RESEARCH **17**

2016-180

WHAT ARE THE CARP VIRUS BIOCONTROL RISKS AND HOW CAN THEY BE MANAGED?

NATIONAL CARP CONTROL PLAN

Assessment of options for utilisation of virus-infected carp

This suite of documents contains those listed below.

NCCP TECHNICAL PAPERS

1. Carp biocontrol background
2. Epidemiology and release strategies
3. Carp biocontrol and water quality
4. Carp virus species specificity
5. Potential socio-economic impacts of carp biocontrol
6. NCCP implementation
7. NCCP engagement report
8. NCCP Murray and Murrumbidgee case study
9. NCCP Lachlan case study

NCCP RESEARCH (peer reviewed)

Will carp virus biocontrol be effective?

1. 2016-153: Preparing for Cyprinid herpesvirus 3: A carp biomass estimate for eastern Australia
2. 2018-120: Population dynamics and carp biomass estimates for Australia
3. 2017-148: Exploring genetic biocontrol options that could work synergistically with the carp virus
4. 2016-170: Development of hydrological, ecological and epidemiological modelling
5. 2017-135: Essential studies on Cyprinid herpesvirus 3 (CyHV-3) prior to release of the virus in Australian waters
6. 2020-104: Evaluating the role of direct fish-to-fish contact on horizontal transmission of koi herpesvirus
7. 2019-163 Understanding the genetics and genomics of carp strains and susceptibility to CyHV-3
8. 2017-094: Review of carp control via commercial exploitation

What are the carp virus biocontrol risks and how can they be managed?

9. 2017-055 and 2017-056: Water-quality risk assessment of carp biocontrol for Australian waterways
10. 2016-183: Cyprinid herpesvirus 3 and its relevance to humans
11. 2017-127: Defining best practice for viral susceptibility testing of non-target species to Cyprinid herpesvirus 3
12. 2019-176: Determination of the susceptibility of Silver Perch, Murray Cod and Rainbow Trout to infection with CyHV-3
13. 2016-152 and 2018-189: The socio-economic impact assessment and stakeholder engagement
    Appendix 1: Getting the National Carp Control Plan right: Ensuring the plan addresses community and stakeholder needs, interests and concerns
    Appendix 2: Findings of community attitude surveys
    Appendix 3: Socio-economic impact assessment — commercial carp fishers
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    Appendix 9: Engaging with the NCCP: Summary of a stakeholder workshop
14. 2017-237: Risks, costs and water industry response
15. 2017-054: Social, economic and ecological risk assessment for use of Cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia
    Volume 1: Review of the literature, outbreak scenarios, exposure pathways and case studies
    Volume 2: Assessment of risks to Matters of National Environmental Significance
    Volume 3: Assessment of social risks
16. 2016-158: Development of strategies to optimise release and clean-up strategies
17. 2016-180: Assessment of options for utilisation of virus-infected carp
18. 2017-104: The likely medium- to long-term ecological outcomes of major carp population reductions
19. 2016-132: Expected benefits and costs associated with carp control in the Murray-Darling Basin

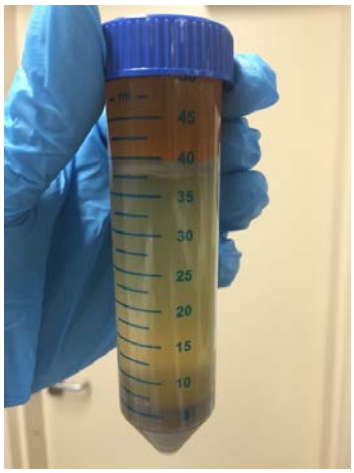
NCCP PLANNING INVESTIGATIONS

1. 2018-112: Carp questionnaire survey and community mapping tool
2. 2018-190: Biosecurity strategy for the koi (*Cyprinus carpio*) industry
3. 2017-222: Engineering options for the NCCP
4. NCCP Lachlan case study (in house) (refer to Technical Paper 9)
5. 2018-209: Various NCCP operations case studies for the Murray and Murrumbidgee river systems (refer to Technical Paper 8)

FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

NATIONAL CARP CONTROL PLAN
RESTORING NATIVE BIODIVERSITY

FRDC 2016/180
Utilisation of Carp Biomass
Final Report



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Options for Utilisation of Carp Biomass
FRDC 2016/180.
2019

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Executive Summary

The National Carp Control Program (NCCP) was formed with an overarching objective to assess the feasibility and potentially manage the release of Cyprinid herpesvirus 3 (CyHV-3) as a biocontrol agent for the invasive carp. Carp, introduced to Australia early in the last century, now make up to 80% of the fish population in some inland waterways. Carp have a strong negative impact on freshwater aquatic environments due to their feeding habits which increase water turbidity and damage aquatic plants; predation; and, competition with native species for food.

In assessing the feasibility of using Cyprinid herpesvirus 3 (CyHV-3) as a biocontrol agent it was recognised that there would need to be an examination of waste utilisation options for the large volumes of fish biomass that would be potentially produced. Whilst land fill was an option, a strategy for re-use/recycling was recommended. Hence this project, FRDC 2016/180: Options for Utilisation of Carp Biomass was subsequently funded under the NCCP. Initially, in project development, a broad scope encompassing utilisation options, quality issues, harvest, transport and processing logistical challenges, legislative considerations and cost benefit evaluation was suggested. However, the scope was later reduced to the development of options and aligned cost benefit evaluation with harvest/clean-up work to be completed under a different research project and legislative and policy considerations to be determined once feasible options were identified.

Initially a literature review was undertaken to understand the compositional aspects of carp as a raw material and to examine end-use options for the product. Subsequently a series of laboratory based trials were planned and one tonne of frozen, edible grade carp was sourced for these trials. As the carp delivered for the trials was of edible quality, product was subject to aging (1, 2 and 6 days old) to mimic the variation in quality condition likely in waterways following carp death and different carp product formats (e.g. whole fish, minced, cutlets) were developed for different putative outcomes.

Most laboratory trials were undertaken at Curtin University and included enzyme hydrolysis (to produce organic fertiliser or aquafeed); rendering (to produce carp meal and oil), trial as a food source for black solider fly (BSF) *(Hermetia illucens)* Larvae Production, and high pressure pasteurisation (HPP) trials on raw minced product for pet food outcomes. Goulburn Valley Water (GVW) staff also conducted laboratory trials testing the use of carp wastewater as a potential input to biogas production following anaerobic digestion. Composting trials were not undertaken at laboratory scale. Compositional analyses were completed for most end-products. These small scale laboratory trials, whilst plagued by operational difficulties, generally showed that it was possible to produce enzyme hydrolysate, BSF larvae, fishmeal and pet food from fresh and aged carp.

However, possibly the most important outcome from the laboratory trials was understanding the difficulties in undertaking small scale laboratory experiments in share use laboratories with decaying fish. The relevance of such trials in a commercial context was also questioned. Following discussions and agreement from the NCCP managers, it was therefore decided that the project would henceforth focus on undertaking larger scale trials with a significant number of stakeholders and/or commercial companies who had expressed interest, whether direct to the Principle Investigator (PI) or through NCCP, in being involved in this project. NCCP staff worked with the PI to develop agreed parameters to determine which larger scale pilot trials would go ahead with interested parties.

There were small scale trials (< 10 tonnes) which were considered to be suitable for local/community based solutions and were not expected to produce a commercial return, and larger scale trials, larger than 10 tonnes, with large commercial entities. The smaller scale trials were to be simply costed with the larger scale trials subject to independent cost benefit evaluation. It is acknowledged that both pilot small and large scale trials were generally undertaken with carp of relatively good quality, and did not truly

mimic the decayed product that might be more representative of virus infected product harvested from waterways. It must therefore be further acknowledged then that results must be interpreted with consideration of the initial fish quality.

The successful smaller, pilot scale trials included a fermentative hydrolysis process to produce liquid fertiliser, and production of a carp worm tea to instigate vermicomposting used to produce vermacast. In both cases the processes were costed and documents and procedures were drafted to allow possible implementation at community scale. The partners in these projects considered that, assuming management of occupational health and safety issues and/or community engagement issues associated with handling decaying fish, that fish quality/age was unlikely to be an issue for successful processing.

Future Green Solutions (FGS), an Australian company developing sustainable protein sources using BSF larvae for various feed industries, was contracted to undertake further BSF larvae carp growth trials. On the advice of the FGS partner, BSF were cultured on three different feeds including 100% carp mince, 70% mince/30% dry matter and a control diet of 70% vegetables/30% dry matter. Although operational problems persisted, particularly in harvesting, BSF larvae from the 70% carp/30% dry matter treatment were harvested, dried and defatted, and this product was used successfully in ongoing Curtin University post-graduate juvenile barramundi and crustacean feeding trials firstly at ~30% replacement of fish meal protein and later as a 10% supplement to plant and animal based feeds. Despite these results, it was considered that BSF commercial production in Australia was not yet at a level for consideration as a serious commercial option for managing very large carp volumes.

Several pilot trials to investigate decaying carp separation (into solids and wastewater) and anaerobic digestion of the waste water were attempted at the GVW facility. Unfortunately these were not successful due to issues with the separating process. However, the carp procured for this wastewater trial were composted at a local facility and the commercial potential of the process recorded in the cost benefit evaluation.

In summary fermentative hydrolysis and vermacast production have been shown to be technically viable for smaller community based applications, and can be implemented based on the draft methods, protocols and costings that have been provided. Some aspirational carp usage options, including anaerobic digestion and BSF larvae production have been proven at small scale but require further work for larger scale assessment and possible implementation.

As part of the larger scale trials, a 40 tonne trial at Camperdown Compost showed that compost production was possible, with optimisation of process and co-composting material. Product monitoring and compositional analyses also met national and state guidelines. It was considered that the composting methodologies developed could be transferred to other areas, closer to where carp harvest might occur. A preliminary implementation plan for managed, localised composting at such remote sites near where carp aggregation was likely to occur was developed by the industry partners involved in the project.

Hence in regard to larger scale commercial options the composting methods developed during the trials at Camperdown Composting are suggested as the most flexible and scalable option. The product value may be low but the process is likely to be able to use severely degraded product. As well flexibility in scale has been suggested with the option, assuming management, to develop small scale, farm-based, regional operations in remote, difficult to access locations with little infrastructure at or close to the water's edge. Pending consideration of transport approvals, and access and infrastructure availability, better quality fish at larger volumes could be transported to larger scale composting sites, either managed by local councils or commercial entities focussed on developing a possible product for consumer markets.

A commercial enzyme hydrolysis trial was planned and undertaken at SAMPI, Port Lincoln. This facility already processes ~1500 tonnes of tuna waste each year. In short the carp, at three different stages of

deterioration (separated into three final product tanks) were successfully processed through the SAMPI enzyme hydrolysis process. The produced hydrolysate was stored in 1000L containers to check stability - there was little separation, no odour and no precipitation on storage. Based on this outcome as well on the compositional results, use of the product as a fertiliser was therefore considered feasible; albeit at a lower quality (and therefore lower economic return) than the aligned tuna hydrolysate product. A leading aquafeed company also requested and received samples of the carp hydrolysate for use in finfish feeding trials - these results have not been made available due to commercial confidentiality reasons. However, carp hydrolysate was successfully supplemented at 10% inclusion rate in ongoing juvenile barramundi and crustacean feeding trials being conducted by post-graduate students at Curtin University.

Production of a carp enzyme hydrolysate is therefore possible, however at present this option is restricted to low numbers of processing sites, and operators have indicated, that capital assistance would be required to upscale plant capacity to be a credible option for processing of the very large volumes that have been indicated. It is however of note that pending approvals, shore based pickup and transport solutions have been designed by the operator. Although the value of the putative product is higher, so are product specifications and hence, the raw material quality must be at <72 hours post mortality. As well operators would likely require high volume harvest aggregation site with suitable riverside infrastructure to access appropriate volumes.

The large scale rendering option was also shown to be feasible, with 16 tonnes of carp processed through a meat rendering facility. There were no technical issues with the process and meal and oil were produced for analysis. Capacity to undertake the processing was therefore possible, and commercial markets available, however there exist stringent quality specifications and hence product >24 hours post-mortality would likely not be accepted for processing. Similarly to the hydrolysate example, operators would likely also require high volume harvest aggregation site with suitable riverside infrastructure to access appropriate volumes of acceptable raw material. There were concerns raised about the consumer acceptance of pet food products from virus infected raw material.

Following consultation with the various commercial industry partners, an initial Cost-Benefit Analysis (CBA) was conducted on 14 possible supply chain scenarios based on four processing pathways. The report stated that each of the four pathways enables viable commercial scenarios, assuming carp are free at the water's edge. However, the CBA has not been fully developed due to major assumptions that commercial processors seek clarity on. As summarised below, clarity on the following issues will give greater confidence in further development of business models.

These issues include

- Clarify who owns a carp killed by the virus, at the harvest point, and confirm if processors own the final processed product,
- Provide greater detail regarding the quality of virus-killed carp available for removal during the "clean-up". For example, will dead fish initially sink in the water column and will be more difficult to harvest, and at what stage (number of hours after mortality) of deterioration will fish float to the surface of the water column?
- Confirm the definition of virus infected fish for transport and processing, a differentiation is required between "biological waste" and "infectious agent." This clarification has direct implications for regulatory approvals and transport costs.
- Provide clarity on the likely yield and location of top fish aggregation and harvest sites across catchments. What infrastructure and harvest facilities are available at each site? Are there any seasonal constraints on aggregation and harvest at each site? This data will greatly inform investors, and derisk harvest and freight costs for large processors.

- Consider the added benefits and costs that would accrue if large processors (renderers, hydrolysers, large composters) commit to large waste stream forward offtake contracts from the infected waterways. The benefits of contracted multiyear supply of large fish volumes could drive substantial improvements in the viability of scenarios analysed in the CBA.
- Confirm with Federal/State agencies and relevant EPA managers the procedures required regarding transport, remote composting, and related aspects of other processes (e.g. anaerobic digestion),
- Confirm if / how carbon credits impact farm composting values and returns,
- Confirm if / how government subsidies apply to compost sites managed by Landcare / CMA's / Councils,
- Ensure that any virus release strategy policy and planning development is aligned with, and guided by realistic commercial utilisation, supply chain and market demand considerations. Planning for the carp utilisation waste task (minimum 350,000 tonnes per annum (tpa) will require the equivalent services of at least 30 large processors each receiving up to 12,000 tpa of carp waste. This requires significant engagement and coordination with commercial processors and confirmation of sufficient infrastructure needs, to ensure efficient community and commercial outcomes.

In summary, a suite of options for utilisation of the carp biomass have been developed and validated at large scale. It is suggested that such a suite of options, targeted for specific harvest location, logistical challenges and product quality variation, all need to be considered rather than a single, holistic solution for utilisation of the carp biomass. There is ongoing interest by commercial operators in taking part in carp utilisation options, but regional difficulties in handling this product have been highlighted, and infrastructure to manage transport, storage and processing issues associated with the large volumes will be required.

The following next steps are recommended.

- NCCP seeks to gain greater advice and clarity on the policy issues raised in the cost benefit analysis and summarised in the conclusions section above. The CBA can then be further modified based on clarification of some of the uncertainties.

- Identify possible aggregation sites and volumes, then work with nominated commercial industry partners (renderers, remote and larger scale composting management, and hydrolysis entities) and develop recommendations for costed implementation plans (including additional processing, transport and infrastructure requirements) and management of regulatory issues. Interest from these commercial entities in the NCCP is ongoing.

- Many of the utilisation options identified in this study would still be technically viable if fish can be harvested in continuous, large volume scenarios, and consistent product quality and handling protocols were instigated. The economic viability would be contingent on the assumptions on fish cost made in the CBA. It may be worth further investigating such a non-virus release scenario based on the results of this study. It was in this context that it was decided to add a carp seafood export option to the CBA.

It is noted that there are currently three PhD students examining both the carp fed BSF larvae and the carp hydrolysate in finfish and crustacean feeding trials as part of their Curtin University post-graduate research, this work is likely to continue until 2022 and will further inform possible end-use options for the putative commercial processes.

Finally, along with the mandatory milestone results, significant community and research extension of the project outcomes was achieved, with print, television and social media coverage, articles in FISH magazine and presentations at NCCP research and stakeholder events.

1. Introduction

Wild Carp (*Cyprinis carpio*) is a species native to a broad geographic area encompassing Asia and Eastern Europe. However, it was introduced to Australia, possibly on a number of occasions, beginning in the mid-19th century and through the earlier part of the 20th century. The invasive species is now well established throughout the Murray-Darling basin (MDB) and is estimated to make up 90% of the fish biomass in some areas. Carp now also occurs across most of south-east Australia, with isolated populations in Tasmania and Western Australia. The only State/Territory that is currently free of carp is the Northern Territory.

Carp have a strong negative impact on freshwater aquatic environments due to feeding habits which increase water turbidity and damage aquatic plants; predation; and, competition with native species for food. Carp control and management is therefore a legislative imperative for protecting the environment of Australian rivers and streams. Due to these impacts, carp have been declared as noxious species across all affected jurisdictions of Australia with legislative provisions for their management and controls on their release and possession.

The Australian Federal government has developed the National Carp Control Plan (NCCP) which will assess the feasibility and potentially manage the release of Cyprinid herpesvirus 3 (CyHV-3) as a biocontrol agent for the invasive carp. The virus was expected to reduce the carp population by between 70-95% within the first few years. Initial release at breeding sites was expected to wipe out primarily juvenile carp at first, followed by mature fish. It was anticipated bird-life would consume a large portion of the immature carp however deceased mature carp presents an environmental challenge as their decomposition may impact upon water quality. The large mass of deceased carp will require a large scale clean-up and present a unique opportunity to be utilised for fish products. Capture and/or removal of deceased carp as a management strategy creates an issue for disposal of the carcasses. Disposal to landfill is problematic due to odour, but is also counter to waste management strategies that encourage recycling and reuse.

Currently carp are harvested for use in fertiliser, lobster bait and some small volumes for human consumption; however as estimates of the deceased biomass are in the hundreds of thousands of tonnes, other avenues for utilisation warrant further investigation. Compositional analysis, assessment of suitability of CyHV-3 infected fish for processing, pilot scale production trials and subsequent market appraisal were required to realise new product streams. Development of new products utilising the infected deceased carp could potentially assist in the clean-up, reduce disposal costs and generate income for the local economy.

This project. FRDC 2016/180: Assessment of Options for Utilisation of Carp Biomass was therefore developed under the NCCP research program to assess such carp utilisation and new product options.

2 Objective

The overarching objective of the project was:

To identify, pilot and undertake subsequent CBA for developing new processes/products from deceased feral carp (as part of NCCP).

3. Methods

3.1 Literature Review

A first stage in the project was to conduct a literature review. The aims of the literature review were to;

- Review wild carp (*Cyprinus carpio*) nutritional composition, including age, gender and habitat seasonal variability.
- Review current use in edible (quality, shelf-life, fresh/frozen/value-added forms) and inedible products (compost, silage, fertiliser, pet food, animal feed, etc.). Summarise current market prices and availability for all carp products.
- Review and scope of current fish hydrolysates and other aligned lower level fish waste products on the market (this will be an update rather than a full review as a detailed literature review on outcomes for fish waste generally and hydrolysates in particular including current products available on the market was conducted in 2015 as part of FRDC 2013/711.40).
- Evaluate suitability of carp for various products based on nutritional composition and volumes.
- Identify potential issues of using infected, deceased carp in chosen products (e.g. viability of virus, consumer perceptions). Assess potential market expectations for infected carp products.
- Establish which products are most promising and develop methodology for laboratory pilot trials.

3.2 Analyses

All chemical and compositional analyses were outsourced to the National Measurement Institute (NMI) or other nationally accredited commercial laboratories. The analyses generally included proximate analyses (protein, oil, ash and moisture), mineral composition, amino acid composition and fatty acid composition.

Microbiological analyses were outsourced to Mérieux NutriSciences.

3.3 Source of Fish for Trials

For the Curtin University and Future Green Solutions (FGS) trials one tonne of carp were commercially harvested by electro-fishing in NSW. The whole carp were immediately frozen and transported to Western Australia. Permission was sought from the Western Australia Department of Fisheries prior to the transportation. The carp were of human food quality, and it is acknowledged that this quality would be better than deceased carp harvested from waterways, all results must therefore be interpreted with this disclaimer. Portions of this carp volume were also used for trials described in Section 4.3.1.

In sourcing of fish for larger scale trials two approaches were undertaken:

a. When a carp "clean-up operation" was planned then, where possible, fish were harvested and transported for processing with nominated project partners (see Sections 4.3.2, 4.3.4 and 4.4.3).

b. For trials described in Section 4.4.2 a call for carp supply from all licensed commercial carp fishers was made through the NCCP, and three responses were received, the entity with the lowest price were contracted to deliver the fish.

3.4 Laboratory pilot trials.

A range of laboratory scale experiments to assess different utilisation options were conducted and are described in Sections 3.3.2 to 3.3.6. Generally these trials were conducted at Curtin University under the direction of Dr Janet Howieson. The exception was the anaerobic digestion trials (Section 3.4.6) which were conducted at the GVW laboratories, Shepparton, under the direction of Elise O'Keefe. It is noteworthy that some of the laboratory based product development trials undertaken were modified from that presented in the original grant application. These modifications included the inclusion of BSF larvae culture (at request of NCCP funding grants committee); inclusion of GVW anaerobic digestion trials (at request of NCCP Program Manager) and inclusion of pet food subject to HPP (request direct to PI from industry, discussed with NCCP Program Manager and included in milestone reporting). The high moisture extrusion trials were not undertaken due to the equipment in the experimental food processing facility not being suitable for the high moisture and decaying carp based raw material. These changes in product options were discussed with the NCC Project Managers, included in milestone reporting and in presentations at NCCP Research meetings.

3.4.1 Formats and Composition of Carp used for Experimentation

Carp of different storage ages were produced as below:

1. Fresh carp samples were of several formats; thawed whole fish, cutlets produced, using a bandsaw, from frozen carp, at Catalano Seafoods, or a fresh carp mince produced from the frozen whole fish by a pet food manufacturer, Conveniently Raw, through an industrial mincer, with mince either re-frozen in 20 kg plastic tubs with liners (Figure 1) or in 1 kg vacuum packed bags. The cutlets and mince were produced at the commercial facilities after Curtin University laboratory equipment was shown to be not suitable for the necessary processing.

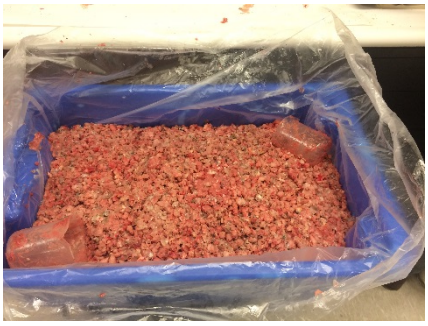

FIGURE 1: **MINCED FRESH WHOLE CARP**

2. 48 hour old carp samples were produced by placing thawed whole fish in ambient tap water in plastic tubs outside for 48 hours (Figure 2). Some 48 hour whole fish were manually cut into approximately one inch cutlets and fed through a garden mulcher to further break up the samples (Figure 3). Cutlets were either used fresh after aging or frozen for later use. 48 hour aged fish were not cut or minced in commercial facilities due to contamination concerns with the deteriorated state of the fish.

FIGURE 2: AGING OF CARP FOR EXPERIMENTS

FIGURE 3: CUTTING OF AGED CARP FOR EXPERIMENTS

3. 144 hour old carp samples were produced by placing thawed carp in ambient tap water in plastic tubs outside for 144 hours. Carp were then sliced with a knife and used fresh for the experiments.

Fresh and aged carp samples were subject to various compositional analyses as described in Section 3.2.

3.4.2 Enzymatic Hydrolysis

Enzyme hydrolysis methods developed as part of FRDC 2013/711.40: New Opportunities for Seafood Processing Waste were used as the basis for the carp enzyme hydrolysis trials.

The initial enzyme hydrolysis trials were completed in a 2 kg Sunbeam sous vide machine. The carp samples tested were fresh carp cutlets and mince, 48 hour aged carp cutlets and 144 hour aged carp cutlets.

The methods used is described below:

1. If necessary frozen cutlets or mince were placed into room temperature water to thaw.
2. 2 - 2.5 kg of carp samples were weighed and placed in a sous vide machine.
3. 2% (w/w of carp sample) alcalase enzyme was mixed into the carp sample
4. The mixture was covered and incubated at 55˚C for at least 2 hours with infrequent stirring until hydrolysis was complete (when all content was liquefied except for bone/scales).
5. The mixture was heated at 95˚C for 1 hour with stirring at 20 min intervals to deactivate the enzyme
6. The hydrolysed solution was sieved to remove bones/scales (which were weighed) and the remaining liquid portion was weighed.
7. The liquid hydrolysed solution was aliquoted into 50 mL centrifuge tubes and centrifuged at 3800 *g* for 10 minutes at room temperature, to separate the different fractions. Percentage composition of different fractions was estimated following centrifugation.
8. Samples of bones/scales; liquid hydrolysate solution and fractions of liquid hydrolysate solution were packaged and frozen for later analysis

Enzyme hydrolysis trials were also completed with fresh carp mince in a 40 kg custom built enzyme hydrolysis unit (Figure 4).

FIGURE 4: CUSTOM MADE ENZYME HYDROLYSIS UNIT

The methods used for the larger volume trial were intended to be the same as for the smaller scale trials but this was not possible as extra water was needed to be added to facilitate hydrolysis. The unit also did not heat or stir properly, hence the hydrolysis process was ceased before completion and finished in the smaller units. The 40kg unit is currently undergoing modification to improve performance, hence no further results are detailed in this report.

3.4.3 Rendering

The rendering process and parameters developed for the laboratory trials was based on information gathered from the literature and also following industry consultation.

The methods used are described below:

1. 1500g of frozen fresh carp mince was thawed.
2. Carp mince temperature was taken to 46˚C before increasing to the processing temperature of 90˚C.The mixture took 58 minutes to reach 90˚C from 46˚C, with the lid on and frequent stirring. Once the processing temperature was reached the mixture was allowed to cook for a further one hour with frequent stirring.
3. Cooked mince was aliquoted into 50 mL centrifuge tubes and centrifuged at 3800 *g* for 10 minutes at 20˚C, to separate meal (solid), stick water and oil components; these fractions were collected individually.
4. Meal (solid) and stick water fractions were dried with frequent mixing in an oven at 80˚C; the concentrated stick water was later mixed into the meal and this mixture was further dried until moisture reached below 10%.
5. Dried meal was ground to a fine powder and stored in air tight containers.
6. Meal was despatched for analyses.

3.4.4 Carp as a Feed Source for BSF Larvae

Preliminary experiments to trial the growth of BSF *(Hermetia illucens)* larvae on various feed mixtures containing carp (including fresh and 48 hour aged carp) were conducted at Curtin University. Whilst acknowledging that 144 hour aged carp would be more typical of deceased carp harvest from waterways, such carp were not trialled due to odour issues in a shared laboratory. BSF larvae is being researched as a

possible protein meal replacement for fish meal in aquaculture feed, and hence there was a request for BSF larvae culture to be included in the project from the NCCP funding committee which approved the funding grant.

10 kg plastic tubs were used for the BSF culture trials, methods are described below:

1. Stocking density was based on container size and stocking density information supplied by BSF larvae supplier FGS.
2. Following advice from FGS various feed mixtures were produced as shown in Table 1. Feed mixtures were percentage by weight. The bran was wheat bran, purchased from a local stock feed manufacturer. The fresh carp mixture was fresh carp mince (Section 2.1) and the aged carp was 48 hour aged carp cutlets (Section 2.1). In addition to the carp based feeds produced, vegetable waste was mixed with 50% w/w wheat bran and used as a control. The vegetable waste was comprised of one portion each of salsa, ratatouille, diced eggplant, shredded asparagus, diced tomato, diced onion, diced veg mix, diced red capsicum, diced green capsicum, basil paste, diced pumpkin, cooked diced tomato and three portions of peeled banana.
3. 7 day old larvae, supplied by FGS and shipped in calico bags, were placed in tubs filled with 1kg of the respective feeds. Following consultation with FGS, larval densities were calculated and intended at 12000 larvae per tub. Unfortunately due to a delay in the delivery of the larvae, upon arrival and inspection a substantial portion of larvae in each bag was deceased (~50%) resulting in lower initial larval densities.
4. The 1kg of feed was replaced at the same time each day (1.30pm) with feeding continuing for 6 days. All frass (larval digestate) was removed on Day 4.
5. Larvae were monitored informally for mortality and growth. Fifteen larvae from each treatment were weighed on Days 1, 4 and 6 and average larval weights calculated.
6. Larvae were harvested on day six. Where possible a minimum 300 g sample of larvae was collected from each tub. Harvest techniques varied with the feed source and are described below.
7. Harvested larvae was stored frozen in a single layer in a sandwich bag. All debris was removed from harvested larvae.
8. A minimum 300 g sample of frass (if possible) was collected at the time of harvest, this was stored frozen for analysis.
9. Frozen larvae and frass were despatched for compositional analyses.

Table 1: Feed Treatments for the BSF Larvae Culture Trials

Container number/composition	Comments
1. 50% veg/50% bran	Mixture of horticulture products and wheat bran
2. 100% fresh carp	Fresh carp mince
3. 100% aged carp	48 hour aged carp cutlets
4. 50% aged carp/50% bran	48 hour aged carp cutlets and wheat bran
5. 100% fresh carp	Fresh carp mince
6. 50% fresh carp/50% bran	Fresh carp mince and wheat bran
7. 50% veg/50% bran	Mixture of horticulture products and wheat bran
8. 50% fresh carp/50% bran	Fresh carp mince and wheat bran
9. 50% aged carp/50% bran	48 hour aged carp cutlets and wheat bran
10. 100% aged carp	Used 48 hour aged carp cutlets

Initially the containers were placed in a 25˚C temperature controlled room. However due to odour issues containers had to be removed to an outside (uncontrolled temperature site) site on Day 1. Growth containers were therefore placed in larger tubs and covered with mesh to prevent invasion by other insects.

When harvesting containers with feed containing wheat bran, initially the top layer of frass was removed manually. The mixture was then sieved to remove as much frass as possible then the remaining material and larvae were placed on a large gauge sieve. Over time the larvae burrowed down through the mesh and fell into the lower chamber, leaving the majority of frass sitting on top of the sieve and the larvae harvested from beneath.

Harvesting of larvae from the 100% fish feed containers proved difficult as the frass was wet and sticky; larvae were slow moving and dispersed throughout the frass. Attempts were made to wash the frass away; this was messy and while it did remove the frass, it left behind a large amount of undigested material mixed with the larvae. Larvae were therefore harvested by removing with tweezers.

3.4.5 Raw Pet Food.

The project lead researcher was contacted by a pet food manufacturer "Conveniently Raw" who were interested in developing a fresh, minced pet food product from the carp. Preliminary fresh carp mince analyses were provided to the company who then requested further investigation including the possibility of reducing bacterial load and extending the mince shelf-life using HPP.

Whole frozen carp were minced at the "Conveniently Raw" facility through an industrial mincer and re-repacked in 1 kg vacuum packed bags and refrozen for the HPP trials. The carp was reported to be best put through the mincer in a semi-frozen state.

The HPP trials were conducted on the Hyperbaric machine located at the commercial Fresh Produce Alliance facility at Manjimup. The 1kg packs of carp mince were subject to pressures of 250, 350, 500 and 600 pounds per square inch (psi) for 3 minutes. Samples (including an untreated control sample) were then assessed for visual colour and subject to microbiological analyses.

3.4.6 Anaerobic Digestion of Carp Wastewater

Elise O'Keefe from GVW, Shepparton conducted a laboratory trial to test the feasibility of co-digestion of the liquid fraction of *Cyprinus carpio* (European Carp) with municipal wastewater. The intent was to determine if carp wastewater could be added as an ingredient in the anaerobic digestion facility currently operated by GVW. The detailed report is available in Appendix 2 with specific methods sections reproduced below.

The experimental phase of the research was conducted by replicating the anaerobic digestion process at the Shepparton Waste Management Facility (WMF)'s High Rate Anaerobic Lagoon (HRAL). To do this, five sealed glass jars were used as rectors, and had eudiometers attached to capture the biogas generated, with water used as the displacement medium (see Figure 5).

FIGURE 5: THE EXPERIMENTAL SETUP FOR THE ANAEROBIC DIGESTION TRIALS.

A 24hr composite sample was taken for the influent wastewater stream coming into the WMF, and the digestate seed for the process was taken from the Shepparton WMF HRAL. Fish liquid was sourced by obtaining fresh dead carp form local recreational fishermen, and then extracting the liquid from the fish. The extraction process involved chopping and mincing the fish, and then hanging the resulting product in a netted bag to drain the liquid overnight.

The reactor jar for the control contained only the 24hr composite wastewater sample and the seed. The other reactor jars contained a mixture of the 24hr composite wastewater sample, the seed, and fish liquid. Magnesium Hydroxide was added to raise the alkalinity above 800 mg/L when required. The volumes, ratios, and variables used are listed in Table 2. Subsequent gas production was measured using the eudiometer.

Table 2. Composition of Samples used for the Anaerobic Digestion Experiment

Variable	UOM	Control	Test 1	Test 2	Test 3	Test 4
Volume	mL	1000	1000	1000	1000	1000
Fish Liquid	%	0%	5%	12%	18%	23%
Fish Liquid	mL	0	50	120	180	230
Wastewater	mL	1000	950	880	820	770
Seed	mL	30	30	30	30	30
Temp	◦C	24.5	24.5	24.5	24.5	24.5

3.4.7 Next Steps from Laboratory Trials

As discussed in the results (Section 4.2) there were many operational and laboratory sharing issues associated with conducting carp trials at Curtin University. There were also concerns with demonstrating feasibility of scale-up of the tested utilisation methods if only laboratory trials were undertaken. Hence, following discussions with NCCP Program Managers, it was decided to enlist the help of many interested stakeholders who had contacted either the NCCP Program Managers or the PI with interest in taking part in trials. It was further decided to divide utilisation options into smaller-scale opportunities (<10 tonnes) which were more likely to be community based and/or (at this stage), non-commercial solutions (Section 3.4) and larger scale solutions (>10 tonnes) in commercially valid operations (Section 3.5). The PI worked with NCCP staff to decide which solutions were to be tested at larger scale. Implicit in being supported to

undertake commercial trials was agreement, within the confines of commercial confidentiality, to provide information for the cost benefit analysis (see Section 3.6), although most operators stated they would rather undertake the trials and assess the results before meeting the CBA consultant.

It was noted during this discussion period with the NCCP, and raised in milestone reports and research meetings that undertaking trials in commercial operations would impact delivery of scientific outcomes which could be published in peer reviewed journals, due to variability in process and inability to do replications etc. However, it was considered and agreed with the NCCP Program Managers that trials developed to show technical and economic feasibility of various utilisation options was the more important output for the NCCP.

3.5 Small Scale Semi-Commercial Trials (<10 tonnes)

3.5.1 Fermentative Hydrolysis

In order to investigate the potential for small scale carp utilization operations at a local, community scale, the processing of minced carp using hydrolysis and anaerobic fermentation was explored in a pilot study, near Canberra, and overseen by Gerry Gillespie, David Hardwick and Dr Sarah Beavis. The inclusion of this trial was at the request of the NCCP Program Manager. The methodology has previously been successfully used to produce fertilizer from kangaroo and pig carcasses following culling operations.

The principal aims of the study were to examine the efficacy of this process with carp, including an assessment of the chemical composition and suitability of the end-product as a fertiliser and to determine indicator pathogen loads before and after processing. The detailed report is available as Appendix 3 with specific methods sections reproduced and/or summarized below.

The stages of the study were:

- Process the carp biomass via hydrolysis/fermentation according to defined specifications
- Analyse the material on completion of processing to determine chemical composition; and
- Prior to, and on completion of, processing establish whether indicator pathogens are present and if so, the efficacy of hydrolysis/fermentation in destroying those pathogens

Background

Fish biomass may be processed anaerobically through a number of stages including hydrolysis, glycolysis and lactic acid fermentation. During this processing the generation of acidity inhibits the growth of other non-desirable organisms, and consequently it has been used historically for food preservation, and more recently in managing animal wastes. Following maceration of the test product, a carbohydrate is added to the mix. This complex carbohydrate is broken down into simpler compounds, with the final step of conversion to lactic acid being achieved through the bacterial action of *Lactobacillus acidophilus.* This bacterium is naturally present in the gut of animals, but can also be added via inoculation using a *lactobacillus* culture. It is this process of acidification which preserves the macerated carcass, and limits decomposition. The product can be stored for a number of months prior to further processing into a final product, such as the proposed fertilizer. The stages of processing are illustrated in Figure 6.

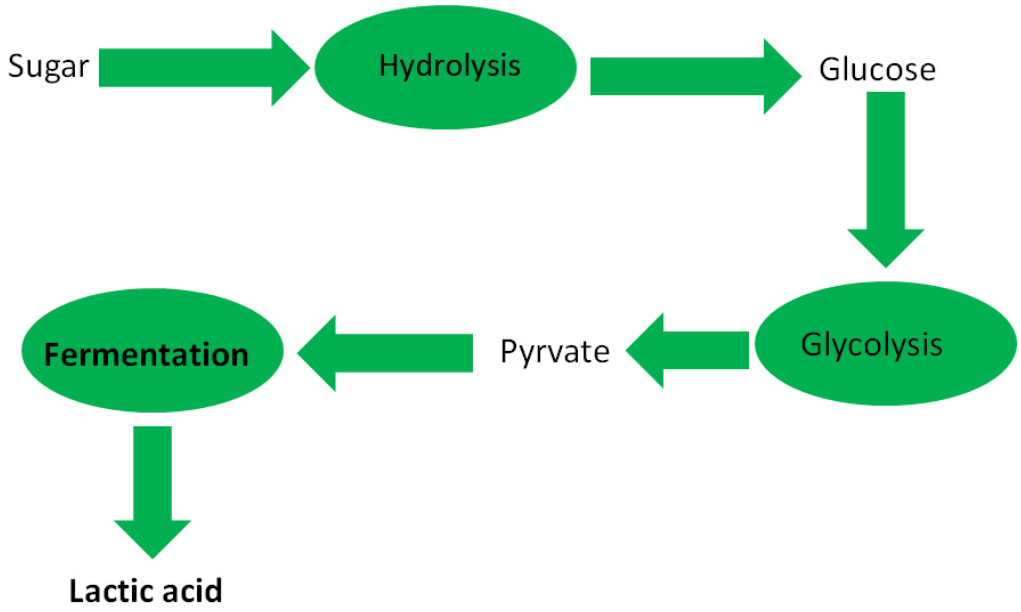

FIGURE 6: STEPS IN THE PROCESS OF HYDROLYSIS, GLYCOLYSIS AND LACTIC ACID FERMENTATION

Methods

Stage 1: Processing

Minced carp material was delivered in combination with a source of sugar (molasses), water and biological inoculant. The biological inoculant (containing *lactobacillus* and comprising a '*base serum*' which is made in a two-step process using rice water and milk); stays stable for 12 months. The dosage of inoculant was 10 ml/L of water. The ratio of biomass to sugar to inoculated water was:

animal tissue: water[1] : molasses : *lactobacillus* stock = 1 : 1 : 0.2 : 0.07

The mixture of carp mince biomass, water, molasses and inoculant was put into a 1000L International Bulking Container (IBC) immediately closed and an air lock fitted. Prior to closure, samples were taken for laboratory analysis of chemical composition, indicator pathogens and *in situ* measurement of pH and temperature. Heating pads and insulation were attached to the sides of the IBC to maintain required temperatures.

Regular measurement of temperature and pH were undertaken.

After 4 weeks, the material was drained off the solids and put into 60L drums. The solids residue could potentially be pumped off for either composting or direct burial. Replicate samples of the final liquid hydrolysate solution were taken for laboratory analysis of chemical composition and indicator pathogens, as well as *in situ* measurement of temperature and pH.

Stage 2: Chemical Analysis

Replicate samples were submitted for analysis at the NATA accredited Environmental Analysis Laboratory (EAL) at Southern Cross University, Lismore, NSW. The analyses represented a standard 'package' provided by EAL for testing materials intended to be used as a liquid fertilizer, and include major and trace nutrients,

[1] Water used should be deionised or milliQ or, alternatively, tap water that has been allowed to stand for several hours for chlorine to escape

pH, electrical conductivity and estimated total dissolved solids. These results were analysed and assessed against the *National Code of Practice for Fertiliser Description and Labelling* (DAFF, 2011). This code is an instrument that ensures consistent standards, specifications and labelling requirements which can be accessed by purchasers and users of fertilisers across all States and Territories of Australia. Compliance with this code will therefore meet the statutory requirements of all States and Territories (DAFF, 2011)

Stage 3: Pathogen Analyses

The risks of contamination of food from irrigation waters, compost or organic fertilisers have been well documented in the literature (Qadir *et al,* 2010; Domingo and Nadal, 2009; Hai *et al*., 2010). To understand the potential risk that may exist, the pathogen indicator species for this study was *E.coli.* Analyses of replicate samples from before and after the process were undertaken at the NSW Department of Primary Industries laboratory in Menangle, NSW.

3.5.2 GVW, Veolia and Western Composting: Anaerobic Digestion and Composting.

In this trial, overseen by GVW, it was decided to attempt to separate whole carp into solid and liquid components by passing through the Veolia sewage separating system. The liquid fraction would then be incorporated into the existing GVW anaerobic digestion ponds, and the solid component despatched to the nearby Western Composting for compost production. Such collaborative trials were planned as the Western Composting, Veolia and GVW facility are all co-located, facilitating ease of product movement between the different entities. The scope of the initial plan is shown in Figure 7.

Trial 1: Approximately two tonnes of carp (from a "clean-up" operation) were transported to the Veolia facility and separation trials commenced. As the separation in the Veolia system was unsuccessful, maceration of the whole fish was also attempted using the Western Composting large scale shredding machine. As separation was not achieved, the remaining carp was added into the Western Composting "tunnel" system for compost production. Some details of this process were recorded by the Western Composting staff.

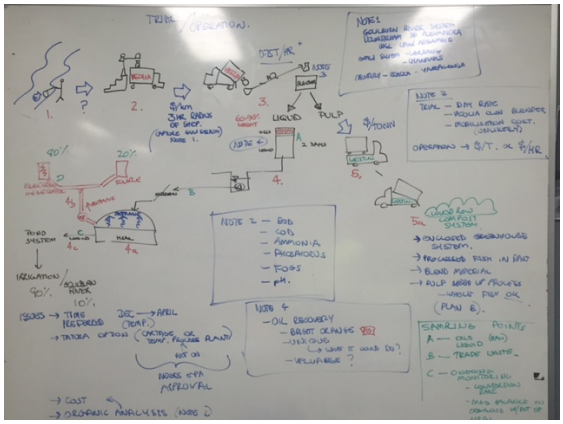

FIGURE 7: SCOPING PLAN FOR TRIAL 1

Trail 2 and 3: Due to the separation issues identified in Trial 1, two further trials were conducted in which an initial mincing step was added to the protocol (see Figure 8). In Trial 2 and Trial 3 approximately 2 tonnes of carp (from a "clean-up" operation) were therefore initially transported to the Daldy Road Knackery for mincing. Two IBC containers was then set up with mesh to suspend the minced material and enable dewatering to occur. The IBC containers were later transported to the Veolia and Western Composting facilities for further processing.

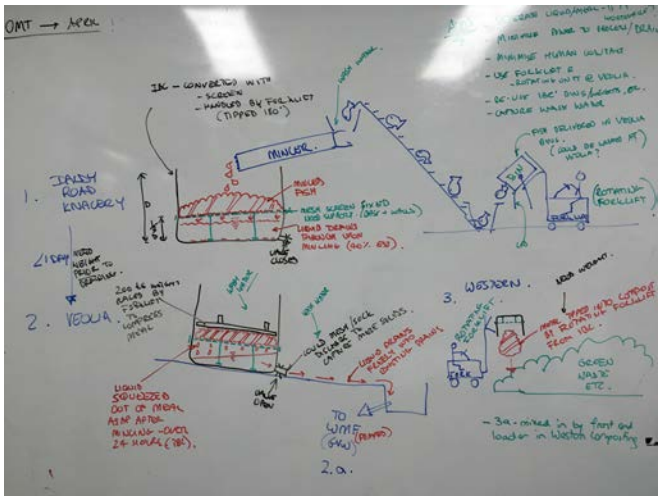

FIGURE 8: SCOPING PLAN FOR TRIAL 2

3.5.3 Carp as a Feed Source for BSF Larvae

Following the Curtin laboratory based trials for carp fed BSF larvae, and the operational challenges, a larger scale growth was proposed to be undertaken at the FGS facility. The trial had the objectives to further determine the suitability of carp biomass as a feed ingredient for BSF larvae. The trial feed mixes were modified from that in the previous Curtin based experiments following discussion of the results with the FGS experts.

At the FGS facility larvae were raised on three feed mixes: 100% minced carp; 70:30% minced carp and poultry/pig feed waste; and 70:30% vegetable waste and poultry/pig feed waste. Three tubs of each treatment were set up with an inoculation rate of 12000 larvae per tub (see Figure 97), frass was removed periodically (Figure 10). At least 15 BSF larvae from each tub were weighed on Days 3 and 6 (Figure 11).

FIGURE 9 CULTURE VESSELS

FIGURE 10: FRASS REMOVED

FIGURE 11: WEIGHING LARVAE

De-frassing occurred twice during this trial (Day 4 and 7).

On Day 4 for 100% carp due to anoxic conditions (Figure 12), the larvae were harvested, whereas for the other two treatment larvae were harvested on Day 7 (Figure 13 and 14). Actual and theoretical harvesting yields were calculated and the larvae from each treatment were washed (Figure 15), packaged and frozen for further processing trials (Figure 16).

FIGURE 12: 100% CARP ANOXIA

FIGURE 13: LARVAE BEFORE HARVEST

FIGURE 14: HARVESTING

FIGURE 15 WASHED LARVAE

FIGURE 16: PACKAGED LARVAE

The BSF larvae from the FGS growth trials were dried, defatted and successfully used in post-graduate research undertaken by two Curtin University Masters of Sustainable Aquaculture students, two PhD students and one post- doctoral scholar. Four feed experiments have been conducted, including three with juvenile barramundi and one with marron.

The objectives of this research were to

1. Identify feasibility of processing (drying, milling, defatting) carp fed BSF larvae.
2. Conduct barramundi and marron feed trials with carp fed BSF meal.

The methods (and results) from this continuing post-graduate research is not reported here, however, a peer reviewed journal article has been accepted for publication in Scientific Reports and three others are in preparation. These papers can be made available to interested readers of this report on request.

3.5.4 Vermicast Production

On request from NCCP program staff, an informal worm tea trial was conducted on 300kg of carp at the Tony's Worm's facility in Victoria, with the intent to facilitate vermicomposting to produce vermicast (a form of compost). Worm tea is reported to be highly active with beneficial aerobic microbes which expedite the decomposition process and minimise/eliminate unpleasant odours.

Three worm tea/vermicomposting trials were set up as well as an untreated control.

The treatments were

1. Non-mixed fish treated with worm tea (Figure 17)
2. Fish “battered” in saw dust and treated with worm tea (Figure 18).
3. Fish “battered” in saw dust and treated with worm tea then covered with additional saw dust (Figure 19).

FIGURE 17: TREATMENT 1

FIGURE 18: TREATMENT 2

FIGURE 19: TREATMENT 3

As the trial was informal no data was collected apart from visual observations.

3.6 Large Scale Semi-Commercial Trials (>10 tonnes)

3.6.1 Enzyme Hydrolysis

A large scale enzymatic hydrolysis trial was conducted at the SAMPI commercial processing facility in Port Lincoln. SAMPI currently conduct enzyme hydrolysis on 1500 tonnes per annum of tuna waste. All final product is sold as organic fertiliser or aqua feed ingredients.

The objectives of the trial were:

- Determine suitability of carp biomass for processing by enzyme hydrolysis.
- Identify optimum enzyme hydrolysis processing conditions for carp, and recoveries.
- Undertake compositional analyses and understand stability and other parameters of the final product.

10 tonnes of fresh then frozen carp were harvested and transported to Port Lincoln. The carp was thawed before the trial: the three thawing protocols were thawing 48 hours before, 24 hours before and on the morning of the trial.

The thawed carp was processed through the SAMPI enzyme hydrolysate system, based on the same methodology used to process 1500 tonnes of tuna waste per annum. This system includes a mincer then pumping to a secondary mincer. Then alcalase enzyme was added to the mince at 2% in heated reaction tanks. After the reaction was complete the bones, scales and other fragments are separated from the hydrolysate through a sieve and the remaining liquid hydrolysate (<5µ) is pumped into the acid tanks where phosphoric acid is added to take the pH to below 3. The finished hydrolysate is then pumped into 1000L IBC’s for storage. Weights and recoveries through the hydrolysate system were calculated. Water was added as necessary and volumes recorded. The final product was subject to appropriate and relevant compositional and nutritional analyses.

3.6.2 Composting

A large scale composting trial was conducted on a licenced EPA premises, owned and operated by Camperdown Composting at 445 Sandy's Lane, Bookaar, Victoria. The detail report is available as Appendix 4 with specific methods sections reproduced and/or summarized below. It is noteworthy that, following discussions, the trial methodology (including monitoring protocols) was developed and approved by the Victorian Environmental Protection Authority prior to the trial commencing (see documentation in Appendix 4).

The method employed in this trial was informed by research into mass mortality composting of chicken carried out by the Victorian Department of Primary Industries and Environment (Wilkinson, 2014) and earlier work by the Victorian Fisheries Authority (2008). Figure 20 shows a schematic of the structure of a compost heap using fish and co-composting materials.

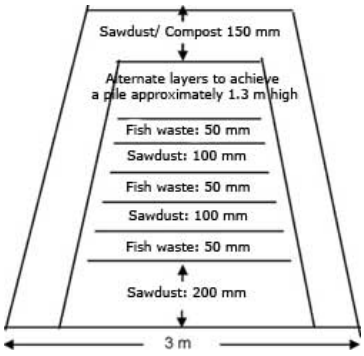

FIGURE 20: SCHEMATIC OF COMPOST STRUCTURE

The four treatments (comprising one row each) to which the fish waste was added were:
1. Compost
2. Compost plus sawdust blend (50:50)
3. Sawdust
4. Straw

These treatments were chosen to mimic natural methods which, if successful, could be transferred to other carp aggregation locations. Fish/co-compost blends were assembled on a 1:2 basis by volume. Earlier chemical analysis of fish frames and whole carp and industry experience with co-composting materials provided sufficient information to inform a starting Carbon: Nitrogen ratio of about 25:1. Each treatment was about $20m^3$.These proportions and starting ratios aimed to ensure rapid composting and suppression of odours. The capping with a layer of co-composting material also aimed to reduce odour. The mechanical turning used a purpose-built compost turner of a type readily available in regional areas. The feedstock's impact on the saleability of the final composted product was also noted, e.g. colour, texture, nutrient level etc.

About 40t of fish and fish carcasses were used in the trial with a corresponding amount of co-composting materials. The fish was made up of 20t of fish frames from the Melbourne market and 20t of carp sourced from Shepparton in regional Victoria. Fish was composted in two principal stages. In Stage 1 composting, the pile was left undisturbed as soft tissue decomposed and bones partially softened. This stage was about 14 days. The compost was then turned and mixed to begin Stage 2 composting, during which time the remains of fish carcasses break down further. Following completion of Stage 2 (~4 weeks), the composting process was completed during a curing phase of up to an additional 6 weeks.

The procedure began with the laying down of a 20–30 cm base layer of an absorbent 'co-composting material' such as sawdust or straw. The main function of this layer was to trap liquids released by the decomposing fish. Once the base layer was in place, fish carcasses were layered between alternating layers of co-composting materials as shown in Figure 20. Alternate layers of fish carcasses and co-composting material were layered on top of the base layer using a skidsteer loader to form a windrow (a type of elongated pile) with dimensions of three to four metres at the base and up to 1.8 m high. Each layer of fish was no deeper than 25 cm with 15 to 20 cm of co-composting materials between each layer. The final windrow was capped with 15 to 20 cm of co-composting material to ensure that all carcasses were covered. The final capping also served as a bio-filter to reduce odours (see Figure 20).

Detailed temperature records were taken. The final treatment – straw – was terminated early in view of excessive odour and poor leachate control. Following the first turning, windrows were capped again with co-composting material to a minimum depth of 10 cm. Further turns were based on temperature and the rate of decomposition.

The carp was composted with a number of different carbon-based feedstocks and combinations as discussed above. Each of four carbon feedstocks formed a separate batch identity to allow comparative temperature, moisture and breakdown data to be collected and collated. This methodology was designed to be replicable using locally available feedstocks e.g. sawdust, mature compost etc. Compost progression was monitored by temperature and visual inspection of piles at turning. The composting process was concluded within a 12 week period.

A late addition to the trial saw the compost only, and sawdust only treatments treated with a *lactobacillus* culture (developed as per Section 3.5.1 and provided by Gerry Gillespie). The *lactobacillus* treatment was applied at row assembly by spraying the culture over the fish and capping material. The odour monitoring by Ektimo provided data on the effectiveness of this treatment.

Leachate emissions were a key area of interest in this trial. The work was carried out on Camperdown Compost's licenced facility in South-West Victoria where the base layer complied with the requirements of EPA's publication 1588 for permeability of subgrade. Given that leachate would not permeate into the subgrade, the piles were evaluated for evidence of leachate below the piles. Consideration was given for collection of leachate (if any) using plastic sheeting but the likelihood of it being ripped up by the compost turner rendered this option impractical. Consideration was also given to the use of a 'full stop'-type device which is used to map wetting fronts in soils under irrigation. Again the impermeability of the subgrade made the installation of such a device impractical. Leachate management used direct observation of the base of the compost piles which were bunded with mature compost to ensure any leachate would be captured and not leave the site.

Following completion of the composting process, samples were dispatched to SESL Australia's NATA-accredited laboratory for testing to Australian Standard 4454 – Composts, soil conditioners and mulches. Finished composts were also tested for total elemental analysis so information on nutrient values could be provided.

Silage, mentioned in the grant application as a possible process to be trialled, was not attempted due to advice from the composting industry partners. The composting consultants advised that silage is generally a product of high rainfall pastures where excess growth is ensiled to sustain animals during periods of low feed availability (i.e. summer). They considered that areas where carp were likely to be extracted are predominantly low rainfall areas, and hence there would be very little if any silage available in those areas; hence the use of straw in the composting trials which would be relatively plentiful in the target areas.

3.6.3 Rendering

16 tonnes of carp from a "clean-up " operation was processed through the meat rendering system at the Manildra Cootamundra processing facility. Meal and oil were produced. Details of the methodology and compositional analysis were not released due to commercial considerations.

3.7 Costings and CBA

On discussion with NCCP Program Leaders a formal cost benefit analyses was only conducted on the commercial scale utilisation options (described in Section 3.5). For the other smaller scale community options (described in Section 3.4), where possible, simple costing was undertaken and recorded.

3.7.1 Basic Costing of Small Scale Options

Formal cost benefit analyses were not completed on the successful small scale, community based options (fermentative hydrolysis (Section 3.5.1) and the vermacost production (Section 3.5.2). This is because it may not be appropriate for these final products to be sold in large volumes commercially, rather they would be provided back to the community/operation which undertook the processing. Commercial partners had raised concerns in the market about such "unregulated" carp products being available commercially. However quotes for equipment and consumables to undertake these small scale community options were obtained as part of final reporting.

Costings were not obtained for the BSF larvae production (Section 3.5.3) as this option was not proven at large scale, and more work is required to establish feasibility (and is ongoing).

3.7.2: Formal CBA of Larger scale trials

Ewan Colquhoun, Ridge Partners, was contracted to undertake the formal CBA.

Following discussions with NCCP Program Managers, the following aspects were incorporated into the development of the cost benefit scope and methodology.

- The CBA will not include the "clean-up" costs, but will commence when the harvested fish is placed in the transport vehicle.
- The CBA will make an assumption that the fish are to be provided for no cost and that the end-product is owned by the processor. This because the policy question of ownership of the carp has not yet been resolved, and needs to be discussed between the states and the NCCP at the relevant committee level. Sensitivity analyses for the cost benefit analysis will be conducted around a processor cost incurred for the provided fish. Another consideration is the suggestion that tonnages be potentially allocated to various end-users after predicted and possibly staged kill events to ensure confidence for the processors in allocating resources for processing (staff, transport and operations).
- For transport we will assume the fish will not be classified as an "infectious agent" but as biological waste. This classification has implications for transport vehicles and licencing and therefore the transport costs.
- The CBA will be completed on the successful commercial trials: the enzyme hydrolysate, composting and render options, and will also consider the anaerobic digestion opportunity. We also included (non-infected) seafood in the analysis as an option necessary so that there is a baseline of data for the "no virus release" scenario.

Fourteen different product outcomes were subject to CBA. The detailed report is available as Appendix 5. Due to the detailed nature of the methodology, including the assumptions, the methods have not been reproduced here, readers are directed to the report.

4. Results and Discussion

4.1 Literature Review

The literature review is attached as Appendix 1. The results of the literature review were used in part to plan the experimental approach.

4.2 Laboratory Pilot Trials.

4.2.1 Aging and Composition of Carp

Carp Aging Observations

During the aging process of carp in water at ambient temperature, degradation of the carp became evident within 24 hours. The water became brown with evidence of bacterial growth obvious after 24 hours. Fish eyes were the first part of the fish to break down. Carp scales became easy to remove after aging. A rotten fish smell was prominent after 48 hours of aging. 30% of the carp had sunk to the bottom of the tub after 48 hours.

Carp aged for 144 hours showed signs of extensive degradation. Eye balls were almost entirely degraded and in some samples not present. A number of fish were bloated and released gas upon handling. The rotten fish smell was noticeably stronger. Carp scales were extremely easy to remove and a portion were found at the bottom of the tub.

Compositional Analyses.

Fresh carp mince and 48 hour aged carp semi-minced cutlets were analysed for proximate composition, fatty acid profile, amino acid profile and trace elements (Table 3). It is acknowledged that only single samples were analysed. It is also noteworthy that the fresh carp samples were whole minced whereas the 48 hour samples did not include the heads, therefore direct comparison of the results is not possible.

Noting the single samples, with the exception of ash, differences for proximate composition between fresh and aged carp samples were not observed. Fatty acid profiles were also similar between samples.

The ash content was notably lower in the 48 hour aged carp sample (2.3 g/100 g) compared to the fresh carp mince sample (5.1 g/100 g) (Table 4). In concurrence with this, calcium, copper, iron, magnesium, phosphorus and sodium were notably lower in carp aged for 48 hours; it is suspected that during aging these elements are washed into the surrounding water, reducing their concentration in the fish. It is also possible that the differences are due to heads not being included in the 48 hour aged sample.

Generally the amino acid profile also showed little differences between samples. The differences in glycine, proline and hydroxyproline can perhaps be accounted for by the lack of heads in the 48 hour aged carp samples. Collagen contains high levels of these amino acids and the head contains a high percentage of collagen based structures. As such a lack of heads in a sample would be expected to result in lower concentrations of these amino acids.

Table 3: Compositional Analyses for Fresh Carp Mince and 48 hour carp cutlets

	Analyte	Fresh carp mince	48 hour aged carp cutlets
Composition (g/100 g)	Moisture	72.6	75.5
	Protein	17.4	18.5
	Fat	5.1	6.3
	Saturated fat	1.4	1.6
	Mono-unsaturated fatty acids (MUSFA)	2.2	2.6
	Poly-unsaturated fatty acids (PUFA)	1.4	2
	Omega 3 fatty acids	1	1.5
	Omega 6 fatty acids	0.4	0.5
	Ash	5.1	2.3
	Total sugars	<1	<1
	Carbohydrates	<1	<1
	Energy	480	550
Saturated Fatty Acids (% of total fat)	C4:0 Butyric	0.1	0.1
	C6:0 Caproic	0.1	0.1
	C8:0 Caprylic	0.1	0.1
	C10:0 Capric	0.1	0.1
	C12:0 Lauric	0.1	0.1
	C14:0 Myristic	2.2	2.2
	C15:0 Pentadecanoic	1	0.9
	C16:0 Palmitic	18.1	16
	C17:0 Margaric	1.5	1.3
	C18:0 Stearic	5.2	4.7
	C20:0 Arachidic	0.1	0.1
	C22:0 Behenic	0.2	0.2
	C24:0 Lignoceric	0.1	0.1
	Total Saturated FA	28.3	25.5
Mono-unsaturated Fatty Acids (% of total fat)	C14:1 Myristoleic	0.1	0.1
	C16:1 Palmitoleic	11.2	11.2
	C17:1 Heptadecanoic	0.1	0.1
	C18:1 Oleic	20.1	19.4
	C18:1 Vaccenic (trans)	8.8	7.4
	C20:1 Eicosenic	3.3	3.1
	C22:1 Cetoleic	0.1	0.1
	C22:1 Docosenoic (Erucic)	0.1	0.1
	C24:1 Nervonic	0.2	0.2
	Total Mono-unsaturated FA	43.7	41.4
Poly-unsaturated Fatty Acid (% of total fat)	C16:4 Hexadecatetraenoic	0.4	0.3
	C18:4 Morotic	0.1	0.1
	C18:2w6 Linoleic	1.9	2.7
	C18:3w6 gamma-linoleic	0.1	0.1
	C18:3w3 alpha-linoleic	1.4	1.6
	C20:2w6 Eicosadienoic	1.1	1.1
	C20:3w6 Eicosatrienoic	0.4	0.4
	C20:3w3 Eicosatrienoic	0.4	0.4
	C20:4w6 Arachidonic	2	1.9
	C20:5w3 Eicosapentaenoic	5.1	6.1
	C22:2w6 Docosadienoic	0.1	0.1
	Omega 3 Fatty Acids	19.5	23.4
	Omega 6 Fatty Acids	7	8.3
	C22:4w6 Docosatetraenoic	1.5	2.1
	C22:5w3 Docosapentaenoic	3.5	4.7
	C22:6w3 Docosahexaenoic	9.1	10.5
	Total Poly-unsaturated FA	26.9	32.1
Trace Elements (mg/kg)	Cadmium	0.01	0.01
	Inorganic Arsenic	0.05	0.05
	Calcium	7900	2300
	Copper	0.87	0.69
	Iron	43	19
	Lead	0.028	0.031
	Magnesium	350	250
	Mercury	0.059	0.087
	Phosphorus	4900	2400
	Potassium	2200	2000
	Sodium (mg/100 g)	100	62
	Zinc	43	50
Amino acids (mg/kg)	Aspartic acid	11000	14000
	Serine	5800	5700
	Glutamic acid	17000	20000
	Glycine	14000	8400
	Histidine	1700	2700
	Arginine	9300	8100
	Threonine	5600	6300
	Alanine	9000	8700
	Proline	9400	6600
	Tyrosine	3000	3700
	Valine	4800	6100
	Lysine	8000	10000
	Isoleucine	4200	5500
	Leucine	8100	10000
	Phenylalanine	4800	5400
	Methionine	3200	3600
	Hydroxyproline	3200	1300
	Taurine	1000	1000

4.2.2 Enzyme Hydrolysis

4.2.2.1 Laboratory Enzyme Hydrolysis Trials.

Four separate 2kg small scale enzyme hydrolysis trials were completed. The hydrolysis was undertaken with the following raw materials (Section 2.1): fresh carp cutlets, 48 hour carp cutlets, 144 hour carp cutlets and fresh carp mince. The methodology used was the same for each trial. An example of the stages of the enzyme hydrolysis process (for fresh carp cutlets) is shown in Figure 21.

Carp cutlets | 1 hour 45 minutes hydrolysis | 2 hour 30 minutes hydrolysis | 5 hour 30 minutes hydrolysis

FIGURE 21: HYDROLYSIS OF FRESH CARP CUTLETS

Combined hydrolysis processing time and recovery results for the different treatments are shown in Table 4. Typically a total of four layers were formed after centrifuging: oil, emulsion (thought to be phospholipids and proteins/peptides with emulsifying properties), liquid hydrolysate and sludge (see Figure 22).

Table 4: Summary of 2kg Laboratory Enzyme Hydrolysis Trials

Hydrolysis Trial	**Fresh Carp Cutlets**	**48 hour carp cutlets**	**144 hour carp cutlets**	**Fresh Carp Mince**
Volume (kg)	2	2	2	2
% liquids	74	81	Not done	86.2
% solids	26	19	Not done	13.8
Time (hours)	5:45	3:40	3:15	2:30
Recovery (%)	90	94.2	Not done	95.0
Temperature (˚C)	50	55	55	55
Comments	**Four layers formed following centrifugation**	**Four layers formed Oil comprised of approximately 10% of the whole hydrolysate, the emulsion layer 5%, the separated hydrolysate 70% and the sludge 15%.**	**No centrifugation due to odour and complaints**	**Typical layers not formed after centrifugation**

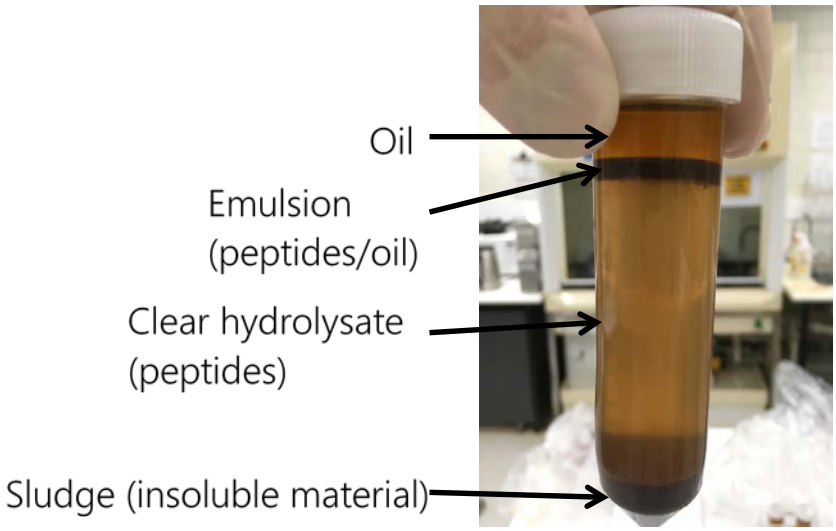

FIGURE 22: ENZYME HYDROLYSED CARP AFTER CENTRIFUGATION

Hydrolysis was faster in the 48 hour aged cutlets when compared to the fresh samples. This may be due to the degraded fish being easier for the enzyme to hydrolyse, the pooled liquid at the base from blood and released moisture may have provided greater surface contact with the cutlets, facilitating the hydrolysis.

Hydrolysis proceeded faster with the 144 hour sample than with fresh or 48 hour aged carp cutlets: this may be due to the significant decay that had occurred during aging. The carp were visibly rotting and had a pungent odour. Hydrolysis reduced the strength of the odour but did not completely remove it. Centrifuging to separate the components was not performed due to the odour making the task difficult to complete.

With the fresh carp mince, hydrolysis was more difficult to gauge by visual assessment compared to when cutlets were hydrolysed. However the mixture did appear to hydrolyse faster than other trials. Upon centrifugation for 10 min at 3800 *g*, in contrast to the fresh and 48 hour cutlet samples, a negligible oil layer was present. Additionally, the emulsion layer, between the oil and separated hydrolysate layers, was not compact and was not fully separated from the separated hydrolysate layer. It was suspected that this may have been due to incomplete hydrolysis.

Compositional Results

Whole (uncentrifuged) hydrolysates from the four small scale trials were analysed for chemical composition (Table 5). Results must be considered acknowledging the single sample size and some of the processing issues.

Generally moisture, ash and protein were quite similar, and values were similar to previous laboratory scale studies with other fish (see Final Report 2013/711.40) and the commercial SAMPI tuna hydrolysate product. Concentrations of the fatty acids of interest (Eicosapentaenoic (EPA) and Docosahexaenoic (DHA)) showed little difference between hydrolysate samples. Similarly, total omega-3 fatty acids were similar between all samples. A key area to be further investigated would be the oxidation of fatty acids with aging as this would be detrimental to the quality of any hydrolysate produced.

Consistent with the values observed in carp mince, sodium concentration appears to decline with increasing aging time. As previously mentioned, this may be the result of sodium washing out of the fish into the surrounding water during aging.

Interestingly, the values for lysine in aged samples appeared lower than those prepared using fresh carp. The loss of lysine from the aged samples may reflect the decarboxylation of lysine into cadaverine, an aromatic compound that is associated with rotting fish (Granata, Flick, & Martin, 2012). Lysine is an essential amino acid for humans and is often the limiting amino acid in animal feeds. Loss of lysine due to decarboxylation would present a loss of protein quality and also a barrier to acceptance, due to the strong putrefied meat smell of cadaverine.

Table 5: Chemical Analysis of Whole Hydrolysates

	Analyte	Fresh mince whole hydrolysate	Fresh carp cutlets whole hydrolysate	48 hour aged carp whole hydrolysate	144 hour aged carp whole hydrolysate
Composition (g/100 g)	Moisture	76.6	78.2	80.7	82.1
	Protein	15.6	14	13.9	13.2
	Fat	5.9	8.3	4.2	6.9
	Saturated fat	1.5	1.1	1.1	1.9
	MUSFA	2.5	3.7	1.9	2.9
	PUFA	1.8	2.4	1.2	2
	Omega 3	1.3	1.8	0.9	1.5
	Omega 6	0.5	0.6	0.3	0.6
	Ash	1.2	0.9	0.8	0.5
	Total sugars	<1	<1	<1	<1
	Carbohydrates	<1	<1	<1	<1
	Energy	480	540	390	480
Saturated Fatty Acids (% of total fat)	C4:0 Butyric	0.1	0.1	0.1	0.1
	C6:0 Caproic	0.1	0.1	0.1	0.1
	C8:0 Caprylic	0.1	0.1	0.1	0.1
	C10:0 Capric	0.1	0.1	0.1	0.1
	C12:0 Lauric	0.1	0.1	0.1	0.1
	C14:0 Myristic	2.2	2.2	2.6	2.5
	C15:0 Pentadecanoic	0.9	1	1	1
	C16:0 Palmitic	16.3	15.7	16.4	17.7
	C17:0 Margaric	1.2	1.2	1.1	1.5
	C18:0 Stearic	4.4	4.4	4.3	4.7
	C20:0 Arachidic	0.1	0.1	0.1	0.1
	C22:0 Behenic	0.2	0.2	0.1	0.2
	C24:0 Lignoceric	0.1	0.1	0.1	0.1
	Total Saturated FA	25.4	24.8	25.7	27.8
Mono-unsaturated Fatty Acids (% of total fat)	C14:1 Myristoleic	0.1	0.1	0.2	0.1
	C16:1 Palmitoleic	12.2	12.6	13.8	12.7
	C17:1 Heptadecanoic	0.1	0.1	0.1	0.1
	C18:1 Oleic	19.7	20.2	20.6	17.8
	C18:1 Vaccenic (trans)	8	8.5	8.1	7.9
	C20:1 Eicosenic	2.9	3	2.8	2.7
	C22:1 Cetoleic	0.1	0.1	0.1	0.1
	C22:1 Docosenoic (Erucic)	0.1	0.1	0.1	0.1
	C24:1 Nervonic	0.1	0.2	0.2	0.1
	Total Mono-unsaturated FA	43.2	44.8	45.6	41.3
Poly-unsaturated Fatty Acid (% of total fat)	C16:4 Hexadecatetraenoic	0.4	0.4	0.4	0.5
	C18:4 Morotic	0.1	0.1	0.1	0.1
	C18:2w6 Linoleic	2.7	2.6	2.6	2.7
	C18:3w6 gamma-linoleic	0.1	0.1	0.1	0.1
	C18:3w3 alpha-linoleic	1.6	0.1	1.7	1.6
	C20:2w6 Eicosadienoic	1	0.9	0.6	1
	C20:3w6 Eicosatrienoic	0.4	0.4	0.3	0.4
	C20:3w3 Eicosatrienoic	0.4	0.4	0.3	0.4
	C20:4w6 Arachidonic	2.2	2.3	1.9	2.4
	C20:5w3 Eicosapentaenoic	6.5	6.1	6.5	7
	C22:2w6 Docosadienoic	0.1	0.1	0.1	0.1
	Omega 3 Fatty Acids	21.9	21.1	20.8	21.1
	Omega 6 Fatty Acids	8.2	7.6	6.5	8.2
	C22:4w6 Docosatetraenoic	1.7	1.3	0.9	1.5
	C22:5w3 Docosapentaenoic	4	3.6	2.8	3.8
	C22:6w3 Docosahexaenoic	9.3	10.9	9.4	8.2
	Total Poly-unsaturated FA	30.5	29.2	27.6	29.7
	Sodium (mg/100 g)	98	72	66	49
Amino acids (mg/kg)	Aspartic acid	12000	12000	13000	9500
	Serine	5700	6300	5800	4100
	Glutamic acid	17000	18000	18000	15000
	Glycine	10000	12000	12000	9400
	Histidine	2500	3000	2500	2000
	Arginine	3200	7100	6200	2500
	Threonine	5300	6000	5700	4000
	Alanine	8500	9100	9000	7500
	Proline	5900	6800	6800	5300
	Tyrosine	1700	5100	4800	1100
	Valine	5700	6500	7900	6000
	Lysine	10000	11000	6300	4800
	Isoleucine	4800	5700	5100	3700
	Leucine	9300	10000	9700	7400
	Phenylalanine	4800	5500	5600	4100
	Methionine	3400	3600	3400	2600
	Hydroxyproline	1000	1100	1600	1400
	Taurine	1200	1400	1300	720

After centrifugation of the whole hydrolysates, the separated hydrolysate layer was extracted for chemical analysis (Table 6) as this would be the product sold for fertiliser or aquafeed. The single sample for analysis is acknowledged.

Similar to the whole hydrolysate samples, the 48 hour aged carp has a lower lysine concentration compared to the fresh carp cutlets sample. Also reflecting the data for whole hydrolysates is lower ash content for the aged sample compared to the fresh sample. In all the separated hydrolysate samples, the oil content is lower than that of the whole hydrolysates. This suggests that, as expected, the bulk of the oil is present in the other layers.

Table 6: Chemical Analysis of Separated Hydrolysate

	Analyte	Fresh mince separated hydrolysate	Fresh carp cutlets separated hydrolysate	48 hour aged carp separated hydrolysate
Composition (g/100 g)	Moisture	83.3	83.9	85.0
	Protein	14	13.5	12.8
	Fat	0.5	0.6	0.6
	Ash	1	1.5	0.7
Amino acids (mg/kg)	Aspartic acid	17000	12000	12000
	Serine	8800	5700	5100
	Glutamic acid	22000	17000	17000
	Glycine	13000	11000	11000
	Histidine	8900	2700	2900
	Arginine	11000	6600	5600
	Threonine	9400	5300	5100
	Alanine	12000	8600	8200
	Proline	10000	6500	6400
	Tyrosine	7300	1100	3100
	Valine	9600	5600	7500
	Lysine	16000	9800	5400
	Isoleucine	9800	5200	4900
	Leucine	13000	9500	9000
	Phenylalanine	11000	4400	5100
	Methionine	8300	2800	2900
	Hydroxyproline	4500	1200	1400
	Taurine	4600	1400	1200

The 2kg trials demonstrated that enzyme hydrolysis of various carp raw materials can produce a product with the potential for use as an organic fertiliser or as an aqua feed ingredient.

As noted in the methods section, the 40kg laboratory scale enzyme hydrolysis trial was unsuccessful. It was generally considered the equipment and protocols in the 40kg pilot plant required significant adjustment to mimic the parameters of the 2kg pilot trials and/or a commercial process. Whilst this equipment adjustment is a long term objective, as discussed previously due to the issues and potential

relevance of laboratory based trials at this point it was decided to proceed with a full commercial trial (see Section 4.4.1) rather than continue with slightly larger scale laboratory based activities.

4.2.3 Rendering

Folloiwng cooking and centrifugation, three laters were observed, an oil layer, a stickwater layer and a solid layer (Figure 23).

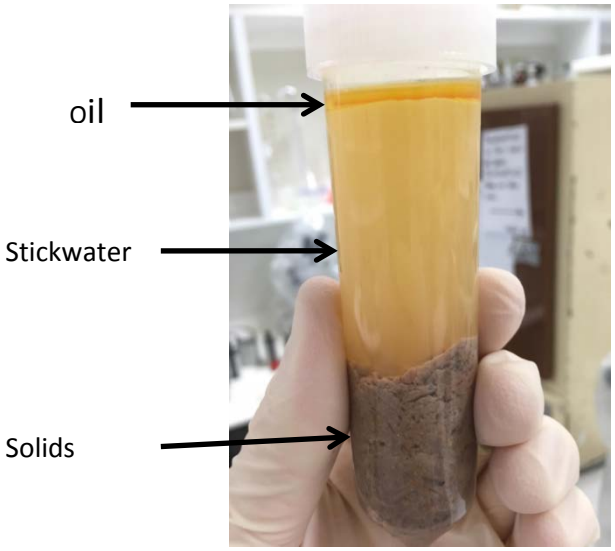

FIGURE 23: FISH MEAL PREPARATION FOLLOWING CENTRIFUGATION

All three layers were separated, the stick water and solids were placed, separately, in an oven at 85˚C for two hours which was then reduced to 70˚C for 17 hours. The reduced stick water and dried solids were then combined and dried at 85˚C for a further 7 hours and 45 minutes (Figure 24). The dry mixture was then ground to a fine powder and stored in a zip lock bag at ambient temperature.

FIGURE 24: DRIED FISH MEAL

Mallard browning of the solids was observed after the first two hours of drying and continued to progress until all solids were brown. On visual examination following centrifugation, the low yield of oil suggested that the rendering process had not lysed the fat cells sufficiently to release the oil. This was confirmed following the chemical analysis of the carp render which showed that fat was still present in the render (Table 7). However the protein, ash and moisture levels were consistent with other commercial fish meal products. As with previous laboratory scale experiments it was decided that any further fish meal development work be conducted, if possible, in a commercial facility.

Table 7: Chemical Analysis of Carp Render

Sample	Carp render
Moisture (g/ 100 g)	5.3
Protein (g/ 100 g)	62.1
Fat (g/ 100 g)	15
Ash (g/ 100 g)	15.2

4.2.4 Carp as a Feed Source for BSF Larvae

There were operational difficulties encountered in the Curtin University BSF trial including significant loss of the initial larvae culture due to courier problems, anoxia partially due to excess moisture and external fly larvae infestation in the 100% carp treatments, and some fungal growth in one of the vegetable treatments. Some of these issues were due to having to move the containers outside due to odour issues. Due to these problems, results must be considered preliminary, and indeed further growth trials were thereafter contracted to FGS (see Section 4.3.3).

Figure 25 is an example of the harvested BSF larvae that was a product of the Curtin University trials.

FIGURE 25: BSF LARVAE FROM FEEDING TRIALS

Table 8 shows the average larval weights from the different feed source treatments on Day 1, 4 and 6. All results were presented as mean ± SE which were subjected to Shapiro-Wilk's and Levene's tests to test the normal distribution. Growth data of BSF larvae were subjected to two-way ANOVA where “diet” and “day” were used as main factors.

There was no significant differences ($p<0.05$) between the weight gains of the larvae fed the different diets for any length of feeding time. The weight of the larvae increased significantly as time progressed regardless of diet. Survival rates between diets could not be assessed due to the harvesting issues.

The larvae collected from the various feed sources were subject to chemical analysis (Table 9). Proximate composition including fatty acids and amino acids composition among BSF larvae fed different diets were compared by one-way ANOVA, followed by tukey multiple comparisons test at $P < 0.05$.

Table 8: BSF Larvae Weights

Container number	Larvae average weight (mg/larvae) (*n*=15 per container) (Day 0 = 49.4)			Two way ANOVA		
	Day 1	Day 4	Day 6	Diet	Day	Diet*Day
50%Veg/50% bran	46.10	137.30	164.30			
100% fresh carp	62.05	141.50	141.05			
100% aged carp	54.50	113.50	151.00			
50% Aged carp/bran	55.00	121.80	168.05			
Fresh 50% carp/50%bran	53.10	122.50	169.90			
ANOVA-P	0.910	0.463	0.360	0.664	0.000	0.200

There was no significant difference between moisture content, protein content, fat, ash and energy between the larvae from the different feed sources. With a few exceptions amino acid compositions also were similar across the different feed treatments. However the fatty acid profile for larvae varied significantly between treatments (Table 9). Of interest are the different concentrations of key omega-3 fatty acids Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) between treatments. Larvae fed the vegetable feed contained negligible quantities of EPA. Comparatively all larvae fed with carp containing feeds contained EPA in their fatty acid profile, with levels in the 50% carp treatments exceeded by levels in the 100% carp feed treatments. These preliminary results suggested that the fatty acid profile of BSF is influenced by the fatty acid profile of the feed. Optimisation of the feed composition could therefore yield high levels of desired fatty acids, even from a low quality, deteriorated feedstock. Such manipulation of the composition of the BSF larvae has implications for aquaculture feed application, and hence the influence of BSF larvae composition with feed source (particularly focussing on carp and other fish products) is being further explored by post-graduate aquaculture students at Curtin University.

Table 9: Chemical Analyses of BSF larvae

	Analyte	BSF 50% Vegetable/50% Bran	BSF 50% Fresh carp/50% bran	BSF 50% aged carp/50% bran	BSF 100% Fresh/aged carp (combined due to harvest issues)
Composition (g/100 g)	**Moisture**	71±1.00	70±0.00	70±0.00	72±1.00
	Protein	16.00±.70	16.80±.10	17.60±0.50	15.75±0.35
	Fat	9.30±0.30	9.05±0.05	8.45±0.55	8.75±0.55
	Saturated fat	5.15±0.05ab	6.35±0.45a	4.75±0.55b	3.85±0.15b
	Ash	2.2±0.00	2.05±0.05	2.25±0.05	2.25±0.35
	Total sugars	1.50±.10a	1.70±.10a	1.55±.05a	1.05±0.05b
	Carbohydrates	1.50±0.50	2±0.00	2±0.00	1.5±0.50
	Energy (kJ)	640.00±10.00	655.00±5.00	645.00±15.00	615.00±15.00
	Mono-trans fats	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
	MUSFA	1.70±0.00b	1.40±0.30b	2.00±0.00b	3.05±0.15a
	Omega 3 FA	0.15±0.00c	0.40±0.00bc	0.50±0.15b	0.95±0.11a
	Omega 6 FA	2.35±0.15a	0.85±0.05b	1.10±0.00b	0.80±0.10b
	Poly-trans FA	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00

	Analyte	BSF 50% Vegetable/50% Bran	BSF 50% Fresh carp/50% bran	BSF 50% aged carp/50% bran	BSF 100% Fresh/aged carp (combined due to harvest issues)
	PUFA	2.45±0.15a	1.30±0.10b	1.65±0.05b	1.80±0.20b
	Trans fats	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
Saturated Fatty Acids (% of total fat)	**C4:0 Butyric**	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
	C6:0 Caproic	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
	C8:0 Caprylic	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
	C10:0 Capric	0.10±0.00	0.10±0.00	0.15±0.05	0.10±0.00
	C12:0 Lauric	14.60±3.00	18.05±0.95	14.55±0.55	9.75±3.75
	C14:0 Myristic	11.15±1.15a	4.25±0.15b	6.55±1.05b	6.10±0.40b
	C15:0 Pentadecanoic	0.55±0.05	1.00±0.00	0.55±0.15	1.00±0.20
	C16:0 palmitic	16.00±0.50a	6.45±0.25b	14.15±2.15a	17.60±0.60a
	C17:0 Margaric	4.55±0.15b	31.30±6.10a	10.15±5.45b	3.60±2.5b
	C18:0 Stearic	4.00±0.20a	2.25±.65b	4.65±0.45a	3.90±0.20a
	C20:0 Arachidic	1.95±0.05ab	1.40±0.10ab	2.40±0.40a	1.30±0.35b
	C22:0 Behenic	2.65±0.15b	5.40±0.30a	3.3±0.50b	1.8±0.50b
	C24:0 Lignoceric	0.10±0.00	0.10±0.00	0.45±0.35	0.10±0.00
	Total Saturated FA	55.40±0.90b	70.15±4.55a	56.25±2.85b	44.45±1.05c
Mono-unsaturated fatty Acids (% of total fat)	**C14:1 Myristoleic**	1.00±0.00b	4.10±.40a	1.10±1.00b	0.55±0.35b
	C16:1 Palmitoleic	2.75±.15bc	1.35±1.25c	5.05±0.55b	10.20±0.20a
	C17:1 Heptadecanoic	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00
	C18:1 Oleic	12.95±.05b	5.60±0.50c	14.35±2.45ab	18.90±0.80a
	C18:1 Vaccenic (trans)	1.00±0.00c	3.45±0.75ab	2.55±0.05b	4.20±0.20a
	C20:1 Eicosenic	0.45±0.05	1.35±1.25	0.60±.10	0.75±.0.05
	C22:1 Cetoleic	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00
	C22:1 Docosenoic (Erucic)	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00
	C24:1 Nervonic	0.1±0.00	0.1±0.00	0.1±0.00	0.15±0.05
	Total Mono-unsaturated FA	18.20±0.20bc	15.70±3.4c	23.60±1.70b	34.70±0.30a
	C16:4 Hexadecatetraenoic	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.05
Poly-unsaturated Fatty Acid (% of	**C18:4 Morotic**	0.1±0.00c	2.15±0.05a	0.90±.10b	0.30±0.10c
	C18:2w6 Linoleic	12.00±.10a	6.05±0.15b	11.75±0.85a	6.35±0.15b
	C18:3w6 gamma-linoleic	0.1±0.00	0.1±0.00	0.1±0.00	0.15±0.05
	C18:3w3 alpha-linoleic	1.50±0.10a	0.80±0.10b	1.05±0.05b	1.05±0.15b
	C20:2w6 Eicosadienoic	0.1±0.00b	2.35±0.15a	0.80±0.40b	0.45±0.25b
	C20:3w6 Eicosatrienoic	0.1±0.00b	0.1±0.00b	0.1±0.00b	0.25±0.05a
	C20:3w3 Eicosatrienoic	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
	C20:4w6 Arachidonic	0.10±0.00c	0.60±0.50ab	0.70±0.10ab	1.60±0.00a
	C20:5w3 Eicosapentaenoic	0.10±0.00c	1.65±0.45b	2.60±0.40b	5.80±0.20a
	C22:2w6 Docosadienoic	0.1±0.00	1.1±0.50	0.1±0.00	0.10±.00
	Omega 3 Fatty Acids	1.50±0.10c	4.60±0.30b	6.20±0.50b	11.25±0.95a
	Omega 6 Fatty Acids	24.90±1.00a	9.50±0.85c	13.30±0.60b	8.85±0.35c

	Analyte	BSF 50% Vegetable/50% Bran	BSF 50% Fresh carp/50% bran	BSF 50% aged carp/50% bran	BSF 100% Fresh/aged carp (combined due to harvest issues)
	C22:4w6 Docosatetraenoic	12.90±0.90a	0.1±0.00b	0.1±0.00b	0.15±0.05b
	C22:5w3 Docosapentaenoic	0.10±0.00c	0.10±0.00c	0.50±.10b	0.90±.10a
	C22:6w3 Docosahexaenoic	0.1±0.00c	0.1±0.00c	1.15±0.05b	3.15±0.45a
	Total Poly-unsaturated FA	26.40±1.10a	14.15±1.15c	19.55±1.15b	20.25±1.25b
	Sodium (mg/100 g)	22±1.00	22±0.00	22.50±1.50	31.50±5.50
Amino acids (mg/kg)	**Aspartic acid**	10400.00±600	10300±700	10500±500	8400±1000
	Serine	5600±600	5950±350	5850±150	4700±0.00
	Glutamic acid	10750±1250	10000±0.00	11000±0.00	9950±1050
	Glycine	6400±700	7150±750	6650±150	5900±400
	Histidine	5150±550	5400±500	4950±250	4850±450
	Arginine	6750±450ab	7600±500a	6650±150ab	5750±50b
	Threonine	5350±450ab	5600±100a	5350±150ab	4600±100b
	Alanine	7250±450	7000±100	7550±150	6200±300
	Proline	9150±500ab	8850±150ab	9500±200a	7850±450b
	Tyrosine	7900±800	9200±800	8300±500	7100±500
	Valine	5950±650	6450±50	6450±250	5250±50
	Lysine	10950±1050	10450±550	10500±500	9000±1000
	Isoleucine	5050±450ab	5600±100a	5150±150ab	4550±50b
	Leucine	9000±700ab	9600±200a	9050±250ab	7850±150b

Different letters in the same columns represent different results by Tukey test ($p < 0.05$).

Two BSF treatment containers were chosen for analysis of the residual frass (extruded digestate produced by the larvae during growth) (Table 10). The 50% fresh carp/50% bran frass contained more protein (19.5 g/100 g) than the sample of frass from a 50% vegetable/50% bran treatment (11.8 g/100 g). This was anticipated given the higher protein content of carp compared to vegetable matter. Frass is being investigated elsewhere for potential for soil amendment/fertilisation (FGS pers. comm.).

Table 10: Chemical Analysis of BSF Frass

Sample	Vegetable frass	50% fresh carp mince frass
Moisture (g/100 g)	44.2	31.2
Protein (g/100 g)	11.8	19.5
Fat (g/100 g)	2.5	3.4
Ash (g/100 g)	4.7	5.2

Despite the experimental issues, the feeding of carp to BSF has produced promising results in regards to the nutritional quality of the larvae. The next stages at larger scale (Section 4.3.3) were designed to include experimental modifications to reduce contamination, improve harvesting success and trial alternative feed compositions. It is acknowledged that the fresh and 48 hour aged carp may not be representative of deceased carp removed from waterways. Also larvae fed on dead carp from waterways

may cause a risk to and for users by transferring excessive amounts of bacteria to the animal being fed. Microbiological testing is therefore advised as the work progresses.

4.2.5 Raw Pet Food

1kg packs of vacuum packed carp mince were produced for the HPP trials. The 1kg packs were subject to pressures of 250, 350, 500 and 600 pounds per square inch (psi) for 3 minutes.

The HPP treatment changed the appearance of the carp mince as is clear from the photographs shown in Figure 26 below. The differences were most apparent at 500 and 600 psi, the red colour changed to brown, indication of changes to the protein structures under the higher pressures.

Control (no pressure)

250 psi

350 psi

500 psi

600 psi

FIGURE 26: CARP MINCE AFTER DIFFERENT HPP TREATMENTS.

Fresh carp mince at 250, 350, 500 and 600 psi and the control was subject to bacteriological assessment on Day 5 after treatment (or Day 8 for the control). Results are shown in Table 11. The control levels were microbiologically unsatisfactory for use as raw pet food, however the HPP produced a clear and significant log reduction in bacterial numbers, particularly at 500 and 600 psi (see Table 11).

In addition the 600psi treatments were held chilled for 20 days and the bacteriological results assessed. BY Day 20 after 600 psi treatment bacteriological levels were still below the control levels (see Table 11).

Table 11: Total Plate Count (TPC) for Control and HPP treated carp.

Treatment	Day 5 (TPC cfu/g)	Day 8 (TPC cfu/g)	Day 13 (TPC cfu/g)	Day 20 (TPC cfu/g)
Control		4.4×10^8		
250 psi	4.3×10^8			
350 psi	2.8×10^8			
500 psi	1.4×10^5			
600 psi	330		1.2×10^5	4×10^6

cfu (colony forming units/g)

The high microbiological numbers as well as the possible issue of nutritional problems associated with the presence of thiaminases in carp, made the use of the untreated minced carp as raw pet food not feasible. Whilst the HPP results in regard to bacterial load reduction (and likely deactivation of thiaminases) and therefore shelf-life extension were promising, the commercial HPP facility at which initial trials were undertaken was closed in March 2018, preventing any further experimentation. It was therefore decided to cease any further work on the pet food utilisation option.

4.2.6 Anaerobic Digestion of Carp Wastewater

The anaerobic digestion laboratory scale trials undertaken by Elise O'Keefe at the Goulburn Valley Water facility are summarised in Appendix 2. Due to the complexity of the experimental reporting, readers are directed to the report for the detailed results. The general discussion and conclusions are reproduced below.

Discussion

Sub-research Question 1: What is the relationship between the ratio of liquid fish waste to wastewater and Biological Oxygen Demand (BOD)/Carbon Oxygen Demand (COD) reduction, suspended solids, volatile suspended solids and volatile fatty acids within the digestion process?

BOD reduction results were inconclusive as the final samples were observed to have a significant amount of suspended solid material which will have increased the overall BOD of the sample. This can be attributed to the small reactor, and thus sample, size. When the reactors were settled and decanted, in an effort to gain enough sample to complete the required analyses, an amount of solids material ended up in the final sample. In futures similar studies, it is recommended that a filtered BOD test be performed, particularly if the process in question involves solids settling before discharge.

Prior to undergoing anaerobic digestion, a linear relationship was observed between volatile fatty acids (VFA) concentration and concentration of fish liquid, this relationship was not observed in the final samples. This was considered unusual and, on reflection, may be attributed to inconsistent sample representation, as discussed above. If the relationship between suspended particles and VFAs is similar to that of suspended particles and BOD, the inclusion of a higher volume of such particles in Test 2 and Test 4 may explain their inconsistency with the other three samples. This is further supported by the observation of a similar pattern in the BOD results.

Ammonia levels in all test reactors showed a significant increase compared to that of the control, however, all were below the inhibitory values of 1700 – 1800 mg/L described by Yenigün and Demirel (2013). Ammonia is generated as a by-product of anaerobic digestion from when proteins are broken down in the process (Akindele and Sartaj 2018). Ammonia is a known inhibitor of anaerobic digestion, however, the biogas yields observed in the test rectors suggests that ammonia had not reached levels that would inhibit methanogenesis.

Alkalinity was maintained in all reactors via the addition of magnesium hydroxide, which was required due to the low alkalinity of the initial influent sample. On reflection, and considering the continuous flow nature of the Shepparton HRAL, it would be beneficial for future study to complete the same experiment with fish liquid waste, influent sample, and a sample from the contents of the HRAL, all representative of the operating system. This would be more representative of the actual system, and, theoretically, assist in stabilising pH.

Suspended solids and volatile solids were unable to be analysed in the final samples due to lack of sample volume, thus the relationship between fish liquid: wastewater ratio and these parameters was not able to

be investigated. Future studies of similar nature should consider the sample size required to thoroughly investigate the water quality, and size the reactors generously to accommodate the required sample size.

Sub-research Question 2: What is the relationship between the ratio of liquid fish waste to wastewater and biogas yield?

A higher biogas yield was observed in all reactors when compared to that of the control reactor. This can be attributed to the increased availability of energy rich molecules as described by Ahring 2003. These results indicate that there is a benefit to the bio digestion of fish waste in that an increased biogas yield, and economic incentive, may be achieved. A linear relationship in the total biogas yield was not observed between fish liquid concentration and biogas generation. However, biogas generation appeared to respond significantly when adjustments were made to alkalinity and pH within the reactors, suggesting that maintaining pH and alkalinity is crucial to biogas production. Given that the fish liquid waste provides high energy feedstock for biogas production, and that an increase in biogas yield was observed in all test reactors compared to that of the control, it could be concluded that an increase in high energy fish liquid waste leads to increased biogas yield. Based on this theory, a linear relationship between percentage of fish liquid waste and biogas yield could have been hypothesised, however, was not observed in this experimental study, possibly due to poor alkalinity control.

Conclusions.

The results observed in this study, suggest that fish liquid waste can be treated via anaerobic digestion and improve biogas yield of the Shepparton WMF HRAL. However, given the high strength nature of the fish liquid waste, several parameters need to be considered and managed in a full scale operation, including VFA: Alkalinity ratio, pH and ammonia. The combining of fish liquid waste with municipal sewage wastewater provides the benefits of dilution which will assist in maintaining VFA: Alkalinity ratios and ammonia at levels low enough to avoid inhibition of the anaerobic digestion process.

It should be noted that dilution ratio is important if there is a limited retention time in the reactor, as is the case with the continuous flow HRAL in Shepparton, as increased loadings generally require increased reaction/retention time. Alkalinity control via chemical addition is recommended in a full scale operation to maintain a Volatile Fatty Acids (VFA)/Alkalinity ratio within the specified ‘safe’ ratio of .1-.3.

Due to uncontrolled and limited retention time at the Shepparton HRAL, a 5% dilution or less is desirable to increase biogas yield whilst still achieving treatment targets.

The results of the laboratory study undertaken by Elise O’Keefe were intended to be used for the proposed larger scale anaerobic digestion trials summarised in Section 4.3.2.

4.3 Small Scale Semi-Commercial Trials (<10 tonnes)

4.3.1 Fermentative Hydrolysis

The results of the fermentative hydrolysis pilot scale trials are summarised in Appendix 3. A portion of that final report is reproduced below. The pilot trial was successful with hydrolysate produced, harvested and tested. Figure 27 shows the inoculant, Figure 28 the experimental set-up and Figure 29 the final product.

FIGURE 27: INOCULANT PRODUCTION

FIGURE 28: EXPERIMENTAL SET-UP

FIGURE 29 PRODUCT MONITORING

pH and Temperature

The pH and temperature were recorded periodically. Results are below in Table 12. pH reduced with retention time.

Table 12: Temperature and pH of Carp Biomass during Fermentation

Date	Product	Ambient Temp	Liquid Temp	pH litmus	pH Manutec
June 5	Carp	10˚C	9˚C	6	6.5
	Food	10˚C	14˚C	6	6.5
June 9	Carp	9˚C	9˚C	5	4.5
	Food	10˚C	14˚C	5	5
June 19	Carp	12˚C	18˚C	5	4.5
	Food	12˚C	14˚C	4	4.5
June 29	Carp	10˚C	9˚C	4	4
	Food	12˚C	14˚C	4	4

Microbiological Monitoring

The results of the microbiological monitoring are provided in Table 13 below. This data was important as the maceration of carp carcasses can be expected to liberate microbes from the fish gut. During hydrolysis and anaerobic fermentation, elevated temperatures should reduce or eliminate these microbes.

Table 13: Concentrations of FaecalCcoliform Units and *E.coli* Prior to and after Processing Macerated Carp by Hydrolysis and Anaerobic Fermentation

	Unit	Replicate 1	Replicate 2	Replicate 3
Pre-processing				
Presumptive*				
Thermo-tolerant faecal coliform	cfu/100ml	200	180	280
Confirmed**				
Thermo-tolerant faecal coliform	cfu/100ml	120	180	280
E.coli	cfu/100ml	120	180	280
Post processing				
Presumptive*				
Thermo-tolerant faecal coliform	cfu/100ml	<100	<100	<100
Confirmed**				
Thermo-tolerant faecal coliform	cfu/100ml	<100	<100	<100
E.coli	cfu/100ml	<100	<100	<100

* Mathematically estimated number of viable microbes by conducting a series of dilutions, then plating and incubating for a standard of period time and temperature.
** Determined either by counting after dilution, plating and incubation or a direct plate count.
cfu (colony forming unit)

The results need to be assessed against Table 14, summarising the Australian standards for irrigation waters.

Table 14: Threshold values for thermotolerant coliforms in irrigation waters used for food and non-food Primary production

Intended use	**Concentration of thermotolerant faecal coliforms**
Raw human food crops in direct contact with irrigation water (e.g. via sprays, irrigation of salad vegetables)	<10 cfu / 100 mL
Raw human food crops not in direct contact with irrigation water (edible product separated from contact with water, e.g. by peel, use of trickle irrigation); or crops sold to consumers cooked or processed	<1000 cfu / 100 mL
Pasture and fodder for dairy animals (without withholding period)	<100 cfu / 100 mL
Pasture and fodder for dairy animals (with withholding period of 5 days)	<1000 cfu / 100 mL
Pasture and fodder (for grazing animals except pigs and dairy animals, i.e. cattle, sheep and goats)	<1000 cfu / 100 mL
Silviculture, turf, cotton, etc. (restricted public access)	<10 000 cfu / 100 mL

Adapted from ARMCANZ, ANZECC & NHMRC (2000)

cfu: colony forming units

It should be noted that the NSW recommended standard for E. coli in waters that may be applied to food crops with edible skin or that may be eaten uncooked is <126cfu/100ml (NSW DPI, 2017), suggesting that there may be some inconsistency, if the National guidelines are not the point of reference.

The results show that the potential use of fish waste, derived from feral carp, as a liquid foliar fertiliser generally should not pose a risk to human health, if used on food crops.

Project Summary

The results of this project indicate that fermenting carp biomass using a simple field based hydrolysis technique has the potential to create a biofertiliser/biostimulant product that can be used in agriculture. At the same time this method can help solve a significant environmental hazard by safely removing carp biomass from river systems and landscapes.

Results also show that the carp hydrolysis product has safe levels of indicator pathogens. In Lactobacillus-based fermentation the lowering of pH is a key regulator of pathogens in this process. *(Beavis 2014).* The exception is the levels for raw human food crops with which it has direct contact, however, given that the product would be applied at a 1:100 dilution, the results of this pilot trial indicate that use as a foliar fertiliser would not be a risk to human health, due to concentrations being well within the relevant guidelines. Therefore, undiluted, the product meets the relevant national water quality guidelines for water being applied to food and non-food crops,

4.3.2 GVW, Veolia and Western Composting: Anaerobic Digestion and Composting

Based on the analysed moisture content of the fish being 75%, it was expected that separation of the fish into solid and wastewater components at the Veolia facility would produce a 65% liquid/35% solids recovery rate.

Trial 1

Approximately two tonnes of carp were transported to the Veolia facility for trial in an operational sewage separation unit. Maceration of the fish was also attempted using the Western Composting large scale shredding machine. Both trials were unsuccessful in separating the carp into solid and liquid components. Therefore all fish waste was transferred to the Western Composting "tunnel" system for compost production. Anaerobic digestion trials on the carp liquid wastewater were therefore not able to be undertaken.

However two tonnes of carp were delivered to Western Composting facility. Technical details were: Batch T224048, >7 days in tunnel 2 and ~70^{o}C, and a summary of the composting process by the company operators is reproduced below.

The average temperature inside the tunnels was approximately 70^{o}C over seven day's retention time. As expected, there were bones remaining after pasteurisation, however, most disintegrated after seven days (see Figure 30). There were also some small pieces of meat that remained after pasteurisation, but operators expect this to breakdown completely during maturation. This was the first stage of a six to eight week process, final product was reported to meet Australian standard 4454-2012 for compost. Although it is acknowledged that the quality of fish delivered was high and deceased fish from waterways may have a different outcome, informal discussions with the composting company indicated processing of lower quality fish would still be possible.

FIGURE 30: CARP AFTER 7 DAYS IN WESTERN COMPOSTING COMPOST TUNNEL

Trial 2

Due to the separation issues identified in Trial 1, in the second trial an initial mincing step was added to the protocol. Approximately 2 tonnes of carp were therefore transported to the Daldy Road Knackery, where it was intended that the carp would be minced, left standing to enable dewatering to occur, then the liquid would be transported to the Veolia, GVW and Western Composting site for further separation, with liquid wastewater for anaerobic digestion and the remaining solids for composting.

1.3 tonnes of carp were delivered to the Daldy Road Knackery mincer in tubs. The fish was efficiently minced in around 40 minutes (see Figure 31) and stored in two 1000L cut-down International Bulk Container (IBC) units (Figure 32). Upon mincing and standing, approximately 40% liquid was produced. Due to a delay in the fish being delivered, the mincing occurred late on Friday. Therefore under instructions

from the industry partner, as the facility did not operate on the weekend, the containers were left at the site and on the following Monday morning the two containers were transported to the Veolia site for separation. An attempt was made to free drain the liquid through the valve of the IBC, this did not work effectively as liquid would not drain – it appeared to congeal wit standing, so all content was sent for composting (0.28T)

FIGURE 31: MINCING

FIGURE 32: MINCED PRODUCT STORED IN IBC

The findings from Trial 2 were summarised by Steve Nash, GVW as below:

- To free drain liquid need to do so immediately after mincing
- Following mincing still need some forced separation into liquid and solids, even with prior mincing, as did not drain well.
- Minced fish disappeared into compost upon mixing very quickly – should break down promptly
- Mincer very effective – once mincer unit installed properly would be labour free
- Need to minimise human contact as fish will arrive in varying degrees of decomposition – product is difficult to load and transport, will likely need lined containers set up to enable fish to be tipped into and then able to be tipped into loader for mincer unit.
- IBC Containers need lids and fork lifts need to be able to rotate the containers, for collection and distribution, to minimise human contact.
- IBC units need to be modified to separate liquid and meal, a proposed designed has been scoped.

Trial 3

A third trial was conducted with 1-2 tonnes of fish through the mincer.

In this case the IBC containing the mincer was agitated and draining of liquid occurred immediately. Packages of potential fish burley were also produced from the solids, with intent to run a recreational fisher trial with the burley, however on discussion with the NCCP Manager, these packages were destroyed.

Finding from Trial 3 are summarised below:

- The liquid comes out continually if allowed to drain and agitated – we currently estimate agitation and draining could separate 40% mass by draining liquid.
- We need to be weighing the fish more frequently during the process, such as upon delivery, post mincing, after draining, etc.
- Liquid could have liquid agricultural fertiliser applications

- We need to develop an agitator which can move the minced carp to enable the liquid to be drained over a period of time, estimated to be 2-6 hours. To estimate the effectiveness, the liquid could be captured and measured, or the carp weighed pre, during and post draining. The process used to date is still to labour intensive, and I have attached a sketch of the device that may assist.
- The fish liquid could be discharged into GVW WMF as part of WMF process or used as liquid fertilizer for orchards. – should this be investigated in future and on larger scale?

Despite the commitment of the GVW staff, due to the operational challenges, particularly due to issues in separation of the fish to produce wastewater able to be added to the anaerobic digestion process, GVW trials were thereafter ceased. Nonetheless, following discussions with the GVW staff, estimated costs/recoveries etc from the proposed process were inputted into the CBA (See Results Section 4.5.3).

4.3.3 Carp as a Feed Source for BSF Larvae

Due to the temperature, contamination and harvest issues of the BSF larvae culture trial at Curtin University (Section 4.2.4), the pilot scale-up trial for the BSF culture was sub-contracted to FGS, a semi-commercial operator for BSF larvae production.

Growth Trial Results

The average larvae weights are shown in Table 15.

Table 15: Individual BSF Larvae Weights

Feed	Larvae Weight (g) *			Larvae length (mm)			Larvae width (mm)		
	Day 0	Day 4	Day 7	Day 0	Day 4	Day 7	Day 0	Day 4	Day 7
100% carp	0.041	0.103	ND (anoxia)	11.4	14.9	ND (anoxia)	3.4	3.9	ND (anoxia)
70% Carp	0.043	0.122	0.168	11.8	15.9	17.97	3.43	4.27	4.43
Control	0.046	0.127	0.157	12.4	15.7	17.13	3.3	4.1	4.03

*Average of 20 larvae from each of 3 different tubs.

The total moisture of the 100% carp diet resulted in a substrate that was too wet, and the tubs rapidly became anoxic. This trial was therefore aborted on Day 4. At this stage, the average weight per larvae for 100% carp diet was 0.103g compared to 0.127g and 0.123g for the control and 70% carp diets respectively (see Table 14). This low growth rate, in combination with the larvae exiting the substrate, indicates that the 100% carp diet was not suitable (in its current form) for BSF larvae production.

By day 7 of the trial the average weight per larvae had increased to 0.157g and 0.168g for the control and 70% carp substrates respectively. Therefore, the greatest weight gains were by those larvae raised on the 70% carp substrate. Larvae raised on the 70% carp diet also appeared more 'plump' and 'oily' than those raised on the control substrate. It is worth noting that although the greatest growth weights were achieved in the 70% there were growth and harvesting operational issues with this substrate as described below.

The substrates were prepared based on the current FGS control diet of mixed vegetables and waste pelletised feed. This control diet, at 60% moisture, maintains a 'fluffy' texture during and post digestion by BSF Larvae (BSFL) and is easily separated from larval cultures using a mechanised sieve. For this reason, the mixed 70% carp diet was also prepared to 60% moisture by incorporation of pelletised waste (7.14% moisture) into the minced carp (74.54% moisture). Despite the moisture content remaining the same for the control and 70% carp, the physical properties and digestibility for each substrate were markedly different. The 70% carp substrate formed a dense layer which appeared difficult for the BSFL to 'churn' and digest. As a result, the BSFL formed a dense layer beneath the substrate which was left mostly

undigested. This diet was also virtually impossible to mechanically separate via sieving action. Separation for both the 70 and 100% carp diet was achieved by a combination of manual removal and washing of larvae with water through a 3mm sieve screen.

Due to the inefficiency of harvesting the total final harvest rates (Table 16) are not representative of the actual weight of larvae reared on each substrate. Therefore, a theoretical harvest amount was calculated using the following formula:

Theoretical harvest weight = stocking density x average weight per larvae
Further to this a harvest efficiency (% harvest) could also be calculated by:
% harvest = (100/Theoretical harvest) x Actual harvest

Table 16: Harvest Results

	Actual Harvest^(g)	**Theoretical harvest^(weight x stocking density) (g)**	**Harvest efficiency^ (%)**
100% carp#	393.33	1216	31.8
70:30 Carp	1535	2011.3	76.3
Control	1611.7	1882.3	85.57

^Average of three tubs per treatment # 100% carp harvested Day 4, others Day 7.

It appears from the preliminary trial with carp that the suitability of substrate for BSF larvae digestion and mechanical separation is dependent on many factors; rather than percentage moisture alone.

In order to amend the whole waste carp into a more suitable substrate for BSF larvae several options could be investigated. These may include:

- Dewatering of whole carp prior to mincing.
- Dewatering of whole carp post mincing.
- Mechanical removal of remaining water via a press.
- Addition of dry substrates pending resulting moisture content and physical appearance of dewatered and pressed carp.

As mentioned in Section 3.3.4: BSF larval product from this trial was subject to processing and drying trials and then used successfully as a protein source at various levels in two juvenile barramundi feeding trials and one marron feeding trial undertaken by Curtin University Masters of Sustainable Aquaculture students, postdoctoral scientists and PhD students. Some of this work has been now accepted for publication in a peer reviewed journal article (Chaklader, M.R., Siddik, M.A.B., Howieson, J.R. and Fotedar, R. (2019) Insect larvae, (2019) *Hermetia illucens* in poultry by-product meal for barramundi, *Lates calcarifer* modulates histomorphology, immunity and resistance to Vibrio harveyi. Scientific Reports (in press)), with three other journal articles in preparation, these documents can be made available to interested readers of this report. A new batch of BSF larvae cultured on carp has been produced and post-graduate student aquafeed trials will continue using this product at least until 2022.

4.3.4 Vermicast Production

The informal observations from the worm tea/vermicomposting trials suggested that there was no perceivable odour from the treatments after having been in the open air for many days, especially when compared to the untreated control. The addition of the worms/worm tea resulted in decomposition to

produce vermicast (high grade compost). Worms were also produced (see Figure 33). An observation was that an extra carbon source (saw dust) was needed to increase the total carbon content and therefore decrease the high nitrogen content from the fish.

In a community context, the facility operators suggested that the fish from a fish kill could be mixed with a carbon source (e.g. community clippings/trimmings from council activities) and then worm tea and worms added. Resulting vermicast (high grade compost) could be used for community or farm composting (at no cost/not sold) and the worms also given away or retailed. These options are further described and costed in Section 4.5.2.

FIGURE 33: WORMS FROM CARP TRIALS.

4.4 Large Scale Semi-Commercial Trials

4.4.1 Enzyme Hydrolysis

The 10 tonne enzyme hydrolysate trial was conducted at the SAMPI facility in Port Lincoln. The process is shown below in Figure 34: whole fish before processing; mincing; heated reaction tanks; sieving; liquid hydrolysate product and bones/scales remaining after process. The trial parameters were adjusted on the advice of the SAMPI operational staff.

Whole fish

Mincing

Heated reaction tanks

Sieving equipment

Finished hydrolysate

Bones/scales following separation

FIGURE 34: STAGES IN THE ENZYME HYDROLYSATE TRIAL

A video of the process has also been produced and is available for viewing by interested readers.

The trial was successful, carp hydrolysate was produced, results from the three sets of thawed carp are summarised in Table 17.

Table 17: Summary of Results from SAMPI Enzyme Hydrolysis Trial.

Parameter	Tank 1 (thawed 48 hours prior)	Tank 2 (thawed 24 hours previously)	Tank 3 (added frozen)	TOTALS
Carp weight (kg)	3332kg	3019	3237	9588
Water added (L)	1000	1400	1500	3900
Enzyme added (kg)	2	2.5	3	7.5
pH at sieve	5.54	6.35	6.7	
Acid added (L)	60	90	105	225
Final pH	2.9	2.8	3	
Hydrolysate (kg)	2919.1kg (2780L)	4257.2 kg (4000L)	4726.4kg (4450L)	11902.7 (kg) (11,230L)
Bones (kg)	575	472.4	485	1442.4

Operational Comments from the factory manager

- Water needed to be added during the mincing process to ensure the carp mince could be pumped through the system. Water is not generally added to the process for the tuna processing.
- The entire hydrolysate production system worked well with the carp, with the reaction time and final product appearance being similar to the processing to produce tuna hydrolysate.
- The bones/scales were separated from the hydrolysate and alternative options are being investigated. At the moment the bones/scales are either going for composting or to increase fertility on local farms.
- The hydrolysate was stable after two month storage in the IBC's. The operator reported no separation, calcium lumps, bloating or smell.
- Some of the product has been mixed with the tuna hydrolysate product with the potential for use for large scale fertiliser addition. The carp hydrolysate was pumpable and could be used successfully in this scenario.

Compositional Results

Carp enzyme hydrolysis compositional results are shown in Table 18.

Table 18: Chemical Analysis of Carp Hydrolysate

	Analyte	Carp Hydrolysate Tank 1	Carp Hydrolysate Tank 2	Carp Hydrolysate Tank 3
Composition (g/100 g)	**Moisture**			
	Protein	6.5	6.1	7.9
	Fat			
	Ash			
Metals (mg/kg)	**Arsenic**	<0.1	<0.01	<0.01
	Cadmium	0.023	0.017	0.01
	Calcium	670	660	600
	Cobalt	0.027	0.021	<0.02
	Copper	0.2	3.8	1.8
	Iron	19	18	13
	Lead	<0.03	0.15	0.07
	Magnesium	150	97	96
	Manganese	0.27	0.25	0.19
	Molybdenum	<0.05	>0.05	<0.05
	Phosphorus	11000	15000	13000
	Potassium	1700	1400	1300
	Selenium	0.29	0.3	0.27
	Sodium	810	640	540
	Sulphur	950	1100	1100
	Zinc	17	20	

Comments from the SAMPI marketing staff.

- Fertiliser markets are likely to be available, maybe up to $1/L.
- Aqua feed markets may also be available but due to different oil levels and composition the return is likely to be less than the current tuna hydrolysate price for aqua feed of $1.10 to $1.20/L. Prices of $0.5 to $0.8/L are likely, particularly with the larger volumes likely to be produced. It is noteworthy that a prominent aqua feed company has requested samples of the carp hydrolysate product for trials.
- The impact of the “Virus infected carp products” on the various markets needs to be ascertained. CSIRO virologists have suggested that the low pH will render the virus inactive however this needs to be clarified.
- The market and prices may be affected by the decrease in hydrolysate quality associated with decomposition.

These comments were provided to the consultant, which along with further direct consultation were used as the basis for the CBA (Section 4.5.3).

At the request of a major aquafeed company the carp hydrolysate produced at the SAMPI facility was provided for experimental trials. Results from this trial have not been provided due to confidentiality issues. However PhD's students at Curtin are currently analysing the results from the use of the carp hydrolysate in juvenile barramundi and marron feeding trials. In this case the carp hydrolysate sample was dried before incorporation into feed, the proximate analysis results showed 59.01% crude protein, 12.89% crude lipid, 0.73% moisture and 46.21% ash. Peer reviewed journal articles are in development using results from completed trials and further trials are being planned. This work will be ongoing until 2022. Interested readers can request copies of these documents from the PI.

4.4.2 Composting

The results from the large scale composting trial undertaken at Camperdown Composting are discussed in detail in Appendix 4, with some sections reproduced below. The trial operators also provided significant feedback to the consultant undertaking the CBA (Section 4.5.3).

The four treatments (comprising one row each) to which the fish waste was added were:
1. Compost
2. Compost plus sawdust blend (50:50)
3. Sawdust
4. Straw

Odour
Odour levels were monitored throughout the composting process by Ektimo to provide information on each of the treatment's effectiveness in minimising the odours from the decomposing fish. This was particularly important to determine effective separation distances between potential composting locations and sensitive receptors. Three of the composting blends (the straw blend wasn't monitored due to early termination) had their odour levels monitored at three different stages of the composting process. All the composts were 2-3 weeks old when odour monitoring was commenced. The compost/sawdust blend was also monitored after the third turning at six weeks of age.

The results indicate that the compost blend was the most effective treatment at supressing the odour of the decaying fish after the turning process had been completed. The sawdust blend was just as effective at supressing odour as the compost blend initially however once the pile became disturbed during the turning process the odour levels were slower to reduce back to previous levels. The compost/sawdust blend released the most odour during the first turn. However, odour levels dropped quickly post-turning and after 6 weeks of composting the odour being produced by the compost/sawdust blend had decreased dramatically.

The compost only and sawdust only blends were treated with a *Lactobacillus* culture to help reduce odours as this was claimed to be successful in previous composting trials. It would appear that the *Lactobacillus* treatment was successful in reducing odours as the compost only and sawdust only blends had substantially better readings than the compost/sawdust blend during and immediately post-turning.

Throughout the trial temperature levels were monitored to track the progress of the composting process and to indicate each treatment's relative effectiveness at decomposing the fish carcasses.
The compost/sawdust blend was the quickest at reaching effective compost temperatures followed by the sawdust blend. The sawdust blend was slower than the compost / sawdust blend in reaching higher temperature, however it had the highest recorded temperatures for any blend. The straw blend was the least effective treatment at reaching critical temperature levels needed for composting process to work effectively.

Leachate

Leachate emissions were monitored to determine the effectiveness of each blend in ensuring leachate from the composting process did not affect the surrounding environment. This is important as one of the aims of this trial was to determine if composting of dead fish is achievable on-farm in areas with permeable subsoils. The compost and compost/sawdust blends were the most effective at controlling leachate levels. The sawdust blend didn't absorb leachate as effectively as the two composted blends, due to the relatively high moisture content of the sawdust and consequent low absorbance. Optimising moisture content of all co-composting materials is important to ensure good leachate capture. It is expected that sawdust would provide just as, or more, effective leachate control with lower starting moisture content. The straw blend failed to capture leachate due to its waxy cuticle and low absorbency.

Laboratory Testing

Compost and compost input samples were analysed at SESL's NATA-accredited laboratory. The fish frames and whole fish were analysed to determine their potential to provide sufficient nutrients to the composting process. The finished compost, compost/sawdust and sawdust blends were tested against the AS4454 for composts, soil conditioners and mulches to assess compliance.

The compost blends were also analysed to determine their nutrient status. Full laboratory results are provided in Appendix 4.The fish frames and whole fish had very high levels of macro nutrients making them very suitable inputs to composting. Sodium, chloride and zinc levels were slightly elevated, however this was offset by blending with co-compost materials which have low levels of these elements. Trace elements were generally low except for iron.

The compost blend passed all criteria under the AS4454 except for moisture content and toxicity. The moisture content was only slightly elevated and can be controlled by allowing the compost to mature for longer. The toxicity result reflected that the compost hadn't reached full maturity as also indicated by the high ammonium levels. Additional time would see the high ammonium convert to nitrate with an expected improvement in the toxicity result. The compost blend had acceptable to high levels of all macro nutrients and good levels of trace elements. Chloride levels were high but sodium levels were low. This blend had a good C: N ratio, acceptable levels of organic matter, a slightly elevated EC and a slightly alkaline pH.

The compost/sawdust blend passed all criteria under the AS4454 except for toxicity. Again, the toxicity result indicates that this compost simply needs more time to reach maturity. The compost/sawdust blend had acceptable to high levels of all macro nutrients and good levels of trace elements. Chloride levels were also high but again sodium levels were low. This blend had a good C: N ratio, high levels of organic matter, an acceptable EC and a pH that was close to neutral.
The sawdust blend passed all criteria under the AS4454 except ammonium levels, proportion of large particles, toxicity, cadmium, copper and zinc. To remove larger sizes of sawdust this blend would have to be put through a 5mm sieve to ensure compliance with the standard. The levels of ammonium and toxicity result show that this compost is not yet fully mature. The sawdust itself is likely the source of the contamination, particularly in the case of the elevated copper levels as this is used in some timber treatments. The sawdust blend had acceptable to high levels of all macro nutrients and good levels of trace elements. Chloride levels were also high but not as high as the other treatments. This blend had a poor C: N ratio which is to be expected due to the nature of sawdust and high levels of organic matter. The finished product had an acceptable EC and a pH that was close to neutral.

Discussion

Composting process

Throughout the trial it became apparent that the straw blend is not suitable for this composting process. It didn't capture odour or leachate effectively and was difficult to turn during the composting process even

for experienced operators. It is also relatively expensive and will be in high demand for other agriculture purposes because of the ongoing drought affecting regional Australia.

The sawdust blend showed that it could be effective in composting large quantities of fish carcasses however it was not without its drawbacks. Odour levels were more noticeable on this blend than the two compost blends. Testing showed that this can be managed with application of a *Lacto bacillus* treatment, however this may increase the cost of composting. Testing against the Australian standard showed that the sawdust blend was contaminated with high levels of cadmium, copper and zinc. As the two compost blends didn't have these issues it is likely the source of contamination is from the sawdust itself rather than the fish carcasses. The contamination in the sawdust is possibly from a small proportion of treated timber in the sawdust. The sawdust blend would also have to be sieved to remove large pieces of timber to meet the Australian Standard and market expectations. The finished product looked like dirty sawdust which may affect marketability.

However, if the issues of potential contamination can be managed, i.e. if sawdust is sourced from mills with no risk of treated timbers entering the supply chain, there are potential advantages in using sawdust. Sawdust – unlike the other co-composting materials used in this trial – has a very high starting carbon to nitrogen ratio. This means composting using sawdust requires much higher levels of nitrogenous materials (i.e. fish) to satisfactorily compost. However, it is not simply a matter of adding a lot more fish to the blend as the sawdust would be slow to compost. It does open the possibility of repeated composting of the compost / fish blends. It is likely that having finished the first round of composting, the sawdust / fish blend could be blended with the same quantity of fish for a second, and potentially up to 4-5 rounds of composting. This would result in lower cost and a higher quality product after multiple composting events. This could make sawdust the preferred co-composting material where pulses of fish might be expected from river systems over a period of weeks or months.

The mature compost blend was effective at processing large quantities of fish carcasses. It had the least amount of odour; the finished product had a high nutrient value and was free of contamination. The finished product looked good which is important for marketability. This blend showed the best potential for areas that have sensitive receptors close by as it was the most effective blend at suppressing odour. However, temperatures were slow to reach optimal. This can be managed by increasing the proportion of fish in the starting blend to a 1:1.5 fish to compost ratio.

The compost/sawdust blend was the most effective at processing large quantities of fish as higher temperatures were achieved quickest during the composting process. The finished product has high nutrient value and looks good. The blending of compost and sawdust alleviated the contamination issues associated with the sawdust blend. This blend has the greatest potential to remove the largest numbers of fish, however it produced more odour so may be less suitable in areas with sensitive receptors nearby.

Turner

This composting trial showed that a purpose-built compost turner is effective at managing the composting process. Tractor-driven turners are generally available in rural areas, can be moved between properties quickly and have the ability to turn up 900m3/hr of compost. A consistent end product can be produced without the need for high levels of training associated with more specialised turning equipment.

Conclusion

This trial has shown that there is good potential for composting to form part a multi-faceted management strategy to tackle the huge numbers of fish that will be produced if the carp herpes virus is released. With some modifications, the composting process has the ability to pasteurise the biomass to minimize biosecurity issues, produce a product with suitable nutrients for land application and there is a ready-made

market for the end product. The methodology set out by this trial has the ability to be rolled out to remote areas with appropriate training.

This report has not addressed the logistics of rolling-out on-farm composting across large areas. However, the managers of this trial have considered the matter at depth and a sound conceptual working model has been developed. A cornerstone of this model involves the use of Camperdown Compost's proprietary cloud-based computer system which accurately tracks all aspects of the composting process together with details of inputs and on-farm operations. A training model has also been developed that would engage suitably skilled local staff such as those working for Catchment Management Organisations, Biosecurity Staff from DWELP, or Land Services staff in NSW. These matters are outside the scope of this report but trial managers from SESL Australia and Camperdown Compost are available for detailed discussions as appropriate.

4.4.3 Rendering

The results of the rendering trial are briefly summarized below. Confidentiality issues precluded any further detail on the compositional analysis however the trial operators provided significant feedback to the consultant undertaking the cost benefit analysis (Section 4.5.3).

The operators reported processing 16 T of carp, there was no major issues through the process and approximately 2.7 T of fish meal and 1 T of fish oil were produced.

4.5 Costings and CBA

4.5.1 Logistical Considerations

A common theme in most of the utilisation options (in particular hydrolysate and meal) was concerns about the rapid deterioration in quality of the carp following death in the waterway and therefore impact on transport logistics and final product quality.

This was also particularly observed during the SAMPI trial with the comment from the factory manager was that the thawing of the carp resulted in rapid deterioration and contamination with other organisms (e.g. blowfly larvae). Such contamination was likely to impact product quality and therefore return on the final product.

SAMPI operators believed that carp might need to be minced and stabilised with acid at the source and then transported, or immediately minced and stabilised at the factory site with processing to occur later. This mincing and acid stabilisation to produce "unfinished product" is commonly applied during heavy volume periods of the tuna harvest. The product is then successfully processed at a later date with little or no impact on product quality.

SAMPI Engineers therefore completed a preliminary design for a mobile unit that could undertake this preliminary processing at the fish source. It was considered that such a facility could be used for pre-processing for other potential carp utilisation options.

In short it was considered that such a mobile plant could process 100 tonne a day. It would contain twin 1 tonne ribbon mixers, with acid added and then the product pumped directly to a tanker. Only one pump and an acid pump would be required, also an air compressor, power plant and water. There would need to be separate storage for acid: 100 tonne processing would require 3000l acid daily so dangerous goods storage and licence would need to be addressed. A hopper for feeding the mincer would be set up on roof. Waste water would be kept extremely low, as clean-up water will have pH lowered and go into product but a reliable water source would be required.

The opportunity to develop a mobile first stage processing option to stabilise the product before transport to Port Lincoln was further discussed with SAMPI following the successful enzyme hydrolysis trial. On further logistical examination, it was determined that a mobile plant to mince and acid stabilise carp would have operational feasibility issues due to the following reasons.

- Phosphoric acid is an essential process ingredient but a hazardous item. EPA has stringent requirements around its storage and handling including bunding in case of spillage and use to be at least 500m from any waterways.
- Health and safety would be an issue with a mobile plant in rough terrain, particularly in higher temperatures. Fish degradation and therefore handling issues would need to be addressed. Labour costs would be high.
- Site selection difficult, particularly in regard to truck and tanker access in riverside areas.

Later, SAMPI engineers considered the best transport option was for the units in charge of the carp removal to place harvested fish in $1m^3$ bins and truck by flat top to a point where a chiller road train could be loaded with 40mt. That would be trucked to processing facility. Although truck transport is expensive (~8c/km) once loaded it was considered that transport costs for up to 15 hours would outweigh the costs, hazards and inconveniences of a mobile mincing/stabilising unit with associated labour, acid, terrain and cost of its construction considerations.

4.5.2 Small Scale Semi-Commercial Trials Costings

Fermentative Hydrolysis

The cost of a single processing unit suitable for processing one tonne of carp by the fermentative hydrolysis process described in Section 4.3.1 and based on a mobile four wheeled trailer with shredders and tanks for inoculant, water, molasses and storage was quoted at $12,000.

The research team suggested that the process was feasible in both scalability and efficacy, with the added advantage of the ability to change inputs (e.g. to other waste streams) should carp numbers decline for any reason. This would protect any investment in both the business model and training. An aligned business case had already been developed for feral pigs and is available on request.

A Standard Operating Procedure Manual (SOP) was developed for the fermentative hydrolysis process, with the ability to be used by community based operators. This SOP is attached as Appendix 6.

It is noteworthy that, although not covered in the formal CBA process, there is potentially the opportunity to retail the hydrolysate produced, thereby returning some funding to the community.

Vermicast Production

The company that undertook the worm trials has developed a proposal to dispose of carp with minimum transport costs and return the resultant organic matter into local soils via vermiculture. It was considered this would result in increased soil productivity and water retention. This proposal is part reproduced below.

Assumptions

- That the carp will be collected and treated at various points along the river system. Collection could happen adjacent to the vermicomposting sites. This would allow transport savings.

- That each location would have access to a front end loader and a source of carbon (green waste, sawdust, straw etc.). This proposal would work best if commenced in Autumn. Each site would need approximately 1 square meter of footprint space per tonne of carp, ideally with some sun protection (e.g. on the south side of a row of trees). Access to water would be advantageous but not essential.

The proposal

1. Suitable sites for vermicomposting are identified along the river system. These would be farms that abut the rivers or Council Transfer Stations.
2. The carp are collected/deposited on those sites along with a suitable carbon source in a ratio of 2:1 carbon: carp.
3. Each site would be attended, worm tea applied to the carp which will reduce/eliminate odour and expedite the composting process by adding beneficial aerobic microbiology.
4. Blend the carp and carbon source (using on site front end loader) into an appropriate ratio and dimensions so as to facilitate static, aerobic composting as per carp pilot trials (Section 4.3.4).
5. Instruct points 3 and 4 (farmer/ Council officer) so that they could then repeat the process in the future. A "Farmer Starter Kit" would be produced which would allow the farmer/council officer to create as much vermicast/worm team as they might require in the future from local organic waste.
6. The farmer/council officer would be left with a supply of compost worms to be introduced into the pile at an appropriate time (after good rain)
7. The pile would then require no management for several months (actual time dependent upon weather) or the whole process can be sped up with some management if preferred. Guidance/advice available by phone/e mail to support / guide farmer/council officer.
8. Once the process is complete, the resultant vermicast and worms (which will be approximately 32-64 times as many worms as were initially placed) remain the property of the farmer /council officer so that they will now have a sustainable low cost source of biological fertiliser to improve productivity for their gardens/pastures/crops …. And a potential source of sales for additional income.

Costs
Cost would be $60 per tonne of carp (minimum of 20 tonnes per site). There is no limit (apart from space) as to the volumes that can be processed on each site, and no limit on how many sites could have this process replicated.

Supplied for cost:
Worm Tea
Worms
Farmer Starter Kit (extra $250)
Onsite teaching
Ongoing telephone/e mail support.

Outputs per site.

Approximately the same amount of tonnes of vermicast as there was carp to begin with. Vermicast retails for up to $1000 per tonne or can be used to commence a sustainable program of soil productivity increases with diminishing requirements for time/energy/dollars inputs.

The ability to produce worm tea. Retails for around $2 per litre.

Around 64,000 compost worms that will continue to multiply exponentially if managed appropriately. Worms retail for around $20 per thousand.

4.5.3 Large Scale Commercial Trials CBA

The CBA report is attached as Appendix 5 and the Executive Summary is reproduced below.

Executive Summary

The Australian Government has established the National Carp Control Plan (NCCP) to assess the feasibility and potentially manage the release of *Cyprinid herpesvirus 3* as a biocontrol agent for the invasive fish species, carp. Australian waterways contain 350,000 – 1,000,000 tonnes of carp. Release of a carp-specific biocontrol agent will result in carp mortality across Australia's freshwater ways, triggering a large waste clean-up. The NCCP seeks to understand if and how this biomass can be employed for the benefit of communities, investors and the environment?

This report presents an initial cost-benefit analysis of 14 commercial supply chain scenarios for the beneficial use of waste carp. The project team has been led by Dr Janet Howieson from Curtin University. Based on a global literature review and confidential consultation with Australian waste industry partners, the project identified ten carp utilisation pathways - seafood (live harvest only), rendering, hydrolysis, composting, anaerobic digestion, insect feed, vermiculture, mincing, torrefaction and collagen production. Pathways were then tested in a limited number of pilot trials regarding their efficacy, flexibility, and broad catchment scalability.

Four preferred utilisation pathways were identified across the 14 scenarios and subjected to commercial cost-benefit analyses (Table 19).

Assuming carp are free at the river bank, each of these pathways is commercially viable based on an analysis of operating costs. However, significant policy and commercial investment assumptions must be addressed to confirm any key issues and multiyear capital requirements, before the CBA can be progressed through to a Net Present Value point and related sensitivity analyses.

Table 19: Carp Utilisation Pathways.

Cost Benefit Analysis	1. Carp Seafood	2. Carp Meat Meal	3. Carp Hydrolysate	4. Carp Compost
Products	Niche market wild catch seafood for fresh domestic markets or overseas processing	Meat meal / oil from virus-killed waste carp rendered within 3 days of mortality	Hydrolysate liquid for use in fertiliser, aquafeed, and as a burley in fishing	Compost for use in agriculture, horticulture, and home-gardens.
Multisite Volume	Small (<10,000 tpa)	Large (>50,000 tpa)	Medium (~15,000 tpa)	Large (>100,000 tpa)
Pros	• High value use - fresh or processed fish • Niche urban domestic markets • Asian export markets	• Large domestic and global markets • Existing renderers • Existing EPA approvals	• Large global market • Existing processors • Lower input quality • Existing EPA approvals	• Flexible carp site processing options • Low technology • Large established consumer markets
Cons	• Limited domestic seafood demand	• Processors need supply certainty before they will invest	• Processors need supply certainty before they will invest	• Requires large volumes of external carbon material (green waste)

	• Must harvest live • High processing costs • Lack of supply chain capacity re volume	• Input specifications are stringent • Prefer large volume long term contracts	• Plants are remote from carp catchments • Prefer large volume long term contracts	• May require individual EPA approvals for each individual site.
Est. Net Benefit Range per fish kg	• $0.22 - $0.57 per kg excluding fish cost	• $0.08 - $0.19 per kg excluding fish cost	• $0.07 - $0.11 per kg excluding fish cost	• ($0.09) - $0.44 per kg excluding fish cost

This report recommends NCCP consideration in 12 areas across the following broad issues:

- processors' rights to own and monetise any carp harvested,
- loss of product quality for virus-killed carp in a water column,
- supply chain transit limitations and food safety issues,
- regional harvest site accessibility, yield and viability,
- EPA approvals for compost transfers,
- Planning for staged multiyear mortalities that will greatly boost processor's motivation to invest,
- availability of incentives for on-farm and regional composting.

5 Conclusions

In summary fermentative hydrolysis and vermacast production have been shown to be technically viable for smaller community based applications, and can be implemented based on the draft methods, protocols and costings that have been provided. Some aspirational carp usage options, including anaerobic digestion and BSF larvae production have been proven at small scale but require further work for larger scale assessment and possible implementation.

As part of the larger scale trials, a 40 tonne trial at Camperdown Compost showed that compost production was possible, with optimisation of process and co-composting material. Product monitoring and compositional analyses also met national and state guidelines. It was considered that the composting methodologies developed could be transferred to other areas, closer to where carp harvest might occur. A preliminary implementation plan for managed, localised composting at such remote sites near where carp aggregation was likely to occur was developed by the industry partners involved in the project.

Hence in regard to larger scale commercial options the composting methods developed during the trials at Camperdown Composting are suggested as the most flexible and scalable option. The product value may be low but the process is likely to be able to use severely degraded product. As well flexibility in scale has been suggested with the option, assuming management, to develop small scale, farm-based, regional operations in remote, difficult to access locations with little infrastructure at or close to the water's edge. Pending consideration of transport approvals, and access and infrastructure availability, better quality fish at larger volumes could be transported to larger scale composting sites, either managed by local councils or commercial entities focussed on developing a possible product for consumer markets.

A commercial enzyme hydrolysis trial was planned and undertaken at SAMPI, Port Lincoln. This facility already processes ~1500 tonnes of tuna waste each year. In short the carp, at three different stages of deterioration (separated into three final product tanks) were successfully processed through the SAMPI enzyme hydrolysis process. The produced hydrolysate was stored in 1000L containers to check stability - there was little separation, no odour and no precipitation on storage. Based on this outcome as well on the compositional results, use of the product as a fertiliser was therefore considered feasible; albeit at a lower quality (and therefore lower economic return) than the aligned tuna hydrolysate product. A leading aquafeed company also requested and received samples of the carp hydrolysate for use in finfish feeding trials - these results have not been made available due to commercial confidentiality reasons. However,

carp hydrolysate was successfully supplemented at 10% inclusion rates in ongoing juvenile barramundi and crustacean feeding trials being conducted by post-graduate students at Curtin University.

Production of a carp enzyme hydrolysate is therefore possible, however at present this option is restricted to low numbers of processing sites, and operators have indicated, that capital assistance would be required to upscale plant capacity to be a credible option for processing of the very large volumes that have been indicated. It is however of note that pending approvals, shore based pickup and transport solutions have been designed by the operators. Although the value of the putative product is higher, so are product specifications and hence, the raw material quality must be at <72 hours post mortality. As well operators would likely require high volume harvest aggregation site with suitable riverside infrastructure to access appropriate volumes.

The large scale rendering option was also shown to be feasible, with 16 tonnes of carp processed through a meat rendering facility. There were no technical issues with the process and meal and oil were produced for analysis. Capacity to undertake the processing was therefore possible, and commercial markets available, however there exist stringent quality specifications and hence product >24 hours post-mortality would likely not be accepted for processing. Similarly to the hydrolysate example, operators would likely also require high volume harvest aggregation site with suitable riverside infrastructure to access appropriate volumes of acceptable raw material. There were also some concerns about consumer response to the use of viral infected product in the lucrative pet food market.

In an economic sense, and following consultation with the various commercial industry partners, an initial CBA was conducted on 14 possible supply chain scenarios based on four processing pathways. The report stated that each of the four pathways enables viable commercial scenarios, assuming carp are free at the water's edge. However, the CBA has not been fully developed due to major assumptions that commercial processors seek clarity on. As summarised below, clarity on the following issues will give greater confidence in further development of business models.

These issues include

- Clarify who owns a carp killed by the virus, at the harvest point, and confirm if processors own the final processed product,
- Provide greater detail regarding the quality of virus-killed carp available for removal during the "clean-up". For example, will dead fish initially sink in the water column and will be more difficult to harvest, and at what stage (number of hours after mortality) of deterioration will fish float to the surface of the water column?
- Confirm the definition of virus infected fish for transport and processing, a differentiation is required between "biological waste" and "infectious agent." This clarification has direct implications for regulatory approvals and transport costs.
- Provide clarity on the likely yield and location of top fish aggregation and harvest sites across catchments. What infrastructure and harvest facilities are available at each site? Are there any seasonal constraints on aggregation and harvest at each site? This data will greatly inform investors, and derisk harvest and freight costs for large processors.
- Consider the added benefits and costs that would accrue if large processors (renderers, hydrolysers, large composters) commit to large waste stream forward offtake contracts from the infected waterways. The benefits of contracted multiyear supply of large fish volumes could drive substantial improvements in the viability of scenarios analysed in the CBA.
- Confirm with Federal/State agencies and relevant EPA managers the procedures required regarding transport, remote composting, and related aspects of other processes (e.g. anaerobic digestion),
- Confirm if / how carbon credits impact farm composting values and returns,

- Confirm if / how government subsidies apply to compost sites managed by Landcare / CMA's / Councils,
- Ensure that any virus release strategy policy and planning development is aligned with, and guided by realistic commercial utilisation, supply chain and market demand considerations. Planning for the carp utilisation waste task (minimum 350,000 tonnes per annum (tpa) will require the equivalent services of at least 30 large processors each receiving up to 12,000 tpa of carp waste. This requires significant engagement and coordination with commercial processors and confirmation of sufficient infrastructure needs, to ensure efficient community and commercial outcomes.

In summary a suite of options for utilisation of the carp biomass have been developed and validated at large scale. It is suggested that such a suite of options, targeted for specific harvest location, logistical challenges and product quality variation, all need to be considered rather than a single, holistic solution for utilisation of the carp biomass. There is ongoing interest by commercial operators in taking part in carp utilisation options, but difficulties in handling this product have been highlighted, and infrastructure to manage transport, storage and processing issues associated with the large volumes will be required.

6 Recommendations and Next Steps

While this project (including CBA) is yet to deliver a final CBA output, we believe it has created further value for the NCCP in developing future recommendations for implementable large scale processing options for deceased carp.

This value includes:

- Identification of the four waste processing pathways,
- Identification and ranking by operating cost data of 14 possible pathways for waste carp processing,
- Engagement (by phone and face to face) with selected commercial players able to manage a component (with existing or additional capacity) of the forecast carp waste stream,
- Identification of the difficult issues and assumptions (listed above) that must be addressed by the NCCP before the completion of a professional CBA is possible,
- Confirmation that the commercial players engaged by the team are likely to be motivated to progress detailed discussion subject to satisfactory responses from government regarding their upfront concerns (noted in the issues above),
- A clear understanding of the types of regional commercial partners that need to be approached once we have an accurate picture of where the significant, economically harvestable fish aggregations are to be found across the basin.

The following next steps are recommended.

- NCCP seeks to gain greater advice and clarity on the policy issues raised in the cost benefit analysis and summarised in the conclusions section above. The CBA can then be further modified based on clarification of some of the uncertainties.

- Identify possible aggregation sites and volumes, then work with nominated commercial industry partners (renderers, remote and larger scale composting management, and hydrolysis entities) and develop recommendations for costed implementation plans (including additional processing,

transport and infrastructure requirements) and management of regulatory issues. Interest by such commercial entities in the NCCP is ongoing.

- Many of the utilisation options identified in this study would still be technically viable if fish can be harvested in continuous, large volume scenarios, and consistent product quality and handling protocols were instigated. The economic viability would be contingent on the assumptions on fish cost made in the CBA. It may be worth further investigating such a non-virus release scenario based on the results of this study. It was in this context that it was decided to add a carp seafood export option to the CBA.

It is noted that there are currently three PhD students examining both the carp fed BSF larvae and the carp hydrolysate in finfish and crustacean feeding trials as part of their Curtin University post-graduate research, this work is likely to continue until 2022 and will further inform possible end-use options for the putative commercial processes.

7. Extension and Adoption

Publications/products from the project are summarised in Table 19 below. Along with the milestone reporting significant community and research extension of the project outcomes was achieved, with print, television and social media coverage, articles in FISH magazine and presentations at NCCP research and stakeholder events. The power point presentation for the final project presentation delivered to the NCCP Principle Investigator and Stakeholder meeting in December 2018, is attached as Appendix 7.

Table 19: Extension Activities for the project.

Publication/Product	**Detail**	***Status***
NCCP PI meeting	*Oral Presentation: Options for utilisation of carp biomass*	*Delivered in July 2017*
NCCP PI meeting	*Oral Presentation: Options for utilisation of carp biomass*	*Delivered in October 2017*
NCCP PI meeting	*Oral Presentation: Options for utilisation of carp biomass*	*Delivered in February 2018.*
NCCP PI meeting	*Written Summary*	*Provided for May 2018*
NCCP PI and Stakeholder Meeting	*Oral Presentation: Options for utilisation of carp biomass (Final)*	*Delivered in December 2018*
Media Release	*Prepared by NCCP communications team*	*Approved in May 2017.*
Media Release	*Prepared by NCCP communications team*	*Approved in September 2017.*
Video footage	*Black soldier fly larvae feeding on carp.*	*Filmed in September 2017*
Video and photography footage	*Port Lincoln enzyme hydrolysis trial. Supplied to ABC Landline journalist,*	*Filmed in February 2018.*

	NCARP media consultant and Coretext. .	
Fish Magazine NCCP update	*Discussion with Toby Piddocke.*	*March 2018 issue.*
ABC Landline	*Discussions with Kerry, journalist ABC Landline about commercial trials.*	*April 2018.*
AORA conference	*Presentation on composting trial by Tony Evans and Declan McDonald*	*April 2018 (see Appendix 4)*
Media release	*NCCP media released in April: multiple media responses (3 x radio interviews; 5 x newspaper articles; 1 x television interview)*	*April 2018.*
Fish Magazine NCCP update	*Article*	*September 2018 issue.*
Newspaper Article	*PL Times*	*July 2018*
SOP for Fermentative Hydrolysis	*Pdf document*	*August 2018*
Peer reviewed Journal article	*Chaklader, M.R., Siddik, M.A.B., Fotedar, R. and Howieson, J.R. (2019) Insect larvae, (2019) Hermetia illucens in poultry by-product meal for barramundi, Lates calcarifer modulates histomorphology, immunity and resistance to Vibrio harveyi. Scientific Reports (in press)*	*October 2019*

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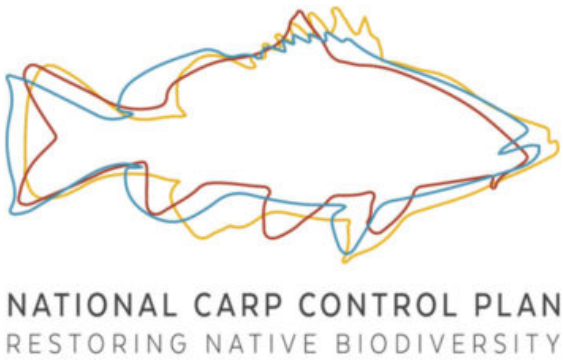
Appendix 1

Assessment of Options for Utilisation of Virus Infected Carp

Literature review

Andrew Tilley, Ewan Colquhoun, Janet Howieson



Contents

1.0 Introduction

Wild carp (*Cyprinus carpio*) are an invasive species found throughout Australian freshwater systems. The species is well established throughout the Murray-Darling basin (MDB) and makes up to 90% of the fish biomass in some areas. The Australian National Carp Control Plan will investigate the release of Cyprinid herpes virus 3 (CyHV-3) as a biocontrol agent for the invasive carp. The virus is expected to reduce the carp population by between 70–95% within the first few years. The large mass of deceased carp will require a large scale clean-up and present a unique opportunity to increase utilisation of this large natural resource.

2.0 Fish waste products available within the Australian market

A number of fish waste products are available in the Australian market, including hydrolysates, fertilisers, aquaculture feed, stock feed and pet food (Table 1.). These product forms typically rely on processes such as hydrolysis, rendering and ensiling to produce the final product. These processes lend themselves to large inputs and typically produce products with a long shelf-life.

Table 1. Summary of fish waste products made in Australia and commercially available

Product Name	**Where Produced**	**Price**	**Price/L**	**Main ingredients**	**Target market/use**
SAMPI Fish Hydrolysate	Port Lincoln	$1.10+ GST/L	1.10	Southern Bluefin tuna waste	Fertiliser, aquafeed
The Green Life Soil Co. Fish Hydrolysate		$85 for 20L $28 for 5L $12 for 1L	4.25 5.60 12	100% pure fish liquid	Fertiliser
ASBS Fish Hydrolysate-Single Origin Fish		$16.50 for 1L $82.75 for 5L $256.10 for 20L $825.00 for 200L $2178.00 for 1000L (Incl. GST and freight)	16.50 16.55 13.63 4.12 2.18	Single origin fish	
ASBS Fish Hydrolysate-Fish and Crustacea		$16.50 for 1L $82.75 for 5L $256.10 for 20L $825.00 for 200L $2178.00 for 1000L (Incl. GST and freight)	16.50 16.55 13.63 4.12 2.18	~ 30% of crustacean shells blended with the fish input. Fish + Crustacean Hydrolysate. 100% natural. No minerals added	Fertiliser
ASBS Triple Fish Hydrolysate		$23.10 for 1L $115.30 for 5L $394.94 for 20L	23.10 23.06 19.75	Mix of 3 different types of fish from Australia (Salmon,	Compost, Compost tea, Soil, Foliage,

		$1320.00 for 200L $2640.00 for 1000L $4848.00 for 2000L (Incl. GST and freight)	6.62 2.64 2.42	Tuna and wild catch). greater diversity with Fatty lipids, proteins, enzymes and minerals	Human and Animal consumption
Yates Uplift Fish Liquid Concentrate					
Agrisense Fish Hydrolysate				Fresh fish waste from Tasmanian aquaculture, produced under a refined cold enzyme process.	Fertiliser
No Frills Hydrofish Fish Hydrolysate	Busselton	$77.75 for 10L $49.90 for 4L $19.81 for 1L	7.77 12.47 19.81	100% Australian processed wild caught Tuna, from a sustainably harvested catch. Using enzyme digestions with no water added.	Fertiliser
Nutritech Farm Saver Liquid Fish	QLD	$80.05 for 20L $668.82 for 200L $2916.32 for 1000L	4.00	Cold water high protein species by product from fish processing.	Foliar spray, fertigation
NatraSol Liquid Fish Hydrolysate	Tas	$55 for 20L $1100 for 1000L + GST and freight	3.34 1.10	100% Tasmanian food grade wild fish species,	
Excel-Crop Liquid Fish Fertiliser		20L $112 (5.60/L) 100L $488 (4.88/L) 200L $699 (3.50/L) 500L $1626 (3.25/L) 1000L $2956 (2.95/L)	5.60 4.88 3.50 3.25 2.95	Aus. fresh sea fish processing waste on the same day of processing. No added water. Using enzyme hydrolysis	Fertiliser
Multicrop Ecofish Plant and Soil Nutrient		$8.99 for 600mL $12.9o for 1L $9.99 for 2L $29.99 for 2.5L Available in 5, 20, 110 and 200L	 12.90 5.00 12.00	Waste table fish	
Omnia		$60 for 20L	3.00	Organic	

Purafish					
BEC Feed Solutions Fish meal (render)	Queensland	No price available, 25 kg bags		Fish market raw material made up from whole fish, fish heads, fish bones and offal.	Stock feed
My Dog: Fish sardines & tuna	Victoria	$12.95 for 12 x 100 g	$10.79/kg	Fish including sardines, tuna and salmon	Dog food

- ASBS: Australian Soil Biological Supplies

2.1 Currently available products containing common carp in Australia

A number of companies harvest carp from the MDB for sale in the local and export markets. The largest company is K & C Fisheries, who harvest over 1000 tonnes per annum. K & C fisheries sell a number of carp products to both the local and export markets (Table 2.). In addition, K &C fisheries supplies carp to Charlie Carp, the sole producer of carp based fertiliser in Australia. The limited scope of common carp utilisation in Australia may provide an opportunity for successful commercialisation of carp products.

Table 2. Carp products currently available on Australian market

Product	**Company**	**Region, country**	**Local/ export**	**Format (Frozen/fresh/ dried)**	**Cost**	**Shelf life**	**Comments**
Whole fish	K & C Fisheries*	Victoria, Australia	Local and export	N/A	N/A	N/A	
Roe	K & C Fisheries	Victoria, Australia	Export	Chilled/salted	N/A	N/A	
Milt (male gonad)	K & C Fisheries	Victoria, Australia	Export	Frozen	N/A	N/A	
Fillets	K & C Fisheries	Victoria, Australia	Local and export	Fresh or frozen	N/A	N/A	Skin/scales on/off
Skin	K & C Fisheries	Victoria, Australia	N/A	N/A	N/A	N/A	Used to make leather
Scales	K & C Fisheries	Victoria, Australia	N/A	N/A	N/A	N/A	Used in some paints
Pituitary glands	K & C Fisheries	Victoria, Australia	N/A	Acetone dried/powder ed	N/A	N/A	Hormone drug to assist females to spawn
Bait	K & C Fisheries	Victoria, Australia	Local	N/A	N/A	N/A	Local crayfish industry
Charlie Carp All-	Charlie Carp**	NSW, Australia	Local and export	Liquid	$10.48 for 1L $9.98 for 2.2L $15.98 for 4.4L	N/A	Minced fish rendered to produce

purpose Fertiliser					Hose pack $44.98 for 5L Also in 20, 25, 200 and 1000L		concentrate

*K & C Fisheries: >1000t p.a. carp harvested

**Charlie Carp: Purchase 150t p.a. from K & C Fisheries

3.0 Factors impacting carp suitability for utilisation

The use of the CyHV-3 infected carp is highly dependent on how quickly the deceased fish can be collected and whether the CyHV-3 virus can be deactivated during processing. If deceased carp cannot be collected in a timely manner, extensive degradation may occur limiting their use in higher value products. Degradation may result in reduced protein and oil quality, increased levels of biogenic amines such as histamine and increased production of unpleasant odours, particularly volatile sulfur containing compounds (department, n.d.). These issues are of particular concern if the biomass is intended for human or animal consumption; in addition to the potential negative health effects (i.e. histamine poisoning), knowledge that products are produced from degraded CyHV-3 infected fish may impact on consumer acceptance of the products.

Market acceptance of products produced from the deceased carp may be negatively impacted by the presence of the virus in processed products, particularly if processed for human consumption. A study on the deactivation of the CyHV-3 virus found that the virus can be completely deactivated by heat treatments above 50˚C for 1 minute (Kasai, Muto, & Yoshimizu, 2005). The study also confirmed that UV irradiation, as well as a number of common disinfectants are effective in deactivating the virus (Kasai et al., 2005). Timely collection of the deceased carp, an appropriate virus deactivation process and clear communication to consumers of the products safety will be critical to ensuring product quality and market acceptance of any developed products.

4.0 Potential processing methods for deceased common carp

4.1 Rendering

By-products from the livestock, poultry and seafood industry are often processed into high protein meal and oil fractions by rendering. The process (Figure 1) involves reducing the feedstock to a consistent particle size, heating, separation of meal and oil, dehydration of the meal fraction and grinding to a consistent size (FAO, 1986).

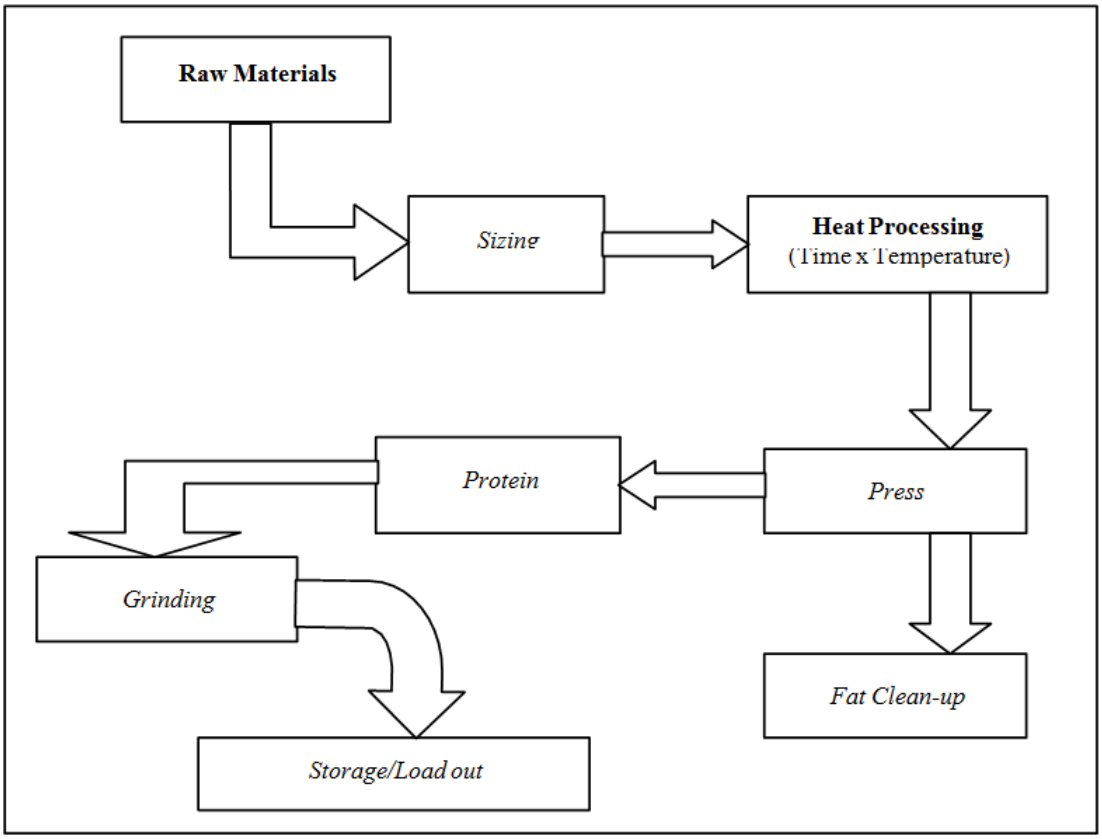


Figure 1. Overview of the rendering process (Meeker & Hamilton, n.d.)

After reducing the particle size, the feedstock is heated to coagulate the proteins and facilitate the separation of oil. This process, depending on the feedstock, typically takes place between 85-145˚C for between 40-90 minutes; in either a batch or continuous cooking unit. This unit operation has the greatest impact upon final product quality, in particular protein and oil quality parameters. During heating the product is pasteurised, improving its shelf-life and helping meet microbial limits (FAO, 1986; Meeker & Hamilton, n.d.; Windsor, 2001).

Once coagulated the mixture is pressed to remove oil and stick water, a mixture primarily composed of water and soluble proteins. The pressed protein meal is then dried and then ground to an even particle size.

A major source of quality loss in fish meal is oxidation during and after processing. Oxidation during processing is managed by controlling processing parameters to minimise unnecessary heating. Post-processing oxidation of fish meal is primarily due to the concentration of and composition of any residual oil in the meal. As such, antioxidants are usually incorporated, during transfer from the dryer to the mill, to inhibit oxidation during storage (FAO, 1986).

The oil, extracted during pressing requires further processing to be suitable for consumption. The steps employed depend on the desired final product but typically include: neutralisation, bleaching, deodorisation, polishing and winterisation. These steps remove unwanted compounds that negatively impact on the stability, functionality and sensory of the oil (Carvajal, 2014).

Variations on the rendering process exist for specific feedstocks and applications. One such process is the exclusion of the oil extraction operation; this process is usually only suitable for low oil content fish (<3% oil) and is often used for small scale operations to reduce capital investment in expensive oil processing equipment (FAO, 1986).

Rendered animal waste is typically used as animal or aquaculture feed. Fish meal, in particular, is frequently used for aquaculture feed formulations as it supplies a source of omega-3 fatty acids which is essential for fish health and marketing purposes (Pike & Jackson, 2010).

4.2 Hydrolysis

A more recent advancement in the use of fish by-products is the production of fish protein hydrolysates. Proteases are used to hydrolyse proteins into soluble proteins, peptides and free amino acids; the process converts whole fish into concentrated peptide liquor. Depending on the enzymes used, particular components such as bones and scales, can be left whole and are easily separated from the liquid hydrolysate. The process also assists in the separation of oil from protein by breaking down the extracellular matrix binding the oil.

Once hydrolysis is complete, the temperature of the mixture is briefly raised to 90-95˚C, in order to deactivate the protease used. This allows for control over the degree of hydrolysis (DH) and subsequently the peptide length profile produced. Controlling the DH allows for certain functional properties to be tailored in the final hydrolysate such as emulsifying, solubility and foaming (Liu et al., 2014). The application of fish hydrolysates in food is becoming more common due to the useful functional properties (Kristinsson & Rasco, 2000).

In addition to providing greater control over the functional properties, protein and oil quality is typically better than that produced by rendering. This is achieved by processing at a temperature of 50-60˚C, lower than the typical temperatures of 85-145˚C used during rendering. Processing at a lower temperature reduces the potential for unwanted thermal degradation of proteins and oil (Carvajal, 2014; Windsor, 2001).

The extracted oil is treated the same as oil extracted during rendering to make it suitable for consumption. However, production of higher quality fish oil is possible due to the lower temperature of processing. Fish oil is a high value product with well documented health benefits due to the high levels of long-chain omega-3 (eicosapentaenoic (EPA) and docosahexaenoic (DHA)) fatty acids present (Lopez-Huertas, 2010). New sources of omega-3 fatty acids are required to supplement existing supplies, both for human consumption and for supplementing aquaculture feed (Pike & Jackson, 2010).

Fish hydrolysates are versatile in their potential applications, such as fertiliser, animal feed, aquaculture feed or functional food ingredients. In addition high quality fish oil can be extracted to diversify the product stream.

4.3 Ensiling

An alternative method for producing liquid fish protein is through the acidification and autolytic hydrolysis of the feedstock. Silage involves reducing the pH of the feedstock to accelerate the hydrolysis of the protein by the feedstock's intrinsic proteinases (Forbes & Sumner, n.d.; Tatterson & Windsor, 2001). This is typically achieved by adding 85% formic acid until a pH below 4 is achieved. Other acids such as hydrochloric and sulfuric acid can be used; however neutralisation of the silage is required before use. An alternative approach is to add a carbohydrate source (i.e. molasses) and inoculate with lactic acid bacteria (LAB) to achieve the required pH reduction. In addition to accelerating hydrolysis, the low pH inhibits unwanted bacterial growth and stabilises the silage (Forbes & Sumner, n.d.; Tatterson & Windsor, 2001).

Silage production is relatively straight forward in comparison to rendering and hydrolysate production. The feedstock undergoes size reduction, acid addition and is then allowed to ferment until the silage converts to liquid. The fermentation time varies depending on feedstock and temperature, ranging from two days to 10+ days in colder weather. While capital investment is cheaper than for rendering, the high moisture content makes transport expensive (Forbes & Sumner, n.d.; Tatterson & Windsor, 2001). Application of fish silage is similar to that of render and hydrolysate. It can be used as a feedstock for livestock and aquaculture or fertiliser.

4.4 Insect production

A promising area of research into waste management is the use of insects such as the black soldier fly (*Hermetia illucens*) (BSF) for bioconversion of organic waste into a functional source of protein, fat and chitin. BSF larvae can feed on a wide variety of organic feedstocks, consuming up to twice their bodyweight per day (Barroso et al., 2017; Magalhães et al., 2017; Waśko et al., 2016). Larvae take approximately 14 days to complete development (Barroso et al., 2017). The combination of a rapid bioconversion rate and reproduction cycle makes them a viable alternative to other protein and lipid sources.

A wide range of applications for BSF have been studied, such as fecal waste management, aquaculture feed, stock feed, biodiesel production and techno-functional ingredients for biotechnology and food production (Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016; Leong, Kutty, Malakahmad, & Tan, 2016; S. Li, Ji, Zhang, Zhou, & Yu, 2017; Magalhães et al., 2017; Spranghers et al., 2017; Waśko et al., 2016).

BSF larvae can contain high levels of lipids up to 30% of the dry weight (Q. Li et al., 2011). The fatty acid profile of BSF lipids can be modified through diet manipulation. In a recent study, it was demonstrated that the inclusion of *n*-3 fatty acids in the diet of BSF larvae can result in the inclusion of these fatty acids in the lipids of the larvae (Barroso et al., 2017). This is a particularly promising concept as it may improve the suitability of BSF larvae as an inclusion in aquaculture feed, which typically include *n*-3 fatty acids to ensure the fish contain these fatty acids.

BSF larvae cuticle contains chitin, a biopolymer typically found in exoskeletons and fungi cell walls (Hamed, Özogul, & Regenstein, 2016). Chitin is a hydrophobic biopolymer which can be *N*-

deacetylated to produce chitosan which is hydrophilic (Hamed et al., 2016). Both forms have a number of applications within the biotechnology and food industries, such as biofilm ingredients, scaffold for tissue regeneration, stabiliser/thickener and antimicrobial/antioxidant ingredient (Hamed et al., 2016). Extraction of chitin from larvae prior to producing aquaculture feed would increase the protein content of the feed while also producing a valuable biopolymer.

4.5 Composting

Remediation of soil for agricultural use typically involves adding in organic matter in the form of compost. Composting is an aerobic biochemical process where organic material is converted into a stable mixture by thermophilic microbes (Schaub & Leonard, 1996). Composting requires a carbon:nitrogen (C:N) ratio of between 25:1 to 35:1 (Schaub & Leonard, 1996); low C:N ratios can result in release of excess nitrogen as ammonia gas, while high C:N ratios can exhaust available nitrogen before composting is complete.

Fish waste is an excellent source of nitrogen, and other essential nutrients, that can be included in the composting process (López-Mosquera et al., 2011). Composting of fish waste requires the addition of a suitable carbon source such as wood chips (Illera-Vives, Seoane Labandeira, Brito, López-Fabal, & López-Mosquera, 2015). The inclusion of wood chips also acts to provide bulk and ensure sufficient aeration of the mixture; this also assists with minimising production of unwanted odours as the fish waste decays. Composting is a well-developed process that can process large quantities of raw material into a stable mixture suitable for storage and then use in agriculture.

5.0 Carp proximate composition

5.1 Protein

Common carp composition is influenced by biological and environmental factors. Carp crude protein concentration has been reported over a wide range (12.9% - 17.9%), however this appears to be primarily influenced by fish body weight (Hasan, Macintosh, & Jauncey, 1997; Mahboob et al., 2015; Mahmoud, Kawai, Yamazaki, Miyashita, & Suzuki, 2007). In a previous study, the influence of bodyweight on crude protein concentration was investigated for both farmed and wild common carp (Mahboob et al., 2015). It was reported that average protein concentration significantly increased with fish size for both farmed and wild carp. Interestingly, this pattern was also observed for crude lipid and ash concentrations but a concomitant decrease in moisture percentage was observed. Hossain, Focken, and Becker (2001) fed carp fry varying diets and observed relatively small differences between final fish crude protein concentrations but significant increases, for all diets, compared to the initial crude protein concentration. This was concurrent with an increase in fish bodyweights as fish developed into fingerlings. A similar study using a different feed composition observed similar results, an initial crude protein concentration of 13.56% increased to 14.7% in the control fish (Siddhuraju & Becker, 2001). While these values represent the whole fish the majority of protein is present in the white muscle, red muscle and collagen.

5.1.1 Collagen

Collagen Type 1 is the major protein constituent of fish skin, scales and bones and has a number of applications in the food and pharmaceutical industries. Collagen can be described by its solubility

under different extraction conditions. Collagen exhibiting low levels of cross-linking is soluble in acid solution; while collagen exhibiting high levels of cross-linking can be solubilised in acid solution with the aid of the enzyme pepsin (Matmaroh, Benjakul, Prodpran, Encarnacion, & Kishimura, 2011). Common carp skin has been reported to contain a high concentration of acid soluble collagen (ASC); the skin was reported to contain 41.3%, the scales 1.35% and the bones 1.06% ASC (Rui Duan, Zhang, Du, Yao, & Konno, 2009). As collagen cross-linking decreases ASC content tends to increase (Rui Duan et al., 2009). The composition of fish collagen varies significantly with environmental conditions; R. Duan, Konno, Zhang, Wang, and Yuan (2010) extracted collagen from common carp scales and reported an increase in thermostability from fish harvested in summer compared to winter. This seasonal difference may be due to variations in water temperature or diet as observed with lipid composition (Guler, Kiztanir, Aktumsek, Citil, & Ozparlak, 2008).

5.2 Lipids

Lipid composition has been a point of focus in fish research, due to the high levels of Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) fatty acids and their well-documented health benefits (Mahmoud et al., 2007). Large variations in crude lipid concentrations (1.09% - 8.4%) and composition have been reported for common carp with seasonality, body weight and diet being significant factors (Guler et al., 2008; Ljubojević et al., 2015a; Mahboob et al., 2015; Yamamoto, Shima, Furuita, & Suzuki, 2003). Crude lipid concentration in common carp is highest in winter and lowest in summer (Guler et al., 2008). A lipid concentration of 1.09% was reported for carp analysed in summer while fish analysed in winter contained 4.45% lipid. Carp analysed in winter contained 41.1% monounsaturated fatty acids (MUFA) and 29.3% polyunsaturated fatty acids (PUFA), whereas fish sampled in summer contained 28.3% MUFA and 42.8% PUFA. These values are considerably different to those reported by Özparlak (2013) who studied common carp from a different location in Turkey. Carp analysed in winter had lower MUFA (36.1%) but much higher PUFA (37.17%) concentrations than those reported by Guler et al. (2008). EPA and DHA fatty acid concentrations decreased from their levels in winter (5.44% and 7.91%, respectively) to 2.93% and 6.49%, respectively, in summer. These changes between studies and seasons may reflect differences in water temperature, populations, fish size when sampled or diet (Özparlak, 2013).

As previously discussed for crude protein, lipid concentration appears to increase as body size increases. Fish between 600 g – 1000 g contained significantly lower lipid concentrations (3.98%) than fish weighing between 1600 g – 2000 g (4.35%) (Mahboob et al., 2015). However, this is difficult to confirm between studies as different feed regimes and compositions are used, which contribute significantly to lipid composition (Ljubojević et al., 2015a; Zajic, Mraz, Sampels, & Pickova, 2013).

Common carp are a major aquaculture species in Eastern Europe and Asia, accordingly a number of studies have investigated rearing conditions including diet. Aquaculture bred carp, fed feed containing fish meal and oil, contain higher levels of DHA and EPA (8.9% and 5.2%, respectively) compared to carp fed feed containing rapeseed oil (4.5% and 2.5%, respectively) (Ljubojević et al., 2015a). While these differences were observed in a controlled environment with a processed feed, similar differences may be observed in wild populations depending on the natural food resources consumed (Arts, Ackman, & Holub, 2001).

5.3 Moisture

The moisture content of common carp can be influenced by factors such as diet and bodyweight (Ljubojević et al., 2015b; Mahboob et al., 2015). Several studies have reported that common carp fed high protein diets exhibit higher fillet moisture contents (Ljubojević et al., 2015b; Trbović et al., 2013). Conversely, fish exhibiting high moisture contents tend to have lower lipid concentrations in the fillet (Trbović et al., 2013). Interestingly, this trend is also observed with increasing bodyweight, which as previously discussed correlates with increasing fillet protein content (Mahboob et al., 2015; Mahmoud et al., 2007). Variations in the moisture content of common carp may have significant implications on predicting yields during processing; for instance high moisture content could result in dilute hydrolysate or silage which could increase transportation costs.

A number of factors influence carp proximate composition such as body weight, seasonal and environmental changes and diet (Guler et al., 2008; Ljubojević et al., 2015a; Mahboob et al., 2015). Given the wide range of environments found in the Murray-Darling Basin (MDB), it is reasonable to assume that variation in common carp composition will be encountered, depending on the area and season of harvest (Brown, Sivakumaran, Stoessel, & Giles, 2005). As part of the development of any waste utilisation streams, detailed proximate analysis of Australian common carp will critical to supporting the development of such streams.

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Appendix 2

Co-digestion of the liquid fraction of *Cyprinus carpio* (European Carp) with municipal wastewater

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Supervisor: Ali El Hanandeh

Date: 31 March 2018

1. Introduction

The species *Cyprinuscarpio* (European Carp) is an introduced pest species in Australian waterways. Since their introduction, they have adapted well to Australian freshwater environments, acting as a predator and competitor for native fish species, and significantly degrading the aquatic environment (NSW Government Department of Primary Industries n.d).

To address this issue, the National Carp Control Plan(NCCP) is being developed to provide recommendations on carp control within Australian waterways. Included in the NCCP is the proposed release of the carp virus, and management of the subsequent clean-up and disposal of carp biomass following release of the virus. Amongst the disposal options being investigated, anaerobic digestion has been of interest due to its ability to process high strength organic wastes, generate biogas to be used as a resource.

Goulburn Valley Water (GVW) were approached by Fisheries Victoria to investigate the option to use one of their existing High Rate Anaerobic Lagoons (HRALs) to process the fish waste. The HRALs are designed to only accept liquid waste, and so GVW proposed a scenario in which the carp biomass be delivered to a local waste processing facility to have liquid and solids separation, after which the liquid is to be pumped to the HRAL, and the solids be transported to a composting facility close by. This scenario was considered to be more economic compared to that of sending the biomass directly to the composting facility.

There is little literature on the anaerobic digestion of *Cyprinus carpio* (European Carp), which may be attributed to the lack of its use in aquiculture and subsequent need for waste disposal. However, there is literature available on the anaerobic digestion of other fish species which has been considered in hypothesis development. With the species considered to be a significant environmental issue in Australian waterways, it is likely that ongoing carp control measures may produce ongoing quantities of biomass for disposal.

Research into the co-digestion of the liquid fraction of carp with a combined stream of industrial and domestic wastewater has been proposed to assess the feasibility of disposing of, and extracting resources from, carp waste via anaerobic digestion.

It is thought that the addition of liquid fish waste will contribute to the anaerobic digestion process and act as a feedstock, increasing biogas generation within the reactor (HRAL). However, it is acknowledged that there will be a likely threshold in which the liquid fish waste stream ratio will be detrimental to the process and potential cause it to become inert.

It is also thought that the co-digestion ratio of municipal wastewater and liquid fish waste will significantly affect the anaerobic digestion process, and that an optimum ratio/ratio range will be determined in the study, as well as a ratio at which the anaerobic digestion process is significantly hindered and/or becomes inert.

Therefore, the aim of this project is to investigate the effect of introducing liquid fish waste into an existing waste stream on the anaerobic digestion process. To enable this, the following research questions have been proposed;

Research Question 1: What is the impact of introducing, and co-digesting, liquid carp waste on the treatment performance of the existing digester?

To answer this, the following sub questions are proposed;

Sub-research Question 1: What is the relationship between the ratio of liquid fish waste to wastewater and biochemical oxygen demand(BOD) reduction, suspended solids(SS), volatile suspended solids (VSS) and volatile fatty acids (VFA) within the digestion process?

Sub-research Question 2: What is the relationship between the ratio of liquid fish waste to wastewater and biogas yield?

Limitations

The study is limited by time, and the seasonal wastewater quality received during the timing of the study. The research project must be completed within the semester and this limits sampling of the wastewater and laboratory tests/experiments to within the March-May period. The wastewater received during this time of the year has a large industrial component due to a large fruit processor's seasonal operation. As such, the findings of the research will not be representative of the HRAL under winter/off peak conditions.

Assumptions

It is assumed that the carp biomass sourced locally is representative of the wider carp population.

Need for Study

Ongoing issues with land rehabilitation, groundwater contamination and greenhouse gas emissions associated with landfill are largely attributed to this option of waste disposal becoming a 'last resort' form of waste management (Agamuthu 2006). Other waste disposal/management options that control or lessen their impact on the environment, as well as offer some form of resource recovery, have become increasingly popular for environmental, economic and social reasons. One such option is anaerobic digestion, which is currently being considered for the disposal of a significant volume of carp biomass during the release of the carp herpes virus.

This presents the immediate need for study to determine the effects of co-digesting the fish waste and municipal wastewater in order to guide a plant scale trial and, ultimately, the implemented operation. Furthermore, there are benefits associated with contributing to the limited existing research on the co-digestion of fish wastes and wastewater from municipal sources to guide the application of this technology in environmentally, economically and socially beneficial waste management operations.

2. Literature review

Anaerobic digestion is a process in which organic matter is broken down in the absence of oxygen, resulting in the production of biogas and digestate(Caruana&Olsen2012).It is widely used in the stabilisation of medium to high strength wastewater, and is a desirable waste treatment option due to its low energy demand and the generation of biogas which may be used as an energy source (Caruana & Olsen2012).

Through the process' four main stages; Hydrolysis, Fermentation/Acidogensis, Acetogenesis and Methanogenesis, organic polymers are broken down into the resultant products of biogas and digestate (see Figure 1)(Li, Park & Zhu, 2011).The main compounds in biogas are methane and carbon dioxide, however, other compounds such as hydrogen sulphide and ammonia are present in small concentrations (Abatzoglou & Boivin, 2009)

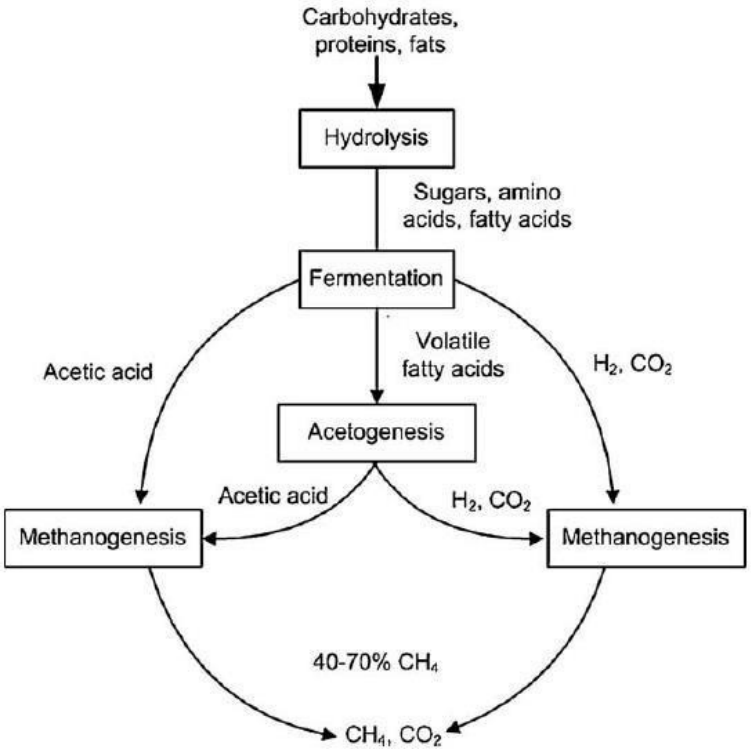

Figure 1. *Process flow of anaerobic digestion (adopted from Li, Park & Zhu, 2011)*

Anaerobic digestion can be used for a single feedstock, or for two or more different feedstock's, with the latter referred to as co-digestion. Co-digestion of different waste streams is considered to increase a plant's economic feasibility by overcoming issues associated with mono-digestions such as low C/N ratios and insufficient nutrient feed (Mata-Alvarez et al. 2014). The most common anaerobic digestion co-substrates found in the literature are sewage sludge, or wastewater, and municipal solid waste (Mata-Alvarez et al. 2014). Mata-Alvarez et al (2014) noted that low organic loads typically experienced in sewage sludge, and the non-used capacity of wastewater treatment plant digesters were big drivers for co-digestion at such sites.

Imbalanced C: N ratios may result in inhibition of the anaerobic digestion process via volatile fatty acid accumulation and high total ammonia nitrogen release(Li, Park & Zhu, 2011). Optimum C:N ratios generally range from 20:1 to 30:1 depending on the feed material to undergo digestion (Li, Park & Zhu, 2011). Fish waste alone is considered difficult to digest anaerobically due to low COD: Nitrogen ratios and inhibition by ammonia (Donoso-Bravo et al. 2015). Because of this, co-digestion of fish wastes with another waste stream is considered necessary (Donoso-Bravo et al. 2015).

Donoso-Bravo et al. (2015) investigated the biodegradability of fish remains, finding that most parts of the fish were readily biodegradable. Fish wastes generally contain high amounts of fats and proteins, making them an ideal energy rich feedstock for biogas production (Solli et al 2014). Documented examples of lipid rich feedstock being added to digesters have demonstrated significantly higher yields in methane, suggesting that economic gains can be achieved in existing operations (Ahring 2003). However, the addition of lipid rich feedstock may inhibit the anaerobic digestion and biogas production process if care is not taken in balancing the co-digestates (Khalid et al. 2011).

The breakdown of such a feedstock during acidiogenisis into volatile fatty acids (VFAs) will consume alkalinity and reduce the pH (Appels et al. 2008). Furthermore, balancing the volumes and ratios of

the co-substrates is important for avoiding overloading and accumulation of nutrients and volatile fatty acids(Solliet al. 2014). Both the free long-chain fatty acids and the high levels of protein, which are characteristic of fish waste create potential for inhibition of the methanogenesis phase of anaerobic digestion (Nges, Mbatia & Bjornsson 2012). A study by Nges, Mbatia and Bjornsson (2012) demonstrated that the co-digestion of fish waste with residue from a crop was effective in mitigating inhibitory effect of lipid rich feedstock via the degradation and/or dilution of the inhibitors to an acceptable level.

Ammonia isknown inhibitor of the anaerobic digestion process, specifically Methanogenesis (Yenigün & Demirel 2013). Yenigun and Demirel (2013) suggest that total ammonia nitrogen reaching 1700 – 1800 mg/L would be inhibitory to the digestion process with acclimated inoculum. However, under conditions in which acclimation of the inoculum had occurred, total ammonia nitrogen levels could reach up to 5000 mg/L before inhibition occurred.

Although there is much literature on the digestion of fish waste and other feed substrates, there is little in the way of investigating the anaerobic digestion and biogas production of just the liquid fraction of fish waste. Fish biomass has been found to be a valuable resource for composting and soil conditioning due to its carbon content and nutrients, including Nitrogen, Phosphorus and Calcium (Illera-Vives et al. 2015). The separation of the solid and liquid fraction of the fish would allow for two beneficial reuse options – composting for the solid fraction, and anaerobic digestion/biogas production for the liquid fraction.

3. Materials and Methods

The experimental phase of the research was conducted by replicating the anaerobic digestion process at the Shepparton WMF's HRAL. To do this, five sealed glass jars were used as rectors, and had eudiometers attached to capture the biogas generated, with water used as the displacement medium (see Figure 2.).

Figure 2. The experimental setup.

A 24hr composite sample was taken for the influent wastewater stream coming into the WMF, and the digestate seed for the process was taken from the Shepparton WMF HRAL. Fish liquid was sourced by obtaining fresh dead carp from local recreational fishermen, and then extracting the liquid from the fish. The extraction process involved chopping and mincing the fish, and then hanging the resulting product in a netted bag to drain the liquid overnight.

Thereactorjarforthecontrolcontainedonlythe24hrcompositewastewatersampleandtheseed. The other reactor jars contained a mixture of the 24hr composite wastewater sample, the seed, and fish liquid. Magnesium Hydroxide was added to raise the alkalinity above 800 mg/L when required. The volumes, ratios, and variables used are listed in Table 1 below;

Table 1. Composition of samples used for the experiment.

Variable	UOM	Control	Test 1	Test 2	Test 3	Test 4
Volume	mL	1000	1000	1000	1000	1000
Fish Liquid	%	0%	5%	12%	18%	23%
Fish Liquid	mL	0	50	120	180	230
Wastewater	mL	1000	950	880	820	770
Seed	mL	30	30	30	30	30
Temp	Degrees C	24.5	24.5	24.5	24.5	24.5
Mixing Energy		Low	Low	Low	Low	Low

pH was measured daily throughout the experiment and, whenpHwasdroppingbelow6.8units (day 5) and biogas production was noticeably dropping, a drop of magnesium hydroxide was added to prevent inhibition of the process.

A sample from the control and each test's wastewater was taken and delivered to the lab for analysis of the test parameters before anaerobic digestion treatment.

The reactors were then placed on a magnetic stirrer in a water bath, with the eudiometer manifold attached. The glassware connections were sealed and the air within the reactor jars and eudiometer was purged with nitrogen gas to create an anaerobic environment.

Daily biogas production for each reactor was obtained by measuring the amount of water displaced by the gas in mL.

The experiment lasted for 11 days, after which the reactors were taken out of the eudiometer and heat bath and left to settle for 1 hour. The supernatant from each reactor was sampled and sent to the lab for analysis. Samples were tested for Alkalinity and VFA at the GVW labviaaKEMAT510 Titrator.

The lab tests for BOD, ammonia and VFAs will be carried out by an external NATA accredited lab using the following methods;

BOD - APHA 5210B (5 day BOD test)
Ammonia - APHA 4500-NH3 H. Ammonia by Continuous Flow Analyser

4. Results

Due to the small rector size, the post digestion sample was too small to perform all analyses on, therefore BOD, VFA, ammonia and alkalinity were prioritised for sample analyses.

BOD reduction within the reactors varied significantly, with only the control showing an acceptable percent reduction (96%). All other rectors either reduced BOD by a low percentage 8-27%, or an increase in BOD was observed (Figures 3 and 4).

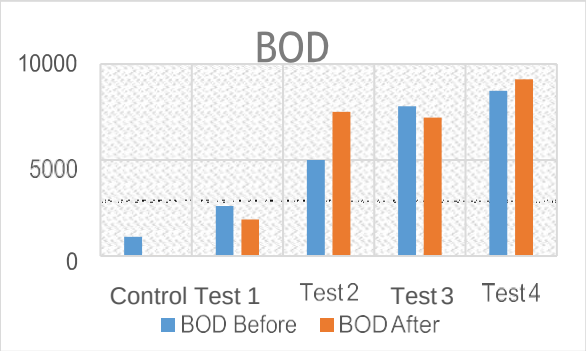

Figure 3. BOD before and after anaerobic digestion

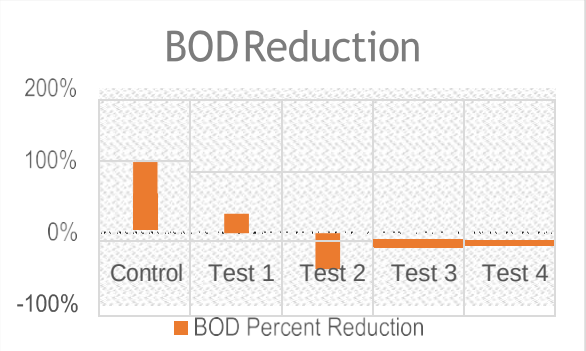

Figure 4. BOD percent reduction over 11 days.

Prior to undergoing anaerobic digestion, a linear relationship was observed between VFA concentration and concentration of fish liquid. Interestingly, a similar relationship was not observed following anaerobic digestion in the final samples (Figure 5).

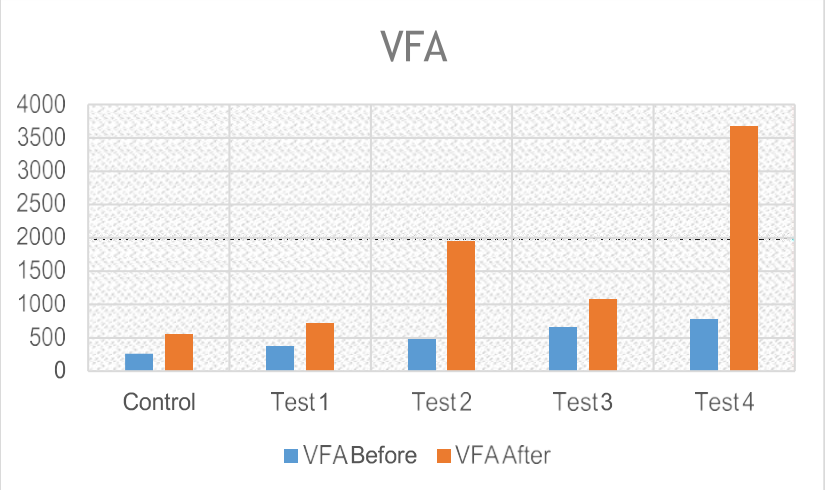

Figure 5. VFA before and after anaerobic digestion.

Alkalinity increased in all test reactors (See Figure 6) which was expected as magnesium hydroxide was added to all samples prior to and during the anaerobic digestion process to avoid inhibition of the process.

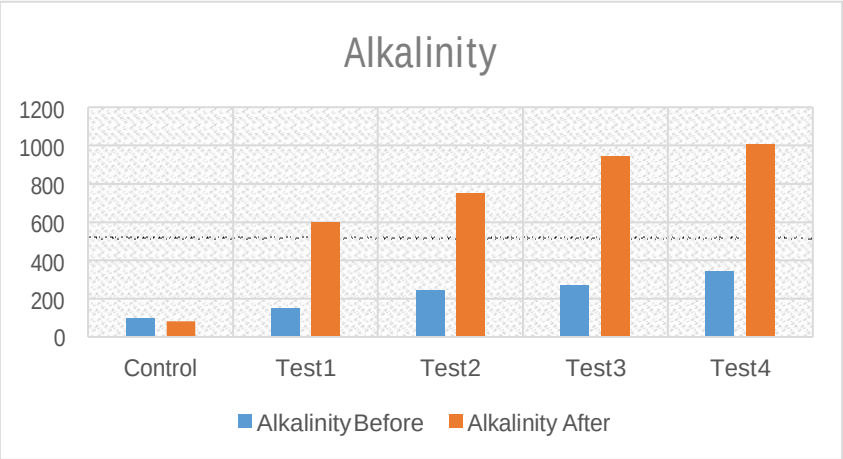

Figure 6. Alkalinity before and after anaerobic digestion.

Ammonia increased throughout the 11 day experimental period in all reactors. Only a 46% increase was observed in the control, with an 87%, 91%, 89% and 79% increase in Test 1, 2, 3 and 4 respectively (Figure 7).

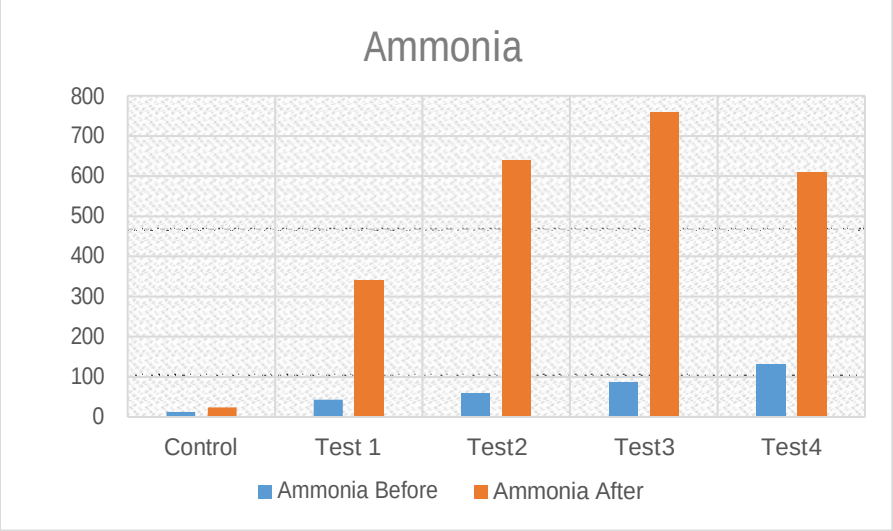

Figure 7. Ammonia before and after anaerobic digestion.

All reactors with fish waste yielded more biogas than that of the control reactor. Figure 8 shows the percent increase in biogas yield, compared to that of the control, for each reactor. On total biogas yield alone, no clear pattern can be observed between fish liquid: wastewater ratio and biogas production, however, further breakdown of biogas production by day (Figure 9) reveals a pattern in biogas production and time.

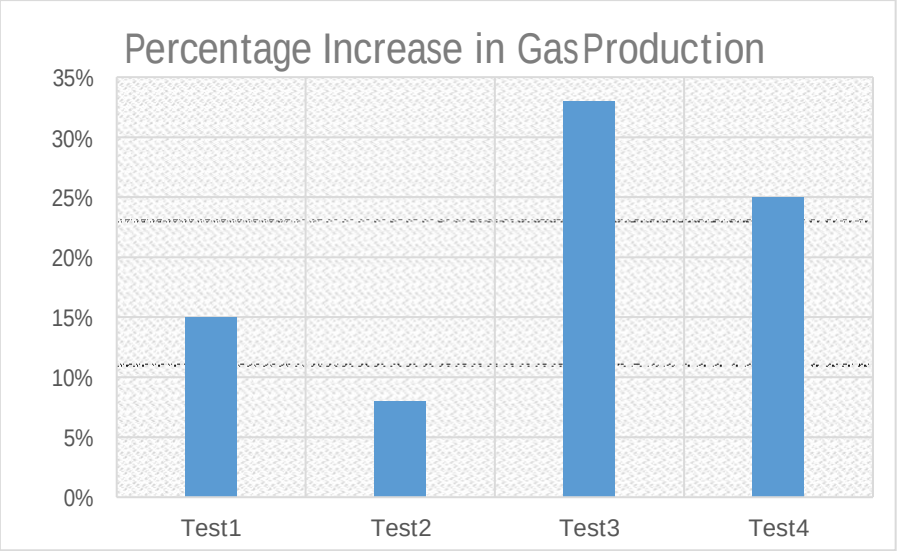

Figure 8. Percentage increase in biogas yield compared to the control reactor.

Figure 6 shows the biogas production from each reactor by day. An increase in biogas production can be seen for Tests 1, 2, 3 and 4 from day 5, which coincides with the day that the alkalinity was adjusted via the addition of magnesium hydroxide. Prior to this, Tests 2, 3 and 4 appear to drop in biogas production as days increase, however, the control appears to increase as days increase. All appear to peak on day 7 before dropping off on day 8.

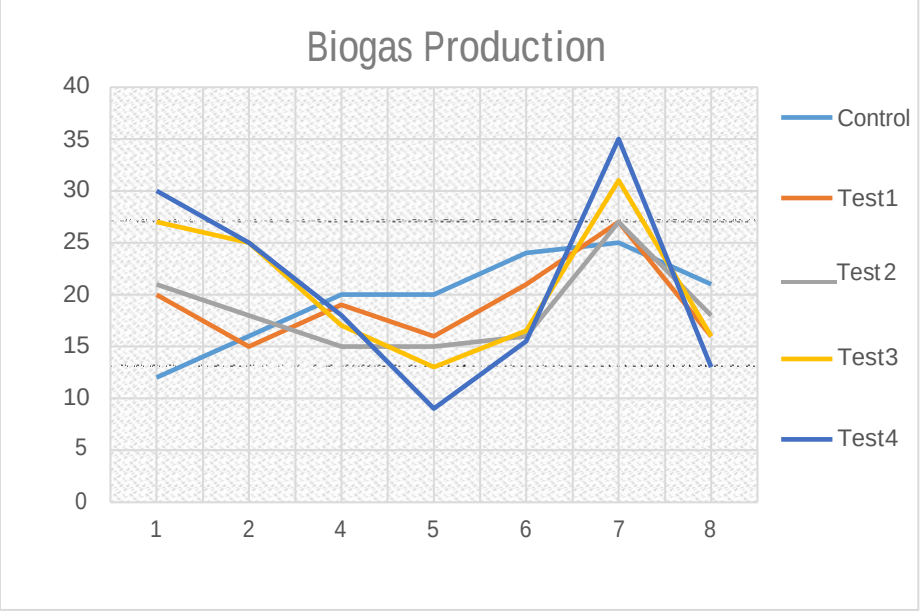

Figure 9. Biogas production by day.

5. Discussion

Sub-research Question 1: What is the relationship between the ratio of liquid fish waste to wastewater and BOD/COD reduction, suspended solids, volatile suspended solids and volatile fatty acids within the digestion process?

BOD reduction results were inconclusive as the final samples were observed to have a significant amount of suspended solid material which will have increased the overall BOD of the sample. This can be attributed to the small reactor, and thus sample, size. When the reactors were settled and decanted, in an effort to gain enough sample to complete the required analyses, an amount of solids material ended up in the final sample. In futures similar studies, it is recommended that a filtered BOD test be performed, particularly if the process in question involves solids settling before discharge.

Prior to undergoing anaerobic digestion, a linear relationship was observed between VFA concentration and concentration of fish liquid, this relationship was not observed in the final samples. This was considered unusual and, on reflection, may be attributed to inconsistent sample representation, as discussed above. If the relationship between suspended particles and VFAs is similar to that of suspended particles and BOD, the inclusion of a higher volume of such particles in Test 2 and Test 4 may explain their inconsistency with the other three samples. This is further supported by the observation of a similar pattern in the BOD results.

Ammonia levels in all test reactors showed a significant increase compared to that of the control, however, all were below the inhibitory values of 1700 – 1800 mg/L described by Yenigün & Demirel (2013). Ammonia is generated as a by-product of anaerobic digestion from when proteins are broken down in the process (Akindele & Sartaj 2018). Ammonia is a known inhibitor of anaerobic digestion, however, the biogas yields observed in the test rectors suggests that ammonia had not reached levels that would inhibit Methanogenesis.

Alkalinity was maintained in all reactors via the addition of magnesium hydroxide, which was required due to the low alkalinity of the initial influent sample. On reflection, and considering the continuous flown nature of the Shepparton HRAL, it would be beneficial for future study to complete the same experiment with fish liquid waste, influent sample, and a sample from the contents of the HRAL, all representative of the operating system. This would be more representative of the actual system, and, theoretically, assist in stabilising pH.

Suspended solids and volatile solids were unable to be analysed in the final samples due to lack of sample volume, thus the relationship between fish liquid: wastewater ratio and these parameters was not able to be investigated. Future studies of similar nature should consider the sample size required to thoroughly investigate the water quality, and size the reactors generously to accommodate the required sample size.

Sub-research Question 2: What is the relationship between the ratio of liquid fish waste to wastewater and biogas yield?

A higher biogas yield was observed in all reactors when compared to that of the control reactor. This can be attributed to the increased availability of energy rich molecules as described by Ahring 2003. These results indicate that there is a benefit to the bio digestion of fish waste in that an increased biogas yield, and economic incentive, may be achieved.

A linear relationship in the total biogas yield was not observed between fish liquid concentration and biogas generation. However, biogas generation appeared to respond significantly when adjustments were made to alkalinity and pH within the reactors, suggesting that maintaining pH and alkalinity is crucial to biogas production. Given that the fish liquid waste provides high energy feedstock for biogas production, and that an increase in biogas yield was observed in all test reactors compared to that of the control, it could be concluded that an increase in high energy fish liquid waste leads to increased biogas yield. Based on this theory, a linear relationship between percentage of fish liquid waste and biogas yield could have been hypothesised, however, was not observed in this experimental study, possibly due to poor alkalinity control.

6. Conclusion

The results observed in this study, suggest that fish liquid waste can be treated via anaerobic digestion and improve biogas yield of the Shepparton WMF HRAL. However, given the high strength nature of the fish liquid waste, several parameters need to be considered and managed in a full scale operation, including VFA:Alkalinity ratio, pH and ammonia. The combining of fish liquid waste with municipal sewage wastewater provides the benefits of dilution which will assist in maintaining VFA: Alkalinity ratios and ammonia at levels low enough to avoid inhibition of the anaerobic digestion process.

It should be noted that dilution ratio is important if there is a limited retention time in the reactor, as is the case with the continuous flow HRAL in Shepparton, as increased loadings generally require increased reaction/retention time. Alkalinity control via chemical addition is recommended in a full scale operation to maintain a VFA/Alkalinity ratio within the specified ‘safe’ ratio of .1-.3.

Due to uncontrolled and limited retention time at the Shepparton HRAL, a 5%dilution or less is desirable to increase biogas yield whilst still achieving treatment targets.

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Appendix 3 Fermentative Hydrolysis Final Report

This report provides a summary of the Carp Biomass hydrolysate project delivered by Soil Land Food for the NCCP program 2018

Background

This project was the result of an initial meeting on 14 March, 2018, between Jamie Allnut, Project Manager for the National Carp Control Program (NCCP) from the Fisheries Research and Development Corporation (FRDC) and Gerry Gillespie from Returning Organics to Soils (ROTS). Subsequent to that meeting the NCCP engaged Soil Land Food to carry out a pilot project to demonstrate an innovative, community-based process for transforming carp biomass, resulting from planned mass eradication of carp from the Murray Darling system and beyond, into a bio-fertiliser using appropriate open-source biotechnology. The bio-fertiliser product could then be used in farming systems across the basin as a soil and crop fertility input. The pilot project aimed to show the potential to address the environmental problem that the disposal of significant tonnages of biomass may cause as carp are progressively killed in Australian river systems. This project was to demonstrate a solution whereby the biomass is transformed into an agricultural fertility input, a plant growth biostimulant/biofertiliser.

The project was executed by a consortium of project partners and was based on the hydrolysate process refined by ROTS and that was used for the development of a Business Plan to control feral pigs in the Aurukun Wetlands area of Cape York. This Business Plan had demonstrated that a pathogen-free foliar product could be produced from animal carcasses and carcasses mixed with food waste and if sold for around $3 per litre, it would enable the person who shot the pig to be paid $1 a kilo for the animal carcass. Given the production of a similar product from other protein sources this financial model remains the same.

Growth trials on the pig product were conducted by CSIRO partner, the Australian Plant Phenomics Facility, with its Plant Accelerator which can control inputs to plants and then digitally scan the plant to determine rate of growth, leaf size and number and plant vigour. In these trials the pig product, named Feraliser, was demonstrated as competitive with four other commercially marketed biostimulants, without added fertiliser. The benefits of biostimulant protein hydrolysates have been demonstrated in numerous research projects around the world. *(Colla et al Protein Hydrolysates as Biostimulants in Agriculture 2015).* These were the central focus of the 2nd Biostimulants Congress held in Florence Italy in November 2015.

Project Partners

The project was auspiced by David Hardwick at Soil Land Food, with input from Gerry Gillespie, ROTS; Dr Janet Howieson, School for Molecular and Life Sciences, Curtin University; Jamie Allnut, NCCP; Dr Sara Beavis, Fenner School of Environment and Society ANU.

Project Process

The objective of the trial and its associated methodology, designed by Dr Beavis, was to deliver a method for processing carp biomass using a fermentation based technique in more isolated communities which is both simple and flexible in terms of methods and equipment; is flexible in terms of inputs; is community based and can be delivered in such a way as to be financially independent. at the same time using a Standard Operating Procedure. Discussions between the project partners led to changes in the size, cost and scale of the trial with the variations including the use of pre-macerated carp and its delivery to Sydney.

Meetings were held with Danny O'Sullivan to finalise the conduct of the trial on his property "Mooncoin", near Queanbeyan NSW on 2 May 2018. A suitable site with water, power and shelter was chosen and a 1000L IBC set up on pallets to enable the later mixing, sampling and extraction of completed product. Small heaters were attached, a fermentation lock fitted and all connections tested to ensure the IBC was fully sealed and water proof. During the month of May 40 litres of Lactobacillus based inoculant was made for the trial.

Dr. Janet Howieson arranged the transport of 14 x 20 kg boxes of macerated fish product from Western Australia to Sydney where it was collected from the RAND refrigerated logistics centre on 28 May. This was transported to "Mooncoin" and transferred from the cardboard boxes into sealable 60 litre plastic containers to enable the fish to thaw in a protected and uncontaminated space.

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The fish was checked after 24 hours but due to low ambient temperatures remained frozen solid. On May 30th the then thawed carp was moved by bucket into the IBC, which had four cat warmer mats attached and was covered in second hand doonas to assist temperature maintenance.

Final quantities in the IBC were:
Carp - 14 boxes of 20 kg each = 280 kg
Molasses - 4 x 13 kg of = 56 kg
LAB inoculant = 40 litres
Water 280 litres
TOTAL 656 LITRES

This process is intended to be used by community groups such as Landcare, Fishing Clubs and Farmer groups some of whom may intend to make it an ongoing enterprise that will aim for a commercial return or at least cover costs. In that case one of the difficulties which could be potentially faced is that if the kill rate for carp was around the estimate of 70%, it is possible that a group which developed a business model based on the fish alone, may find that its source of income would cease once a 70% kill rate was reached. Food scraps are an alternative biomass source in many areas and to this end a mixture of 30 litres of meats, vegetables, fruits and paper, water and molasses with inoculant, was processed through an insinkerator and stored under fermentation lock. This mixture was tested in parallel with the carp tests.

If the process with food once again, proves viable as it had in the previous pig trial, it would mean that there is adequate proof that should a group which has developed a biofertiliser business based on carp biomass, find that they can no longer get carp, they can change their process over to food waste and/or other species of feral animals without harming their business model.

One of the difficulties of conducting this trial during June on the NSW Southern Tablelands was that the ambient temperature often did not rise above 14^0 C. In the first two weeks of June there were five consecutive nights with temperatures of -5^0C.

Dr Beavis had stated in her Methodology for the project that she would have preferred a temperature range similar to that maintained for the pig project which she also oversaw. That project was conducted in November/December in the Southern Highlands of NSW. The temperature range in this trial was well below the preferred range and as a result a satisfactory product will be far more reliant on the combination of pH and the length of time of this trial, which is four weeks. Commencement Date 1st June and completion date 29th June 2018.

However, given the location and timing of this trial, it is also worth considering that once the herpes virus is released it "is generally only observed between a permissive temperature range of 16 and 25 °C" *(Hedrick et al. 1999; Denham 2003; Perelberg et al. 2003; Sano et al. 2004; Terhune et al. 2004; Tu et al. 2004).*

This would indicate that no dead carp will be available in cooler climates for processing in winter and reinforces the need to provide an alternative input in areas where a viable business is to be established.

Product Monitoring

The trial site was visited on 16 of the 28 days over which it was conducted. The pH and temperature were recorded periodically. Results are below in Table 1.

Table 1: Temperature and pH of carp biomass during fermentation - 2018

Date	Product	Ambient Temp	Liquid Temp	pH litmus	pH Manutec
June 5	Carp	10 deg C	9 deg C	6	6.5
	Food	10 deg C	14 deg C	6	6.5
June 9	Carp	9 deg C	9 deg C	5	4.5
	Food	10 deg C	14 deg C	5	5
June 19	Carp	12 deg C	18 deg C	5	4.5
	Food	12 deg C	14 deg C	4	4.5
June 29	Carp	10 deg C	9 deg C	4	4
	Food	12 deg C	14 deg C	4	4

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Note the shifts in pH. The liquid pH was tested with litmus paper and the solids pH with a Manutec soil pH test kit. The litmus strips were tested with pH buffer solution and found to be accurate.

In addition to monitoring pH and temperature as part of process control, samples were taken to test whether the process controlled potential pathogens. Samples were taken before and after fermentation. These samples were tested for indicator pathogens. ASL was the lab used for testing. Dr Sara Beavis assessed the results of the tests and a summary of her observations is provided below.

Dr Sara Beavis - Comments on microbiological testing of carp samples

The potential use of fish waste, derived from feral carp, as a liquid foliar fertiliser should not pose a risk to human health, if used on food crops. Thermotolerant coliforms and E.coli are generally accepted as the most reliable indicators of faecal contamination of water and food for human consumption (Fresh Produce Safety Centre August 2015). These microbes are present in very large numbers in faeces. Australian standards for irrigation waters provide the following thresholds for faecal coliforms (Table 2)

Table 2: Threshold values for thermotolerant coliforms in irrigation waters used for food and non-food Primary production

Intended use	Concentration of thermotolerant faecal coliforms
Raw human food crops in direct contact with irrigation water (e.g. via sprays, irrigation of salad vegetables)	<10 cfu / 100 mL
Raw human food crops not in direct contact with irrigation water (edible product separated from contact with water, e.g. by peel, use of trickle irrigation); or crops sold to consumers cooked or processed	<1000 cfu / 100 mL
Pasture and fodder for dairy animals (without withholding period)	<100 cfu / 100 mL
Pasture and fodder for dairy animals (with withholding period of 5 days)	<1000 cfu / 100 mL
Pasture and fodder (for grazing animals except pigs and dairy animals, i.e. cattle, sheep and goats)	<1000 cfu / 100 mL
Silviculture, turf, cotton, etc. (restricted public access)	<10 000 cfu / 100 mL

Adapted from ARMCANZ, ANZECC & NHMRC (2000)
cfu = colony forming units

It should be noted that the NSW recommended standard for E. coli in waters that may be applied to food crops with edible skin or that may be eaten uncooked is <126cfu/100ml (NSW DPI, 2017), suggesting that there may be some inconsistency, if the National guidelines are not the point of reference.
The maceration of carp carcasses for processing into a foliar fertiliser can be expected to liberate microbes from the fish gut. During hydrolysis and anaerobic fermentation, elevated temperatures should reduce or eliminate these microbes. In this pilot trial, therefore, it was important to establish the level of faecal contamination of the macerated carp prior to the commencement of hydrolysis and fermentation, and again on completion of that process to establish the efficacy of the processing and the suitability of the end product for its designed purpose. Three replicate samples of macerated carp were therefore submitted to ALS Canberra for analysis before and after processing. The results are provided in Table 3 below.

Table 3: Concentrations of faecal coliform units and *E.coli* prior to and after processing macerated carp by hydrolysis and anaerobic fermentation

	Unit	Replicate 1	Replicate 2	Replicate 3
Pre-processing				
Presumptive*				
Thermo-tolerant faecal coliform	cfu/100ml	200	180	280
Confirmed **				
Thermo-tolerant faecal coliform	cfu/100ml	120	180	280
E.coli	cfu/100ml	120	180	280
Post processing				
Presumptive*				
Thermo-tolerant faecal coliform	cfu/100ml	<100	<100	<100
Confirmed **				
Thermo-tolerant faecal coliform	cfu/100ml	<100	<100	<100
E.coli	cfu/100ml	<100	<100	<100

* Mathematically estimated number of viable microbes by conducting a series of dilutions, then plating and incubating for a standard of period time and temperature.

** Determined either by counting after dilution, plating and incubation or a direct plate count.

Project Summary

The results of this project indicate that fermenting carp biomass using a simple field based hydrolysis technique has the potential to create a biofertiliser/biostimulant product that can be used in agriculture. At the same time this method can help solve a significant environmental hazard by safely removing carp biomass from river systems and landscapes.

Results also show that the carp hydrolysis product has safe levels of indicator pathogens. In Lactobacillus-based fermentation the lowering of pH is a key regulator of pathogens in this process. *(Beavis 2014).* Therefore, undiluted, the product meets the relevant national water quality guidelines for water being applied to food and non-food crops, with the exception of raw human food crops with which it has direct contact. However, given that the product would be applied at a 1:100 dilution, the results of this pilot trial indicate that use as a foliar fertiliser would not be a risk to human health, due to concentrations being well within the relevant guidelines. The project results are also consistent with Kansas State University's project work on using hydrolysis as a means to control human pathogens in animal biomass. *(Kansas State University)*

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NSW DPI, 2017 Wash water safety fact sheet https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0009/433674/Wash-water-safety.pdf)

Carcass Disposal: A Comprehensive Review *(Kansas State University - USDA Carcass Disposal Working Group - August 2004)*

Appendix 4

Carp on-farm Composting Trial

31st August 2018

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Introduction

Over the coming years the planned release of the carp herpes virus into the Murray-Darling basin has the potential to produce hundreds of thousands of tonnes of fish mortalities. To avoid the potentially huge environmental impacts from that volume of decaying fish, an effective management strategy is required. On farm composting of fish carcasses is part a multi-faceted management strategy. It can be deployed in remote areas, process large volumes quickly, can take oversupply form other management options (fish fertilising and anaerobic digestion), and provide good environmental outcomes. SESL Australia was commissioned to partner a trial with Camperdown Compost to determine the efficacy of composting dead carp using open windrow composting. The trial monitored compost temperature, moisture content, odour and leachate. Post-trial testing was conducted to determine the composts' nutrient status and to ensure compliance with AS4454-2012.

Location

The trial was conducted on a licenced EPA premises, owned and operated by Camperdown Compost at 445 Sandys Lane, Bookaar Victoria (Figure 1). All data was collated and input into cloud based software, demonstrating how data could be collected and managed from remote locations. This data will be useful in assisting future planning and seeking EPA approvals should the trial methodology be expanded. It will also demonstrate how the trial can be replicated and managed at diverse geographical sites.

FIGURE 1: **SITE LOCATION**
CLIENT: Camperdown Compost Company
PROJECT REF: C8080.B42507.Q6406 CC GME
GPS: Easting: 38.1028 Northing: 143.441 Datum 141 mAHD

Trial Methodology and Timelines

The method employed in this trial is informed by research into mass mortality composting carried out by the Victorian Dept of Primary Industries and Environment (Wilkinson, 2014) and earlier work by the Victorian Fisheries Authority (2008). Figure 2 shows a schematic of the structure of a compost heap using fish and co-composting materials following the methods cited above. The four treatments (comprising one row each) are:

1. Compost
2. Compost plus sawdust blend (50:50)
3. Sawdust
4. Straw

Fish / co-compost blends were assembled on a 1:2 basis by volume. Earlier chemical analysis of fish frames and whole carp, and industry experience with co-composting materials provide sufficient information to inform a starting C:N ratio of about 25:1. Each treatment was about 20m^3.

These proportions and starting ratios aimed to ensure rapid composting and suppression of odours. The capping with a layer of co-composting material also aimed to reduce odour.

The mechanical turning used a purpose-built compost turner of a type readily available in regional areas.

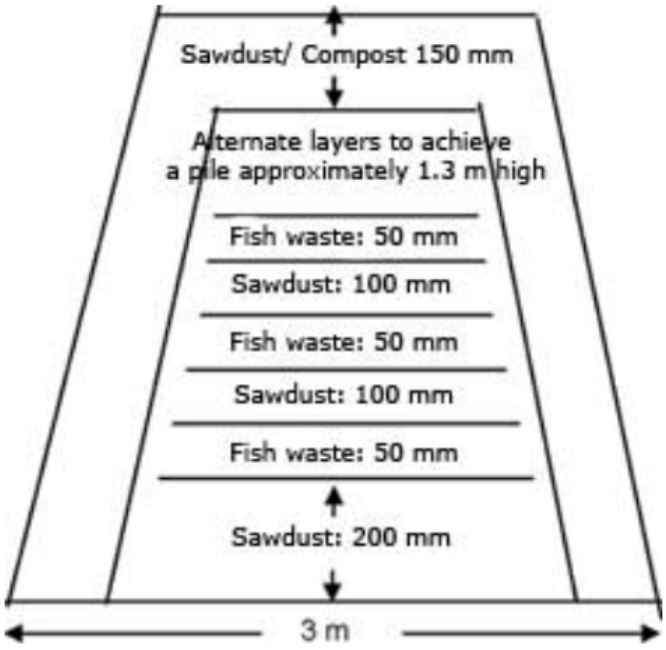

Figure 2. Schematic of compost windrow (VFA, 2018)

The feedstock's impact on the saleability of the final composted product was also noted, e.g. colour, texture, nutrient level etc.

About 40t of fish and fish carcasses were used in the trial with a corresponding amount of co-composting materials. The fish was made up of 20t of fish frames from the Melbourne market and 20t of carp sourced from Shepparton in regional Victoria.

Fish was composted in two principal stages. In Stage 1 composting, the pile was left undisturbed as soft tissue decomposed and bones partially softened. This stage allowed about 14 days. The compost was then turned and mixed to begin Stage 2 composting, during which time the remains of fish carcasses broke down further. Following completion of Stage 2 (~4 weeks), the composting process moved to completion during a curing phase of 6 weeks.

The procedure began with the laying down of a 20–30 cm base layer of an absorbent 'co-composting material' such as compost, sawdust or straw. The main function of this layer was to trap liquids released by the decomposing fish. Once the base layer was in place, fish carcasses were layered between alternating layers of co-composting materials as shown in figure 2. Alternate layers were constructed on the base layer using a skid-steer loader to form a windrow (a

type of elongated pile) with dimensions of three to four metres at the base and up to 1.8 m high. Each layer of fish was no deeper than 25 cm with 15 to 20 cm of co-composting materials between each layer. The final windrow was capped with 15 to 20 cm of co-composting material to ensure that all carcasses are covered. The final capping also served as a bio-filter to reduce odours (see Figure 2).

Each of four carbon feedstocks formed a separate batch identity to allow comparative temperature, moisture and breakdown data to be collected and collated. This methodology was designed to be replicable using locally available feedstocks e.g. straw, sawdust, and mature compost.

Temperature monitoring and starting moisture contents are critical. Compost progression was monitored by temperature and visual inspection of piles at turning. The composting process was concluded within a 12 weeks period.

Detailed temperature records are shown in Appendix B. The aim of any composting is to achieve temperatures in excess of 55°C. The compost treatment failed to reach this minimum temperature due most likely to the relative maturity of the compost and lack of labile carbon. Other treatments achieved minimum temperatures. The final treatment – straw – was terminated early in view of excessive odour and poor leachate control. Following the first turning, windrows were capped again with fresh co-composting material to a minimum depth of 10 cm. Further turns were based on temperature and the rate of decomposition.

A late addition to the trial saw the compost only, and sawdust only blends treated with a *lacto bacillus* culture. The *lacto bacillus* treatment was applied at row assembly by spraying the culture over the fish and capping material. Kim Russell and Gerry Gillespie of Resource Recovery Australia use the culture in static pile anaerobic composting in NSW and claim it significantly reduces odours. Gerry Gillespie provided the culture. The odour monitoring by Ektimo provides data on the effectiveness of this treatment.

Leachate from compost piles has the potential to negatively impact ground and surface waters, and may contribute to odour emissions. This trial was carried out on Camperdown Compost's licenced facility in South-West Victoria where the base layer complies with the requirements of EPA's publication 1588 for permeability of subgrade. Given that leachate through the subgrade will not occur, the piles were evaluated for evidence of leachate below the piles. Consideration was given for collection of leachate (if any) using plastic sheeting but the likelihood of it being ripped up by the compost turner rendered this option impractical. Consideration was also given to the use of a 'full stop'-type device which is used to map wetting fronts in soils under irrigation. Again the impermeability of the subgrade made the installation of such a device impractical. Leachate management used direct observation of the base of the compost piles. Piles were bunded with mature compost to ensure any leachate would be captured and not leave the site.

Following completion of the composting process, samples were dispatched to SESL Australia's NATA-accredited laboratory for testing to Australian Standard 4454 – Composts, soil conditioners and mulches. Finished composts were also tested for total elemental analysis to provide information on nutrient values.

Results

Odour

Odour levels were monitored throughout the composting process by Ektimo P/L. Information on the effectiveness of each treatment in controlling odour is important to determine effective separation distances from other land uses. Refer to the Ektimo report (Appendix A) for full analysis of the odour results.

Three of the composting blends (the straw blend wasn't monitored due to early termination) had odour levels monitored at three different stages of the composting process (Table 1). All the composts were two weeks old when odour monitoring commenced. The compost/sawdust blend was also monitored after the third turning at six weeks of age.

Time of Monitoring During the Composting Process	Unit of Odour Measurement	Compost Blend	Compost/Sawdust Blend	Sawdust Blend
Before 1st Turn	**OUV/min**	<85	<315	<80
During 1st Turn	**OUV/min**	370,000	1,000,000	270,000
After 1st Turn	**OUV/min**	<80	5900	13,000
After 3rd Turn	**OUV/min**	N/A	<90	N/A

Table 1. Summary of odour testing results undertaken by Ektimo.

The results show that the compost blend was the most effective at supressing odour after the turning process had been completed. Initially the sawdust blend was just as effective at supressing odour as the compost blend. However once the pile was disturbed by turning, odour levels were much slower to return to previous levels. The compost/sawdust blend released the most odour during the first turn. However, odour levels dropped quickly post-turning and after 6 weeks of composting the odour produced by the compost/sawdust blend had decreased dramatically.

The compost only and sawdust only blends were treated with a *lacto bacillus* culture. The *lacto bacillus* treatment may have reduced odours as the compost only and sawdust only blends had substantially lower readings than the compost/sawdust blend during and immediately post turning.

Throughout the trial temperature levels were monitored to track the progress of the composting process and to determine each treatment's relative effectiveness at decomposing the fish carcasses. For full temperature results see Appendix B.

The compost/sawdust blend was the quickest to reach effective compost temperatures. The compost only and sawdust only blends heated similarly but the sawdust achieved the highest temperatures for any blend. The straw blend was the least effective treatment at reaching critical temperatures needed for composting.

Leachate

Leachate emissions were monitored to determine the effectiveness of each blend to control leachate impacts on the surrounding environment. One of the aims of this trial was to determine if composting of dead fish is achievable on-farm in areas with permeable subsoils. The compost and compost/sawdust blends were the most effective at controlling leachate. The sawdust blend didn't absorb leachate as effectively as the compost only and compost/sawdust blends due to the relatively high moisture content of the sawdust and consequent low absorbance. Optimising moisture content of all co-composting materials is important to ensure good leachate capture. It is expected that sawdust would provide just as, or more, effective leachate control with lower starting moisture content. The straw blend failed to capture leachate due to its waxy cuticle and low absorbency.

Laboratory Testing

All compost and co-composting materials were analysed at SESL's NATA accredited laboratory. The fish frames and whole fish were analysed to determine their potential to provide sufficient nutrients to the composts. The finished compost, compost/sawdust and sawdust blends were tested against the AS4454 for composts, soil conditioners and mulches to ensure compliance. The compost blends were also analysed to determine their nutrient status for agronomic value. Full laboratory results are provided in Appendix C.

The fish frames and whole fish had very high levels of macro nutrients making them very suitable inputs to composting. Sodium, chloride and zinc levels were slightly elevated, however this was offset by blending with co-compost materials with low levels of these elements. Trace elements were generally low except for iron.

The compost blend passed all criteria under the AS4454 except for moisture content and toxicity. The moisture content was only slightly elevated and can be controlled by allowing the compost to mature for longer. The toxicity result reflected that the compost hadn't reached full maturity. This was also indicated by the elevated ammonium. Additional time would see the high ammonium convert to nitrate with an expected improvement in the toxicity result. The compost blend had acceptable to high levels of all macro nutrients and good levels of trace elements. Chloride levels were high but sodium was low. This blend had a good C:N ratio, acceptable levels of organic

matter, a slightly elevated EC and a slightly alkaline pH.

The compost/sawdust blend passed all criteria under the AS4454 except for toxicity. Again, the toxicity result indicated that this compost simply needs more time to reach maturity. The compost/sawdust blend had acceptable to high levels of all macro nutrients and good levels of trace elements. Chloride levels were also high but again sodium levels were low. This blend had a good C:N ratio, high levels of organic matter, an acceptable EC and a pH that was close to neutral.

The sawdust blend passed all criteria under the AS4454 except ammonium levels, proportion of large particles, toxicity, cadmium, copper and zinc. The larger sizes of sawdust was due to clumping of sawdust which is not particularly troublesome. If available, this blend could be put through a 5mm sieve to ensure compliance with the standard. The levels of ammonium and the toxicity result show that this compost is not yet fully mature. The sawdust itself is likely the source of the cadmium and copper contamination, particularly in the case of the elevated copper as this is used in some timber treatments. The sawdust blend had acceptable to high levels of all macro nutrients and good levels of trace elements. Chloride levels were also high but not as high as the other treatments. This blend had a poor C:N ratio which is to be expected due to the very high C:N ratio of sawdust. Organic matter was high, EC was acceptable, and pH was close to neutral.

Discussion

Composting process

Early in the trial it was apparent that the straw blend was not suitable for this composting process. It didn't capture odour or leachate effectively and was difficult to turn even for experienced operators. It is also relatively expensive and will be in high demand for other agriculture purposes because of the ongoing drought affecting regional Australia.

The sawdust blend showed that it could be effective in composting large quantities of fish carcasses; however it was not without its drawbacks. Odour levels were more noticeable with this blend. Testing showed that application of a lacto bacillus treatment could help, however this may increase the cost of composting. Testing against the Australian Standard showed that the sawdust blend was contaminated with cadmium, copper and zinc. As the two compost blends didn't have these issues it is likely the source of contamination is from the sawdust itself rather than the fish carcasses. The contamination in the sawdust is possibly from a small proportion of treated timber in the sawdust. The sawdust blend require sieving to meet the Australian Standard and market expectations. The finished product looked like dirty sawdust which may affect marketability.

However, if the issues of potential contamination can be managed, i.e. if sawdust is sourced from mills with no risk of treated timbers entering the supply chain, there are potential advantages in

using sawdust. Sawdust – unlike the other co-composting materials used in this trial – has a very high starting carbon to nitrogen ratio. This means composting using sawdust requires much higher levels of nitrogenous materials (i.e. fish) to satisfactorily compost. However, it is not simply a matter of adding a lot more fish to the blend as the sawdust would be slow to compost. It does open the possibility of repeated composting of the compost / fish blends. It is likely that having finished the first round of composting, the sawdust / fish blend could be blended with the same quantity of fish for a second, and potentially up to 4-5 rounds of composting. This would result in lower cost and a higher quality product after multiple composting events. This could make sawdust the preferred co-composting material where pulses of fish might be expected from river systems over a period of weeks or months.

The mature compost blend was effective at processing large quantities of fish carcasses. It had the least amount of odour and the finished product had a high nutrient value and was free of contamination. The finished product looked good which is important for marketability. This blend showed the best potential for areas that have sensitive receptors close by as it was the most effective at suppressing odour. However, temperatures were slow to reach optimal. This can be managed by increasing the proportion of fish in the starting blend to a 1:1.5 fish to compost ratio.

The compost/sawdust blend was the most effective at removing large quantities of fish as higher temperatures were achieved quickest in this blend. The finished product has high nutrient value and looks good. The blending of compost and sawdust alleviated the contamination issues associated with the sawdust blend. This blend has the greatest potential to remove the largest numbers of fish, however it produces more odour so may not be suitable in areas with sensitive receptors nearby.

Turner

This composting trial showed that a compost turner mounted on the back of a tractor is effective at managing the composting process. Tractor-driven turners are generally available in rural areas, can be moved between properties quickly and have the ability to turn up 900m^3 of compost per hour. A consistent end product can be produced without the need for high levels of training associated with more specialised turning equipment.

Conclusion

This trial has shown that there is good potential for composting to form part a multi-faceted management strategy to tackle large quantities of fish mortalities if the carp herpes virus is released. With some modifications, the composting process has the ability to pasteurise the biomass to minimize biosecurity issues, and produce a product with an attractive nutrient profile for agriculture. The methodology set out in this trial can be rolled out to remote areas with appropriate training.

This report has not addressed the logistics of rolling-out on-farm composting across large areas. However, the managers of this trial have considered the matter in depth and a sound conceptual working model has been developed. A cornerstone of this model involves the use of Camperdown Compost's proprietary cloud-based computer software which accurately tracks all aspects of the composting process together with details of inputs and on-farm operations. A training model has also been developed that would engage suitably skilled local staff such as those working for Catchment Management Authorities, Biosecurity staff from DWELP, or Land Services staff in NSW. These matters are outside the scope of this report but trial managers from SESL Australia and Camperdown Compost are available for detailed discussions as required.

Declan McDonald

Senior Soil Scientist

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RIDGE PARTNERS
CONSULTANTS AND ADVISERS

APPENDIX 5

CARP WASTE UTILISATION OPTIONS

A Final Report to

Curtin University, WA

October 2019

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Figures

Executive Summary

The Australian Government has established the National Carp Control Plan (NCCP) to assess the feasibility and potentially manage the release of *Cyprinid herpesvirus 3* as a biocontrol agent for the invasive fish species, carp. Australian waterways contain 350,000 – 1,000,000 tonnes of carp. Release of a carp-specific biocontrol agent will result in carp mortality across Australia's freshwater ways, triggering a large waste clean-up. The NCCP seeks to understand if and how this biomass can be employed for the benefit of communities, investors and the environment?

This report presents an initial cost-benefit analysis of 14 commercial supply chain scenarios for the beneficial use of waste carp. The project team has been led by Dr Janet Howieson from Curtin University.

Based on a global literature review and confidential consultation with Australian waste industry partners, the project identified ten carp utilisation pathways - seafood (live harvest only), rendering, hydrolysis, composting, anaerobic digestion, insect feed, vermiculture, mincing, torrefaction and collagen production. Pathways were then tested in a limited number of pilot trials regarding their efficacy, flexibility, and broad catchment scalability.

Four preferred utilisation pathways were identified across the 14 scenarios and subjected to commercial cost-benefit analyses:

Cost Benefit Analysis	1. Carp Seafood	2. Carp Meat Meal	3. Carp Hydrolysate	4. Carp Compost
Products	Niche market wild catch seafood for fresh domestic markets or overseas processing	Meat meal / oil from virus-killed waste carp rendered within 3 days of mortality	Hydrolysate liquid for use in fertiliser, aquafeed, and as a burley in fishing	Compost for use in agriculture, horticulture, and home-gardens.
Multisite Volume	Small (<10,000 tpa)	Large (>50,000 tpa)	Medium (~15,000 tpa)	Large (>100,000 tpa)
Pros	• High value use - fresh or processed fish • Niche urban domestic markets • Asian export markets	• Large domestic and global markets • Existing renderers • Existing EPA approvals	• Large global market • Existing processors • Lower input quality • Existing EPA approvals	• Flexible carp site processing options • Low technology • Large established consumer markets
Cons	• Limited domestic seafood demand • Must harvest live • High processing costs • Lack of supply chain capacity re volume	• Processors need supply certainty before they will invest • Input specifications are stringent • Prefer large volume long term contracts	• Processors need supply certainty before they will invest • Plants are remote from carp catchments • Prefer large volume long term contracts	• Requires large volumes of external carbon material (green waste) • May require individual EPA approvals for each individual site.
Est. Net Benefit Range per fish kg	• $0.22 - $0.57 per kg excluding fish cost	• $0.08 - $0.19 per kg excluding fish cost	• $0.07 - $0.11 per kg excluding fish cost	• ($0.09) - $0.44 per kg excluding fish cost

Assuming carp are free at the river bank, each of these pathways is commercially viable based on an analysis of operating costs. However, significant policy and commercial investment assumptions must be addressed to confirm any key issues and multiyear capital requirements, before the CBA can be progressed through to an Net Present Value point and related sensitivity analyses.

This report recommends NCCP consideration in 12 areas across the following broad issues:

- processors' rights to own and monetise any carp harvested,
- loss of product quality for virus-killed carp in a water column,
- supply chain transit limitations and food safety issues,
- regional harvest site accessibility, yield and viability,
- EPA approvals for compost transfers,
- Planning for staged multiyear mortalities that will greatly boost processor's motivation to invest, and
- availability of incentives for on-farm and regional composting.

1. Introduction

a. Background

Wild or common carp *(Cyprinus carpio)* are an invasive species found throughout Australian freshwater systems. The species is well established throughout the Murray-Darling Basin (MDB) and makes up to 90% of the fish biomass in some areas. Recent research has confirmed that the carp biomass comprises between 350,000 – 1,000,000 tonnes Australia wide, subject to catchment and regional rainfall and flooding events which promote carp infestation. Carp are damaging the ecology of Australia's freshwater ways (e.g. increased turbidity adversely impacts native water plants) and out-competing native fish species for food.

The National Carp Control Plan (NCCP) has been established by the Australian Government to assess the feasibility and potentially manage the release of Cyprinid herpesvirus 3 (CyHV-3) as a biocontrol agent for invasive carp. The virus was originally predicted to reduce the carp population by between 70±95% within the first few years.

The carp waste stream volume subsequently available is yet to be established by NCCP biomass and virus epidemiology experts. The volume and time scale will depend on the release strategy implemented. As a basis for commercial analysis, this study assumes the fish waste volume will be very large in the first few years after virus release, and at a lesser volume each year thereafter. A plateau volume may be reached within a decade. While birds in the catchment will consume a portion of deceased carp floating on waterways, it is expected that large scale carp mortality and decomposition will impact upon water quality and the environment. The large mass of deceased carp will require a large-scale clean-up, presenting a unique opportunity to utilise fish waste. Reduction in the time period between fish mortality and removal/harvest will reduce environmental impacts (decomposition and putrification, odour, oxygen extraction from waterways) and also improve the input quality of the carp waste stream to processors.

Currently carp are harvested on a small scale for various uses including human consumption as seafood, fishing bait, and for fertiliser production. However, as the potential carp waste stream resulting from virus release is very large other avenues for utilisation warrant further investigation. Compositional analysis, suitability of CyHV-3 infected fish for processing, pilot scale waste processing trials and subsequent commercial[1] product and market appraisal has been undertaken to identify best use options. Further development of new products utilising the infected deceased carp will assist in the clean-up, reduce disposal costs and generate regional and rural jobs across the catchment.

It is important to note that the proposed use options developed in this study, may still be relevant even if the carp virus is not released.

[1] The term "Commercial" used in this report refers to the activity of buying, selling or trading of any good or service by a firm, agency, organisation or person for direct or indirect economic gain. For-profit entities will typically undertake commercial transactions (rendering or seafood processing) primarily for direct financial gain. Not-for-profit entities, government agencies (state and local), Landcare Australia, Catchment Management Authorities, Community based organisations and other NGOs will typically undertake commercial transactions (waste management, water quality improvement, infrastructure development) that involve a greater indirect economic gain that benefits all members of a community. Both the direct financial values and a broader indirect economic value are embraced by any commercial activity described in this report.

This project (FRDC 2016/180 Assessment of Options for Utilisation of Virus Infected Carp) is managed by Dr Janet Howieson, a fisheries waste management expert based at Curtin University, Perth WA. The work undertaken in this Cost-Benefit Analyses project has been undertaken as a separate subcontract, based on consultation with Dr Howieson and relevant waste industry partners and investors.

A full social cost benefit analysis was beyond scope of this project.

b. Objectives

The overarching project objectives are:

1. To identify, pilot and undertake subsequent cost benefit analysis (CBA) for developing new processes/products from deceased feral carp (as part of National Carp Control Plan).
2. Contribute to relevant sections within the National Carp Control Plan detailing potential uses of dead carp biomass.
3. Articulation of potential uses of carp biomass, including costs and potential markets, to inform a cabinet submission.

c. Methodology

Dr Howieson's literature review, industry consultation and pilot trials have identified and trialled a range of waste utilisation solutions.

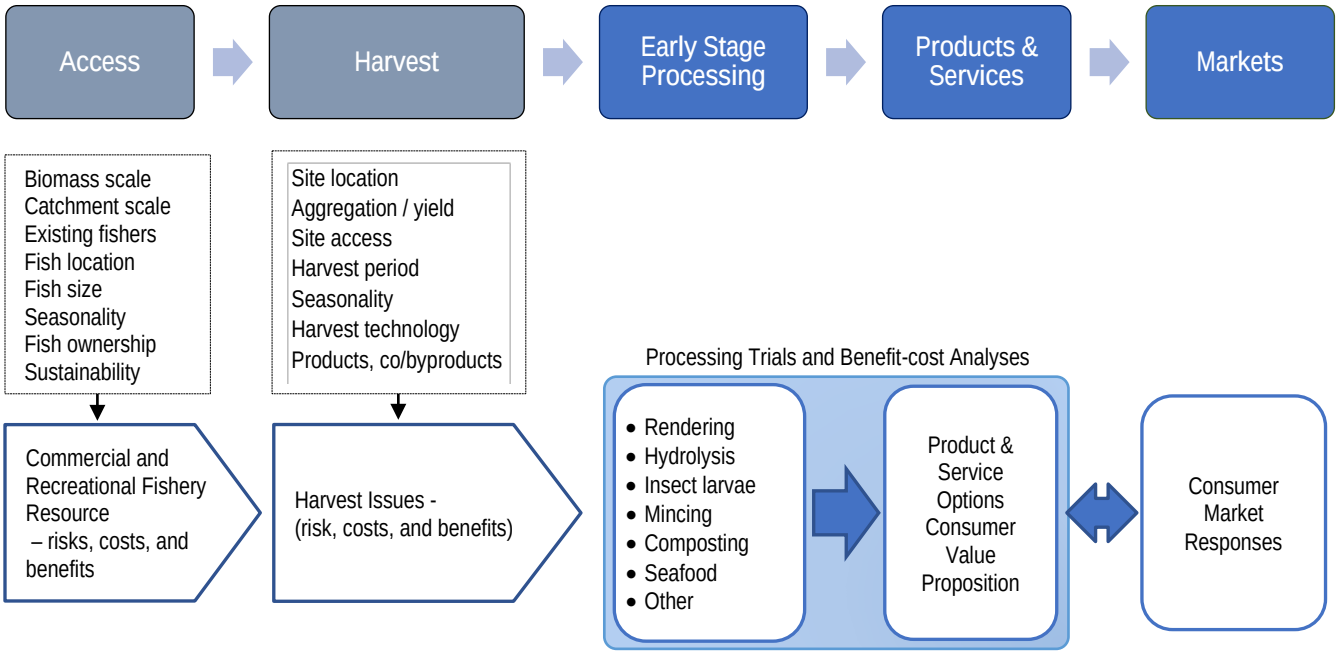
This research has established a short list of preferred carp waste treatment approaches that offer all stakeholders an appropriate utilisation outcome. The range of possible options has assessed solutions that encompass:

- Waste utilisation options for commercially harvested carp (i.e. fish mortality that is independent of the proposed virus release), and carp that have been killed by the virus,
- Broad geographic site locations for fish aggregation, fish harvest and processing; logistics related to all parts of the proposed processing chain; seasonality of carp aggregations across the catchment,
- The capacity and suitability of existing urban and regional infrastructure to support large scale carp waste utilisation, including road freight systems, and capital-intensive processing facilities (e.g. rendering to produce Meat meal and oil - MMO, hydrolysis, composting, waste water treatment, anaerobic digestion, knackeries, cogeneration, seafood processing and exporting facilities),
- New technologies (e.g. feeding waste to insect larvae, vermiculture, torrefaction) that may offer innovative waste management solutions,
- Pathways for regional industries (dairy, horticulture, agriculture) and communities to engage and invest in carp waste treatment and mitigation,
- Flexible, scalable and cost-effective waste treatment approaches that would be appropriate in remote parts of the catchment.

Figure 1 illustrates the project's supply-chain based methodology. The methodology implemented by the project team included:

- Identification of commercial experts and processors in both the Early Stage Processing and Products & Services elements of the supply chain,
- Small scale laboratory trials of carp waste processing options,
- Working confidentially with each chain partner to understand their existing supply chain and processing activities,
- Designing and conducting batch trials of carp product to determine process feasibility and outcomes,
- Confirming the proposed optimal carp processing activities, related supply chain activities and volumes, and carp waste product and service specifications,
- Documenting indicative scenarios, related process costs and benefits, and market values.

FIGURE 1. PROJECT METHODOLOGY



A range of waste treatment solutions were assessed. These treatment solutions generally divide into two separate utilisation options:

- Small scale (<10 tonnes of carp waste) community-based solutions (fermentative hydrolysis, vermiculture, etc) which are not expected to be commercially viable. These utilisation options are typically community based, have low input volume requirements, low capital investment requirements, and are small scale.
- Large-scale commercial options (composting, enzymatic hydrolysis, rendering, anaerobic digestion).

The small-scale solutions were costed but not subject to a cost benefit analysis in this project.

2. Broad Scale Carp Commercial Utilisation

a. Long List of Processing Options

Initial research undertaken by the project team has identified a preliminary list of ten carp waste treatment / product output approaches that could utilise the proposed carp waste stream, including:

i. Rendering – products are meat meal and meat oil (MMO) used in pet foods, aquafeed and livestock feeds,
ii. Hydrolysis – product is a hydrolysate liquid used as a fertiliser in agriculture, as fishing burley, or as an aquafeed ingredient,
iii. Composting – product is compost used in broadacre and intensive agriculture, and gardens,
iv. Anaerobic digestion (AD) – products are compost inputs (solids), and waste water as an input to methane production,
v. Seafood – products is processed finfish (whole, gilled & gutted, head-off & gutted) for human consumption,
vi. Insect feed – product is (black soldier fly) insect larvae that are used for aquafeed and livestock feeds,
vii. Vermiculture – product is vermicompost and related liquid fertiliser,
viii. Mincing - as a process to produce pet food, or as a preliminary stage to separate carp solids and liquids,
ix. Torrefaction – product is an alternative to inorganic fertiliser for use in agriculture,
x. Collagen – product is collagen extracted from fish scales and bones.

The research and collaborative trials also confirmed that some utilisation approaches may be adopted in series to optimise product outcomes. For example, mincing was identified as an early stage process that would improve the market appeal and value of petfood, enhance the efficiency of the hydrolysis process, and enable separation of solids and liquids prior to composting or anaerobic digestion.

b. Relevant Commercial Issues

Identification of a broad list of potential carp processing and product options (listed above) was a critical step in determining the carp biomass utilisation choices. Moving from broad potential options to target commercial options is challenging and subject to complex economic, social, regulatory and regional community variables.

We need to understand the broader dynamic issues at play in Australia's large carp infested basins including for example, the Murray Darling Basin MDB, (illustrated in Figure 2) so we can inform and refine our commercial utilisation, processing and product choices for waste carp. At the fundamental resource allocation and investment level, this means we must use the same approach to frame the cost benefit analyses that support these choices.

The following brief discussion identifies a number of critical issues relevant to the design and completion of the CBA :

i. Who owns the fish?

Using the MDB example, the basin spans five legislative jurisdictions (ACT, NSW, VIC, SA, QLD), some of which maintain fishery management regulations that specify the commercial harvest of live carp (ACT, NSW, SA only), as well as management of EPA agencies that regulate biologically active waste such as hydrolysate or compost.

Under the terms of relevant recreational or commercial fishing licences the harvested fish becomes the property of the licensed fisher at the harvest point. Currently, licensed fishermen in QLD and VIC can not capture and harvest live carp. The commercial sale of fish harvested under a recreational license is illegal in all Australian jurisdictions.

FIGURE 2. MAP OF MURRAY DARLING BASIN

Alternately, if the virus (or other biocontrol) is released by Commonwealth-State agreement and a carp that is killed by the virus is subsequently removed from the water column (dead carp may not always float on the water surface), who owns that fish? Does the harvester of a virus-killed carp have a right to commercially trade, process or monetise that fish for personal financial or community economic gain? Is a commercial price paid for this right?

Answers (and related policy frames) to these "property right" questions are fundamental to the carp utilisation cost benefit analyses context, as they clarify the extent to which down-stream carp processors will invest capital (financial and human) in securing access to supplies of carp. Large processors (e.g. rendering and hydrolysate) will not fully integrate carp waste streams into their long-term processing strategies unless these supply rights and issues are clearly defined.

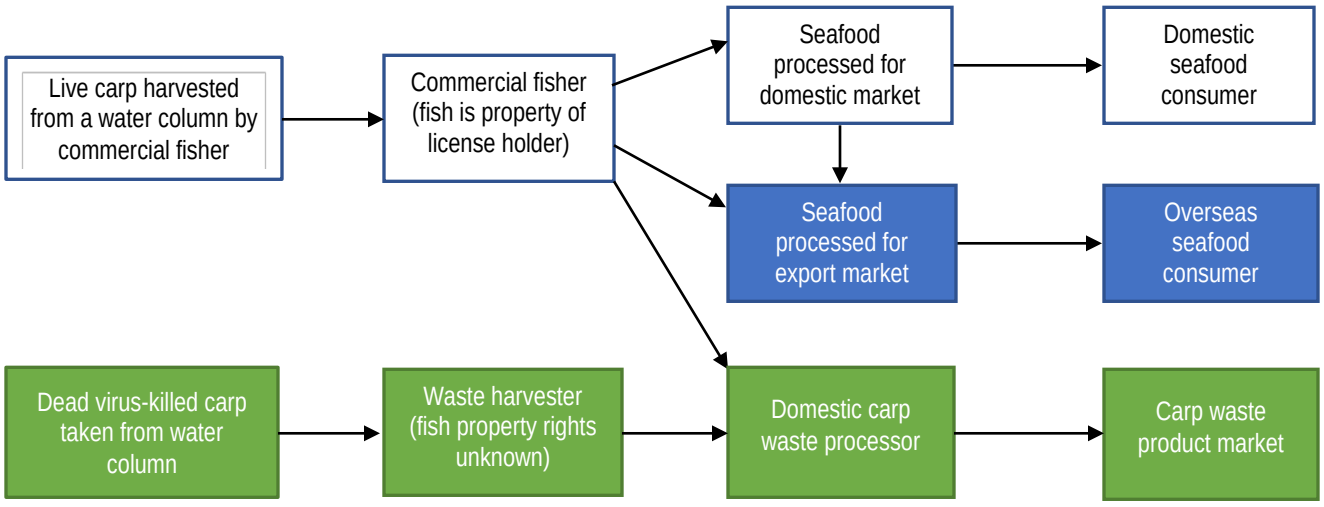
ii. Ongoing Commercial Fishery or Once-off Clean Up Strategy?

The NCCP aims to "help recover the health of Australian waterways and aquatic biodiversity". The Program will undertake a "risk assessment[2]" to determine whether biological control is likely to be viable for carp in Australia. If a bio-control is released, what is the strategic, spatial, temporal and volumetric profile (i.e. risk related carp volume) that will be available for processing? This risk-based approach is pursued in the following discussion.

Before investing in the carp utilisation opportunity, a commercial carp "investor" (especially those that are capital intensive) will want clear advice related to a number of risks identified below in order to derisk their investment.

- *Fishery Management or Waste Utilisation* - Under Australian seafood regulations in all jurisdictions, domestic common carp entering the seafood supply chain must be harvested by licensed commercial fishers as illustrated in Figure 3.

FIGURE 3. FISH STREAMS: FISHERY MANAGEMENT V VIRUS-KILLED WASTE



[2] NCCP Carp Fact Sheet September 2017

A carp that is captured live for seafood (even if harvested as a waste mitigation strategy) will therefore be the property of a commercial fisher at the harvest point. It will progress through the chain to a domestic or export seafood market. However, commercial fishers can also sell their catch to a domestic waste stream processor.

It is yet to be confirmed if a commercial fisher who identifies early stage virus infection in a commercially harvested carp is able to sell that carp (asymptomatic) to a domestic processor for export as seafood. (The Cyprinid herpesvirus 3 (CyHV-3) is specific to carp and there is no human food-consumption risk. The project team understands asymptomatic fish are sold commercially as seafood in Indonesia.) The assessment of the seafood market risk and commercial market utility of carp that carry evidence of the virus is yet to be clarified.

Harvesters who remove virus-killed carp from the water column must direct that fish to a non-waste stream processor who will service a waste product market.

- *Strategic multiyear risk* – Will a known supply volume of carp waste (live or dead) be available every year? For how many years will commercial volumes of waste carp be available? Will the biocontrol drive a once-off carp waste volume that needs to be processed (possibly subsidised by governments), or will there be annual ongoing supply of carp waste (that offers a commercial investment proposition) at lesser but significant volume? Some waste-processor investors will be keen to contract long term waste supply volumes that can be tuned to optimise their waste stream mix specifications. For example, a small renderer or dedicated fish hydrolysate plant may choose a specific mix of input waste species (beef, pork, tuna, kangaroo) in combination with carp to establish a new output product specification in a meat meal (MMO) or liquid fertiliser market place. Will it be viable for a processor to invest additional capital over many years?

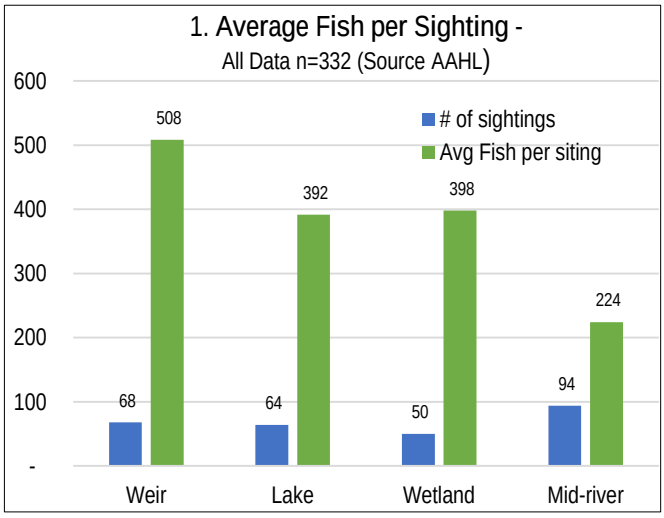
  Clearly the answers to these questions are somewhat dependent on the virus release strategy that may be implemented.

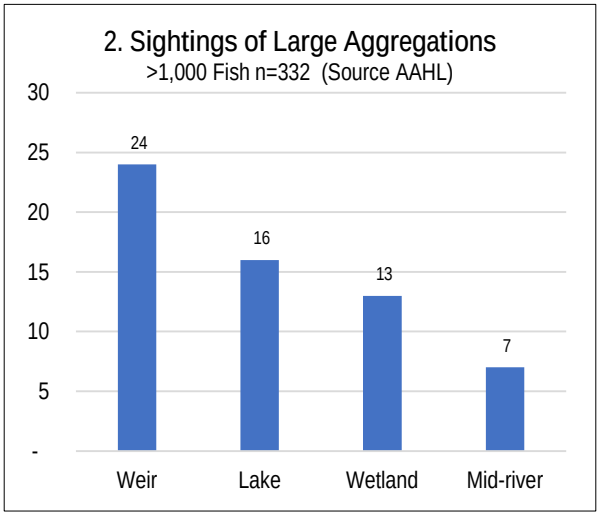
- *Spatial risk* – Australian catchments are geographically large (the MDB is ~2,000 klms long), meaning that the freight task is a big cost for carp removal (carp comprise >70% water at harvest). If we assume a minimum initial annual freight task involving 350,000 tonnes of waste carp at a very conservative multi-stage road-freight rate of $0.10 per kg, the cost of freight is $35 million.

  The optimum long-term processing solutions for carp across the basin will be those that are able to access, harvest, and process carp waste, and then cost-effectively deliver processed carp products to communities or commercial markets. The release of the virus will result in carp mortality that is often remote from these centres and their related infrastructure (roads, secure and serviceable river access points, labour sources, state and local government resources, and resources managed by Land Care and Catchment Management Authorities). Who will be responsible for the waste management task in remote areas of the basin?

- *Harvest point* - Where in the basin/catchment/waterway will the live or dead fish be available in optimum aggregations for harvest at high CPUE (Catch Per Unit Effort) rates, for freight uplift? As with all commercial fisheries, the cheapest fish to catch and commercially process is the one at the end of the jetty. There are therefore very strong commercial (i.e. freight) incentives for waste processors to only harvest carp that are near to harvest infrastructure such as jetties, concrete pads, roads and weirs. The project team notes that there are existing restrictions on wild catch fishing from or near weirs in some jurisdictions.
- *Temporal risk* – When will the proposed harvest occur? Will there be additional risks such as seasonal floods that may change the carp harvest CPUE, or competing uses for available infrastructure (e.g. road freight allocated to cart wheat during peak season, etc) at peak harvest times. And will the timing of the harvest complement or compete with other input supplies that the plant currently receives (e.g. abattoir or tuna aquaculture waste streams to rendering and hydrolysate plants are often seasonal and capacity to accept waste carp may not be available at some peak periods). Logistics options will dictate costs.
- *Volume supply risk* - How many tonnes of carp will a specific carp aggregation/harvest point yield? How many separate aggregations (i.e. harvest points) will be required to supply the minimum waste stream volume required for commercial viability of a processor? Where are these aggregation harvest points, how will their respective harvest yield change as the waste stream declines, what harvest infrastructure (e.g. road train concrete pads) is available there, and what will be the freight cost to move that waste product to the processing plant? Figure 4 presents early data developed by CSIRO (Australian Animal Health Laboratory) in 2018 from crowd sourced survey responses. While this data is useful, the CBA requires site specific data for locations (across the basin) and volumes from commercial fishers and experienced catchment watchers to adequately answer these supply-risk questions.

FIGURE 4. FISH AGGREGATIONS





iii. Infrastructure, Markets and Community Services

Commercial processing of carp waste will require appropriate infrastructure (harvest, freight and processing), efficient distribution channels, viable markets (to either return a commercial profit, or recover unsubsidised costs), and community services that are sustainable and efficient.

Figure 2 confirms there are many regional cities, towns and communities across the Murray Darling basin. Other carp-infested basins would present similar demographic trends. This urban/regional diversity is underpinned by additional diversity in:

- Land uses that offer markets for carp waste products – broadacre livestock grazing; intensive livestock operations such as pigs, poultry and dairy; broadacre cereals and pulses; horticulture and irrigated cotton and rice; urban centres that include seafood consumers and significant companion animal populations, etc,
- Labour and human capital availability,
- Road and rail freight infrastructure.

Access to fixed infrastructure (harvest vessels, jetties, etc) and mobile infrastructure (small and large road freight systems) will be critical to efficient and prompt capture of waste carp from the water column and despatch to a processing facility. For some processors (e.g. seafood processors and some renderers) any delay in this transit time will be critical to achieving the minimum quality specifications set for their processed output products.

From consultation with the partner waste stream processors, it is clear most have well established market offtake contracts for their end products. For example:

- Regional and urban renderers have contracts with buyers of petfood and products for companion animal sectors, aquafeed, and industrial users,
- Hydrolysate plants have supply offtake contracts with fertiliser merchandisers who break bulk, repackage and distribute to consumer markets. (The Charlie Carp hydrolysate processor based in Deniliquin, NSW is one well established branded processor).
- Compost companies supply regional (consumer product packs to retailers) and fertiliser (bulk volumes) distributors who service the dairy farming, home garden markets or specialist horticulture sectors.

Seafood processors build, invest-in and maintain similar commercial offtake agreements. Carp seafood supply contracts exist between commercial fishers and processors (e.g. Sydney Fish Market, and buyers at Melbourne and Adelaide fish markets – collectively process approximately 250-300 tonnes per year), but there are very few carp seafood contracts that commit volume to other domestic or export markets.

Many Australian marine wild fisheries are underutilised (e.g. Blue grenadier, squid, sardine, leather jackets, luderick, Australian salmon). However, over the last decade there is mounting evidence that rising demand for seafood in the Asian middleclass is driving increased interest and investment in Australian fisheries exports to those markets. Other major drivers for this increased investment include the weak Australian currency (relative to the US$ and Yuan), the number of new Free Trade Agreements that Australian has signed across emerging Asian economies, and the global recognition that Australian fisheries are clean, food safe, sustainable, and

among the best managed in the world. On the back of this market momentum, the opportunity exists for freshwater carp to be exported to Asia in larger volumes as a basis for direct seafood consumption and or value adding to manufactured seafood products. In addition, a number of existing niche seafood processors, traders and exporters are exporting carp roe to Germany.

Carp compost processing offers a range of flexible and community-based processing scenarios, including:

- Large private or local government fixed-site plants based in regional centres that co-process large volumes of urban and regional green waste, and waste carp,
- Small remote on-farm sites across catchments where land holders can access fish and secure the benefits of batched compost for spreading on farms,
- Point source emergency response sites across catchments where an anaerobic digestion-composting strategy can cost-effectively and promptly respond to a fish-kill, and hold carp waste volumes in suspension until a processing solution becomes available (e.g. site based composting).

Community or catchment-based coordination of these activities can be via local governments, regional Landcare groups, CMA's or other organisation in catchment communities.

There is need for a significant management and coordination plan to be established to align the waste management task with the proposed staged release of the virus, catchment by catchment. This Plan will dictate many of the critical assumptions that the CBA now needs.

3. Targeted Processing Options

a. Key Drivers of the Waste Value Proposition

Based on the preceding discussion the project team has identified four preferred processing options for commercial-volume carp waste streams.

These options are preferred (by the project team) based on their anticipated capacity to commercially capture, leverage, mitigate and/or resolve the issues, risks and challenges identified in the preceding discussion. Desk research, confidential discussion with industry parties, and joint pilot trials has identified the following short list of processing options that were progressed and subjected to benefit costs analyses.

FIGURE 5. TARGET PROCESSING AND UTILISATION OPTIONS

1. Seafood	2. Meat Meal + Oil (MMO) (Rendering)	3. Hydrolysate (Hydrolysing)	4. Compost + cogeneration
A. Quality & Value			
• High input quality • High product value • Export	• High input quality • Moderate product value • Domestic	• Average input quality • Low product value • Export and domestic	• Average input quality • Low product value • Domestic
B. Logistics			
• Small volume • Single site processing (medium freight costs)	• Moderate-High volume • Single site processing (medium freight costs)	• Moderate-High volume • Single site processing (high freight costs)	• Low-High volume • Multi-site processing (low freight costs) • Emergency fish kills – anaerobic digestion response
C. Commercial Products			
• Whole fish • Gilled & gutted • Headed & gutted • Roe • Heads – lobster bait	Ingredients for: • Aquafeed • Petfood • Livestock feed	• Agriculture fertilisers and treatments • Recreational fishing • Aquafeed ingredient • Industrial ingredients	• Agriculture markets (chemical fertiliser replacement) • Urban markets • Pelletised • Granulated

The table identifies three levels of waste / product assessment for each of the four utilisation options. Waste is not always waste, is the key point. Quality and value considerations are critical to seafood processing (e.g. food safety standards) and therefore attract a high market value per kilogram. Similarly, some rendering firms require very high and strict input quality specifications in line with their consumer market specifications (e.g. pet foods), while other renderers servicing lower value industrial markets will accept a lesser quality waste input stream. At the cheaper end of the spectrum, low quality specifications for inputs to composting mean that the output products also carry a low commercial market value.

The logistic input and output tasks vary according to the waste utilisation option.

Seafood processing will be a small volume outlet for carp based on the estimated sales margins available from exports and overseas manufacture. Currency volatility is therefore a significant commercial risk.

Many industrial waste processors and rendering plants are already located across the MDB at small and large single-site, capital-intensive processing factories. Their demand capacity ranges up to 15,000 tonnes per annum, but their freight costs will vary according to carp aggregations, harvest point yields, harvest accessibility, and road freight distances. At $0.10 per kilo this is an average cost per site in the order of $1.5 million.

Composting offers a very flexible waste utilisation option for large volumes (up to 15,000 tpa per site), and for small low-capital on-farm batch-based composting (~500 tpa per site). Composting sites can be close to the river bank, and require minimal capital investment and are therefore easily replicated across a catchment.

One option considered in the pilot trials was to mince whole carp and separate the solid and liquid waste streams. The solids would be sent to a composter and the liquids to a waste water (anaerobic digestion) cogeneration plant connected to the Australian electricity grid. While second stage (after initial waste processing) freight costs for each stream must be controlled, the separation of these streams results in a net gain in two ways:

- Lower composting charges per tonne of carp harvested (i.e. via a lower gate fee for the same harvest volume of fish), and
- Higher methane yields from carp-infused anaerobic digestion of liquids.

Anaerobic digestion is also a processing feature suited to remote emergency fish kills, where a rapid response is required to capture and hold large volumes of carp biomass in suspension until it can be processed locally via composting or other means.

The main commercial products are identified below for each of the target processing and utilisation options.

b. Pros and Cons of Target Utilisation Options

Figure 6 summarises the pros and cons for the four main utilisation options as well as the supplementary role that Anaerobic Digestion can offer.

FIGURE 6. TARGET PROCESSING OPTIONS - PROS AND CONS

Process Option	Pros	Cons
1. Rendering	• Large domestic and global markets exist for rendered meat meal and meat oil (MMO) products including pet foods and aquafeeds, • Many commercial rendering plants (large and small) exist in urban, and regional centres across the MDB and other catchments, servicing the livestock and feral animal industries (e.g. kangaroo, wild goats), • Rendering plants can efficiently process very large volumes of livestock or carp waste material, • Existing rendering plants will already hold all necessary operating permits and EPA approvals and meet environmental/ community standards.	• Rendering plants are capital intensive, long term investments. New ventures are unlikely to be developed while the location and volume of carp waste remains uncertain. Existing plants in catchments near carp harvest points are therefore most cost-effective. • Plant input specifications for rendering are quite stringent, requiring carp to be delivered within 12-24 hours of mortality. • Rendering plants are most cost effective when input wastes are forward contracted and scheduled in large volumes over annual cycles.
2. Hydrolysis	• Hydrolysate (liquid output from enzymatic hydrolysis) is basis for large global market for a very effective agricultural inoculant and fertiliser, with additional use as a recreational fishing burley or ingredient to aquafeeds, • There are several hydrolysate plants in Australia, including large processors of fishery wastes, • Waste input specifications allow a longer (1-3 days) post mortality period for fish wastes, • Large plants can efficiently process very large volumes of livestock or carp waste material, • Existing plants already hold necessary EPA approvals and meet community standards.	• Hydrolysis plants are capital intensive, long term investments. New ventures are unlikely to be developed while the location and volume of carp waste remains uncertain. • There are no large existing plants near carp harvest points. • Large hydrolysis plants are most cost effective when input wastes are forward contracted and scheduled in large volumes over annual cycles.
3. Composting	• Composting offers flexible and attractive carp processing options: o process volume can be small or large scale, o process sites can be urban, regional or remote, o minimal technology and new capital investment is required, o option to partially replace chemical fertilisers on-farm with locally produced compost, o sites can be farm based, or managed by local governments or Landcare/CMA managers, o compost products are well established in markets across broadscale agriculture, intensive and mixed farming, and regional and urban home-gardening.	• Requires a large readily available supply of carbon material, uncontaminated green waste, or sawdust to be input to the composting process, initially and progressively. • Subject to the jurisdiction, may require individual EPA approvals for each individual composting site.
4. Anaerobic Digestion	• Anaerobic digestion offers processing solutions at two levels: o capability to respond to large emergency fish kills by "harvest and hold" of waste carp in plastic liners, until composting or alternate process capacity is available, o in conjunction with waste water treatment facilities currently operated by local governments across regional Australia, anaerobic digestion offers a pathway to methane production at scale and therefore electricity cogeneration, • Separation of solids and liquids by mincing will enhance digestion and electricity cogeneration. • There are a number of large regional cities near carp infested catchments that already operate waste water treatment and anaerobic digestion plants.	• Waste water treatment plants are are capital intensive, long term investments. New ventures are unlikely to be developed while the location and volume of carp waste remains uncertain. • Waste water treatment / anaerobic digestion processors will require preliminary mincing of carp waste to separate solids and liquids.
5. Seafood	• Highest value use of available carp biomass, • Carp is a white fish meat available as a base for fresh, chilled, frozen processed or manufactured seafood (e.g. fish patties), • Carp is a seafood species that is already well established in Asian export markets serviced by Australian based processors.	• Carp seafood is generally unknown in domestic consumer markets and is currently accepted as a very small seafood niche market. • Carp seafood for Australian consumers will need to be harvested live. While the proposed carp virus has no human food consumption risk, the harvesting of carp killed by the virus will potentially not be an acceptable seafood source in consumer markets. • High processing costs for Australian based seafood supply chains competing in global carp seafood markets. • Seafood supply chains do not have the capacity to harvest, receive, process and distribute large volumes of carp in domestic or export markets.

4. Cost Benefit Analysis

a. Operational Cost Analysis

The project has developed a detailed spreadsheet analysis to compare the costs and benefits for fourteen commercial waste utilisation scenarios. This analysis does not include additional multiyear capital investment.

The analysis compares net monetary returns (estimated annual commercial sales margins less operating cost) for each of ten commercial industry partners across the fourteen waste utilisation scenarios.

Long term multi-year discounted cash flow investment return analyses have not been undertaken in this CBA for a number of reasons, including:

- The multiyear carp waste stream profile volumes are currently too uncertain, and related capital investment requirements to accommodate the known carp volume are therefore unknown. Any assessment of long-term investment performance on this basis would be very misleading.
- The ownership rights and processor risks associated with virus-killed fish are not clear and therefore cannot yet be factored into prices or returns,
- The level of subsidy available from governments for some scenarios (e.g. local composting, impacts of carbon credits, local government support) is unknown. The long-term commercial investment performance of these scenarios by private entities or community organisations would therefore also be misleading.

The spreadsheet model developed for this project is transferable (on negotiable terms) and can be used to assess the feasibility of other carp waste utilisation options not covered in this document.

A more detailed discussion of the general assumptions used, is as follows:

b. Assumptions

The following assumptions have been made by the project team in developing the target processing options and related Cost Benefit Analyses (CBA).

Figure 7 summarises the target utilisation options, and the respective value proposition drivers for a commercial value proposition. Each option has been tested across a number of cost benefit analyses suited to its specific supply characteristics.

The bottom line in the table lists the annual forecast tonnage (wet harvested weight) of carp for each of the fourteen scenarios addressed by the CBA. Seafood processing will require around one export container per month (480 tpa), while a large rendering, hydrolysing or composting plant will draw around 12,000 - 15,000 tpa of carp waste. To put this in context, the scale of the carp utilisation waste task (minimum 350,000 tpa previously noted) will require the equivalent services of around 30 large processors each receiving 12,000 tpa of carp waste.

Assumptions are as follows:

1. Processors proposing to invest in the carp waste utilisation task will face a range of issues and risks (briefly discussed earlier in this report in Chapter 2). The opportunity exists to receive and process carp waste as a single year once-off opportunity, or as a processing activity over a number of years. The current lack of details regarding the carp waste stream profile (multiyear risk related volume) means the logistics task is not yet planned, and longer term processor viability is very uncertain.

FIGURE 7. KEY ASSUMPTIONS

Utilisation Option	SEAFOOD	MEAT MEAL + OIL	HYDROLYSATE	COMPOST + Cogeneration
a. Commercial processing partner	• Seafood Processor	• Rendering Plant	• Hydrolysing Plant	• Composting + optional fish mincing to separate solids and liquid + optional anaerobic digestion (AD) for methane to cogeneration
b. Commercial products and markets	• Frozen seafood for export, • Coproducts (roe, heads)	• Dried fish meal and fish oil, • For aquafeed & livestock	• Liquid hydrolysate, • For agri & industrial	• Compost material for domestic market • Methane gas for domestic power cogeneration
c. Fish input quality required; Product output quality and markets	• Edible input quality - foodsafe • Edible quality - foodsafe • Export seafood; domestic bait	• High input quality • Moderate quality- petfood • Domestic & export	• Avg input quality • Low product quality • Domestic & export	• Avg input quality • Low product quality • Domestic
d. Processor benefits and Limitations	• Small volume • Single site processing • Commercial processing only	• Medium-high volume • Single site processing • Commercial processing only • Product cost & quality subject to mix of livestock inputs	• Med-high volume • Single site processing • Commercial processing only	• Low-high volume • Single or multisite processing in urban precinct or remote site • Flexible capacity to respond promptly to emergency fish kills • Potential engagement by Landcare, CMAs and local government • May require additional EPA approvals, and government subsidies
e. Harvest seasonality risk	• Not applicable	• Subject to requirements, harvest may need to align with other seasonal livestock	• Harvest to fit in with tuna and other seafood processing	• Remote on-farm composting near rivers, lakes etc is subject to wet season access. Some fixed site composting may be seasonal subject to availability of green waste carbon material.

CBA scenario	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Processor capacity	Medium	Medium	Medium	Large	Small	Large	Medium	Large	Small + cogen.	Medium + cogen.	Medium	Medium + AD	Medium	Large
Processor site	Urban	Urban	Urban	Region	Region	Urban	Region	Region	Region	Region	Remote	Remote	Region	Region
Product: format / quality	Whole	Gilled & gutted	Head off & gutted	Prem-ium	Average	Average	Average	Average	Average	Average	Average	Average	Average	Average
Carp input wet weight (tpa) per processor site	480	480	480	6,000	6,000	6,000	6,000	15,000	3,000	6,000	6,000	5,000	6,000	12,000

2. A consequence of this forward supply uncertainty is processors' likely inability to contract long-term input volumes, and therefore to estimate any necessary capital investment required to facilitate this forward waste volume. Two of the pilot trial commercial partners identified additional upfront capital expenditure required to facilitate their waste processing capacity, ranging from $260,000 to $600,000 for each processing company. In the absence of a satisfactory forward contract for waste supply volume they will seek this capital as a grant from government.

3. Carp aggregate in sufficient volumes at or near harvest service points to enable cost-effective collection, and freighting to a processing facility,
4. The time delay between carp mortality - harvest - processing is minimised to less than 72 hours in all cases (in extreme cases less than 24 hours),
5. The average weight per fish is 2.6 - 3.5 kg wet,
6. Fish that are killed by the virus when harvested are unencumbered and available for commercial processing,
7. Fish are available across all identified carp infested catchments at the water's edge,
8. Fish are available free of charge to the processor loaded in a road freight vehicle at the water's edge, ready to transport to a processing facility,
9. Current seasonality impacts on processors (i.e. availability of other input wastes, flooding in the catchments, etc) are modest and will not greatly affect the demand profile for waste carp across a year,
10. Discussions with commercial industry partners have been confidential, and any data provided to the project team will remain confidential to the project team. The CBA is therefore based on best estimates for each scenario as at November-December 2018.
11. Carp seafood exports will trade at a USD/AUD rate of $0.75,
12. Freight costs are forecast based on discussion with commercial partners, on a cost per kilometre per tonne basis. This approach will give a more accurate forecast of the real costs for diverse scenarios. Freight rates vary according to scenarios at four levels:
    - Local catchment freight and logistics charges for travel up to ~50 klms. This would potentially be in small fish bins (20 kg containers for seafood, or 1 tonne bins), or short-haul semitrailer movements,
    - Long-haul freight of waste carp to a processing centre on main high ways in semitrailers,
    - Specific second stage freight for selected output products (i.e. fish waste from a seafood processor),
    - Other input-material freight (e.g. sawdust and urban green waste) is costed at a CIF delivered rate to the processor.
13. Estimated processor yields are identified for each scenario based on discussion with industry partners. These may vary significantly subject to the quality specification targeted by the specific processor in each scenario (e.g. seafood processing yields (whole, G&G, H&G), and rendering yields).
14. Secondary freight costs have been included for minced carp solids and liquids directed to other processors, and for coproducts/byproducts sold CIF (e.g. seafood fish viscera waste streams, carp heads as lobster bait, and roe).
15. Disposal costs have been included where relevant for such things as waste bone and powder from the hydrolysing process.
16. Composting costs and returns are more complex as the range of waste utilisation scenarios is broader that other target processes. Where relevant these costs and revenues include carbon input costs (e.g. sawdust, or green waste), compost site preparation, monitoring by regulators and experts, farmer and staff training, and use of proprietary site management software.

17. GST impacts have been ignored in all figures. Seafood will not attract GST but all other non-food product options for domestic sale will incur a GST charge on sale.

c. Why Seafood has been included as a Utilisation Option

This project is about the beneficial utilisation of the carp waste biomass, that will result from release of the herpes virus. All utilisation options (identified in Chapter 2. a) except seafood are directly subject to carp mortality resulting from the virus.

During the development of the processing pilot trials and subsequent cost benefit analyses, the project team came to the view that the analyses would be deficient without reference to a comparison with a seafood utilisation option. The carp seafood utilisation scenario is likely to achieve the highest gross sales value per kg for carp, a modest net financial return for seafood processors, but (on current estimates) the process will use only a very small volume of fish. Based on current sales forecasts (processing yield, costs and margins), seafood carp will not make a significant contribution to resolving the "carp control" problem, but with regulatory approval, and the right supply chain and partners could be a significant and viable long-term utilisation option.

The carp seafood option that exists today (via commercial fishers), is not subject to the release of the virus or other biocontrols and, we understand, will continue to exist during and after the release of the proposed virus. It therefore requires an initial cost benefit analyses as part of this project.

To investigate this carp seafood option the project team worked with an experienced and motivated commercial seafood processor based in Australia, and an Asia based buyer/seafood manufacturer prepared to consider a significant monthly shipment of frozen processed carp.

d. CBA Findings

Figures 8 and 9 summarise the CBA findings for the fourteen utilisation scenarios. These data and analyses are confidential and specific to the commercial waste utilisation scenarios developed with commercial partners. They should not be used as a guide for any other purpose. They do not present a final CBA analysis based on an NPV.

These estimates are based on best available data and the assumptions described in this report. As agreed with NCCP Executives the harvest cost of carp landed into a transport vehicle at the water's edge is not included in these estimated cost and benefit findings.

Each group of scenarios highlighted in colour, compares the costs per kilogram of input waste carp for each of the various scenarios relevant to the target utilisations, as follows:

1. Seafood - blue bars:
   - Whole fish,
   - Gilled and gutted fish,
   - Head off and gutted fish.
2. Meat meal – green bars:
   - Premium grade quality output - rural processor,
   - Low grade quality output – rural processor,
   - Medium grade quality output – Melbourne based processor.

3. Hydrolysate:
   - Low volume processor,
   - High volume processor,

4. Compost:
   - Minced fish compost + liquids cogeneration - low volume,
   - Minced fish compost + liquids cogeneration – high volume,
   - Whole fish compost – rural remote batched processor,
   - Whole fish compost - remote anaerobic digestion processor,
   - Whole fish compost - low volume fixed processor,
   - Whole fish compost - high volume fixed processor.

The **cost per kilogram of input carp ranges from $0.08 / kg to $2.59 / kg.** In parallel, the black or red bars estimate the **net benefit or loss (before tax) for each scenario ranging from a loss of $0.09 / kg to a surplus of $0.57 / kg of input carp.**

FIGURE 8. CARP WASTE UTILISATION - CBA SUMMARY OF FINDINGS

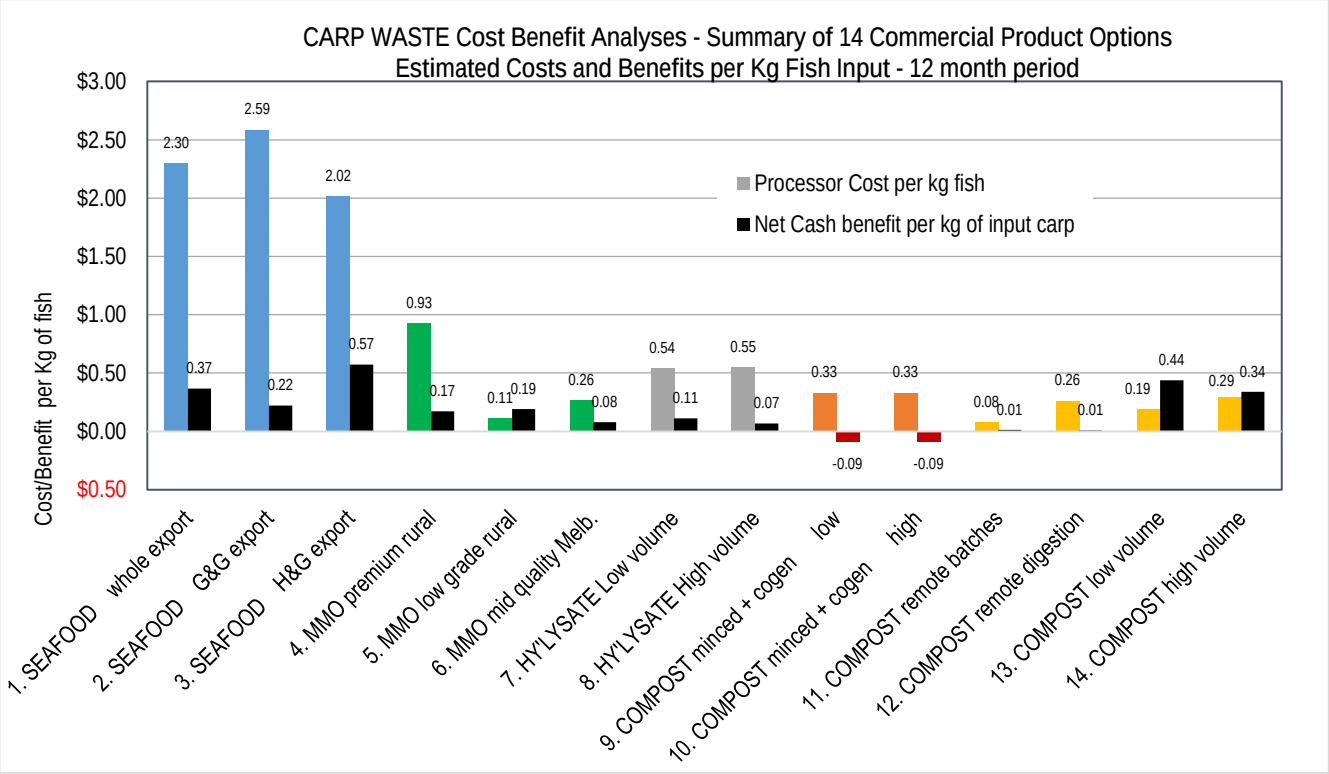


Figure 9 also presents more detail regarding the assumptions for each utilisation scenario.

One obvious question arises: Would any of these 14 utilisation options be commercially viable even if the virus is not released? All 14 utilisation options are feasible today (from a technical, harvest, and supply chain perspective) and are not dependent on the proposed release of the virus. So why aren't processors investing privately now to build capacity to access and process carp biomass? Discussion with large processors and

supply chain partners (across rendering, hydrolysing, and composting) suggests that their investment risks and uncertainty arise from a number of sources (some previously noted):

- Processors' lack of awareness of the NCCP's common carp control issue and related potential commercial solutions being assessed by government,
- Lack of political or government confirmation that a biological control will or will not be approved for release, and a release plan published,
- It is not yet clear from NCCP how the impacts of a virus release will be staged or manifest. Will release be a once-off virus release that kills the vast bulk of fish across catchments in the initial years, or will release of the virus be staged over multiple years and so enable planned long-term investment by processors in harvest capacity? Is it to be a carp supply "clean-up" scenario, or a carp "managed fishery" scenario, or a combination of both?
- Input costs of carp (i.e. cost of fish aggregation access, harvest, and freight to the processing site) for a processor are too high at present to enable viable commercial processing supply chains to markets. Release of a carp control vector will confirm that the government (on behalf of the public and the environment) is investing in carp utilisation and eradication. Such a release will switch the carp supply chain value proposition from the current "commercial market pull" approach that must achieve a positive profit margin, to a new "environmental public push" approach. A processors' primary driver for carp utilisation and investment will therefore change from a singular focus on commercial market returns, to a balanced approach where processors' returns will be partially subsidised to invest in carp utilisation and eradication. Under this balanced approach, subsidies could potentially relate to fish input cost reimbursement, incentives to farmers to replace existing chemical fertilisers with locally produced carp compost, or subsidies to local governments and CMAs to establish and support local regional composting ventures.
- The rights to access and process virus-killed carp over multiyear investment horizons are not yet confirmed by government.
- Product specifications are not yet established for carp-based products, therefore related downstream market scale and competitive issues are yet to be determined. For example, an aquafeed manufacturer will not be able to determine its commercially optimum processing input volumes until it has secured regulatory approvals and has negotiated contracts for supply with downstream customers.

e. Analysis Risks & Gaps

The CBA's initial findings are best estimates drawn by the project team from desk research, industry partner discussions, collaborative pilot processing trials and internal analysis. They do not yet enable a complete CBA.

The project has drawn a broad range of private waste processors and value chains across the Australia's largest carp-infested catchment, into likely scenarios that would process carp at various levels of throughput. These are "operating cost" scenarios and therefore do not including changes in long term capital values or investment returns.

These CBA findings are therefore only indicative costs and benefits for each scenario based on the assumptions listed in this report and volumes of carp available for processing. Due to the significant uncertainty related to point source harvest costs and carp ownership, the analyses assume that the harvest cost of the carp is not included in the estimated analysis. Completion of the CBA requires early agreement on key policy and investment issues.

Recommendations

The project team has identified a number of findings from the CBA, including gaps that inhibit our understanding of the optimum carp utilisation pathways. The project team recommends the NCCP consider the following issues:

1. Clarify who owns a carp killed by the virus, at the harvest point, and confirm if processors own the final processed product,
2. Confirm deactivation parameters for the proposed virus to ensure that virus infected fish products are safe and commercially acceptable for their respective proposed uses,
3. Confirm that virus exposed or infected fish with lesions are acceptable regarding product integrity, for seafood or other processed products. This risk relates to both single year and multiyear commercial harvest of asymptomatic seafood.
4. Provide greater detail regarding the quality of virus-killed carp available for removal during the “clean-up”. For example, will dead fish initially sink in the water column and be more difficult to harvest, and at what stage (number of hours after mortality) of deterioration will fish float to the surface of the water column?
5. Confirm the definition of virus infected fish for transport and processing: ”biological waste” rather than ”infectious agent?” This clarification has direct implications for regulatory approvals and transport costs.
6. Consider minimum specifications for carp product quality (e.g. carp seafood exposed to the virus) to ensure markets are fully informed and consumers are not at risk.
7. Confirm the yield and location of top fish aggregation and harvest sites across catchments. This data will greatly inform investors, and derisk harvest and freight costs for large processors.
8. Consider the added benefits and costs that would accrue if large processors (renderers, hydrolysers, large composters) commit to large waste stream forward offtake contracts from the infected waterways. The benefits of contracted multiyear supply of large fish volumes could drive substantial improvements in the viability of scenarios analysed in the CBA.
9. Confirm with Federal/State agencies and relevant EPA managers the procedures required regarding transport and remote composting, and related aspects of other processes (e.g. anaerobic digestion),
10. Confirm if/how carbon credits impact farm composting values and returns,
11. Confirm if/how government subsidies apply to compost sites managed by Landcare / CMA’s / Councils,
12. Ensure that any virus release strategy policy and planning development is aligned with, and guided by realistic commercial utilisation, supply chain and market demand considerations. Planning for the carp utilisation waste task (minimum 350,000 tpa) will require the equivalent services of at least 30 large

processors each receiving up to 12,000 tpa of carp waste. This requires significant engagement and coordination with commercial processors to ensure efficient community and commercial outcomes.

FIGURE 9. CARP WASTE UTILISATION - OPTIONS, COSTS AND FINANCIAL BENEFITS

Carp Utilisation Options	SEAFOOD	MEAT MEAL + OIL	HYDROLYSATE	COMPOST + Cogeneration
a. Commercial processing partner	• Seafood Processor	• Rendering Plant	• Hydrolysing Plant	• Composting + optional fish mincing to separate solids and liquid + optional anaerobic digestion (AD) for methane to cogeneration
b. Commercial products and markets	• Frozen seafood for export, • Coproducts (roe, heads)	• Dried fish meal and fish oil, • For aquafeed & livestock	• Liquid product, • For agricultural and industrial	• Compost material for domestic market • Methane gas for domestic power cogeneration
c. Fish input quality required; Product output quality and markets	• Edible input quality - foodsafe • Edible quality - foodsafe • Export seafood; domestic bait	• High input quality • Edible quality - petfood • Domestic & export	• Avg input quality • Industrial product quality • Domestic and export	• Avg input quality • Industrial product quality • Domestic
d. Processor benefits and Limitations	• Small volume • Single site processing • Commercial processing only	• Medium-high volume • Single site processing • Commercial processing only • Product cost & quality subject to mix of livestock inputs	• Med-high volume • Single site processing • Commercial processing only	• Low-high volume • Single or multisite processing in urban precinct or remote site • Flexible capacity to respond promptly to emergency fish kills • Potential engagement by Landcare, CMAs and local government • May require additional EPA approvals, and government subsidies

Cost – Benefit Scenario	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
Processor capacity	Medium	Medium	Medium	Large	Small	Large	Medium	Large	Small + cogen.	Medium + cogen.	Medium	Medium + AD	Medium	Large
Processor site	Urban	Urban	Urban	Region	Region	Urban	Region	Region	Region	Region	Remote	Remote	Region	Region
Product - format/quality	Whole	Gilled & gutted	Head off & gutted	Premium	Average	Average	Average	Average	Average	Average	Average	Average	Average	Average
1. Carp input wet weight (tpa) per processor site	480	480	480	6,000	6,000	6,000	6,000	15,000	3,000	6,000	6,000	5,000	6,000	12,000
2. Average fish wet weight (kg)	3.5	3.5	3.5	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
3. Est. no. of fish per year ('000)	137	137	137	2,308	2,308	2,308	2,308	5,769	1,154	2,308	2,308	1,923	2,308	4,615
4. Est. avg. freight input cost ($/t)	300	300	300	30	60	64	95	95	40	40	35	35	92	92
5. Other material inputs (t)	nil	nil	nil	nil	nil	nil	nil	nil	788	1,575	3,000	800	4,500	9,000
6. Product: Carp seafood (t)	480	425	316											
Carp seafood roe (t)		11	11											
Bait - fish heads (t)			110											
Carp meat meal (t)				4,500	1,560	1,320								
Carp oil (kl)				684	684	456								
Hydrolysate (kl)							4,920	12,300						
Compost (t)									1,890	3,780	3,591	9,000	10,800	21,600
7. Additional CAPEX ($'000)							260	600	317	317				
8. Est. Cost ($/kg fish)	$2.30	$2.59	$2.02	$0.93	$0.11	$0.26	$0.54	$0.55	$0.33	$0.33	$0.08	$0.26	$0.19	$0.29
9. Est. Net Benefits ($/kg fish)	$0.37	$0.22	$0.57	$0.17	$0.19	$0.08	$0.11	$0.07	-$0.09	-$0.09	$0.01	$0.01	$0.44	$0.34

5. Sensitivity Analysis

The Cost-Benefit Analysis is based on best estimates of carp availability, supply and price, and supply chain costs for fourteen individual carp utilisation scenarios. As previous described, some scenarios have been readily costed to existing processing supply chains and facilities, while others (e.g. farm based composting, emergency anaerobic digestion, methane cogeneration) are based on proposed waste processing streams yet to be developed in unknown locations across the catchments.

Data in each scenario is supported by confidential discussion with industry partners and pilot trial processing data. This analysis is only at the prefeasibility level of development and not in any way representative of a comprehensive feasibility or CBA of the respective scenarios that would be expected by an investor or supporting agency. Detailed investor feasibility analyses or business planning will only be possible when the fish harvest, supply and forward contracting questions (previously discussed in this report) have been addressed. Therefore, comprehensive sensitivity and comparison analysis is not yet possible nor meaningful for these fourteen scenarios.

However, there are at least two critical (and therefore sensitive) cost – benefit variables that can be considered in a meaningful way: freight cost, and cost of fish. Freight costs have been included in the CBA, but fish costs have not.

a. Freight

As previously noted, freight is a significant component of the carp utilisation task. This cost is subject to the high water content of fish, and the large and often remote geography where carp harvesting and processing will be undertaken. Freight costs per scenario will range from $140,000 to $1,500,000 per year, or $30/tonne to $300/tonne. Freight costs will consume a large portion of the revenue generated from fish sales, ranging from 13% to 55%.

Planning undertaken prior to the release of the virus should therefore focus on ways and means to increase the efficiency of the freight task across all processing scenarios.

b. Cost of fish

It has been noted previously that the cost of the fish (harvested by a commercial fisher, or as a result of virus-induced mortality) has been excluded from the cost-benefit analyses. There are many complex variables that impact the harvest and collection process for carp (e.g. fish aggregation location, harvest point yield and duration, infrastructure availability, seasonality, etc). This cost is very uncertain.

However, a simple assumption regarding rising fish input costs will inform our understanding of the sensitivity of this variable. Figure 10 identifies for each scenario the estimated cost price of fish at the water's edge that will reduce commercial operating benefits to nil. Black numbers are estimated annual commercial benefits above zero – red numbers are estimated benefits below zero. The table suggests that a cost of fish above 20 cents per

kilogram at the water's edge will eliminate any commercial gain from most scenarios. These figures should be used with caution as a guide only.

FIGURE 10. SENSITIVITY TO FISH HARVEST COST

	SEAFOOD			MEAT MEAL + OIL			HYDROLYSATE		COMPOST					
Fish Cost $/kg	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
0.00	176,000	106,379	274,673	1,033,200	1,150,200	472,200	674,820	1,019,250	-272,550	-545,100	75,757	40,756	2,628,000	4,056,000
0.05	152,000	82,379	250,673	733,200	850,200	172,200	374,820	269,250	-422,550	-845,100	-224,243	-209,244	2,328,000	3,456,000
0.10	128,000	58,379	226,673	433,200	550,200	-127,800	74,820	-480,750	-572,550	-1,145,100	-524,243	-459,244	2,028,000	2,856,000
0.15	104,000	34,379	202,673	133,200	250,200	-427,800	-225,180	-1,230,750	-722,550	-1,445,100	-824,243	-709,244	1,728,000	2,256,000
0.20	80,000	10,379	178,673	-166,800	-49,800	-727,800	-525,180	-1,980,750	-872,550	-1,745,100	-1,124,243	-959,244	1,428,000	1,656,000
0.25	56,000	-13,621	154,673	-466,800	-349,800	-1,027,800	-825,180	-2,730,750	-1,022,550	-2,045,100	-1,424,243	-1,209,244	1,128,000	1,056,000
0.30	32,000	-37,621	130,673	-766,800	-649,800	-1,327,800	-1,125,180	-3,480,750	-1,172,550	-2,345,100	-1,724,243	-1,459,244	828,000	456,000
0.35	8,000	-61,621	106,673	-1,066,800	-949,800	-1,627,800	-1,425,180	-4,230,750	-1,322,550	-2,645,100	-2,024,243	-1,709,244	528,000	-144,000
0.40	-16,000	-85,621	82,673	-1,366,800	-1,249,800	-1,927,800	-1,725,180	-4,980,750	-1,472,550	-2,945,100	-2,324,243	-1,959,244	228,000	-744,000
0.45	-40,000	-109,621	58,673	-1,666,800	-1,549,800	-2,227,800	-2,025,180	-5,730,750	-1,622,550	-3,245,100	-2,624,243	-2,209,244	-72,000	-1,344,000
0.50	-64,000	-133,621	34,673	-1,966,800	-1,849,800	-2,527,800	-2,325,180	-6,480,750	-1,772,550	-3,545,100	-2,924,243	-2,459,244	-372,000	-1,944,000
0.55	-88,000	-157,621	10,673	-2,266,800	-2,149,800	-2,827,800	-2,625,180	-7,230,750	-1,922,550	-3,845,100	-3,224,243	-2,709,244	-672,000	-2,544,000
0.60	-112,000	-181,621	-13,327	-2,566,800	-2,449,800	-3,127,800	-2,925,180	-7,980,750	-2,072,550	-4,145,100	-3,524,243	-2,959,244	-972,000	-3,144,000
0.65	-136,000	-205,621	-37,327	-2,866,800	-2,749,800	-3,427,800	-3,225,180	-8,730,750	-2,222,550	-4,445,100	-3,824,243	-3,209,244	-1,272,000	-3,744,000
0.70	-160,000	-229,621	-61,327	-3,166,800	-3,049,800	-3,727,800	-3,525,180	-9,480,750	-2,372,550	-4,745,100	-4,124,243	-3,459,244	-1,572,000	-4,344,000

Appendix 6: Carp Biomass Fermentation

Standard Operating Procedure

This document outlines the steps required to effectively process carp biomass into an agricultural biofertiliser/biostimulant using hydrolysis and lactic acid fermentation. By following these instructions a product that meets the *National Code of Practice for Fertiliser Description and La belling can be achieved.*

Contents

Important

The processing of feral carp for use as a biofertiliser/biostimulant requires a number of steps that will result in a consistent and safe quality product if followed. There are two main WHS risks are associated with this process:

1. The risk of the product containing biological pathogens
2. The risk of workers getting injured while making the product through activities including heavy lifting and operation of equipment; and/or direct physical contact with bones.

Therefore any person or group intending to follow this procedure on-farm should therefore conduct a risk assessment, identify any risks and put in place Safe Work Procedures to protect workers prior to commencing manufacturing. Wearing protective clothing according to WHS protocols is required, including during sampling of material for analysis.

Recipe

Carp biomass fermentation requires the following ingredients:

- **Clean water** – non chlorinated
- **Carp biomass** – needs to be shredded or macerated to less than 10mm pieces if possible
- **Molasses**
- **LAB Serum Inoculant** – the procedure uses a Lactobacillus based inoculant called LAB Serum. Contact details for making this inoculant are at the end of this document. Alternate commercially made Lactobacillus based inoculants are available to use. Confirm with the supplier that they are Lactobacillus based. Common terms for these type of products include “EM” or “Lacto”.

General proportions of ingredients used in this process are:

- 1 part - Carp Biomass
- 1 part – Clean water
- 0.10 to 0.15 parts - Molasses
- 0.15 to 0.2 parts - Lab Serum Inoculant

Batch Recipes

200 L Barrel		1000L IBC	
Carp Biomass	80 L*	High Protein Biomass	400 L*
Water	80 L	Water	400 L
Lab Serum	16 L	Lab Serum	80 L
Molasses	11 L	Molasses	55 L

* Recipe is by volume not by weight.

Equipment/Resources

Loading equipment	A tractor with forks, skid steer loader or a backhoe are needed to lift and shift containers/tanks and materials.
Receiving Tanks/Drums	These are needed to store carp biomass prior to maceration/shredding.
Pallets	These can be used as a platform for bulk containers and make it easy to shift and store materials during the process.
Macerator/ Process Shredder	Used to macerate or shred the carp biomass into fine pieces or a pulp.
Pump/Hoses/Fittings	Used to pump biomass or final product slurry from one tank to another if required. Sump pumps work well.
Molasses	Key ingredient that provides energy to the fermentation.
Water	Key ingredient. It should be clean and non-chlorinated.
Inoculant^ (Lab Serum)	Key ingredient that provides fermenting bacteria to the ferment. In this recipe *Lactobacillus* bacteria are used. See note at end of this Table for more details on the inoculant.
Fermentation Tanks/Drums	Used to ferment the biomass. It needs to have a fermentation lock attached to it so it can be sealed from oxygen but release gas as the fermentation proceeds. IBCs (1000L shuttles) are the easiest to use. 200L drums are also OK for smaller scale production.
Hand-held pH meter	Used to monitor the pH during the fermentation process. A probe or litmus paper kit is best.
Thermometer	Used to measure temperature during the fermentation process. Ideally a probe thermometer designed for liquids is best.
Sample Bottles	Used to take samples for sending to the lab for analysis of final product. Urine sample bottles are ideal. They can be purchased from most pharmacies.
Heating system (optional)	In cold temperatures the fermentation may not happen. If making the product in a cold winter then some kind of heating is needed. Either heat the room or heating mats can be wrapped around the fermentation tank.
Monitoring Record Sheet	Used to keep track of temperature and pH during the fermentation process.
Analysis Laboratory*	When the fermentation process is finished then a sample of the product needs to be tested to ensure it contains no pathogens and to measure its nutrient levels.

^ **Inoculant** – *This procedure uses a Lactobacillus based inoculant called LAB Serum. Contact details for making this inoculant are at the end of this document. Alternate commercially made Lactobacillus based inoculants are available to use. Confirm with the supplier that they are Lactobacillus based. Common terms for these products include "EM" or "Lacto". If using a commercial product then it is recommended to do a trial batch to confirm the effectiveness of the product before commencing large scale production.*

* **Analysis Laboratory** – *It is important to verify that the final product is safe and contains nutrients. It is recommended to use a NATA accredited laboratory. The analysis needs to test total nutrients and biological pathogens at a minimum.*

Standard Operating Procedure

Stage	Step	Procedure
Pre-Production		
	1	**Set up equipment and clean**. Ensure all equipment is ready, clean and in working order before starting the fermentation.
	2	**Confirm biomass source**. Check and validate the source of carp that you will be using. Check quantity, age and contamination issues. Fresh carp, within 24 hours, or frozen carp, are best. All containers for transporting the carp should be clean.
Production		
	3	**Receive carp biomass.** Take delivery of carp and check it is ready to use. If it is frozen it will need to be defrosted.
	4	**Process shred carp biomass.** The physical loading of carp biomass into a process shredder should be handled by either a skid steer load or backhoe with a three-way bucket. Once loaded into the shredder the carp is macerated without further human input. Transfer shredded carp biomass directly into the fermentation tank if possible. Add some of the water while macerating if needed. Grind the carp to size fractions equal to or less than 10mm in diameter to optimize contact with, and absorption of, lactic acid.
	5	**Mix molasses, water & inoculant.** Now add the correct quantities of these ingredients to the carp biomass in the fermentation tank. Mix well. The quantities of each ingredient will depend on your batch size. See the *Batch Recipes in the Recipe Section of this SOP*.
	6	**Sample & test mixture - 1.** Now take a sample of the mix and send to the laboratory for pathogen analysis. The sample should be approximately 150ml. Use non-latex gloves for hand protection. The material should be placed into a labeled and dated urine sample bottle and sent via Express Post to a NATA accredited laboratory for analysis of *E.coli* and *thermo-tolerant fecal coliforms.* Then test the mixture for pH and temperature and record these on a Monitoring Record Sheet.
	7	**Sample & test mixture - 2.** Then test the mixture for pH and temperature and record these on a Monitoring Record.
	8	**Set the fermentation.** Now seal the fermentation lock and check the fermentation tank is airtight. Make sure the fermentation is located where daily temperature changes are minimized. If the bin is located where low temperatures occur (for example in cool temperate zones during winter), then use a heated room or use electric heating pads on the outside of the bin to maintain required temperatures for fermentation. In extremely hot conditions keep in shaded are if possible.
	9	**Ferment & Monitor.** The fermentation should now start. During this time the batch should be monitored for pH and temperature. Initially, daily recording of temperature and pH should be undertaken. This is because most bacteria are killed within two days when temperatures are between 30 and 40^{O}C, and most viruses are killed at these temperatures after 5 days. The aim is to maintain these high temperatures for five days to ensure any pathogens present are killed, and to have records available that provide evidence for such conditions having been reached. After one week you can monitor the ferment every 3 -4 days.
	10	**Finalise fermentation.** The fermentation should be finished after 4 weeks. At this time check that the product has no strong odour and is not putrid. Do a final pH and temperature check.
Post- Production		
	11	**Product sample & test – Biological pathogens.** Now get two duplicate samples of

		the liquid with labeled and dated urine sample bottles. The sample should be approximately 150ml. Use non-latex gloves for hand protection. Send via Express Post to a NATA accredited laboratory for analysis of *E.coli* and *thermos-tolerant fecal coliforms*. It is important to take duplicate samples for quality control.
	12	**Product sample & test – Liquid fertiliser.** Now get two more duplicate samples of the liquid with labeled and dated urine sample bottles. The sample should be approximately 150ml. Use non-latex gloves for hand protection. Send these to Environmental Analysis Laboratory (EAL) at Southern Cross University, Lismore, NSW. For a *liquid fertiliser test*. This test includes major and trace nutrients, pH, electrical conductivity and estimated total dissolved solids. These results can then be assessed against the *National Code of Practice for Fertiliser Description and Labeling*^ (DAFF, 2011).
	13	**Decant & Store Product.** Now separate off the liquid product and store in sealed, airtight and clean containers. Store in a cool place. Any solids can be composted or used as a soil amendment. If no other options are available then bury them.
	14	**Clean & Store equipment.** Finally clean and rinse all equipment and store it safely.

^This code ensures consistent standards, specifications and labeling requirements which can be accessed by purchasers and users of fertilisers across all States and Territories of Australia. If the product complies with this code it will meet the statutory requirements of all States and Territories (DAFF, 2011)

Further Resources

- LAB Serum Recipe – contact Gerry Gillespie at ROTS
  or David Hardwick at Soil Land Food
- EAL Laboratory – For information on testing.
- Technical information on animal biomass fermentation

This SOP was written by Dr Sara Beavis. Editing and review by David Hardwick – Soil Land Food and Gerry Gillespie – ROTS. **It is based on the best available technical knowledge however** Soil Land Food and ROTS accept no liability arising out of interpretations and actions based on this article, for any loss, damage or injury. The user takes this information on these terms. This procedure was developed as part of the Carp Biomass Hydrolysate Project, part of the National Carp Control Program (NCCP) 2018. The project acknowledges the support of Dr Sara Beavis, CSIRO; Jamie Allnut, FRDC; and Dr Janet Howieson, Curtin University. All photos are by David Hardwick & Gerry Gillespie.

Appendix 7

Final Presentation on FRDC 2016/180 to NCCP.

FRDC

National Carp Control Plan

2016/180:Assessing Options for Utilisation of Virus Infected Carp

Janet Howieson and Ewan Colquhoun

NATIONAL CARP CONTROL PLAN
RESTORING NATIVE BIODIVERSITY

Project Objectives

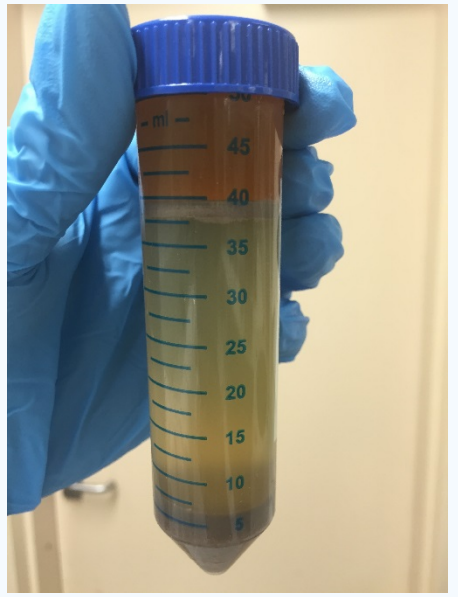
- To identify, pilot and undertake subsequent cost benefit analysis (CBA) for developing new processes/products from deceased feral carp (as part of National Carp Control Plan).

- Contribute to relevant sections within the National Carp Control Plan detailing potential uses of dead carp biomass (draft by June 2018).

- Articulation of potential uses of carp biomass, including costs and potential markets, to inform a cabinet submission (due September 2018).

OPTIONS ARE ASSETS

- **Rendering** (fish meal and oil)
- **Hydrolysis** (Acid, Enzyme or Fermentative) (fertiliser, feed, pet food, fish oil extraction, etc.)
- **Composting/soil amendment**
- **Anaerobic Digestion (Wastewater or Whole fish) (Biogas?)**
- **Feeding Insect (Black Soldier Fly)Larvae** (aqua feed; useful fine chemicals)
- **Vermiculture** (vermiconmpost etc)
- **Raw Minced Pet food**
- **Torrefaction (>350˚C)** (fertiliser additive)
- ****Human Food (seafood and/or swim bladders/roe etc)**
- **Scales: Collagen/ w Collagen**



AN EARLY SHIFT IN PROJECT THINKING:

a. Moved quickly From Laboratory to Pilot Scale Commercial Trials

b. Consider separation of options into smaller scale community based options (product not sold but made available to community /council) v large scale commercial options (BCA on these options)

Semi-Laboratory Options Pilot Tested (non-viable/feasible at this stage)

1. Feeding of Carp to Black soldier Fly Larvae to assess as possible aquaculture feed ingredient. : First barramundi feeding trial completed with carp Fed insect meal as fish meal replacement.

2. Raw Minced Pet Food: Heavy bacteriological load, thiaminases, community perceptions of virus infected product; lack of expertise to undertake nutrition trials

3. Torrefaction (fertiliser additive): Investment issues for commercial partners, therefore large scale trial did not go ahead (small scale trial results are available).

4. Collagen: difficulty in extracting from scales/bones (student work to continue as commercial operations do exist in Europe.

Smaller Scale/Community Based Options Tested and Costed

1. **Fermentative Hydrolysis**: successful 700kg trial, including testing of liquid fertiliser Product. Standard Operating Procedures formalised; mobile/stand alone unit costed and also ongoing operational costs. Could be implemented now by relevant communities/councils.'




2. Vermicompost; successful 300kg trial; worm tea, worms and suitable carbon source; Produce vermicompost as fertiliser replacement. Costed proposal for multiple managed sites and products.


Larger Scale Commercial Options Tested and Costed

1. 20 tonne rendering trial to produce fish meal and oil trial at commercial premises: SUCCESSFUL

2. 10 tonne enzyme hydrolysis trial at commercial premises: SUCCESSFUL

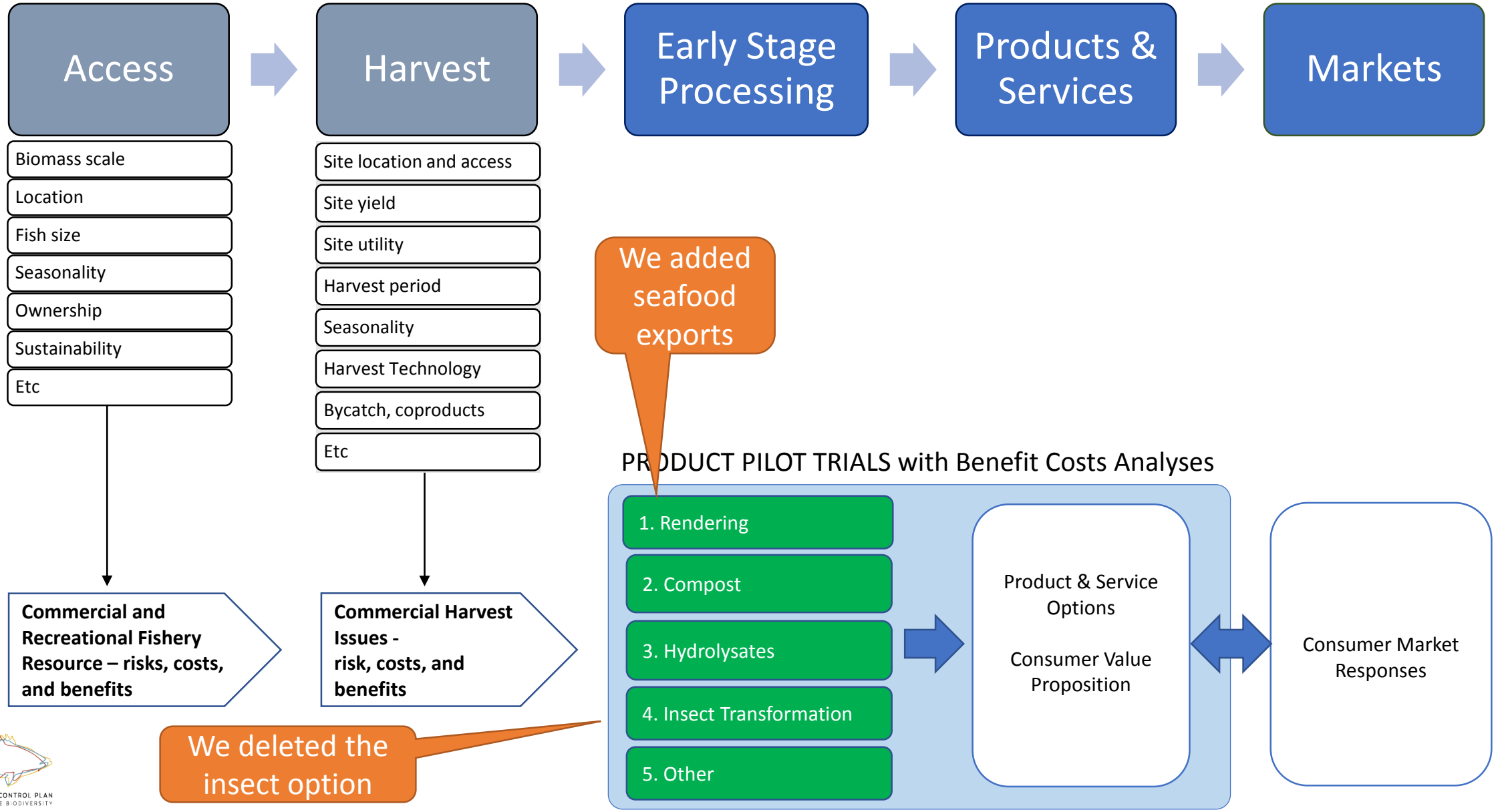
3. 40 tonne composting trial at commercial premises: SUCCESSFUL Different substrates and methodologies. All monitoring as suggested by EPA. (theoretical scenario to apply results to smaller scale remote site applications).

Satisfactory Compositional Analysis and stability.

4. 2-5 tonne mincing, separation (wastewater and solids) and composting trial completed with commercial operators. Intent was to use wastewater in anaerobic digestion and gas production plant. PARTIALLY SUCCESSFUL (MINCING and COMPOSTING but not WASTEWATER ANAEROBIC DIGESTION OPERATIONALLY IMPLEMENTED) (note that optimising of gas production from carp wastewater was undertaken at small scale.

5. Theoretical anaerobic digestion (emergency response) scenario: NOT TESTED.

1. Source and Commercial Use of Carp



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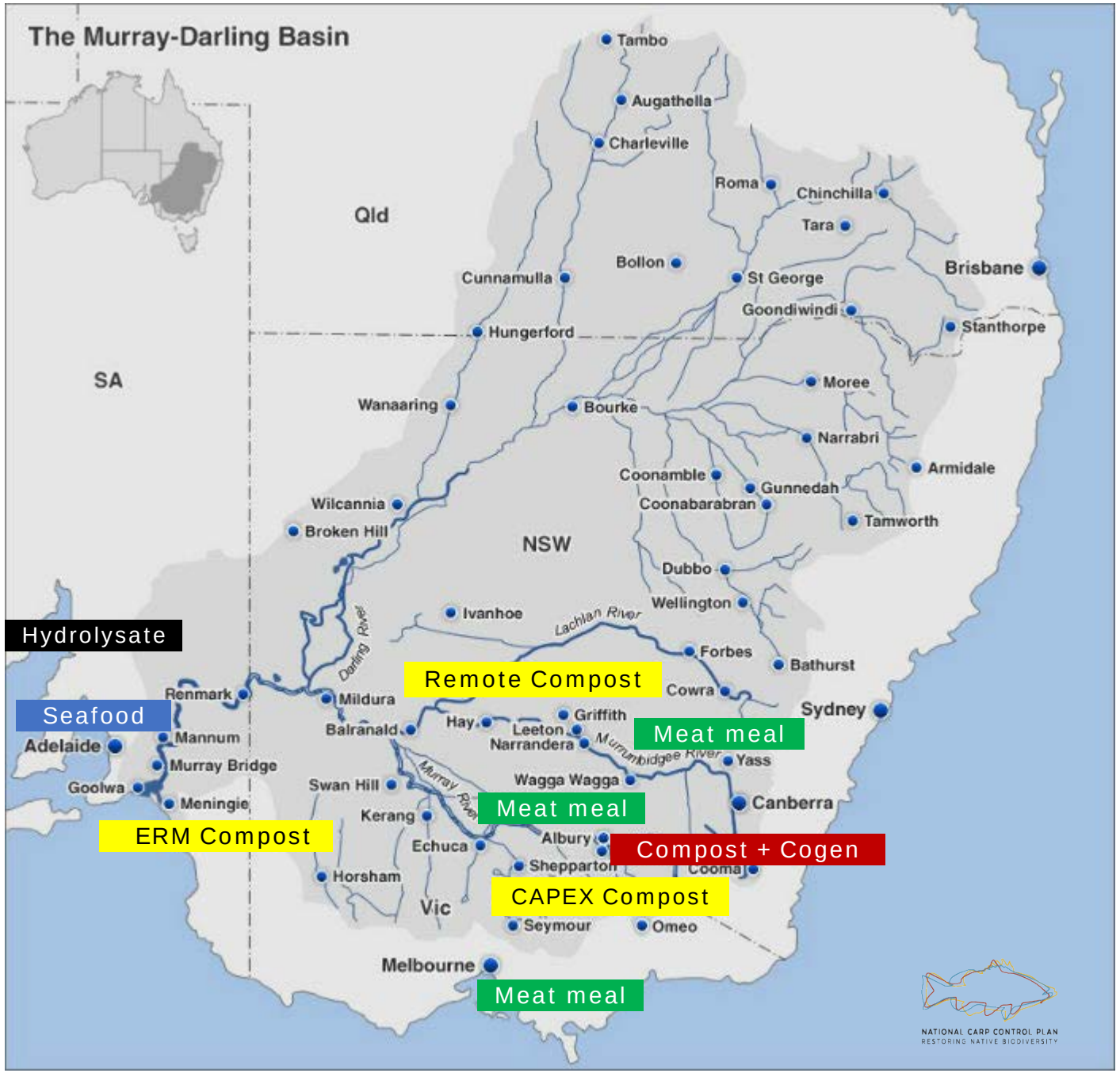
Processors, Products & Places

1. Seafood	2. Meat Meal + Oil	3. Hydro - lysate	4. Compost + cogeneration
Quality & Value			
• High input quality • High product value • Export	• High input quality • Moderate product value • Domestic	• Avg. input quality • Low product value • Export & domestic	• Avg. input quality • Low product value • Domestic
Logistics			
• Small volume • Single site processing (Med freight)	• Mod-High volume • Single site processing (Med freight)	• Mod-High volume • Single site processing (High freight)	• Low-High volume • Multi site processing (Low freight) • ERM AD events
Commercial Products			
• Whole fish • Gilled & gutted • Headed & gutted • Roe • Heads – lobster bait	Ingredients for: • Aquafeed • Petfood • Livestock feed	• Agriculture fertilisers & treatments • Recreational fishing • Industrial ingredients	• Agriculture markets (chemical replacement) • Urban markets • Pelletised • Granulated



BCA (Benefit Cost Analysis) Assumptions

- Carp aggregate in sufficient volumes to service cost effective harvesting (weirs) for all processing options
- BCA considers 2 perspectives: once-off response + ongoing carp markets (non-virus)
- Harvest:
  1. achieved within minim post mortality period (avg wt 2.6-3.5kg)
  2. fish are unencumbered and available for commercial value adding
  3. fish available across catchments, pre-harvested and loaded into transport options, at the waters edge, free of charge
  4. seasonality - hydrolysate (tuna season); composting (site access)
- BCA:
  1. Best Estimates over 12 mths - 14 products (11 confidential discussions)
  2. all costs/receipts from river bank to product sale. Fish cost = $0.
  3. new CAPEX in excluded. Exports @A/US $0.75. Single year of analysis
  4. sensitivity analyses.

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Why we included Seafood options

- Seafood?? – unknown option (non virus release option)
- An experienced and motivated commercial processor was identified
- Large volume export customers identified in China and Vietnam for frozen carp products
- Export sensitive - $A/US = 72c
- Post virus strategy - need to develop long term value added carp seafood markets
- Alternate large scale utilisation option for consideration if virus not released
- Ongoing criticism of “options” project development and outcomes if not considered

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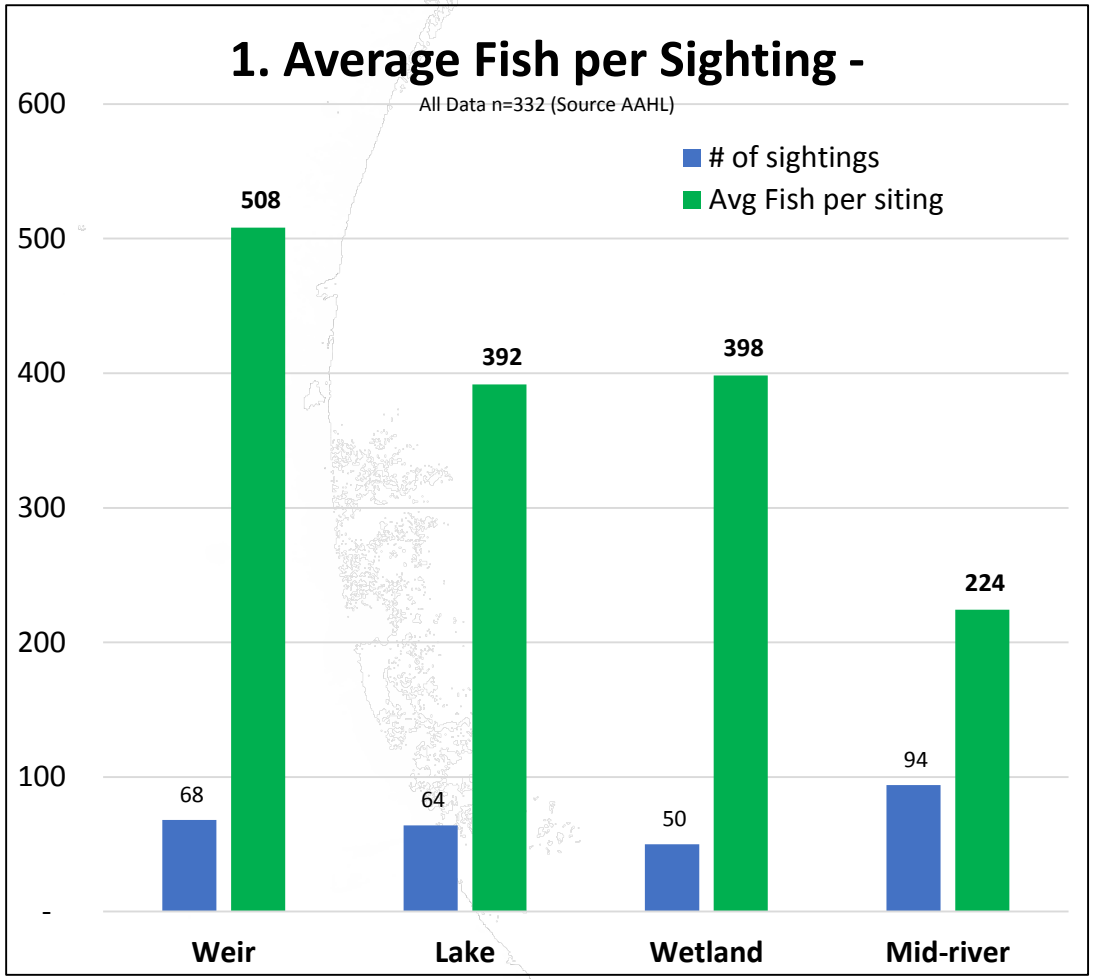
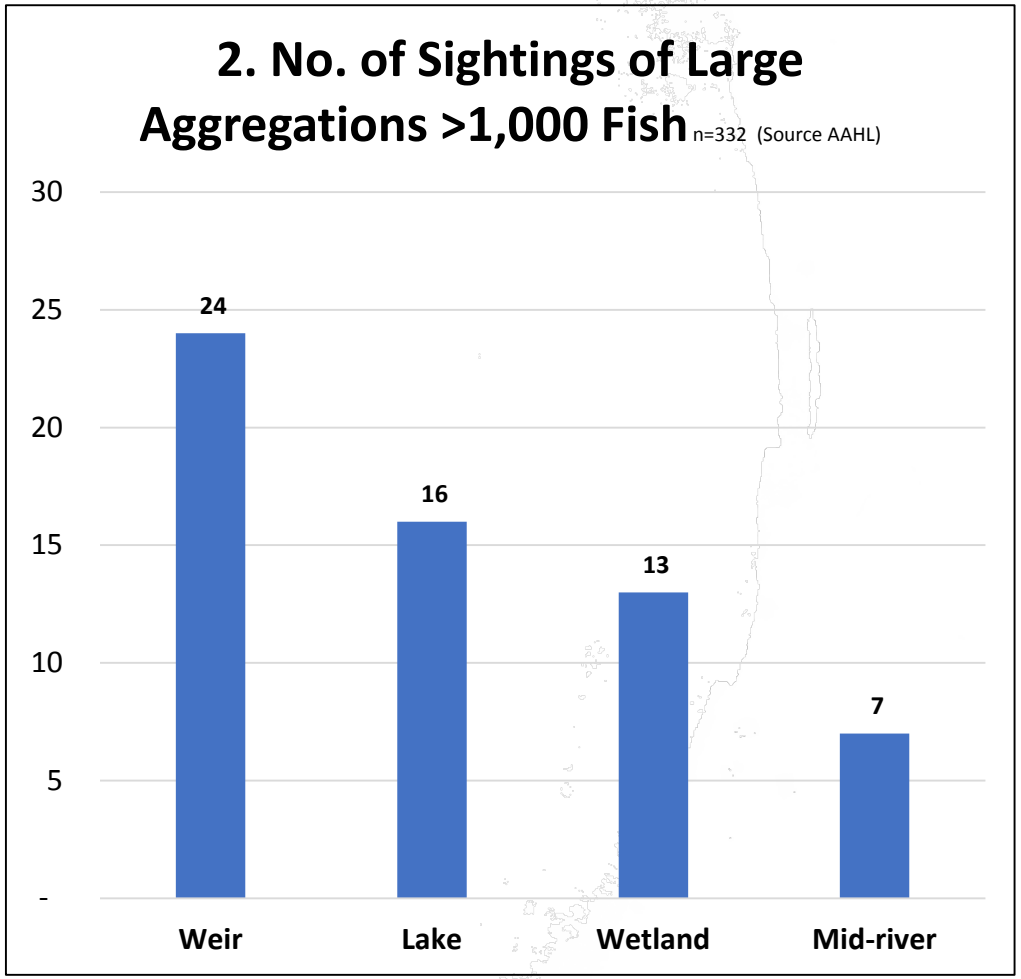
Fist Aggregations (Source: CSIRO/AAHL – crowd sourced data)





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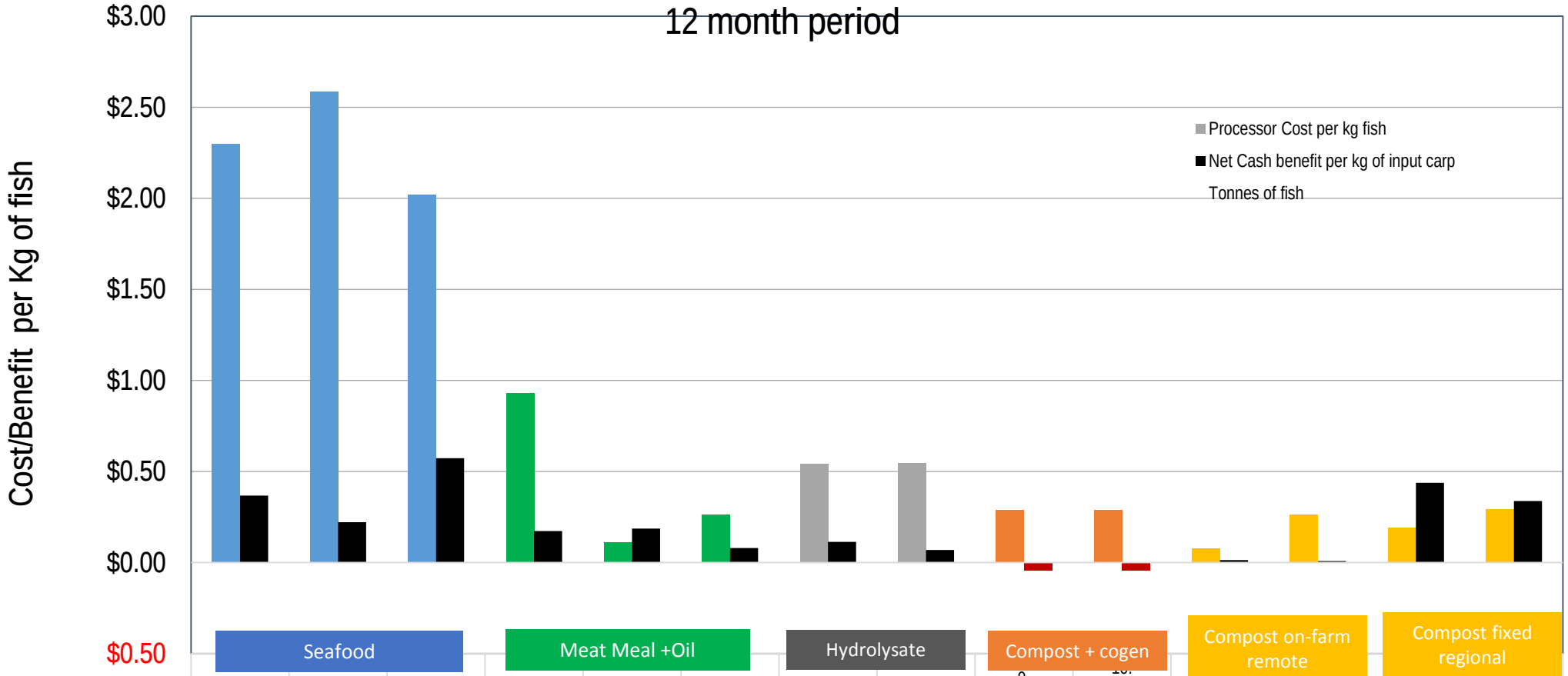
Utilisation Costs V Net Benefits of Carp Utilisation

Summary of 14 Commercial Product Options
12 month period



	1. SEAFOOD whole export	2. SEAFOOD G&G export	3. SEAFOOD H&G export	4. MMO premium rural	5. MMO low grade rural	6. MMO mid quality Melb.	7. HY'LYSATE Low volume	8. HY'LYSATE High volume	9. COMPOST minced + cogen low	10. COMPOST minced + cogen high	11. COMPOST remote batches	12. COMPOST remote digestion	13. COMPOST low volume	14. COMPOST high volume
Processor Cost per kg fish	$2.300	$2.586	$2.019	$0.930	$0.110	$0.264	$0.544	$0.547	$0.288	$0.288	$0.077	$0.262	$0.192	$0.292
Net Cash benefit per kg of input carp	$0.367	$0.222	$0.572	$0.172	$0.186	$0.079	$0.112	$0.068	-$0.047	-$0.047	$0.013	$0.008	$0.438	$0.338
Tonnes of fish	480	480	480	6,000	6,000	6,000	6,000	15,000	3,000	6,000	6,000	5,000	6,000	12,000

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What happens if processors have to pay for the fish?

Cost of Carp/kg	1. SEAFOOD whole export	2. SEAFOOD G&G export	3. SEAFOOD H&G export	4. MMO premium rural	5. MMO low grade rural	6. MMO mid quality Melb.	7. HY'LYSATE Low volume	8. HY'LYSATE High volume	9. COMPOST minced + cogen low	10. COMPOST minced + cogen high	11. COMPOST remote batches	12. COMPOST remote digestion	13. COMPOST low volume	14. COMPOST high volume
$ -	$176,000	$106,379	$274,673	$1,033,200	$1,116,000	$472,200	$674,820	$1,019,250	-$140,250	-$280,500	$75,757	$40,756	$2,628,000	$4,056,000
$ 0.0500	$152,000	$82,379	$250,673	$733,200	$816,000	$172,200	$374,820	$269,250	-$290,250	-$580,500	-$224,243	-$209,244	$2,328,000	$3,456,000
$ 0.1000	$128,000	$58,379	$226,673	$433,200	$516,000	-$127,800	$74,820	-$480,750	-$440,250	-$880,500	-$524,243	-$459,244	$2,028,000	$2,856,000
$ 0.1500	$104,000	$34,379	$202,673	$133,200	$216,000	-$427,800	-$225,180	-$1,230,750	-$590,250	-$1,180,500	-$824,243	-$709,244	$1,728,000	$2,256,000
$ 0.2000	$80,000	$10,379	$178,673	-$166,800	-$84,000	-$727,800	-$525,180	-$1,980,750	-$740,250	-$1,480,500	-$1,124,243	-$959,244	$1,428,000	$1,656,000
$ 0.2500	$56,000	-$13,621	$154,673	-$466,800	-$384,000	-$1,027,800	-$825,180	-$2,730,750	-$890,250	-$1,780,500	-$1,424,243	-$1,209,244	$1,128,000	$1,056,000
$ 0.3000	$32,000	-$37,621	$130,673	-$766,800	-$684,000	-$1,327,800	-$1,125,180	-$3,480,750	-$1,040,250	-$2,080,500	-$1,724,243	-$1,459,244	$828,000	$456,000
$ 0.3500	$8,000	-$61,621	$106,673	-$1,066,800	-$984,000	-$1,627,800	-$1,425,180	-$4,230,750	-$1,190,250	-$2,380,500	-$2,024,243	-$1,709,244	$528,000	-$144,000
$ 0.4000	-$16,000	-$85,621	$82,673	-$1,366,800	-$1,284,000	-$1,927,800	-$1,725,180	-$4,980,750	-$1,340,250	-$2,680,500	-$2,324,243	-$1,959,244	$228,000	-$744,000
$ 0.4500	-$40,000	-$109,621	$58,673	-$1,666,800	-$1,584,000	-$2,227,800	-$2,025,180	-$5,730,750	-$1,490,250	-$2,980,500	-$2,624,243	-$2,209,244	-$72,000	-$1,344,000
$ 0.5000	-$64,000	-$133,621	$34,673	-$1,966,800	-$1,884,000	-$2,527,800	-$2,325,180	-$6,480,750	-$1,640,250	-$3,280,500	-$2,924,243	-$2,459,244	-$372,000	-$1,944,000
$ 0.5500	-$88,000	-$157,621	$10,673	-$2,266,800	-$2,184,000	-$2,827,800	-$2,625,180	-$7,230,750	-$1,790,250	-$3,580,500	-$3,224,243	-$2,709,244	-$672,000	-$2,544,000
$ 0.6000	-$112,000	-$181,621	-$13,327	-$2,566,800	-$2,484,000	-$3,127,800	-$2,925,180	-$7,980,750	-$1,940,250	-$3,880,500	-$3,524,243	-$2,959,244	-$972,000	-$3,144,000
$ 0.6500	-$136,000	-$205,621	-$37,327	-$2,866,800	-$2,784,000	-$3,427,800	-$3,225,180	-$8,730,750	-$2,090,250	-$4,180,500	-$3,824,243	-$3,209,244	-$1,272,000	-$3,744,000
$ 0.7000	-$160,000	-$229,621	-$61,327	-$3,166,800	-$3,084,000	-$3,727,800	-$3,525,180	-$9,480,750	-$2,240,250	-$4,480,500	-$4,124,243	-$3,459,244	-$1,572,000	-$4,344,000

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Gaps & Risks

- NCCP: 1. clarify who owns a carp killed by the virus, at the harvest point, and confirm processors own the final, processed product.

  2. confirm deactivation parameters for virus to ensure that virus infected fish products are safe for relevant use.

  3. confirm top fish aggregation and harvest sites across catchments (this will greatly decrease freight costs for large processors).

  4. confirm definition of virus infected fish for transport and processing: ”biological waste” rather than ”infectious agent?” (this will have implications for transport costs)

  5. uncertainty re acceptability of virus infected fish to the processor preharvest. (lesions, product integrity, ??)

- NCCP: Large capital intensive processors (render, hydrolysers, large composters) will seek to contract multiyear supply of large volumes of carp based on clear specs.

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Gaps & Risks

- Federal/state agencies to confirm relevant EPA considerations/procedures re transport and remote composting (and aspects of other processed eg anaerobic digestion).
- This BCA: 1. provides prefeasibility guidance – detailed analysis must be undertaken to support NCCP decisions

  2. yet to confirm if/how carbon credits impact farm composting

  3. yet to confirm if/how government subsidies apply to composting managed by Landcare Australia/CMA's/Local Governments

  4. final details of cost-benefits of cogeneration
- Product quality minimum specification: if plethora of similar carp products enter the market (concern by some commercial operators).

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Summary of BCA Findings (prefeasibility only)

1. Multiple commercially viable options exist for carp utilisation, including:
   - Composting – base quality for broadacre/urban use, or in value-added formats
   - Rendering - meat meal + oil, as mixed inputs to animal feeds
   - Hydrolysate - liquid fertilisers and treatments, recreational fishing burley
   - Possibly export Seafood (whole, G&G, H&G, roe) or bait (heads) – risk is $US/A
2. Carp are ~70% water - so wet freight is expensive. Must identify large harvestable aggregations of fish - critical to viability (location + volume) – currently unknown
3. Fish input quality is critical for seafood value, and important for render markets
4. Capital intensive (meat meal, hydrolysate, cogeneration, seafood) processing is very efficient. BUT compost solutions suit both remote agri and urban markets.
5. Composting offers greatest flexibility – processing scale, timing, location, cost effectiveness, community and local government engagement. Needs subsidies.

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Questions?

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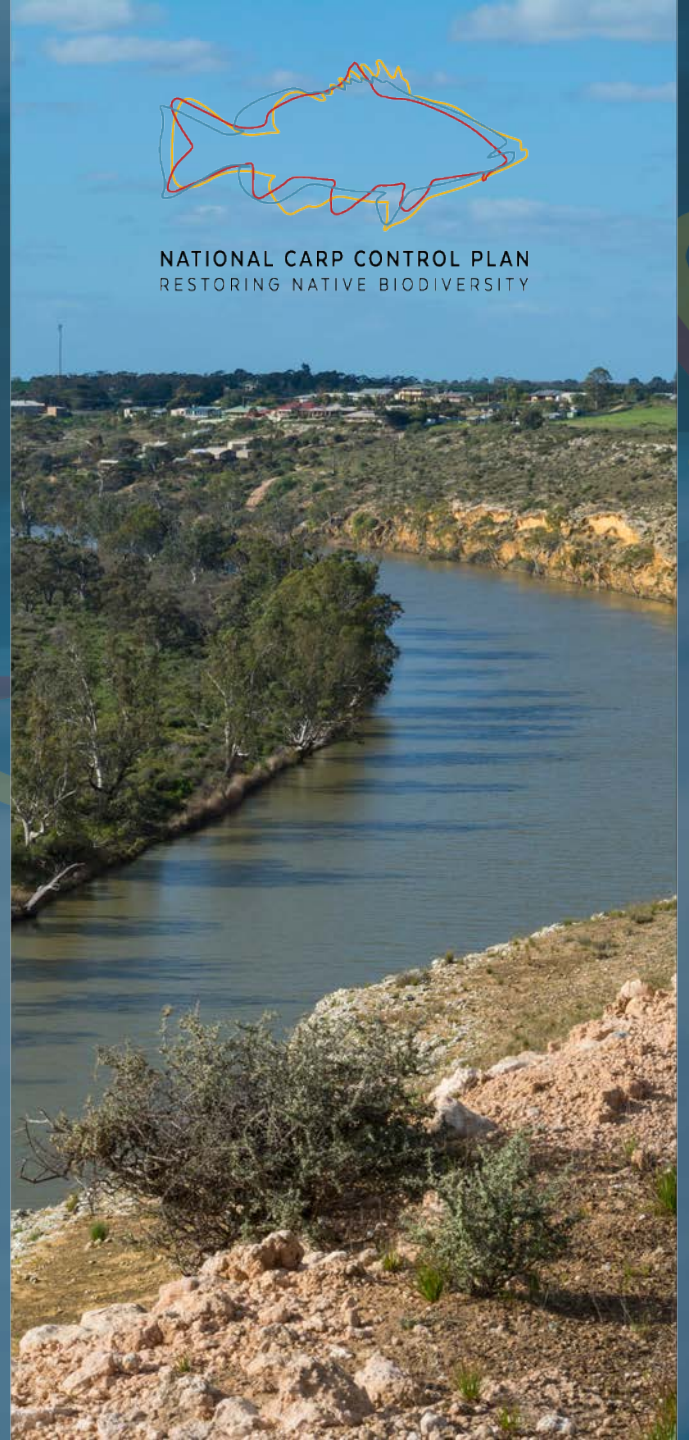
FRDC



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The National Carp Control Plan is managed by the
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