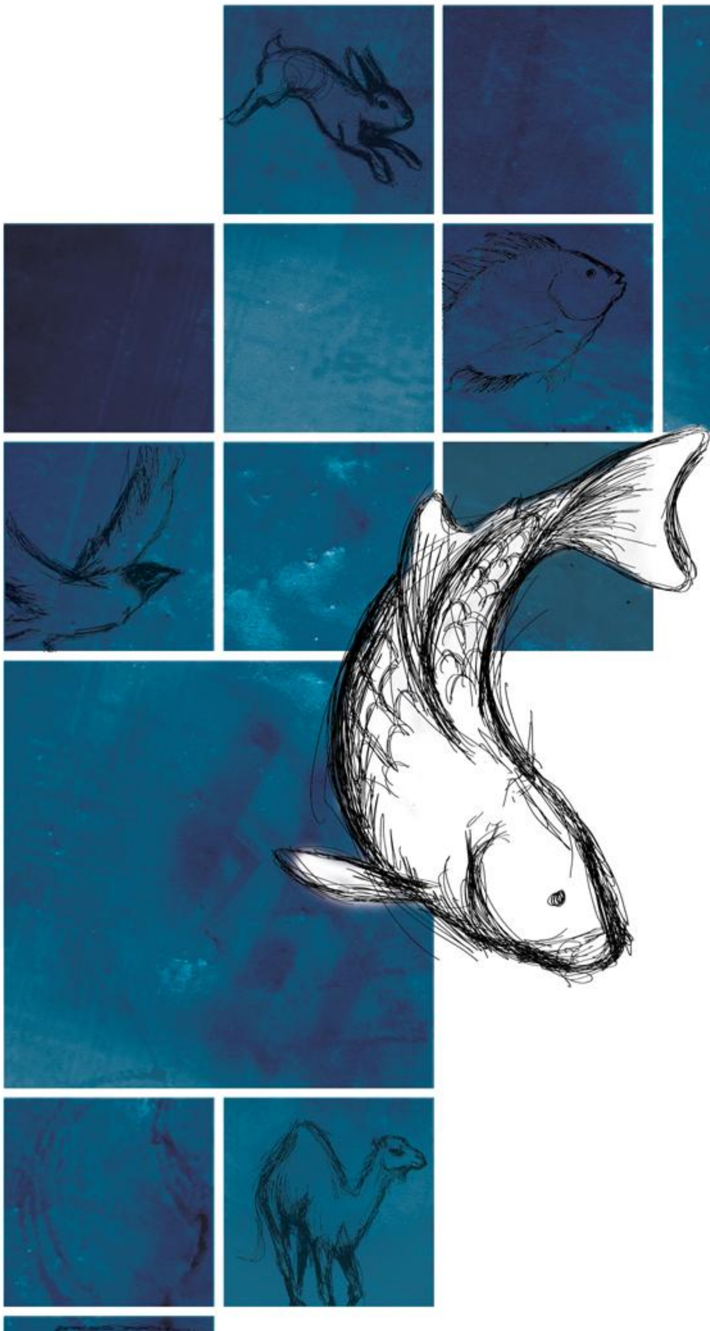

PESTSMART

Validating the age of carp from the northern Murray-Darling Basin

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Summary

Being able to accurately age carp is important for modelling population dynamics and potential response to various control strategies. This study examined the use of oxytetracycline (OTC) and otolith (ear bone) sampling to determine the formation of bone growth rings and in turn estimate the age of carp populations in the northern Murray–Darling Basin (MDB). OTC leaves a mark in bony tissue that can be used as a reference point to analyse the formation of subsequent growth rings, known as 'check marks'. The number of check marks that appear over a known time period can then be used to determine the age of the fish. In carp populations in the southern MDB, check marks are known to form annually. However, whether this occurs in the subtropical environment of the northern MDB had not been examined before this project. Validating this ageing method will enable population modelling to be applied to the whole MDB and assist in carp management.

Over 200 adult and 200 young of the year (YOY) carp were captured by electrofishing from lagoon and river sites in the Macintyre River catchment near Goondiwindi, Queensland. Adult fish were treated with OTC in late summer and autumn 2007 and tagged for individual identification with passive integrated transponder tags and dart tags. YOY fish were treated with OTC in July 2007. A sub-sample of both adult and YOY carp were held in tanks, while the remainder were released into two small lagoon habitats.

Captive adult fish were held for up to 15 months before recapturing for otolith analysis. Two or three YOY tank-held fish were sacrificed each month from August 2007 to April 2008. Tagged adult fish from the lagoons were collected by electrofishing between 356 and 605 days after release. The lagoons were sampled quarterly for YOY-sized fish, and then at monthly intervals over the spring, when completion of a check mark was expected to occur.

Otoliths were removed from all sacrificed carp. An OTC mark was not visible in the majority of YOY carp. However, it was possible to follow the formation of the first annual check in the tank-held YOY fish. Otolith sampling confirmed that the annular check is laid down through November into early December. Growth past the annular check was observed in early January. The majority of adult carp formed check marks after the OTC reference point consistent with annual formation. The exceptions were five carp (13.5%) that were observed to have two complete check marks form within a period when only one check mark would be expected. Therefore, caution should be applied when estimating age at maturity by plotting fish length against age and excluding outliers (whose length is shorter than expected for a given age compared to the majority of fish). This will improve the precision of such estimates. These outliers are likely to be those fish that lay down more than one check per annum.

Overall, the otolith growth check method was validated for estimating carp age in northern populations and will enable age estimates from northern MDB populations to be confidently used for carp population modelling over the entire MDB.

1. Introduction

The introduced pest fish carp, *Cyprinus carpio*, has invaded the majority of the Murray–Darling Basin (MDB) and various coastal catchments in south-eastern Australia and parts of south-western Australia (Koehn 2004, Morgan et al 2004). There are also recent reports of carp from the tropical Fitzroy system in Queensland (Greg Ringwood, Fisheries Queensland, personal communication). Carp are now the most abundant large-bodied fish in the MDB (Stuart and Jones 2006). Carp can increase water turbidity, damage aquatic plants, undermine river banks and increase water nutrient concentrations (Koehn et al 2000, Smith 2005). Such damage could threaten endangered species and alter ecosystems (Koehn et al 2000). It has been concluded that the large biomass of carp in many of Australia's lowland rivers must contribute to river degradation (Smith 2005).

Being able to accurately age carp is important for modelling carp population dynamics. Models such as 'CarpSim' (Brown and Walker 2004) can help predict effects of different management methods for carp. Valid age estimates are needed to calculate age at maturity, growth and mortality rates (Brown et al 2004). These parameters can vary between catchments due to different environmental conditions. Within the MDB, ageing validation has only been completed for carp populations in the southern catchments (Brown et al 2004, 2005, Vilizzi 1998, Vilizzi and Walker 1999). There is a need to examine the ageing of northern carp populations to see if, for example, growth is faster and age at maturity is earlier in the northern MDB. Validating the age-estimation method will enable population models to be applied to the whole of the MDB.

In the southern MDB catchments, carp populations are known to grow slowly during the winter months then faster in spring, resulting in formation of annual check marks in their otoliths (Brown et al 2004, 2005; Vilizzi 1998; Vilizzi and Walker 1999a,b). However, in the northern MDB populations, where the climate is more sub-tropical, it was not certain if checks also form annually. The aim of this project was to determine whether checks form annually in these carp populations and to determine the timing of check formation.

2. Methods

Marking of carp

Over 200 adult and 200 young of the year (YOY) carp were captured by electrofishing from lagoon and river sites in the Macintyre River catchment near Goondiwindi. Adult fish were treated with oxytetracycline (OTC) in late summer and autumn 2007. YOY fish were treated with OTC in July 2007. OTC leaves a mark in bony tissue that fluoresces under ultraviolet light. This mark is used as a reference point as it is formed at a known point in time. The number of check marks subsequently laid down after the OTC mark over a known time period can be used to determine the frequency of check formation.

Adult carp were injected intramuscularly with OTC at a dosage rate of 50 mg/kg body weight. For smaller adults, the entire dose was injected into the dorsal muscle, but for larger fish the dose was split between the dorsal muscle and the base of the pelvic fin. Softer tissue at the base of the pelvic fin would accommodate larger doses without any back-leakage. YOY carp were held in a buffered solution of 0.5 g/L OTC in a 1000-L tank for 24 hours. All OTC-treated adults were tagged with both dart and passive integrated transponder (PIT) tags for individual identification. The majority of OTC-treated fish were stocked into two small lagoons near Goondiwindi, in the northern MDB. Temperature loggers (Hobo® Pendant) were used to record temperatures at hourly intervals in the lagoon systems for the period that fish were at large. A sub-sample of 36 adult and 40 YOY carp were held in tanks at Deception Bay.

Maintenance of tank fish

Adult carp were maintained in two 5000 L tanks on a recirculating freshwater system, with mechanical and biological filtration including UV treatment of the water. YOY were held in a 1000 L tank and given a 5% water change daily after feeding and a 20% water change one day per week. Tanks were covered with netting to prevent fish jumping out. Feed holes were cut into the netting. Faecal waste and uneaten food were siphoned daily from the bottom of the tank. All tanks were maintained at ambient temperature. A temperature logger (Hobo® Pendant) was used to record the temperatures at hourly intervals in a representative tank system.

During summer, fish were fed daily on commercial pellet and crumble diets (Ridley’s Aquafeed for native fish) at a rate of about 2% body weight per day. The YOY diet was supplemented with HBH tropical® fish food flakes, and adult carp were occasionally fed cut frozen prawns. During the winter, carp were less active and were not fed at least three days out of every week. Carp appeared fully satiated at the normal feed rates and less food was eaten during the cooler months.

Sacrificing of tank-held fish and collection of lagoon fish

Captive adult fish were held for up to 15 months. Two or three YOY tank-held fish were sacrificed each month from August 2007 to April 2008 by sedation with Aqui–S, and quick killing with a priest tool. Most adult fish were held for the full 15-month period before being sacrificed the same way. Two adult fish were held for just 12 months, as they were captured from the wild later than the other tank-held fish. Otoliths were removed from all sacrificed fish (see below).

The lagoons were sampled quarterly for YOY-sized fish, and then at monthly intervals over the spring period, when completion of an increment was expected to occur. Boat electrofishing and fine mesh fyke nets were used to catch carp. A seine net was also hauled through snag-free areas of one of the lagoons. Unfortunately, carp in the appropriate size class were difficult to catch, so only tank-held fish were used to estimate the timing of completion of the first check mark. It is possible that predation by pelicans, cormorants darters and other water birds may have impacted on numbers of YOY carp released into the lagoons. Large Murray cod were also found to be present in one of the lagoons.

Tagged adult fish from the lagoons were collected by electrofishing between 356 and 605 days after release. These fish were sacrificed by sedation with Aqui–S and quick killing with a priest tool. Recreational anglers also captured some tagged carp. Due to local publicity about the research project, anglers contacted the Department of Primary Industries and Fisheries (now the Department of Employment, Economic Development and Industry, DEEDI) to report tagged fish captures and stored the tagged carp in a freezer until collected by DEEDI staff.

Removal and preparation of otoliths

Fish were measured, weighed and sexed where possible. These details and the date of sacrifice, sample location (tank or lagoon) and any tag numbers were recorded. Asteriscii otoliths were removed from the carp using a modified version of the 'up through the gills method' (Secor et al 1991). The dorsal occipital process and lateral processes were cut through using bone cutters and the prootic bullae was levered dorsally until it broke, exposing both asteriscii otoliths. Otoliths were removed, washed and then dried for up to three weeks. Otoliths were then placed in silicon moulds and embedded in Polyplex 61–209 casting resin. Once the resin was dry, the blocked otoliths were sectioned on a Buehler Isomet ™ low-speed saw to a thickness of 400 μm. Sections were mounted on microscope slides using polyester resin and covered with a cover slip.

A subsample of YOY otoliths were sent to Fish Ageing Services Pty Ltd for sectioning and mounting. These otoliths were prepared in a three-stage process to achieve transverse sections approximately 30–60 μm thick. Using thermoplastic glue (Crystalbond), the otolith was arranged with the posterior end facing away from the slide edge. Under reflected light, the primordium was positioned carefully in line with the slide edge. Using 400 grit wet/dry paper, the posterior side was ground down to the edge of the glass slide. A finer grade of wet/dry paper (1200 grit) was used to remove deep scratches. The exposed edge was further polished with 5 μm lapping film.

The slide was reheated to loosen the glue adhesion and the remaining otolith half was removed. The ground face was attached to a second heated slide using Crystalbond and left to cure. The anterior side of the otolith was ground until growth increments were visible from the primordium to the edge of either the dorsal or ventral side. During this stage, the preparation was continually checked to prevent over-grinding. The preparation was polished using progressively finer grade lapping film (15 μm to 3 μm). To improve increment clarity and definition and reduce surface refraction, immersion oil was used to cover the preparation before reading.

Otoliths and slides were kept out of direct light to ensure the OTC marks did not suffer any degradation.

Viewing of otoliths

Slide-mounted otolith sections were viewed on a Nikon Microphot FXA TM ultraviolet microscope to inspect for presence or absence of an OTC mark. A Fuji digital camera mounted on the microscope was used to take digital images. Images were examined for numbers of complete annuli past the ultraviolet mark by two DEEDI staff. The readers were consistent regarding the number of complete annuli past the UV mark.

Images and 38 otolith sections were also sent to Fish Ageing Services for ageing as per Brown et al (2003). Readability was rated between 1 and 5. A score of 1 indicated the otoliths were read with a high degree of confidence, whereas a score of 5 indicated that an otolith could not be aged. All otoliths were aged and checked for a fluorescent OTC mark. The number of complete annuli past the UV mark was recorded. Twenty of the otoliths were re-read for a comparison of age determination. Average percent error was calculated at 4.97%.

YOY otoliths not sectioned by Fish Ageing Services were viewed whole under reflected light to determine the timing of the first check formation. Sectioned YOY otoliths were viewed by Fish Ageing Services for reading of daily increments. If daily increments could be read, then this would provide a more precise indication of the timing of the formation of the first check mark.

3. Results

Water temperatures

Annual variation in the water temperature between winter and summer was slightly greater in the lagoon environment compared to the tanks at Southern Fisheries Centre, but winter minima were similar. One lagoon data logger went missing — possibly stolen. The second data logger was recovered. The minimum recorded winter temperature in the lagoon environment was 14.8 ºC and the maximum summer temperature was 31.5 ºC. Falling water levels may have stranded the lagoon data logger above water level in early summer. This led to some highly variable daily readings that were not considered to be water temperatures. The minimum recorded winter tank temperature was 15.3 ºC and the maximum recorded summer tank temperature was 29.0 ºC.

YOY otoliths

In the majority of the YOY carp, OTC marks did not form in their otoliths. Only three captive YOY fish had confirmed OTC marks. Recaptures of potential YOY fish in the wild were low, and the lack of an OTC mark made it impossible to confirm if they were YOY fish or not. The tank-held fish were able to be used to follow through formation of the first annular check as demonstrated in Figures 1 to 6.

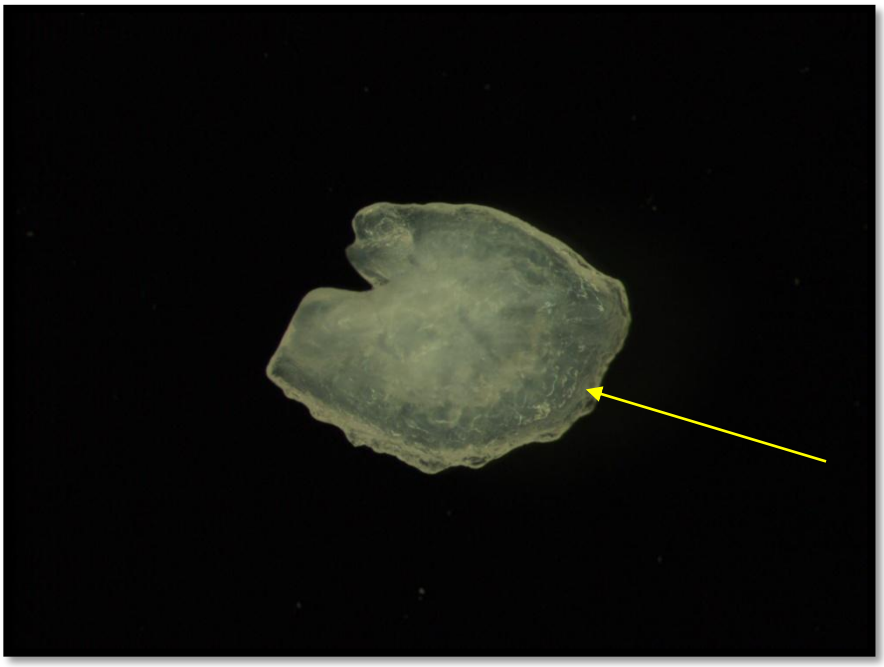

Figure 1. Whole otolith from YOY fish sacrificed 25/10/2007 — approximately 9 months old. No complete annular check is visible. An annular check can be seen starting to be laid down (arrowed) on the edge.

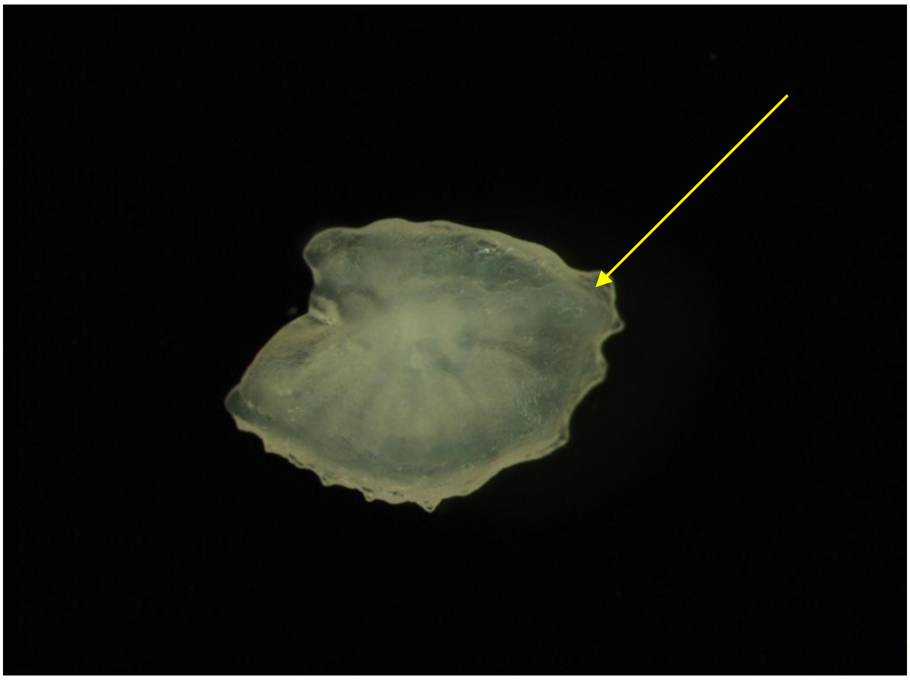

Figure 2. Whole otolith from YOY fish sacrificed 03/12/2007 — approximately 11 months old. Note the formation of the first annular check extending along the edge of the otolith.

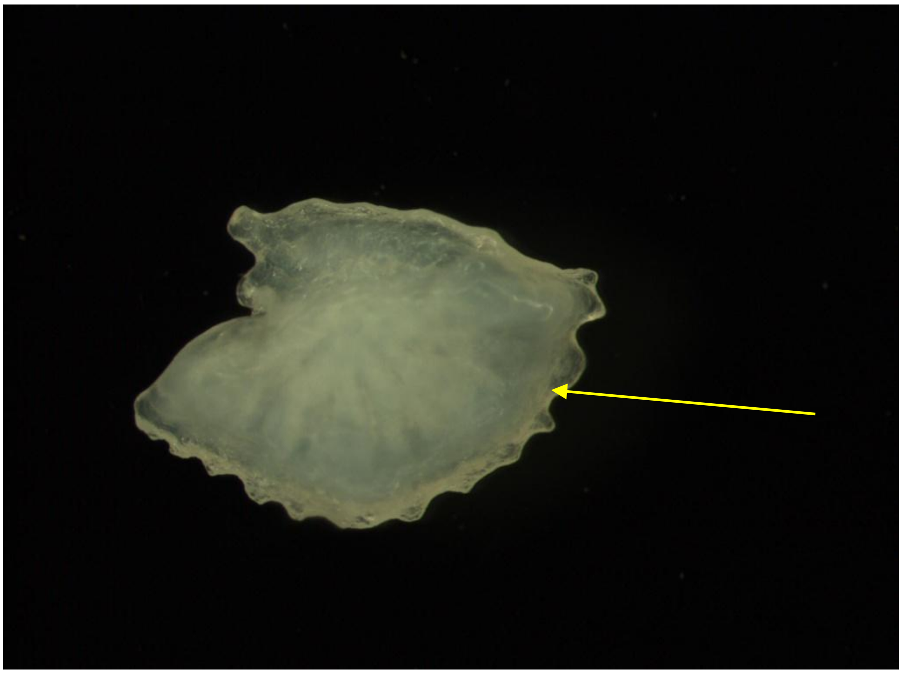

Figure 3. Whole otolith from YOY fish sacrificed 04/01/2008 — approximately 1 year old. The annular check has formed (arrowed) and there is growth past the annular check.

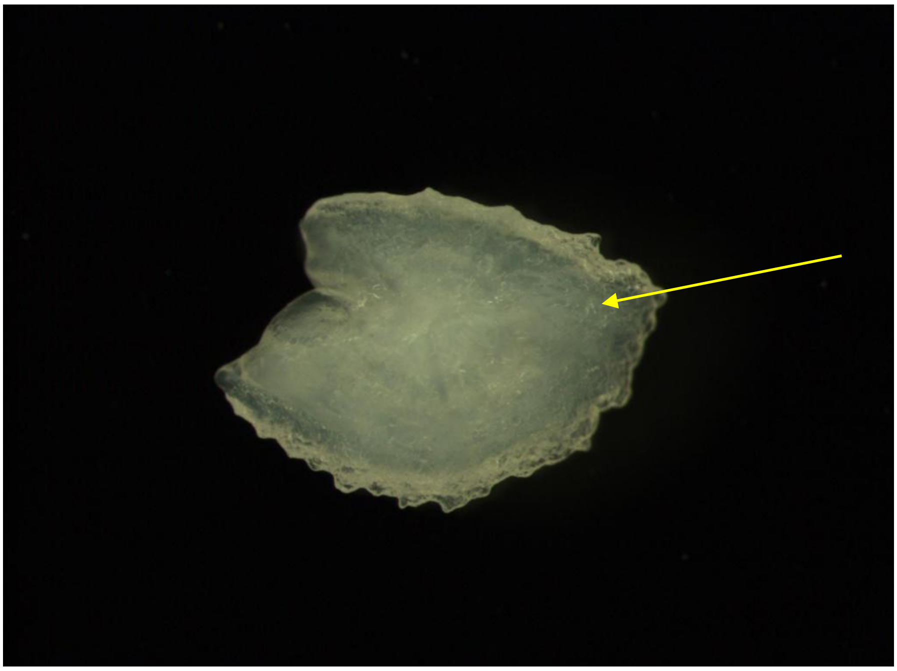

Figure 4. Whole otolith from YOY fish sacrificed 08/02/2008 — approximately 13 months old. The annular band can be seen (arrowed), but is not clearly defined.

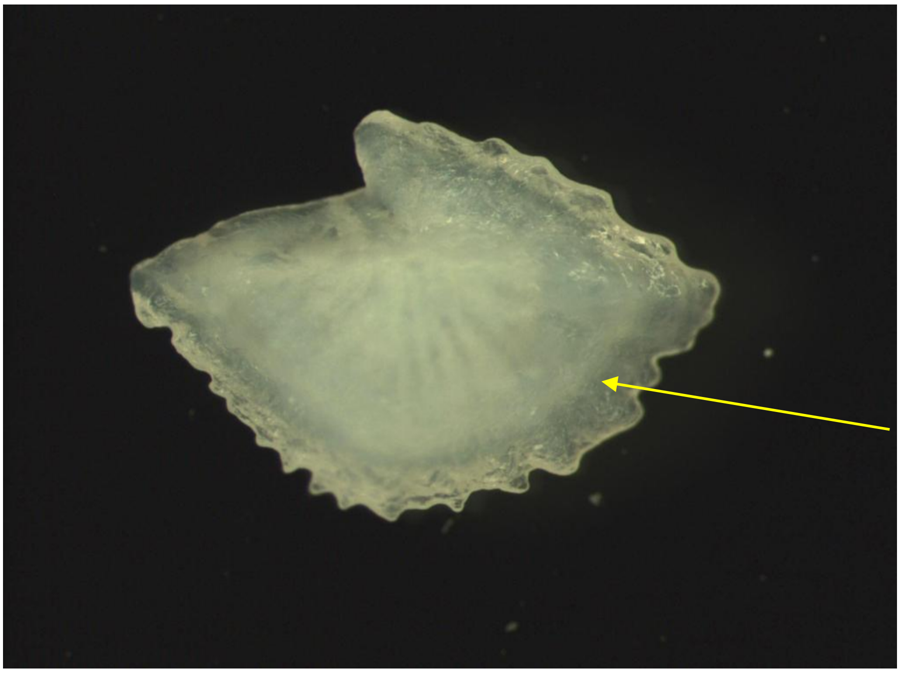

Figure 5. Whole otolith from fish sacrificed 06/03/2008 — approximately 14 months old. Note that the first annular check (arrowed) appears to be quite opaque. This may not appear as pronounced if the otoliths are sectioned.

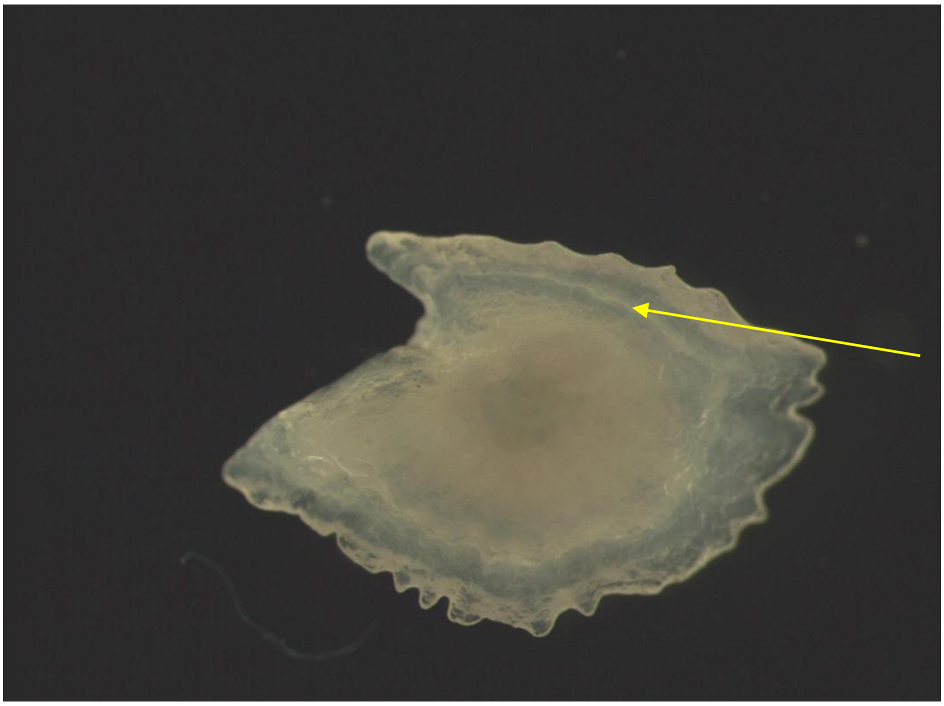

Figure 6. Whole otolith from YOY fish sacrificed 04/04/2008 — approximately 15 months old. The annular check is now very well defined. Inside the annular check (arrowed) the opaque primordium followed by a clear 'growth' section is visible.

From three YOY fish sacrificed in late October (one is illustrated in Figure 1) it was apparent that an annular check was beginning to form. As shown in Figure 2, it is clear that the annular check was laid down through November into early December. Growth past the annular check was observed in early January (Figure 3) and continued through the summer months into autumn (Figures 4, 5 and 6).

Fish Ageing Services were unable to provide confident daily increment readings from sectioned YOY otoliths. Annual checks were apparent in sectioned YOY otoliths and the results were generally consistent with those observed for whole otoliths. However, in sectioned otoliths the checks appeared to be further from the edge (Figure 7) than those in whole otoliths. This suggests that check formation could be slightly earlier than interpreted from whole otoliths. A check can be observed on the edge of a sectioned otolith from an older fish recaptured in early October (refer to section on adult fish below — Fish number Cc056).

Figure 7. Thin sectioned otolith from YOY carp sacrificed 3/12/2008. Arrows indicate the position of the first assumed annual opaque zone (check mark). This is consistent with whole otolith observations, but the check appears further from the edge in sectioned otoliths.

Adult and sub-adult otoliths

Table 1 shows the number of check marks observed to have formed in adult carp after the OTC mark, in comparison to the number of check marks expected. This information is presented together with sex, length data, days at liberty and estimated age of fish at the time of sacrifice. The majority of carp formed check marks after the OTC mark consistent with annual formation. That is, fish that were at large for only one winter and sacrificed in autumn formed one complete check, and fish at large for two winters and sacrificed in spring formed two check marks. However, five carp (13.5%) had two complete check marks form within a period when only one check mark was expected (Table 1). This biennial check formation was observed in three tank-held fish and two fish at liberty in a lagoon. No carp formed fewer check marks than expected. Figures 8 and 9 show OTC-marked otoliths from fish that formed one and two check marks respectively as would be expected from annular formation. Figures 10 and 11 show examples of otoliths with more checks formed than expected.

Table 1. Age estimates and characteristics of OTC-marked carp used in the ageing validation experiment. The number of checks observed after OTC marking is compared to the number that was expected.

ID#	Fork Length	Total Length	Sex	Age	Readability	Location	Days at large after OTC marking	Observed checks past OTC mark	Expected checks past OTC mark
Cc041	450	485	M	12	3	lagoon	355	1	1
Cc028	347	390	F	6	3	lagoon	360	1	1
Cc037	418	470	M	4	3	lagoon	360	1	1
Cc030	458	512	?	9	4	lagoon	360	2	1
Cc036	525	565	?	7	4	lagoon	360	1	1
Cc034	442	488	M	8	4	lagoon	361	1	1
Cc033	555	600	F	8	3	lagoon	361	1	1
Cc048	515	560	M	9	4	tank	365	1	1
Cc047	535	584	F	8	4	tank	365	1	1
Cc043	333	370	M	3	3	lagoon	433	1	1
Cc042	423	474	?	6	3	lagoon	434	1	1
Cc029	470	525	M	12	1	lagoon	437	1	1
Cc031	417	449	M	12	2	lagoon	438	1	1
Cc004	400	448	M	5	3	tank	461	1	1
Cc021	400	445	M	7	3	tank	461	1	1
Cc007	415	464	M	3	4	tank	461	2	1
Cc005	490	541	F	13	4	tank	461	1	1
Cc006	500	553	F	11	3	tank	461	1	1
Cc019	519	575	M	9	4	tank	461	1	1
Cc009	574	635	F	9	3	tank	461	2	1
Cc018	581	630	F	13	2	tank	461	1	1
Cc002*	390	432 est.	M	4	4	tank	462	1	1
Cc010	399	448	F	3	3	tank	462	1	1
Cc044	399	445	M	5	3	tank	462	2	1
Cc012	403	451	M	5	4	tank	462	1	1
Cc045	410	459	F	6	4	tank	462	1	1
Cc020	410	455	F	5	4	tank	462	2	1
Cc049	412	463	M	6	3	tank	462	1	1
Cc011	461	514	F	11	3	tank	462	1	1
Cc050	480	527	M	15	3	tank	462	1	1
Cc016	492	540	M	9	3	tank	462	1	1
Cc003*	517	572 est.	M	9	4	tank	462	1	1
Cc001*	517	572 est.	M	14	4	tank	462	1	1

ID#	Fork Length	Total Length	Sex	Age	Readability	Location	Days at large after OTC marking	Observed checks past OTC mark	Expected checks past OTC mark
Cc013	560	610	M	12	3	tank	462	1	1
Cc014	563	615	F	12	3	tank	462	1	1
Cc046	595	655	F	not aged	5	tank	462	not aged	1
Cc015	613	675	F	12	3	tank	462	1	1
Cc017	655	710	F	13	3	tank	462	1	1
Cc057	355	393	F	5	4	lagoon	528	1	1 (2)
Cc058	373	413	M	5	3	lagoon	528	1	1 (2)
Cc095	355	400	?	4	3	lagoon	564	1	1 (2)
Cc096	375	420	M	5	3	lagoon	564	2	1 (2)
Cc056	434	473	M	6	2	lagoon	605	**2** (e)	1 (2)

Notes:
*Carp Cc001, Cc002 and Cc003 had damaged tails. Total length is estimated based on the mean ratio of fork length to total length.
(e) Indicates a check mark formed near the edge of the otolith.
(2) Indicates that it is possible that a second annual check mark after the OTC mark might have been completed, but the time of sacrifice was during spring when a second check mark may not yet be completely formed, therefore either one or two checks post the OTC mark could be expected.

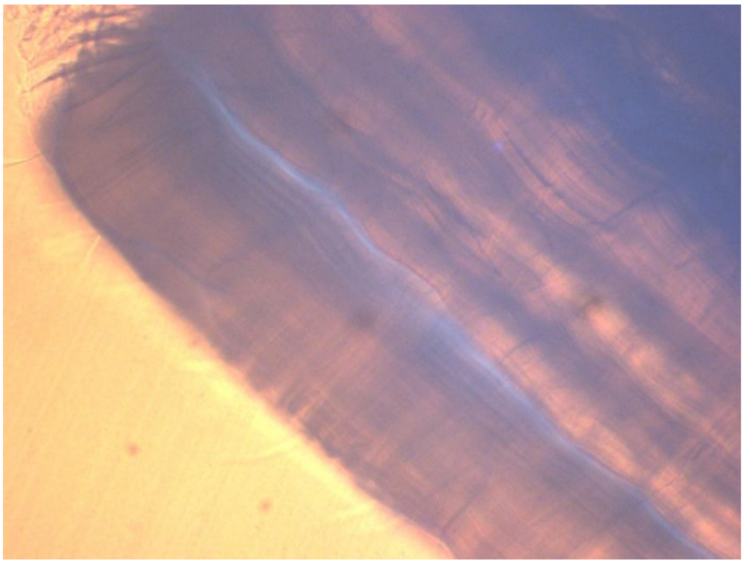

Figure 8. 100x magnification of a sectioned adult carp otolith (Cc031) with epi-fluorescence. Note one complete band after the OTC mark, and the wide growth band after the check mark. Photograph courtesy of Fish Ageing Services. This fish was OTC marked in February 2007 and recaptured in April 2008.

Figure 9. Image of a sectioned adult carp otolith (Cc056) viewed under transmitted light. Note check mark just after fluorescent mark (green rectangle inset) followed by a wide growth band and a second check mark forming near the edge. This fish was at large for 604 days (OTC marked in April 2007 and recaptured in October 2008).

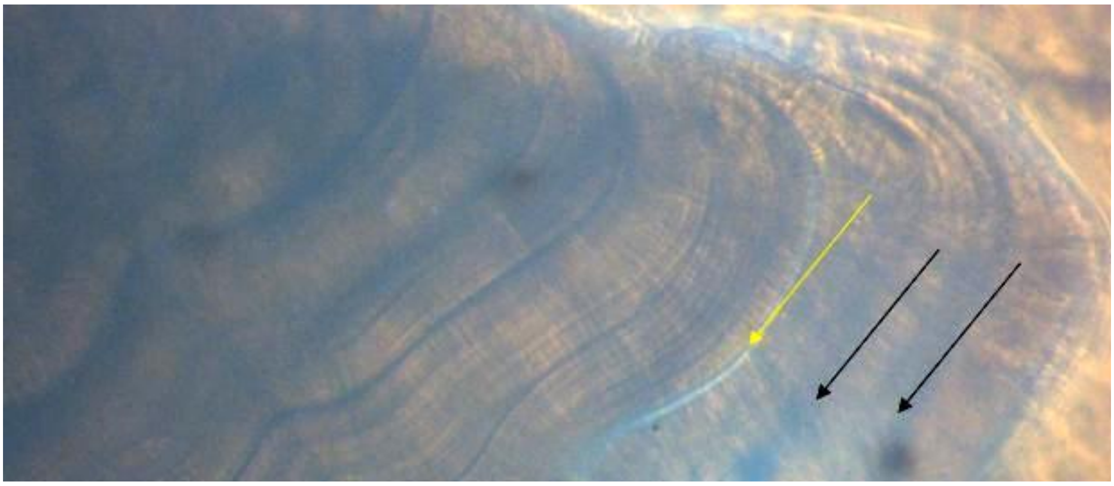

Figure 10. Image of a sectioned adult carp otolith (Cc009). Magnification 100x with epi-fluorescence. Note two check marks (black arrows) after the OTC mark (yellow arrow) even though this fish had been at large for only 461 days.

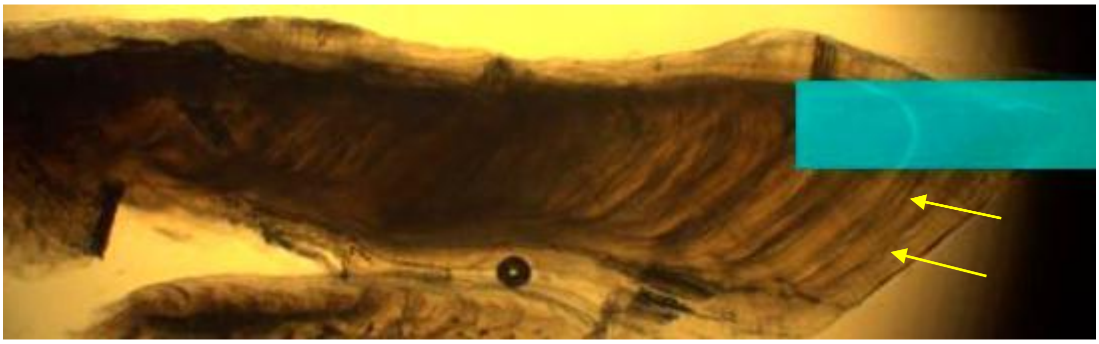

Figure 11. Image of a sectioned adult carp otolith (Cc044) viewed under transmitted light, showing further evidence of two check marks forming after the OTC mark (green rectangle inset) in a fish at large for less than two years (462 days).

4. Discussion

The age validation results suggest that age estimation as used in southern MDB carp populations is likely to also be correct for carp in sub-tropical Queensland. However, some exceptions to this were observed and these are further discussed below.

YOY carp used in this study were probably spawned in spring or early summer. These fish were OTC marked in July (16/07/2007) and then sacrificed over the next 10 months to identify the time at which the first check mark was formed. It was expected that the otolith would include an opaque primordium, followed by a translucent initial summer growth zone as the juvenile carp grew through the ‘fingerling’ stage. This would be expected to be followed by an opaque slow winter growth check before a translucent (under reflected light) area as the fish grew in the warmer months. Figure 6 shows this ‘classic’ opaque primordium, clear zone and opaque annular check. It appears that timing of annular check formation is very close to, but slightly earlier than, that reported by Brown et al (2004) for carp in Victoria.

One complete zone formed in most tank-held fish kept for more than 12 months. The majority of tank fish were sacrificed in June. Some showed signs of beginnings of a new check forming on the edge (which might be expected during slowed winter growth), but formation was incomplete. Some carp, including two wild fish and three tank-held fish had evidence of biennial check formation with growth past the second check. Hutchison et al (2005) also found evidence for biennial check formation in 2.5% of long-finned eels in south eastern Queensland. It was thought that a second check could have formed in some eels during stressful summer conditions (eg high temperatures and low water levels). Biennial checks have been observed in carp in a large impoundment in South Africa (Winker et al 2010). One check is formed during the winter slow growth period, and the second forms in summer. The second check formation in South African carp has been attributed to spawning impacting on somatic growth (Winker et al 2010). Therefore, biennial checks are only likely to form in mature fish, but not in immature fish, complicating age estimation.

It is uncertain why some carp in the current study formed more than one check per annum. Possible causes include direction of significant resources away from somatic growth towards gonad development, or stress factors such as increased parasite load and high water temperatures during summer months impacting on somatic growth in some individuals. The direction of resources away from somatic growth and towards gonad development has some merit, as none of the otoliths from YOY carp held in tanks showed any evidence of biennial check formation during the period they were held.

The results of this current study suggest that in the majority of cases age estimation is likely to be correct for carp in sub-tropical Queensland, but in up to 12% of carp ages could be overestimated due to biennial check formation. Plotting age against length (assuming all fish originated from the same habitat) could be used to detect outliers and to eliminate those fish that may be laying down more than one check per year. It is not known if the number of checks laid down by an individual carp can vary from year to year. Further research would be needed to determine this.

5. Conclusion

The majority of carp in the northern MDB lay down a single otolith check each year, but a minority of fish lay down two otolith checks each year. Therefore, some caution is needed when estimating carp age at maturity. Plotting length against age and excluding outliers (where length is shorter than expected for a given age compared to the majority of fish) will improve the precision of such estimates, as outliers are likely to be those carp that lay down more than one check per annum.

6. Acknowledgements

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