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Use of circuli spacing on scales to discriminate hatchery and wild barramundi, *Lates calcarifer* (Bloch)

C. G. BARLOW *Fisheries Research Branch, Department of Primary Industries, Walkamin Research Station, Walkamin, Queensland, Australia*
B. A. GREGG *Texas Parks and Wildlife Department, Fish Hatcheries Branch, Austin, Texas, USA*

Abstract. A study was conducted to determine if hatchery-reared and wild barramundi, *Lates calcarifer* (Bloch), could be distinguished by the patterns of circuli spacing on the scales. Proprietary software and digitizing equipment was used to obtain measurements of circuli spacing within one millimetre of the focus of the scales. Discriminant analyses separated the groups with up to 83% accuracy. As the technique utilizes innate tags laid down in response to the rearing environment, it has considerably more potential for evaluating the efficacy of large-scale enhancement programmes than do traditional physical tagging techniques.

Introduction

Stocking hatchery-reared fingerling fish is a widely practised management technique for enhancing recreational fisheries stocks and restoring populations of endangered species. Determining the efficacy of such programmes depends on recognizing the hatchery fish after stocking. In the past, fingerling fish have been marked by mutilation or excision of fins, by insertion of micro-wire tags, and with chemicals (for instance, stains, dyes and tetracycline antibiotics) applied either externally or internally (Wydoski & Emery 1983). These techniques are subject to error from mortality and tag loss and are quite labour intensive.

As early as 1913 it was realized that growth patterns on scales could be used to identify races of salmonids (Gilbert 1913, cited in Henry 1961). Subsequent studies showed that the rearing environment (analogous to the racial history) effectively induced an innate tag, as a consequence of the high correlation between environmental conditions, growth rates of fish and scale growth (Henry 1961; Major, Mosher & Mason 1972; Doyle, Talbot & Nicholas 1987). Later developments led to methods for discriminating populations of various salmonid species by analysing patterns of scale shape and circuli spacing using discriminant function techniques (Amos, Anas & Pearson 1963; Cook & Lord 1978). These methods have recently been shown to be capable of distinguishing hatchery and wild striped bass, *Morone saxatilis* (Walbaum), stocks, and even assigning cultured fish to their hatchery of origin (Ross & Pickard 1990). The development of specific software applications has facilitated use of the methods, which require extremely sensitive measurements of shape and distance.

The aim of the present study was to determine if patterns of circuli spacing on barramundi, *Lates calcarifer* (Bloch), scales could be used to distinguish hatchery and wild

Correspondence: Mr C.G. Barlow, Fisheries Branch, Department of Primary Industries, PO Box 46, Brisbane, Queensland 4001, Australia.

fish. The barramundi is a catadromous centropomid distributed from the Indo-west Pacific, south-east to northern Australia and north to Taiwan (Greenwood 1976). In several south-east Asian countries (where it is known as sea bass) it is produced for farming, while in Australia it is reared for farming and enhancement of recreational fisheries (Copland & Grey 1987). If valid, this discrimination technique would be of considerable benefit to the management of barramundi in areas where hatchery fish are released to supplement wild stocks.

Materials and methods

Scale origin and preparation

The wild fish were from the Cairns region (17°S, 146°E), northern Queensland. A scale set was obtained from approximately 120 fish collected between August 1979 and May 1980. The total lengths (TL) of the fish ranged from 150 to 450 mm.

The hatchery fish were bred at the Northern Fisheries Centre (NFC), Cairns, in November 1988. Larval rearing was conducted in saltwater tanks at the NFC. When 20 days old (spawning = day 0, hatching = day 1), the fish (about 10 mm TL) were transferred to fresh water at the Walkamin Research Station. They were reared indoors until approximtely 20 mm TL, then stocked into a pond. Scale samples were collected from 100 fish (range 120–220 mm TL) in March 1989.

Scales from both wild and hatchery fish were taken from the region immediately posterior to the pectoral fin. Scales were washed in water and about five non-regenerated scales from each fish were mounted between glass microscopé slides.

Data acquisition

Data were acquired using the Optical Pattern Recognition System (OPRS) software, microcomputer, video camera and monitor, frame grabber, digitizer pad and mouse (Biosonics 1989). The video camera was attached to a microscope (2 × objective). The frame grabber transformed video images of scales to digital images, which were displayed on the monitor. Linear distance on the image display was calibrated with a stage micrometer. All measurements were obtained using the mouse and menu-driven software.

The cleanest scale from each set was chosen for image analysis. Measurements were conducted along two 1-mm lines (Fig. 1), creating two data sets. The first set, herein termed straight-line data, was derived along a line located adjacent to the radii, where the circuli curve and are most widely spaced. The other, herein termed 45° reference-line data, was from a line at 45° to the first, or at approximately 90° to the anterior–posterior axis. Because the distance from the centre of the focus to the first complete circuli varied between scales, the origin of the measuring line was located just inside the first circulus. The programme automatically located the circuli by differences in luminance, although manual control was necessary to mark correctly circuli with inadequate contrast between light and dark zones, such as at overlapping or broken circuli.

To examine the relationship among circuli formation, TL and age, 10 scales from each of 43 hatchery-reared fish were examined. Circuli were counted on each scale from the focus to

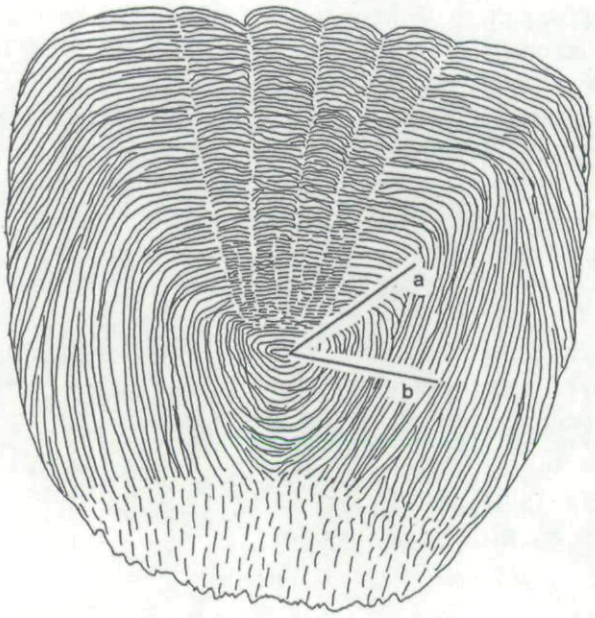

Figure 1. Diagrammatic representation of a barramundi scale. Straight-line data were derived along line a and 45° reference-line data along line b.

the margin adjacent to the radii. The fish were 25–38 days old and from 11 to 40 mm TL (scales had not formed in fish smaller than 11·0 mm TL). Because of the small size of these fish, it was not possible to take the scales from a confined area on the body.

Data analyses

Discriminant function analysis developed by Cook (1982) and Cook & Guthrie (1987) and available as part of the OPRS software package (Biosonics 1989) was conducted to separate hatchery from wild barramundi. The procedure uses a jack-knifing technique, termed 'leaving-one-out' by Lachenbruch (1967, 1975), to calculate the discriminant function. This iterative procedure uses the total data set as the training and test set and produces better estimates with less bias than other commonly used techniques. Discriminant function coefficients calculated for all variables in each type of analysis were the basis for selecting variables to enter into the discriminant function analysis. Results of the analysis were displayed as stock separation estimates in an error rate classification table.

The relationship between the number of circuli, TL and age was evaluated using regression analysis. The number of circuli used in the analysis was taken as the mean of the number of circuli on the 10 scales taken from each fish.

Results

Initial screening of the scales from the wild fish showed that scales from fish larger than approximately 350 mm TL were unreadable with the equipment available. The thickness of the scales and the irregularly positioned pigment spots interfered with light transmittance,

effectively disrupting the pattern of circuli spacing. After rejection of the unreadable scales, analysis was conducted on a set of 880 + wild fish (150–350 mm TL). The set comprised 68 fish from the 1978/79 spawning season and 20 fish from the 1979/80 spawning season.

Determining the position of circuli on scales from wild fish smaller than 350 mm TL and from all the hatchery fish (120–220 mm TL) was readily accomplished.

The basic measures analysed for these scales were various sets of single, double and triple intercirculus distances. For the paired circulus measures, distances between circuli were combined two at a time beginning at the centre of the scale (e.g. pair 1 = (1 + 2), pair 2 = (3 + 4) . . .). With triplet measures, the circuli distances were combined three at a time, from the centre.

Separation of hatchery from wild barramundi scales was examined using combinations of variables that displayed high negative or positive unstandardized discriminant coefficients. The following combinations of circuli distances were tested.

Straight-line data

A/ Singles 13, 14, 15, 16, 18

B/ Pairs (7–8), (9–10), (13–14), (15–16) ,

C/ Triplets (1–3), (4–6), (7–9), (13–15), (16–18)

45° Reference-line data

D/ Pairs (1–2), (9–10), (13–14)

E/ Pairs (1–2), (3–4), (9–10), (13–14)

F/ Triplets (1–3), (7–9), (13–15), (16–18)

The straight-line luminence data provided better separation than did the 45° reference-line data, particularly for triplets and pairs (Table 1). The straight-line data were also easier to derive, as the positioning of the line, adjacent to the radii, was simple, and the space of the circuli was clearer and with less overlapping than along the 45° reference-line.

Using the triplet combination, 83% of the hatchery scales and 82% of the wild scales were correctly classified (17 and 18% errors, respectively). The paired circuli combination was separated with 83% correct for hatchery scales and 77% correct for wild scales. The classification rates for the 45° reference-line luminance data ranged from 74 to 77% of hatchery scales classified correctly to 74 to 76% of the wild scales correctly scored.

Table 1. Percentage of wild and hatchery barramundi correctly identified using data derived from the spacing of circuli on scales and linear discriminant analysis.

Test	Wild (n = 88)	Hatchery (n = 100)	Composite
Straight-line data			
Test A (Singles)	68	68	68
Test B (Pairs)	77	83	80
Test C (Triplets)	82	83	83
45° reference-line data			
Test D (Pairs)	74	77	76
Test E (Pairs)	75	77	76
Test F (Triplets)	76	74	75

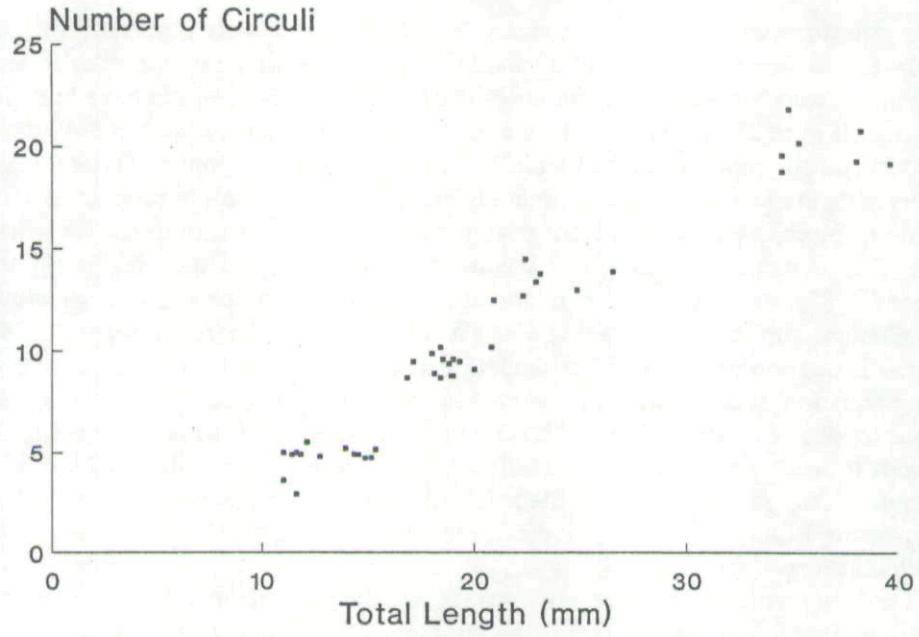

Figure 2. Relationship between the number of circuli on scales and TL of 43 fingerling barramundi 25–38 days old. Each datum is the mean of 10 scales. The relationship is described by $y = -2{\cdot}76 + 0{\cdot}63x$ ($r^2 = 0{\cdot}94$), where y = number of circuli and x = TL (mm).

Multiple linear regression analysis revealed that 94% of the variation in the number of circuli was attributable to TL ($P < 0{\cdot}001$, partial $r^2 = 0{\cdot}9413$). However, adding age to the equation did not improve its descriptive power ($P > 0{\cdot}05$, partial $r^2 = 0{\cdot}0047$). The relationship between the number of circuli and TL was described by the linear equation $y = -2{\cdot}76 + 0{\cdot}63x$, where y = number of circuli and x = TL (mm) (Fig. 2).

Discussion

The separation of hatchery from wild scales achieved with these data (83% for hatchery and 82% for wild) was encouraging, especially since only luminance line data were used. This compares favourably with other stock discrimination investigations using the same technique. For instance, Schwartzberg & Fryer (1989) separated natural and hatchery stocks of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), with 80–94% accuracy; Cook & Lord (1978) separated three riverine stocks of sockeye salmon, *Oncorhynchus nerka* (Walbaum), with 64–80% accuracy; Whaley (1988) separated a lake strain and riverine strain of brown trout, *Salmo trutta* L., reared under virtually identical hatchery conditions with 94–98% accuracy. Ross & Pickard (1990) used scale shape information analysed as Fourier transformations in addition to variables associated with circuli spacing to obtain 87–91% accuracy in separating yearling hatchery and wild striped bass stocks.

It is noteworthy that certain of the circuli distances repeatedly occurred in the sets of variables tested. For example, circuli 13 and 14 occurred in all of the six variable sets analysed and circuli 15 and 16 were found in four of the six. Circuli 13–16 would have been formed when the fish were 25–30 mm TL, which would have coincided with the first 5–10 days after the hatchery fish were transferred from the laboratory to the ponds. This suggests that hatchery fish might have been incidentally marked during that portion of the rearing procedure. Other research has demonstrated that scales of brown trout can be marked by varying the temperature during egg incubation and rearing of the alevins (Skurdal & Andersen 1985), and that sudden temperature shifts can induce banding on otoliths of juvenile chum salmon, *Oncorhynchus keta* (Walbaum) (Volk, Schroder & Fresh 1987).

Initial formation of scales of barramundi occurred at about 11 mm TL, and thereafter circuli formation was related to length. In walleye, *Stizostedion vitreum* (Mitchill), squamation occurs at about 20 mm TL (Glenn & Mathias 1985), while for three species of salmonids it occurs at 37–42 mm TL (Bilton 1988). The comparatively small size at which barramundi forms scales, coupled with the fact that juveniles can be reared over a wide range of temperatures (at least 24–32°C), indicates that barramundi may be particularly amenable to batch marking of scales.

If it proved possible to induce permanent tags in the patterning of the circuli on scales of hatchery-reared fish, many of the questions associated with the assessment of stocking programmes (in particular, management techniques, estimates of post-stocking survivals and population sizes) could be more readily answered. In fact, because of the problems inherent in individually tagging large numbers of small fish, we consider that the use of scale pattern analysis has greater potential for the quantitative evaluation of some stocking programmes than do traditional physical tagging methods.

One obvious problem with barramundi, however, is that circuli on scales from fish larger than 350 mm TL could not be read with the present equipment. The difficulty experienced in reading circuli on scales from large barramundi apparently does not apply to similar-sized fish with smaller scales, such as salmonids. Consequently, the potential for separating hatchery-reared fish from wild fish using patterns of circuli spacing may be better for species with small scales (for instance, red drum, *Sciaenops ocellatus* L.) than for those with large scales. Within Australia, an ideal subject for investigation would be small-scaled species such as freshwater cods, *Maccullochella* spp., and silver perch, *Bidyanus bidyanus* (Mitchell).

Since the present study was restricted to investigation of scale circuli pattern analysis as a means of discriminating hatchery from wild barramundi, only early growth zones were examined. Other workers have shown that analysis of the shapes of whole otoliths or scales can also be used to differentiate stocks or genetic races (Jarvis, Kludowski & Sheldon 1978; Riley & Margraf 1983; Bird, Eppler & Checkley 1986; Maceina & Murphy 1989). In Australia, the barramundi resource consists of a series of genetically different stocks which show little intermixing (Shaklee & Salini 1985; Russell & Garrett 1988). Scale or otolith shape analysis (also a function within OPRS software) may provide an alternative means of separating these stocks or delineating the composition of other multi-stock assemblages.

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