PESTSMART

Exploitable biological vulnerabilities of common carp

Susan L. Gehrig
Leigh A. Thwaites

Invasive Animals CRC

An Australian Government Initiative

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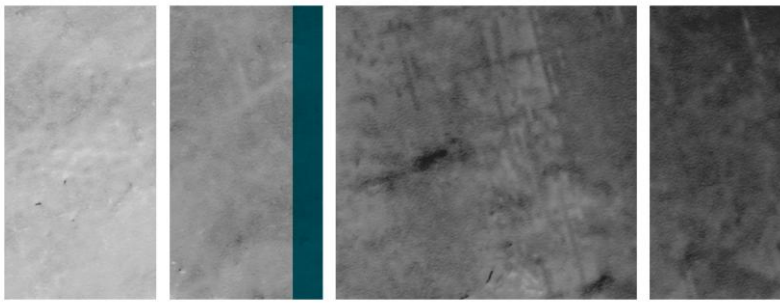

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South Australian Research and Development Institute (SARDI)

SARDI Aquatic Sciences
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An Invasive Animals CRC Project

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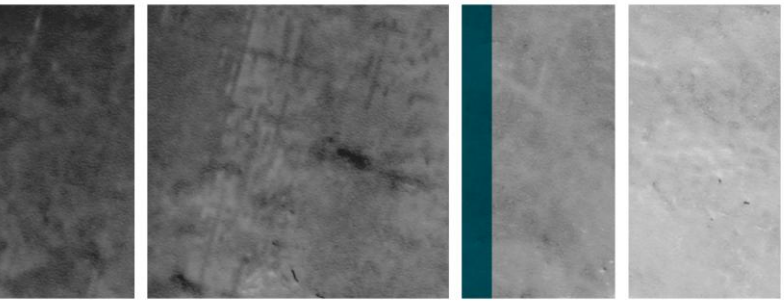

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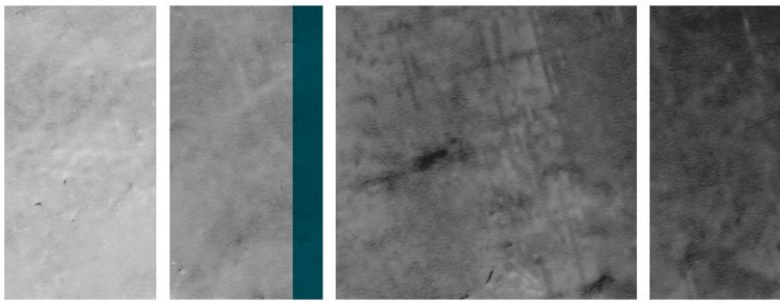
Front cover image: Installation of the Lake Bonney Wetland Carp Seperation Cage (SARDI Aquatic Sciences)



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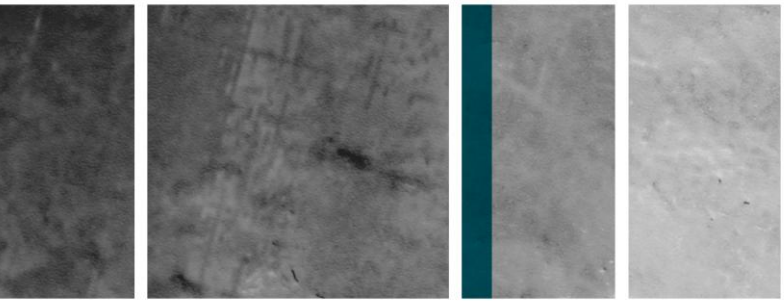

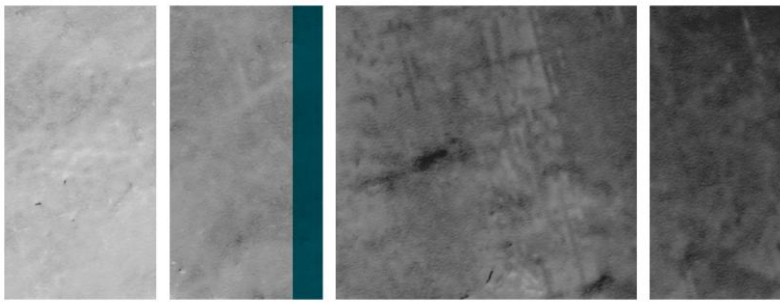

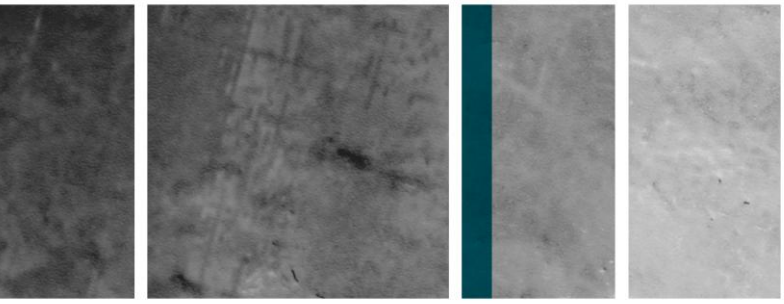

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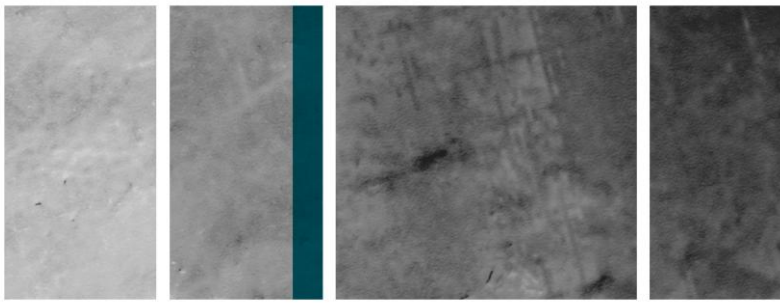

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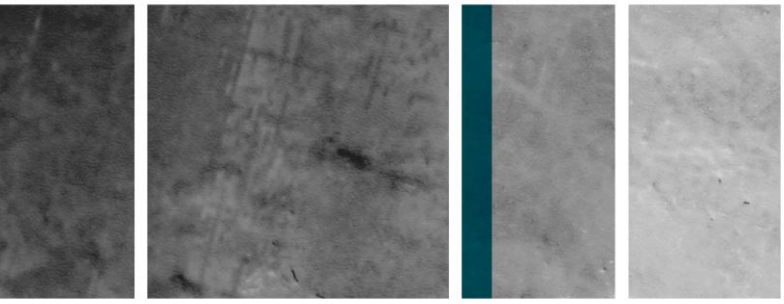


Summary

This project synthesises the outputs of various projects supported by the Freshwater Products and Strategies Program of the Invasive Animals Cooperative Research Centre (IA CRC) in order to assess weaknesses of carp that can be exploited for their control.

Some of the key vulnerabilities identified that may contribute to carp management in Australia include:

1. Limited number of carp spawning sites

It was found that although adult carp populations were widespread and abundant across the MDB; these populations were supported by a limited number of areas where juveniles were present (*Project 1,* Carp spawning hotspots in the MDB). This suggests carp reproduction is localised and restricted to a relatively small number of 'hotspots' within the MDB. Such identification of hotspots allows carp control to be targeted at a key number of recruitment sources rather than scattered over tens of thousands of river kilometres.

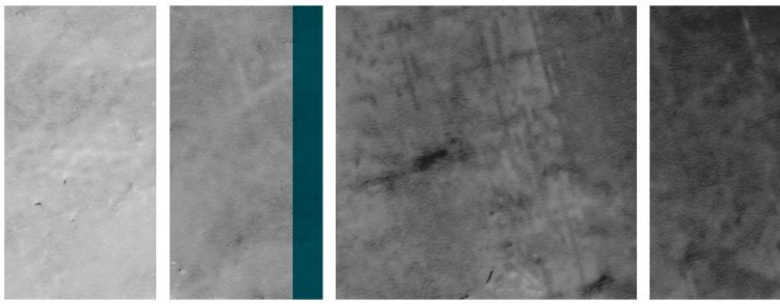
At the local scale it provides potential for:

- targeted control of adult carp migrating towards spawning areas (ie spawning aggregations),
- exclusion of adults from spawning areas,
- and control of dispersing juveniles from spawning areas.

Conventional physical control methods tend to be effective on small spatial scales or for short timeframes, but in conjunction with Integrated Pest Management (IPM) programs these spawning hotspots may provide a focus for a carp biocontrol agent release following spawning events, which would improve carp management at the regional scale. For example, hotspots may provide ideal target areas for the release of transgenic genes ('daughterless gene technology') or vectors carrying other potential biological control agents (eg Koi herpes virus), 'Judas' fish (radio-tagged male fish that are used to track carp aggregations) or installation of wetland carp separation cages (CSC) for carp removal. If similar long-term databases exist, the information could be applied for detecting carp recruitment hotspots in other catchments within Australia and elsewhere.

2. Limited carp movement

It was found that adult carp move at relatively small scales between sub-catchments in the MDB (*Project 2*, Carp movement and migration in the MDB), particularly in low-flow conditions. The limited nature of catchment scale dispersal movements throughout this study suggests that adult carp have limited capacity to colonise connected sub-catchments in large numbers during low flows and that colonisation most likely occurs from i) the progeny of a small number of dispersing adult carp or ii) dispersing juveniles. Limited adult carp movement suggests there is strong potential for using cost-effective, targeted physical, chemical and/or biological control strategies at local and regional scales since control of adult carp may be sustained in certain areas by consistent methods that prevent either re-colonisation of juveniles or the reproduction of new colonisers.



3. Innate behaviours

Further research confirmed that carp have innate behaviours, such as juvenile and adult carp migrate annually between river and wetland habitats for spawning from early August onwards (*Project 4*, Optimised wetland carp separation cages). During spawning times, carp were attracted to flowing water and moved upstream towards the source of the flow. Carp also had an innate ability to push past or jump over barriers, even in shallow waters < 40 cm. Therefore carp control strategies that focus on intercepting and harvesting carp at wetland entrances are particularly desirable as migrating carp are vulnerable to trapping. Thus, at the local scale there is potential to exploit these innate behaviours, especially through physical control methods such as:

- carp exclusion screens (CES),
- CES with integrated push trap element,
- wetland carp harvesting systems,
- and a combination of all (or some) of the aforementioned controls.

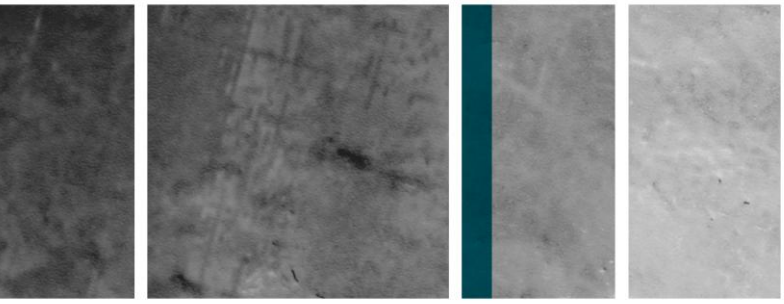
At the regional scale, identification of the triggers (e.g. flows) that might cue spawning migrations may provide more foci for the release of a carp biocontrol agent as part of organised IPM programs.

4. Genetic structure

Within the Murray-Darling Basin, three discernible strains of carp were identified in Project *5* (Population genetics of common carp in the MDB); descendant from the European/central-Asian subspecies *Cyprinus carpio carpio*. Most importantly the three strains were found in distinct locations within the regional scale. The identification of specific locations for carp strains within catchments builds upon the evidence that there are discrete management units that could be routinely targeted at the local scale for carp control programs.

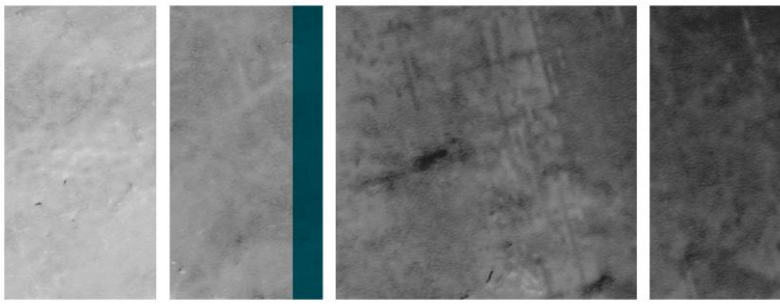
In addition, from a pest control context, the ability to control the expression of genes that influence sex expression in carp could allow for the manipulation of sex ratios or in particular create a sex bias that leads to population extinction. *Project 6* (Sex Determination and Differentiation in carp), found that there are a number of genes that play a role in early larval development in carp. In particular, the *dmrt1* gene is a critical male-differentiating factor in carp, exclusively expressed in the gonad and indicating an early role in the sex-determining pathway. Thus the gene product *dmrt1* is a strong candidate for daughterless technology in carp because it could potentially direct ectopic gene expression in developing gonads of genetic females, resulting in their sex reversal to males and creating a sex bias in carp populations.

Although both methods are in early stages of development, carp populations could decline significantly in 20 years and reach pseudo-extinction within 30 years with a successful release of daughterless gene technology. Conversely, conventional physical/chemical control methods are generally only effective on small spatial scales or for short timeframes. Nonetheless, such conventional tools are still important because significant barriers often exist to the employment of biological control options arising from the obvious technological issues, such as host specificity, the substantial resource investment required, as well as public perceptions and regulatory requirements.



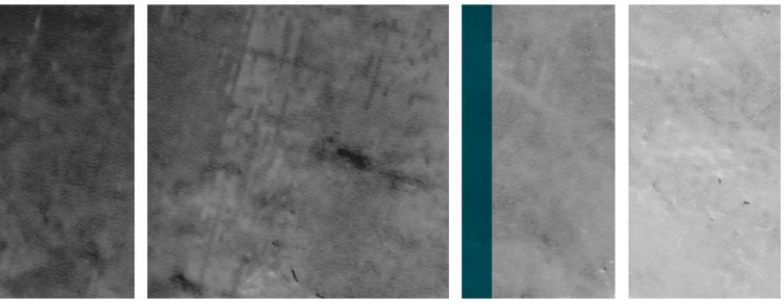
Finally, while no immediate insights into the vulnerabilities of carp were identified in the following projects, they contribute to the knowledge of carp reproductive biology, which is critical for modelling population dynamics and for assessing potential responses of carp populations to various control strategies. For instance it was assumed that most carp form one complete growth check per year (e.g. carp populations in the southern MDB); however *Project 3* (Carp age validation in the northern MDB) found that the majority of carp from the northern, subtropical region of the MDB form one complete growth check per year, but a small minority form two complete growth checks per year. In addition, *Project 7* (Early Gonad Development in the Common Carp) shows that both differentiated gonochorists (where the larvae develop directly into males and females) and undifferentiated gonochorists (where individuals develop ovotestis) co-exist in carp, although most are differentiated gonochorists, forming either an ovary or testis. This suggests an effective reproductive strategy in common carp that may be a significant contributing factor to their successful recruitment in the MDB.

Identifying discrete management units within catchments (Project 5) or locating specific 'hotspot' spawning sites (Project 1) provides strong potential for targeted, refined localised control programs. Especially if known, innate behaviours of carp (e.g. annual spawning migrations), are exploited with the use of targeted physical control methods, such as carp harvesting systems (Project 4). For instance, carp harvesting systems could be used at wetland inlets to prevent entry of adult carp into potential 'sink' hotspots, or alternatively used to minimise dispersal of juvenile carp from 'source' hotspot locations (Project 1). If localised targeted control efforts are organised, integrated and consistent, carp control would in turn be enhanced at the regional scale, which is the key to carp control at the Australia-wide scale. However, further research on carp population genetics (Project 5), hotspot locations (Project 1) and carp movement (Project 2) at the broader Australia-wide spatial scale is needed in order to identify a network of discrete management units that could then be targeted and prioritised under certain circumstances (temporal spawning, recruitment) thus allowing even further refinement of control strategies. Furthermore, more information and data on carp reproductive and development biology is needed to assess population dynamics and genetic structure as this information is key to enhancing carp management strategies and assessing the potential carp responses to carp control programs.



Acronyms and abbreviations

CAT	Coded Acoustic Transmitters
CES	Carp exclusion screens
CPUE	Catch per unit effort
CSC	Carp separation cage
DCP	Daughterless Carp Project
dmrt1	*doublesex* and *mab*-3 related transcription factor 1
dpf	days-post-fertilisation
FCA	Factorial Component Analysis
FFRD	Freshwater Fish Research Database
FL	Fork length
IA CRC	Invasive Animals Cooperative Research Centre
MDB	Murray-Darling Basin
MDBA	Murray-Darling Basin Authority
NSW	New South Wales
OTC	oxytetracycline
PCR	Polymerase chain reaction
PIT	Passive integrated transponder
SL	Standard length
TL	Total length
YOY	young of the year



Document scope

This report provides a synthesis of knowledge outcomes from various research projects that were selected and supported by the Freshwater Products and Strategies Program of the Invasive Animals Cooperative Research Centre (IA CRC), including additional and relevant information from Australian literature. The primary aim of this synthesis report is to consider the weaknesses in carp biology that have been identified by the IA CRC projects and that could be exploited for control purposes.

This review supports Goal #4 of the IA CRC in 'reducing carp and other pest fish impacts' and the associated target of providing 'a capacity to deliver improved quality and availability of inland water through reduced impacts and rates of spread of carp and other pest fish species'.

The research projects that were selected by the IA CRC were:

Project 1: Carp spawning hotspots in the MDB. Project Leader: Dean Gilligan, Industry and Investment, New South Wales.

http://www.invasiveanimals.com/research/phase1/goals/goal-4/4f5/

Project 2: Carp movement and migration in the MDB. Project Leader: Paul Brown, Victorian Department of Primary Industries.

http://www.invasiveanimals.com/research/phase1/goals/goal-4/4f6/

Project 3: Carp age validation in the northern MDB. Project Leader: Michael Hutchinson, Department of Employment, Economic Development and Innovation, Queensland.

http://www.invasiveanimals.com/research/phase1/goals/goal-4/4f5/

Project 4: Optimised wetland carp separation cages. Project leader: Ben Smith, South Australian Research and Development Institute (SARDI) Aquatic Sciences.

http://www.invasiveanimals.com/research/phase1/goals/goal-4/4f12/

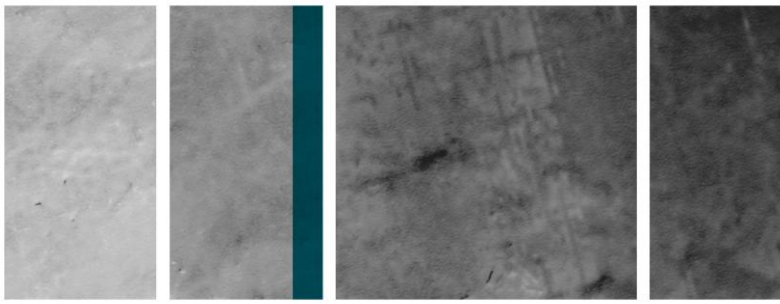
Project 5: Population genetics of common carp (*Cyprinus carpio*) fry in different recruitment areas in the Darling-Basin (MDB), Australia. PhD candidate: Gwilym Haynes, University of Sydney.

http://www.invasiveanimals.com/about-us/people/students1/gwylim-haynes/

Project 6: Sex Determination and Differentiation in carp, *Cyprinus carpio*. PhD candidate: Megan Barney, University of Tasmania.

http://www.invasiveanimals.com/about-us/people/students1/megan-barney/

Project 7: *Early Gonad Development in the Common Carp* Cyprinus carpio *(L.) at 20°C and 25°C*. Honours candidate: Janina Beyer, University of Tasmania



Document structure

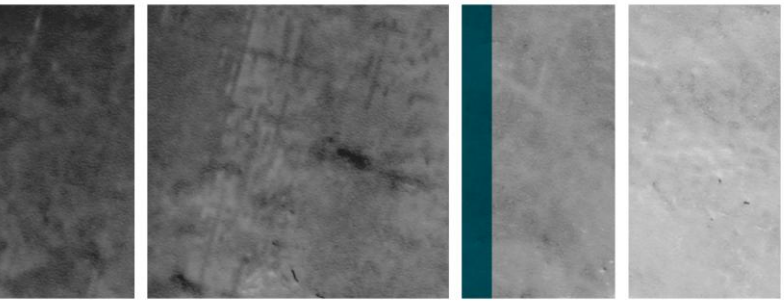
As a synthesis of information, this report is relatively brief. Readers are encouraged to access the final reports and associated documents or to contact the primary researchers for detailed project information, such as methods, experimental design and analyses.

A short synopsis of major concepts relevant to each research project is presented as a prelude. Information relevant to each research project is then provided under the following headings:

- Project publications (if applicable)
- Project summary
- Rationale and objectives
- Outcomes
- Vulnerabilities and application
- Suggested future work.

Final synthesis and concluding remarks are provided in the discussion at the end of the report. This section highlights how this review contributes knowledge for transforming carp and pest fish management from current ad hoc arrangements to integrated management over large regions, with targeted tactical interventions in key hotspots.

For a full list of projects within the Freshwater Products and Strategies Program ('Freshwater'), refer to *http://www.invasiveanimals.com/research/goals/*. The majority of the Freshwater carp projects are incorporated under Goal #4.



Introduction

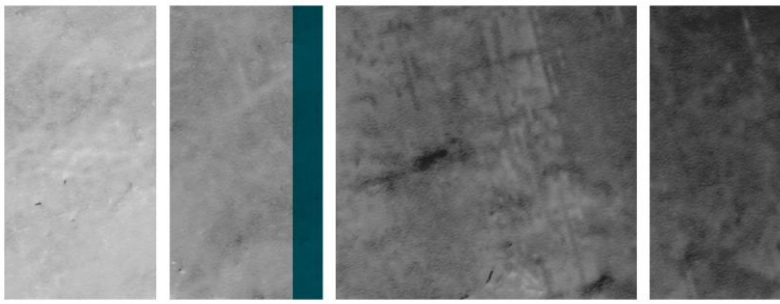
Common carp (*Cyprinus carpio* L.; Family Cyprinidae) are teleosts, or ray-finned fish, and are regarded as powerful invaders of Australian waterways (Koehn 2004). In 2004, expenditure on carp invasions was estimated to be ~AU$15.8 million dollars annually; $2 million was allocated to carp management, $2 million to research and the remainder to remediation of environmental impacts (McLeod 2004). Carp have spread to all Australian states except the Northern Territory, but have the potential to invade all permanent freshwater habitats (Koehn 2004). According to Koehn (2004), areas of particular concern include:

- eastern and southeastern coastal rivers of the mainland,
- Tasmanian waterways,
- drainages along the north coast,
- and Lake Eyre and Bulloo–Bancannia drainages in central Australia.

Carp are habitat generalists, associated with all manner of aquatic habitats (Stuart and Jones 2002; Nicol et al 2004), such as:

- main channels,
- floodplain lakes,
- anabranches,
- wetlands,
- swamps,
- billabongs,
- irrigation channels,
- macrophyte stands,
- open water,
- overhanging riparian vegetation zones,
- undercut banks,
- and woody debris.

They also prefer mid-latitude, low-altitude, lentic environments with silty substrate and access to shallow vegetated areas for spawning (Smith 2005). Carp are often found in degraded habitats that reflect sustained, human-induced impacts (Cadwallader 1978; Koehn 2004). In these instances, carp may dominate catch numbers and biomass; hence, native species diversity may be low (Gehrke et al 1995; Gehrke and Harris 2000, 2001).



Carp can tolerate broad environmental conditions (see Koehn 2004; Smith 2005). They are capable of withstanding:

- temperatures ranging from 2 to 40.6ºC,
- pH ranging from 5 to 10.5,
- high turbidity,
- moderate salinities,
- high toxicant loads,
- and low dissolved oxygen levels.

Carp are omnivorous, mainly feeding on aquatic invertebrates, aquatic plants and algae by sifting through sediments (Jhingran and Pullin 1988; Billard 1999; McLeod and Norris 2004). Male and female carp are long-lived (up to 28 years), mature relatively early (≈300–350 mm Total Length; TL) compared with similar-sized native fish and highly fecund (≈100 000 eggs per kg) (Brown et al 2003).

'Invasive' species such as carp are able to establish, reproduce and disperse within an ecosystem, but how they impact or disturb an ecosystem is complex (Ehrlich 1989; Whitney and Gabler 2008). For instance, although often associated with disturbed environments (Cadwallader 1978; Koehn 2004), carp also behave as ecosystem engineers within their invaded territories, indirectly and/or directly altering resource availability by physically destroying submerged vegetation. This physical alteration of habitat, according to King et al (1997) and Crooks (2002), may indirectly affect resident biota via:

- an increase in water turbidity,
- algal blooms,
- damage to river banks,
- loss of aquatic vegetation,
- alterations to the trophic cascade of ecosystems,
- and subsequent declines in native fish numbers; usually as a consequence of the above impacts.

Furthermore, identifying environmental impacts of carp and quantifying their extent is complicated by the difficulties of discerning them from other human-induced factors that degrade waterways and affect native fish populations, such as flow regulation, irrigation and land clearing (Smith 2005).

Some approaches to vertebrate pest management may include a variety of physical, chemical and/or and/or biological control methods (

Table 1, adapted from Kerr 2007; Saunders et al 2010). Other approaches may be to introduce a known exotic predator, exotic virus or a genetically modified virus. These, however, are generally considered to be some of the least desirable and acceptable methods to employ (Kerr 2007).

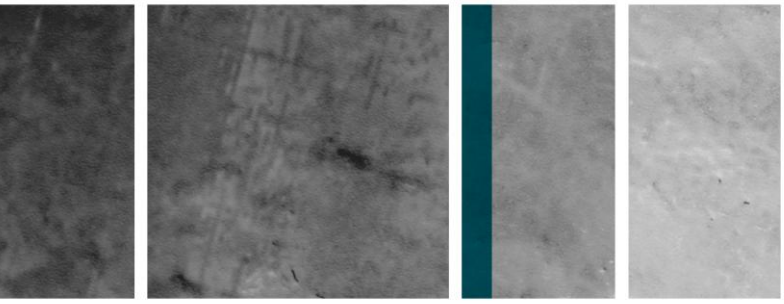


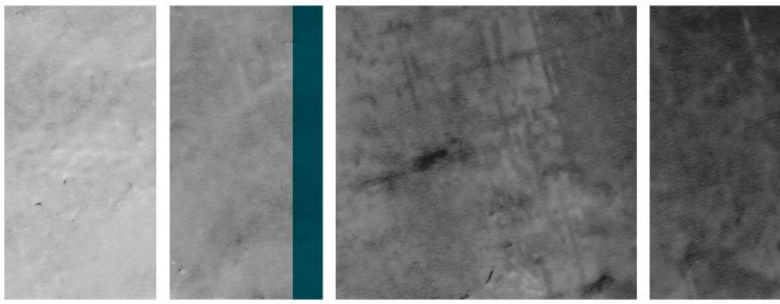
Table 1. Examples of various approaches to vertebrate pest control/management.

Physical/chemical options	Biological options
• do nothing • environmental remediation (pests in disturbed areas only) • physical removal • water-level manipulations • commercialise • use species-specific biocides, or non-specific biocides	• augment native predators • augment native pathogens • develop genetic manipulation of pest only • introduce exotic parasites • develop and introduce exotic diseases • genetic manipulation of native species

Some of the methods in Table 1 have been used on carp within Australian waterways. To date, however, attempts to control carp have been rather ad hoc and localised with the exception of control efforts in Tasmania (Fulton and Hall 2008). A range of techniques used to control carp and their associated environmental impacts are discussed in Shields (1958), Gervai et al (1980), Berg et al (1997), Koehn et al (2000), Propst and Gido (2004), Taylor et al (2005), Stuart et al (2006a), Stuart and Jones (2006b), Thwaites and Smith (2010), Thwaites et al (2010). These include;

- commercial and recreational harvest,
- environmental rehabilitation,
- water level manipulation,
- biomanipulation (eg stocking with predatory fish),
- exclusion with screens or barriers,
- poisoning and biological control,
- bioacoustics and bubble barriers,
- and genetic manipulation.

In terms of physical removal methods, some state governments (eg NSW) have introduced incentive schemes, such as offering commercial fishing licences to parties who could demonstrate the ability to catch and sell carp (Kick 2001). Although physical removal as a population management tool is not practical at a whole-of-basin scale (Brown and Walker 2004), recent advances in targeted harvesting technology have improved efficiency in some instances. One example is the use of 'Judas' fish in Tasmania, where a number of males were radio-tagged to detect aggregations and to determine habitat preference patterns and behaviours (Diggle et al 2004). Other examples are the Williams' carp separation cage (CSC), a low-cost, automated trap/drafting device designed for fishways (Stuart et al 2006a), and push traps, which have recently been adapted for wetlands (Thwaites and Smith 2010; Thwaites et al 2010; Conallin et al in press).



Currently, two alternative biological control options are being evaluated: Koi herpes virus (KHV) and daughterless carp gene technology. The latter involves the use of 'autocidal' genetic techniques, based on the inheritance of transgenes through males to either sterilise females or convert them into functional males (Smith 2005; Thresher 2007). Although both methods are in the early stages of development, carp populations could decline significantly in 20 years and reach pseudo-extinction within 30 years with a successful release of daughterless gene technology. Conversely, conventional physical/chemical control methods are generally only effective on small spatial scales or for short timeframes (Thresher 2007). Nonetheless, such conventional tools are still important because significant barriers often exist to the employment of biological control options arising from the obvious technological issues, such as host specificity, the substantial resource investment required, as well as public perceptions and regulatory requirements (Saunders et al 2010).

Ideally, vertebrate pest control should focus on the dual objectives of maximising efficacy and minimising non-target hazards (O'Brien 1986). Hence, efforts are now directed to a more coordinated/integrated approach to carp research and management, especially in the areas of detection, prevention, rapid response and education. With the support of the Murray–Darling Basin Authority (MDBA), the IA CRC established a program to specifically collect critical information on carp biology to discover weaknesses and vulnerabilities to develop effective carp control mechanisms.

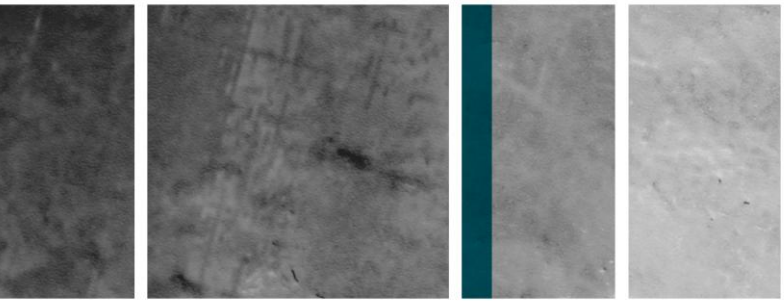
Aims

The overarching aim of this synthesis report is to consider the weaknesses in carp (*Cyprinus carpio* L.) biology and ecology that were identified by the IA CRC research projects and that may be exploited for control purposes.

This review specifically supports IA CRC Goal #4: 'reducing carp and other pest fish impacts' and the associated target of providing 'a capacity to deliver improved quality and availability of inland water through reduced impacts and rates of spread of carp and other pest fish species'.

Research Scope

This report provides a synthesis of the key knowledge outcomes from research projects that were fully supported by the Freshwater Program of the IA CRC. In particular, we present knowledge outcomes from four completed research projects (4.F.5: Project 1, Carp spawning hotspots in the MDB; 4F.6: Project 2, Carp movement and migration in the MDB; 4.F.11: Project 3, Carp age validation in the northern MDB and 4.F.12: Project 4, Optimised wetland carp separation cages), two completed PhD projects (Population genetics of common carp in the MDB, Haynes 2009; Sex determination and differentiation in carp, Barney 2010) and one completed Honours thesis (Early gonad development in common carp, Beyer 2005). We also included a wider search of information from the Australian literature, such as peer-reviewed articles in scientific journals, fishery and/or departmental reports and unpublished manuscripts.



Project 1: Carp spawning hotspots in the MDB

Prelude

Reproductive biology of common carp in Australia

The remarkable reproductive ability of carp is a major factor in its success, and so understanding its reproductive biology is a crucial step in controlling carp recruitment in Murray–Darling Basin (MDB) waterways (Smith and Walker 2004a). Carp are asynchronous, multiple-batch spawners with various breeding seasons (Sivakumaran et al 2003b; Smith and Walker 2004b). Eggs become mature by the end of winter, and reproduction begins in early spring. Not all females spawn at once, and gonads of individuals include a range of developmental stages (Sivakumaran et al 2003; Smith and Walker 2004b). Although all oocytes of a matured batch are generally spawned at once, up to 20% may be retained for repeat spawning (Smith and Walker 2004b). After spawning and absorption of residual oocytes, rematuration of the ovaries takes at least 2–4 months (optimal is 60 days at 20°C, Billard 1999). Females of 47 cm produce about 300 000 sticky, adhesive eggs, which are laid on shallow vegetation (Jhingrand and Pullin 1985).

Carp spawning may occur when mean water temperatures and photoperiods exceed minimum thresholds (15–16°C and 10 h light) and where there is access to suitable habitat, such as submerged vegetation in shallow, lentic environments (Hume et al 1983; Smith and Walker 2003a, 2003b). In South Australia, temperature and light thresholds are reached annually from about mid-September to March/April (6–7 months), which correlates with the known spawning period of carp (Smith 2004; Smith and Walker 2004a, 2004b). In the Barmah–Millewa Forest, Victoria, climatic conditions are similar to South Australia, and carp also spawn from mid-September to April (Sivakumaran et al 2003). Hence, individual females may spawn two batches of eggs per annum in these locations: once at onset and another three to four months later when gonads have rematured. Even in lakes Crescent and Sorell, Tasmania, female carp may spawn up to two batches of eggs per annum when temperature and light thresholds are reached from November to March (Donkers 2003; Day et al 2004).

Carp generally occupy two broad habitat types: shallow wetland habitats during spring through autumn and deep water habitats during winter. Shallow habitats enable feeding, spawning and the replenishment of populations via recruitment (Smith and Walker 2004b; Stuart and Jones 2006b). Deep habitats maintain warmer stable temperatures compared to surface waters (Johnsen and Hasler 1977; Inland Fisheries Service 2008; Penne and Pierce 2008). Migrations between these two habitats occur annually (Penne and Pierce 2008).

Project Summary

In the main channel of most rivers, suitable spawning habitat, such as submerged vegetation in lentic environments, is scarce. Hence, offchannel water bodies are major point sources for carp recruitment (Stuart and Jones 2001; Stuart and Jones 2002). It has been suggested that carp exhibit source-sink population structure at broad scales (Pulliam 1996; Driver et al

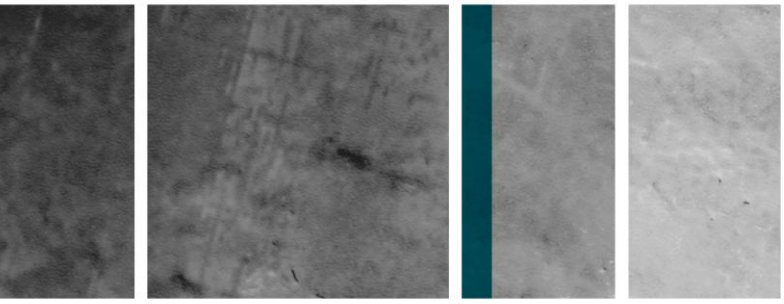
2005): the most significant source populations are represented by unregulated lowland rivers, but the sink populations are represented by slope zones of catchments. Studies at finer scales in the mid-reaches of the Murray River, southeastern Australia, suggest that carp's reproductive activity and recruitment is not widespread but restricted to a single large floodplain wetland system (Gilligan and Schiller 2003; Crook and Gillanders 2006; Stuart and Jones 2006b).

Identifying hotspots for reproduction and recruitment within the MDB (see Figure 1 for general study region description) will help to:

- explicitly define spatial structure of carp populations
- specify the population units that contribute the most to recruitment (ie source versus sink populations)
- allow specific areas to be prioritised for focused carp control efforts, especially as part of an integrated pest management strategy.

The CarpSim modelling (discussed in further detail in Project 2) demonstrates the likely impact of carp management at specific key spawning/recruitment sites within a river system.

Figure 1. Map of Murray-Darling Basin (MDB) catchment showing major river reaches. Source: http://www.connectedwaters.unsw.edu.au/articles/2008/05/supermodelling-murray)



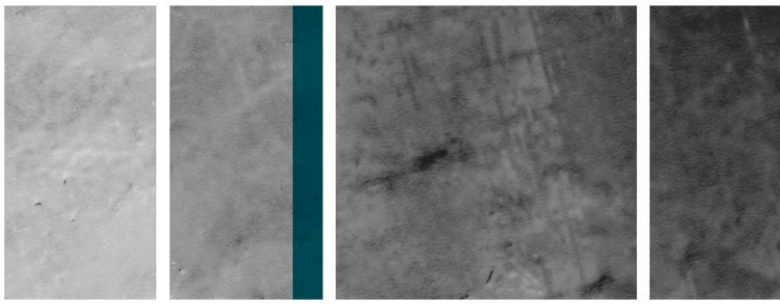
Rationale and objectives

Data from several individual projects were used for Project 1, resulting in a substantial longterm data set. Since 1990, the government fisheries research agency in NSW has routinely collected data on the abundance and size structure of carp populations, using a consistent electrofishing procedure. The electrofishing protocol consists of between 10 and 20 minutes of single-pass electrofishing time, using either backpack or boat-mounted electrofisher units. Data from these surveys were collated within the NSW Freshwater Fish Research Database (FFRD). A second source of data was available from the MDBA Sustainable River Audit (SRA) project (Davies et al 2010), which not only also used a consistent electrofishing protocol but was applied at a whole-of-basin scale. All together, the datasets were collected from 1677 sampling locations, on a total of 3294 sampling occasions within the MDB. Between 1st January 1990 and 3rd August 2011, 68% of these locations were sampled once, 14% were sampled twice and the remainder were sampled 4-10 times. The majority of samples were collected from October to April each year, although some winter samples were also collected.

Data relating to sampling location, electrofishing effort, catch and length data from the FFRD and SRA databases were extracted. Then abundances were standardised to provide catch per one minute of electrofishing effort (CPUE) per sampling location. Length data were used to determine carp population structure: *young-of-the-year (*YOY) (<151 mm Fork Length [FL]); subadult (>150 mm and <301 mm [FL]) and adult (>300 mm [FL]) size classes. The total CPUE was multiplied by the proportion of YOY, subadult and adult individuals in the sample to estimate the CPUE of each size class. For the YOY size class, a second dataset was derived. This dataset excluded all data collected from valleys that experienced a major flood prior to sampling, or during the preceding spring–autumn period. The CPUE data were analysed for each size class to identify spatial clusters of high values (ie hotspots) and spatial clusters of low values (ie coldspots), using the Getis-Ord G_i^* Hot Spot Analysis Tool in ArcGIS (Fischer and Getis 2010). The dataset at the valley scale (n = 21 valleys) was further analysed. Goulburn and Broken valleys and the Upper Murray and Mitta Mitta valleys were merged prior to analysis due to zero YOY catches in one of the paired valleys.

Outcomes

Carp were present within 64.6% of locations sampled (ie captured at 1083 sites out of 1677). Where carp were present, YOY were caught at 636 (58.7%) and subadults at 669 (61.7%) sites. Adults were present at the majority of locations (85.4%), and 252 (23%) sites contained only adult fish. Likewise, 158 (14.6%) sites had only YOY and subadult fish. Where carp were captured, the distribution and abundance of YOY were heavily skewed - over 90% of the YOY captured were caught at 34 (3.14%) sites and 50% at 15 (1.38%) sites. Subadult abundances were also similarly skewed. Where carp were captured, over 90% of subadults were caught at 178 (16.4%) sites and 50% at 16 (1.48%) sites. There was a positive correlation between the CPUE of subadults and that of the YOY at each location, and between the CPUE of adults and that of subadults. However, there was no significant correlation between the CPUE of adults and that of the YOY at each location.

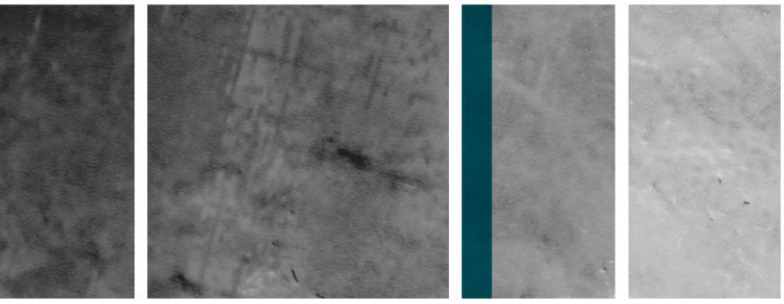


Hotspots at the Basin wide scale

There were three 'hotspots' with high YOY abundance within the MDB (Table 2, Figure 2*)*. If flood-affected data (ie times when major floods occurred within 12 months of sampling) were excluded, the Darling River hotspot was not identified and an additional four hotspots for the YOY were detected (Table 2).

Table 2. List of the most significant hotspots, with high young-of-the-year (YOY) carp (*Cyprinus carpio*) abundance within the MDB (based on Gilligan et al unpublished). Hotspots are listed in order of descending size. When flood-affected data (ie major floods had occurred within one year of sampling) were removed, the Darling River hotspot was not significant but four other hotspots were (shaded in grey).

Hot spot	Areas inclusive
Barwon River	Upstream of the town of Bourke and the lower reaches of its tributary valleys, including: Bogan River, Macquarie River, Castlereagh River, Namoi River, Gwydir River, Border Rivers, Moonie River and the distributaries of the lower Condamine–Culgoa River.
Murray Riverina	The Murray River and its anabranches between Tocumwal and Nyah and including the Barmah–Millewa Forest, Koondrook–Perricoota–Gunbower Forest, Werai Forest and the entire Edward–Wakool anabranch system, as well as the lower reaches of Loddon and Avoca Rivers.
Darling River	Between Tilpa (north) and Burtundy (south) including the Menindee Lakes system.
Great Cumbung Swamp	Great Cumbung Swamp and lower Lachlan River upstream to Booligal.
Lower Murrumbidgee River	Murrumbidgee River between Hay and Redbank Weir.
Lachlan River	Single site near Lake Cargelligo.
Willandra Creek	Single site near Roto.



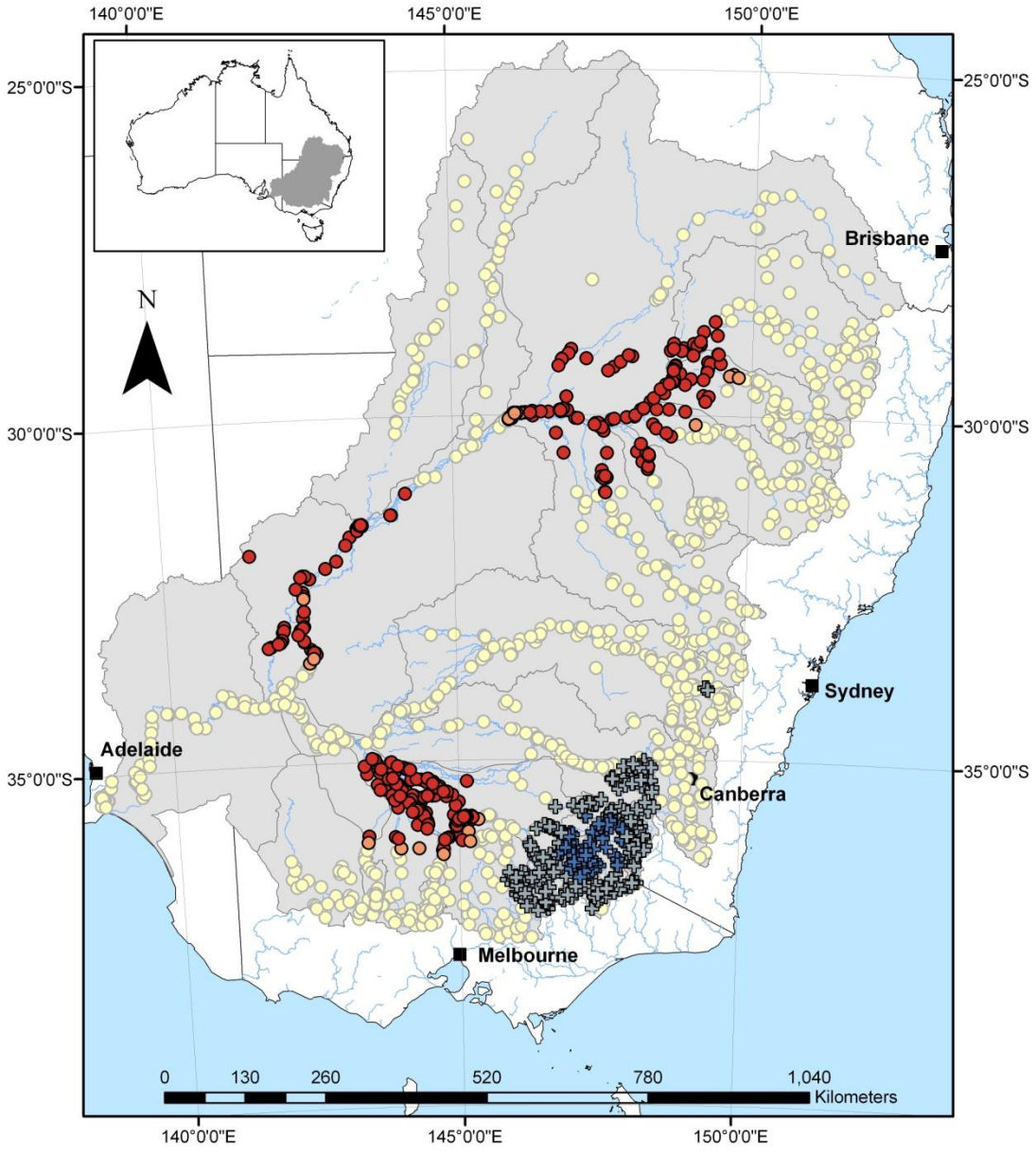
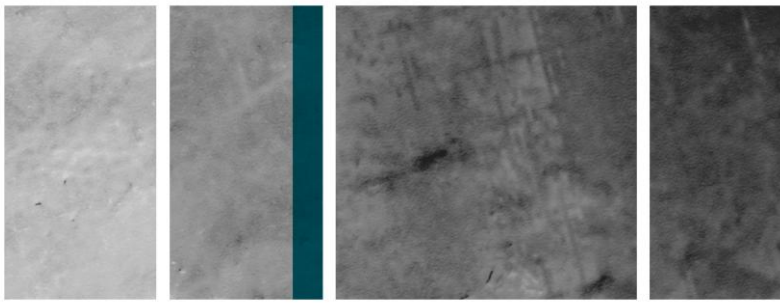


Figure 2. Distribution of young-of-the-year (YOY) carp (*Cyprinus carpio*) abundance within the MDB. Red points represent statistically significant hotspots; blue/grey crosses represent statistically significant coldspots and yellow represent non-significant sites. Orange points represent sites that have higher-than-average YOY abundance at the local valley scale but are not significant at the basin scale (from Project 1).



Overall, there were inconsistencies in the size and distribution of YOY and subadult hotspots. The total area of subadult hotspots in the southern basin was much larger than the two or three YOY hotspots (Figure 3*)*. For subadults, the Barwon River hotspot was still present, but the area had greatly reduced in extent and intensity, compared to the region that was significant for the YOY (Table 2). Similarly, the upper extent of the Murray Riverina hotspot had retracted downstream. Subadults were no longer abundant in the lower Goulburn–Broken, Campaspe, Loddon and Avoca valleys, although the downstream limit had expanded as far as Chowilla, South Australia (Figure 3).

Table 3. List of significant hotspots with high subadult carp (*Cyprinus carpio*) abundance within the MDB (based on Project 1). Hotspots are listed in order of descending size.

Hot spot	Areas inclusive
Barwon River	The Barwon River upstream of Bourke and the lower reaches of its tributary valleys, including: Bogan River, Macquarie River, Castlereagh River, Namoi River and the distributaries of the lower Condamine–Culgoa River.
Murray Riverina	Murray, Edward and Wakool Rivers and anabranches west of (downstream) of about Barham and extending into Chowilla.
Darling River	Near Tilpa (north) to Murray–Darling confluence (south) including Menindee Lakes system and now merged with Murray Riverina hotspot.
lower Murrumbidgee River	Lower Murrumbidgee River and Lowbidgee floodplain downstream of Redbank Weir and merged with Murray Riverina hotspot.

Likewise, the Darling River hotspot had expanded downstream to the Murray–Darling River confluence and merged with the Murray Riverina hotspot. In the lower Murrumbidgee Valley, either subadults from the Murray Riverina hotspot had expanded upstream, or the YOY in the lower Murrumbidgee hotspot had shifted downstream to occupy the Lowbidgee region and merge with the Murray Riverina subadult population. The size, number and distribution of hotspots for adult carp abundance were markedly different to those for YOY and subadaults, tending to be detected in regions where sub-adult or YOY classes were less abundant (Figure **4**).

Hotspots at the valley wide scale for YOY

Valley-scale hotspot analyses identified 12 significant YOY hotspots: nine were encompassed within those identified within the larger, basin-scale analysis. In particular three within the Central Murray Riverina hotspot, one within the Darling River hotspot and one with the lower Lachlan–Great Cumbung Swamp hotspot. Three new hotspots were also identified: one within the Lower Murray River (between Lake Victoria and Chowilla), a second in Lake Brewster (Lachlan Valley) and the third in the Wimmera Valley (Victoria).

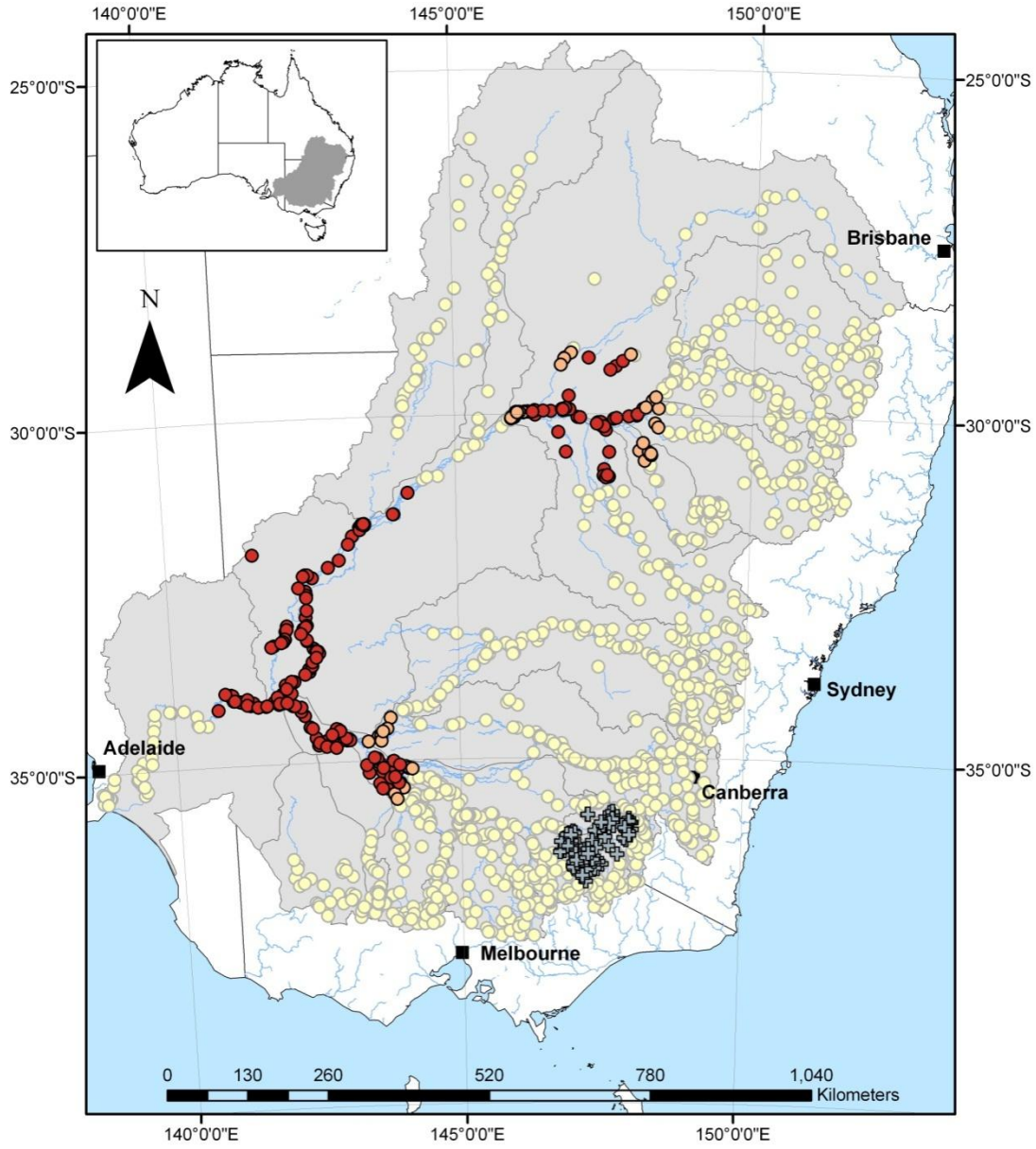


Figure 3. Distribution of subadult carp (*Cyprinus carpio*) abundance within the MDB. Red points represent statistically significant hotspots; blue/grey crosses represent statistically significant coldspots and yellow represent non-significant sites. Orange points represent sites that have higher-than-average subadult abundance at the local valley scale, but which are not significant at the basin scale (from Project 1).

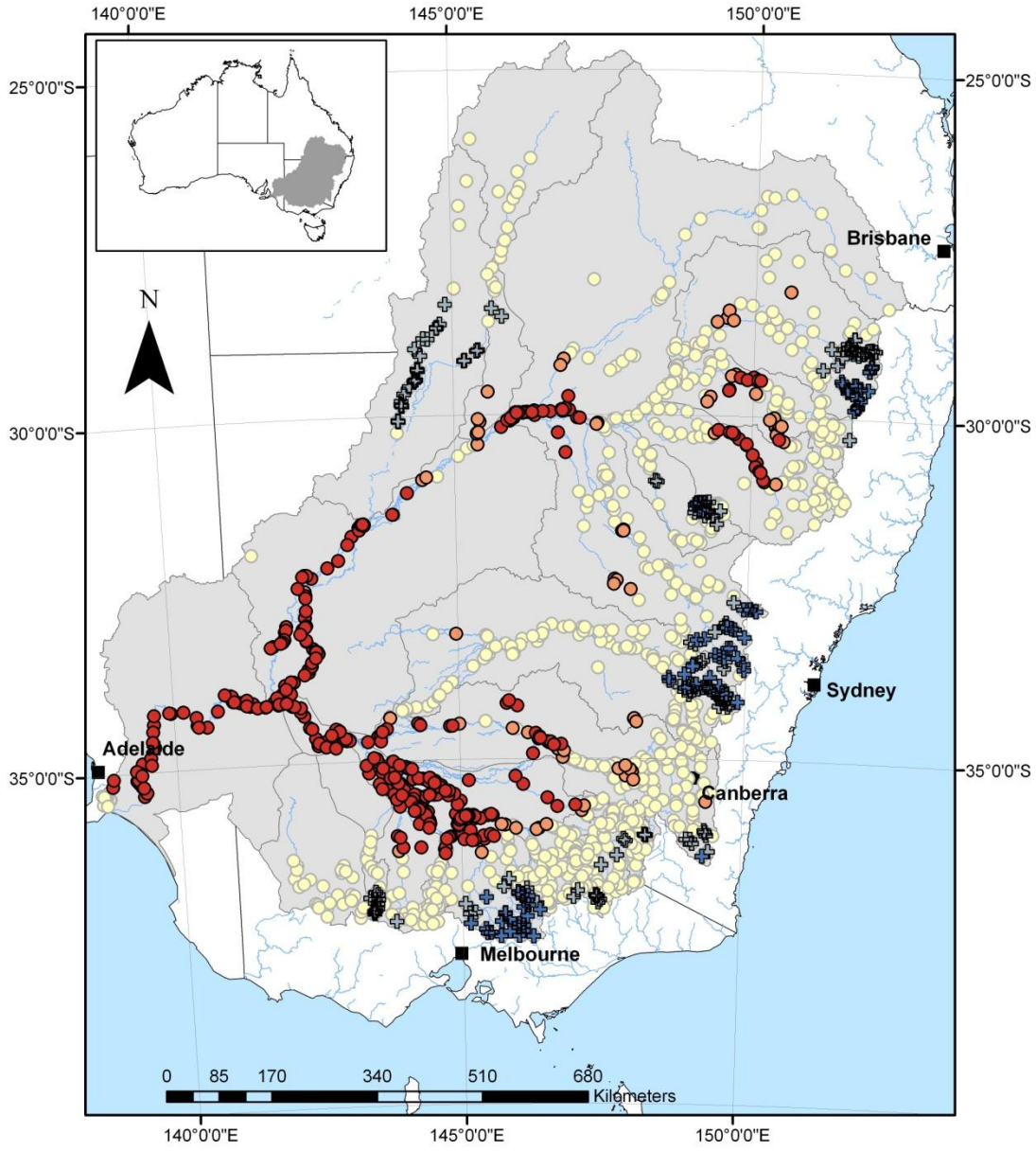
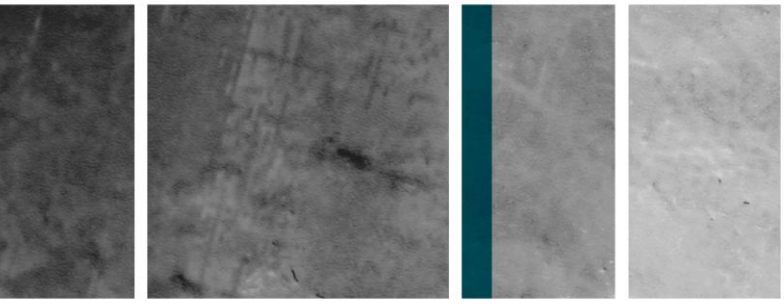


Figure 4. Distribution of adult carp (*Cyprinus carpio*) abundance within the MDB. Red points represent statistically significant hotspots; blue/grey crosses represent statistically significant coldspots and yellow represent non-significant sites. Orange points represent sites that have higher-than-average adult abundance at the local valley scale but are not significant at the basin scale (from Project 1).



The distribution and abundance of the three size classes of carp in the MDB was spatially clustered, meaning that carp did not consistently use or occupy all available habitats. For instance, no YOY or subadult carp were caught in approximately 40% of the locations where adult carp were captured. Of great significance to carp management, the juvenile-size classes, which are the focus of management interventions, were concentrated within basin hotspots; large areas were presumably not used for spawning (ie source-sink population structure) and would not warrant management activities or efforts. Adult carp were widespread and abundant at a limited number of reproduction/recruitment sites.

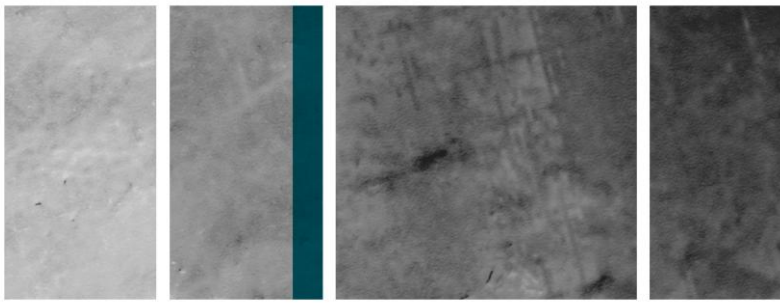
Another key finding was the identification of three primary recruitment hotspots across all of the MDB in:

- the Barwon River region (including tributaries and floodplains),
- the central Murray Riverina (including Barmah–Millewa, Koondrook–Perricoota, Gunbower and Werai Forests),
- and the Darling River from its mid to lower reaches (incorporating Menindee Lakes system).

The data were reanalysed to ensure that the pattern identified was not coincidentally a reflection of these sites being intensively sampled following the recent major flooding experienced across the MDB between August 2010 and May 2011 (MDBA 2011). When all YOY data collected within 12 months of a major flood event were excluded, the Barwon River and central Murray Riverina hotspots still had high YOY abundances regardless of flooding. In contrast, the Darling River hotspot was no longer apparent when flood-affected data were removed. The high abundances of YOY carp in the Darling River hotspot may indicate that larval or small juvenile carp disperse downstream from the Barwon River hotspot when a flood occurs. Thus, recruitment only occurs during flood conditions.

Vulnerabilities and application

The key vulnerability of carp identified from this work is that recruitment is localised to a small number of hotspots within the MDB. The identification of reproduction/recruitment hotspots could allow carp control to be targeted at key recruitment sources at the local scale, rather than scattered, uncoordinated control over tens of thousands of river kilometres. This helps develop integrated carp management strategies across the MDB. Furthermore, the identification of carp hotspots allows for targeted control of adult carp migrating towards spawning areas (ie spawning aggregations), exclusion of adults from spawning areas and the control of dispersing juveniles from spawning areas. Identification of carp hotspots may improve the efficacy of physical, chemical and/or biological control strategies. For example, hotspots may provide ideal target areas for the release of transgenic genes ('daughterless gene technology') or vectors carrying other potential biological control agents (eg Koi herpes virus), 'Judas' fish (radio-tagged male fish that are used to track carp aggregations) or installation of wetland CSC for carp removal. If similar long-term databases exist, the information from this project could also be applicable for detecting carp recruitment hotspots in other catchments within Australia and globally.



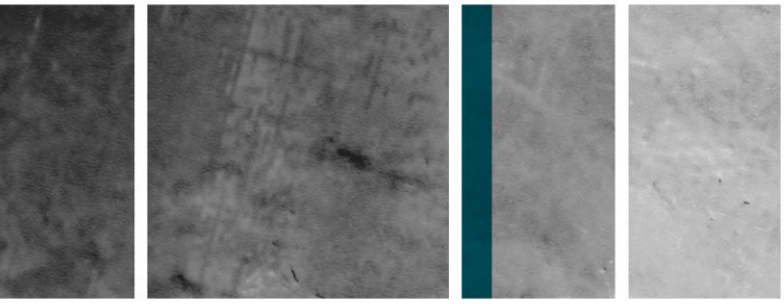
Suggested future work

Throughout the study period, the southeastern MDB region was subjected to ongoing drought conditions (Bond et al 2008; Smith et al 2009b; Conallin et al 2010). Hence, in this study, data from outside of NSW were largely collected during this drought. This potentially influenced the results because minimal rainfall and low-flow conditions may mean some potential spawning hotspots were not identified. It is recommended that more data be collected to provide greater spatial (ie waterways outside of NSW) and temporal (ie post-drought) coverage so that a comprehensive survey of potential MDB carp spawning hotspots can be determined. As a minimum, it is highly recommended that this exercise be repeated following each round of SRA sampling.

All state agencies within the MDB possess the resources/equipment to apply carp larval sampling strategies. This enables immediate capitalisation of sampling opportunities when they arise, especially in areas that were not sampled during Project 1. The NSW Department of Primary Industries is committed to applying the larval sampling strategy to fill in the remaining gaps within NSW catchments when opportunities arise.

Otolith microchemistry has also been successfully used to identify hotspots and coldspots (Crook and Gillanders 2006), especially as this method is able to quantify the proportion of the total population that originated from sampled nursery habitats. Thus, this method could be applied across the remainder of the MDB. In addition, Shibata et al (2011) determined the carbon stable isotope ratios of basal food webs to mark differences between main lakes and tributary lagoon sites. Stable isotopic signatures of individual fishes collected from lagoon sites were then compared to confirm whether they were residents of lagoons, or recent immigrants from the main lake system.

Larvae could also be sampled for hotspot locations along with native species, especially those of recreational or cultural significance, such as Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*). This is because the use of hotspots by native fish species may complicate, or make unfeasible, the use of some pest control strategies in these locations.



Project 2: Carp movement and migration in the MDB

Prelude

Population dynamics

How migratory animals use their habitat network provides valuable insights for predicting population dynamics at local and regional scales (Shibata et al 2011). Evidence from other vertebrate pest (eg mouse, rabbit and fox) population control and modelling studies suggests that population spatial structure and movement rates are strong determinants in the success of control measures (Brown and Robertson 2008).

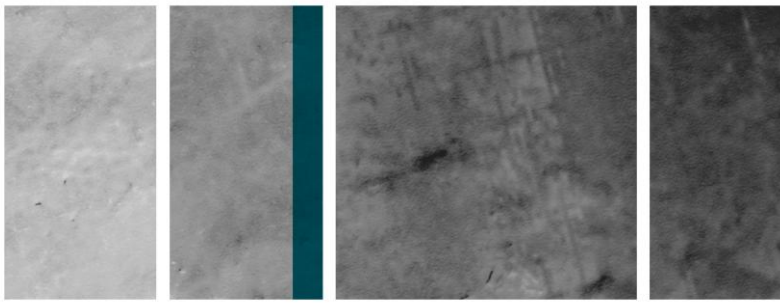
In 2004, the Victorian Department of Primary Industries in collaboration with the Pest Animal Control CRC (precursor to the IA CRC) developed the simulation software CarpSim (Brown and Walker 2004). CarpSim is open-access software that uses information about age at maturity, growth rates and movement to evaluate various carp management strategies. It has since been revised to incorporate spatial variation, environmental stochasticity and connectivity among carp populations (information and download available from: http://vro.dpi.vic.gov.au/dpi/vro/vrosite.nsf/pages/pest_animals_carpsim). In CarpSim, the MDB can essentially be defined as a multicompartment model with spatially segregated carp stocks. Carp stocks are defined using independent parameter sets, user-input emigration rates and customised movement and connectivity data. CarpSim modelling suggests the outcomes of carp management options may be sensitive to movement and connections (ie emigration and immigration) between carp stocks, which have not been quantified across MDB 'management units' (Brown and Robertson 2008); see Haynes (2009) for a list of management units.

Project summary

For some time, studies of carp movement in the MDB were contradictory. For instance, an early tagging study concluded that carp were essentially non-migratory, making only short, random movements (Reynolds 1983). However, three types of movement by carp have since been identified:

- large-scale, upstream dispersive movements through fishways (Mallen-Cooper et al 1995; Stuart and Jones 2002; Stuart et al 2006a, 2008)
- lateral movements from the main river channel to shallow, lentic habitats for spawning (Stuart and Jones 2006a, 2006b; Smith et al 2009a; Conallin et al 2010; Thwaites and Smith 2010, Thwaites et al 2010)
- the downstream transport of carp larvae and juveniles (ie larval drift) from wetland habitats after high-flow events (Gilligan and Schiller 2003).

This project aimed to improve knowledge in this area and increase understanding of carp movement and migration.



Rationale and objectives

The apparent longitudinal and lateral movement of carp as well as larval drift was spatially limited. Consequently, carp may not move within or between stocks. Thus, Project 2 established a monitoring network throughout the main tributaries of the MDB system to help clarify the extent, timing and variation in the large-scale movement of adult carp among MDB stocks.

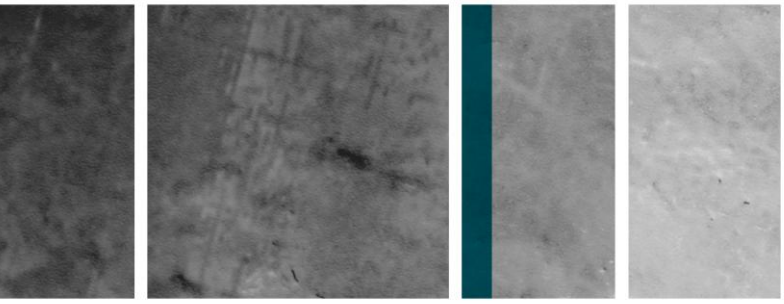
The specific aims and methods of Project 2 were to:

- quantify spatial and temporal movement patterns of adult carp between stocks and MDB management units (over time scales relevant to their life history)
- understand MDB stock structure and evaluate the biological (eg sex, size) and environmental (river flow and water temperature) triggers for potential or actual movement
- confirm locations/times when carp movement behaviour may be exploited for management purposes (eg when carp may aggregate for spawning and/or overwintering, or as they pass though river fishways)
- integrate the efforts of multiple state and federal agencies in marking and recapturing individual carp.

To achieve the above aims, the movements of carp were recorded using two types of tags: individually coded acoustic transmitters (CAT) and passive integrated transponder (PIT) tags. CAT tagged individuals (n=236) were released at seven locations within the MDB system at the site of capture (Table 4).

Table 4. Location and date of capture and release of 236 carp implanted with coded acoustic transmitters (CAT).

River	Location	Latitude	Longitude	Release dates (n)
Murray	Junction of Ovens River at Bundalong	-36.051200°	146.198300°	February 2007 (12), January 2008 (11)
Murray	Barmah forest	-35.955000°	144.958000°	February 2007 (12), January 2008 (21)
Murray	Swan Hill	-35.331790°	143.563830°	July 2007 (28), January 2008 (35)
Darling	Menindee, downstream of main weir	-32.396266°	142.432000°	September 2007 (14), March 2008 (15)
Darling	Tilpa	-30.942280°	144.415390°	August 2007 (11)
Darling	North Bourke	-30.055203°	145.951786°	August 2007 (16)
Barwon	Mungindi	-28.976140°	148.982231°	August 2007 (12), March 2008 (27)



The length of tagged individuals ranged from 246 to 670 mm. Subsequent movements of tagged carp were detected on 21 pairs of acoustic receivers that were installed at strategic positions along 20 rivers (Murray, Darling, Kiewa, Ovens, Goulburn, Broken, Campaspe, Loddon, Murrumbidgee, Edwards, Wakool, Lachlan, Barwon, Bogan, Bukara, Castlereagh, Macquarie, Gwydir, Namoi and Warrego; see Figure 1) and on receivers fitted to 14 fishways along the Murray River and its tributaries. PIT tag data were retrieved from the MDBA's Fishways Monitoring System. Data on acoustic detections were also shared with collaborating agencies running projects with compatible equipment in the Murray River near Echuca (Victorian Department of Sustainability and Environment) and the Wakool–Edwards River system in the Riverina (NSW Industry & Investment).

Outcomes

Up until August 2011, a total of five PIT tagged carp were detected at fishway monitoring stations. The paired acoustic receivers had logged 2.3 million transmitter detections from a total of 83 individual carp. The majority of the redetected carp (n=70) were recorded within the original sub-catchment in which they were caught and released. Interestingly, all of the tagged carp released in the Darling system remained in the Darling or associated tributaries. The same results were detected for the tagged individuals released into the Murray system. Overall, only 5% (n=13) of the total number of tagged carp moved into a different river sub-catchment from the original sub-catchment in which they were released (eg Murray, Ovens, Lower Darling, Upper Darling, Barwon). In 2011, there were 195 tags still transmitting, and 100 of these will continue transmitting to any remaining compatible acoustic receivers until 2017 to provide data to update CarpSim.

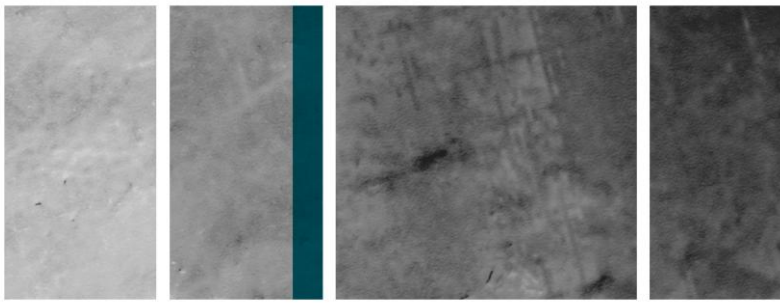
Vulnerabilities and application

A key vulnerability of carp identified here is that tagged, adult individuals moved at relatively small scales between sub-catchments during all flow conditions, suggesting adults have limited capacity to colonise connected sub-catchments in large numbers. Such colonisation must therefore come from:

- the progeny of small numbers of adult dispersing carp,
- and/or, dispersing juveniles (<246 mm TL).

Owing to such small-scale movements, this study did not identify any locations/times when carp could be vulnerable to trapping or management. The low numbers of tagged fish recorded at fishways is a consistent problem with large-scale fish movement research, as release sites may not be located in regions where fishway PIT tag detectors are common.

Catchment scale control of adult carp may therefore be sustained if re-colonisation by juveniles and the reproduction of new colonisers is prevented. The lack of movement was initially thought to reflect the effects of reduced flow and variability caused by river regulation, which was further exacerbated during the study period by ongoing drought conditions across southeastern Australia (Bond et al 2008; Smith et al 2009a, 2009b; Conallin et al 2010). However, little movement was also recorded in the Darling and Murray catchments (following large flows) in the final stages of the data-collection period. That is,



the large-scale movements predicted at the onset of this study, including those by female carp, were not more apparent when flows increased. Data collected from Project 2 will provide an important comparative baseline for all future studies. This study also provides input for CarpSim to simulate realistic levels of stock interaction and carp control activities, including those in the IA CRC's carp management demonstration sites.

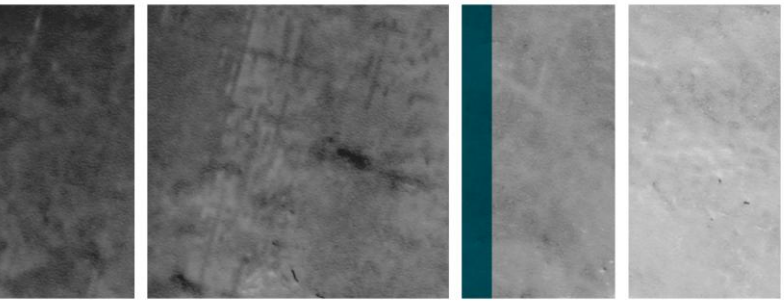
Suggested future work

It is recommended that this research be continued to encompass the range of environmental variability inherent in large-scale systems. For example, further research could capture potential, sporadic longitudinal movements by carp that may result from higher flows, including overbank floods.

The number of tagged carp and release sites should be increased (≥ 500 and ≥ 15 respectively) to increase the depth and breadth of information and the spatial resolution. The monitoring network (ie acoustic receiver pairs and PIT tag detector stations) should be expanded to include catchments where no monitoring currently exists, such as Murrumbidgee, Lachlan and South Australian Murray, especially in sections of the river where fishway PIT tag receivers are currently installed. Monitoring should also focus on sites of interest, such as hotspots identified by Project 1, or areas where carp are currently aggregating in the main channel. The reported low-movement rates can be further validated in the future by periodic ground-truthing of the status of any CAT-tagged carp that are not moving; ie confirming tags are still present on individuals.

Similarly, pairing PIT tag detectors on fishways with acoustic loggers would confirm that PIT-tagged carp approach fishways and still contain the acoustic tag. It would also be informative to compare data from this project, such as release location, methodology and timing, with data for PIT-tagged carp tagged during MDBA's fishway monitoring.

Finally, all available data and environmental correlates (eg flows, temperature), including data from years of higher flow (as they become available), should be scrutinised to highlight triggers for movement, or maybe to explain why the tagged carp are not moving.



Project 3: Carp age validation in the northern MDB

Project publications

Hutchinson M, Chilcott K, Norris A and Stewart D (2012). Validating the age of carp from northern Murray-Darling Basin. PestSmart Toolkit publication, Invasive Animals Cooperative Research Centre, Canberra, Australia.

Prelude

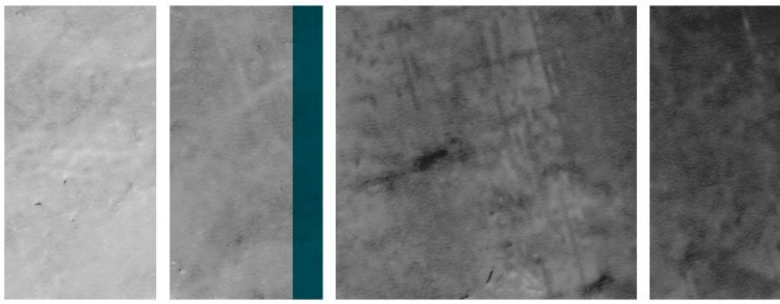
Population dynamics of carp in Australia

Accurate age data are critical to fisheries management, allowing assessment of population structures and informing carp population models, such as CarpSim (Brown and Walker 2004). Age data can include age at maturity and other growth transitions, such as spawning, hatch and settlement dates, rates of growth, mortality and recruitment and morphometric relationships. Age is estimated based on the number of growth increments in otoliths, scales, opercles, fin rays and vertebrae. Under a light microscope, growth increments in otoliths appear as paired concentric, translucent (light) and opaque (dark) bands, known as 'check marks'. Each ring typically represents one year's growth for mature fish or one day for YOY fish aged 0 (ie fish in their first growing season) (Smith 2005a; Smith and Walker 2003b).

Validation is an essential criterion of age-based studies, ensuring that age estimates reflect true ages. Methods for age validation include the use of known-age fish, marginal increment ratio analysis (MIRA), edge type analysis (ETA) and capture-mark-release-recapture (CMRR) (Campana 2001). In South Australia and Victoria, MIRA, ETA and CMRR have been used with fish aged up to approximately 14 years (Vilizzi and Walker 1999; Smith and Walker 2003b; Brown et al 2004), confirming that the observed increments were annual increments. In South Australia, the time of annulus formation in fish one year or older was estimated as November/December for otoliths and October/November for scales and opercular bones (Vilizzi and Walker 1999).

Project summary

Until recently, the ageing of carp in Australia had only been validated for southern temperate populations, where annual check marks were formed by slow growth during winter months, which was followed by increased growth in spring (Brown et al 2004). However, at the onset of Project 3, it was unknown if check marks also formed annually in the otoliths of carp from northern MDB populations, which experience a more subtropical climate and reduced seasonal variation in air/water temperatures. Thus, this project investigated whether check marks formed annually in the otoliths of northern carp populations and estimated the timing of check formation.



Rationale and objectives

The key objectives were to:

- determine whether check marks form annually in northern MDB carp populations,
- and estimate the timing of check formation.

Determination of check formation in northern MDB carp populations

A total of 200 adult carp were captured by electrofishing from lagoon and river sites in the Macintyre River catchment, Goondiwindi, Queensland (ie northern MDB). Individual fish were then marked by intermuscularly injecting oxytetracycline (OTC: a broad-spectrum antibiotic) at a dosage rate of 50 mg/kg body weight. Carp were then tagged with dart and PIT tags, and the majority were released into small (≤ 1 ha area; ≥ 6 m depth), natural lagoon sites near Goondiwindi. Tagged adult fish were collected via electrofishing 356–605 days after release. A total of 12 marked, tagged adult carp were recaptured from the natural lagoon sites. Individuals were measured, weighed and sexed where possible. Asterisci otoliths (ie smaller of the two otoliths found in carp) were removed from individuals using a modified version of the 'up through the gills method' (Secor et al 1991). In addition, several other tagged OTC marked carp were recaptured by anglers at the same lagoon sites where they were originally released, up to two years after release, and supplied to the research team. A subsample of 36 adults was placed in 5000 litre laboratory tanks (Deception Bay, Brisbane) and kept for 15 months before the evaluation of OTC marks and check marks on the otoliths. Otoliths were removed, washed and dried for three weeks. They were then placed in silicon moulds, embedded in casting resin, sectioned and slide mounted. Otolith sections were then aged as per Brown et al (2004).

Timing of first check formation

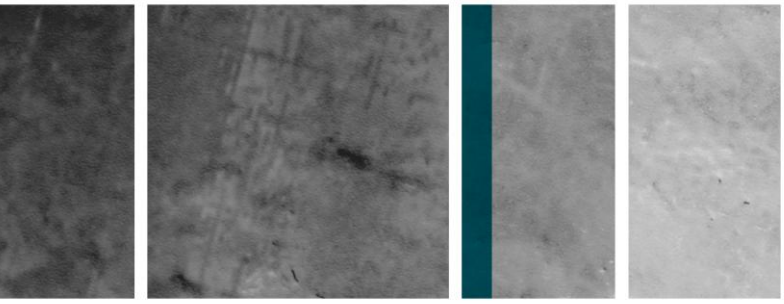
In July 2007, 200 YOY carp were captured by electrofishing from lagoon/river sites in the Macintyre River catchment, Goondiwindi. They were placed in a buffered solution (0.5 g/L OTC) for a 24-hour period. YOY were then released into small, natural lagoon sites near Goondiwindi. Lagoons were then sampled (via electrofishing, fine-mesh fyke nets or seine nets where possible) once per season, and then at monthly intervals over spring when completion of an increment was expected to occur. However, no fish in the appropriate size classes were recaptured.

A subsample of 40 YOY was also held in 1000 litre laboratory tanks (Deception Bay, Brisbane) and each month, two or three tank-held YOY were sacrificed from August 2007 to April 2008 to estimate the timing of the first check formation. Laboratory tanks were maintained at ambient temperature, and loggers recorded temperatures at hourly intervals.

Outcomes

Water temperature

Annual variation in water temperatures in winter and summer was slightly greater in the natural lagoon environment (minimum in winter = 14.8 °C; maximum in summer = 31.5 °C) compared to the laboratory tanks (minimum = 15.3 °C, maximum = 29 °C).



Young-of-the-year (YOY) otoliths

The OTC reference mark was not found in the majority of YOY carp. Only three captive YOY carp had confirmed OTC marks and an annular check was beginning to form in October. Recaptures of potential YOY carp in the wild were low. However, tank-held carp could still be used to follow through the formation of the first annular check, where the check was laid down from November to early December. Growth past the annular check was observed in early January, and continued through the summer months into autumn.

Adult and subadult otoliths

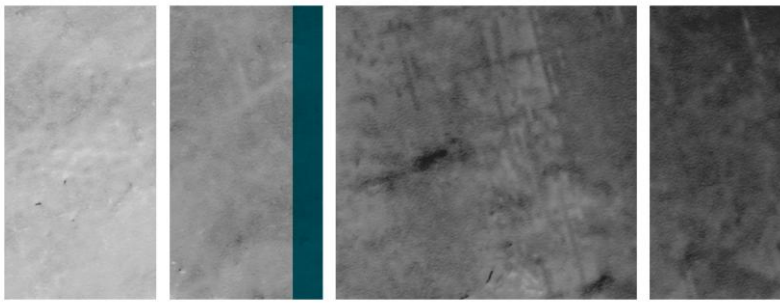
The majority of adult and subadult carp formed check marks consistent with annual formation. Fish at large for only one winter and sacrificed the following autumn formed one complete check. Some fish at large for two winters and sacrificed in spring formed two check marks. However, five carp (13.5 % of the total sample) had formed two complete checks within one winter. This biannual check formation was observed in three tank-held fish and two lagoon-released fish. No carp formed fewer checks than expected.

Vulnerabilities and application

No definitive vulnerabilities were identified from this research, but the results enhance understanding of carp population dynamics, which is critical for assessing potential responses to carp control strategies. In particular, it was found that the majority of subtropical carp in the northern MDB formed one complete check per year, and the first check was formed by one year of age. However, a small minority of subtropical carp formed two complete checks per year. Thus, some caution is required when estimating age at maturity for northern populations of carp in the MDB compared to southern populations. It is recommended that total length be plotted against age and then outliers be excluded. Outliers may include individuals where length is shorter than expected for a given age compared to the majority of fish. Such outliers are likely to be those fish that form more than one complete check per year. With caution, these adjusted results could then be comparable to carp in populations in the southern MDB, although this remains untested. Results of this study are also transferrable to carp in the adjacent Logan–Albert River catchments, which highlights that this knowledge could be extended to greater spatial scales.

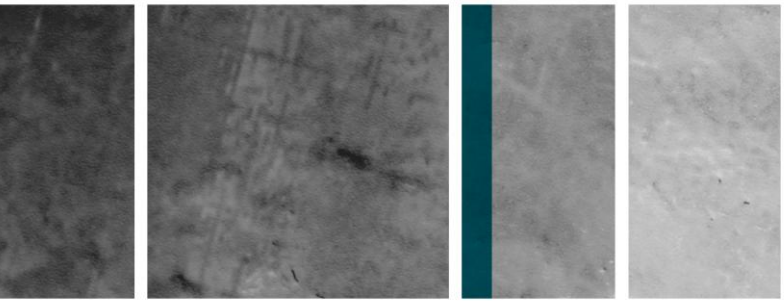
The application of knowledge from this work supports:

- comparisons of age-specific biological traits (eg maturity, growth rates and fecundity),
- carp population assessments (eg examination of population age structures to relate recruitment success with biotic and abiotic factors),
- modelling and assessment of:
    - spatial and temporal variability in population dynamics between northern and southern catchments of the MDB,
    - and the likely effects of carp management strategies for populations in various locations.



Suggested future work

Populations of carp in the northern MDB should be sampled to determine age at maturity and duration of the reproductive season to provide further knowledge on carp population dynamics and structure across their geographical range in Australia.



Project 4: Optimised wetland carp separation cages

Project publications

Smith BB and Thwaites LA (2007). *Carp Spawning Migrations and Identification of Possible Sensory Attractants*: a scoping report for the Invasive Animals Cooperative Research Centre. SARDI Aquatic Sciences Publication Number F2007/000712-1. SARDI Research Report Series Number 226. Prepared by the South Australian Research and Development Institute (SARDI) Aquatic Sciences, Adelaide. 25 pp.

Thwaites LA, Fleer D and Smith BB (2007). *Conceptual Development of a 'Finger' Style Pushing Trap for Common Carp*. SARDI Aquatic Sciences Publication Number F2007/000790-1. SARDI Research Report Series Number 238. SARDI Aquatic Sciences, Adelaide.

Smith BB, Thwaites LA and Conallin AJ (2009). *Guidelines for the Selection and Implementation of Carp Management Options at Wetland Inlets: a Test Case for South Australia*. Prepared by the SARDI Aquatic Sciences for the Invasive Animals Cooperative Research Centre (IA CRC), Canberra.

Thwaites LA, Smith BB, Decelis M, Fleer D and Conallin A (2010). A novel push trap element to manage carp (*Cyprinus carpio* L.): a laboratory trial. *Marine and Freshwater Research* 61:42–48.

Thwaites LA and Smith BB (2010). *Design and installation of a novel wetland carp harvesting set-up at Lake Bonney, South Australia.* A summary report for the South Australian Murray-Darling Basin Natural Resources Management Board, Invasive Animals Cooperative Research Centre and the Murray-Darling Basin Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000295-1. SARDI Research Report Series No. 469. 58pp.

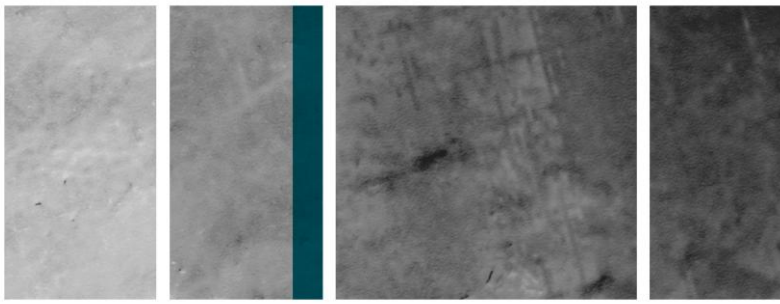
Thwaites LA (2011). *Proof of concept of a novel wetland carp separation cage at Lake Bonney, South Australia.* A summary report for the Invasive Animals Cooperative Research Centre and the South Australian Murray-Darling Basin Natural Resources Management Board. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2011/000086-1. SARDI Research Report Series No. 530. 38pp.

Conallin AJ, Smith BB, Thwaites LA, Walker KF and Gillanders BM (2012). Environmental water allocations in regulated lowland rivers may encourage offstream movements and spawning by common carp, *Cyprinus carpio*: implications for wetland rehabilitation. *Marine and Freshwater Research* 63:865-877.

Prelude

Habitat use of common carp in Australia

Carp occupy two broad habitat types: shallow wetlands in spring/autumn and deeper water habitats in winter. They migrate between these habitats annually (Penne and Pierce 2008).



However, migration pathways may be blocked by barriers, such as weirs or carp exclusion screens (CES), at wetland inlets (French et al 1999). When confronted by barriers, migrating carp have an innate desire to jump over or push through them. Field observations suggest that these behaviours are:

- persistent during the day and night,
- seen in mature carp of diverse sizes,
- and so vigorous that they typically lead to severe anterior dorsal wounds.

In contrast, native freshwater fishes of the MDB are either not able to, or simply do not, leap from the water (Stuart et al 2006a). There are no reports of native freshwater fish attempting to push through barriers to migration (Thwaites and Smith 2010). Thus, the innate jumping and pushing behaviours of carp could be used to trap and separate them from native fish.

Project summary

The key focus of this study was to capitalise on the innate jumping and pushing behaviours of carp to develop and trial a carp push trap and a Wetland Carp Seperation Cage for use at wetland entrances, so that carp may be separated from native fish for efficient harvesting. Intercepting and harvesting carp at wetland entrances is particularly desirable as migrating carp are vulnerable to trapping. Controlling the access of mature carp to wetlands would lead to reduced recruitment and environmental impacts (Smith 2005; Miller and Crowl 2006; Matsuzaki et al 2009). At the onset of Project 4, trials of carp jump traps had been restricted to river fishways (Stuart et al 2006a, 2006b) and a pushing trap for carp had not been investigated.

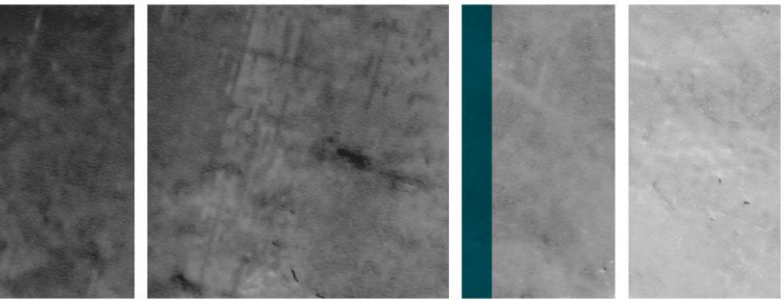
Rationale and objectives

Specific aims of this study were to evaluate:

- the patterns (timing, frequency, duration) of carp and native fish movement through wetland inlets,
- design options and applications for a carp push trap element,
- the application of existing fishway CSC technology for trapping and removing jumping carp at wetland inlets,
- and modifications needed to incorporate a carp push trap element and ‘optimised’ cage cladding (ie jail bar mesh, with 31 mm apertures between bars) into the design of a wetland CSC.

Outcomes

The offstream movement patterns of carp and native fishes were assessed between the Murray River channel and six perennially inundated wetlands in South Australia from August to November, 2006 (Conallin et al 2010). Variable movements of juveniles and adults were



detected among wetlands despite the shared river reach and the relative proximity of the wetlands to each other. Although there was no consistent directionality in the movements, direction has been previously observed for carp under higher flow conditions. It was speculated that directional movements may become more apparent when river flows increased, especially since recent drought conditions throughout the MDB had resulted in reduced entitlement flows to South Australia at the time of the study (Bond et al 2008; Smith et al 2009a, 2009b).

A conceptual design for a carp pushing trap was developed (Thwaites et al 2007) and proven (Thwaites et al 2010). This work determined the pushing capacity of carp (ie about twice their body weight), compared five trap designs and successfully demonstrated the passage of carp through the preferred 'finger style' carp push trap element in a laboratory trial.

Wetland CSCs were tested which incorporated jumping and pushing trap elements in the inlet (capturing fish moving with the flow) and outlet creeks (capturing fish moving against the flow) to Banrock Station wetland, South Australia, during June to December, 2008 (Conallin et al 2012). Major findings included:

- catches in the outlet cage comprised >99% of the total catch, potentially because the physical and chemical properties of the outflowing wetland water attracted aquatic fauna from downstream areas (after Smith and Thwaites 2007; Elkins et al 2009), whereas there was no 'attraction flow' at the inlet,
- carp comprised >95% of the catch at the outlet,
- large-bodied native fishes including bony herring (*Nematalosa erebi*) and golden perch (*Macquaria ambigua*) represented <3% of the catch at the outlet and they mostly began migrating after carp, when water temperatures exceeded 20°C,
- >150 turtles (*Chelodina longicollis* and *Chelodina expansa*) were captured — none died and most had climbed the central jumping baffles, so turtle escape chutes at the rear of all carp cages are suggested for incorporation into future designs,
- individual carp were capable of pushing, even in shallow waters (<40 cm depth) with no obvious preference to push or jump - the complementary function of both trapping components is useful especially in shallow waters where jumping may be prevented but pushing behaviour may still be exploited,
- and the exploitation of both jumping and pushing led to the separation of approximately 90% of carp that entered the carp cages - weekly range during the survey period was 42–100%.

As a result of the work conducted at Banrock Station, optimsed wetland CSC's were designed and trialed within the inlet culvert of Lake Bonney (South Australia) during two environmental water allocations in spring/summer 2009 and 2010 (Thwaites and Smith 2010; Thwaites 2011). The optimsed wetland CSC included:

- technology to separate carp ≥ 250 mm TL from native fish using jumping (Stuart *et al*. 2006) and pushing trap elements (Thwaites *et al*. 2010),

- cage cladding (mesh; vertical jail bar design, with a 31 mm aperture between bars) designed to permit the unimpeded passage of small and medium sized native fish, while impeding the passage of carp ≥ 250 mm TL (Thwaites *et al*. 2010; Hillyard *et al*. 2010),
- infrastructure to mechanically lift and automatically funnel captured fish into trailer-mounted fish bins (
- Figure **5**). When the jumping and pushing trap elements are in use, there are two cage sections (Holding Zone and Carp Cage, Figure 6) and these should be able to be emptied independently. For example all fish in the Holding Zone (potentially including carp and native fish) would be emptied first, so that native fishes can be returned to the water,
- a modular WCSC design permitting straightforward management changes. For example, when fish aggregations comprised entirely of carp or commercial species (e.g. bony herring in South Australia) are present, the central baffle of the cage (comprising the jumping and pushing trap elements) can be removed, and the cage can be used as a large scoop,
- and compliance with Australian design and OHS&W legislation.

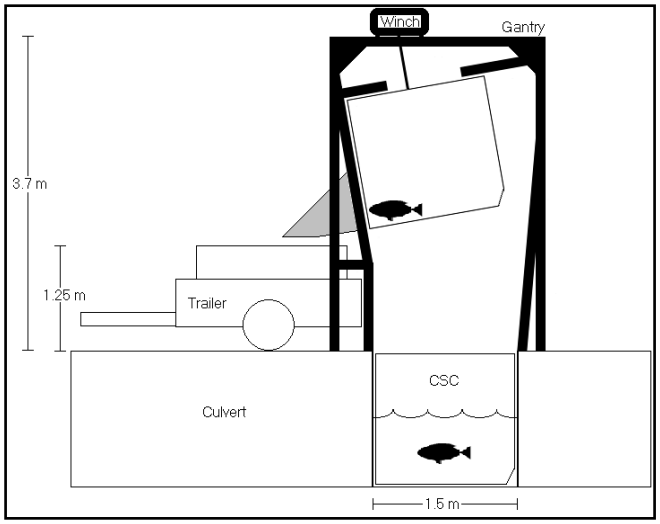
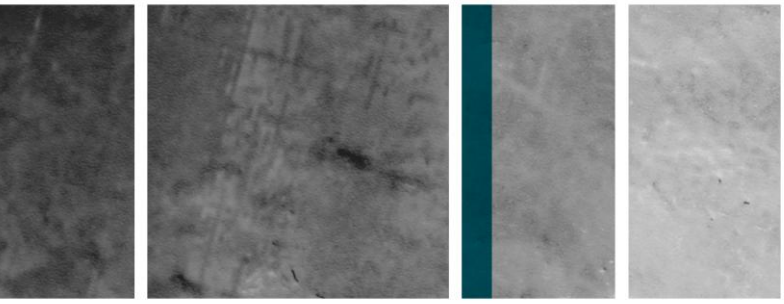


Figure 5: Schematic representation of the conceptual Lake Bonney WCSC lifting infrastructure.



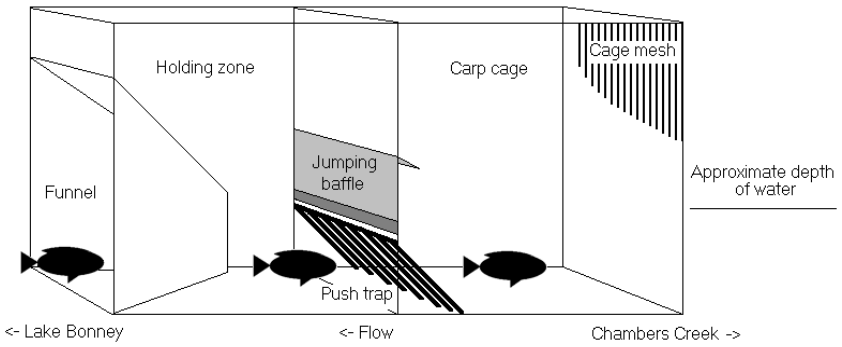
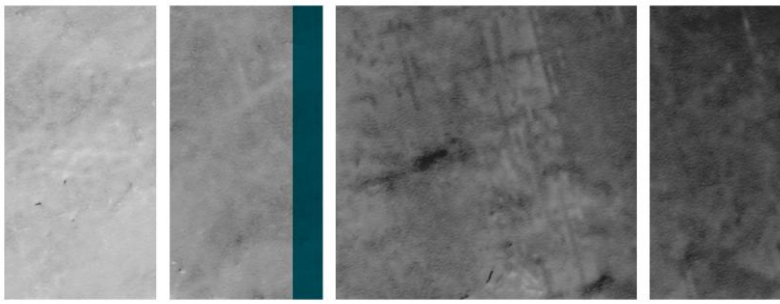


Figure 6: Schematic representation of the Lake Bonney WCSC showing the entrance funnel, holding zone, jump/push trap element, carp cage and jail bar cladding with 31 mm aperture (cage mesh).

Although large numbers of carp attempted to migrate from the lake during the water allocation in 2009, the wetland CSC was not tested that year due to a lengthy commissioning process (Thwaites and Smith 2010). However, in 2010, Lake Bonney received a further 25 GL water allocation and the Invasive Animals Co-operative Research Centre (IA CRC) and South Australian Murray-Darling Basin Natural Resources Management (SA MDB NRM) Board supported a "proof-of-concept" trial of the WCSC. Thus, WCSC operation commenced on the 28th September 2010 and continued for 4 weeks.

Carp and bony herring were the only fish species expected to enter the cage so the central baffle (comprising jumping and pushing trap elements) was removed to allow the cage to operate as a large scoop trap. The lower 30 cm section of the upstream cage cladding was constructed using a jail bar configuration (with a 31 mm aperture between the bars) to allow unrestricted passage of bony herring, while restricting the passage of carp ≥ 250 mm TL.

During the 2009 water allocations, large carp aggregations formed rapidly within the inflow plume and persisted for approximately 2 months, however this response was not observed during the 2010 allocation. In particular, no large carp aggregations were observed adjacent to the cage, within the weir pool or within the inflow plume, but a total of 529 carp (mean total length = 607.6 mm ± 8.1 S.E.; mean weight = 4400 g ± 155 S.E.; width ≈ 83 mm, > 2 tonnes carp) were captured over 13 harvesting events. By-catch included a total of 356 bony herring (mean total length = 433.3 mm ± 3.4 S.E.; mean weight = 931.2 g ± 26.7 S.E.; width ≈ 45 mm), 2 goldfish, 1 golden perch and 4 birds. The recorded by-catch highlights the need to assess the resident native fauna assemblage of a site before the installation of any carp trapping infrastructure. Thus, if iconic or high value species are encountered, then appropriated design modifications and management protocols are required to ensure carp infrastructure has minimal or no impact.



Vulnerabilities and application

This work confirms and highlights that carp posess distinct, innate behaviours that could be exploitable vulnerabilities. These behaviours include:

- predictable, annual migrations between river and wetland habitats for spawning, starting early August,
- being attracted to flowing water, especially at spawning times, leading to upstream movement towards the source of the flow,
- and having an innate ability to jump and push past migration barriers.

Thus, there is potential to exploit these behaviours to improve the efficacy of control programs by:

- optimising the use of CES (Hillyard et al 2010),
- optimising CES with integrated push trap element (Smith et al 2009b; Thwaites et al 2010),
- optimising wetland carp harvesting systems (Thwaites and Smith 2010),
- or using a combination of the above (Smith et al 2009b).

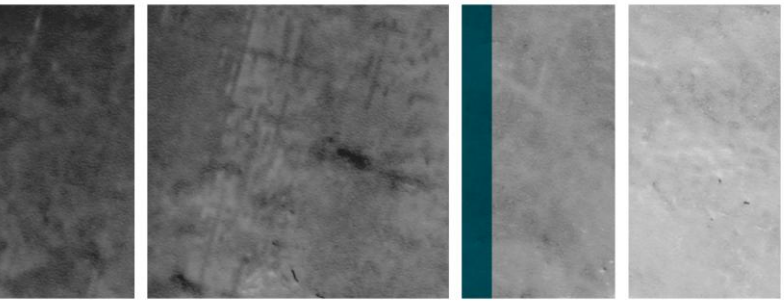
To ensure successful outcomes in relation to managing carp at local scales (e.g. wetland inlets), Smith et al (2009b) already considered how exploitation of innate carp beahviorus may be used for control purposes by developing a set of guidelines. The guidelines not only summarise the vulnerabilities and control options described above, but also higlights that careful planning and detailed knowledge (e.g. baseline fauna and flora surveys) of the site in question are needed before undertaking action. This will allow community groups and wetland managers to determine whether carp management interventions are worthwhile. The guidelines also allow managers to choose the best management option for their particular wetland type providing that a wetland management plan was already developed and carp management was a high enough priority to warrant action.

Suggested future work

Project 4 proposes that several critical, logistical issues must be addressed by future research on physical control options for carp management at specific, local wetland inlets. These include:

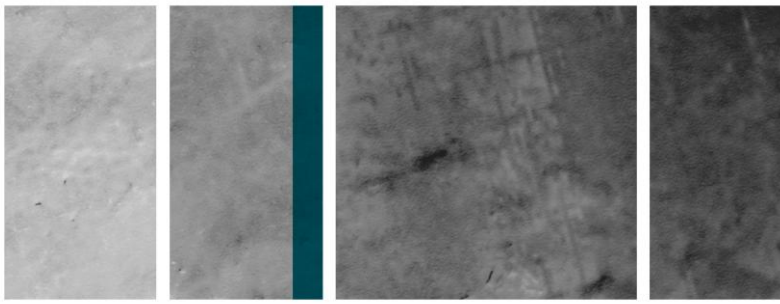
- the development of operational protocols,
- understanding site-specific impacts on native flora and fauna,
- fouling potential,
- and maintenance schedules for wetland carp harvesting systems and optimised carp-exclusion screens.

Another logistical issue requiring further investigation is the feasibility and realistic cost of carp cages, the associated infrastructure and their ongoing maintenance and management.



Future research areas for consideration include:

- the movements of carp out of wetlands to ‘overwintering’ spots in the main river channel - for example, how/whether to incorporate a one-way carp push trap element in CES to allow adult carp to move out of permanent wetlands for overwintering,
- the passage requirements of other native vertebrate species – how to allow their movement (eg tilted screens with a portion of the screen constructed like a ramp and with different mesh that enables turtles to better traverse),
- the density-dependent factors regulating carp recruitment - for instance, considering carp are highly fecund (producing 0.5–1.5 million eggs per female) and recruitment is closely tied to the ‘carrying capacity’ of wetlands and environmental factors, only a few carp broodstock may produce the same number of recruits as a large number of broodstock,
- and the differences in lateral movements and habitat use of carp and native species under higher flow regimes and pre-drought patterns of lateral and longitudinal connectivity.



Project 5: Population genetics of common carp in the MDB

Project publications

Haynes GD, Gongora J, Grewe P, Gilligan DM, Moran C and Nicholas FW (2011). Cryptic hybridization and introgression between invasive Cyprinid species *Cyprinus carpio* and *Carassius auratus* in Australia: implications for invasive species management. *Animal Conservation* 15(1): 83-94.

Haynes GD, Gilligan DM, Grewe P, Moran C and Nicholas FW (2010). Population genetics of invasive common carp *Cyprinus carpio* L. in coastal drainages in eastern Australia. *Journal of Fish Biology* 77(5): 1150–1157.

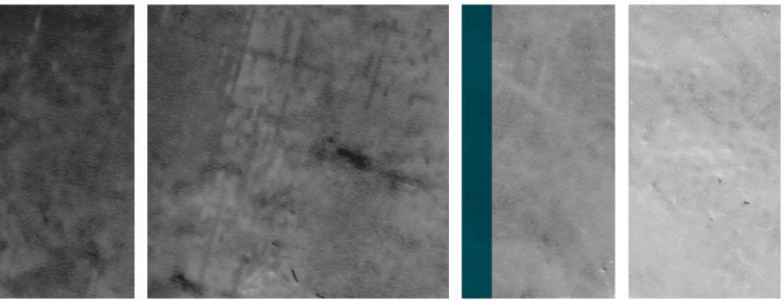
Haynes GD, Gilligan DM, Grewe P, and Nicholas FW (2009). Population genetics and management units in invasive common carp (*Cyprinus carpio* L.) in the Murray–Darling Basin, Australia. *Journal of Fish Biology* 75(2): 295–320.

Haynes GD, Gongora J, Nicholas FW and Zenger K (2009). Rapid identification of maternal lineages in common carp (*Cyprinus carpio L.*) using Real-Timer PCR and high resolution melt-curve analysis. *Aquaculture* 287: 59–66.

Haynes G (2009). *Population Genetics of Common Carp (*Cyprinus carpio *L.) in the Murray-Darling Basin*. PhD thesis. Faculty of Veterinary Science, The University of Sydney, Sydney, New South Wales, 186 pp. (*.pdf available at: http://www.feral.org.au/?p=44505)

Prelude

Carp (*Cyprinus carpio* L.) are indigenous to Asia and Western Europe and can be separated into three to four distinct subspecies and innumerable aquaculture and ornamental strains (Kohlmann et al 2003, 2005; Zhou et al 2004; Mabuchi et al 2005, 2008). Carp were independently introduced into Australia on a number of occasions following European settlement and are believed to have been established in the MDB by as early as the 1920s. Carp were widespread, but present only in low densities in the MDB until the mid-1970s, when there was a rapid and unprecedented explosion of carp numbers. The Boolara strain of carp was introduced into the Basin in the late 1960s, allegedly an aquaculture strain illegally imported from Germany. Wide-scale flooding events in the mid-1970s provided abundant habitat for carp spawning and led to the population explosion. Previous population genetic studies on carp in Australia identified four strains: Prospect, Boolara, Yanco and Japanese Koi. Although interbreeding has been recorded between the Yanco and Boolara strains, there is no evidence to suggest that it could not occur between other existing carp strains. Hybridisation between carp and goldfish (*Carassius auratus*) has also been detected in the MDB, but the level of introgression between the two species had not been quantified prior to this study. Furthermore, although the genetic structuring of carp within the MDB has been identified, the patterns related to this structuring were not clearly defined.



Project summary

This project aimed to comprehensively characterise the population structure and level of genetic diversity of carp in the MDB, as well as discern the history of carp introduction and subsequent spread throughout the MDB. Another key aim was to identify barriers to gene flow throughout the MDB in order to propose potential management units for carp control programs. Several secondary projects were undertaken to:

- focus on the potential to determine the origin of different strains of carp that have been introduced to Australia by comparing local strains with wild carp (sourced from European stocks),
- investigate the population genetics of the carp popualations in separate waterways on the east coast of Australia (ie Sydney Basin and Hunter, Parramatta and Hawkesbury-Nepean Rivers),
- optimise polymerase chain reaction (PCR) protocols for amplifying microsatellite DNA loci in both carp and goldfish,
- characterise the level of introgression between feral carp and goldfish in the MDB,
- and develop a protocol for the screening of sequence variants in the mitochondrial control region using real-time PCR and high-resolution melt-curve analysis technology.

Key findings from this project are as follows:

- the presence of the Prospect strain in the MDB was confirmed,
- the extent of population genetic structuring in the Basin was characterised,
- the disparate distribution of the different stains as a result of human-mediated dispersal was confirmed,
- and the cryptic introgression between goldfish and carp was described.

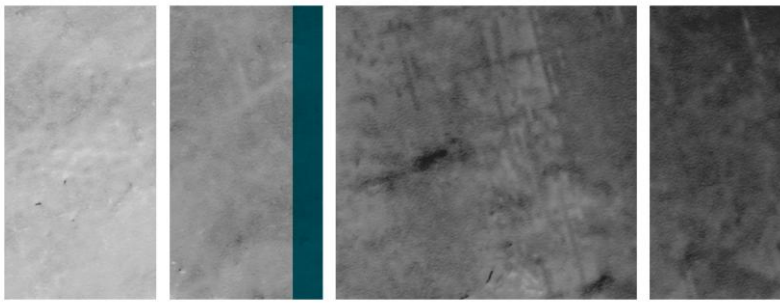
Despite being recently introduced, carp can exhibit population structuring within a single river basin, and this structuring is consistent with the population not yet being in mutation-drift-migration equilibrium and gene flow playing a larger role than genetic drift in shaping genetic structure.

Rationale and objectives

Population genetics can be used to research the population history and connectivity of carp in Australia to inform management programs. Population genetics can be defined as the study of changes in allele or gene frequencies in space and time and can be used to:

- identify population structure (ie the presence of subpopulations),
- quantify the genetic differences between subpopulations,
- and estimate effective population sizes and migration rates (ie gene flow).

In addition, phylogenetic analyses can be used to infer the history and origin of different populations.



Common carp were collected by electrofishing (March 2004 to October 2006) and a fin clip from each individual was immediately placed in 70% ethanol. A minimum of 30 individuals were collected from above and below major dams within every major river catchment in the MDB. Carp were also collected from Prospect Reservoir (source of the 'Prospect' strain) in the Sydney Basin and three rivers (Hunter, Parramatta and Hawkesbury–Nepean Rivers) on the east coast of Australia. Japanese Koi carp and domestic mirror-scaled carp were sourced from aquaculture facilities. To ascertain patterns of the history of introduction and colonisation of carp in the MDB, wild carp were also sourced from various rivers in the UK and the River Danube in Germany. Russian Ropsha strain carp were obtained from a live gene bank in the Czech Republic to determine whether strains found in Australia may originate from Europe (or not). In addition, to characterise the level of introgression between carp and goldfish in the MDB, goldfish were opportunistically collected along with carp from the MDB and sourced from local pet stores in Sydney. Additionally, 23 suspected carp-goldfish hybrids in the MDB were collected (identified based on having aberrant barbells around their mouths). All carp were characterised for 14 microsatellite DNA loci. All goldfish and suspected hybrids were characterised for five of the 14 microsatellites that could be PCR-amplified reliably in both carp and goldfish. Population genetics was analysed using a range of tests, including:

- Mantel tests to detect isolation-by-distance patterns of genetic structure,
- Bayesian assignment tests,
- Factorial Component Analysis (FCA),
- various genetic diversity indices (from published data on common carp in their native range, other invasive species of freshwater fish, and freshwater fish in general),
- tests for departures from mutation-drift equilibrium indicative of population bottlenecks,
- and tests to identify genetic discontinuities across the landscape.

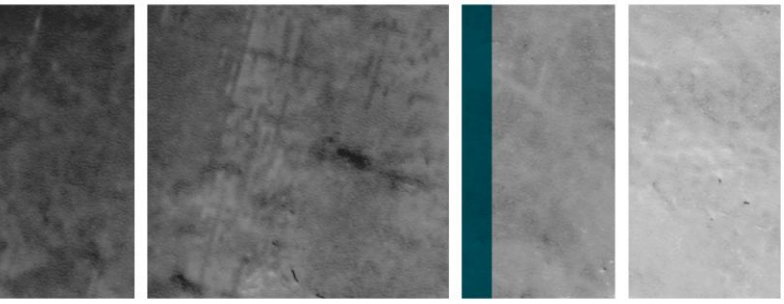
The mitochondrial DNA control region was also sequenced in a selection of carp and goldfish and all suspected hybrids. Phylogenetics was also analysed.

Outcomes

The Prospect, Yanco, Boolara and Koi strains were readily identified in the FCA and Bayesian analyses using microsatellite DNA loci.

The Prospect, Yanco and Boolara strains accounted for the majority of genetic variation within the MDB with some minor contributions from the ornamental Japanese Koi carp strain. Although the Koi strain was already known to belong to the east-Asian carp subspecies (*Cyprinus carpio haematopterus*), comparison between these strains and the European and Russian carp indicated that the Prospect, Boolara and Yanco strains were all descended from European carp, thereby belonging to the European/central-Asian carp subspecies (*Cyprinus carpio carpio*).

The history of introduction and colonisation of carp in the MDB was evident from the distribution of the different strains and from historical records of flooding events, carp introductions and presence in different parts of the Basin. The Prospect strain was



widespread, consistent with its early introduction during colonisation and large expansion during the 1950s floods. The Boolara strain was similarly widespread but was known not to have entered the MDB until the late 1960s. Both Boolara and Prospect strains rapidly expanded during large-scale floods of 1974–1975, which led to their widespread distributions. This expansion was also possibly aided by heterosis (ie hybrid vigour) as a result of mating between the two strains. Distinctively, the Yanco strain was scarce in some regions but abundant in other regions in the MDB. This suggests that this strain dispersed after the expansion in range of the Prospect and Boolara strains. The expansion of the Yanco strain may have also been constrained by the presence of weirs and natural barriers. Ornamental Koi carp strains were found in low numbers at a number of sites in the MDB, and had contributed little to the overall carp population. Most regions had multiple strains present and no evidence of recent population bottlenecks, which implies that the invasiveness of carp may be associated with high levels of genetic diversity.

Although the MDB is often divided into distinct catchment regions for management purposes, such as water purchasing (eg http://www.environment.gov.au/water/policy-programs/entitlement-purchasing/index.html), to assist future carp control programs, the author proposed 15 management units within the MDB. The units were based on:

- the presence of the four different strains,
- the detection of genetic discontinuities between sites where carp were sampled,
- and known barriers to carp dispersal (Figure 7).

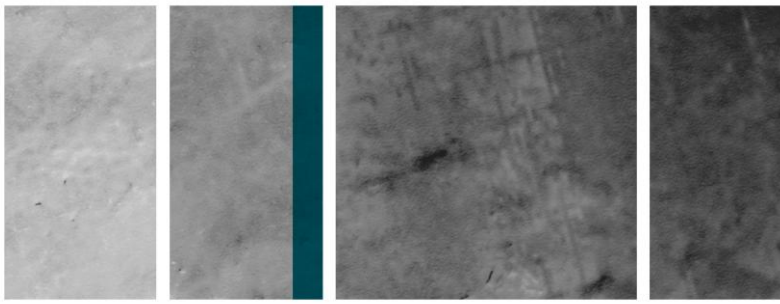
Each management unit corresponds to the presence of man-made impoundments (barriers), naturally limited flows (hydrology) and catchment boundaries. The management units, however, do not take into account:

- the ongoing construction of fishways (Stuart et al 2008),
- and the possibility of changed flow management that increases connectivity between populations in various regions.

Both of these limitations could render some management units obsolete. Connectivity between population management units could be minimised by the inclusion of Williams' CSC to reduce carp movements and to harvest carp at river fishways (Stuart et al 2006b).

Using the same 14 microsatellite loci that were used to study carp populations in the MDB it was found that different strains of carp were present in each of the populations from the east coast of Australia (ie Hunter, Parramatta and Hawkesbury–Nepean Rivers). Thus each population was established by a series of independent, man-made introductions rather than natural colonisation between river basins. Notably, ornamental Koi carp were detected in the Hunter River and were the dominant strain in the Parramatta River. This indicates that the Koi strain is capable of surviving in the wild and establishing invasive populations.

PCR was optimised for five microsatellite loci in both carp and goldfish. Introgression between the two species in the MDB was quantified using the Bayesian analyses in the STRUCTURE and NEWHYBRIDS programs and FCA and direct inspection of microsatellite alleles in the 23 suspected hybrids identified in the field. All analyses confirmed the mixed nuclear-genome



ancestry of all 23 putative hybrids: with 20 classified as F1 (first generation) hybrids and three classified as F2 (second generation) or backcrossed. Cryptic mixed ancestry was also detected in 14 individuals from the MDB phenotypically identified as carp, three domestic Koi carp and one individual from the MDB identified as a goldfish. Phylogenetic analysis of the mitochondrial control region revealed that 19 of the 20 F1-generation hybrids had goldfish mitochondrial DNA (ie a goldfish mother). Thus, carp–goldfish hybridisation was biased in favour of male carp mating with female goldfish. However, too few individuals and loci were analysed to resolve this trend with any certainty. The presence of F2 and backcrossed individuals indicates that carp-goldfish hybrids are not always sterile and gene flow is occurring between the two species. This is of concern as it may introduce new adaptive alleles (eg genes for disease resistance, or genes that are not affected by daughterless carp genetic constructs) into invasive carp populations.

Finally, a protocol was developed for screening sequence differences in the mitochondrial DNA control regions between different strains of carp and goldfish. This protocol employed real-time PCR and high-resolution melt-curve analysis to identify slightly different melting temperatures of different mitochondrial sequences.

Vulnerabilities and application

The discovery that three strains of carp are found in specific, discrete locations at the Murray Darling Basin catchment scale allows for the integration of localised targeted control efforts to enhance carp control at the regional scale. While carp control strategies can be divided into physical/chemical and biological approaches as described in Table 1, this genetic population research highlights how the MDB could be divided into 15 discrete management units based on known man-made barriers to dispersal, catchment boundaries, hydrology, genetic discontinuities between carp at different locations and the presence or absence of the Koi and Yanco strains (

Figure **7**). These proposed management units can then assist control strategies and programs by:

- identifying specific target areas where physical removal methods will be most effective (smaller management units with limited immigration),
- identifying regions from which areas could be recolonised after control (upstream management units or within same management unit),
- and predicting which regions can be affected by release of biological controls, such as genetically modified daughterless carp or carp-specific diseases (downstream management units).

The discovery of a low level of introgression between carp and goldfish, however, could compromise the implementation and efficacy of biological control strategies, especially since it is inevitable that domestic goldfish individuals may find their way into waterways, then survive and reproduce. If diseases are released to control carp, to which the goldfish are immune, certain individual alleles conveying immunity carried by goldfish could spread rapidly through the wild carp population, negating the effectiveness of the disease. In addition, the success of daughterless-gene technology may be undermined because goldfish

could act as a genetic diversity reservoir of functional copies of the modified gene(s).

This study also confirmed that ornamental Koi carp were surviving in the wild and were capable of establishing viable populations. This is of concern for carp management, as keeping, breeding and selling of Koi is still legal in some states in Australia.

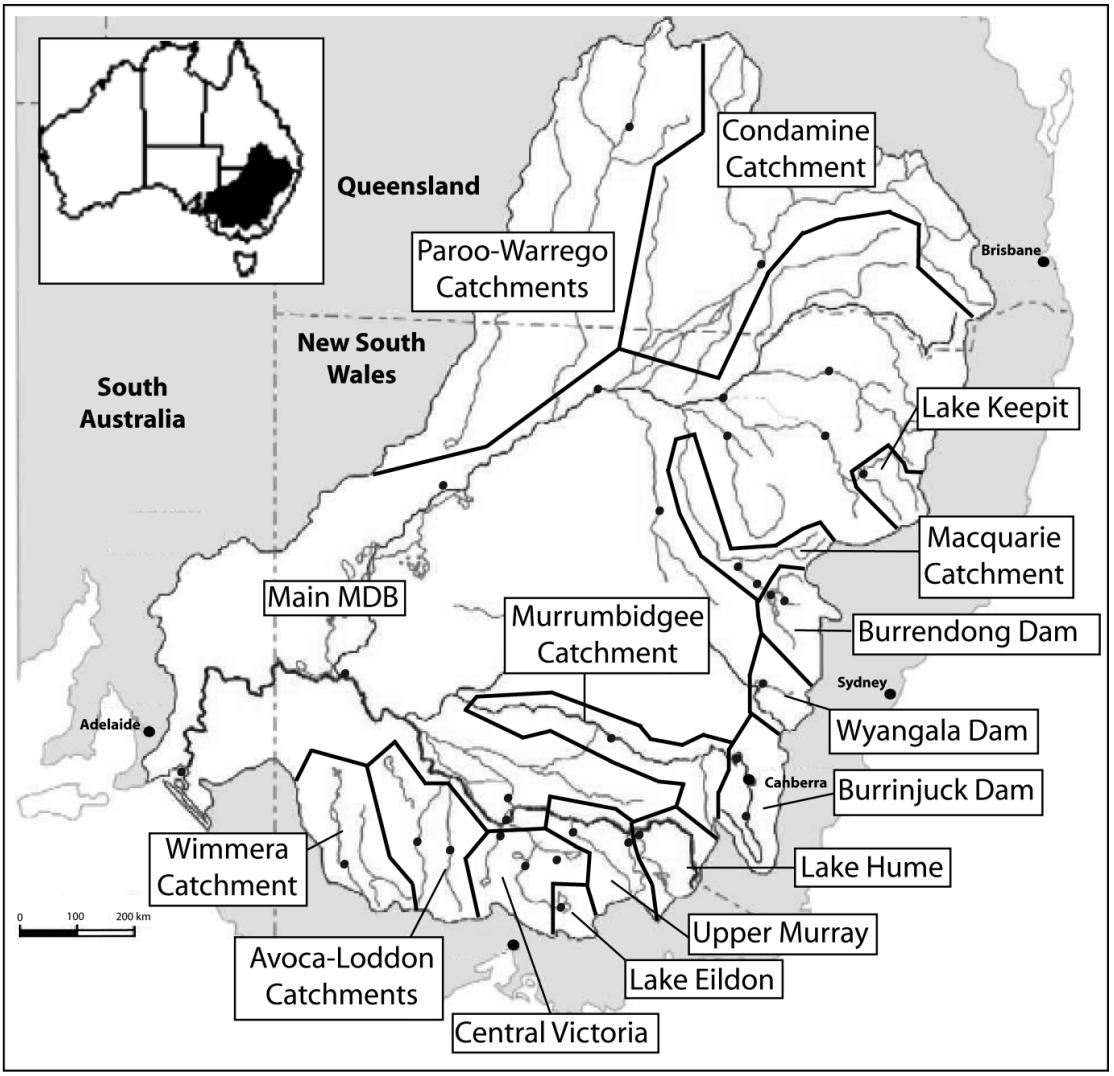
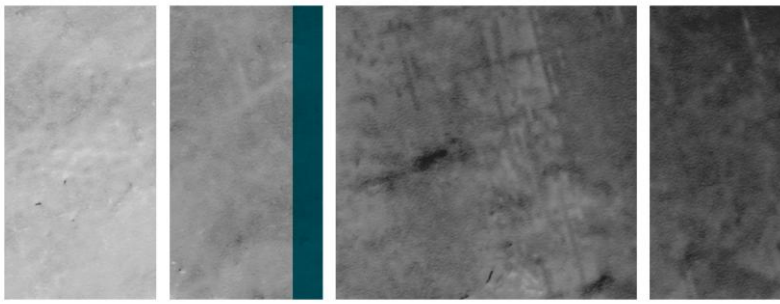


Figure 7. Proposed management units for carp in the Murray–Darling Basin by Haynes (2009). Units are based on genetic discontinuities and geographic barriers to dispersal (see Haynes et al 2009b).



Suggested future work

There is scope to improve the accuracy and power of the population genetic inferences presented here by scoring more genetic markers and including additional outgroups' populations. Potential outgroups include:

- additional Prospect strain carp from Prospect Reservoir or Potts Point Reservoir,
- populations of Boolara strain carp that have not had the opportunity to breed with other strains (available in Gippsland, Victoria),
- and additional east-Asian and European carp populations.

Such additional research may allow new management units to be identified and delimited and could shed new light on the history of carp introduction and expansion in Australia. The accuracy and power of the inferences about carp-goldfish introgression could similarly be improved by including more microsatellite DNA markers that can be PCR-amplified and genotyped for both species and sampling goldfish more widely throughout the MDB.

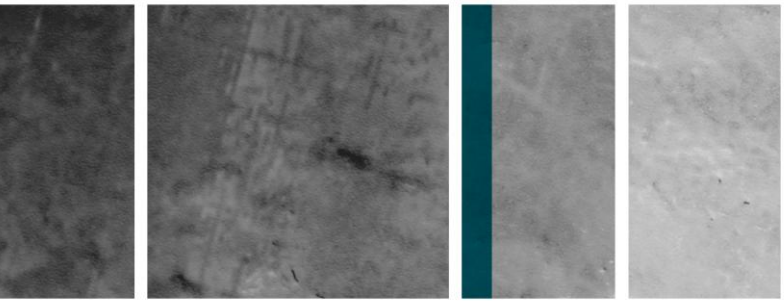
Numerous coastal drainages are infested with carp. Sampling and population genetic analyses of the carp in these drainages could inform management practices by:

- identifying colonisation pathways,
- identifying if and where ornamental Koi carp are being released into waterways,
- and delimiting management units.

Such studies should emphasise between-catchment population genetic structure, rather than the within-catchment structure investigated in this PhD.

The presence of an introduced population of crucian carp (*Carassius carassius*) has recently been confirmed in the Campaspe River in the MDB. Genetic investigation of this species could clarify the full range of this species in the MDB, using mitochondrial DNA loci and microsatellites that can be PCR-amplified in carp, goldfish and crucian carp. Morphological identification of crucian carp is difficult as they are easily confused with wild goldfish, but genetic identification allows identification of introgression between crucian carp, carp and goldfish.

Improving flow management may increase connectivity between carp populations in some regions and render some of the proposed management units obsolete. Therefore, it would be beneficial to conduct similar investigations during post-flood conditions.



Project 6: Sex determination and differentiation in carp

Project publications

Barney M (2010). *Molecular Investigations on Sex Determination and Differentiation Pathways in the Common Carp,* Cyprinus carpio. PhD thesis, University of Tasmania, Hobart, Tasmania, 217 pp.

Barney, M. L., Patil, J. G., Gunasekera, R. M. and Carter, C.G. (2008). Distinct cytochrome P450 aromatase isoforms in the common carp (*Cyprinus carpio*): Sexual dimorphism and onset of ontogenic expression. General and Comparative Endocrinology **156**(3): 499-508.

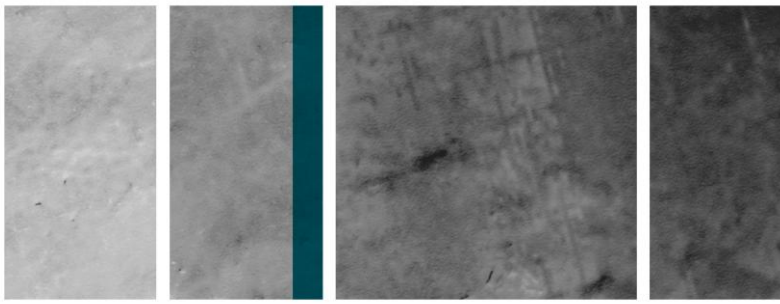
Prelude

Daughterless gene technology

Several carp control methods have been explored in Australia (Roberts and Tilzey 1996), including the use of 'daughterless' technology. Such technology uses a heritable sex-ratio manipulation predicted to cause localised extinction of carp populations (Hamilton 1967; Werren et al 1981). The Murray–Darling Basin Commission, now the MDBA, supported the IA CRC and its research partner CSIRO Marine Research to undertake the Daughterless Carp Project (DCP) as a tool to control common carp in the basin. The DCP focuses on genetically manipulating and skewing the sex ratio of common carp populations in favour of males by introducing exclusively functional males. The aim is to introduce multiple copies of a daughterless gene into common carp, which would be periodically released into the wild. Copies of the gene are carried by males and introgressed though the populations, resulting in male-biased sex ratios and ultimately a drastic reduction of female offspring. This in turn would potentially lead to localised extinction of carp populations. The research described here forms part of this project, as it explores potential novel candidate genes in the sex-determination pathway of common carp that may be targeted to manipulate sex ratios.

The DCP technology concept arose from the widespread use of chemical aromatase inhibitors to create single-sex lines of fish for aquaculture (Thresher 2007). Aromatase is the enzyme responsible for female development. If aromatase is inhibited, all embryos develop as fully functional phenotypic males irrespective of their genotypic sex (XX or XY) (Piferrer et al 1994). Despite being the initial target for this eradication method, the aromatase genes had not been fully described for carp.

Little is known about mechanisms of sex determination and differentiation in common carp. The expression of sex is generally governed by two processes: sex determination (genetically) and sex differentiation (male/female phenotype). Sex determination refers to the mechanisms that direct sex differentiation. Sex differentiation refers to the development of ovaries or testis from an undifferentiated or bipotential gonad (Hayes 1998). Sex differentiation also includes sexually influenced development of morphology, behaviour and biochemical secondary sex characteristics. Although morphologically distinct sex



chromosomes cannot be identified in common carp (Kirpichnikov 1981), sex determination is thought to be of the XX/XY system with conventional diploid offspring yielding 1:1 sex ratios (Manzoor and Satyanarayana 1989; Komen et al 1992; Cherfas et al 1994). In teleosts (ie ray-finned fish), however, sex differentiation is complex because it is influenced by a balance of genetic, steroidogenic and environmental factors (Devlin and Nagahama 2002).

Project summary

To assist in the development of genetic control mechanisms in common carp, this PhD project investigated the molecular pathway of sex determination and differentiation in carp. Candidate genes known to be involved in sex determination and differentiation processes in carp, namely ovarian *cyp19a* and *foxL2* genes, brain *cyp19b* gene and testis *dmrt1* and sox9 genes were investigated using molecular cloning. Spatial expression of these genes was analysed in adult tissues and several genes were identified and described for the first time. In addition, the effect of temperature on gene expression and final sex ratios and the onset and timing of expression were determined during early ontogeny and through larval development at two temperatures (20ºC and 25ºC).

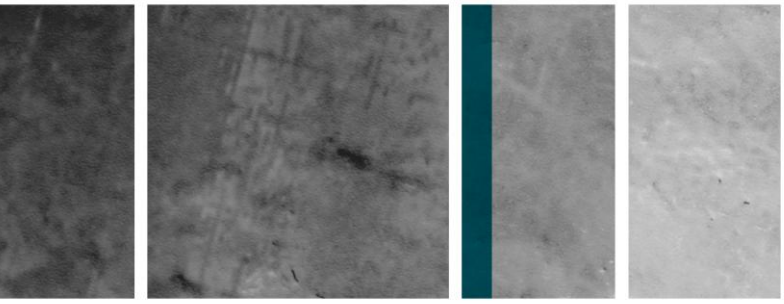
Rationale and objectives

The primary gene target for the daughterless approach is the ovarian isoform of cytochrome P450 aromatase gene (*cyp19a*) based on its essential role in oestrogen synthesis and ovarian development. Oestrogen is required for many functions and in many tissues. Thus, it is important to know where c*yp19a* is locally expressed and co-expressed with the brain isoform. Such understanding helps determine potential undesired or compensatory effects of 'switching off' this gene. As aromatase was the primary target for the DCP, this study also aimed to identify and clone the promoter region of both aromatase isoforms, namely the ovarian (*cyp19a*) and brain (*cyp19b*) isoforms to determine potential regulatory elements involved in transcription. Forkhead box L2 (*foxL2* gene) is one such regulatory element of aromatase. This project describes, for the first time, the cloning and expression of this gene in adult and developing common carp. The cloning and expression profiles of two other genes involved in male development, *dmrt1* and *sox9,* are also reported here.

Exploring the molecular pathways that lead to ovary and testis development in teleost fish will result in greater understanding of sex determination and differentiation in all vertebrate species. More specifically, this will allow greater understanding of a potential "Achilles' heel", or vulnerable aspects of sexual development, that may be used to control sex ratio and, in turn, pest species. Examples would be controlling phenotypic sex by inducing or repressing expression of critical genes involved in development of either females (*cyp19a*) or males (*dmrt1*). More generally, the ability to manipulate sex ratios of carp or other fish is of benefit to:

- pest control, where biasing can result in extinction,
- and aquaculture, where single-sex populations can improve production efficiency.

The overall aim of this project was to enhance the understanding of the mechanisms underpinning sex determination and differentiation in carp by:



- selecting, cloning and characterising candidate genes in sex determination and differentiation,
- determining sex- and tissue-specific expression of candidate genes,
- examining timing and onset of candidate genes and expression patterns through gonadal differentiation,
- exploring the effect of two different rearing temperatures (20ºC and 25ºC) on gene expression and resultant sex ratios,
- and analysing potential interactions of candidate genes and consequences of these interactions.

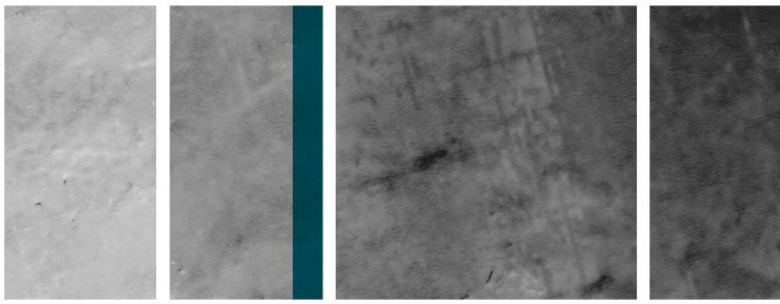
Outcomes

There were two isoforms of the cytochrome P450 aromatase gene in carp: ovarian (*cyp19a*) and brain (*cyp19b*). Based on the level of CYP19 gene expression, the brain was the main site of aromatase synthesis, predominantly the brain isoform. Also expressed highly in the brain were isoforms of sex-determining genes described for other organisms (Schultheis et al 2006). In particular, both isoforms of SRY (sex-determining region Y), -box 9 (*sox9*) and forkhead box L2 *(foxL2)*, were expressed; with the former possibly having a role in testicular differentiation (Klüver et al 2005) and the latter in ovarian differentiation (Baron et al 2005) in higher vertebrates. Within the gonad, *cyp19a* and *foxL2* were predominantly expressed in the ovary. In the testis, '*doublesex* and *mab-3* related transcription factor 1' (*dmrt1*) was primarily expressed. Ontogenic expression indicated that *cyp19a* and *sox9a* were maternally inherited. Female critical *cyp19a* showed sexually dimorphic expression only in fish larger than 20 mm with warmer conditions (25ºC) suppressing expression. This suggests a male-skewed final sex ratio, indicating that differential expression may be a result of sex differentiation rather than a cause.

Conversely, expression of *cyp19b* peaked prior to carp hatching, possibly indicating that sexual differentiation occurs first in the brain before the gonads are present. Expression of *dmrt1*, critical for male development, peaked soon after fertilisation in the 25ºC treatment, indicating an early role in the sex-determining pathway. Peak expression of *sox9* genes and *foxL2* occurred prior to hatching with consequent expression failing to show any sexually dimorphic expression. This suggests that these genes play a role in early larval development in the species but not sex differentiation.

Vulnerabilities and application

From a pest control context, the ability to control the expression of genes that influence sex expression in carp could allow for the manipulation of sex ratios or in particular create a sex bias that leads to population extinction. Barney (2010) found that there are a number of genes that play a role in early larval development in carp. In general, the two isoforms of the cytochrome P450 aromatase gene — *cyp19a* and *dmrt1* — were accurate markers of ovarian and testis differentiation respectively. Thus, the ability to influence the expression of these genes may allow manipulation of sex ratios of common carp and other fish. This could be applied in a pest control context, where population sex bias can result in extinction. It could



also be useful for aquaculture, where use of single-sex populations can improve production efficiency.

The maternal inheritance of the *cyp19a* gene suggests it plays a key role in the early development in both male and female individuals in carp. In addition, adults showed expression of *cyp19a* in the testis, as well as in the ovary albeit at lower levels. The *dmrt1* gene was a critical male-differentiating factor in carp and other teleost fishes. The *dmrt1* was exclusively expressed in the gonad and initiated at mid-blastula transition, indicating an early role in the sex-determining pathway. The gene product *dmrt1* is a strong candidate for daughterless technology in carp because it could potentially direct ectopic gene expression in developing gonads of genetic females, resulting in their sex reversal to males.

Suggested future work

Further research will improve our understanding of the interactions of genes involved in sex determination in teleosts, and more specifically, carp. Protein studies and cell culture work would be useful to determine which of the potential aromatase *cyp19a* or *cyp19b* promoter sites are active.

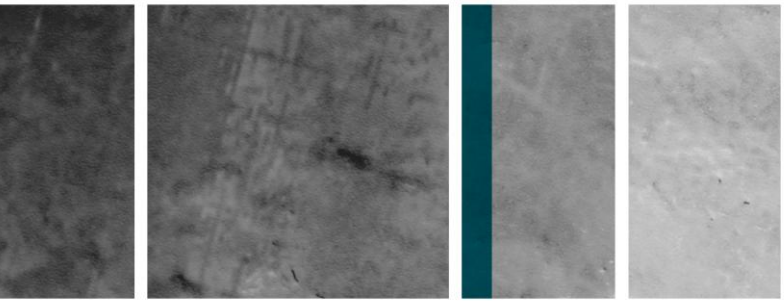
The role of *cyp19a* in the testis of common carp also requires more research, especially because the repression of *cyp19a* to produce exclusively male-bearing offspring needs to be heritable for DCP technology. Predominant *cyp19a* expression in the ovary suggests that this gene is a viable DCP candidate, although low levels of expression were observed in other tissues, particularly brain tissue. Inadvertent blocking of *cyp19a* expression in domains other than the ovary requires further investigation before it can be used for large-scale production of daughterless carp lines for pest control.

Overall, this research suggests that *cyp19a* and *dmrt1* are good molecular markers of female and male differentiation respectively. Nonetheless, the development of a sex-specific marker will be useful for early sexing in aquaculture and for testing the genetic sex of genetically-manipulated offspring produced by daughterless technology. Techniques such as Random Amplified Polymorphic DNA (RAPD) or Amplified Fragment Length Polymorphism (AFLP) need to be explored to identify such a marker.

The development of a gonad-specific carp microarray would also allow greater understanding of concurrent changes in sex-determining factors through early larval development or sex-reversal using exogenous steroids.

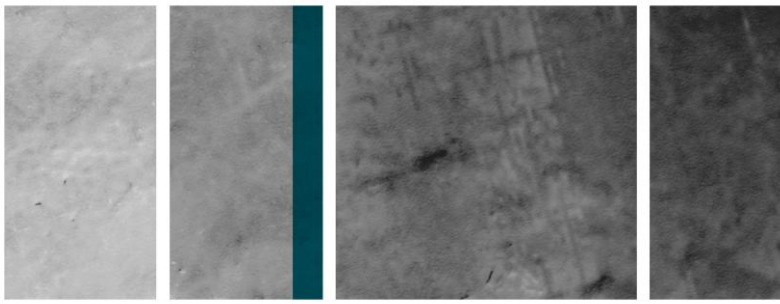
Other candidate genes

Owing to the conserved nature of genes involved in sex determination and differentiation, it is possible to examine fully described genomes to determine factors that may be involved in these processes in different teleost fish. Another potential regulator of *cyp19a* is the transcription factor Dax1, through its repressive action on the SF-1-mediated transactivation of the *cyp19a* promoter (Wang et al 2001). In fish, Dax1 has also been found to downregulate both SF-1 and *foxL2*-mediated *cyp19a* expression in Japanese medaka ovarian follicles (Nakamoto et al 2006).



Environmental sensitivities

Environmental factors can influence sex determination. For instance, endocrine disruption studies have shown that aromatase is an environmentally sensitive gene. Further investigation is recommended to clarify how environmental factors, such as temperature regime, impact reproduction in carp and other fish.



Project 7: Early gonad development in the common carp

Prelude

The considerable reproductive ability of carp is a major contributor to its success, and so understanding carp reproductive biology is important to control recruitment in MDB waterways (Smith and Walker 2004a, 2004b). Teleosts (ie ray-finned fish) have a diverse array of sex determining mechanisms and modes of sexuality (Potts and Wootton 1984). Understanding gonad development, cell movements, interactions between cell types and differentiation and specialisation of cells in the ovary and testis will provide key knowledge for the development of daughterless gene technology. Understanding carp gonad development and the influence of temperature on sex differentiation and sex ratios may help identify vulnerable aspects of biology that can be exploited in carp control.

Project summary

To enhance our understanding of carp gonad development and whether temperature influences sex differentiation, this Honours project examined early gonad development in the Australian-reared common carp. Changes in gonads were recorded in response to temperature treatments that best represented the lower to higher limits of optimum temperatures for carp (eg 21˚C and 25˚C, respectively). It was found that carp were undifferentiated gonochorists, and most individuals formed either an ovary or ovotestis, where the ovotestis appeared to further develop into a testis. Some individuals, however, seemed to develop testis directly without a transitional state, hence suggesting that the coexistence of carp as undifferentiated and differentiated gonochorists is possible.

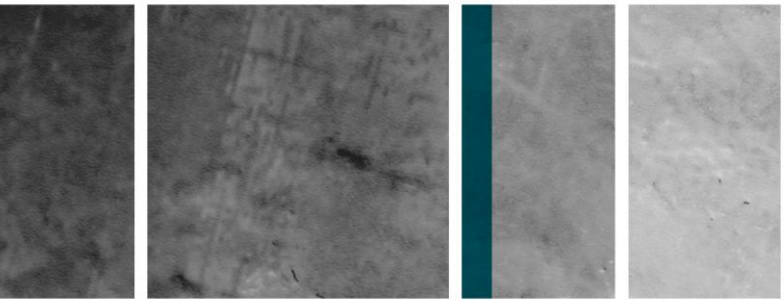
Rationale and objectives

The aims of this project were to:

- determine the pattern of sex differentiation in Australian-reared carp, *Cyprinus carpio* (L.), at 20˚C and 25˚C,
- and document the change in gonads over time.

Within the operational constraints of the hatchery, two selected temperatures (21˚C and 25˚C) were chosen to represent the lower and higher limits of the optimum temperatures for carp. The morphological development of carp larvae over the course of the experiment was also examined.

Carp were spawned and reared at K and C Fisheries in Victoria. Embryos were initially incubated at a median temperature (23 ± 0.52˚C) for up to five days-post-fertilisation (dpf). Histological samples were prepared to determine the stages of gonad development. Sampling commenced the day after fertilisation, with daily sampling up to five dpf. On the fifth day, larvae were placed into their respective temperature treatment tanks, after which they were randomly sampled at weekly intervals for 16 weeks. At each sample time, fish weight, total



length (TL) and standard length (SL) were recorded. On 120 dpf, the remaining fish were weighed and measured. An additional 60 fish from each temperature treatment were sexed using the aceto-carmine staining technique (Guerrero and Shelton 1974).

Outcomes

Sexual differentiation in carp has previously been reported to proceed along:

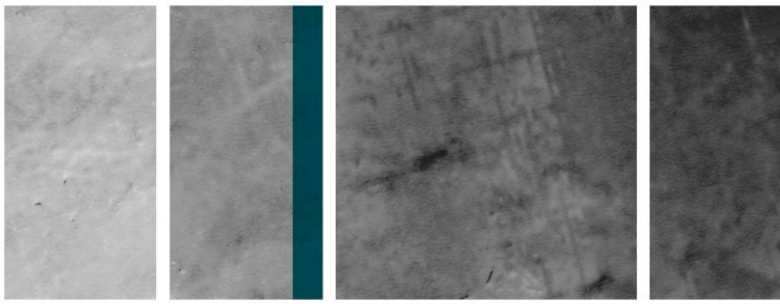
- a differentiated gonochorist pathway, where the larvae develop directly into males and females (Parmentier and Timmermans 1985; Komen et al 1992),
- and an undifferentiated gonochorists pathway of juvenile hermaphroditism, where the individuals initially develop ovarian tissue (Davies and Takashima 1980; Komen et al 1992).

The results observed in this study support the latter hypothesis, with most individuals forming either an ovary or ovotestis. Some individuals, however, contained only spermatic tissue without the presence of oocytes or remnants of disintegrating oocytes. This suggests they developed testis directly without a transitional state. It is possible that both differentiated gonochorists and undifferentiated gonochorists co-exist in carp.

The timing of gonad differentiation varied between the two temperature treatments. Temperature appears to have a minimal effect on carp growth up to 72 dpf, but from then on a larger difference was observed, with larvae in the 25°C treatment significantly larger than in the 20°C treatment. Higher temperatures resulted in faster growth rates, thereby increasing the rate of gonad development. Ovarian differentiation occurred in carp from 2 cm SL, which also correlated with larval metamorphosis into juveniles. Testicular differentiation occurred with carp above 3 cm SL. However, temperature did not have an effect on sex ratios at temperatures between 21°C and 25°C.

The lack of difference in the sex ratios between the temperature treatments may have been because:

- the carp in the 20°C treatment were not fully differentiated, and therefore, a sex ratio for the lower temperature cannot be confirmed, especially when compared to the higher temperature treatment,
- a skew in the sex ratio may not have occurred because the 25°C incubation temperature may not have been high enough to induce heat stress (ie oocyte apoptosis),
- and the temperature in the two treatments fluctuated with changes in daily air temperatures, resulting in the actual difference between the two treatments being often less than 5°C.

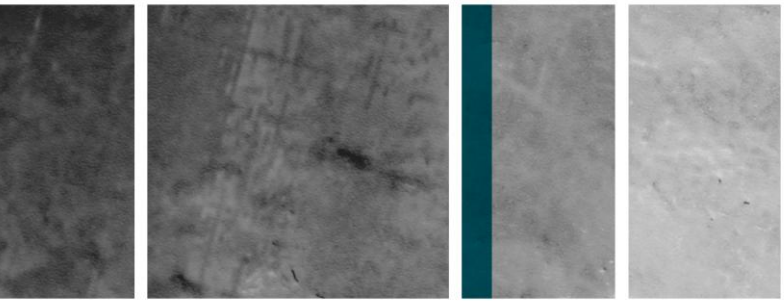


Vulnerabilities and applications

The results from this research need to be further explored before vulnerabilities and applications to carp control can be identified. Although a relationship between carp size and sex differentiation (where ovarian differentiation occurred in carp size ≥2 cm SL but testicular differentiation occurred in carp size ≥3 cm SL) was identified in common carp, it is unlikely to have practical applications, especially in the field.

Suggested future work

The effect of temperature on sex ratio is not conclusive from this work, but another study suggests that higher temperature should skew sex ratios towards males in the common carp (Nagy et al 1981). The results from this study did not support this hypothesis, and the influence of temperature on sex ratio in the carp requires further investigation. Whether ovotestis formation is a result of environmental influences or from inherited genetics also remains unsolved. Although this study suggests that temperature may not play a significant role, the ovotestis occurred in both the 20°C and 25°C treatments. Hence the formation of ovotestis may be a result of differences in genetic backgrounds (Komen et al 1992) or of the combined influences of hormones and temperature (Nagy et al 1981). Therefore, determining why carp mature via differentiated gonochorists or undifferentiated gonochorists may help identify reasons for their reproductive and recruitment success.



Discussion

The projects reviewed above present some key outcomes that may help transform carp and other pest fish management from potentially *ad hoc* measures to those that are integrated over large regions with strategic interventions in targeted hotspots. Although no specific set of traits/attributes can universally predict invasiveness across all taxa and biomes, the attributes of invasiveness within specific taxa and specific habitats/regions have been extensively researched (Kolar and Lodge 2001). Koehn (2004) collated a list of the previously known attributes of carp as invasive species, and this table has been modified to include the key vulnerabilities highlighted through the IA CRC research projects reviewed in this report.

Some of the contributions of the above projects to carp management in Australia include:

- the identification of specific target areas (eg hotspots: Project 1) and potential management units in the MDB (Project 5),
- and the demonstration that carp make limited, small-scale movements in low-flow conditions (Project 2).

These two outcomes may allow the targeted use of physical, chemical and/or biological control strategies. Efficacy of carp control may increase if targeted control capitalises on the use of physical infrastructure (eg carp harvesting systems) that can exploit identified carp behaviours. Exploitable behaviours include those of adult and juvenile carp described in Project 4:

- annually migrating between river and wetland habitats for spawning from early August onwards,
- being attracted to flowing water and moving upstream towards the source of flow,
- and having an innate ability to push past and/or jump over migration barriers.

Additionally, carp harvesting systems and separation cages could be used to help limit the dispersal of juvenile carp from source hotspot locations identified in Project 1.

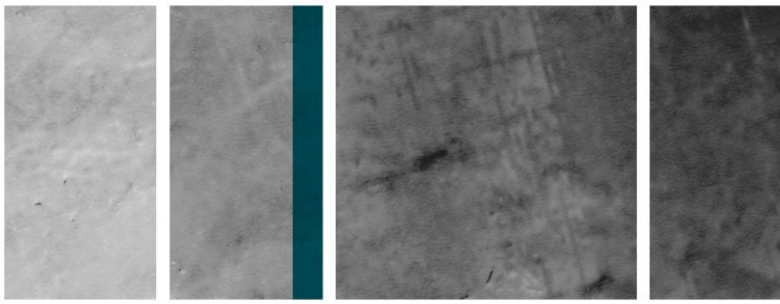
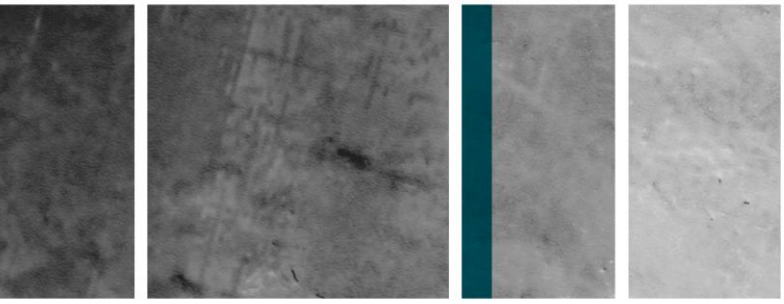


Table 5. Attributes of carp and details of new knowledge gained from reviewed research projects.

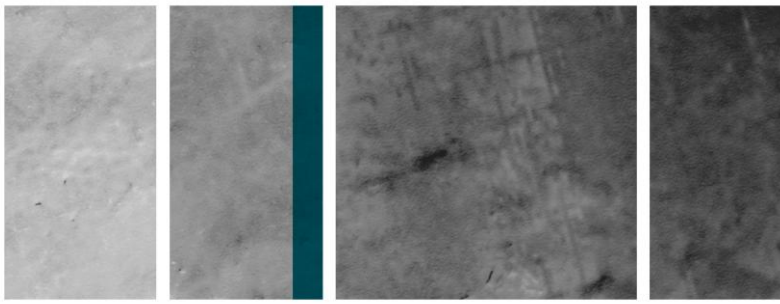
Attribute	Details (provided from Koehn 2004)	References	Details of new knowledge	References
Global invasion history, wide distribution and abundance	Introduced and successfully established throughout Europe, Asia, Africa, North America, South and Central America, Australia, New Zealand, Papua New Guinea and some islands of Oceania.	Lever (1996)		
Wide environmental tolerances	Temperature tolerance ranges 2 to 40.6°C, salinity tolerances up to about 14‰ (where 0.4 is seawater salinity), pH 5.0 to 10.5, oxygen levels as low as 7% saturation and generally occurring in most types of freshwater habitat.	Horoszewicz (1973); Ott et al (1980); Crivelli (1981); Hellawell (1986); Howes (1991)		
High genetic variability	Three genetic strains in Australia.	Shearer and Mulley (1978)	Four genetic strains were identified in MDB: Prospect, Boolara, Yanco and Japanese Koi. Two major strains, Prospect and Boolara, were distributed across the entire MDB, while a third strain, Yanco was locally abundant and Japanese Koi was rare. Interbreeding exists between Yanco and Boolara (and possibly exists between all strains). Interbreeding between carp and goldfish occurs, producing fertile hybrids. However, they may have reduced levels of fertility.	Project 5
Early sexual maturity	Males mature at one year, females at two years.	Brumley (1996)	Early gonad development in carp follows two pathways: undifferentiated gonochorism (where individuals develop ovotestis) and differentiated gonochorism (where the larvae develop directly into males and females). Both types co-exist in Australian populations, but they appear skewed towards the undifferentiated gonochorism. Two isoforms of the cytochrome P450 aromatase gene are responsible for ovarian (*cyp19a*) or testis (*dmrt1*) formation. There is a direct relationship between carp size and sex differentiation; ovarian differentiation occurred in carp ≥2 cm and testicular differentiation in carp ≥3 cm.	Project 6; Project 7
Short generation time	Two to four years			



Attribute	Details (provided from Koehn 2004)	References	Details of new knowledge	References
Growth rates	In southern, temperate MDB carp populations, the first annual increment is at age one, then every one to two years thereafter.	Brown et al (2003); Vilizzi and Walker (1999); Smith and Walker (2003b)	In northern, subtropical MDB carp populations, the first annual increment is at age one, then every year thereafter. Higher temperatures speed up gonad development.	Project 3; Project 7
Rapid development	Hatching of eggs is rapid (two days at 25°C) and newly hatched carp grow very rapidly.	Balon (1975); Adamek (1998); Vilizzi and Walker (1999)		
High reproductive capacity	Highly fecund broadcast spawners with egg counts as high as two million per female.	Balon (1975); Banarescu and Coad (1991)		
Broad diet	omnivore/detritivore	Hume et al (1983)		
Gregariousness	A schooling species	Cadwallader and Backhouse (1983)		
Possessing natural mechanisms of dispersal	A mobile species with fish moving between schools. Dispersal can also occur with the downstream drift of larvae. Rates of transfer can be affected by conditions such as flooding.	Harris (1997); Koehn and Nicol (1998); Stuart et al (2001)	Overall, the presence of carp larvae was scarce in the MDB, and most of the larval recruitment was localised to a small number of hotspot locations. In southern, temperate regions of the MDB, adult carp predictably migrate between river and wetland habitats for spawning, starting early August. Carp are attracted to flowing water and move upstream to a source of flow, especially at spawning times. Adult carp move at relatively small-scales within and between sub-catchments during low-flow conditions.	Project 1; Project 4 Project 2; Project 4
Commensal with human activity	Bred as an ornamental and aquaculture species used as bait and sought by some anglers	Li and Moyle (1993); Lever (1996); Koehn et al (2000)		

Considerations

Although several projects have largely contributed to the ability to identify potential target areas at the local scale for carp management, the research scope needs to be extended to encompass greater scales (regional to Australia-wide) and a wider range of environmental variables. For instance, Project 1 provided a comprehensive set of carp spawning hotspots within the region of the Murray–Darling catchment investigated, however this study could be extended across the entire MDB. Likewise, although spawning activity was happening at a specific site, it is uncertain whether drifting larvae from elsewhere can still recruit to another location. These considerations are particularly important, especially because of the level of



intra-basin variability in carp populations found in age validation Project 3 and carp strain populations Project 5.

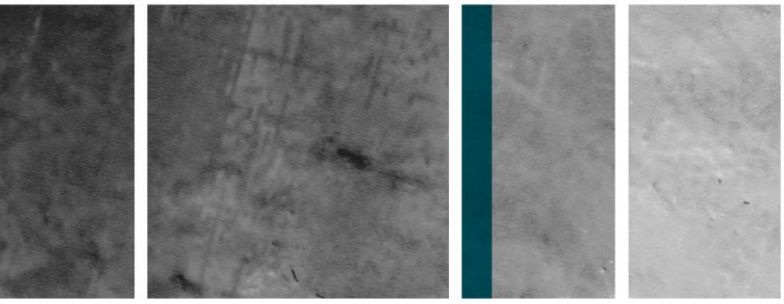
Furthermore, from 1996 to 2010, the MDB was subjected to the most severe drought in recorded history (Bond et al 2008).It is unknown whether sampling during this low-flow period biased the outcomes of several projects. For instance, the investigation into carp movement within the MDB (Project 2) only assessed low-flow conditions. More specifically, variable movements, or lack of directionality, of juvenile and adult carp among wetlands were detected under low-flow conditions only. Thus, differences may exist in movement and habitat use under higher flow regimes. Additionally, the small-scale movement of tagged individuals means that locations/times when carp may be vulnerable to trapping or management have not been identified. Increased connectivity may occur under high-flow conditions, and this may render the delineation of the MDB management units, which were based on the distinct distribution of different carp strains, obsolete (Haynes 2009).

One of the primary targets of restoration ecology is to restore or maintain resilient ecological processes and services over relatively long temporal scales (Young 2000). This concept is complementary to, but not necessarily equivalent to, conservation biology targets: to provide stopgap protection of remnant populations. That is, despite the efforts to constrain or stop the negative impacts of carp populations, carp management activities may not sufficiently focus on the maintenance of native diversity and protection (Palmer et al 2009). Hence, the complementary inclusion of native species within further investigations is recommended. For instance, carp movements (Project 2) could also be assessed in conjunction with the movement of native species of recreational or cultural significance, such as Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*) to maximise the outcomes from the available infrastructure. Likewise, larval sampling of carp at proposed hotspots (Project 1) could also include sampling of native species of recreational or cultural significance to maximise outcomes.

Another key consideration is the resources and feasibility of physical carp control strategies. For instance, Project 4 highlighted that the following needs to be carefully scrutinised:

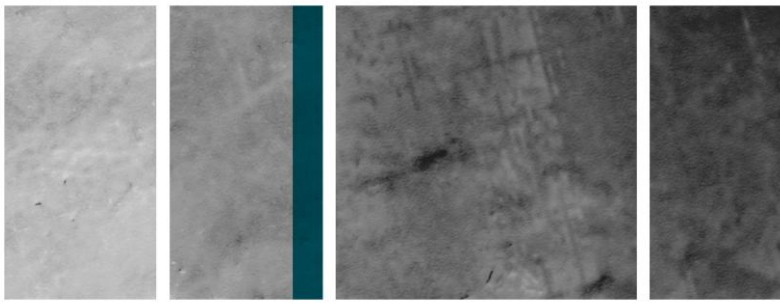
- the resources involved in the development of sound operational protocols, fouling potential and maintenance schedules for wetland carp harvesting systems and optimised CES,
- the realistic cost of developing and installing carp cages and other associated infrastructure and ongoing maintenance and management,
- and passage requirements of other native vertebrate species and how best to allow movement.

Additionally, the complementary function of both jumping and push trapping components improves efficacy of catches using this technology, although habitat conditions may mean that only one behaviour can be exploited. For instance, catches in the push trap are higher in shallow water where jumping is prohibited. In this case, the efficacy is reduced to some extent.



The student research of Projects 5, 6 and 7 enhanced the knowledge of the development biology in common carp. Acquirement of this knowledge is a crucial first step involved in the process of 'daughterless carp technology'. This is because it is essential to locate the specific candidate genes for genetic manipulation, especially before proceeding with other steps, such as the construction of a daughterless gene, generating transgenic carp and determining the level of fertility and heritability. Despite many positive advances, these research projects also highlighted key complexities. For instance, Project 5 showed that interbreeding between invasive common carp strains (*Cyprinus carpio*) and goldfish (*Carassius auratus*) occurred in the MDB. This consequently produced fertile offspring although they may have reduced levels of fertility. Introgressive hybridisation, resulting in the exchange of genetic material via backcrossing, is capable of providing novel genetic combinations (Anderson 1949 cited in Whitney and Gabler 2008). Therefore, introgression between carp and goldfish could reduce the efficacy of biological controls. For instance, goldfish could act as a reservoir for genetic diversity for highly invasive and destructive carp, which essentially undermines the efficacy of daughterless gene technology. Furthermore, if diseases to which goldfish are resistant are released to control carp, then individual alleles conveying immunity, carried by goldfish, can spread rapidly through the carp population and negate the effects of the disease. This may also inhibit other biological control options, such as the potential use of Koi herpevirus (KHV, Cyprinid herpevirus-3) as a biological control agent. Outbreaks of the disease have occurred in both farmed and wild populations of carp and areas associated with high mortality (70–100%) and a high degree of host specificity. However, at this stage, it appears that only common carp (includingKoi carp) are affected (Bretzinger et al 1999; Perelberg et al 2003; Haenen and Hedrick 2006) and juvenile carp are particularly sensitive to the virus (Perelberg et al 2003).

In addition, Project 6 showed that environmental factors could influence sex determination. For instance, through endocrine disruption studies, aromatase is known to be an environmentally sensitive gene. Therefore, further investigation into inter-annual variation of expression may help understand the impacts of environmental factors, such as temperature, on reproduction of carp and other fish. Finally, Project 7 suggested that the possible coexistence of undifferentiated and differentiated gonochorism may provide a powerful reproductive strategy in the common carp, contributing to their successful recruitment in the MDB.



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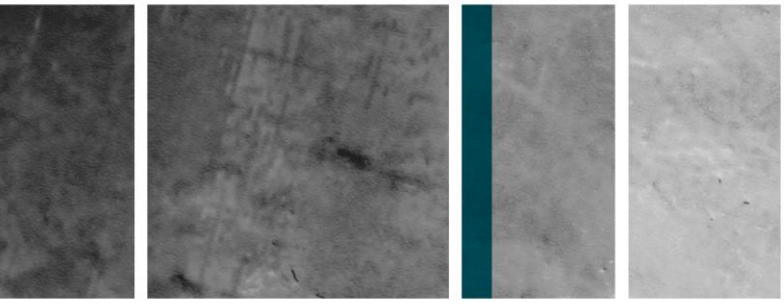

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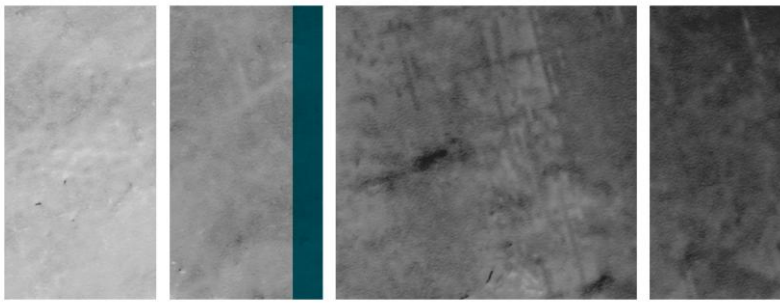

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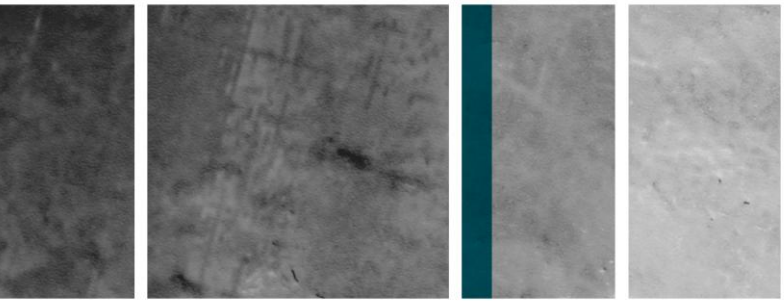

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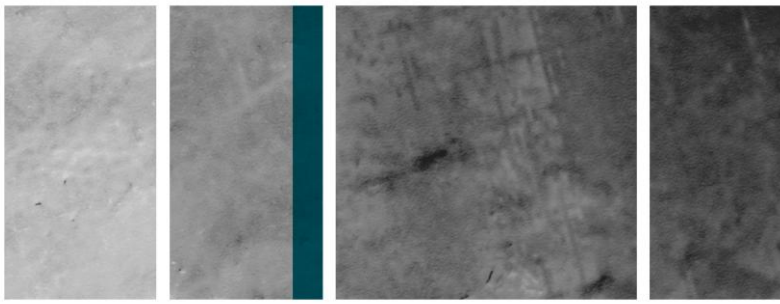

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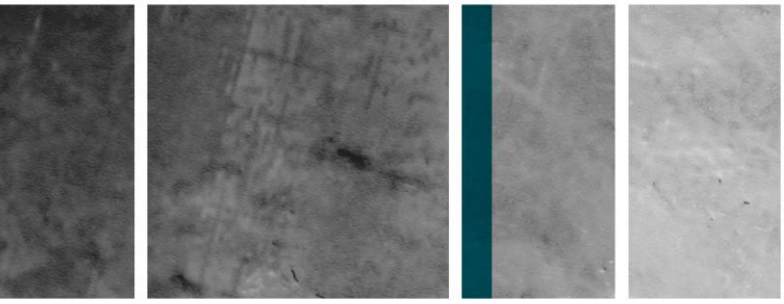

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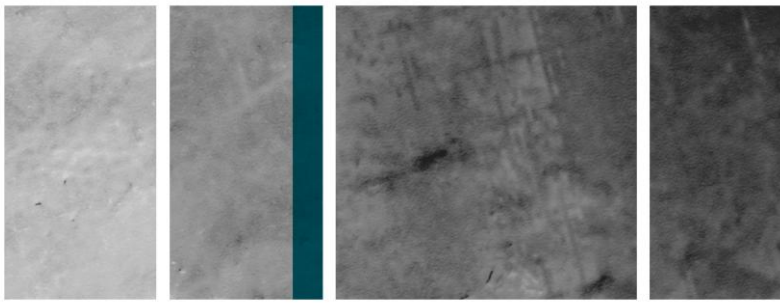

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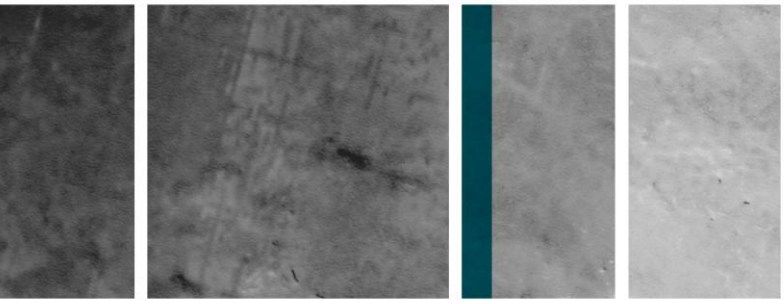

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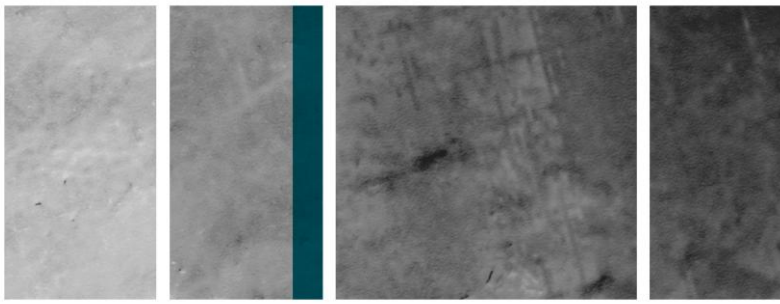

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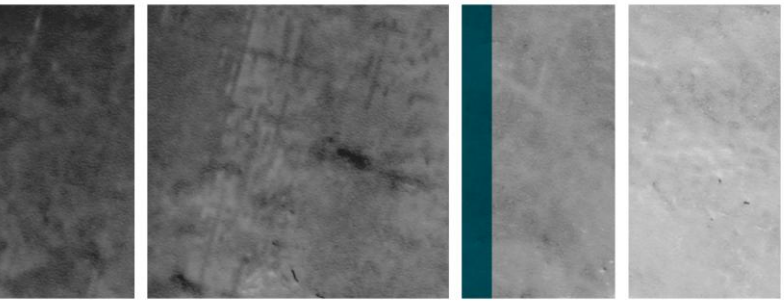

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