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Scale stocking checks to differentiate between hatchery-reared and wild mulloway *Argyrosomus japonicus*

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Abstract Scales from hatchery-reared, stocked and wild mulloway *Argyrosomus japonicus* (Temminck and Schlegel) captured in the Georges River, and a library of mulloway scales from coastal New South Wales, were examined for the presence of scale checks. Checks specific for hatchery-reared fish were present in 100% of recaptured hatchery-reared mulloway; the origin of which was confirmed by the presence of a chemical mark in the otolith or fin spine. Up to 7% of wild mulloway were incorrectly classified as hatchery-reared on the basis of these checks. An abrupt reduction in salinity from 35 to 5 and 6 days starvation successfully induced checks in the scales of hatchery-reared mulloway. The marking efficiency for stocking checks was comparable to that obtained using oxytetracycline hydrochloride, and supports the short-term use of scale stocking checks to evaluate mulloway stock enhancement programmes in Australia.

KEYWORDS: mulloway, re-stocking, scale increment, stock enhancement, stocking checks.

Introduction

Current fish culture techniques allow the rearing of large numbers of fish for release to supplement or enhance existing wild fish stocks (Munro & Bell 1997; Taylor, Palmer, Fielder & Suthers 2005b). Responsible enhancement projects include robust monitoring programmes and adaptive management to facilitate ongoing improvements in success, but these rely on suitable methods to identify hatchery-reared fingerlings on recapture (Blankenship & Leber 1995). Whilst coded wire tagging and genetic marking represent the ideal marking techniques for stock enhancement projects (Bartley, Kent & Drawbridge 1995; Brennan, Leber, Blankenship, Ransier & DeBruler 2005), both methods are expensive to set up through either large capital investment in tagging apparatus or characterisation of selectively neutral polymorphic loci. At the pilot stage, enhancement studies may be financially constrained, so other methods of marking are required to evaluate initial enhancement efforts. Such methods include otolith marking with chemical stains, and circuli patterns on scales (Barlow & Gregg 1991).

Mulloway *Argyrosomus japonicus* (Sciaenidae; Temminck and Schlegel) is an apex predator common across the Pacific and Indian Oceans, including South Africa (Griffiths 1996), China and the southern coast of Australia (Taylor, Laffan, Fielder & Suthers 2006b). The species is fast growing and heavily targeted by both commercial and recreational fishers in Australia. Consequently, mulloway is subject to declining catches in most southern Australian states (Silberschneider & Gray 2005; Taylor *et al.* 2005b), and is now the subject of pilot stock enhancement research along the mid-northern coast of New South Wales, Australia.

Scale increments may be deposited on a daily basis (Kingsford & Atkinson 1994), but ageing of sub-yearling fish using scales may be confounded by incongruent rates of circuli deposition. Scale formation does not usually commence at hatch in sciaenids (Bridges 1971), and the lack of a regular relationship between numbers of sub-yearly increments and time has prevented use of scale circuli for back calculation of size-at-age for age-0+ a fish (Szedlmayer & Able 1992). Circuli deposition on ctenoid teleost scales is thought to

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be affected by abiotic conditions, ontogenetic stage, food supply and growth rate (Jobling 2002). Consequently, the regularity of the hatchery environment has produced uniformity in circuli increment widths, which can distinguish hatchery reared fish post-release from wild fish using discriminant functions (Butcher, Mayer, Willet, Johnston & Smallwood 2003). The hatchery environment has also produced notable features within the scale as a product of hatchery events such as a change in rearing environment, temperature or salinity, or feeding regime (Humphreys, Park, Reichle & Mattson 1990).

The objectives of this study were to evaluate the effectiveness of scale stocking checks to identify hatchery-reared mulloway in the Georges River, Sydney, Australia and test whether it is possible to experimentally manipulate rearing conditions to produce discernable checks in the scales of mulloway. The Georges River is an urbanised estuary in the south-western metropolitan area of Sydney, New South Wales, Australia. The estuary receives pollution from residential and industrial sources (Birch 1996), and has been a recreational fishing haven since 2001. Pilot mulloway stocking research commenced in the Georges River in 2003 to address a potential recruitment limitation (Taylor *et al.* 2006b).

Methods

Rearing, transport and stocking of mulloway

Mulloway were spawned at Port Stephens Fisheries Centre, New South Wales in 2003 and 2007. Fish were reared using extensive hatchery techniques (Fielder & Bardsley 1999) at a salinity of 35 and pH ~8. In 2003, approximately 20 000 mulloway [79.8 ± 4.8 mm TL (mean ± SD)] were chemically marked in oxytetracycline (OTC) (Taylor, Fielder & Suthers 2005a). Fish were starved for 24 h prior to transport by road to the Georges River, New South Wales (33 °58′ S, 151 °00′ E), and released at two sites with salinities of ~25. Thirty five fish were held in 1 m^3 floating cages at the river and fed maintenance rations for 7 days post release. After 7 days the fish were euthanized and their scales used to estimate the relative radius from the scale focus within which to search for any stocking associated checks. In 2007, fish were reared under conditions identical to those above, and transported by road to the Sydney Institute of Marine Science Aquarium Facility. Mulloway were held in 1000-L tanks (salinity of 35, pH ~8) on a flow-through aquarium system prior to experimental manipulation.

Field trials

The stocking sites within the Georges River were sampled intermittently for 1.5 year post release. Mulloway were captured using an otter trawl (6 m mouth, 6 mm cod-end mesh-size; towed at ~1 m s^{-1}), monofilament gill nets (100 m long, 7 cm mesh size), or through the recreational fishery. Fish were freshly frozen upon capture, and later thawed, and otoliths and five scales removed from the distal side of the body where the tip of the pectoral fin touched the torso. Catch-and-release recreational fishers removed scales from the same region of the body, and detached and retained the first anal fin spine for detection of the chemical mark. Otoliths and anal fin spines were embedded in epoxy resin, sectioned and chemical marks visualized using an Olympus BH-2 microscope with RFC fluorescence attachment as described previously (Taylor *et al.* 2005a).

Experimental manipulation to induce stocking checks

Factors contributing to the deposition of scale checks in mulloway were tested in an enclosed system using the following factorial (n = 3): Salinity, ambient (35) or reduced (5); and feeding regime, fed, or starved for 6 days (Table 1). Twelve, 40-L aerated rectangular tanks were prepared on a flow-through aquarium system at the Sydney Institute of Marine Science Aquarium Facility. Fifteen mulloway [60.9 ± 9.4 mm TL (mean ± SD)] were added to each tank, and left to acclimate to new conditions for 24 h. Salinity was lowered from 35 to 5 in the reduced salinity treatment over 3 h, and water to the ambient salinity treatment turned off. Water was exchanged once per day for 3 days in all tanks, and then tanks were returned to flow-through and salinity allowed to return to ambient levels for the remainder of the growout. Feed was withheld simultaneously from the starved treatment as described in Table 1.

Image and data analysis

Scales from hatchery-reared and recaptured mulloway, wild mulloway, and a library of historical scales from coastal New South Wales were compared for the presence of stocking checks using image analysis. Five scales from each fish were cleaned and mounted flat between two glass slides. Digital images of the three best scales for each fish (scales that had dissolved foci, or any form of damage were excluded) were captured using a SPOT Flex digital camera mounted on an

Table 1. Summary of experimental manipulations performed to induce checks in scales of mulloway *Argyrosomus japonicus*

Treatment tank	Day 1	Day 3	Day 6	Day 14	Mean (SE) % of fish with check
Ambient salinity, starved	Feed withheld		Normal feeding regime resumed	Experiment concluded	83 (9)
Ambient salinity, fed				Experiment concluded	19 (11)
Reduced salinity, starved	Salinity reduced, feed withheld	Salinity returned to ambient	Normal feeding regime resumed	Experiment concluded	95 (4)
Reduced salinity, fed	Salinity reduced	Salinity returned to ambient		Experiment concluded	83 (9)

Olympus SZ-CTV dissecting microscope, between 1.1× and 6.3× magnification. Incremental distances, radii, and distance and number of increments to the start and finish of each check were measured along the longest axis using Image Pro v. 5.1 (Media Cybernetics). Checks were subjectively identified by not more than five closely spaced circuli, and identification was supported by the presence of dead-end or deformed circuli within the check (see Humphreys *et al*. 1990).

Arcsin transformed percentage presence of stocking checks, and untransformed stocking check duration data (total number of increments within check) were evaluated for the experimental manipulation using a single factor ANOVA to compare the four treatments (Table 1). Regression analysis was performed on biometric and scale radius data to evaluate relationships between scale growth and age and length of mulloway. All data analysis was performed using SPSS (SPSS, Chicago, IL, USA).

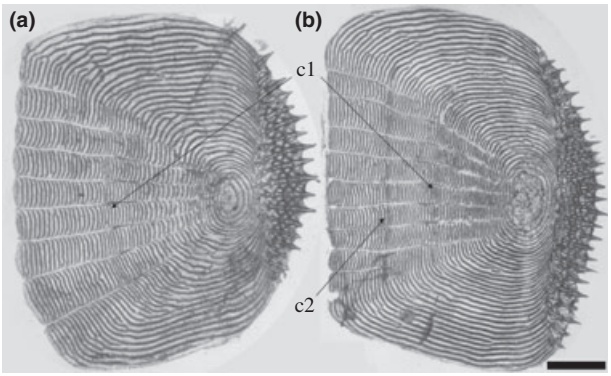

Figure 1. Mulloway *Argyrosomus japonicus* scale checks in a 135 mm TL wild (a) and 133 mm TL hatchery-reared mulloway recaptured 60 days post stocking (b) (c1 denotes check 1; c2 denotes check 2). Scale bar represents 250 μm.

Results

Natural and stocking related scale checks in mulloway

Two checks were identified in the scales of 35 hatchery-reared fish ongrown at the release site (Fig. 1). The first check occurred at a distance of 667 ± 149 μm (Check 1; mean ± SD), and the second check occurred at 1014 ± 191 μm (Check 2; mean ± SD. from the scale focus. Consequently, a maximum radius of 1200 μm from the scale focus was used to search for checks in wild and recaptured mulloway. Checks mostly comprised two increments, but included up to five increments in some fish. Of the 93 fish captured from the Georges River during the sampling period, 81 were of hatchery origin (stocked), as confirmed by the presence of an OTC mark in the otolith. Ninety-four percent of stocked fish had Check 1 present within the search radius, and all stocked fish had Check 2 present, which was determined to be characteristic of hatchery origin (Table 2). Eighty seven and 83% of fish from the Georges River and coastal scale library respectively had Check 1 present, while 7 and 5% respectively had Check 2 present within the search radius (Table 2). The presence of a second check within the 1200 μm search radius will correctly classify all fish of hatchery origin, but will, therefore, misclassify up to 7% of wild fish as of hatchery origin.

The total number of circuli increments for same age hatchery reared fish formed a significant positive linear relationship with fish TL ($r = 0.768$, $P < 0.01$; Fig. 2a). The number of scale increments formed a logarithmic relationship ($r = 0.823$; $P < 0.01$) with fish age (Fig. 2b), indicating the absence of a one increment-per-day relationship.

Induction of scale checks in mulloway by experimental manipulation

Manipulation of salinity and feeding regime produced discernable checks in the scales of hatchery-reared mulloway, and the presence of the checks was significantly different between treatments ($ANOVA_{3,47}$

Table 2. Presence of stocking checks in 93 recaptured fish from the Georges River during 2003 and 2004, and a historical library of wild mulloway scales from coastal New South Wales

Cohort	Chemical mark (%)	Size range stocked (Mean ± SD mm)	Size range analysed (mm)	Proportion (%) of catch	Percentage with check 1	Check 2 (% with stocking check)	Agreement between origin† and check 2 (%)
2003	100	79.8 ± 4.8	104–470	87	94	100	100
Wild	–	–	133–490	13	87	7	93
Library	–	–	93–476	–	83	5	95

†Origin refers to whether the fish originated from the hatchery as identified by the chemical mark, or whether the fish were wild as identified by the absence of the chemical mark.

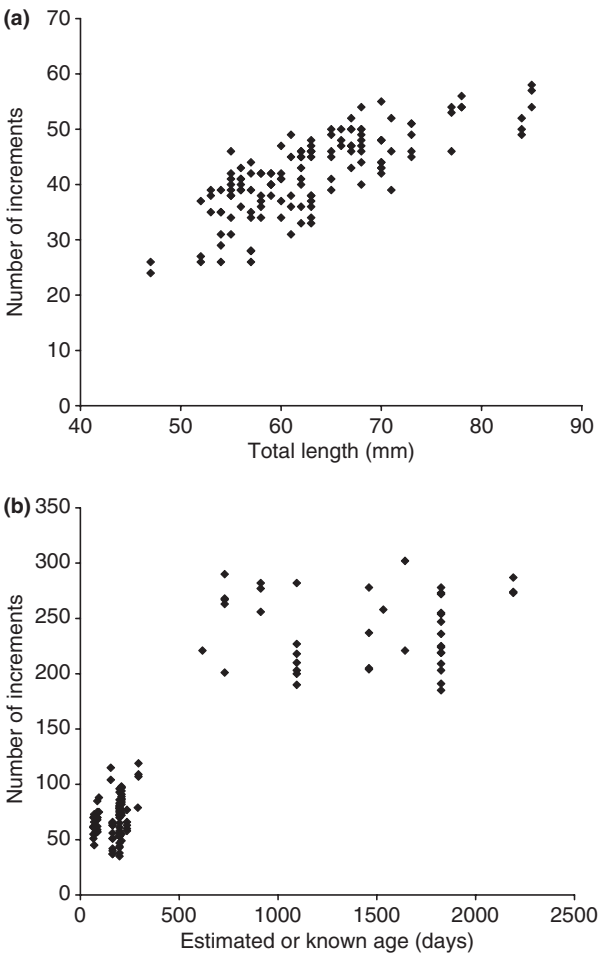

Figure 2. Relationship between the number of increments and total length of same-age fish (a), and number of increments and age (b), for mulloway *Argyrosomus japonicus*.

$P < 0.01$). The number of scales with checks (Table 1) was significantly less in the non-starved, ambient salinity treatment, than the starved, ambient salinity treatment (Bonferroni, $P < 0.01$), the starved, reduced salinity treatment (Bonferroni, $P < 0.01$), and the fed, reduced salinity treatment (Bonferroni, $P < 0.01$). There was no significant difference between the duration of checks for the four treatments ($ANOVA_{3,47}$ $P = 0.25$).

Discussion

The presence of a stocking related check (Check 2) in the scales of *Argyrosomus japonicus* allowed classification of recaptured, hatchery-reared mulloway in 100% of cases tested. The method is best applied in combination with other marks, particularly for initial characterisation of the stocking check; but in the absence of alternative marks for verification the presence of a stocking check should still provide a reliable indication of origin in stocked fisheries. There is potential for misclassification using this method as a second check occurred within the 1200 μm search radius in up to 7% of wild fish. The presence of an earlier check in the scales of both wild and hatchery-reared mulloway (Check 1) may relate to an ontogenetic transition between habitats or target prey, as observed in *Salmo salar* L. for the transition between stream and marine environments (Heidarsson, Antonsson & Snorrason 2006).

Previously, marking methods for evaluation of mulloway enhancement relied solely on the use of OTC, but OTC marks in the otoliths and anal fin spines of mulloway stocked in 2003 had deteriorated after 3 years post-release (M. D. Taylor, unpublished data). Identification of a stocking check on recaptured mulloway may allow recaptured fish with questionable OTC marks to be correctly classified by origin, but the clarity of the stocking check with increasing time after release should be evaluated. The simple presence/absence method presented here has some advantage over classic discriminant function analysis (e.g. Silva & Bumguardner 1998), as mathematical formulae and measurement of incremental distances are not required.

Sub-annual scale circuli were not deposited daily in *Argyrosomus japonicus* (Fig. 2b), which is consistent with studies on other species (Szedlmayer & Able 1992). Circuli number and spacing in teleost scales are principally related to body growth (Kingsford & Atkinson 1994; Borkholder & Edwards 2001), and the positive relationship of increment number with size for same age mulloway (Fig. 2a) support the suggestion that circuli in mulloway scales are laid down in response to growth rate, rather than age. Despite the lack of a 1:1 relationship between increment deposition and daily age, reliable back-calculation to size-at-release may be possible with mulloway scales where a clear and permanent check is present, following the methods developed for salmonids (Henderson & Cass 1991; Heidarsson *et al*. 2006) and bluegill × sunfish hybrids (Klumb, Bozek & Frie 2001).

A reduction in salinity for 3 days or starvation for 6 days were sufficient to produce a discernable mark in the scales of hatchery-reared mulloway, and these regimes can be applied exclusively or in combination to induce scale checks prior to release. Repeated applications of this method may allow deposition of multiple marks in mulloway scales for discrimination of different cohorts, as seen in *Oncorhynchus keta* (Walbaum) otoliths (Volk, Schroder & Fresh 1990). The results from the scale marking experiment indicate the scale checks observed in stocked fish are likely because of a period of adaptation to the wild environment, 24 h starvation of hatchery-reared mulloway prior to transport to the release site, a drop in salinity between hatchery and wild conditions, or a combination of these factors. The period of adaptation to conditions in the wild can take several weeks (Kristiansen & Svåsand 1992), but gut content analysis shows released mulloway begin consuming wild prey within 1 day post release (Taylor *et al*. 2006a). The relatively rapid behavioural adaptation in mulloway is evident in the brief 1–2 increment duration of stocking checks observed here.

Evaluation of stock enhancement programmes is underpinned by the ability to identify stocked fish upon recapture. The process of stocking mulloway will produce discernable scale checks at the time of stocking, and the deposition of such checks can be induced and controlled by manipulation of salinity and feeding regime. These marks allow for the reliable classification of recaptured hatchery-reared mulloway, but their use in other enhancement programmes should be evaluated in terms of the classification accuracy required, the objectives of the enhancement programme and the longevity of the mark.

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