-reared juveniles to wild harvest fishing grounds. This has slowed the advancement of stock enhancement in SA, in part because, prior to considering production of F1 cohorts, the Western Zone Abalone Industry seek to trial release of FX juveniles given their availability and low price. These FX juveniles are readily available and inexpensive because Yumbah Aquaculture produces surplus Greenlip Abalone each year that would be otherwise culled.

Objectives

There were three objectives for this project. These were:

1. Use single nucleotide polymorphism (SNPs) to compare the genetic diversity and population structure of wild Greenlip Abalone and FX abalone;
2. Assess the genetic suitability of using FX abalone for release into wild populations at sites across the SA Western Zone Fishery, ensuring that the genetic structure and diversity of wild populations is maintained, including an expert-based, genetics risk assessment workshop; and
3. Undertake a preliminary cost-benefit analysis to inform a 'stop/go' decision.

Methods

Objective 1 – Use SNPs to compare the genetic diversity and population structure of wild and FX Greenlip Abalone

Tissue samples were collected from eight sites in South Australia containing wild Greenlip Abalone (The Gap, Taylor Island, Anxious Bay, Point Westall, Flinders Island, Bairds Bay, Tiparra Reef and Avoid Bay; termed 'wild'; Figure 1 in Appendix 1) and from three cohorts of FX (i.e., progeny with unknown generation) Greenlip Abalone from the general population at the Yumbah Abalone Aquaculture hatchery near Port Lincoln. Details are provided in Appendix 1. Briefly, live abalone were placed in a tub of seawater, the foot and tentacles were allowed to fully extrude, and tentacle(s) were then snipped and placed immediately into a 1.5 mL Eppendorf tube filled with 100% ethanol and stored on ice (n = 60 animals sampled per site/cohort). Dissection tools were thoroughly cleaned and sterilised between samples, and ethanol was changed once prior to storing the samples at -20 °C.

A total of 550 samples (n = 50 samples per site, 10 samples per site held in 'reserve') were submitted to Diversity Arrays Technology (DaRT) for processing and analysis (see Appendix 1 for details). To identify differences between wild and FX Greenlip Abalone, DaRT provided the following analyses of SNPs: distance dendrograms, principal component analysis (PCA) plots by site and by wild/hatchery abalone, average allelic richness, expected heterozygosity and pairwise comparisons by site of the fixation index.

Objective 2 – Assess the genetic suitability of FX abalone for release into wild populations, including an expert-based, genetics risk assessment workshop

A 2-day, expert-based, abalone release genetics risk-assessment workshop was held on 1 and 2 July 2021 to provide an avenue for information exchange, identification of key risks around abalone release and refinement of a genetics risk-assessment method for abalone release (see Appendix 2 for Agenda). Over 30 participants were invited to attend the workshop including expert geneticists, abalone scientists, fishery and policy managers, abalone industry members and hatchery/farm representatives. The DaRT methods and results (from Appendix 1) were presented in detail to workshop participants, alongside presentations on genetic risks from abalone release in Australia (Dr Adam Miller, Dr Lachlan Strain) and New Zealand (Dr Tom McGowan).

A draft, likelihood-consequence genetics risk-assessment method, which had been developed as a tool to assist with identifying the genetic risks of F1 and/or FX abalone release into wild populations, was presented to workshop participants. Using the information provided in the presentations, and that from the subject matter experts attending, each component of the draft method was assessed, evaluated and discussed by workshop participants. This included refinement of the overarching management objective, along with the likelihood and consequence factors, their score definitions and weightings, the proposed risk matrix and potential risk rankings.

The refined genetics risk assessment was applied to five hypothetical case studies (see Appendix 2 for Agenda) to evaluate suitability and appropriateness of resulting scores and potential risk rankings. The hypothetical case studies included an experimental release of FX abalone in The Gap, a commercial subtidal aquaculture release of FX in Anxious Bay, re-establishment of Roe's Abalone (*Haliotis roei*) stock following a mortality from a marine heatwave in Western Australia (Strain et al. 2019), and large-scale commercial releases of FX or F1 (from diverse and replenished broodstock) across the Western Zone.

Throughout the case studies application, the risk assessment was further reviewed and refined through recommendations from workshop participants.

Objective 3 – Preliminary cost-benefit analysis

The model

A preliminary, cost-benefit model was developed to estimate survivorship, biomass, and value of a cohort of Greenlip Abalone released as juveniles. This analysis assumes all surviving released juveniles are harvested. Estimation of survivorship, biomass and value requires multiple model parameters including growth, survival, weight-at-length, beach price, cost to buy and release juveniles, and cost of fishing (Table 1). Profitability of juvenile abalone release was compared to two alternative investment scenarios: foregoing catch, and financial investment. The model is interfaced for stakeholder adoption as a user-friendly, Microsoft Excel spreadsheet, and the calculation steps were validated through parallel coding in R statistical software. For the base-case model, parameters were calculated or obtained from the best available data sources (see Table 1). Because these may be incomplete and over/underestimated, there are several parameters that can be modified by the user of the spreadsheet tool (see Table 1), which then change the financial and biological outcomes. This enables accessibility and ease-of-use by industry decision-makers, who can update the inputs, and obtain more refined outcomes, as new information becomes available. The model parameters, and whether they are fixed or variable, and the alternative investment scenarios are detailed below.

Growth

To estimate growth parameters, a dataset of 910 length measurements associated with a tag-recovery program of Greenlip Abalone conducted at the entrance to Mitlers Cove, The Gap, between 2002 and 2004 (SARDI unpublished data) were used. The GROTAG growth algorithm (Francis 1988), coded in R, was used to obtain the parameters of the von Bertalanffy (1938) growth model that represented the best fit to the dataset. This algorithm also accounts for seasonality in growth.

From these growth parameters, the time taken for the cohort to grow from release (i.e., length of 40 mm) to harvest (i.e., length of 150 mm) was determined. This was 7.71 years. This parameter is fixed.

Survival

Survival rates observed in the study of released Greenlip Abalone in Western Australia by Hart and Strain (2016) were adopted for the base case. Survival was parameterised in two components. Firstly, Hart and Strain (2016) report that average survival among 24 release locations during the first 6 months from release is 38%. Secondly, since Hart and Strain (2016) report that overall survival from release to harvest is 10%, it is implied that survival from 6 months to harvest is $^{10\%}/_{38\%} = 26\%$. Thus, we assume that 38% of released abalone survive the first 6 months, and 26% of abalone that survived to six months then also survive until harvest. These values correspond to instantaneous rates of mortality (M) of 1.94 and 0.187, respectively. These parameters can be modified by the user of the spreadsheet tool.

Weight-at-length curve

At a particular length of abalone, the weight was estimated using a standard allometric weight-length relationship:

$$\text{Whole weight} = a \times [\text{Shell length}]^b$$

Stobart et al. (2020, Table 6.8) list the parameters of the weight-length relationship for a series of unpublished datasets of Greenlip Abalone in South Australia. These parameter estimates were pooled using meta-analysis, with a random-effects model to allow for heterogeneity across studies (Borenstein et al. 2009). Constant variance was assumed across studies, and since only sample size was reported, studies were weighted by sample size. Independence in estimates of each parameter was assumed, and the analysis was undertaken in the R package *meta* (Balduzzi et al. 2019). This resulted in pooled parameter estimates of $a = 2.09 \times 10^{-4}$ and $b = 3.06$. This corresponds to a 150 mm abalone weighing 830 g. These parameters are fixed.

Beach price

Following a meeting with industry representatives, a beach price of $60/kg whole weight for 150 mm Western Zone Greenlip Abalone was used. This parameter can be modified by the user of the spreadsheet tool.

Cost to buy and release juveniles

The cost to buy and release juvenile Greenlip Abalone in South Australia is not yet determined. In Western Australia, Hart et al. (2013c) assume that a 40mm individual costs $1.32 to buy and release, and this is the value used in the base-case model. This parameter can be modified by the user of the spreadsheet tool.

Total dollars invested

The potential investment levels for releasing juvenile Greenlip Abalone in South Australia are yet to be discussed and will depend on the outcomes of this study. In the base model, $100,000 is used. This value is divided by the cost to buy and release juveniles (above) to determine the total number of juveniles released. This parameter can be modified by the user of the spreadsheet tool.

Alternative investment choices

Categorising stock recovery as an investment, other investment scenarios can be considered and compared. Two are included here: foregoing catch and financial investment.

Cost of fishing

In the Western Zone in 2018-19, the average variable cost of fishing per licence was $293,835 (BDO EconSearch 2020). In calendar year 2019, the total combined catch of abalone (i.e., including both Greenlip Abalone and Blacklip Abalone) was 117.13 t meat weight. Assuming a 3:1 ratio of whole weight to meat weight, this corresponds to a total combined catch of 351.39 t whole weight. Across 22 licences, this equates to an average combined catch of 15.97 t whole weight. Dividing the average variable cost by the average combined catch, the cost of fishing was estimated at $18.39/kg whole weight, and is the value used in the base-case model. This parameter can be modified by the user of the spreadsheet tool.

Foregoing catch

Under a scenario of foregoing catch, which also increases stock biomass, the return from investing the same dollar amount into reducing catch for one year, rather than releasing juveniles, is estimated. Simply put, this essentially 'buys' an amount of adult abalone in the water, which would not have remained there otherwise. This scenario is included because it represents a relatively low-risk method for increasing the biomass of the abalone population, compared to the much higher risk, and much lower outcome certainty,

from releasing juveniles. The second value of M (0.187) is assumed. Since the wild Greenlip Abalone in this scenario grow from legal minimum length (i.e., 145 mm shell length (late adulthood)) for an additional year (to 151 mm shell length, and thus to a length similar to the size at harvest of the modelled released cohort (150 mm shell length)), the survival over that year was estimated as $e^{-0.187} = 82.9\%$. This parameter can be modified by the user of the spreadsheet tool.

Financial investment

Under this second alternative investment scenario, the return from a low-risk financial investment (e.g., term deposit in a bank) using a standard compound growth formula, rather than releasing juveniles, is estimated. This scenario yields a financial return but makes no contribution to the recovery of abalone biomass. A time step that matches the stock recovery scenario (i.e., 7.7 years from juvenile abalone release to harvest) and a 3% interest rate were assumed. While larger discount rates have been used in the economic analyses of Australian abalone fisheries by Hart et al. (2013c) and BDO EconSearch (2020), a lower rate was used to represent a conservative financial investment.

Table 1. The model parameters and base-case values used for preliminary cost-benefit analyses.

Parameter	Base-case value	Source	User-changeable?
Cost per juvenile to buy and release ($)	$1.32	Hart et al. (2013c) & AIASA	Yes
Total dollars invested ($)	$100,000	-	Yes
Cost to fish 1 kg whole ($ / kg)	$18.39	Calculated from data in BDO EconSearch (2020)	Yes
Proportional survival over first 6 months	38%	Hart and Strain (2016)	Yes
Proportional survival from 6 months to 150 mm	26%	Calculated from data in Hart and Strain (2016)	Yes
Proportional survival from 145 mm for 1 year	83%	Calculated from data in Hart and Strain (2016)	Yes
Beach price ($/kg whole)	$60.00	Industry consultation	Yes
Weight at legal length 145 mm (kg whole)	0.748 kg	Calculated from data in Stobart et al. (2020)	Not recommended
Time from planting to 150 mm (years)	7.7 years	Calculated from SARDI unpublished data	Not recommended
Weight of an abalone at 150 mm (kg whole)	0.823 kg	Calculated from data in Stobart et al. (2020)	Not recommended
Length 1 year after foregoing catch (mm SL)	151 mm	Calculated from SARDI unpublished data	Not recommended
Weight of an abalone at 151 mm (kg whole; i.e., 1 year growth from 145 mm	0.841	Calculated from data in Stobart et al. (2020)	Not recommended
Bank interest rate (%)	3%	-	Yes

Results

Objective 1 – Use SNPs to compare the genetic diversity and population structure of wild and FX Greenlip Abalone

Using SNPs, the genetic diversity and population structure of wild Greenlip Abalone was found to be different from hatchery FX abalone (Figures 2-4 in Appendix 1). The distance dendograms highlighted that wild Greenlip Abalone had a higher level of genetic diversity than FX abalone, with the reduced diversity observed for the FX abalone suggesting a level of inbreeding in the hatchery cohorts. Wild and FX abalone also formed distinct clusters in the PCA plots with no overlap, portraying clear differences in the genetic structure of both groups. Values for average allelic richness and expected heterozygosity were slightly higher for wild populations compared to FX abalone (Figures 5 & 6 in Appendix 1), providing further support to the observation of increased genetic diversity in wild populations and potential inbreeding in FX abalone. There was also clear differentiation between wild and FX abalone from the fixation index pairwise comparisons (Figure 7 in Appendix 1), with a general lack of differentiation within the wild sites and a moderate level of differentiation among the FX abalone cohorts.

Objective 2 – Assess the genetic suitability of FX abalone for release into wild populations, including an expert-based, genetics risk assessment workshop

The primary result of the two-day, expert-based abalone release, genetics risk assessment workshop was a risk-based method to assist evaluation of genetic risks associated with juvenile abalone release (Appendix 3). The approach encapsulates, in a likelihood (i.e., probability of the hazard occurring; see Table 2) – consequence (i.e., magnitude of the outcome if the hazard occurs (i.e., potential change in the genetic integrity of the population through displacement of the native gene pool by released individuals, under the precautionary presumption that increased difference between released and native abalone would result in a more serious consequence; after Hindar et al. 1991, Li et al. 2004, Roodt-Wilding 2007, Blanchet et al. 2008, Laikre et al. 2010, Danancher and Garcia-Vazquez 2011, Grant et al. 2017, O'Sullivan et al. 2020; see Table 2)) framework, the key genetic risk factors around abalone release that were identified during information exchange and discussion of the DaRT results (Appendix 1) by over 25 workshop participants (Appendices 2 & 4).

During information exchange, Dr Adam Miller provided essential information on maximising genetic diversity, minimising genetic risks associated with captive rearing and the importance of matching genes to local environments through understanding the population genetics of wild stocks. Dr Lachlan Strain and Dr Tom McCowan presented examples of existing genetic risk management, including application of policy where available, for juvenile abalone release in Western Australia and New Zealand. The DaRT results, presented by Dr Sarah Catalano and Dr Andrzej Kilian, showed clear genetic differentiation between wild and FX Greenlip Abalone (Appendix 1). Other topics discussed included the potential for introducing more tolerant genotypes into local environments (e.g., heat-tolerant genotypes), the impact of hatchery broodstock and breeding practices on release animals and the application and relevance of DaRT results to abalone release in South Australia.

Each of these presentations and discussions were vital in refining the draft genetics risk assessment, with significant progress made (Table 2): the overarching management objective and key hazards being assessed were clarified, consequence and likelihood risk factors were reviewed and modified, and score descriptors were revised. In addition, workshop participants (1) noted there were multiple potential

'pathways to harm' (Raybould 2006, Ankley et al. 2010) through which the release of hatchery-reared juvenile abalone could result in adverse outcomes on the genetic integrity of existing wild stocks (e.g., gametes from released abalone mix with gametes from native abalone), and (2) considered each consequence and likelihood risk factor score should have an equal weighting in the aggregated consequence and likelihood scores (i.e., across factors) for testing case studies.

Table 2. A comparison of the genetics risk assessment method before (Draft) and after (Refined) discussions and evaluation by the workshop participants.

Component	Draft (Before)	Refined (After)
Overarching Management Objective	Maintain genetic sustainability in metapopulations of Wild Abalone stocks in South Australia	Preserve genetic integrity in wild abalone stocks in South Australia
Key Hazard	N/A	Genetic integrity (diversity, local adaptive traits, resilience, fitness) reduced in South Australian wild abalone stocks, interpreted as a 'Change in genetic structure of wild abalone populations'
Likelihood factors	• Release location (Subtidal aquaculture (structures) vs Restocking (reef)) • Release number (Small vs Large; single vs multiple) • Dispersal potential (Disconnected vs Fully Connected) • Distance to wild abalone (Within/Adjacent vs Distant) • Harvest size (Before maturity vs Fully mature)	• Spatial scale * • Release number* • Distance to wild abalone (Within/Adjacent vs Distant) • Harvest size (Spawning potential)
Consequence factors	• Release location density (High vs Low) • Release location genetic diversity (High diversity vs Low diversity) • Broodstock origin (Fresh from release location vs Existing farmed) • Offspring genotype relative to release location genotype (Similar vs Dissimilar) • Number of spawners contributing to offspring (<5 vs >20)	• Existing wildstock density (High vs Low) (catch as proxy)* • Representative contribution to offspring from adults* • Seed stock* • Genetic base of released juveniles (broodstock founders)*
*Definitions of likelihood and consequence scores were also amended		

The revised risk assessment was applied to five hypothetical case studies, which led to further refinement. The scores assigned for each likelihood and consequence factor (including the rationale), and the weighted likelihood and consequence scores for each of the five case studies using the risk assessment (Appendix 3) are provided in Table 3. Scores were assigned by workshop participants, based on the information made available to them at the workshop. The information made available to the workshop participants is provided in Appendix 4 for case studies 1-3. Case studies 4 and 5 were included at the end of the workshop and designed to show contrast between large-scale releases of well-managed F1 versus FX juveniles. Information for these latter case studies was provided verbally to workshop participants.

There are 12 steps to the risk assessment. Applying the risk assessment shown in Appendix 3 and using case study 1 (experimental release of FX abalone in The Gap) as an example, the 12 steps are:

1. Assign a score for likelihood factor 'spatial scale'. This factor reflects the spatial extent of the release. Given abalone undertake limited movement, the likelihood of released individuals contributing to the gene pool of wild abalone stocks increases with the spatial extent of the release. Scores range from 1 (small part of coastline in a single fishing ground, interpreted as release over 100's of metres) to 4 (widespread release along coastline, interpreted as releases in >50% of fishing grounds). A score of 1 was assigned to experimental release in The Gap because the experimental release would be localised to a very small part (<1%) of the fishing grounds at The Gap.
2. Assign a score for likelihood factor 'release number'. This factor reflects the total of the number of releases and the number of individuals released (i.e., cumulative). The likelihood of released individuals contributing to the gene pool of wild abalone stocks increases with increasing numbers of released individuals. Scores range from 1 (<5,000 individuals) to 4 (>200,000 individuals). A score of 1 was assigned because the experimental release would be 4,500 individuals.
3. Assign a score for likelihood factor 'distance to wild abalone'. This factor reflects the degree of spatial separation between the release location(s) and wild abalone stocks. The likelihood of released individuals contributing to the gene pool of wild abalone stocks increases with reduced distance between the release location and wild abalone stocks. Scores range from 1 (>3 km away) to 4 (<1 km away). A score of 4 was assigned because the experimental release site would be in the commercial fishing grounds at The Gap. If 'distance to wild abalone' is unknown, apply highest score.
4. Assign a score for likelihood factor 'harvest size'. This factor reflects the spawning potential of the released juveniles prior to harvest. The likelihood of released individuals contributing to the gene pool of wild abalone stocks increases with increasing harvest size. Scores range from 1 (immature, $<L_{50}$) to 4 (fully mature, $>L_{95}$; i.e., capable of producing a high number of viable gametes). A score of 4 was assigned because the released individuals are not to be recaptured and are expected to reach full maturity. If 'harvest size' is unknown, apply highest score.
5. Integrate the four likelihood factors to calculate the overall likelihood score, by multiplying each of the four likelihood factor scores by their weighting and summing. Thus, for case study 1, the overall likelihood score is (1 * 0.25) + (1 * 0.25) + (4 * 0.25) + (4 * 0.25) which is 2.5. This step is built into the risk assessment spreadsheet.
6. Assign a score for consequence factor 'wildstock density'. This factor reflects the resilience (measured in catch volume/density as these are the most readily available data sources) of the wild abalone stocks to genetic introgression. The consequence of released individuals contributing to the gene pool of wild abalone stocks increases with decreased resilience. Scores range from 0 (high producing area, interpreted as having a mean annual catch >5 t meat weight or no abalone present (e.g., areas in WA where abalone stocks experienced mass mortality during a marine heatwave; see Strain et al. (2019)) to 4 (very low producing area, interpreted as having a mean annual catch <1 t meat weight). A score of 1 was assigned because mean annual Greenlip Abalone catches from The Gap over the past five years were about 4.5 t. If 'wildstock density' is unknown, apply highest score.
7. Assign a score for consequence factor 'broodstock pairings contributing to released stock'. This factor reflects the number of broodstock pairings contributing to the released individuals. The

genetic consequence of released individuals contributing to the gene pool of wild abalone stocks increases as broodstock pairings become fewer, because the fewer parents that are used, the more restricted the gene variation of released individuals. Score is either 0 (offspring from multiple, interpreted as >10, adult pairings) or 4 (offspring dominated from single adult pairing / unknown). For the release scenario at The Gap, a score of 4 was assigned because the number of adult pairings in the FX abalone was unknown. Higher scores should be assigned with increased uncertainty in 'broodstock pairings contributing to released stock'.

8. Assign a score for consequence factor 'generations in captivity of released stock'. This factor reflects the number of generations in captivity. The consequence of released individuals contributing to the gene pool of wild abalone stocks increases with captive timeframes. Score is either 1 (F1 – reflecting expected loss of genetic diversity through hatchery breeding) or 4 (FX). A score of 4 was assigned because the released individuals were FX. Higher scores should be assigned with increased uncertainty in 'generations in captivity of released stock'.
9. Assign a score for consequence factor 'broodstock founders contributing to released stock'. This factor reflects the turnover rate of the adults used to produce the released individuals. The consequence of released individuals contributing to the gene pool of wild abalone stocks increases with a lower replacement rate. Scores range from 0 (100% new appropriate wild broodstock, >30 contributing parents) to 4 (0% new appropriate wild broodstock and/or <5 contributing parents). A score of 4 was assigned because no new wild broodstock were used to produce the FX abalone. Higher scores should be assigned with increased uncertainty in 'broodstock founders contributing to released stock'.
10. Integrate the four consequence factors to calculate the overall consequence score, by multiplying each of the four consequence factor scores by their weighting, and summing. Thus, for case study 1, the overall likelihood score is (1 * 0.25) + (4 * 0.25) + (4 * 0.25) + (4 * 0.25) which is 3.25. This step is inbuilt to the risk assessment spreadsheet.
11. Multiply the overall likelihood and consequence scores, and round up to the nearest whole number, to obtain the risk score. Thus, for case study 1, the risk score is 2.5 * 3.25 which is 8.125 which is then rounded up to a risk score of 9. This step is inbuilt to the risk assessment spreadsheet.
12. Use the risk score to consider the potential risk ranking and risk management/mitigation options that may be appropriate to reduce risk. In this example, the risk score of '9' translated to a potential risk ranking of 'high'.

In the risk assessment, likelihood scores range from 1 to 4, and consequence scores from 0 to 4. Thus, the potential range of risk scores is from 0 to 16. Five potential risk ranking categories – from negligible to extreme – were proposed (Appendix 3).

While workshop participants identified that the "potential risk rankings" component of the risk assessment (see Appendix 3) would require further work – as these were neither (1) calibrated against real-world outcomes because data and evidence to do this are rare (Hayes 2022), nor (2) able to be fully considered at the workshop – the five case studies used to test the refined risk assessment did result in a range of risk scores that reflected the levels of risk intuitively perceived by the experts present (Table 4). Overall, the workshop provided an approach that serves as a valuable foundation and provides a structure for further consideration and refinement, along with adaptation to suit local application and use, by regulatory agencies.

Objective 3 – Preliminary cost-benefit analysis

The spreadsheet

The user interface (Microsoft Excel spreadsheet; Figure 1) has been developed with base-case values. However, for many parameters, there is the ability for the user to specify input values (see Table 1), notably in the "Key Inputs" section, as several parameters may change regularly and need amendment (e.g., cost per juvenile to buy and release (base case: $1.32), total dollars invested (base case: $100,000), and cost of fishing (base case: $18.39/kg)). These key inputs are used in background calculations in the "Under the Hood" section (Figure 1 and detail in Figure 2). The calculations are then used to determine the "Extra Biomass" section, coloured green, which shows the additional biomass gained in each of the three scenarios. That biomass then leads to the corresponding economic outcome in the "Net Increase in Value of Investment" section, coloured gold. The economic value is given in dollars gained (or lost) from the initial investment for each of the three scenarios considered (stock recovery by juvenile release, 'foregoing catch', 'financial investment'). For the 'stock recovery' and 'foregoing catch' scenarios, the "Net Increase in Value of Investment" assumes that the biomass is harvested for a revenue, and then the initial investment is subtracted from that revenue. A graph also gives a visual illustration of these same figures. In this way, the user interface offers the user a rapid way to examine how the economic and biomass outcomes, as estimated by the model, change under a variety of key inputs.

Outcomes

The outcomes of the base-case, cost-benefit analysis model (single investment of $100,000; Figure 3) show juvenile release resulting in a gain in biomass of 6,211 kg after 7.7 years, which has a net landed value gain of $158,444 (i.e., initial investment subtracted). This compares favourably to both alternate scenarios ('foregoing catch': 1 year, 1,555 kg biomass increase, net loss of $35,314; 'financial investment': 7.7 years, 0 kg biomass change, net gain of $25,581).

However, the outcomes are highly dependent on the input parameters. For comparison, for the same $100,000 investment, but with both the purchase costs per released juvenile and the cost of fishing increased by 50%, juvenile release results in a 4,141 kg biomass gain after 7.7 years (i.e., 33% lower because fewer juveniles are released for the $100,000 investment) and a net landed value gain of $34,222.

This still compares favourably to both the 'foregoing catch' (1 year, 1,555 kg biomass increase, net loss of $49,616) and 'financial investment' (7.7 years, 0 kg biomass change, net gain of $25,581) scenarios.

The mortality values applied also substantially influence the outcomes. If survival over the first 6 months is reduced to 19% (i.e., half the corresponding base-case value), with other base-case parameters unchanged, the gain in biomass from juvenile release is estimated at 3,106 kg after 7.7 years, with a net gain of $29,222. This still compares favourably to both the 'foregoing catch' (net loss of $35,314) and 'financial investment' (net gain of $25,581) scenarios.

Table 3. The results from each case study using the genetics risk assessment method developed at the workshop. Scores (bold font) assigned for each likelihood (dark grey shading) and consequence (light grey shading) factor, the weighted likelihood and consequence scores and the risk score (rounded up to nearest whole number) for each of the five case studies are shown. Also shown is the rationale for the assigned score.

Case Study	Likelihood factors				Weighted likelihood score	Consequence factors				Weighted consequence score	Risk Score (Likelihood Score * Consequence Score)
	Spatial scale	Release number	Distance to wild abalone	Harvest size		Wildstock density	Broodstock pairings contributing to released stock	Generations in captivity of released stock	Broodstock founders contributing to released stock		
1. Experimental release of FX abalone in The Gap	**1** (single small release area)	**1** (<5,000 released)	**4** (release site in commercial grounds)	**4** (no planned recapture)	2.5	**1** (commercial ground)	**4** (unknown, highest score assigned)	**4** (FX)	**4** (no new wild broodstock used)	3.25	9
2. Commercial subtidal aquaculture release of FX in Anxious Bay	**1** (single small release area)	**4** (>200,000 released)	**4** (release site in commercial grounds)	**4** (harvest after full maturity)	3.25	**1** (commercial ground)	**4** (unknown, highest score assigned)	**4** (FX)	**4** (no new wild broodstock used)	3.25	11
3. Re-establishment of Roei stock following a heat wave mass mortality in Western Australia	**2** (multiple small release areas)	**3** (>50,000 released)	**4** (release site in previous commercial grounds)	**4** (no planned recapture)	3.25	0 (previous productive commercial grounds, no Roei stocks in release area)	**0** (multiple breeding pairs)	**1** (F1)	**3** (only new wild broodstock used – but from distant source)	1	4
4. Large-scale commercial release of FX into key fishing grounds of the Western Zone	**4** (multiple large release areas)	**4** (>200,000 released)	**4** (release site in commercial grounds)	**4** (no planned recapture)	4	**1** (commercial grounds targeted for release)	**4** (unknown, highest score assigned)	**4** (FX)	**4** (no new wild broodstock used)	3.25	13
5. Large-scale commercial release of F1, into multiple key fishing grounds, from a diverse and replenished broodstock	**4** (multiple large release areas)	**4** (>200,000 released)	**4** (release site in commercial grounds)	**4** (harvest after full maturity)	**4**	**1** (commercial grounds targeted for release)	**0** (multiple breeding pairs)	**1** (F1)	**0** (only new wild broodstock used)	0.50	2

Table 4. *Results from each case study, using the genetics risk assessment method developed at the workshop. Results based on the information made available to the workshop participants to undertake the risk assessment and provided in Appendix 4 for case studies 1-3. Case studies 4 and 5 were included at the end of the workshop and designed to show contrast between large-scale releases of well-managed F1 versus FX juveniles. Information for these latter case studies was provided verbally to workshop participants. The "potential risk rankings" and "potential residual risk rankings" components are in grey font as 'indicative'. While these were not fully considered at the workshop, the five case studies used to test the refined risk assessment did result in a range of scores and outcomes that reflected the levels of risk intuitively perceived by the experts present.*

Case Study	Risk score	Potential risk ranking	Potential controls to reduce risk score	Residual risk score if all potential controls applied	Potential residual risk ranking if all controls applied
1. Experimental release of FX abalone in The Gap	9	High	Release site away from wild stocks; use of F1 offspring from appropriate breeding program	1	Low
2. Commercial subtidal aquaculture release of FX in Anxious Bay	11	High	Release site away from wild stocks; fewer juveniles released; released juveniles recaptured before sexual maturity; use of F1 offspring from appropriate breeding program	1	Low
3. Re-establishment of Roei stock following a heat wave mass mortality in Western Australia	4	Low	None identified	4	Low
4. Large-scale commercial release of FX into key fishing grounds of the Western Zone	13	Extreme	Fewer, smaller, release sites away from wild stocks; fewer juveniles released; use of F1 offspring from appropriate breeding program	1	Low
5. Large-scale commercial release of F1, into key fishing grounds of the Western Zone, from a diverse and replenished broodstock	2	Low	None identified	2	Low

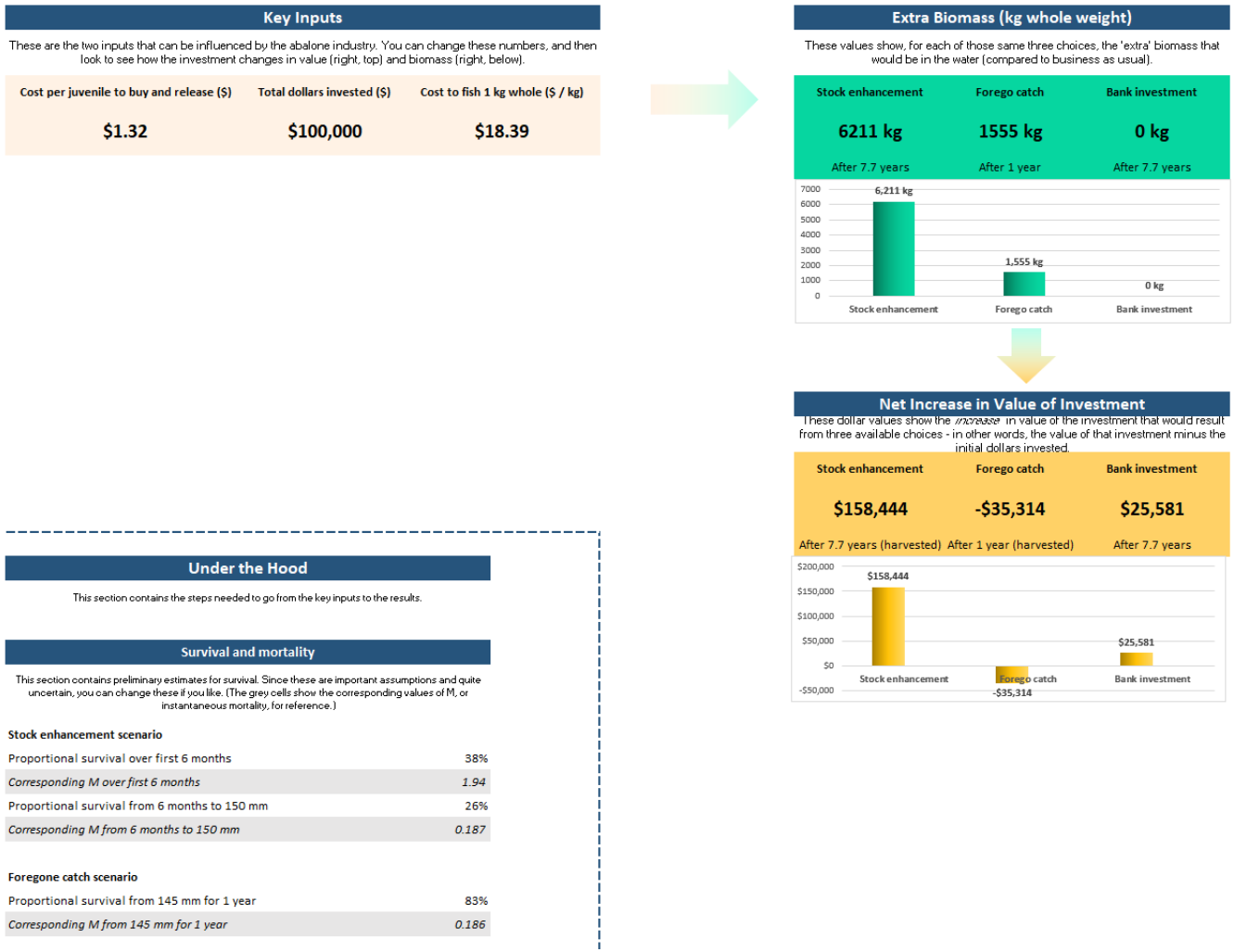

Figure 1. *Screenshot of the cost-benefit analysis model user interface, which implements the preliminary version of the stock recovery model as a Microsoft Excel spreadsheet*.

Under the Hood

This section contains the steps needed to go from the key inputs to the results.

Survival and mortality

This section contains preliminary estimates for survival. Since these are important assumptions and quite uncertain, you can change these if you like. (The grey cells show the corresponding values of M, or instantaneous mortality, for reference.)

Stock enhancement scenario

Proportional survival over first 6 months	38%
Corresponding M over first 6 months	*1.94*
Proportional survival from 6 months to 150 mm	26%
Corresponding M from 6 months to 150 mm	*0.187*

Foregone catch scenario

Proportional survival from 145 mm for 1 year	83%
Corresponding M from 145 mm for 1 year	*0.186*

Fixed inputs

These are inputs that the abalone industry can't really influence. You can change these if you like, but keep in mind they may be difficult to change in reality.

Beach price at 150 mm ($/kg whole)	60

Stock enhancement scenario

Time from planting to 150 mm (years)	7.7
Weight of an abalone at 150 mm (kg whole)	0.82982

Foregone catch scenario

Years of foregone catch	1
Weight at legal length 145 mm (kg whole)	0.74805
Length 1 year after foregoing catch (mm SL)	151
Individual weight 1 year after foregoing catch (kg whole)	0.84065

Bank investment scenario

Bank interest rate (%)	3

Intermediate calculations

These are the intermediate steps that Excel uses to take the inputs and calculate the return on investment.
Caution: Changing these steps may make Excel behave incorrectly.

Stock enhancement scenario

Total number of abalone released	75758
Survival from seeding to 150 mm (%)	9.9%
Number surviving to 150 mm (# individuals)	7485
Combined weight when abalone reach 150 mm (kg whole)	6211
Revenue from survivors ($)	$372,666
Cost to harvest survivors ($)	$114,222
Landed value of survivors ($)	$258,444

Foregone catch scenario

Weight of catch foregone (kg)	1667
Individuals of catch foregone	2228
Individuals that survive the year	1849
Combined weight of survivors (kg whole)	1555
Landed value from finally harvesting foregone catch ($)	$64,686

Calculating financial value ($) from each scenario

Net increase in value of investment from **stock enhancement** ($)	$158,444
Net increase in value of investment from **bank** ($)	$25,581
Net increase in value of investment from **foregone catch** ($)	-$35,314

Calculating additional biomass (kg) from each scenario

Additional biomass under **stock enhancement** after 7.7 years (kg)	6211
Additional biomass under **foregone catch** after 1 year (kg)	1555
Additional biomass under **bank investment** after 7.7 years (kg)	0

Figure 2. *Screenshot of the background calculations in the "Under the Hood" section of the stock recovery model*.

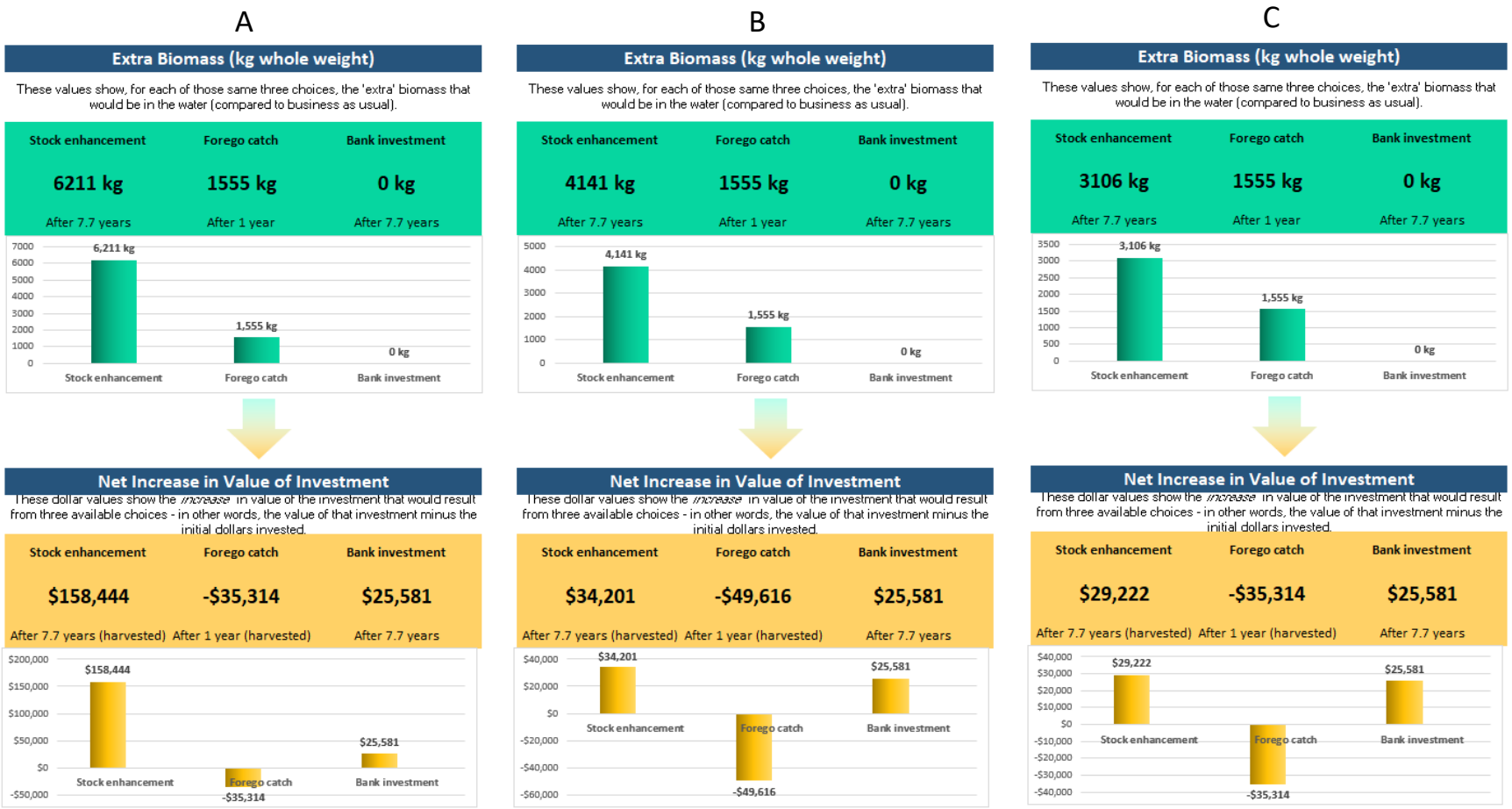

Figure 3. *Key inputs, extra biomass and net increase in value of investment for the (A) base-case and sensitivity tests, including (B) cost of juveniles and fishing 50% higher and (C) initial survival 50% lower scenarios*.

Discussion and Conclusions

Wild abalone catches have declined by 50% nationally and 39% in South Australia over the past 20 years (Status of Australian Fish Stocks Reports). In South Australia, most of this decline was for the Western Zone Blacklip Abalone Fishery, but the Western Zone Greenlip Abalone stocks have also been in decline, and catches have reduced to 15% below the long-term catch of 78 t (Stobart and Mayfield 2021). Obtaining information that informs the release of juvenile Greenlip Abalone, including directly into commercial Greenlip Abalone grounds, could facilitate substantial increases in yield through stock recovery and associated Gross Value of Production. This is because abalone is a high-value product; small increases in volume translate to large increases in value, in turn supporting regional economies and communities.

This diverse project has been pivotal in making progress towards releasing juvenile Greenlip Abalone to support stock recovery in South Australia. The project has addressed several key needs that currently represent a "bottleneck" to progressing restocking areas of the Western Zone Abalone Fishery where Greenlip Abalone are depleting, with biomass levels well below carrying capacity and historical levels (Stobart and Mayfield 2021). The key industry need was to assess release of juvenile Greenlip Abalone in the Western and Central Zones to evaluate its long-term economic viability. To support this important industry goal, the key Government need was for data to underpin the release policy – aimed at ensuring a low risk to wild abalone populations through adequate mitigation of disease and genetic risk associated with stock recovery and sub-tidal aquaculture activities (after Sandoval-Castillo et al. 2017) – and the opportunity to enable the review, and potentially to amend, the Livestock Notice requiring, for sub-tidal aquaculture, the released abalone to be F1 descendants of local broodstock if there is a population of wild abalone within 1 km, noting that this principle has also been applied to the release of hatchery-reared juveniles to wild harvest fishing grounds.

The project focused on three key first steps required to 'pave-the-way' to restocking trials and programs using hatchery-reared abalone. These were (1) comparing genetic differences between wild and hatchery-reared (FX) Greenlip Abalone; (2) developing a genetic risk assessment as a new method for assessing genetic risks associated with release of abalone; and (3) delivering a preliminary cost-benefit analysis model of release of juvenile Greenlip Abalone with an easy-to-use interface, to facilitate use by industry members to inform investment in abalone release.

Undertaking the genetic analyses to compare wild and FX Greenlip Abalone was an important step in determining the suitability of the hatchery-reared FX abalone for re-stocking. The potential advantages for the WZ Abalone Industry in using FX juveniles are their availability and low price, given that Yumbah Aquaculture produces surplus Greenlip Abalone each year that are culled. This study, which used SNPs to examine differences in genetic diversity and population structure between wild Greenlip Abalone at eight sites and from three hatchery cohorts from Yumbah Aquaculture, demonstrated substantial differences between the wild and farmed Greenlip Abalone. Results were consistent across genetic analyses – including distance dendrograms, PCA, allelic richness, expected heterozygosity and pairwise comparisons – and across three genetic marker sets spanning over one order of magnitude in the number of markers. This indicates a high level of confidence and robustness in the results. Notably, samples from wild and FX Greenlip Abalone formed distinct clusters in the distance dendrograms, PCA plots had no overlap, and average allelic richness and expected heterozygosity were higher for wild samples. The latter indicates

potential inbreeding in the FX Greenlip Abalone, though it may also reflect founder/broodstock capture and/or deliberate selection effects. The key finding from the genetic analysis was that the genetic diversity and population structure of wild Greenlip Abalone is different from hatchery FX Greenlip Abalone. No direct information on the likely change in genetic structure of wild stocks from fishing (e.g., selective removal of fast-growing individuals) were available. However, the high heterozygosity from the SNP analyses in the wild abalone suggest a low impact (Appendix 1).

The national, two-day, genetic risk assessment workshop yielded an expert-based and objective method for assessing the genetic risks associated with juvenile abalone release. Workshop participants (n > 25; spanning expert geneticists, abalone scientists, fishery and policy managers, abalone industry members, and hatchery/farm representatives) agreed that the relevant, overarching management objective – to which the risk assessment should be applied – was to "Preserve genetic integrity of wild abalone stocks in South Australia", with the key hazard to be managed being "Genetic integrity (e.g., diversity, local adaptive traits, resilience, fitness) reduced in South Australian wild abalone stocks", which was interpreted as 'change in genetic structure of wild abalone populations', because no information on the genetic integrity sub-components was available. The risk assessment takes a likelihood (i.e., probability of released individuals contributing to the population gene pool and impacting the genetic integrity of the population) – consequence (i.e., magnitude of the potential change in the genetic integrity of the population through displacement of the native gene pool by released individuals, under the precautionary presumption that increased difference between released and native abalone would result in a more serious consequence; after Hindar et al. 1991, Li et al. 2004, Roodt-Wilding 2007, Blanchet et al. 2008, Laikre et al. 2010, Danancher and Garcia-Vazquez 2011, Grant et al. 2017, O'Sullivan et al. 2020) approach consistent with the International Standard for risk management (ISO 31000:2018). Workshop participants considered information from the series of presenters and identified four likelihood and four consequence risk factors. Score descriptions for each of the eight factors were developed, and their relative importance implemented via a weighting. Higher scores reflect higher risk, and a higher weighting would reflect increased importance of that risk factor. The risk score (i.e., the consequence score multiplied by the likelihood score) range can be used to consider the potential risk ranking and alternate risk management/mitigation options that may be needed/appropriate to reduce risk.

During the workshop, the risk assessment was used to assess genetic risk across multiple, diverse, hypothetical case studies. Amongst others, these case studies included the experimental release of FX Yumbah juveniles at The Gap in South Australia and a commercial release of F1 progeny from non-local broodstock to re-stock *Haliotis roei* following heat-wave mortalities in Western Australia. The case studies were useful in refining the risk assessment, which, in re-application following modification, yielded risk scores and risk rankings generally consistent with the expert-based, intuitive approach. Notably, the risk score increases if you have higher release numbers, release into (or adjacent to) existing wild abalone stocks, and the released stock is FX (Li et al. 2004, Blanchet et al. 2008). The converse applies: lower risk scores eventuate from releasing few F1 juveniles produced using new and genetically appropriate (i.e., local) broodstock with a large number of contributing parents (Gutierrez-Gonzalez and Perez-Enriquez 2005, Roodt-Wilding 2007, Le Vay et al. 2007) into a high density of, or distant from, existing wild abalone stocks (i.e., there would be a low proportional contribution to the gene pool from released abalone).

Release approaches that result in lower risk scores inform potential controls to reduce the genetic risks associated with higher risk scores. For example, the risk score for the experimental release of FX Greenlip Abalone in The Gap could be reduced from 9 to 1 by having the release site away from wild stocks and

using F1 offspring from an appropriate breeding program (Table 4). Given that three of the four consequence factors relate directly to the genetic 'quality' of the juvenile Greenlip Abalone being released, the most effective control for reducing risk is to release F1 offspring from a comprehensive breeding program explicitly designed to minimise the genetic differences between released farmed juveniles and native wild stock. These differences can be measured prior to release.

Several potential improvements to the risk assessment were identified during the workshop. First, while noting the five case studies used to test the refined risk assessment did result in a range of risk scores and potential risk rankings that reflected the levels of risk intuitively perceived by the experts present, these were unable to be fully considered at the workshop. The second is the scoring guidelines, particularly for release number. Based on the experience from Western Australia, these guidelines may be overly conservative (Drs Anthony Hart and Lachlan Strain, personal communication) and could be adjusted upwards to reflect expected survivorship to maturity or harvest. However, this would result in the risk assessment being less conservative. The third area for further consideration is the weighting for each of the likelihood and consequence risk factors. The current version of the risk assessment has each of these equally weighted. An alternative would be to have uneven weighting, with an increased weighting applied to two consequence risk factors (e.g., generations in captivity of released stock and broodstock founders contributing to released stock; Dr Adam Miller, personal communication). In addition to these three potential improvements, the risk assessment could be simplified through the amalgamation of overlapping consequence factors (e.g., generations in captivity of released stock and broodstock founders contributing to released stock; Dr Marty Deveney, SARDI, personal communication).

If juvenile abalone are released, pre- and post-release monitoring should occur to inform the assumptions and suitability of the risk assessment. Where practical, this monitoring should include (1) genetic differences between wild (including broodstock) and the released juvenile abalone; (2) measurement of survival, growth and maturity of released juveniles; and (3) periodic evaluation of any genetic changes in abalone at the release site(s) that may have occurred (e.g. Sekino et al. 2005). Consistent with the methods used in this study, SNPs analyses to inform genetic diversity and population structure, distance dendrograms, PCA, allelic richness, expected heterozygosity and pairwise comparisons would be suitable to monitor genetics. These three monitoring components should be used to identify and inform potential amendments to the risk assessment during the required periodic reviews, that are necessary as information on juvenile abalone release impacts improves. Notably, if a genetic impact from release is detected, this information should then be used directly to review, and potentially modify, the risk assessment. This additional information should also be used to review the definitions for each likelihood and consequence factor, factor weightings, and the link between risk score and risk rating. Reviewing the link between risk score and risk rating, along with risk management and mitigation options that may be needed/appropriate to reduce risk, is important because, as data/evidence were rare, the risk scores were unable to be calibrated against real-world outcomes, making identification of acceptable levels of adverse effects (and, thus, risk acceptance) difficult (Hayes 2022). Future review of the risk assessment should also (1) consider and, where appropriate include, representation from more diverse stakeholders than were at the workshop, such as the community, recreational fishers, indigenous groups and the hospitality industry and, (2) further develop and document 'pathways to harm'.

Reflecting the need for periodic review and improvement of the risk assessment, application of the version developed in this project should be undertaken in a precautionary manner (with higher scores assigned for each factor as uncertainty increases; after Hayes 2022) that reflects known uncertainties and

idiosyncrasies (after Hayes 2022). For example, discretising the likelihood factor 'release number' (scores range from 1 for <5,000 individuals to 4 for >200,000 individuals) can result in either (1) different scores for this factor from small changes in release number (e.g., releasing 4,999 and 5,001 individuals results in scores of 1 and 2, respectively) or (2) the same score for large changes in release number (e.g., releasing 200,001 or 2 million individuals both result in a score of 4). For the first of these, factor score increases without an appreciable change in risk while for the latter, factor score is unchanged with an appreciable change in risk. Additionally, there may be some uncertainty associated with measures within the risk assessment (e.g., wildstock density) that might require further information for assigning a factor score (e.g., surveys) or by using a conservative approach; notably, higher scores should be assigned for each factor to address uncertainty. Secondly, the translation from the risk scores to the potential risk rankings were neither fully considered at the workshop nor calibrated against real-world outcomes because data and evidence to do this are rare (Hayes 2022). This means the risk scores that should be associated with each risk ranking are uncertain and yet to be determined and, thus, the risk score that translates to acceptable levels of adverse effects is unknown. Until these data and evidence become available, a conservative approach would be to have lower risk scores resulting in higher risk rankings and/or applying risk mitigation options (e.g., fewer individuals released, selecting release locations further away from wild abalone) even at lower risk scores and potential risk rankings. Thirdly, the risk assessment was largely developed for commercial fishing grounds in South Australia. As there are differences among jurisdictions – including in risk appetite and the need/level for additional risk controls for release of juvenile abalone – local adaptation of definitions for assignment of likelihood and consequence factor scores is required. Subsequently, the links among risk score, potential risk rankings and risk management/mitigation options also require consideration. Despite these challenges, the workshop has provided a risk-assessment method that serves as a foundation framework for undertaking genetic risk assessments that allows structured consideration of genetic risk of releasing hatchery-reared, juvenile abalone and can be considered, refined/adapted and then used by regulatory agencies. Reflecting the localised recruitment life-history trait of abalone, a risk assessment may not be necessary if the proposed release site is >30km from wild abalone stocks.

In the third key component of this project, a preliminary, cost-benefit analysis model, for assessing release of juvenile Greenlip Abalone as a strategy to aid recovery of Greenlip Abalone stocks in South Australia was developed. To aid ease of use by industry – the primary end user – this model was built in Excel with a simple user interface. The model uses inputs on growth and survival following release, costs of release and fishing, and price of harvested Greenlip Abalone. Costs and benefits are measured in both biomass and dollars and are compared directly with two alternative investment choices: foregoing catch and a financial investment. A key feature of the model is the ability for the user to specify important model parameters, allowing them to be updated with changing circumstances (e.g., beach price) or new information (e.g., local survival rates of released juveniles). This important feature facilitates (1) a range of scenario testing, (2) model longevity, and (3) transferability across the Australian abalone fisheries.

Similar to Hart et al. (2013a,b,c), the base case model, which used the best-available data and information, yielded a strong net positive outcome. With a single unborrowed (i.e., cost of capital is zero) investment of $100,000, the model estimates show juvenile abalone release resulting in a gain in biomass, assuming all surviving released juveniles are harvested, of approximately 6 t at around 8 years post-release. The net gain in value was estimated at about $160,000, which compares favourably to both alternate scenarios of 'foregoing catch' and 'financial investment'. Notably, stock rebuilding will need a large number of juvenile

abalone releases, likely over several years, resulting in a high, cumulative total cost. The total cost, and consequently the net benefit, will be strongly influenced by the cost of capital (Prince 2013).

However, the outcomes from the model are sensitive to several key input parameters. One of these influential inputs is the proportion of released juvenile Greenlip Abalone surviving to harvest size. While the base case model uses a value of 10%, an overall average from the work of Hart and Strain (2016) in Western Australia, this parameter value is uncertain for South Australia. For example, the growth-rate estimates for South Australia are slower than those for Western Australia (Mundy 2020). This implies a longer time to reach harvest size in South Australia, potentially resulting in longer exposure to natural mortality (M; e.g., predation) and reduced survivorship. Moreover, Hart and Strain (2016) reported large variability in survival over the first 6 months among the 24 sites of initial releases. By contrast, they found relatively little difference in survival of Greenlip Abalone after the first 6 months. If the survival proportion over the first 6 months is reduced to 19% (i.e., half the base-case value and well within the range of values, from about 10-70% observed by Hart and Strain), with other parameters unchanged, the gain in biomass from juvenile release is estimated at 3 t over 8 years, with a net gain in value of approximately $30,000. This is substantially lower than the base-case, indicating the importance of both early-stage survival as a determinant of release success, and the need to measure post-release survival to reduce uncertainty in the model. Conversely, following ecological theory, the lower average water temperatures in a key release location – The Gap – compared to the study sites in Western Australia, may reduce abalone metabolism (Gillooly et al. 2001), thus potentially improving survival rate per year (McCoy and Gillooly 2008) but the slower invertebrate growth at lower temperature results in a longer time on the bottom to reach legal size and potentially to experience mortality as noted above. With these trade-offs, survival rate is one of the most difficult, but vital, quantities to predict.

Three other influential input parameters are growth rate, costs to buy and release each juvenile, and the cost of fishing. For the first of these, the fixed estimate of time to grow from release to harvest size in the base case model (8 years) may be an overestimate. This is because the growth rate data were obtained from Greenlip Abalone in a relatively sheltered area of The Gap, which may be associated with slower growth rates than other areas of The Gap into which release of juveniles would be more likely. One disadvantage for release into this deeper, faster flowing channel of The Gap – which highlights the role that release habitat plays in recovery – is that dispersal of released juveniles is likely to be higher, meaning they will be harder to find 6 months later, with under-estimation potentially biasing estimates of survival. Given the level of parameter influence on the model outcomes, the design feature allowing the user to specify a high number of model parameters (that should include growth rate in future iterations to account for estimate uncertainty and/or growth variation among locations), thereby enabling these model parameters to be updated with changing circumstances or new information, is an important feature. Thus, the key output from this component of the project is a cost-benefit analysis model that will be a useful tool for industry to evaluate the merits of potential future abalone releases for fisheries recovery under varying circumstances.

In conclusion, this project has delivered three key steps to facilitating juvenile abalone release as a method to support stock recovery in South Australia:

1. Genetic differences were identified between wild and FX Greenlip Abalone.
2. A genetics risk-assessment that constitutes an appropriate method for assessing genetic risks associated with juvenile abalone release was developed. Importantly, with further refinement and local adaptation, it can be used to assist with policy development and application, and is a

significant step towards evaluating the genetic risks associated with the release of juvenile abalone in South Australia.

3. An appropriate modelling framework for cost-benefit analysis has been developed, and the key biological and economic inputs have been derived. Designed for the end user, this tool will facilitate industry understanding of the economic impacts of the release of juvenile abalone under varying circumstances.

Implications

This project has demonstrated clear differentiation between wild Greenlip Abalone collected from numerous sites across South Australia, and hatchery-reared FX Greenlip Abalone obtained from Yumbah Aquaculture. The risk assessment delivered through the national genetic risk assessment workshop, built in Excel, provides a proposed method for assessing the genetic risks associated with juvenile abalone release, enabling the risks of using readily available FX Greenlip Abalone for stock recovery and/or subtidal aquaculture to be compared with the risks of using F1 Greenlip Abalone, bred from appropriately-managed broodstock. The project has also delivered a cost-benefit analysis model, built in Excel, with a user interface to aid ease of use by industry, for assessing release of juvenile Greenlip Abalone as a strategy to aid stock recovery. Both the risk assessment and cost-benefit tools can be readily adapted nationally to inform abalone stock-recovery strategies. The genetic risk assessment can also be used to inform in-sea, abalone aquaculture.

Further development

This project was established as the first phase of an intended multi-phase project. The proposed Phase 2 comprises a natural extension, focused on in-water release and monitoring. Within Phase 2, there are three proposed stages and seven proposed objectives:

Stage 1

Stage 1 is focused on undertaking a pilot study to develop baseline skills for industry and produce a procedure and training materials for the release of juvenile Greenlip Abalone that will also guide Stage 2. There is one proposed objective for Stage 1:

1. Undertake a pilot release of juvenile Greenlip Abalone, including development of training material for industry to undertake juvenile Greenlip Abalone releases.

Stage 2

Stage 2 is focused on expanding the pilot study, consolidating baseline skills for industry and testing release methods and Greenlip Abalone type (i.e., F1 and FX). There are four proposed objectives for Stage 2:

2. Test the effect of alternate release methods on release success;
3. Test the effect of F1 vs FX juvenile Greenlip Abalone on release success;
4. Assess commercial viability of releasing juvenile Greenlip Abalone to accelerate stock recovery in the Western Zone; and

5. Consolidate skills for Industry in release of juvenile Greenlip Abalone and monitoring, including procedure and training material revision as required.

Stage 3

Stage 3 is focused on a commercial release of F1 Greenlip Abalone into an area of the Central Zone where Greenlip Abalone occurred historically. There are two proposed objectives for Stage 3:

6. Apply the tools, knowledge and information obtained through Phase 1 and Phase 2 (Stages 1 and 2) to a release of F1 juvenile Greenlip Abalone in the Central Zone; and
7. Use the data obtained from Objectives 1-6 in Phase 2 to update the assessment of commercial viability of releasing juvenile Greenlip Abalone for stock recovery in SA.

Information acquired in the proposed Phase 2 will be important for several reasons. Firstly, it will arm industry with practical, in-water experience, knowledge and skills in juvenile abalone release. While these skills may not immediately be applied to attempt a large-scale stock recovery, they will help to 'future-proof' the industry against, for example, impacts associated with climate change. Secondly, the data acquired will enable a more sophisticated cost-benefit analysis, potentially including (1) improved survival and growth estimates that would be more accurate and locally relevant; and (2) influence of different seeding and harvest sizes on the economic viability of stock recovery.

In addition to the proposed Phase 2, there would also be benefit in further considering, refining, and then using, the genetic risk-assessment as a tool for assisting the assessment of juvenile Greenlip Abalone releases for stock recovery and subtidal aquaculture. Two data gaps were also identified: (1) genetic differences between F1 and FX progeny and between parent and F1 progeny, and (2) adaptive genotypes (functional correlates) across ecological gradients (e.g., climatic/habitat specific genotypes; after Sandoval-Castillo et al. (2017)).

Extension and Adoption

This is a small project that has had substantial ongoing interaction with stakeholders, including PIRSA Fisheries and Aquaculture, the Western Zone Abalone Industry, the Central Zone Abalone Industry, Dinko Tuna Farmers and the University of Adelaide. Milestone Progress Report 1 was distributed broadly, including to project participants and the Abalone Council Australia (ACA). There was a diverse participation at the genetics risk-assessment workshop, with all attendees receiving copies of the DaRT report, and other supporting materials. Summaries of the project were provided to the AIASA Executive on numerous occasions via regular telephone conversations and at in-person meetings (e.g., 27/5/21, 10/6/21, 23/8/21). A summary of the project was presented to the Western Zone Abalone Industry on 16/9/2021. This included discussions on a potential Phase 2. The risk assessment has also been used by PIRSA to inform decision making on two recent juvenile abalone release applications. There will be a broad distribution of the Final Report, which will include AIASA, ACA, Dinko Tuna Farms and Yumbah Aquaculture.

Project materials developed

Two pieces of 'Project Materials' were developed. These were:

1. Consequence-likelihood risk-assessment as a method for assisting evaluation of genetic risks associated with juvenile abalone release for stock recovery or subtidal aquaculture; and
2. Cost-benefit analysis spreadsheet toolkit

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Appendices

Appendix 1: DaRT report

Report for service DHal21-5894

Diversity Arrays Technology Pty Ltd

Background

This document provides the results of analyses on data generated for service DHal21-5894 ordered by SARDI Aquatic Sciences. A total of 550 Greenlip Abalone (*Haliotis laevigata*) samples (tentacles) were collected from 11 "sites" in South Australian waters. Fifty Greenlip Abalone samples were collected per "site". The "sites" were eight wild populations and three Yumbah hatchery cohorts (Figure 1, Table 1).

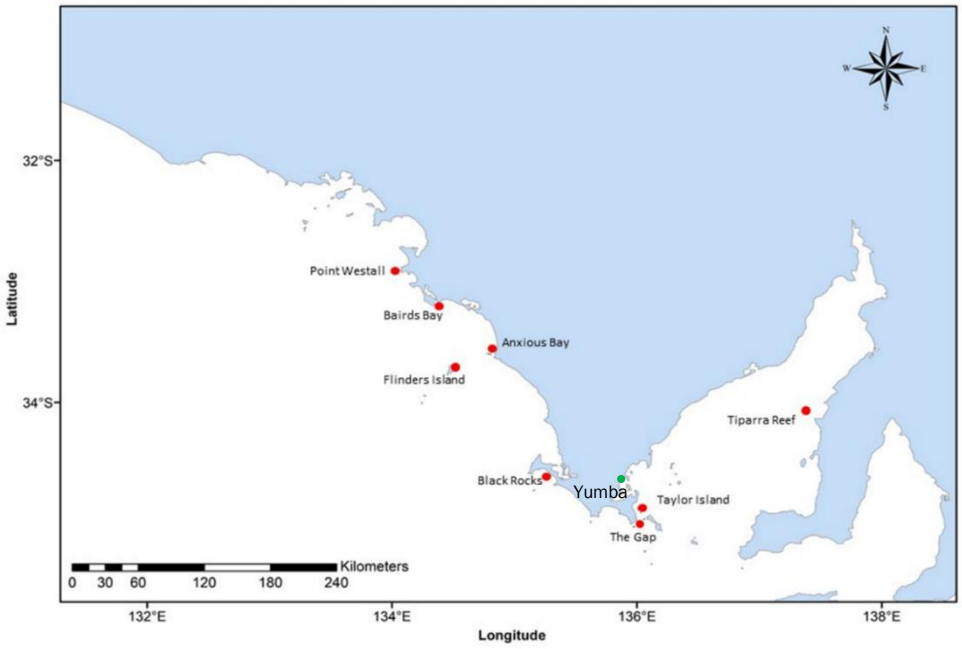

Figure 1. *Collection sites for abalone samples in South Australian water, including eight wild populations (red dots) and three Yumbah hatchery cohorts (green dot).*

Table 1: *Abalone sample collection sites, site abbreviations (used in subsequent figures) and sample IDs.*

Collection site	Site abbreviation	Wild/hatchery	Sample IDs	Total samples/site
Point Westall	PoiW	wild	A181-A230	50
Bairds Bay	BaiB	wild	A481-A530	50
Anxious Bay	AnxB	wild	A541-A582, A584-A591[b]	50
Flinders Island	FliI	wild	A421-A470	50
Avoid Bay	BlaR	wild	A121-A170[a,b]	50
The Gap	TheG	wild	A1-50[a]	50
Taylors Island	TayI	wild	A61-110	50
Tiparra Reef	TipR	wild	TR1-TR50	50
Yumbah 0.5g	Y0.5	hatchery	A361-A410	50
Yumbah 10g	Y10	hatchery	A301-A350[b]	50
Yumbah 50g	Y50	hatchery	A241-A287, A290-A292[b]	50

[a] Samples A35 (The Gap), A41 (The Gap) and A167 (Avoid Bay) did not amplify and were not included in the final DaRT dataset. The final dataset contained 547 out of 550 samples.

[b]Duplicate samples (sampling of the same abalone individual) as evident from Figure 2 dendogram: Anxious Bay A586+A581, Avoid Bay A123+A125, Avoid Bay A156+A157, Anxious Bay A551+A552; and hatchery - Yumbah 10g A317+A318, Yumbah 50g A259+A260, Yumbah 10g A319+A320.

Methods

Following subsampling into 96-well plates, DNA was extracted from the abalone tissue samples (tentacles) using the NucleoMag kit (MACHEREY-NAGEL). For the lysis step, samples were overlaid with 50µL of T1 Buffer and 6.25µL of proteinase K. The plate was centrifuged briefly (30-60 sec at 1000 rpm) to ensure the tissue sample was completely submerged in the solution. Samples were then digested overnight at 60 °C, before being centrifuged for 10 min at 3000 rpm and the clear lysate transferred to a new deep well plate.

DNA was bound to NucleoMag B-beads using a suspension of 6µL beads in 90µL MB2. The plates were continuously agitated to prevent the beads from settling. Samples were then transferred to the Tecan T100 robot (T100), with the final extraction steps (washing and elution into Elution Buffer) performed on the T100 using 96 tips head and a DArT PL script.

DNA extracts were processed using a *Haliotis* optimised DArTseq assay, with digestion/ligation reactions following Kilian et al (2012) but replacing a single PstI-compatible adaptor with two different restriction enzyme overhangs (Sansaloni et al, 2011). The PstI-compatible adapter was designed to include the Illumina flowcell attachment sequence, sequencing primer sequence and “staggered”, varying length

barcode region, similar to the sequence reported by Elshire et al (2011). The reverse adapter contained the flowcell attachment region and SphI-compatible overhang sequence. Only "mixed fragments" (PstI-SphI) were effectively amplified in 30 rounds of PCR using the following reaction conditions:

1. 94 °C for 1 min
2. 30 cycles of: 94 °C for 20 sec
   58 °C for 30 sec
   72 °C for 45 sec
3. 72 °C for 7 min

After PCR, equimolar amounts of amplified product were bulked and applied to c-Bot (Illumina) bridge PCR followed by 77 cycles of sequencing (single reads) on the Illumina Hiseq2500. Sequences generated from each lane were processed using proprietary DArT analytical pipelines. In the initial pipeline, poor quality sequences were removed, with greater filtering parameters applied to the barcode region compared to the rest of the sequence, ensuring the assignments of the sequences to specific samples (based on the "barcode split") was reliable.

Filtering was performed on the raw sequences using the following parameters:

Filter	Filter Parameters
-Barcode region	-Min Phred pass score 30, Min pass percentage 75
-Whole read	-Min Phred pass score 10, Min pass percentage 50

Approximately 2,750,000 sequences per sample were used in marker calling. Identical sequences were collapsed into "fastqcoll files" which were "groomed" using DArT PL's proprietary algorithm which corrects low quality base from singleton tag into a correct base using collapsed tags with multiple members as a template. The "groomed" fastqcoll files were used in the secondary pipeline for DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). For SNP calling, all tags from all libraries included in the DArTsoft14 analysis are clustered using DArT PL's C++ algorithm at the threshold distance of 3, followed by parsing of the clusters into separate SNP loci using a range of parameters, including the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating true allelic variants from paralogous sequences. In addition, multiple samples were processed from DNA to allelic calls as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers.

Marker calling quality was validated by high average read depth per locus (average across all markers was over 35 reads/locus). In addition, Average Reproducibility for all reported markers was calculated using 85 technical replicates generated for 547 samples (three submitted samples did not yield any DNA, see Table 1 for sample IDs). The Average Reproducibility is calculated as a fraction of allele calls which are consistent among the technical replicates (libraries) generated from the same DNA samples in a fully

independent manner. Reproducibility fraction is calculated for each of the two alleles and averaged for the marker.

Results

DArT standard thresholds were applied for the report of ~64,500 SNP markers with average read depth of approximately 40X, call rate close to 80% and average reproducibility of 99.5%. This indicates above average quality of the data.

As we observed a significant tail of rare alleles, we trimmed these markers, along with those that had a reproducibility below 100%, resulting in a final dataset of 23,750 SNP markers. A new (Hamming) distance matrix was generated, and additional downstream analyses were carried out on this dataset. This additional selection increased the average read depth to approximately 50X and included only those markers with 100% reproducibility across 85 technical replicates.

Examination of the distance matrix revealed that there were seven duplicate samples (same individual accidently sampled twice, see Table 1 for sample IDs) as the distance between those pairs was between 0.001 and 0.002. The fact that we detected some difference in marker calls among samples from the same individual is not surprising, as technical replicates from the same DNA extracts are slightly less stringent than the biological replicates from separate extractions and sampling. These seven sample duplications (which unintentionally serve as an internal control) are visible in Figures 2a and 2b, with four duplicated samples collected from wild populations and three collected from the hatchery cohorts (see Table 1 for sample IDs).

Figure 2a highlights a clear genetic separation between wild and hatchery samples (two distinct clades; wild in red, hatchery in green), with greater genetic diversity (distance between samples) observed for wild population samples compared to the hatchery cohorts. For the wild populations, there is clear mixing of genetic information among the collection sites, however samples from Tiparra Reef (in blue, Figure 2b) appear to be more distinct, with separation from the remaining 7x wild populations. Mixing of genetic information is also evident between the three hatchery cohorts, with some level of inbreeding apparent (lower distances between hatchery samples compared to wild samples) (Figure 2b).

The same observations were recorded from the PCA plot, with clear separation of wild (red) vs. hatchery (green) samples (Figures 3 & 4), and no separation of individual populations/cohorts within both the wild and hatchery groups, apart from the Tiparra Reef samples (Figures 3 & 4). Note that one wild sample (A563, Anxious Bay) is observed in-between the wild and hatchery clusters (Figures 3 & 4) and was also grouped within the hatchery clade (Figure 2a & b). This is most likely a mis-labeled/mis-handled hatchery sample.

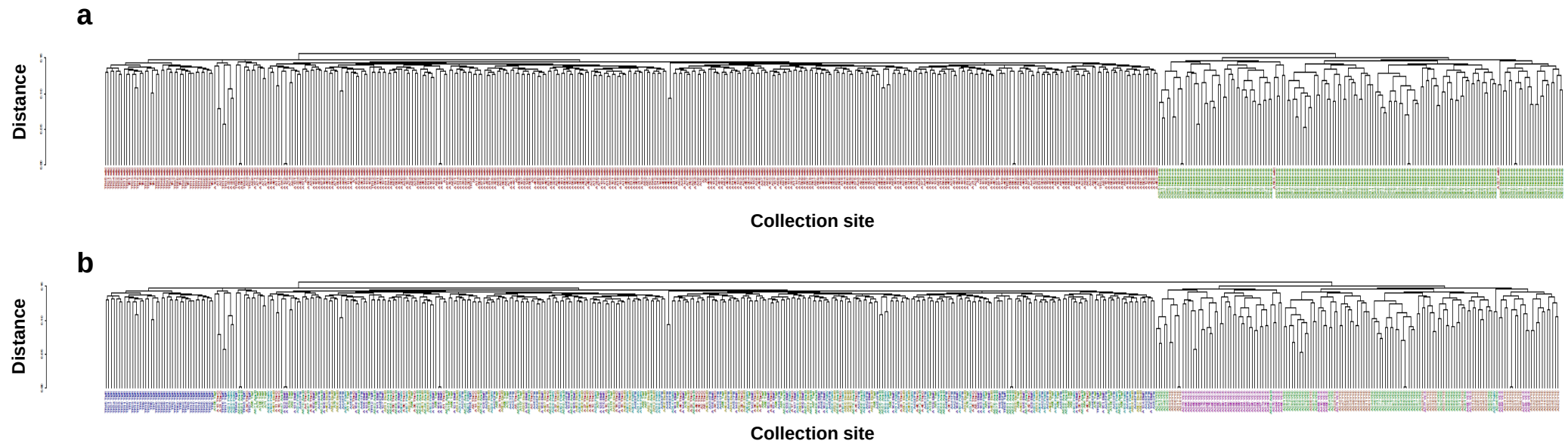

Figure 2. *Dendrogram of 547 abalone samples based on 23,750 SNPs with 100% average reproducibility, colour-coded according to a) wild (red) vs. hatchery (green) and b) each of the 11 collection sites as distinct colours (TipR, dark blue; TayI, light green; TheG, brown; PoiW, aqua; AnxB, dark green; BaiB, olive; BlaR, purple; FliI, blue; Y0.5, pink; Y10, light brown; Y50, green; refer to Table 1 for site abbreviation explanations).*

Figure 3. *PCA plot of 547 abalone samples based on 23,750 SNPs with 100% average reproducibility, colour-coded according to wild (red) vs. hatchery (green). Plot labels presented (e.g., A342) are sample IDs (refer to Table 1 for details).*

Figure 4. *PCA plot of 547 abalone samples based on 23,750 SNPs with 100% average reproducibility, colour-coded according to each of the 11 collection sites as distinct colours (TipR, dark blue; TayI, light green; TheG, brown; PoiW, aqua; AnxB, dark green; BaiB, olive; BlaR, purple; FliI, blue; Y0.5, pink; Y10, light brown; Y50, green). Plot labels presented (e.g., A342) are sample IDs (refer to Table 1 for details).*

To examine allelic richness (number of allelles), the Allelic Richness value (AR) was calculated using the full stringent marker set with all 23,750 SNPs and plotted for each sample collection site (Figure 5a). Wild populations had slightly higher AR compared to hatchery cohorts, indicating raised allelic diversity and potential for adaptability and persistence (Greenbaum et al 2014), with Tiparra Reef samples, consistent with previous findings, being the most diverse (Figure 5a). When only high AR markers were considered (AR >1.2 across all populations; 7870/23,750 SNP markers), the same pattern was observed with greater allelic richness in wild compared to hatchery samples, although Tiparra Reef samples were (marginally) lower than others within wild populations (Figure 5b). Across both marker sets, the Y50 (Yumbah 50g) hatchery cohorts recorded the lowest AR, indicating this cohort had the lowest allelic diversity and reduced potential for adaptability and persistence (Figure 5a, b).

Similar to the AR finding, the Expected Heterozygosity (H) (gene diversity based on the number of allelles and abundance/evenness of the allelles), calculated using the full stringent marker set with all 23,750 SNPs, highlighted slightly higher H values and hence raised genetic diversity for wild samples compared to lower H values and hence lower genetic diversity for the hatchery samples (Figure 6). The Y50 (Yumbah 50g) hatchery samples again recorded the lowest H representing the lowest genetic variability. The result of lower H observed for the hatchery cohorts further supports the notion that there is some level of inbreeding occurring in these samples.

To quantify population/cohort differentiation, a Fixation Index (FI, pairwise) analysis was undertaken. The resulting matrix (Figure 7) is consistent with the previous findings, with clear differentiation of wild populations from hatchery cohorts evident by FI increasing between 10x to almost 100x between these groups. Within the 8x wild populations, a lack of differentiation was observed for all sites except Tiparra Reef. The comparisons between the Tiparra Reef wild population and the three hatchery cohorts presented the highest FI value, highlighting these samples are the most distinct from one another. Some level of differentiation was also observed among the three hatchery cohorts, although this was about half of what was observed between the hatchery cohorts and wild populations (e.g., FI ~0.06 between hatchery cohorts, FI ~0.10 between hatchery cohorts and wild samples) (Figure 7).

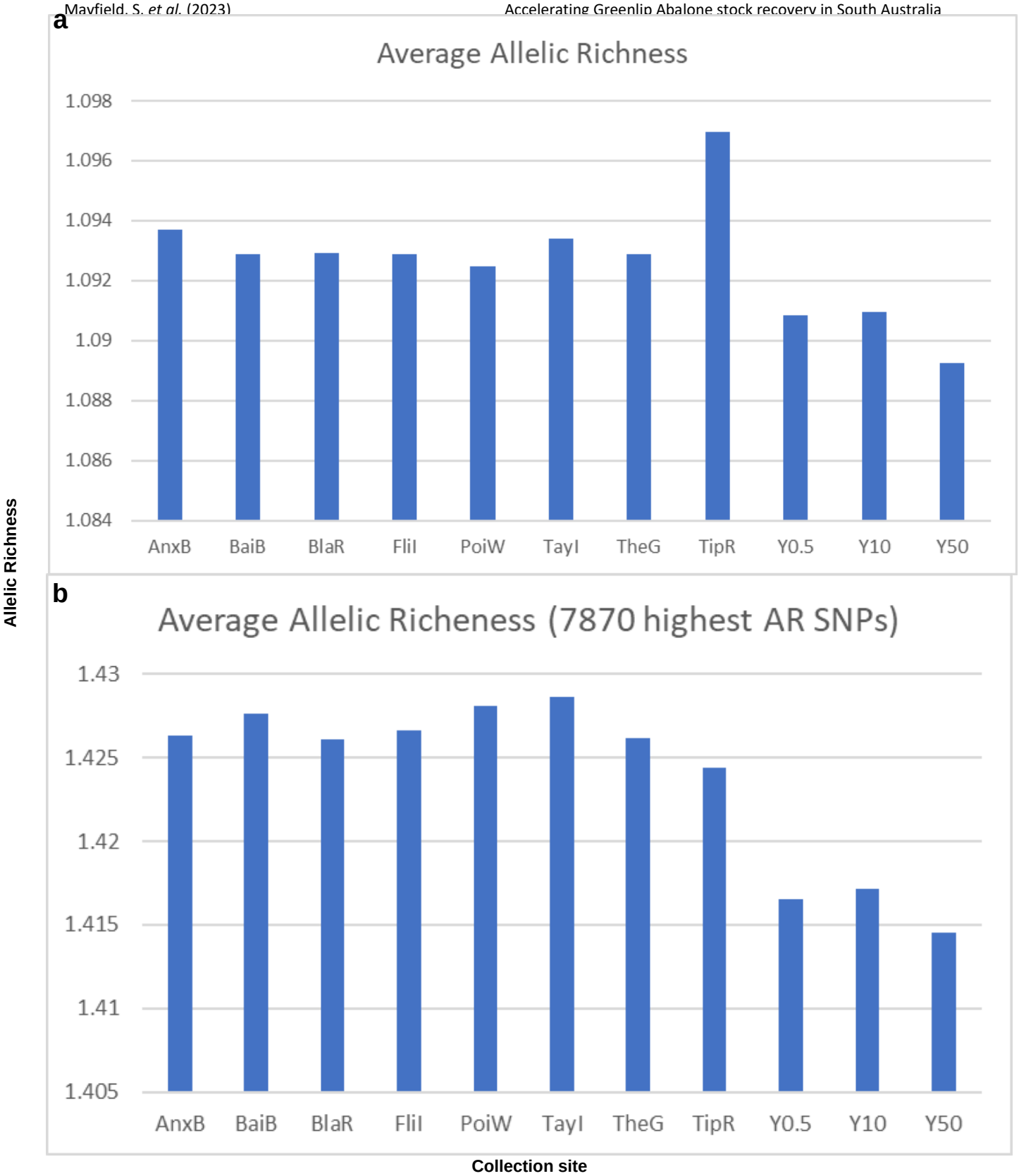

Figure 5. *Average Allelic Richness in the wild populations and hatchery cohorts using a) the full stringent marker set of 23,750 SNPs and b) high AR markers only (AR >1.2; 7870 SNP markers).*

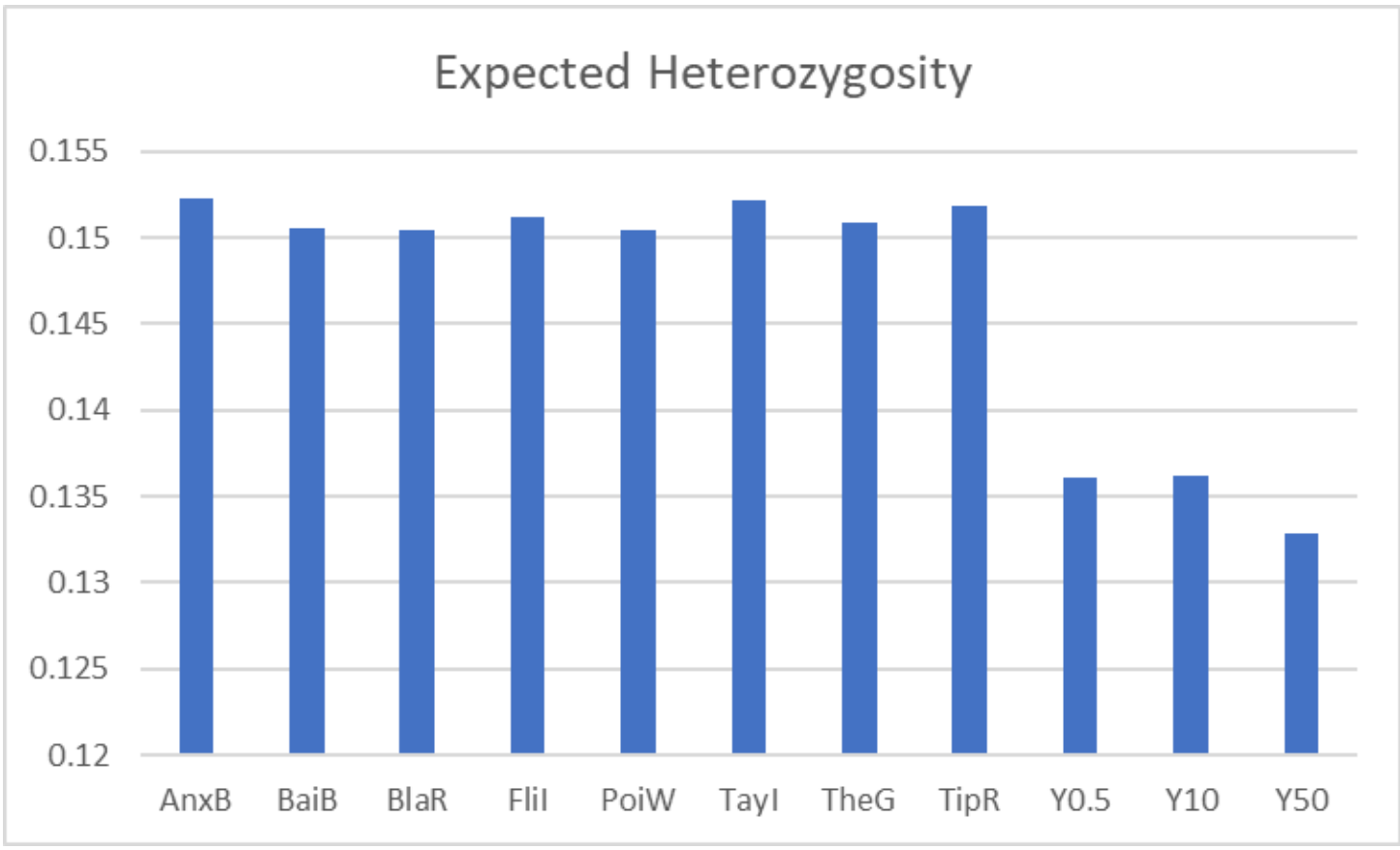

Figure 6. *Expected Heterozygosity in the wild populations and hatchery cohorts analysed using the full stringent marker set of 23,750 SNPs.*

	PoiW	BaiB	AnxB	FliI	BlaR	TheG	TayI	TipR	Y0.5	Y10	Y50
PoiW											
BaiB	0.001142										
AnxB	0.004632	0.003540									
FliI	0.002684	0.002154	0.003603								
BlaR	0.002662	0.002312	0.003966	0.002789							
TheG	0.003332	0.001463	0.003975	0.002613	0.002634						
TayI	0.002092	0.000573	0.002212	0.002414	0.001745	0.001451					
TipR	0.029233	0.027567	0.026295	0.028017	0.027363	0.027813	0.027548				
Y0.5	0.101862	0.100640	0.098156	0.099486	0.100843	0.100616	0.098990	0.121838			
Y10	0.101443	0.100758	0.097753	0.099866	0.101722	0.101275	0.098610	0.123592	0.066117		
Y50	0.104711	0.102990	0.102133	0.103838	0.103415	0.104609	0.101797	0.125131	0.074909	0.045499	

Figure 7. *Fixation Index (FI, pairwise) analysis result. The values are colour coded using conditional formatting function with green highlighted values representing a lack of differentiation and dark red highlighted values representing the highest differentiation between the compared population pairs.*

Conclusion

1. There is a high level of genetic diversity in Greenlip Abalone wild populations, with a large frequency of rare alleles, especially those contributed by the Tiparra Reef population.
2. Wild populations have a higher level of genetic diversity compared to hatchery cohorts, represented by higher (albeit small) values of Allele Richness and Expected Heterozygosity.
3. Hatchery cohorts presented a greater frequency of samples with high genetic similarity compared to wild populations, suggesting an increased level of inbreeding.
4. There is clear genetic separation between wild and hatchery populations with limited genetic differentiation among wild populations (restricted to TipR against the other wild populations) and slightly higher differentiation among the three hatchery cohorts.
5. All analyses reported in this document were performed using three levels of marker selection spanning over one order of magnitude in the number of markers. The results were consistent across these marker sets, giving further support to the key findings and highlighting the robustness of the data.

References

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Greenbaum G, Templeton AR, Zarmi Y, Bar-David S. (2014). Allelic richness following population founding events – a stochastic modeling framework incorporating gene flow and genetic drift. PLoS One, 9(12): e115203.

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Pritchard JK, Stephens M, Donnelly P. (2000). Inference of population structure using multilocus genotype data. Genetics. 155(2):945-59.

Sansaloni C, Petroli C, Jaccoud D, Carling J, Detering F, Grattapaglia D, Kilian A. (2011). Diversity Arrays Technology (DArT) and next generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus. BMC Proceedings 5(Suppl 7):P54.

Appendix 2: Genetics risk assessment workshop agenda

Agenda

FRDC 2020/116: Expert-based, abalone-release, genetics risk-assessment workshop (a COVID-SAFE event)

Date / time: Thursday 1 and Friday 2 July 2021, 9:30pm – 4:30pm

Location: Conference Room, South Australian Aquatic Sciences Centre and MS Teams

Informal dinner: Glenelg Surf Club, Colley Terrace, Glenelg, Thursday, 1 July, 6pm (attendees' own cost)

Terms of reference: Appendix 1

Attendees:	AgCommunicators: Belinda Cay (Facilitor) PIRSA: Dr Stephen Mayfield, Dr Belinda McGrath-Steer, Dr Sarah Catalano, Dr Ben Stobart, Dr Matthew Bansemer, Dr Annabel Jones, Dr Katherine Heldt (minute taker/scribe/COVID Marshall) Deakin University: Dr Adam Miller University of Melbourne: Dr Nick Robinson Tasmanian Seafoods: Dr Anton Krsinch James Cook University: Dr Jan Strugnell DPIRD: Dr Lachlan Strain, Dr Anthony Hart, Rhiannan Jones AIASA: Dr Nicole Hancox, Jonas Woolford, Kane Williams, Thomas McNab Dinko Tuna Farms: Amrik Singh Aulakh Yumbah Aquaculture: Dr Tom Hyde, David Connell CZ Abalone Industry: Michael Tokley FRDC: Dr Chris Izzo DaRT: Dr Andrzej Kilian Abalone managers and researchers: Dr Rowan Chick, Dr Craig Mundy NT Fisheries: Dr Thor Saunders, Dr Eliza Kimlin VFA: Travis Baulch Paua Industry Council: Dr Tom McGowan

Session Agenda		
No.	**Agenda Item**	**Officer**
1.	**Acknowledgement:** *"We would like to acknowledge this land we meet on today is the traditional lands for Kaurna people and that we respect their spiritual relationship with the country. We also acknowledge the Kaurna people as the custodians of the Adelaide region and that their cultural and heritage beliefs are still important to the living Kaurna people today."*	Belinda Cay

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2.	**Welcome, apologies, background and objectives of the workshop Day 1 – 09:30-10:00**		Belinda Cay
	2.1	Welcome, apologies and introductions	Belinda Cay
	2.2	Management of COVID risk	Dr Katherine Heldt
	2.3	Project Background	Dr Stephen Mayfield
	2.4	Workshop objectives and scope	Dr Stephen Mayfield
3.	**Information exchange & identification of key risks Day 1 – 10:00-12:00**		Belinda Cay
	3.1	Reseeding and translocations: A genetic perspective	Dr Adam Miller
	3.2	WA – Genetic risk management for juvenile release	Dr Lachlan Strain
	3.3	WA – Risk management in practice: Ocean Grown Abalone	Dr Lachlan Strain
	3.4	NZ – Genetic risk management for juvenile release	Dr Tom McCowan
	3.5	Discussion	Belinda Cay
4.	**Genetics Risk Assessment of juvenile release Day 1 – 12:00-16:30**		Belinda Cay
	4.1	Overview of risk assessment – Likelihood and Consequence	Dr Ben Stobart
	4.2	What's the risk: identification of Risk Factors	Belinda Cay
	4.3	Likelihood component – risk factors, weighting and score definition	Belinda Cay
	4.4	Consequence component – risk factors, weighting and score definition	Belinda Cay
	4.5	Risk matrix, risk ratings and risk mitigation	Belinda Cay
	4.6	Discussion	Belinda Cay
	End of Day 1		

5.	**Recap of day 1** **Day 2 – 09:30-10:00**		Belinda Cay Dr Stephen Mayfield
6.	**Case studies: application of DRAFT Risk Assessment** **Day 2 – 10:30-13:30**		Belinda Cay
	6.1	Genetic testing of wild and Hatchery-reared Greenlip Abalone: sampling, DaRTSeq methods/QC checks/analyses/results	Dr Sarah Catalano & Dr Andrzej Kilian
	6.2	Review and discussion of genetic testing results/findings	Belinda Cay
	6.2	Case study 1: Experimental release of Yumbah juveniles at The Gap (SA)	Belinda Cay Dr Stephen Mayfield
	6.3	Case study 2: Commercial release of Yumbah juveniles at The Gap (SA)	Belinda Cay Dr Stephen Mayfield
	6.4	Case study 3: Phase 1 commercial release of Yumbah juveniles to Abitats in Anxious Bay (SA)	Belinda Cay Dr Stephen Mayfield
	6.5	Case study 4: Commercial release of F1 Greenlip Abalone to Foul Bay – local broodstock (SA)	Belinda Cay Dr Stephen Mayfield
	6.6	Case study 5: Commercial release of F1 Greenlip Abalone to The Gap – Anxious Bay broodstock (SA)	Belinda Cay Dr Stephen Mayfield
	6.7	Case study 6: Commercial release of F1 to re-stock Roei – non-local broodstock (WA)	Belinda Cay Dr Lachlan Strain
	6.8	Review Risk Factors, weightings and scores; modify if required and recommend risk assessment	Belinda Cay
7.	**Strategies and controls to mitigate risk** **Day 2 – 13:30-1400**		Belinda Cay
8.	**Identification of genetic R&D needs to support releases** **Day 2 – 14:00-1500**		Belinda Cay
9.	**Workshop summary and conclusions** **Day 2 – 15:00-16:00**		Belinda Cay
10.	**Next steps & Workshop Closure** **Day 2 16:00-16:30**		Belinda Cay

Appendix 1 – Terms of Reference

ToR 1: *Membership* – All workshop attendees
ToR 2: *Behaviour* – Communicate in an open, collaborative and respectful manner that facilitates the aim of the workshop and enhances the outcomes of FRDC Project 2020/116
ToR 3: *Aims* – Develop an objective, likelihood-consequence, genetic risk-assessment framework for assessment of genetic risk from juvenile abalone release; Assess 6 case studies using the risk assessment framework.
ToR 4: *Process* – Consider, in relation to juvenile abalone release, (1) results of DaRTSeq data analysis, (2) information on approaches to managing genetic risks, and (3) strategies (controls) to mitigate risk.
ToR 5: *Out of scope* – This workshop is not considering non-genetic factors associated with juvenile abalone release (e.g. disease risk, cost-benefit).
ToR 6: *Outcome* – Recommendation of an appropriate likelihood-consequence, genetic risk-assessment framework for assessment of genetic risk from juvenile abalone release to PIRSA to inform policy.

Appendix 3: Screenshots of the genetics risk assessment for assisting evaluation of genetic risks associated with juvenile abalone release using Case Study 1

Likelihood

Likelihood factors	Weighting	Likelihood	Likelihood score	Evidence for likelihood score
Spatial scale	0.25	1	0.25	Juveniles to be released into small part of one mapcode
Release number (cumulative)	0.25	1	0.25	Number of juveniles to be released is 4,500
Distance to wild abalone (fishable populations)	0.25	4	1.00	Low numbers (not fishable density) of Greenlip Abalone at release site
Harvest size	0.25	4	1.00	No intended recapture of released juveniles
Total weighting	1	Total likelihood score	2.5	

Score descriptions for likelihood factors	Score 1	Score 2	Score 3	Score 4
Spatial scale	Small part one fishing ground (100's of m)	Throughout one fishing ground	>10% of fishing grounds	>50% of fishing grounds
Release number (cumulative)	<5,000	5,000-50,000	50,000-200,000	>200,000
Distance to wild abalone (fishable populations)	>3km-30km	2-3km	1-2km	<1km
Harvest size	Immature ($<L_{50}$)	Early maturation ($<L_{75}$)	Maturing (L_{75}-L_{95})	Fully mature ($>L_{95}$)
if no information, apply highest score				

Consequence

Factors influencing consequence	Weighting	Consequence	Consequence score	Evidence for consequence score
Wildstock density	0.25	1	0.25	Mean commercial catch last 5 years between 4 and 5 t/yr
Broodstock pairings contributing to released stock	0.25	4	1	Juveniles to be released are from Yumbah. Number of broodstock pairings unknown. Highest consequence score assigned
Generations in captivity of released stock	0.25	4	1	Juveniles to be released are from Yumbah. Number of generations in captivity unknown. Highest consequence score assigned
Broodstock founders contributing to released stock	0.25	4	1	Juveniles to be released are from Yumbah. Broodstock founders contributing to released stock unknown. Highest consequence score assigned
Total weighting	1	Total consequence score	3.25	

Factors influencing consequence - score descriptor	Score 0	Score 1	Score 2	Score 3	Score 4
Wildstock density	>5 t MW / no fishable stock	>4 t MW	>3 t MW	>1 t MW	<1 t MW
Broodstock pairings contributing to released stock	Dispersed (e.g. Multiple pairs, >10)	NA	NA	NA	Dominated (e.g. few pairs, <2)
Generations in captivity of released stock	NA	F1	NA	NA	FX
Broodstock founders contributing to released stock	100% new appropriate wild broodstock, >30	80% new appropriate wild broodstock,	75% new appropriate wild broodstoc	50% new appropriate wild broodstock	F1/F2, 0% new appropriate wild broodstock and/or <5 contributing parents
if no information, apply highest score	For "Genetic Bases... (Factor 4) : if objective is to recover depleted area, increased distance from release site should be reflected in higher consequence score particularly if environmental gradient				

Appendix 3 continued:

Risk Score = Consequence * Likelihood

	Risk Score
Consequence X Likelihood (raw)	8.125
Consequence X Likelihood (rounded up)	9

Potential Risk Matrix

	Consequence Score				
Likelihood Score	0 (Insignificant)	1 (Minor)	2 (Moderate)	3 (Severe)	4 (Catastrophic)
1 (Remote)	0 (Negligible)	1 (Negligible)	2 (Low)	3 (Low)	4 (Low)
2 (Rare)	0 (Negligible)	2 (Low)	4 (Low)	6 (Moderate)	8 (Moderate)
3 (Possible)	0 (Negligible)	3 (Low)	6 (Moderate)	9 (High)	12 (High)
4 (Likely)	0 (Negligible)	4 (Low)	8 (Moderate)	12 (High)	16 (Extreme)

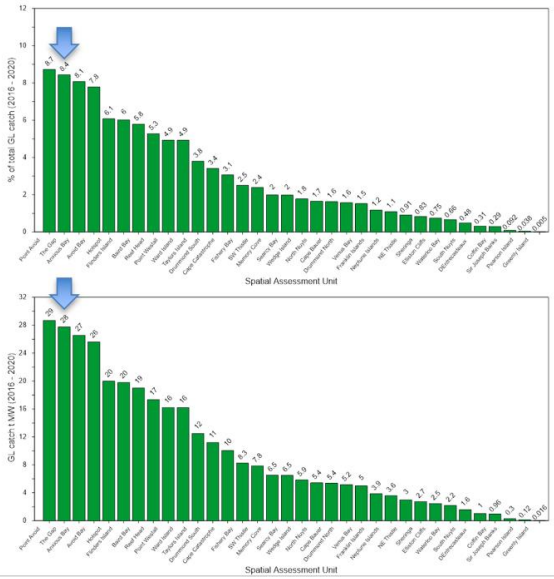
Potential Risk Rankings

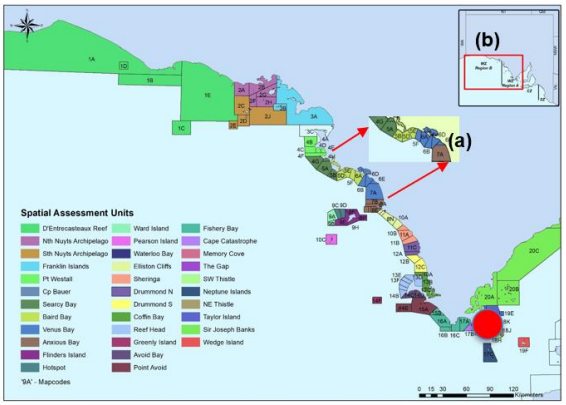
Risk score	Potential Risk ranking
0-1	Negligible
2-4	Low
5-8	Moderate
9-12	High
>12	Extreme

Appendix 4: Information available to inform application of the risk assessment to Case Study 1

Case Study 1

Experimental release of Yumbah juveniles at The Gap (Phase 2, Objective 4)





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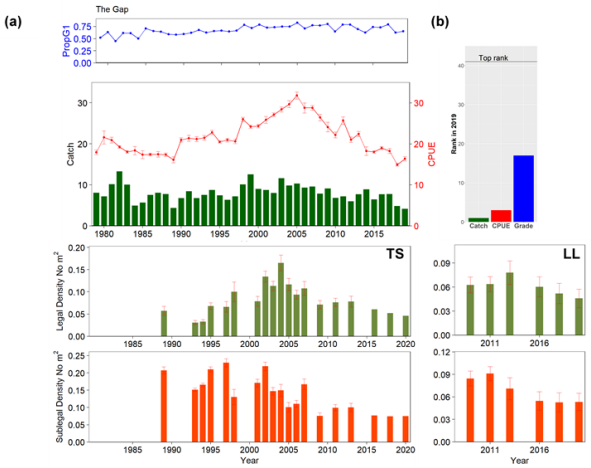
Case Study 1

Experimental release of Yumbah juveniles at The Gap (Phase 2, Objective 4)



Objective is experimental trial

Indicative release date late 2021

4,500 individuals from existing Yumbah stock

3 size classes (20-40mm, 41-60mm, 61-80mm)

1 location, on reef with Greenlip Abalone

3 release methods (hand, square tube, semi-permanent)

No targeted harvest planned

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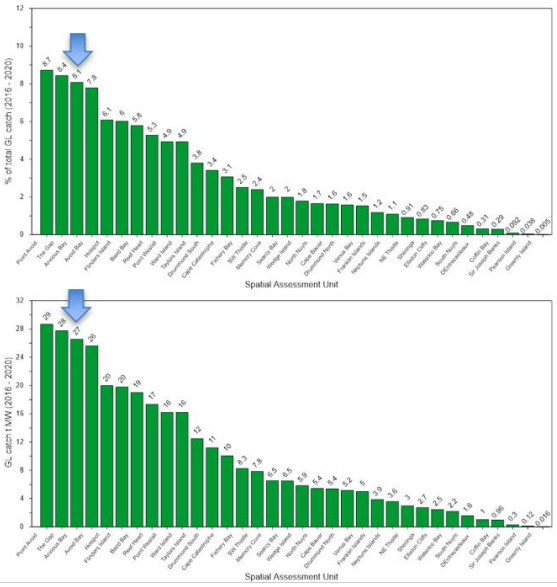
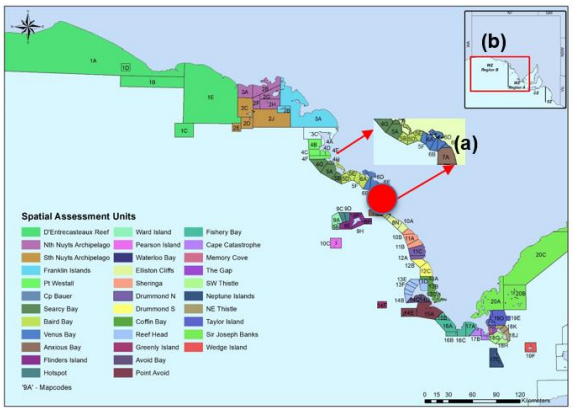
Case Study 2

Phase 1 commercial release of Yumbah juveniles to Abitats in Anxious Bay





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Case Study 2

Phase 1 commercial release of Yumbah juveniles to Abitats in Anxious Bay

Yellow = abalone fishing grounds

Blue shading = lease

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Case Study 2

Phase 1 commercial release of Yumbah juveniles to Abitats in Anxious Bay

Abitat design not finalised – 2021 used to trial options

~2,000 Abitats in water by August 2021

Projected total Abitats in lease area = 20,000 (2030)

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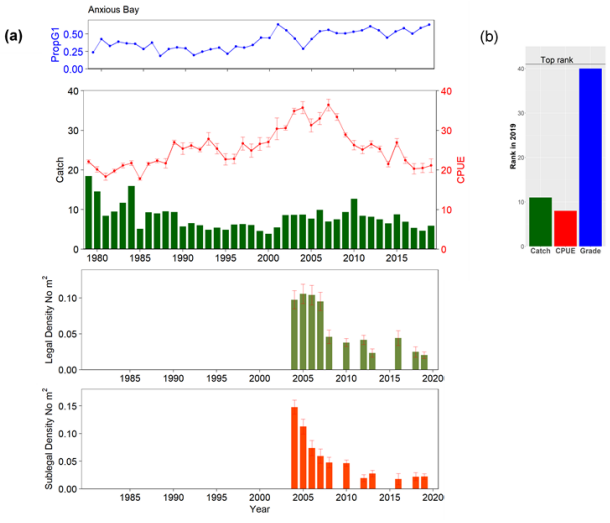
Case Study 2

Commercial release of Yumbah juveniles to Abitats in Anxious Bay



Phase 1 objective – experimental trial of Abitats

Indicative release date late 2021

1,000,000 individuals from existing Yumbah stock

1 size class (40mm)

Released to ~20,000 Abitats within lease boundary

Lease adjacent to reef with Greenlip Abalone

Harvest planned at 120mm; L_{50} = 77mm

Phase 2 objective – commercial production with 10,000 Abitats

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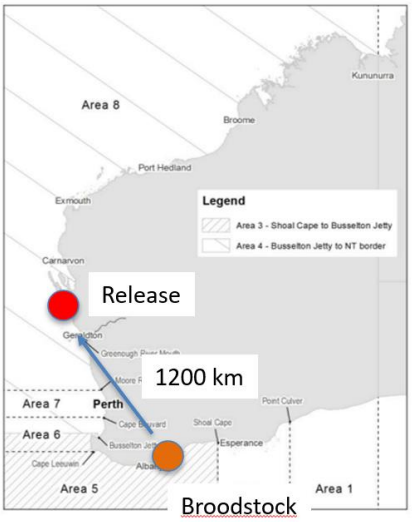
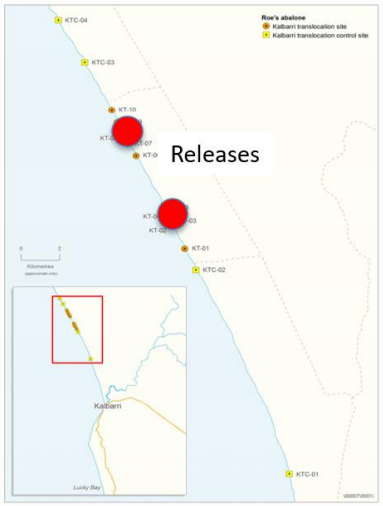
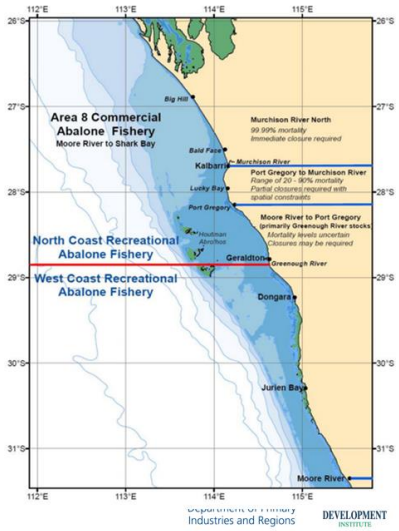
Case Study 3

Commercial release of F1 juveniles at Kalbarri (South Coast broodstock)





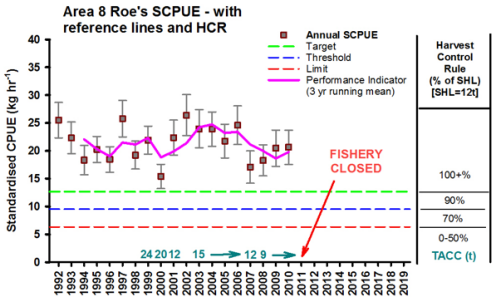


Case Study 3

Commercial release of F1 juveniles at Kalbarri (South Coast broodstock)

Area 8 commercial catch (t) from 1991-2010

Area 8 Regions	Catch (t)	Percentage (%)
Big Hill to WA/NT Border	6	2
Murchison River to Big Hill	329	88
Port Gregory to Murchison River	9	2
Moore River to Port Gregory	29	8
Total	373	100



Multiple releases over 3 years (total released = 77,364)
1st year = 15,446, 2nd year = 32,788 and 3rd year = 29,130

F1 individuals from South Coast broodstock
Approx. 75 adults (50 female, 25 male) per year
Different broodstock each year

1 size class (20mm) for all releases

Release module trial for 1st years release
Hand release method for 2nd and 3rd years releases

Establish founder populations at selected sites
Previous Roe's abalone populations

No targeted harvest planned
Population conservation

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