Proceedings of the National Carp Control Workshop

5 – 6 March 2003, CSIRO Discovery Canberra

Pest Animal Control CRC Limited

MURRAY-DARLING BASIN COMMISSION

Edited by Keryn Lapidge

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June 2003

Pest Animal Control CRC

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Acronyms and Abbreviations

AFFA	Department of Agriculture, Fisheries and Forestry - Australia
ANU	Australian National University, Canberra ACT
ARI	Arthur Rylah Institute for Environmental Research, Heidelberg VIC
BA	Biotechnology Australia
BRS	Bureau of Rural Sciences
DPI	Department of Primary Industries
dsRNA	Double-stranded RNA
ESC	Embryonic stem cells
GFP	Green fluorescent protein
GMO	Genetically modified organism
ICM	Integrated Catchment Management Policy (MDBC).
MAFRI	Marine and Freshwater Resources Institute, Department of Primary Industries, VIC
MDB	Murray-Darling Basin
MDBC	Murray-Darling Basin Commission
MDFRC	Murray-Darling Freshwater Research Centre, Albury NSW
NCTF	National Carp Task Force
NFS	Native Fish Strategy (MDBC)
NMS	National Management Strategy for Carp Control (MDBC)
PAC CRC	Pest Animal Control Cooperative Research Centre, Canberra ACT
PGC	Primordial germ cell

Background

'Daughterless Technology' is a concept developed by the CSIRO under a Chief Executive-sponsored project addressing the wider concept of 'Sterile Ferals'. CSIRO's Division of Marine Research has demonstrated that genes controlling sexual development of fish can be blocked to exclusively produce males.

The Murray-Darling Basin Commission has included further development of 'daughterless' technology as part of its 50-year Native Fish Strategy. The program is a blue-sky area of relatively high-risk research. However, the possible benefits of such a novel method of invasive species control warrant further development.

The Murray-Darling Basin Commission (MDBC) has appointed the Pest Animal Control Cooperative Research Centre (PAC CRC) as Project Coordinator of the Daughterless Carp Control Research Program. PAC CRC has been developing immunocontraceptive controls for the fox, rabbit and mouse for a decade.

The National Carp Control workshop was jointly convened by the MDBC and the PAC CRC to engage both the public and scientific communities in the 'daughterless carp' program. It was to serve as a forum for public comment on the project as proposed, and to ensure all interested parties have the opportunity for input. Contributions from scientists and Institutions were made to identify key research issues and ensure the best possible project design. The objectives of the workshop were:

- **To inform interested people of the proposed 'daughterless carp' developments.**
- **To identify and involve interested members of the community and researchers in the project.**
- **To identify additional measures (research or otherwise) necessary for successful implementation of 'daughterless carp' technology.**

Carp in Australia

Taken from Koehn, J., Brumley, A. and Gehrke, P. (2000) *Managing the Impacts of Carp*. Bureau of Rural Sciences (Department of Agriculture, Fisheries and Forestry – Australia), Canberra.

Introduction and Spread

Carp (*Cyprinus carpio*) were first introduced into ponds in Australia in Victoria (1859) and New South Wales (1865) but these did not spread to the wild. Carp were released into the wild during the 1900s by the Acclimatisation Societies of Victoria, New South Wales and Western Australia but did not become widespread.

During the early 1960s, carp populations originating from hatchery-produced fish became established in south-eastern Victoria at Morwell, the La Trobe river and Lake Wellington. Evidence of ecosystem damage by carp in North America raised concerns about potential problems in Australian waters. Carp were subsequently declared noxious and an eradication program was undertaken by the Victorian Department of Fisheries and Wildlife to kill all carp in farm dams with poisons.

The widespread distribution of carp in Australia began in 1964 when fish bred at a fish farm at Boolarra, in Gippsland, Victoria were released into the Murray River at Lake Hawthorn near Mildura. Large floods in 1974-75 accelerated the spread of carp in the Murray-Darling Basin. Carp have also been introduced at numerous sites due to the deliberate use by anglers of live carp for bait or with the purpose of establishing new carp fisheries and perhaps as a contaminant of releases of native fish.

Carp are now the most abundant large freshwater fish in the Murray-Darling Basin and are the dominant fish species in many fish communities in south-eastern Australia. The species is widespread in rivers, lakes, dams and estuarine systems in Victoria, New South Wales, South Australia and the Australian Capital Territory. More limited populations occur in Queensland, Western Australia and Tasmania.

Impacts

Carp have potential economic impacts for both public and private sectors by lowering water quality and damaging aquatic habitats. Industries affected include water suppliers, agriculture, commercial and recreational fisheries and tourism. Carp feeding activity increases water turbidity and damages aquatic plants. Direct impacts on native fish fauna are the subject of much speculation but less well documented and declines in native fish populations in many areas had already begun prior to the expansion of carp.

Taxonomy

Native to China, carp are the largest member of the Family *Cyprinidae* in Australia and are closely related to, and often confused with goldfish. There are genetic differences between the strains of carp in China and Europe, but carp from both continents are recognised as belonging to the same species. There is little genetic variation among carp in south-eastern Australia with the Boolara strain the most abundant and common in the Murray-Darling Basin. Populations of the Koi strain (a selectively bred ornamental carp variant originally from Japan) also occur in Western Australia.

Life Cycle

Male carp mature at 1-3 years while female carp mature at 2-4 years and may produce more than one million eggs per year. Carp can migrate at any time of year and spawning usually occurs in late spring or early summer. Females may spawn several times in one season. Development is rapid with eggs hatching in two days at 25°C and six days at 18°C. Juvenile carp frequent shallow floodplains and river channels, and mortality may exceed 98% in the first year. Survival of carp appears to be density-dependent. Carp have been reported to live for more than 15-17 years, growing to 18-26 kg although it is thought they can live much longer. Growth rates of carp vary greatly between different regions depending on temperature, food availability and population density.

Habitat

Carp have broad environmental tolerances and thrive in habitats that have been disturbed by human activities. They occupy a range of habitats and live in low-altitude or rivers or standing waters in mid-latitude regions. The ability of carp to survive periods of poor water quality gives the species a competitive advantage over many native fish species in Australia. Increased availability of suitable habitats for spawning and recruitment during floods means that recruitment success is often much greater in years with large floods. Habitat disturbances such as sedimentation, bank and land clearing, river flow alteration etc, have meant a loss of habitat for native fish and the creation of habitats in which carp thrive.

Feeding

Carp are omnivores and feed by filtering small particles from the water or by sieving food items from the bottom sediments. This behaviour can noticeably stir up fine sediments and increase water turbidity.

Introduction

Tony Peacock, CEO, Pest Animal Control CRC

Pest Animal Control CRC

The purpose of the Pest Animal Control Cooperative Research Centre (PAC CRC) is to develop practical, cost-effective and socially acceptable products and strategies to reduce pest animal damage in Australia. The development of immunocontraceptive controls for the fox, rabbit and mouse have so far been the priority of the PAC CRC, which is now extending it's expertise in these areas to carp control in partnership with the MDBC. Based on the work with foxes, rabbits and mice to date it is clear that biological control methods work and are a viable strategy for pest animal management.

Pest Animal Control CRC

Fertility control, such as employed in the 'daughterless carp' program is a promising technology for several species. However technical challenges remain and the social challenges associated with genetic modification technologies are probably greater than the technical ones.

Biological Control

Biological control methods for pest animal species have proved successful in the past. Much of the Australian landscape would look very different today without the myxoma and calici viruses. In dollar terms the impact of the calici virus (RHV) has had a direct benefit of around $2.2 billion to Australia at a cost of approximately $151 million.

Looking ahead, the PAC CRC is already involved in the development of promising fertility control methods for several pest animal species. For mouse and rabbit these are in the form of disseminating viruses targeting zona pellucida proteins vital for reproduction. Viral fertility control mechanisms are also under development for the fox.

Social Challenges

As with any new technology, certain social challenges must be overcome before any pest animal control program can be implemented successfully. This is particularly true for strategies involving biological control and genetic modification. In order to minimise potential problems down the track, it is imperative that the specific social challenges surrounding the Daughterless Carp program be taken into account right from the start. The PAC CRC has previous experience with biological control mechanisms for pest animal species and some of the specific community concerns likely to arise from this type of project are:

- **"Biological control is not humane".**
  The success of the myxoma virus on rabbit populations is strong evidence against this argument. It was highly effective, relatively inexpensive and easy to implement, target specific, embraced widely and had a sustained impact.

- **"Genetically Modified Organisms (GMOs) are not acceptable"**
  Public opinion to this issue is highly personal and ranges from total opposition to total embracement, usually depending on what the organism is and how it has been modified. Most people have a sliding scale of acceptable genetic modification and may consider GMOs for the purposes of energy conversion, forestry or pharmaceuticals more acceptable than genetically modified foods, animal or human cloning. The challenge for us is to anticipate opposition and to provide the community with the information necessary to identify where pest control (and more specifically the Daughterless Carp project) fits on their sliding scale.
- **"One person's pest is another person's endangered species"**
  In Spain, the rabbit is native and valued. Researchers there are developing a disseminating vaccine for RHD and myxomatosis. The rabbit is considered essential for the survival of the Spanish Imperial Eagle and the Iberian Lynx.
- **"The risks outweigh the benefits"**
  'Risk' is defined somewhere between an extreme attitude to risk (eg bungee jumping) and trying to avoid risk at all costs. Risks may also tend to be more immediate while real benefits may be long-term.

Project Proposals

- PAC CRC calls for Carp project proposals by 1 May 2003 for funding consideration.
- Funding is limited and competitive.
- Co-investment in projects is encouraged.
- Funding may be considered for a start from 1 June 2003 and must be completed by 30 June 2005.

- Standard forms (MS Word and Excel) should be used.

Management Arrangements & Terms of Reference

The Murray Darling Basin Commission (MDBC)

MDBC as the principal program investors hold ultimate responsibility for the program. The Daughterless Carp Program falls within the MDBC Native Fish Strategy. The Native Fish Strategy is a major initiative involving a large range of measures related to restoration of native fish populations from their current 10% of pre-European settlement levels to 60% over a 50-year period. The Daughterless Carp project is the single biggest invasive species program within the Strategy.

The MDBC has contracted **PAC CRC Ltd** to deliver the project against agreed milestones. MDBC will instigate reviews and set budgets as appropriate to their overall objectives. All budgets from the Commission are provided on a single-year basis, with a good faith statement regarding out-years.

MDBC Daughterless Carp Consultative Group

MDBC will coordinate communication with a high-level group to ensure key organisations are informed of progress. This group will consist of several Commissioners and Executive of MDBC, Chiefs of relevant CSIRO Divisions and CEOs of key State Government Agencies. The Reference Group will rarely be convened but will be kept up to date with project progress to ensure developments can be built into the planning of their own agencies.

MDBC Native Fish Strategy Manager (Jim Barrett)

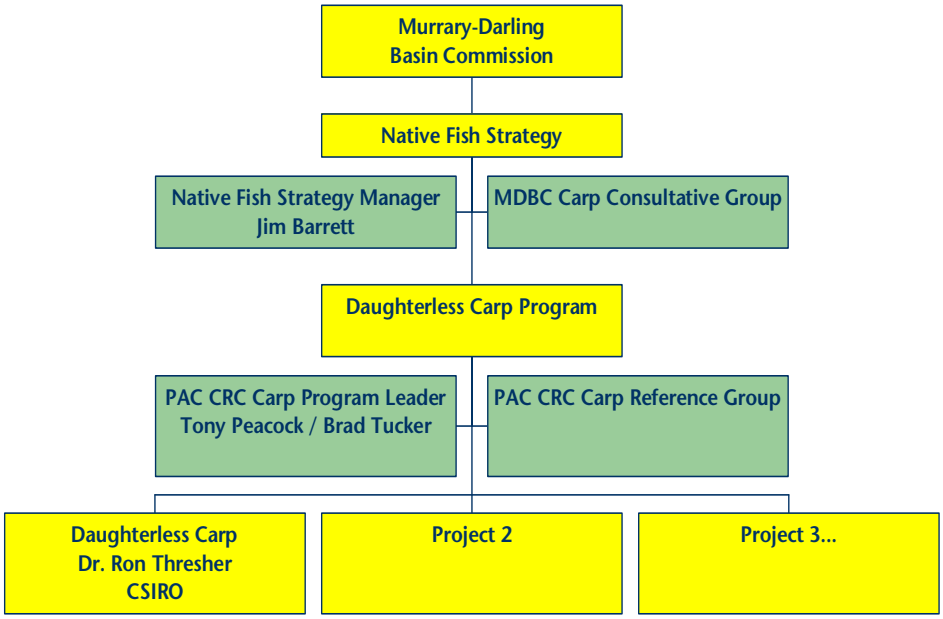
Communications between MDBC and PAC CRC Ltd will be through the Native Fish Strategy Manager.

PAC CRC Board and Executive

The Daughterless Carp Program has full status as a Program within the PAC CRC. Thus it becomes Objective 1.5 *Genetic technology for the long-term reduction of carp in the context of Integrated Pest Management.* As such, program status is reported to the Board on a regular basis through the CEO. The Program is expected to contribute to CRC Key Performance Indicators. The Carp Program Leader will report monthly to the CRC Executive.

Daughterless Carp Program Leader

The Carp Program Leader develops budgets, monitors progress and is responsible for the conduct of the Program. He/she reports against the contracted milestones to the MDBC and monthly to the PAC CRC Executive.



PAC CRC Daughterless Carp Reference Group

Because of the complex scientific nature of this work, spanning a range of disciplines, the Carp Program Leader will establish a Daughterless Carp Scientific Reference Group. The Group's Terms of Reference are as follows:

- Consider the scientific progress of the Daughterless Carp Program.
- Comment on the progress of the Program through detailed examination of data.
- Consider the relevance of the Program and its components in terms of:
  - The Murray-Darling Basin Commission's Native Fish Strategy
  - The current scientific status of similar work world-wide.
- Suggest changes in general direction and/or specific direction of individual projects.

The Reference Group will consist of six members and will be serviced through the PAC CRC Carp Program Leader. The PAC CRC CEO in consultation with the MDBC will appoint members.

Project Leaders

Individual projects within the Program will be commissioned by the CRC against specific milestones. Project Leaders will have responsibility for the conduct of their respective projects and will report against milestones to the CRC Carp Program Leader.

Program Communications

All participants in the Daughterless Carp Program recognise the importance of adhering to a formalised Communications Plan for the work. The key concerns include management of expectations (eg. expectation that this work will 'fix' the carp problem in the next few years); potentially conflicting points of view (eg. commercial fishing) and, in particular, the use of genetic modification for pest fish control.

The CRC's Communication Manager is available for advice and assistance in all communications of the work. Statements from the CRC or from MDBC must follow their normal approval processes. Contractors should avoid making comment unless approved by the Carp Program Leader in advance. The CRC and MDBC Communication Units will need to liaise closely to ensure consistent and accurate information. A Communication Plan will be completed within three months of project commencement.

The Murray-Darling Basin Commission 50-year Native Fish Strategy

Jim Barrett, Murray-Darling Basin Commission

Native Fish Strategy (NFS)

The Native Fish Strategy (NFS) is a response to key threats to native fish management in the Murray-Darling Basin (MDB). These threats include artificial regulation of water flow; habitat degradation; reduced water quality; barriers to fish passage; introduced fish species; fisheries exploitation; the spread of disease; inappropriate translocation and stocking of fish.

Native fish populations have declined because of these threats, and are currently at 10% of pre-European settlement levels. NFS seeks to ensure viable fish populations are sustained within the Basin. All species and fish communities across the MDB are included in the NFS.

The specific goal of the NFS is to rehabilitate native fish communities in the Murray-Darling Basin back to 60% of estimated pre-European settlement levels after 50 years of implementation. Carp management strategies are included as part of a wider integrated program including habitat restoration and environmental flows to achieve this goal.

The NFS will be developed & implemented under Integrated Catchment Management Policy (ICM) to ensure it is "a process through which people can develop a vision, agree on shared values and behaviours, make informed decisions and act together to manage the natural resources of their catchment". There is also a strong emphasis on community involvement in the NFS with participation by individuals and communities, including indigenous communities, encouraged.

The current status of native fish in the MDB:

- Localised extinctions
- 8 of 35 species are nationally threatened
- Decline in status of 'flagship' species
- Loss of most commercial fisheries
- Declines in recreational fishing success
- Presence of 11 alien species

The control of alien fish species is a priority of the Native Fish Strategy. As a result the NFS seeks to

- support implementation of National Management Strategy for Carp Control
- develop rapid response system for new alien species
- ensure consistency in alien species management
- identify and fill knowledge gaps of alien species
- investigate biotechnological approach of alien species management (inc. daughterless carp technology)
- encourage and build on existing community actions

The use of demonstration reaches for trial of rehabilitation techniques is also a priority of the NFS. These should be degraded but fixable stretches of river in each state that allow demonstrable results from rehabilitation actions and have the ability to address several threats or ecological issues concurrently. To be effective demonstration reaches should demonstrate visibility, profile and access to the public; demonstrate community support for rehabilitation; and allow testing of scientific hypotheses and measurement & monitoring of results.

National Management Strategy for Carp Control (NMS)

Linked to the NFS is the National Management Strategy for Carp Control (NMS), the goals of which are to:

- Prevent the spread of carp
- Reduce the impacts of carp to acceptable levels
- Promote environmentally and socially acceptable application of carp eradication and control programs
- Improve community understanding of the impacts of carp and the management strategies to counteract those impacts
- Promote the cost efficient use of public resources in carp eradication and control programs

Carp possess the usual bio-physical attributes of a successful invasive species and eradication of carp in Australia is not feasible with current technologies. Effective carp control will require a combination of techniques, applied as a "package" and management plans should focus on reducing *impacts*, not merely *density*, of carp to acceptable levels. Direct carp control will be ineffective if applied in isolation of other restorative measures. Management plans need to be integrated with other relevant plans such as nutrient, fisheries, algal and vegetation management.

While it is imperative to prevent the further spread of carp, it is difficult to distinguish the impacts of carp from other (largely human-induced) damage. Further, the direct and indirect interactions between carp and other aquatic fauna remain poorly understood.

Management should be based upon a catchment or sub-catchment approach where the outcomes are clearly defined and related to the desired environmental and primary industry outcomes for the area. Areas of focus should be locations where carp can be easily controlled; with high biodiversity values; high water quality values; probability that action will have positive and long-term benefits.

Establishing management units and ranking for priority (A Guide for Carp Management Groups):

- **Step 1.** Divide region into non-carp (A) and carp (B) areas.
- **Step 2.** Divide (B) into management units.
- **Step 3.** Identify eradication units.
- **Step 4.** Determine biodiversity & water quality units. Rank units.
- **Step 5.** Rank units for vulnerability to carp.
- **Step 6.** Multiply vulnerability score with the higher of the biodiversity or water quality primary score to develop the **PRIORITY RANK**.
- **Step 7.** Reassess rankings using Norton criteria and secondary factors to determine whether carp management is practicable.

Carp Control Options

There are several levels of carp management options including eradication, containment, sustained management and no action.

Essential criteria for successful eradication are that:

- Pests can be killed at a faster rate than they can replace themselves.
- Immigration can be prevented.
- All reproductive individuals are at risk.

It is also desirable that the pest can be monitored at low densities; that there is a supportive socio-political environment; and that the high costs of eradication are justified. If all criteria can't be fulfilled total eradication is impossible. Containment and sustained management options are more feasible.

Current chemical carp control methods usually take the form of poisons (piscicides or non-piscicides) delivered directly in-stream or via baits. Biological control techniques include predation, pathogens, fertility control and genetic manipulation.

Physical control involves fish removal (commercial fishing, electrofishing) or exclusion (barriers, fish traps). Habitat modification practices such as water level draw-down, flow management, removing or adding structural elements and changing water quality can also be used as control mechanisms.

Integrated pest management principles:

- Define the problem in terms that measure impacts or damage (not reduction of numbers)
- Identify the scope of the problem
- Identify the range of control techniques that need to be applied to reach the objective
- Monitor and evaluate

MDBC Expectations

The Daughterless Carp project is an important tool within the National Management Strategy for Carp Control, which in turn sits within the Native Fish Strategy and the Integrated Catchment Management Policy of the MDBC.

MDBC expectations of the Daughterless Carp program are:

- To legitimise Daughterless Carp as a natural resource management issue.
- To foster confidence within the jurisdictions regarding the benefit, versus cost, of Daughterless Carp technologies.
- To have confidence in the community's understanding and perception of the issue.
- To be across the current knowledge with respect to the population dynamics of wild carp; and have the ability to address any deficiencies in knowledge.
- To ensure that there are no legal or logistical impediments to release.
- To continue to monitor community perceptions.
- To assess the impact of daughterless carp on (i) overall river health, (ii) native fish populations and (iii) carp populations.
- To assess the transferability of this approach to other alien species.
- To integrate daughterless carp into a conceptual model of outcomes for fish populations in the Basin.

Conclusions

Effective carp management in Australia will depend on:

- the commitment of key stakeholders to provide ownership of the issue;
- the support of government at all levels;
- including commitment to resource innovative technologies & sustained management;
- a critical mass of "showcase", implemented local carp management plans;
- integration with a package of other interventions (NFS) and
- integration with other plans (ICM).

A review of the commercial use of carp

Keith Bell, K&C Fisheries, Sale Victoria

This is a general commercial picture of the carp industry of Australia – some history, statistics, where the industry has gone since 1966 and what the future may hold.

Founded in 1984 by Keith and Cate Bell, K & C Fisheries is the largest wild-catch carp harvesting and process company in the Southern Hemisphere. The company processes in excess of 1000 tonnes of carp per annum and exports to Europe, the Middle East and the Pacific Region. K & C Fisheries constitutes 75–80% of the Australian carp industry tonnage and in 1999-2000 carp was the largest single fishery in Victoria (30% total tonnage). Carp domestic markets include fresh table fish, rock lobster bait, raw ingredients for fertiliser and stock feed, and dried and smoked specialty products.

Photo: K. Bell, K&C Fisheries

1966	First carp identified in the Gippsland Lakes but illegal to sell to market.
1970	Carp allowed to be marketed, but not promoted well at this time.
1974	Sold to the rock lobster bait market, Melbourne and Sydney Fish Markets. Approx. 1 tonne / week sold.
1998	Export to Poland.
1999	Export to Fiji.
2000	Export to Poland and Israel.
2002	Market export to Poland and roe to Germany.
2003	New markets in Germany and Malaysia.

K & C Fisheries operate under a permit system in Victorian waters and under licence in New South Wales utilising seine fishing, electro-fishing and specialist traps dependent on location and fishing conditions.

The Australian Carp Industry consists of about 70 licensed carp fishermen operating in three states. In New South Wales there are currently 19 Carp/Yabbie licences, in Victoria there are 35 licences and permits and in South Australia there are also 35 river and lakes licences. Several of these licences are predominantly catching native fish and yabbies and approximately 8 are exclusively carp.

K & C Fisheries operates 8 vessels up to 10.5 mtrs, 5 vehicles including a specialist tipping truck and currently employs 10 staff including management. K & C Fisheries also operates a twin engine Cessna 337 available for fish spotting, survey, crew and equipment transport, and it own mobile freezer. The majority of the rest of the industry has only much smaller equipment such as dinghies and utes.

Our processing factory at Sale has Food processing accreditation with the Australian Quarantine and inspection service licensed for Carp export. K & C Fisheries set the bench mark standards for carp export processing. The freezer capacity of the facility is in excess of 360 tons.

At this stage there are only 3 others companies in Australia registered to export carp for human consumption.

Photo: K. Bell, K&C Fisheries

Company turn over is in excess of $A1 million per annum with $1.4 million invested in infrastructure. Exports generate approximately $700 000 annually.

The total industry turnover last year was only $A1.7 million, mainly due to the drought and a much smaller biomass of fish.

Commercial carp fishing is one of the methods of eradication at this stage. K & C Fisheries and other commercial fishers have been utilised for these operations because of the local knowledge and expertise.

Markets could realise the following values if industry was able to supply enough carp:

Domestic:

- Cray bait for the Victorian and South Australian crayfish industries: 300-1800 ton p.a. $150000.
- Human consumption via the Melbourne, Sydney & Adelaide fresh fish markets: 200 ton p.a. $250 000.
- Value added products: 50 ton p.a.
- Pet food industry: up to 600 ton p.a. $300 000.
- Fertiliser industry: 80 ton p.a. $56 000.
- Lots of little niche markets, eg SA Carp Sausages.

Export Markets:

- Poland with a potential demand of 400 ton p.a. $1.2 million.
- Middle East with a potential demand of 1200 ton p.a. $3 million.
- Carp Roe into Europe: 80 ton p.a. $500 000.
- Carp trunks into Europe: 700 ton p.a. $1.5 million.
- Pet food industry: 20 000 ton p.a. instead of pilchards and anchovies $6 million.

If supply of product could be maintained through the year, the value of K & C Fisheries export markets would be in excess of $A 6.8 p.a, not including the pet food industry which could be as much as $6 million on it's own.

The world consumption of Carp is approx 1.8 million tons per annum, mainly in China (1.3 million tones). Nearly all carp produced overseas for market is farmed. Australia produces less than .001% of the world carp market.

Over the past 17 years much of K & C Fisheries efforts have been directed at perfecting fishing equipment and techniques to enable cost effective harvesting and the establishment of sufficient market outlets to ensure company viability. Unprocessed carp is a low value commodity therefore efficient harvesting of large tonnages combined with cost-effective transport and processing is paramount. Fish quality must also be maintained to a very high standard.

K & C Fisheries in its operation in the Gippsland lakes has achieved these goals however in recent times high salinity levels in the Gippsland lakes due to the prolonged drought have severely impacted on the companies ability to target sufficient fish to meet current and potential demands. Fishing potential in others parts of Victoria and New South Wales has been investigated as the opportunities have presented themselves. Fishing operations conducted in other parts of Victoria and New South Wales, have highlighted difficulties in maintaining fish quality and cost effective operations when operating in remote areas away from the company's processing establishment. With this in mind it is understandable that most of the remote fishers can't even recover costs on small amounts of fish.

When looking at production costs, the carp industry doesn't only help the fisher and the environment but is also a big help to the rest of the community and government. K & C Fisheries support up to 10 families directly and the rest of the industry could of supporting between 4-70 families directly. With industry earning $1.7m/year, this goes through the community with a value of approx $5.1 million on a dry year.

The following costs must be considered when evaluating the business potential of a carp fishery:

- Harvesting costs: licences, nets, labour, vehicles, boats, issuance, QAP factors.
- Storage costs: refrigeration plant and running costs, factory approved by govt/AQIS, QAP factors.
- Marketing: transport, commission, handling unknown factors, chemical analysis.
- Packaging costs: cardboard boxes, plastic crates, ice slurry, plastic bags/liners, pallets.

Photo: K. Bell, K&C Fisheries

Industry Concerns are:

- The long term tenure of the carp industry due to confusing government policies:
  - New South Wales closed native fin fishery and encouraged fishers to set up to harvest Carp. So were does the Daughterless Carp project leave them?
  - Victoria – has shown a neutral approach and have only permitted people who have shown their own initiative.
  - South Australia has a by catch fishery although it tried to go the same way as New South Wales closing its Native fin fishery and leaving carp.

    No peak body within the industry.
- Sustainability mainly reliant on environmental conditions and good management.

Questions and Comments from Audience:

Q. *Is there any fish in the Gippsland Lakes other than carp? What happens if you remove the carp?*
A. Yes - the Gippsland lakes has an estuarine fishery catching other fish e.g. Bream, mullet, whiting, & salmon. Due to the drought and the high rising salinty levels in the last 2 years, not many carp have been caught in the actual lakes - only in the rivers leading into the lake. If you remove the carp it will help highlight the more serious environmental problems and the lack of returning native fish species.

Q. *Is farming of carp in the future a potential means of compensating the carp industry if carp are successfully eradicated?*
A. Very few people will enter the carp industry now – they are not prepared to invest if they can't see any return. Farming of carp not seen as feasible. K & C Fisheries are keen to be part of the Daughterless Carp program - it requires a source of fish (which K & C could supply).

Q. *How would the release of a genetically modified carp impact on the industry?*
A. All exports to European Union countries would cease. They won't accept any possible GM product.

Q. *How can carp be successfully promoted to the Australian market?*
A. Can't see carp for human consumption taking off here. Australian's not big fish eaters anyway, we are also spoilt for choice and the carp industry got off on the wrong foot at the start – the poor perception of carp by the Australian public goes back to the 1960s.

Q. *What would the impact of a carp bounty be?*
A. Won't get rid of the last carp. The economic equation always balances out. E.g. Tasmania – the industry will collapse to a certain point but won't totally eradicate the fish.

The science of producing daughterless technology; Possibilities for population control using daughterless technology; maximising the impact of carp control.

Ron Thresher and Nic Bax, CSIRO Marine Research

Ron Thresher

Pest Control Options

Genetic technology offers potential opportunities to get into pest control at a generic level. Many traditional pest control/eradication procedures are unacceptable in the case of carp – e.g. generalist predator, genetically modified virus. A gene technology approach that affects only the target species is rated more acceptable than classic biocontrol.

"Unfit" genes won't spread easily in a population but daughterless technology affects the sex ratio at population level even though individuals are themselves fit and healthy.

Daughterless Technology

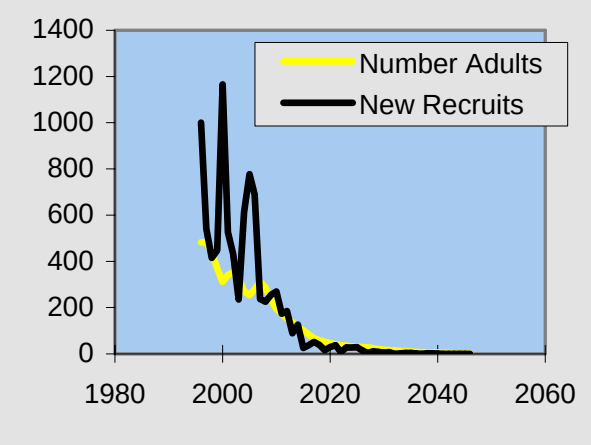
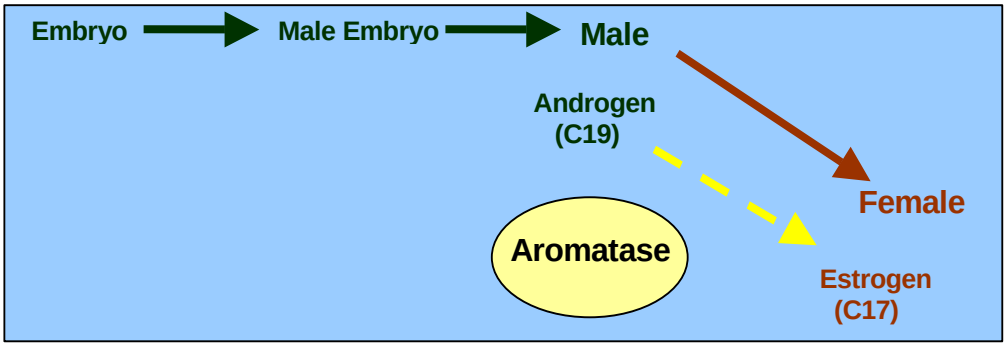


Figure: Effect of replacing 5% of wildtype recruits each year with daughterless carriers.

1. Daughterless technology is similar to the concept of meiotic drive which has been around since the mid 1960s. [Hamilton (1967). *Science* 1853: 537-539].
2. Daughterless technology involves an engineered genetic construct using species-native genes that is inheritable and that biases offspring sex ratios towards males.
3. Models indicate that replacing 5% of wild type recruits each year with daughterless carriers would show a significant decrease in population levels by 2020 and near extinction by 2030.
4. Stocking rate of daughterless carriers and recruitment variability impact on the length of time before an effect on population numbers is seen. A greater effect could be seen if daughterless technology is used in combination with physical removal (fishery).

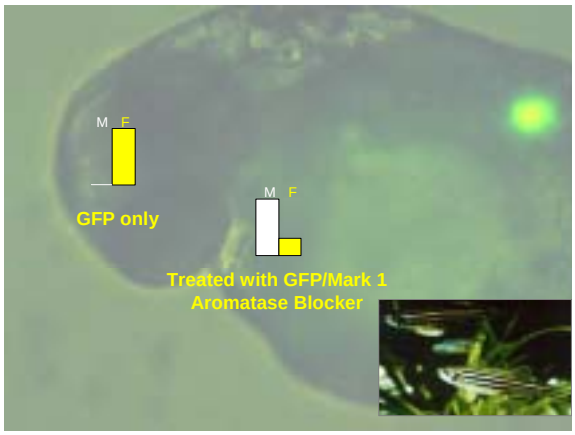
Fish Sexual Development



Embryo to male is the 'default' pathway in fish sexual development. Female development is initiated by estrogen which is produced from androgen by the action of the enzyme **aromatase**.

The daughterless construct involves a genetic duplication to shut down aromatase action blocking the androgen ⇓ estrogen pathway and thus stopping the diversion of male development to female development.

- The construct includes the aromatase gene blocker, a selectable marker gene (GFP), a female specific promoter (PGC – expressed in gonads).
- Lab results on zebrafish so far show that the promoter is only turned on in females (construct without aromatase blocker gene) and that the blocker gene produces 83% viable males.



Where we stand

- The basic daughterless technology appears to work.
- Models and theory are intuitively reasonable, physiology is right, and the recombinant technology is consistent with other studies.
- Broad public good will.
- No integration and no direct tests at population level. Carp specific constructs also need to be engineered and tested.
- Not melded with carp population dynamics/ecology.
- Logistics – what is the reality of stocking even 1% of the wild type population as daughterless carriers?

Project Objectives

Outcome: a "solution" to the carp problem.

- To have an Integrated Pest Management Plan ready to implement in 7 years or less.
- Daughterless as the core eradication technology.

Daughterless Carp Technology Business Plan

Medium term (7-year) Objectives:

- Ready to apply biotechnology to efficient carp control.
- Determine optimal release and monitoring strategies (population models, adequate pd&g data, IPM).
- Communication and, by implication, approval to commence trials
- Effective project management
- Decision point at end of 7 years

Intermediate (3 year) Objectives:

- Develop daughterless technology to a practical stage – does it work, reliably and safely?
- Is daughterless cost-effective, relative to other options
- Is it logistically feasible – stocking strategies, hatchery operations
- Decision point at end of 3 years

Population and Recombinant Genetics

- Build, test and integrate daughterless constructs Marks II and III in *Gambusia*
- Determine minimum and maximum copy numbers, confirm inheritance, stability, silencing and effects on F1, F2 etc.
- Detailed behavioural studies on neomales, relative to wild-type males – growth and survival, competitiveness, fecundity.
- Multi-generation trials in stable lab populations, using different constructs, stocking rates, etc.
- Build an "optimised" carp construct.
- Trials using cell lines and cloning to speed up integration.

Population Models

- Build realistic models for *Gambusia*.
- Population extinction? What are the dynamics at highly skewed sex ratios?
- Test model predictions against lab trials.
- Refine models and predict dynamics in field.
- Explore theoretically alternative approaches.
- Begin development of more realistic carp models.

Hazard Analysis

- Test species specificity of constructs – hybridisation, horizontal gene transfer.
- Undertake hazard analysis to identify potential environmental risks.

Broader Issues

- Realistically parameterised but simple carp population model for the MDB, data and other needs.
- Carp Population Dynamics Group.
- Cost-benefit analysis of alternative carp-control measures.
- Formal risk analysis.
- Recommended (interim) IPM strategy for carp in the MDB.

Nic Bax

Stages of Modelling

1. Development and testing of genetic control techniques on a carp-like population (simulation).
2. Risk assessment (costs/benefits) of alternative integrated pest management programs on specific carp populations, representing the full diversity of carp populations existing in the MDB (data fitting).
3. Monitoring of performance of control strategies once implemented (adaptive management).

Developing and testing on a carp-like population:

- Test performance of existing genetic techniques (in combination with other control techniques)
- Modify expected performance of existing genetic techniques based on new experimental information gained from laboratory and pond trials (e.g. changes in mating behaviour of manipulated fish).
- Identify biological approached that would speed introgression of the transgenic individuals (e.g. accelerated growth, selective removals).
- Develop new theoretical approaches to the genetic control of carp.

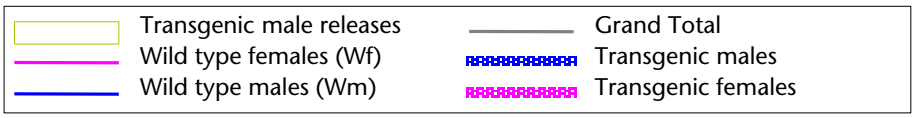
Model characteristics

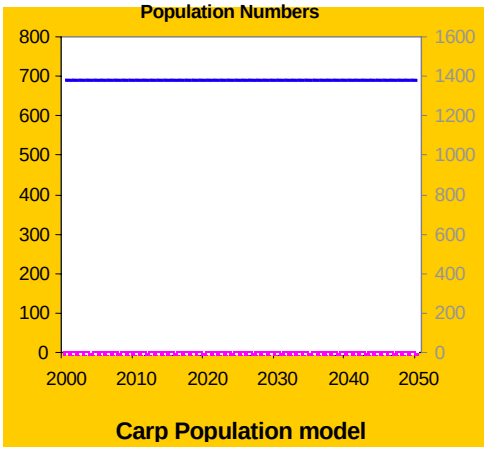
- Emphasis on detailed representation of individual behaviour and genetics
- A realistic range of population characteristics including various levels of spatial interconnection
- Not a detailed model of the MDB population(s)
- Used to design laboratory and pond trials
- Model(s) and parameters updated following results of laboratory and pond trials

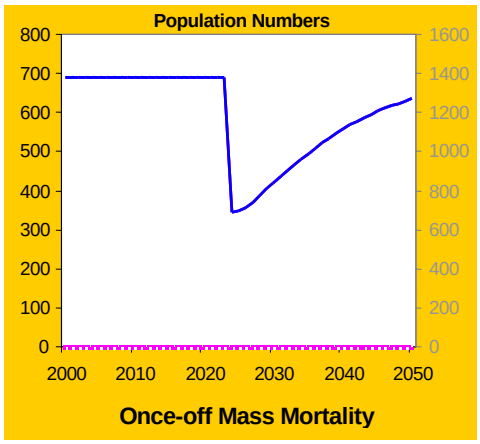
First model of a carp population

Assumptions:

- max age 15
- 50% maturity 3
- 95% maturity 5
- Recruitment 1000
- Equal sex ratio
- Compensation low



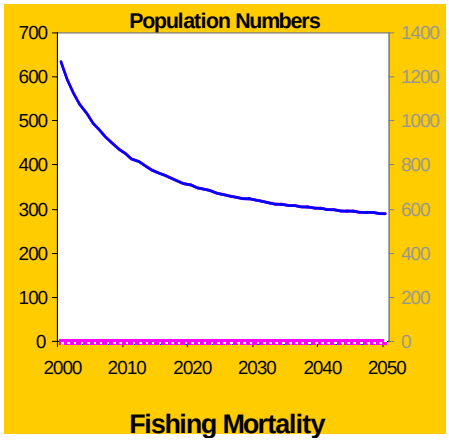


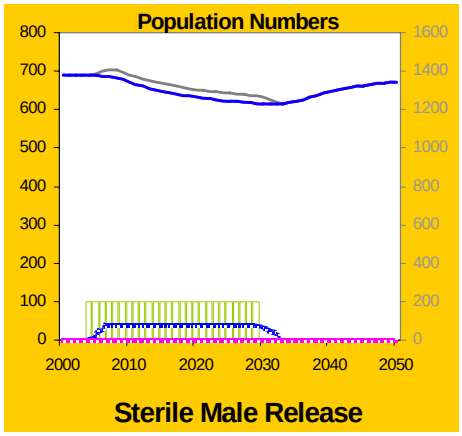


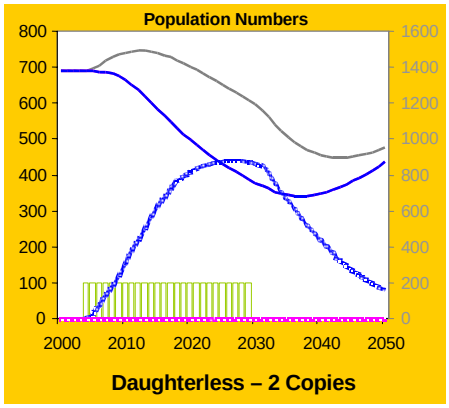
Once off mass mortality – instantaneous population decline of 50% in 2025

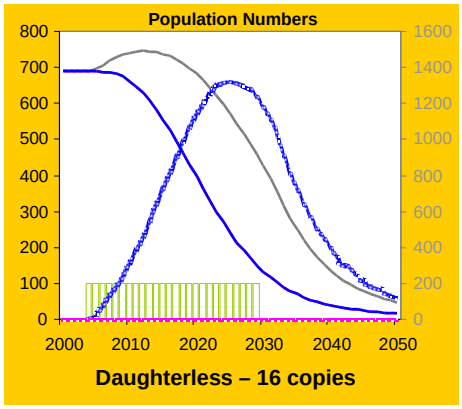
Fishing mortality – continuous mortality at 1/3 rate of natural mortality. Population declines then reaches new equilibrium.

Sterile male release – typically need to release many more males than are naturally recruited to make any impact in population numbers.









Daughterless construct – model predicts significant population decline with repeated releases over 25 years. The model is sensitive to copy number of the daughterless construct – the higher the copy number the greater the population decline. Density dependence is also a factor with recruitment decreased when populations are large.

Improving effectiveness of genetic approaches

- Stage-specific lethality
- Female-biased gene
- Inducible mortality
- Increase introgression rate
- Improved fitness – Trojan gene
- Genetically engineered underdominance to drive gene into the population
  - If transgene can be driven into the population to a level of 50%, it is likely to continue to fixation by itself.
  - Model predicts the transgene will be driven into the population if between 2-3% of recruitment is daughterless carriers.
  - May increase the effectiveness of daughterless approach

Public perception of genetic technologies

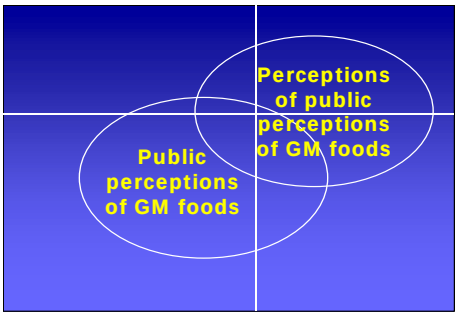
Janine Young, Biotechnology Australia

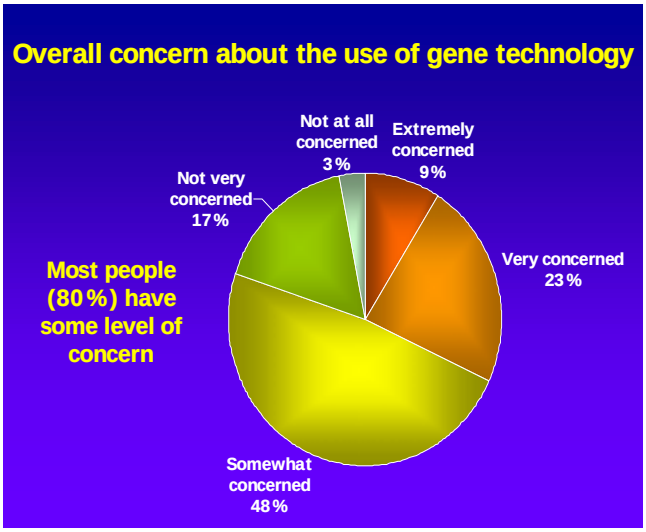
Janine Young has been a Communication Officer for Biotechnology Australia since politely declining a research career after a PhD in Immunology from the ANU. She also has a Science Communication degree and subsequently worked under the auspices of AusAID at the National Science Museum of Thailand.

Biotechnology Australia (BA) aims to create an environment where members of the public are able to make informed decisions on the applications, uses and future of biotechnology, through the provision of balanced and factual information, explaining the benefits and risks of the technology.

There is so much information available that is inaccurate, or biased in one direction or another that finding balanced information can be difficult. In order to be able to deliver this information in the best way possible, it's often useful, if not essential, to know what it is people think in the first place. Another part of our work involves regularly surveying Australians to find out what, and how, they think about biotechnology-related issues. It is well known that this is a rather fraught process, so we use a number of different methods, including telephone polls followed by focus groups.

We have found that the perception of public perception of GM issues is actually quite different from real public perceptions. There is definitely an air of concern – but things are not as bad as they are made to appear when you ask on an individual basis.





So what do we know about people's concerns towards gene technology in general and what is driving those concerns?

It is clear that the majority (80%) of Australians express at least some level of concern regarding the use of gene technology - but there are big differences in their levels of concern.

Of course, answers will vary when you directly ask: Do you have concerns about eating GM foods? Their level of awareness of GM might have been quite low and their concern might also have been relatively low, but a direct question will bring this forward in their consciousness.

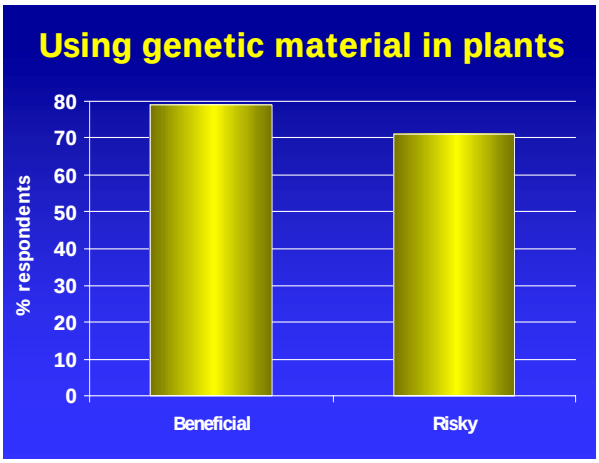
In response to this question, most people say 'yes'. But when we map those concerns against others, we can see that GM foods is the least concerning issue when compared with other food-specific issues such as pesticide use and food poisoning. This effect is even more evident when GM foods are compared with other issues such as climate change and nuclear waste.

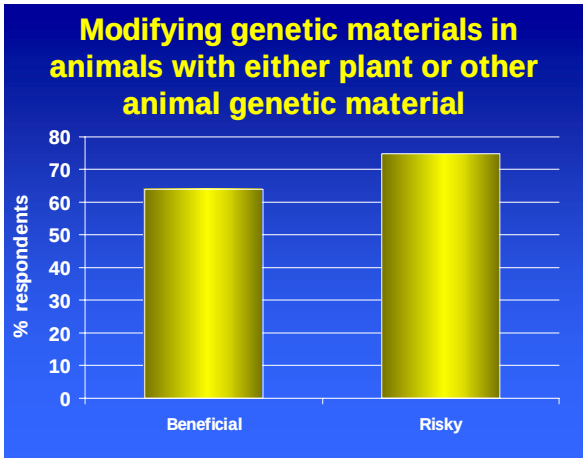
The same patterns have been noted recently in the UK, with a survey by the University of East Anglia showing that concern is relative and likely not the hysteria we're given the impression of.

So, given that people are concerned, on various levels, what are they concerned about?

The clear winner is health concerns, followed by environmental issues. Ethical concerns are not far behind, and then issues of labelling.

We know that modifying the genetic material of plants has higher perceived benefits than risks. People are apparently reasonably comfortable with plant material being transferred between plants.



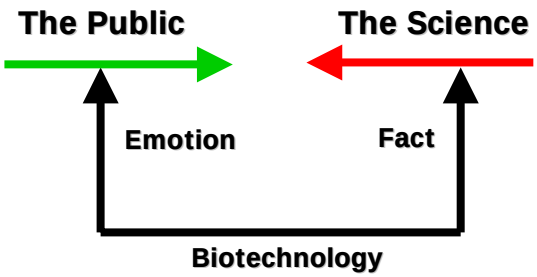


But when you cross animal and plant genes, people are much less happy. This is perceived to have higher risks than benefits.

An interesting addendum is that we have seen acceptance for GM medicines fall slightly in the last couple of years at the same time as GM foods acceptance has risen. There is apparently an increasing danger that big pharmaceutical companies may eventually be perceived in the same way as big agrochemical companies. As an example, we have already seen Aventis and Bayer separate into two companies with separate activities. When we asked what people think about various applications of stem cells in health and medical applications of biotechnology, we found that overall, there is a feeling that most of these applications are acceptable. This changes dramatically, however, when it comes to human cloning. High support instantly becomes over 80% unacceptability.

This is also seen when considering the reasons for undertaking the various applications. When used for purposes that are considered to be worthwhile and fixing a serious condition, there is around 70% acceptability. When the application is considered to be trivial or cosmetic, however, you see the flip again from high acceptability to almost equal, if not stronger, unacceptability.

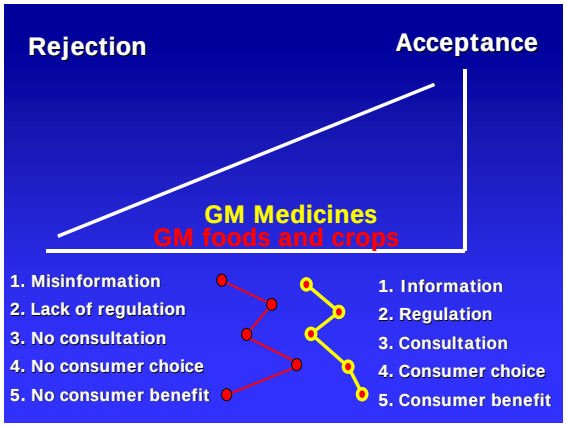
We also know that the biotechnology debate, particularly in the area of agriculture, has been dominated by largely emotional arguments from groups opposed to the technology. This has been countered by facts from scientists.



This clash of fact and emotion does not work to allay fears of the public stuck in the middle. This is very important as, in understanding the public it is useful to know what they think - but it is more vital to know how they think.

For any new technology to be adopted by society it generally has to pass a five-fold test:

- The public must feel they have enough information and that it is credible
- There must be adequate and effective regulation in place
- The public must feel as though they have been consulted on the new technology
- There must be a choice – you can take it or leave it
- There must be some clear and obvious benefit to the consumer, whether it be financial or other.



Currently GM foods and crops in Australia are reasonably strong on regulation and consumer choice. However, opponents are very strong on pushing misinformation and saying that regulation is not strong enough. There are also currently no consumer benefits and consultations are negligible.

GM medicines, on the other hand are reasonably strong on nearly all of the five criteria.

But if you disagree with something, as driven by your internal ethical barometer, no amount of information, regulation or any of the other criteria is going to change your mind. We saw this recently with the stem cell debate. There were very strong negative voices that could see the potential benefits but still came away saying it was just plain wrong.

So, concerns are driven by risk, safety and ethical issues. This is a complex process and varies from person to person.

One of the major findings relevant to attitude formation we have discovered over time is that people are becoming more sophisticated in regard to how they make decisions about accepting or rejecting a technology.

It is no longer a simple risk benefit analysis, as people want to know both about the outcome and the process of the technology. They ask: who benefits from the technology? The community or a company? And is the process harmful or harmless to the environment? This is well-illustrated by the various health and medical applications we asked people about – cosmetic and trivial uses were not acceptable.

The public are least likely to approve of applications that:

- Benefit a company over the public;
- Involve gene transfer from species that are not closely related;
- Are not undertaken for a societal benefit;
- Are perceived as being possibly harmful to people or the environment;
- Were not developed with perceived consultation; and
- Is a food over a non-food application

Applications that the public are most likely to approve:

- Have direct consumer benefits;
- Have a gene modification within the organism, or from an organism that is closely related;
- Have direct societal benefits or align with societal values;
- Are perceived as being not harmful to people or the environment,
- Were developed with perceived consultation; and
- Are not a food or used in foods

Key Lessons

- Concerns must be understood in context
- GM concerns are as much driven by process and ethics as outcomes
- GM foods and crops are moving away as 'hot' topics. Health & medical applications are new 'hot' topics
- Current GM food concerns will apply as much to both pharma-crops and medical applications as to GM foods and crops.

Community Awareness and Involvement

Adrian Wells, National Carp Task Force

Adrian Wells has a background in horticulture, the media, rural counselling, community development and rural education. He currently works with the Murray Darling Association and is based in Albury-Wodonga. Adrian is a founding member of the National Carp Task Force and is project manager for the Murray Valley Trail. He was recently appointed chairman of the Community Stakeholder Group for the Murray-Darling Basin Commission's *Native Fish Strategy*. Last year, Adrian received a Community Services Award from the Victorian Government for services to education, including environmental education.

The terms community consultation, community engagement, community involvement and community participation are written, spoken and embraced enthusiastically by politicians, government agencies, and communities. And in recent years, there have been excellent examples of genuine and effective community consultation leading to good natural resource outcomes. However, there have also been occasions when the concepts have been misunderstood, badly managed, manipulated, or even completely ignored.

Sometimes, those who so enthusiastically promote community consultation need to stop and think occasionally about what the term *community* actually means.

Individuals can belong to a number of communities (eg. family, locality, work, organisations, etc.) and move amongst those communities daily in different ways. Each of those communities can be quite distinct, have different processes and specific aims. These groups or communities cannot be simply lumped under the term '*the community'* and then be a target for some sort of consultation process.

One of the great strengths of the integrated catchment management approach across the Murray-Darling Basin is the strong commitment to community participation and the strong community-government partnership. The commitment by the Commission, the Ministerial Council and Basin governments to involve the community in catchment management is clearly documented. However, in practical terms, engaging the community in natural resource management issues is not simple or easy. It takes time, patience and resources which sometimes there is an unwillingness to invest.

The involvement and participation by the community not only produces better outcomes on all sorts of issues, but it also gives the community interest and ownership of those outcomes. Such activities also acknowledge, and draw on, the wealth of community wisdom, skills and knowledge residing along our waterways.

In community meetings along the Murray River about the Murray-Darling Basin Commission's *Native Fish Strategy,* many participants were recreational fishers. It was enlightening to listen to some of the very astute observations of these people who spend a lot of time quietly waiting, listening and observing. These people don't see themselves as 'experts' but the observations and insights that they provided at those meetings reflected great expertise, not just about fish but about the state of the rivers, condition of trees and vegetation, placement of snags, numbers of birds, and so on. And they were also able to make a very clear link between improving native fish and environmental flows.

Not only were they passionate about native fish, eradicating carp as well as showing an interest in the daughterless carp concept, but these recreational fishers want to be involved in some way from day one.

The Community Advisory Committee of the Murray-Darling Basin Ministerial Council not only wants to ensure on-going involvement and participation by the community in the implementation of the *Native Fish Strategy,* but also wants the community to be involved in the debate about what sort of fish might be eventually put back into the rivers to produce the daughterless carp. The Committee's view is that you cannot just come up with a genetically-engineered fish and assume that the community will be happy to let such fish lose in the river, even if it does mean the end of carp.

Again, it was the Committee's very strong view that the community needs to be consulted, involved and engaged from day one, not when the research results start to look promising.

The daughterless carp program falls within the Commission's *Native Fish Strategy.* As a key component of that strategy is developing a partnership between governments and community, such a partnership must be reflected in the daughterless carp project as well.

It is also important not to forget that the community played a key role in the mid-1990's in getting carp back on the political (and funding) agenda, This was achieved by a number of community forums including the major carp summit at Renmark in 1995. The outcome of that summit was the establishment of the National Carp Task Force by the Murray Darling Association. Over the years, the Carp Task Force undertook community forums on carp; produced high quality education materials; distributes a national carp newsletter *Cyprinus*; provided community representation on the Carp Control Coordination Group; supported communities to remove carp from wetlands; and supported business plans to exploit carp. The Carp Task Force also played a key role in convincing the Murray-Darling Basin Ministerial Council to take a much stronger leadership role in the management of carp.

It is worth noting that the Carp Task Force was established by a Local Government/community organisation. It would be disappointing if its accumulated knowledge, expertise and networks were ignored and not used in the daughterless carp project.

At a workshop on daughterless carp held in Melbourne about 18 months ago, a number of community people urged the development of good communication and consultative strategies to fully engage the community who, at the end of the day, are the ones who will have to live with the consequences of this research. I left the workshop assuming that a commitment was given that a community engagement process would be included in the business plan for the project. I also assumed that this would translate to community participation in managing the project

There are fish scientists who work within the Murray-Darling Basin who actively encourage community involvement in their research and on project steering committees with positive results for the project and the community from day one. This is reflected in the acknowledgment sections of their reports and are usually backed up verbally by the community who speak warmly and enthusiastically about this partnership approach – almost to the point when you could be forgiven for believing that it was their research project and the results belong to them personally (although at the end of the day, that is the goal).

Interestingly, while the integrity and value of CSIRO's research results are rarely questioned in the community, community involvement and participation with CSIRO projects out in the field is sometimes perceived as somewhat lacking.

A report to the MDBC last year identified partnerships and participation as defining characteristics of integrated catchment management in the Murray-Darling Basin. Such community - government partnerships were also characterised as being integral right from the start, not something that was added along the way. The report noted that this was very different from most other approaches, particularly scientific approaches, and reflects changing attitudes regarding the roles played by stakeholders in natural resource management.

There has been a marked shift from the time when governments were regarded as the administrators, researchers were regarded as the experts, and communities were regarded as the passive recipients. We are now in an era of true community engagement, consultation and partnership approaches.

The daughterless carp project must reflect those trends and demonstrate a true partnership approach. The project must acknowledge the wealth of community knowledge, including that of Indigenous people, and tap into the increasing community aspirations that they be included in these types of projects.

In the introductory material for this workshop, it notes that there are many and varied interests involved in carp management and this workshop will ensure that all interested parties have the opportunity to input. The challenge is to give the community every confidence that the input will extend for the life of the project.

Taking Action: the role of modelling in informing decision-making

Charley Krebs, CSIRO Sustainable Ecosystems

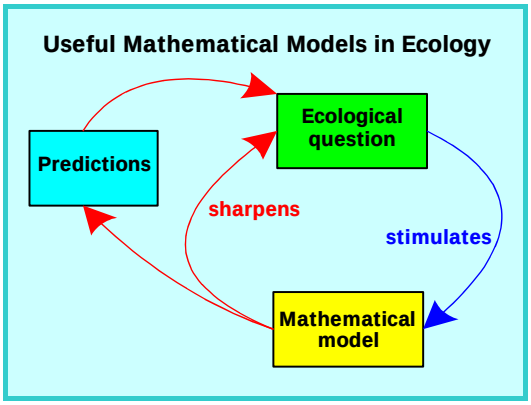
Too much emphasis is being placed on modelling in the Daughterless Carp Project. It is an exciting project but is being developed with too little ecological information. There seems to be no ecological information on the role of carp in the ecosystem. What controls carp recruitment needs to be determined for accurate modelling.

- Models are essential for science but they are **hypotheses**. A great variety of models are possible (verbal, analytical, simulation).
- All models must make some **predictions** and have **testable assumptions**.
- Verbal models are usually looked down on as archaic.
  Analytical models have largely failed ecologists trying to solve practical problems.
  Simulation models and complex systems are in vogue.

Numerical models are often said to be verified, validated or confirmed and we should use these words carefully: [Oreskes *et al.* (1994): *Science* 263:641-646]

- **Verification** = to establish as true. This is impossible to achieve in open systems.
- **Validation** = contains no flaws, input parameters and auxiliary hypotheses are correct.
- **Confirmation** = agrees with the data. BUT some data agree and some do not.

Models in the real world can only be confirmed and should be kept simple to maximise testability. The best models are often the ones that don't work as you can then ask 'why?' If they do work you can't be sure whether you were just lucky.



It is far easier to construct models than to gather data. This has induced an asymmetry into ecology that has been nearly fatal. The assumptions of models are rarely stated clearly or worried about. Even more rarely are they tested empirically.

Fisheries models have in general been an unmitigated disaster for fisheries around the world as they assume a simple biology in an equilibrium world. Recruitment models have been extensively developed for fish populations. However only 38% of published models have been confirmed in subsequent work [Myers 1998, *Reviews in Fish Biology and Fisheries* 8: 285-305]. Much of fisheries management has been misled by unconfirmed models. The standard fisheries modelling argument that there is a simple monotonic relationship between stock size and recruitment is probably not true for any fish population. There are too many other variables that affect recruitment.

Recommendations:

- Use models that have measurable parameters.
- Build in feedback loops to check on model predictions.
- Remember the assumptions.

Remember:

- Mathematicians have a higher status than ecologists.
- Computers run faster than intelligent thought.
- Keep talking to the field ecologists.

Q. Can models help the communication of questions between geneticists and ecologists?

A. Models have a heuristic role. They point out the variables that may be useful but I question the validity of using them in the real world without extensive confirmation and testing of assumptions.

Workshop – Gene Technology Project Development

Facilitator: Richard McLoughlin, Fisheries Victoria, DPI Victoria

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Objectives:

1. Provide detailed information on the planned gene technology projects.
2. Gain comments on issues associated with planned projects.
3. Identify potential collaborators and/or alternative projects.
4. Identify issues that may require further research.

This workshop discussed the status of gene technology projects currently underway that directly impact on the development of daughterless carp. Key objectives of gene technology projects over the next 2-5 years were then discussed and agreed upon. It should be noted that these objectives must be in line with those spelt out in the MDBC agreement. The forum then produced a list of proposed projects and debated how they should be prioritised in the context of the key objectives.

Current Projects:

- Finalisation of proof of concept for *Gambusia* as a model organism – CSIRO Marine.
- Definition of concept: 1. Develop genetic constructs
  2. Constructs stably integrated into fish (*Gambusia*) and inherited in Mendelian manner.
- Current work in China on carp embryonic stem cell (ESC) lines.

Key Objectives of Gene Technology next 2-5 yrs:

- Development of genetically modified (GM) carp for laboratory trials.
- Demonstrated heritability and stability of GM fish (*Gambusial*/carp) in lab.
- Continual development of models for carp.
- Development of comprehensive deployment plan.
- 'Stop/Go' analysis and decision rules.
- Identify locations for field trials and address bio-containment issues.

Key Projects next 2-5 yrs:

1. Review of meiotic drive as the basis for the technology, with respect to carp population dynamics.
2. Development of a carp specific genetic construct.
3. Audit of population genetics of carp with a focus on aromatase.
4. Development of an effective morphological marker.
5. Risk Assessment projects. E.g. effect of aromatase blockers on fitness.

Questions and Comments: (Q=Question, R= Response, C=Comments)

Q. Do we need a review of meiotic drive as the basis for the technology? There has been a long history of proposals for different pests based on this concept but nothing has proceeded to practical application. Are we relying on old research too much? A literature review on applications of the meiotic drive concept and a full understanding of the potential pitfalls is needed.

R. Early experiments based on meiotic drive involved putting mutations into genes and caused too many other problems, e.g. unfit individuals.
The blowfly project failed because there was too much migration and it was impossible to put out enough individuals to make a difference.
There was much debate about the necessity and priority of such a review.

Q. Is the fact that the current work is on *Gambusia* not carp driven by the fact that the work is carried out in Tasmania. Would carp work be better done somewhere warmer.

R. Using *Gambusia* as a model organism is driven more by the long generation time of carp than anything else.

C. There is evidence that the generation time to maturity of carp can be increased by keeping them in warmer water.

Q. Is a morphological marker really necessary? There may be links to fitness in the environment depending on which marker is chosen.

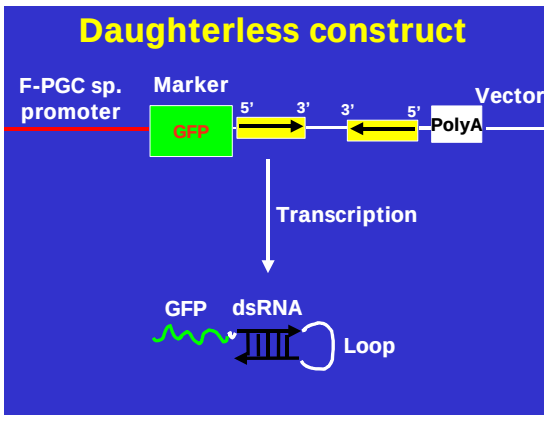
C. Any morphological marker needs to be a dominant one.
Possible problems with using morphological markers if the construct is under the control of a sex specific / cell specific promoter?
Debate over whether markers should be morphological or genetic.

Brief Presentation by Jawahar Patil, CSIRO Marine Research, Tasmania

Mechanisms underlying sex determination and differentiation are complex. This is compounded in fish by environmental influences, however it is a known and exploited fact that the aromatase enzyme is essential to female gonadal development. No aromatase, no ovary development.

Whereas mammals normally have a single copy of the aromatase gene for both brain and ovary function, fish have two copies enabling selective manipulation of ovary function but not brain function.

Double stranded RNA (dsRNA) or RNAi are employed as the chief mode of disruption or silencing of the aromatase gene. The genetic construct (Mark I, diagram right) contains a female primordial germ cell (PGC) specific promoter, a green fluorescent protein (GFP) marker gene and head to head copies of a segment of the aromatase gene which form a hairpin loop upon transcription thus creating a dsRNA.



Project Objectives for *Gambusia* and Carp:

- To establish reliable gene transfer technology in *Gambusia* - there is little evidence of previous successful gene transfer in fish libraries.
- Isolation and cloning of ovarian aromatase in carp and *Gambusia*.
- Isolation, cloning and characterisation of female/male PGC specific promoter
- Building, testing and integrating Mark I, II constructs - regions of lower homology may be an area to focus on for species specificity
- Identifying / engineering a co-segregating morphological marker - desirable for easy selection of transgenic fish.
- Population genetic studies

Transformation of primary blastocysts and embryonic stem cell (ESC) mediated gene transfer may be a potential tool for manipulating carp. Use of ESC lines allows targeting and selection of gene insertion as well as in vitro characterisation of insertion events. Such a technique could be particularly relevant to the daughterless carp project given the long generation time of the fish and reliance on multiple integration of the construct. ESC and/or nuclear transplantation work is already being carried out in several fish species and carp cell lines are currently being developed in China.

Brief Presentation by Peter Grewe, CSIRO Marine Research, Tasmania

Genetic issues to be tackled for producing daughterless carp:

- Appropriate and alternative genes
- Optimizing constructs (Mark I, II..etc)
- Alternative Silencing Mechanisms
- Species Specificity (affects on non target organisms)
- Transient vs. integrated construct
- Multiple copies in the genome

"Daughterless" technology involves the development of a genetic construct using species-native genes (aromatase) that is heritable and that biases off-spring sex ratios towards males.

Preliminary results in zebrafish have demonstrated that expression of the GFP marker gene in the Mark I construct is confined to females and that treatment with the Mark I aromatase blocker produces significantly more males than females. Results so far, however, are based entirely on transient expression and the construct has not yet been shown as heritable or integrated into the genome.

Integration vs transient issues:

- ⊄# Transient primarily a quick test of promoter, used for experiments to date due to logistical constraints.
- ⊄# Integration location (using flanking sequences of specific genes)
- ⊄# Copy number and Silencing
- ⊄# Stability (Generation carry over……..tank experiments)
- ⊄# Affect on viability (overall fitness and reproductive cap.)

Transformation techniques:

- ⊄# Micro-injection
- ⊄# Electroporation
- ⊄# Lipofection
- ⊄# Gene gun
- ⊄# Pantropic Retroviral Vectors and Lentivirus-based Systems
- ⊄# ±Transposable elements

Integration methods to focus on:

- ⊄# Electroporation may work well for carp
- ⊄# Lipofection
- ⊄# Pantropic Retroviral Vectors and Lentivirus-based Systems
- ⊄# ±Transposable elements

Workshop – Communication and Risk Management

Facilitators: Jenifer North, CSIRO and David Dall, Pestat Ltd

Objectives:

1. Identify the communication needs of the Daughterless Carp Program.
2. Propose a process for meaningful involvement of the community in the Daughterless Carp Program.
3. Brainstorm the possible risks associated with the Daughterless Carp Program.
4. Identify processes for further identifying and managing risks associated with the program.

Communication Needs

- Define audiences:
  - Science community
  - Various communities outside the MDBC.
- Define key overall message(s) – e.g. tool in toolbox.
  - Make sure there are no mixed messages. A central coordinating body is needed to construct messages.
  - Continuous messages.
- Put carp in overall environmental / MDBC context and Native Fish Strategy.
- How the Daughterless Carp Program can be used to benefit overall river health.
- Communicate carp not whole problem.
- Balancing of perceived benefits / risks.
- Awareness of community attitudes to fish species.
- Fit into MDBC overall communications strategy.

Community Involvement

- Need to define different communities and their concerns. The community is not one mass, individual consultative programs may be needed to tackle different communities.
- Use National Carp Task Force (NCTF) as a channel. Newsletter could be a good tool to expose / inform the daughterless carp project to different communities.
- Important to target schools because of the long-term (20-50 yrs) nature of this project. Potential to do this through CSIRO Education Programs.
- Identify other sources of partner community involvement that could be used (e.g. Landcare, Rivercare).
  Workshops with groups to convey information.
- Consider internet as a tool, especially for younger people & schools.
- Involve Catchment Management Authorities, MC Community Advisory Committee.
- Wider communities – non MDB – in case we wish to spread the technology.
- Include scientific community, politicians.

Risk

- Technical
- Social / Regulatory
- Commercial
- Environmental (to/from)
- Unknown

Specialist Community	General Community
Demonstrating progress Adequate project design Data collection / management Hybridisation with goldfish etc New introductions "immune"	Other fish species Expectation: success / failure Credibility "making it worse" (more fish, unrelated causes) Lack of clear understanding Risk to people / animals from GM carp ⇓ Public Health issues. Long time frame to visible results. Ecological balance / food web.
Compensation: GMO release ⇓ instant effect Aquarium industry / Koi carp Angling / recreational fishers	

Workshop – Integrated Pest Management

Facilitator: Peter Gehrke, CSIRO

Objectives:

1. Identify past and current projects relevant to positioning the daughterless carp project as part of an integrated pest management program.
2. Conduct a SWOT analysis of the carp control situation in Australia assuming 100% success of the daughterless carp genetic technology.
3. Outline projects that would greatly enhance the implementation of daughterless carp technology, should it be successful.

Past and current projects:

<u>Addresses problem of defining objective:</u> ≠ Work on local recruitment patterns (Ivor Stuart, ARI) ≠ Population dynamics (Paul Brown, MAFRI) ≠ National Carp Management Strategy ≠ Spawning aggregations – targeting (local fishers) <u>Tool Development Projects:</u> ≠ Managing spawning behaviour ≠ Carp removal programs ≠ Selective traps ≠ Pheromone attractants (US) ≠ Poisons and pellets ≠ Fish exclusion dividers <u>Type of Management:</u> ≠ Carpbusters (QLD)	<u>Implementation:</u> ≠ Population dynamics (Paul Brown, MAFRI) ≠ Population structure (Ben Smith) ≠ Downstream migration (Dean Gilligan, NSW Fisheries) <u>Monitoring and Evaluation:</u> ≠ Ecological responses (Dean Gilligan, NSW Fisheries) ≠ Tasmanian studies – including sex bias ≠ Analysis of commercial catch data ≠ Environmental correlations and impacts (Patrick Driver?) ≠ Assessment of recovery (Pilby Ck, Banrock Station).

SWOT Analysis of carp control:

STRENGTHS	WEAKNESSES	OPPORTUNITIES	THREATS
⋕ Interdisciplinary approach. ⋕ Hypothetical potential for eradication. ⋕ Biological control. ⋕ Efficacy of research with respect to other pest species. ⋕ Public support ⋕ Interaction with other methods. ⋕ Long time frame.	⋕ Interdisciplinary approach. ⋕ GMO Issues (Likelihood of gene jumping). ⋕ Need to add lots more carp to reduce numbers. ⋕ Cost of production. ⋕ Interaction with other methods. ⋕ Long time frame. ⋕ Long term funding. ⋕ Opportunities for private involvement.	⋕ Consultation – opp. For experimental testing & ecological understanding. ⋕ IP development and commercialisation ⇓ Aquaculture industry. ⋕ Use natural systems to rear GM juveniles. ⋕ Ecological recovery from impacts. ⋕ Enhance d'less carp technology by other tools and routine management.	⋕ Political issues (Only 1 state needed to block whole thing). ⋕ Effects of endocrine disruptors, pesticides, and environmental estrogens on sex bias manipulation. ⋕ Ecological consequences for predators / birds / fish. ⋕ Long time frame. ⋕ Intergenerational values ⋕ Bad management of project components. ⋕ What to stop / pursue?

Other potential projects:

- ⋕ Ecological and behavioural patterns to develop release strategies (to minimise release numbers etc).
- ⋕ Heritability (of genetic construct) over successive generations.
- ⋕ Control process for recruitment ⇓ Determination of juvenile survival.
- ⋕ Design active Adaptive Management Plan, as per carp strategy.
- ⋕ Optimisation of cost / benefit assessment of all control techniques
- ⋕ Reversibility of environmental impacts of carp.
- ⋕ Reversibility of daughterless carp solution in case of 'worst possible scenario'.

Workshop – Release Strategies: what research is needed?

Facilitators: Wayne Fulton, MAFRI

Objectives:

1. Identify past and current projects relevant to release of daughterless carp into the Murray-Darling system.
2. Agree on the six technical factors most likely to lead to success or failure of a release of daughterless carp into the Murray-Darling system.
3. Describe the knowledge you believe is vital prior to a release.
4. Describe the infrastructure you believe is vital prior to a release.

Past and current projects:

- Population dynamics, modelling (Paul Brown, MAFRI)
- Movement, spawning patterns, seasonal aggregations in Murray river (Ivor Stuart, ARI)
- Larval drift / dispersal; 7-8 years data on stock recruitment (Dean Gilligan, NSW Fisheries)
- Sanjeev Srivastava (ANU)
- Carp spawning – South Australia
- Ecological data from Victoria (Paul Humphries, MDFRC)

Components of a release strategy for daughterless carp:

- How many ⇓ what size, how old?
- Where and when?
- Population dynamics ⇓ recruitment, age, growth, biomass etc.
- Movement / distribution.
- Monitoring, feedback.
- Fish production.

Knowledge required:

- Population parameters – environmental effects, climate
- Movement – present distribution, main sources of recruitment, impacts of barriers
- Spatial variability
- Fish community structure

Infrastructure required:

- Fish production
- Hatchery infrastructure, dependent on dispersal
- Determining numbers for release, timing of release, size.
- Agency communication
- Monitoring – introgression, ecosystem impacts.

Matching R&D needs and capacity

Tony Peacock, Pest Animal Control CRC
Gary Jones, Freshwater Ecology CRC

Needs and capacity

- ⋕ Identify projects
- ⋕ Identify people
- ⋕ Identify interdependencies of projects
- ⋕ Manage as a program, not individual projects
- ♥ # The outcome is reduction in carp damage, not new knowledge.

What are the critical knowledge risks to the success of the project?

- ⋕ Population dynamics of carp – how successful will the introduction be?
- ⋕ Broader ecological implications / certainty of improvement.
- ⋕ Policy / legal framework / financial / economic issues.
- ⋕ Communication and managing stakeholder perception / ownership.

Critical Path Analysis

A. Daughterless gene construct (Gambusia)	**A. Depends on:**
B. Model improvement	**B. Depends on: [E]**
C. GMO communications strategy	**C. Depends on:**
D. Daughterless gene construct (Carp)	**D. Depends on: [A]**
E. Reproductive fitness	**E. Depends on:**
F. Release strategy	**F. Depends on: [A], [D], [B]**
G. IP strategy	**G. Depends on: [A]**

Priority projects will be those on the critical path to reduction in impacts of carp using daughterless technology. Many projects, while desirable, are not critical.

Conclusions

Kevin Goss, Murray-Darling Basin Commission

- Importance of relationship between MDBC, PAC CRC and other stakeholder groups for the success of the overall project.
- Environmental damage / stress is Murray-Darling is presently high – major fish kills, river red gums showing stress.
- Daughterless carp is part of the Native Fish Strategy, which is nested in The Living Murray program, which sits under Water for the Environment.
- The MDBC has the capacity to get knowledge in front of the policy decision makers. It is a flexible and strategic funder and is prepared to spend money on issues of importance. The MDBC invests in a broad range of fish research.

MDBC Daughterless Carp Consultative Group and PAC CRC Carp Reference Group Joint Meeting, 7 March 2003, Canberra

The Daughterless Carp Project is one of the tools essential for improving river health under the MDBC Native Fish Strategy. Areas that need to be addressed include:

Certainty of Improvement

- How much would a "daughterless" tool help river health?'
- Success indicators
- Genetic / competitive advantage

Population Dynamics

- How would daughterless carp react / act on entry to a river?
- Includes fitness, gene spread, expression
- How will the dynamics of the river (temp, flows) affect carp?
- Fragmentation

Success Framework

- Policy
- Social
- Political
- Skills
- Legal
- Technical
- BCA
- Economic
- Financial
- Knowledge
- Intellectual Property (freedom to operate)

Program Structure; Resources Allocated; Potential Co-investors

Project Initiation

⇔

Project Implementation

⇔

Project Control Framework

⇔

Project Closure

- Projects for priority now (i.e. starting 1 June 2003) should be tailored to years 1-3 taking into account research and data that is already available.
- Expectations for gene technology (Ron Thresher's lab) need to be scaled down in line with reduced resources and / or more resources need to be provided (PhD students / full time personnel).
- A strong Communications Strategy is important for implementation and flow of the project. A clearly defined technology and research direction is needed to present to the public to instil confidence in the project. Money needed here to facilitate Genetic Research and Development Strategy and Integrated Pest Management. Have to ensure that we only try to sell what can be achieved.
- BRS pest animal program is currently not involved with daughterless carp and is a potential source of funding.
- Terms of reference used throughout workshop:
  Proof of Concept – proof that "daughterless" technology works.
  Risk Assessment – differs in use. MDBC view 'risk' in financial terms whereas the scientific perspective refers to 'risk' in terms of genetics and heritability of daughterless technology.
- Next Meeting of the PAC CRC Carp Reference Group: 12 – 13 May 2003 Sale, Victoria.

Review of Membership

MDBC Daughterless Carp Consultative Committee

Organisation	Member/s
MDBC	Kevin Goss
Fisheries Departments	Richard McLoughlin (Vic); Steve Dunn (NSW); Will Zacharin (SA); Peter Neville (QLD)
PAC CRC	Tony Peacock
AFFA	Ian Thompson
National Carp Task Force	Adrian Wells
CSIRO	Steve Morton (Corp); Joanne Daly (Ento); Tony Haymet (Marine); John Williams (CLW).
EA (Environment Australia)	Conall O'Connell

PAC CRC Carp Reference Group

The PAC CRC has established a Carp Reference Group to guide the management of the Daughterless Carp Program. At the initial meeting it was decided that:

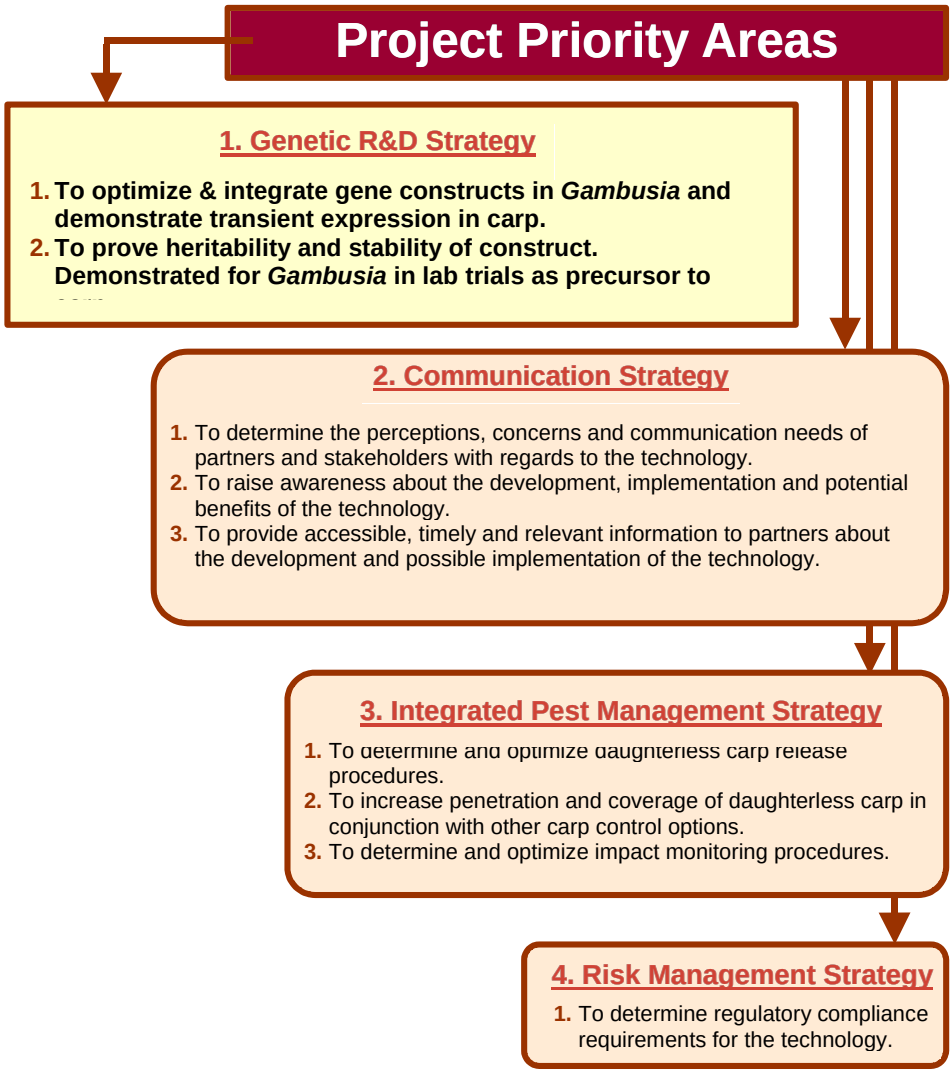
- The 'skills base' necessary for the group is over simplified as 'scientific' skills in the Terms of Reference. It was decided the skills should include communication and fish industry skills and knowledge. The word 'scientific' will be removed from the group's name.
- The reference group will meet at least three times in the first year to get projects running smoothly. It will have a slightly increased membership to allow direct involvement of the Fisheries R&D Corporation and the New Zealand Department of Conservation in the project.

Organisation	Member/s
MDBC	Jim Barrett
K&C Fisheries	Keith Bell
NZ Dept. Conservation	Lindsay Chadderton
Fisheries Departments	Wayne Fulton (MAFRI); Dean Gilligan (NSW)
PAC CRC	Tony Peacock; Steve Lapidge; Brad Tucker
CRC Freshwater Ecology	John Harris
University of	Peter Koopman
National Carp Task	Adrian Wells
FRDC	Patrick Hone
CSIRO	Peter Gehrke (Land & Water)

Workshop Outcomes

The program has now officially begun with a contract signed between the Murray-Darling Basin Commission and the Pest Animal Control Cooperative Research Centre (PAC CRC) for project development and delivery. PAC CRC has in turn commissioned the major genetic development project with CSIRO's Division of Marine Research in Hobart.

Following the National Carp Control Workshop in Canberra, a further call for projects is underway. The main new project priority areas for the daughterless carp are outlined below (a later call for more generic research into societal issues associated with pest animal control is likely).



Other concerns exist regarding the competitiveness of daughterless carp. For example: will aromatase blocking mean that brain development is affected, possibly inhibiting reproductive fitness? Will it be necessary to build in an approach that makes daughterless carp hyper-competitive on release in order to aid gene flow? Would it be better to release a large number of fingerlings or grow them out to a greater weight prior to release?

The policy, regulatory and legal framework for release of daughterless fish

Project Terms of Reference

The Pest Animal Control Cooperative Research Centre is managing a program on behalf of the Murray-Darling Basin Commission aimed at developing 'daughterless' carp for release into Australian rivers, as a means of ultimately controlling carp populations.

The Daughterless Carp program, although only in its earlier stages, raises many issues of concern to stakeholders. These include factors that relate to the policy, regulatory and legal frameworks for release.

The Pest Animal Control Cooperative Research Centre requests project proposals to assist in development of an appropriate assessment of the issues named above as they relate to implementation of Daughterless fish technology. Some issues we consider relevant are identified below.

Policy
Trans-boundary issues; Relative importance of invasive fish strategies within resource management programs; Current GM policies of Australian and New Zealand jurisdictions; Jurisdictional restrictions on pest fish release.

Regulatory
Research compliance with the requirements of, for example, the Office of the Gene Technology Regulator (OGTR); Requirements for field testing and release in relation to the OGTR.

Legal
Issues associated with requirements of the Biological Control Act and the Gene Technology Act, and any other Acts deemed relevant; Possible compensation requirements in relation to a field release; Legal risks associated with the role of a proponent of the technology; Freedom to operate in relation to intellectual property.
The successful applicant will be required to provide a report that describes the current situation in relation to each of the policy, regulatory and legal issues identified. The report will also identify specific actions necessary in order to conduct a release of daughterless pest fish, should this ever be technically feasible. Applicants should provide a statement of their approach and capability, specifically setting out a price, timeframe and deliverables to the Pest Animal Control CRC. There are no restrictions on eligibility to submit an application. However, preference will be given to organisations providing co-investment in the proposed project, and/or those that have a strategic interest in the Daughterless Carp program, and/or those that are members or associates of the Pest Animal Control CRC or the Freshwater Ecology CRC.

Perceptions, concerns and communication needs of stakeholders in regards to daughterless fish

Project Terms of Reference

The Pest Animal Control Cooperative Research Centre is managing a program on behalf of the Murray-Darling Basin Commission aimed at developing 'daughterless' carp for release into Australian rivers, as a means of ultimately controlling carp populations.

The Daughterless Carp program, although only in its early stages, raises many issues of concern to stakeholders. These include, but are not limited to:

- the use, or possible use, of genetic technology to affect wild populations;
- the value of carp to commercial or recreational fishers;
- little or no perception of benefit from city communities unfamiliar with river health issues;
- issues related to possible trans-boundary transfer of daughterless carp to other countries;
- perceptions and expectations that daughterless technology offers a 'silver bullet' solution to both carp incursions and river health.

The Pest Animal Control Cooperative Research Centre requests project proposals to assist in developing a Communications Plan for the Daughterless Carp program. We are seeking proposals that:

- benchmark the Australian public's attitude to carp, and gauge the level of knowledge in relation to the range of control measures. Any proposed study should be repeatable, produce clear guidelines for PAC CRC to fulfill information needs of the community, and be comparable with similar studies conducted by Biotechnology Australia.

- identify key stakeholders in the program, specifically seek to assess their attitude to the program and recommend communication needs and information requirements for each stakeholder.

Applicants should provide a statement of their approach and capability, specifically setting out a price, timeframe and deliverables to the Pest Animal Control CRC. There are no restrictions on eligibility to submit an application. However, preference will be given to organisations providing co-investment in the proposed project, and/or those that have a strategic interest in the Daughterless Carp program, and/or those that are members or associates of the Pest Animal Control CRC or the Freshwater Ecology CRC.
Applications must be received by cob 1 May 2003.

Contact Details

Presenters

Tony Peacock
Pest Animal Control CRC
PO Box 284, Canberra ACT 2601
Ph: (02) 6242 1768
Fax: (02) 6242 1511
Email: tony.peacock@csiro.au

Jim Barrett
Murray-Darling Basin Commission
L5, 15 Moore St, Canberra ACT 2601
Ph: (02) 6279 0154
Fax: (02) 6248 8053
Email: jim.barrett@mdbc.gov.au

Keith Bell
K&C Fisheries Pty Ltd
PO Box 1269, Sale VIC 3850
Ph: (03) 5144 1616
Fax: (03) 5144 1616
Email: bellcarp@netspace.com.au

Ron Thresher
CSIRO Marine Research
50 Castray Esplanade, Hobart TAS 7001
Ph: (03) 6232 5378
Fax: (03) 6232 5485
Email: Ron.Thresher@csiro.au

Nic Bax
CSIRO Marine Research
50 Castray Esplanade, Hobart TAS 7001
Ph: (03) 6232 5341
Fax: (03) 6232 5485
Email: nic.bax@csiro.au

Janine Young
Biotechnology Australia
GPO Box 9839, Canberra ACT 2601
Ph: (02) 6213 6386
Fax: (02) 6213 6952
Email: janine.young@industry.gov.au

Adrian Wells
National Carp Task Force
PO Box 359, Albury NSW 2640
Ph: (02) 6021 3655
Fax: (02) 6021 2025
Email: awells@dragnet.com.au

Charley Krebs
CSIRO Sustainable Ecosystems
PO Box 284, Canberra ACT 2601
Ph: (02) 6242 1623
Email: Charles.Krebs@csiro.au

Kevin Goss
Murray-Darling Basin Commission
L5, 15 Moore St, Canberra ACT 2601
Ph: (02) 6279 0120
Fax: (02) 6249 6139
Email: kevin.goss@mdbc.gov.au

Gary Jones
CRC for Freshwater Ecology
Bldg 15, University of Canberra ACT 2601
Ph: (02) 6201 5168
Fax: (02) 6201 5038
Email: pa@lake.canberra.edu.au

Project Officer – Carp research

Brad Tucker
Pest Animal Control CRC
PO Box 284, Canberra ACT 2601
Ph: (02) 6242 1547
Fax: (02) 6242 1511
Email: bradley.tucker@pestanimal.crc.org.au

Pest Animal Control Cooperative Research Centre
c/ – CSIRO Sustainable Ecosystems
Barton Highway Crace Canberra ACT Australia
GPO Box 284 Canberra ACT 2601 Australia
Phone: + 61 2 6242 1768
Fax: + 61 2 6242 1511
Email: office@pestanimal.crc.org.au
Web: www.pestanimal.crc.org.au