



FRDC

FISHERIES RESEARCH &
DEVELOPMENT CORPORATION

FINAL REPORT

An Impact Assessment of Investment in FRDC Project 2014-022:

**Developing a rapid molecular identification technique to
improve egg production based fish biomass assessment**

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January 2023

FRDC Project No 2016-134

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ISBN [Insert ISBN/ISSN – researcher to obtain]

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FRDC Project 2016-134**

2022

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Acknowledgments

Agtrans Pty Ltd (Agtrans Research) and ACRE Economics Pty Ltd (ACRE Economics) would like to thank Patrick Hone (Managing Director), Jennifer Marshall (Cross-Functional Facilitator) and Carolyn Stewardson (Research Portfolio Manager) of the Fisheries Research and Development Corporation for facilitating contact with relevant project personnel and for their guidance and feedback throughout the Impact Assessment process.

Specific acknowledgments for helpful comments on earlier drafts and for supplying additional information are made to:

- Richard Saunders, Fisheries Scientist, Principal Investigator
- Samuel Stone, Fisheries Manager, Department of Primary Industries and Regions, Government of South Australia

Abbreviations

ABS	Australian Bureau of Statistics
CBA	Cost-Benefit Analysis
CRRDC	Council of Rural Research and Development Corporations
DAWR	Department of Agriculture and Water Resources
DEPM	Daily Egg Production Method
DNA	Deoxyribonucleic Acid
FRDC	Fisheries Research and Development Corporation
NT	Northern Territory
NTDPIR	Northern Territory Department of Primary Industries and Resources
OCS	Office of the Chief Scientist
PCR	Polymerase Chain Reaction
PVC	Present Value of Costs
QDAF	Queensland Department of Agriculture and Fisheries
R&D	Research and Development
RD&E	Research, Development and Extension
SARDI	South Australian Research and Development Institute

Executive Summary

Surveys of the eggs and larvae of fish can provide a low-cost method for monitoring fish species populations and their communities. Such surveys can provide information on spawning locations, recruitment and changes in fish populations and biomass.

One survey method is the Daily Egg Production Method (DEPM). While this approach is fishery independent, the approach has some limitations as it is dependent on spawning behaviour and the distribution of spawning fish, the mortality of fish eggs, and the distribution and abundance of fish eggs. Collecting these various datasets is challenging and results are dependent on egg identification.

The Fisheries Research and Development Corporation (FRDC) funded Project 2014-022 that developed a molecular approach to identify fish eggs utilising a flow cytometer and multiplex bead array. This approach targeted five important species in northern Australia including Goldstripe Sardinella, Spotted Sardine, Black Jewfish, Spanish Mackerel and Grey Mackerel.

The new method identified eggs of different target species from plankton collected in the wild, but the success rate varied from 50% to 100%. In addition, a series of issues were identified that potentially limited the application of the method in the context of DEPM and egg identification. The principal issues were associated with the method of preserving the DNA, as DNA amplification and egg staging were both impacted by the ethanol-based preservation trialled.

The recommendation from the project was that, given the difficulties encountered during development and testing, existing approaches are preferred to that investigated in this project for future monitoring.

Total funding for the investment in Project 2014-022 over the period 2014 to 2019 totalled \$830,011 in present value terms. The FRDC investment costs over the same period were \$279,263 in present value terms. Given the results of the project, no attempt was made to value any impacts from the investment.

Introduction

The Fisheries Research and Development Corporation (FRDC) required an annual series of impact assessments to be carried out on a sample of completed investments from the FRDC research, development, and extension (RD&E) portfolio. The assessments were required to meet the following FRDC evaluation reporting requirements:

- Reporting against the FRDC 2015-2020 RD&E Plan and the Evaluation Framework associated with FRDC's Statutory Funding Agreement with the Commonwealth Government.
- Annual Reporting to FRDC funding partners and other stakeholders.
- Reporting to the Council of Rural Research and Development Corporations (CRRDC).
- Reporting RD&E impact and performance to FRDC levy payers and other fisheries and aquaculture stakeholders as well as the broader Australian community.

In April 2017, FRDC commissioned Agtrans Pty Ltd (Agtrans) to undertake the annual impact assessments for RD&E projects funded under the FRDC 2015-2020 RD&E Plan and completed in the years ended 30 June 2016 to 2020 (FRDC Project 2016-134). Between 2016/17 and 2020/21, four series of annual impact assessments were completed. Each of the four series of assessments included a set of 20 randomly selected FRDC RD&E investments as well as an aggregate analysis across all 20 investments evaluated in each year. Published reports for the annual FRDC evaluations can be found at: <https://www.frdc.com.au/frdc-project-impact-assessments-benefits-research>.

The fifth and final series of impact assessments under Project 2016-134 was for a set of FRDC RD&E investments completed in the year ended 30 June 2020, the final year of the FRDC 2015-2020 RD&E Plan. As in previous years, the fifth series of impact assessments included 20 randomly selected FRDC RD&E investments. The 20 investments had a total value of approximately \$5.30 million (nominal FRDC investment) and were selected from an overall population of 81 FRDC investments worth an estimated \$17.66 million (nominal FRDC investment) where a final deliverable had been submitted in the 2019/20 financial year.

The 20 RD&E investments were selected through a stratified, random sampling process such that investments chosen spanned all five FRDC Programs (Environment, Industry, Communities, People and Adoption), represented approximately 30.0% of the total FRDC RD&E investment in the overall population (in nominal terms), and included a selection of small, medium, and large FRDC investments (total nominal FRDC investment of \leq \$50,000, \$50,001 to \$250,000, and $>$ \$250,000 respectively).

Project 2014-022: Developing a rapid molecular identification technique to improve egg production based fish biomass assessment was randomly selected as one of the 20 RD&E investments completed in 2019/20 for evaluation in the fifth series of annual impact assessments (2019/20 sample). The current report presents the Project 2014-022 analysis and findings.

Method

The annual impact assessments of FRDC RD&E investments followed general evaluation guidelines that are now well entrenched within the Australian primary industry research sector including Research and Development Corporations, Cooperative Research Centres, State Departments of Agriculture, and some universities. The approach includes both qualitative and quantitative assessment components that are in accord with the current [guidelines for impact assessment](#) published by the CRRDC (CRRDC, 2018).

The evaluation process utilised an input to impact continuum RD&E project inputs (costs), objectives, activities, and outputs were briefly described and documented. Actual and expected outcomes, and any actual and/or potential future impacts (positive and/or negative) associated with project outcomes then were identified and described. The principal economic, environmental, and social impacts were then summarised in a triple bottom line framework and validated through consultation with expert personnel and review of published literature.

Once impacts were identified and validated, an assessment then was made about whether to quantify/value any of the impacts in monetary terms as part of the project-level analysis. The decision to value an impact identified was based on:

- Data availability and information necessary to form credible valuation assumptions,
- The complexity of the relevant valuation methods applicable given project resources,
- The likely magnitude of the impact and/or the expected relative value of the impact compared to other impacts identified, and
- The strength of the linkages between the RD&E investment and the impact identified.

Where one or more of the identified impacts were selected for valuation, the impact assessment used cost-benefit analysis (CBA) as a principal tool. The impacts valued therefore were deemed to represent the principal benefits delivered by the project investment. However, as not all impacts were valued (based on the selection criteria), the investment criteria estimated for the project investment evaluated are likely to represent an underestimate of the true performance of the FRDC project. No impacts were valued for Project 2014-022.

The qualitative and quantitative analysis processes, data sources, assumptions, specific valuation frameworks (where applicable), and evaluation results were clearly documented and then integrated into a written report.

Project Background

Background

Ichthyoplankton surveys provide a low-cost method for monitoring fish species populations and their communities as fish inhabit the upper water column during their early life. This can provide information on spawning locations, recruitment and changes in fish populations and biomass. A common survey method is called the Daily Egg Production Method (DEPM). The method has been applied to a range of species including the assessment of the spawning biomass of Australian sardines. While the approach is fishery independent, the approach has some limitations as it is dependent on spawning behaviour and the distribution of spawning fish, the mortality of fish eggs, and the distribution and abundance of fish eggs. Collecting these various datasets is challenging, including the egg survey, being in turn, dependent on egg identification.

Egg identification has traditionally been undertaken on egg morphology, but this is not straightforward for some species where egg features are not easily visible and species-unique. The approach is most difficult when eggs cannot be clearly demarcated between families (e.g. Snapper), or between closely related fish species. In some cases mis-identification of species via morphological identification has led to inaccurate fishery assessment and subsequent management.

Molecular methods for identifying fish eggs include sequencing methods that develop species-specific probes or the application of in-situ hybridisation. Such methods require the preservation of DNA of the fish egg, and most molecular methods are reliant on a polymerase chain reaction (PCR). The preservation of DNA can be difficult as water samples containing the fish eggs also contain other organisms that can interact with preservatives, such as ethanol, lowering preservation efficacy and distorting PCR results.

Rationale for Project 2014-022

FRDC Project 2014-022 was funded to develop a molecular approach to identify fish eggs utilising a flow cytometer and multiplex bead array. This bead array identification method targeted five important species in northern Australia including Goldstripe Sardinella (*Sardinella gibbosa*), Spotted Sardine (*Amblygaster sirm*), Black jewfish (*Protonibea diacanthus*), Spanish Mackerel (*Scomberomorus spp.*) and Grey Mackerel (*Scomberomorus semifasciatus*). In addition, three other species were included (Mackerel tuna (*Euthynnus affini*), Large Scale Grunter (*Terapon theraps*), and the Eightband butterfly fish (*Chaetodon octofasciatus*).

Project Details

Summary

Project Code: 2014-022
Title: <i>Developing a rapid molecular identification technique to improve egg production based fish biomass assessment</i>
Research Organisation: James Cook University, in collaboration with Queensland Department of Agriculture and Fisheries, and the Northern Territory Department of Primary Industry and Resources.
Principal Investigator: Richard Saunders, Adjunct Research Fellow, James Cook University
Period of Funding: June 2014 to January 2019
FRDC Program Allocation: Environment 60%, Industry 10%, People 30%

Objectives

1. To develop a novel high-throughput, low cost DNA based egg identification method for important fish species in northern Australia
2. To assess the application of the technology developed for use in the daily egg production method for biomass estimation.

Logical Framework

Table 1 provides a description of the project in a logical framework developed for the evaluation.

Table 1: Logical Framework for FRDC Project 2014-022

Activities	<p><u>Selection of identification method</u></p> <ul style="list-style-type: none">• The method selected was a multiplex bead array method; this is where species-specific probes are developed and bound to beads that fluoresce when passed through a flow cytometer if bound to the target species DNA.• This method allowed for the identification of multiple species at the one time as differing probe-bead combinations could be used to identify different fish species. <p><u>Development of probes and bead arrays</u></p> <ul style="list-style-type: none">• Species-specific probes were developed for a range of northern Australia fish species including Goldstripe Sardinella (<i>Sardinella gibbosa</i>), Spotted Sardine (<i>Amblygaster sirm</i>), Black jewfish (<i>Protonibea diacanthus</i>), Spanish Mackerel (<i>Scomberomorus spp.</i>) and Grey Mackerel (<i>Scomberomorus semifasciatus</i>). In addition, three other species were included (Mackerel tuna (<i>Euthynnus affini</i>), Large Scale Grunter (<i>Terapon theraps</i>), and the Eightband butterfly fish (<i>Chaetodon octofasciatus</i>).• The probes were tested against identified tissue for each of the species.• The bead array was developed and tested against a small number of wild collected fish eggs.• The bead array method was applied to identify eggs of several species in a larger scale egg survey.
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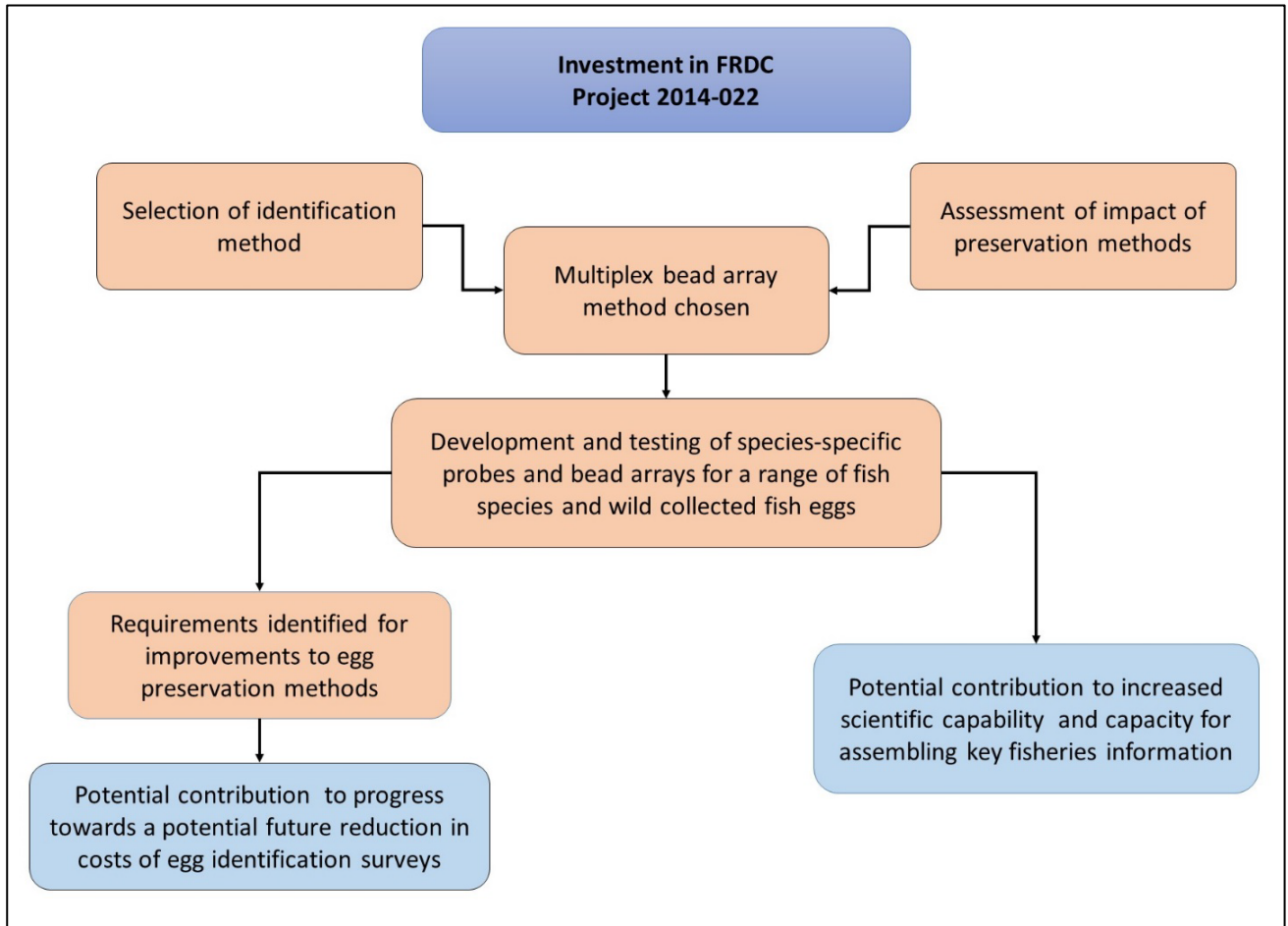
	<p><u>Assessment of impact of preservation methods</u></p> <ul style="list-style-type: none"> As molecular methods usually require preservation in ethanol, consideration of the impact of the preservation method on egg staging vs DNA amplification was given consideration, as ethanol preservation is conducive to the latter, while formalin preservation is conducive to the former. However, reliable identification (acceptably low rate of false negatives) appeared to require a combination of these (Jennifer Marshall, pers. comm., 2022).
Outputs	<ul style="list-style-type: none"> A multiplex bead array method was developed successfully. This bead array method allowed the potential identification of multiple fish species from a population of different fish species at the one time; this was possible via a different probe-bead combinations for each species. The method identified eggs of different target species from plankton collected in the wild, but the success rate varied from 50 to 100%. The principal issues identified as potentially limiting the application of the method in the context of DEPM and egg identification were associated with the method of preserving the DNA; DNA amplification and egg staging were both impacted by the ethanol-based preservation method (e.g. provision of a limiting rate of false negative results). The best preservation method for egg staging was using 5% formalin, but this was the worst performing method for DNA amplification. An ethanol-based preservation method is essential for DNA based identification and this also allows for some egg staging, but limits critical embryonic development identification (Jennifer Marshall, pers. comm., 2022). The recommendation from the project was that, given the difficulties encountered during development and testing, that there are better approaches to use than the one investigated in this project (Richard Saunders, pers. comm., 2022).
Outcomes	<ul style="list-style-type: none"> In the context of biomass assessment, the project recommendations included a caution against the use of this method as a principal egg identification method without addressing the associated issues identified. The South Australian Research and Development Institute (SARDI) recently developed an alternative method for fish egg identification utilising in-situ hybridisation. This approach worked well but required injection of individual eggs which reduced its efficiency dramatically. Project 2014-022 recommended the in-situ hybridisation approach would be better if a single species was being targeted (as is usually the case) but that future research was needed to focus on dechoriation of eggs (a method by which a barrier is formed to protect the embryo from preservatives) to make the SARDI method more efficient (Richard, Saunders, pers. comm., 2022). Dechoriation would be beneficial also for the work undertaken in Project 2014-022, in order to make the initial PCR more likely to succeed (Richard Saunders, pers. comm., 2022). The method for egg identification applied in Project 2014-022 worked but relied on complex chemistry with multiple steps, the failure of any one of which resulted in a false negative result. This problem could be overcome with more investment and at the scale of implementation but there was little appetite in Queensland for using egg production-based assessments at the time. In the Northern Territory (NT) there was some enthusiasm for developing the small pelagic fishery at the inception of the project, but this has since waned (Richard Saunders, pers. comm., 2022).

	<ul style="list-style-type: none"> • Comments from a South Australian representative (Sam Stone) follow: <ul style="list-style-type: none"> ○ To apply the DEPM to small pelagic fish (sardine, blue mackerel, jack mackerel, redbait), SARDI undertake a morphology-based method that is validated using standard genetic techniques. The methods used on these species have been well tested and are trusted (e.g. by the Australian Fisheries Management Authority and fishers in the small pelagic fishery). ○ The technique described in FRDC project 2014-022 is one that has not been tested extensively, and it is noted that genetic techniques have improved since FRDC Project 2014-022 was funded.
Impacts	<ul style="list-style-type: none"> • A potential contribution to progress towards a future reduction in costs of egg identification surveys. • A potential contribution to increased scientific capability and capacity of scientists with respect to assembling key fisheries information.

Pathway to Impact

A diagram describing the simplified pathways to impact for the investment in Project 2014-022 is provided in Figure 1.

Figure 1: Pathway to Impact for Project 2014-022



Nominal Investment

Table 2 shows the annual investment made in Project 2014-022 by FRDC, James Cook University (JCU), the Queensland Department of Agriculture and Fisheries (QDAF) and the Northern Territory Department of Primary Industry and Resources (NTDPIR) as indicated in Table 2.

Table 2: Annual Investment in Project 2014-022 (nominal \$)

Year ended 30 June	FRDC (\$)	James Cook University (\$)	QDAF and NTDPIR (\$)	TOTAL (\$)
2014	36,000	0	0	36,000
2015	54,730	52,029	62,432	169,191
2016	16,300	52,029	61,053	129,382
2017	32,091	52,029	90,696	174,816
2018	0	0	0	0
2019	18,000	0	0	18,000
Totals	157,121	156,087	214,181	527,389

Source: FRDC Project Agreement, FRDC Financial Acquittal

Program Management Costs

For the FRDC investment, the cost of managing the FRDC funding was added to the FRDC contribution for the project via a management cost multiplier (x1.179). This multiplier was estimated based on the share of 'employee benefits' and 'supplier' expenses in total FRDC expenditure reported in the FRDC's Cash Flow Statement (FRDC, 2017-2021). This multiplier then was applied to the nominal investment by FRDC shown in Table 2. A multiplier of 1.00 was used for administration and management costs for James Cook University and the two government agencies.

Real Investment and Extension Costs

For purposes of the investment analysis, the investment costs of all parties were expressed in 2020/21-dollar terms using the Implicit Price Deflator for Gross Domestic Product (ABS, 2021). No additional costs of extension were included as the outcomes and impacts were largely driven by project activities including communication carried out within the project.

Impacts

Table 3 provides a summary of the principal types of impacts identified in Table 1 and categorised using a triple bottom line framework into economic, environmental and social impacts.

Table 3: Triple Bottom Line Categories of Principal Impacts from Project 2014-022

Economic	<ul style="list-style-type: none">• A potential contribution to progress towards a future reduction in costs of egg identification surveys.
Environmental	<ul style="list-style-type: none">• Nil
Social	<ul style="list-style-type: none">• A potential contribution to increased scientific capability and capacity of scientists with respect to assembling key fisheries information.

Public versus Private Impacts

The potential impacts identified in this evaluation are related to the potential for future long-term improvements in effective management of some Australian fisheries, benefiting potentially both public and private sectors. Some social impacts may be delivered in the short-term via the increased potential scientific knowledge gained by fisheries scientists.

Distribution of Private Impacts

Any long-term private impacts will be captured via any future contribution the project may make to improved egg identification surveys and the potential use of such surveys in future fisheries management. Such potential private benefits likely will be shared across fishery supply chains according to associated supply and demand elasticities.

Impacts on Other Australian Industries

It is expected that there would be negligible impacts on other Australian primary industries.

Impacts Overseas

Any impacts overseas will be largely associated with potential use of the scientific knowledge generated by the project.

Match with National Priorities

Australian Agriculture, Science, and Research Priorities

The Australian Government's National Science and Research Priorities and Agricultural Innovation Priorities are reproduced in Table 4. Project 2014-022 indirectly contributed to National Science and Research Priority 1. Further, the RD&E investment is likely to contribute indirectly to Agricultural Innovation Priority 2 through a contribution to improved decision making associated with fish biomass estimates in the future, improving industry economic and environmental sustainability in the long-term.

Table 4: Australian R&D Priorities

Australian Government	
National Science and Research Priorities¹	National Agricultural Innovation Priorities²
<ol style="list-style-type: none"> 1. Food – optimising food and fibre production and processing; agricultural productivity and supply chains within Australia and global markets. 2. Soil and Water – improving the use of soils and water resources, both terrestrial and marine. 3. Transport – boosting Australian transportation: securing capability and capacity to move essential commodities; alternative fuels; lowering emissions. 4. Cybersecurity – improving cybersecurity for individuals, businesses, government, and national infrastructure. 5. Energy and Resources – supporting the development of reliable, low cost, sustainable energy supplies and enhancing the long-term viability of Australia’s resources industries. 6. Manufacturing – supporting the development of high value and innovative manufacturing industries in Australia. 7. Environmental Change – mitigating, managing, or adapting to changes in the environment. 8. Health – improving the health outcomes for all Australians. 	<p>On 11 October 2021, the National Agricultural Innovation Policy Statement was released. It highlights four long-term priorities for Australia’s agricultural innovation system to address by 2030. These priorities replace the Australian Government’s Rural Research, Development and Extension Priorities which were published in the 2015 Agricultural Competitiveness White Paper.</p> <ol style="list-style-type: none"> 1. Australia is a trusted exporter of premium food and agricultural products by 2030. 2. Australia will champion climate resilience to increase the productivity, profitability, and sustainability of the agricultural sector by 2030. 3. Australia is a world leader in preventing and rapidly responding to significant incursions of pests and diseases through futureproofing our biosecurity system by 2030. 4. Australia is a mature adopter, developer, and exporter of digital agriculture by 2030.

FRDC National RD&E Priorities

Through extensive consultation, the FRDC 2015-2020 RD&E Plan identified three national RD&E priorities to focus and direct FRDC investments. The three FRDC national RD&E priorities were:

1. Ensuring that Australian fishing and aquaculture products are sustainable and acknowledged to be so.
2. Improving productivity and profitability of fishing and aquaculture.
3. Developing new and emerging aquaculture growth opportunities.

Project 2014-022 indirectly addressed FRDC national RD&E priority 1 by potentially contributing to improved industry economic and environmental sustainability in the long-term because of better decision making associated with future fish biomass estimates in the future.

¹ Source: 2015 Australian Government *Science and Research Priorities*. <https://www.industry.gov.au/data-and-publications/science-and-research-priorities>.

² Source: 2021 National Agriculture Innovation Policy Statement. https://www.awe.gov.au/agriculture-land/farm-food-drought/innovation/research_and_development_corporations_and_companies#government-priorities-for-investment.

Valuation of Impacts

Neither of the impacts in Table 3 were valued due to the limited success of the project and a lack of information available on which to base credible assumptions on any follow-up project.

The impacts identified in Table 3 were not valued for the following reasons (Table 5):

Table 5: Reasons for Not Valuing Impacts

Impact/Potential Impact	Reason why Impact Not Valued
A potential contribution to progress towards a future reduction in costs of egg identification surveys.	There was no evidence of any further pursuit of the progression of the technique at the time of this evaluation. Further, there would have been difficulty in assessing the likelihood, cost and success of any future project to improve the method.
A potential contribution to the increased scientific capability and capacity with respect to assembling key fisheries information at a species level for fisheries management purposes.	This contribution was not valued in monetary terms due to the difficulty of developing credible assumptions regarding the project and the extent of capability and capacity built and its future usefulness.

Results

All past costs were expressed in 2020/21-dollar terms. All costs were discounted to 2021/22 using a discount rate of 5%.

Investment Criteria

Investment criteria were estimated in accordance with the guidelines of the CRRDC (CRRDC, 2018). Tables 6 and 7 show the investment criteria estimated for different periods of costs for the total investment and FRDC investment respectively. Note that, as no impacts for this project were valued, the investment criteria reporting are restricted to the Present Value of Costs (PVC).

In the interests of consistency with other project analyses, aggregation and reporting, the PVC was reported for the length of the investment period plus for different periods up to 30 years from the last year of investment (2018/19). Thus, the PVC was the same for each period.

Table 6: Investment Criteria for Total Investment in Project 2014-022

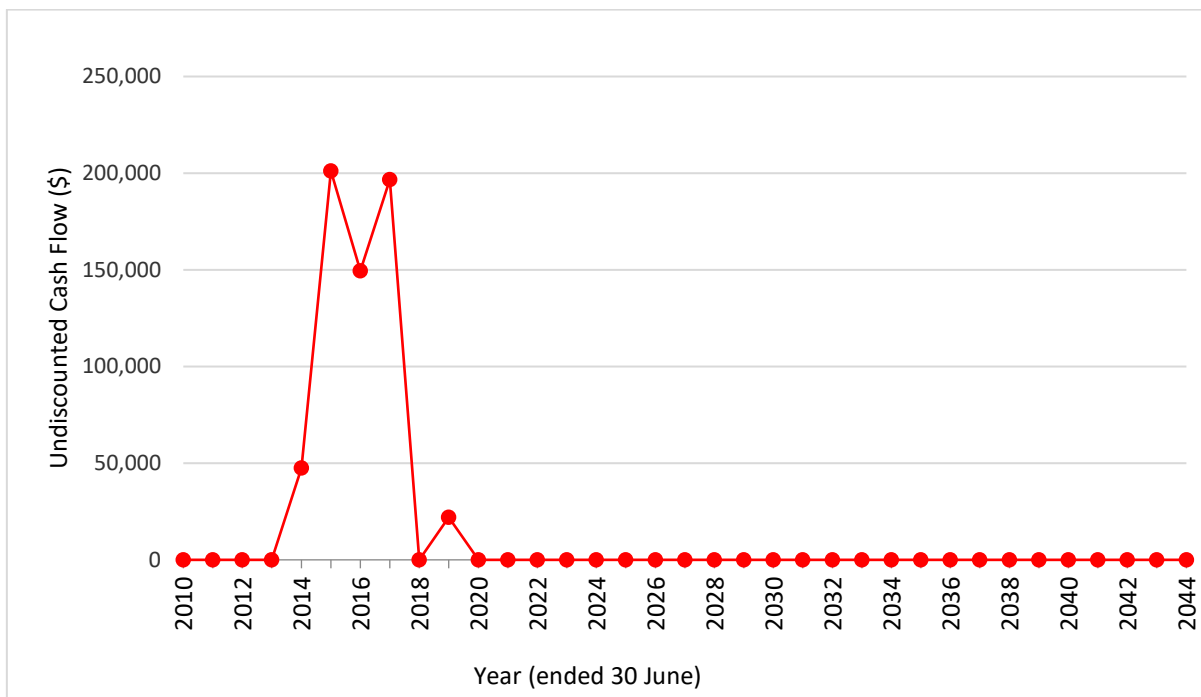
Investment criteria	Number of years from year of last investment						
	0	5	10	15	20	25	30
Present value of costs (\$)	830,011	830,011	830,011	830,011	830,011	830,011	830,011

Table 7: Investment Criteria for FRDC Investment in Project 2014-022

Investment criteria	Number of years from year of last investment						
	0	5	10	15	20	25	30
Present value of costs (\$)	279,263	279,263	279,263	279,263	279,263	279,263	279,263

The annual undiscounted cost cash flow for the total investment for the duration of the investment period is shown in Figure 2.

Figure 2: Annual Undiscounted Cash Flow of Total Costs



Conclusions

Total funding for the investment in FRDC Project 2014-022 over the period 2014 to 2019 totalled \$830,011 in present value terms. The FRDC investment costs over the same period were \$279,263 in present value terms.

The method for egg identification applied in Project 2014-022 relied on complex chemistry with multiple steps, the failure of any one of which resulted in a false negative result. This problem potentially could be overcome in future with more investment, but there has been no request for further investment that has progressed the method.

The evaluation has identified two impacts of the investment:

- A potential contribution to progress towards a potential future reduction in costs of egg identification surveys, if such methods are pursued in the future.
- A potential contribution to increased scientific capability and capacity with respect to assembling key fisheries information at a species level for fisheries management purposes.

However, given the uncertainty of any future investment, no attempt was made to financially value either of the two potential impacts identified.

Glossary of Economic Terms

Cost-benefit analysis:	A conceptual framework for the economic evaluation of projects and programs in the public sector. It differs from a financial appraisal or evaluation in that it considers all gains (benefits) and losses (costs), regardless of to whom they accrue.
Benefit-cost ratio:	The ratio of the present value of investment benefits to the present value of investment costs.
Discounting:	The process of relating the costs and benefits of an investment to a base year using a stated discount rate.
Internal rate of return:	The discount rate at which an investment has a net present value of zero, i.e., where present value of benefits = present value of costs.
Investment criteria:	Measures of the economic worth of an investment such as Net Present Value, Benefit-Cost Ratio, and Internal Rate of Return.
Modified internal rate of return:	The internal rate of return of an investment that is modified so that the cash inflows from an investment are re-invested at the rate of the cost of capital (the re-investment rate).
Net present value:	The discounted value of the benefits of an investment less the discounted value of the costs, i.e., present value of benefits - present value of costs.
Present value of benefits:	The discounted value of benefits.
Present value of costs:	The discounted value of investment costs.

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