Mar. Freshwater Res., 1996, **47**, 757–61

Use of Scale Patterns to Evaluate Stocking Success of Silver Perch, *Bidyanus bidyanus* (Mitchell), Released at Two Different Sizes

Daniel J. Willett

Southern Fisheries Centre, PO Box 76, Deception Bay, Qld 4508, Australia.

Abstract. A study was conducted to determine relative success of stocking silver perch (*Bidyanus bidyanus*) at two different sizes: 35 mm and 50 mm total length. By purposeful manipulation of growth rates of pre-stocked fish it was possible to induce unique markers in scale circulus patterns, thus differentially marking the two size-groups. The scale circulus data were acquired with automated video-digitizing equipment. Both size-classes were stocked into two south-eastern Queensland impoundments. Relative stocking success after two years was quantified by sampling surviving silver perch and using discriminant function analysis to assign individuals to their respective size-at-release groups. Relative survival of fish stocked at 35 mm and at 50 mm was not significantly different in either impoundment. Post-stocking growth rates of 35 mm fish were not compromised due to their smaller size at release. From a management perspective, this suggests that 35 mm is a more cost-effective release size of silver perch than 50 mm. This trial demonstrates the feasibility of using scale circulus patterns to differentially mark multiple groups of fingerlings for such controlled experiments.

Introduction

Hatchery-reared silver perch fingerlings have been stocked into rivers and impoundments in Queensland since 1986 as part of the State Government's Recreational Fishery Enhancement Programme (Wager 1994). These releases have been important for augmenting natural reproduction and for establishing and maintaining fisheries where no natural reproduction is possible. Little is known, however, about the size at which it is most efficient and cost-effective to stock the fish. The current Queensland Government policy requires hatcheries to grow fingerlings to a minimum size of 50 mm total length (TL) for stocking. If survival of fish released at a smaller size (e.g. 35 mm) were found to be comparable, the cost of stocking fish could be significantly reduced.

The lack of information on the relative success of stocking silver perch at different sizes may be due to several methodological problems. Because natural systems cannot be replicated, truly controlled comparisons require that different size-groups be introduced in the same waterway simultaneously. Previous studies have lacked this internal control. Stocking different sized fish in alternate years or in different lakes is not satisfactory because physical or biotic characteristics may vary significantly between localities and years, affecting the strength of each group or year-class of fish (Jennings and Philipp 1992).

If different size-groups are to be introduced concurrently into a waterway, some form of marking system is required to differentiate the cohorts. Traditional marking methods such as external tags are labour-intensive since every fish has to be marked individually, and they can affect survival and growth (Wydoski and Emery 1983). In addition, these techniques cannot be used to mark small fingerlings. Methods suitable for marking small fingerlings, such as immersion in tetracycline to stain otoliths, require fish to be killed upon recapture so that marks can be read. However, by manipulating growth rates of pre-stocked fish, unique natural markers can be induced in scale circulus patterns without the deleterious effects of these other marking methods.

Scale circulus pattern analysis is a well established technique for discriminating between stocks (Anas and Murai 1969; Major *et al.* 1972; Lear and Misra 1978; Barlow and Gregg 1991; Schwartzberg and Fryer 1993; Willett 1993). The technique is based on the high correlation between growth rates of fish, caused by genetic and environmental factors, and the circulus spacing on scales. In silver perch, variation in water temperature by as little as 5°C can induce detectable differences in circulus spacing and allow discrimination between groups (Willett 1994). This method offers scope for differentially marking groups of pre-stocked fish. The objective of the present study was to use scale pattern recognition to determine the relative stocking success (post-stocking survival and growth rates) of silver perch released at two different sizes.

Materials and Methods

Scale Origin and Study Sites

Silver perch used in this study were spawned at the Sunland Fish Hatchery, Pomona, Queensland, during the 1992–93 breeding season. Two-day-old larvae were grown in a prepared pond until scale formation commenced. The pond contained abundant zooplankton including cladocerans and copepods, suitable as food for the silver perch

1323-1650/96/050757$05.00

(Cadwallader and Backhouse 1983). In order to obtain marked cohorts, a sample of approximately 1200 fish (mean TL: 20·5 ± 0·90 mm) was then collected by seine-net, transported to the Southern Fisheries Centre, Brisbane, and released into a 2000-L tank. Water temperature in the tank was ambient and fish were fed an artifical diet consisting of a minimum 50% crude protein, 10% crude fat, and 7% crude fibre (Aqua-feed Products Australia, Brisbane, Queensland). Food was delivered six times daily through an automatic feeder.

By the time these tank-reared fish had reached a mean length of 33·6 ± 1·92 mm, fish remaining in the pond were harvested (mean length 50·0 ± 2·86 mm). This growth difference was the result of environmental manipulation to ensure that scale patterns of the two groups would be sufficiently different to allow their discrimination, and it was presumably due to superior growing conditions in the hatchery pond. Tank-reared fish are hereafter referred to as the 'small' size-class, whereas pond-reared fish are the 'large' size-class.

For each size-class, 250 individuals were released at dawn on 4 February 1993 into each of two impoundments on a farm at Caboolture, Queensland. The first impoundment (Lake 1), had a surface area of approximately 20250 m^2. The second (Lake 2) was approximately 23625 m^2. Lake 1 was an older impoundment with larger invertebrate populations than the more recently constructed Lake 2. Both impoundments were chiefly used as water storage for crop irrigation but contained large populations of spangled perch (*Leiopotherapon unicolor*) and freshwater eels (*Anguilla reinhardtii*). Catfish (*Tandanus tandanus*) were abundant only in Lake 2. Cormorants (*Phalacrocorax* spp.) were also commonly seen in the vicinity of the lakes and these birds are known to prey on juvenile silver perch (Barlow and Bock 1984).

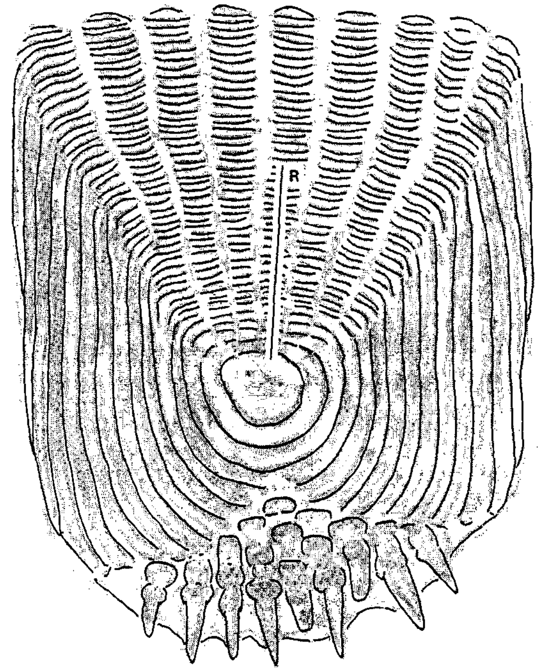

Fig. 1. Diagrammatic representation of a silver perch scale. Circulus spacing data were derived along the radial line (R).

Data Acquisition and Analysis of Reference Samples

In order to establish a set of scale features that varied between small and large size-class fish, it was necessary to conduct analyses on reference scale sets from both groups. The identified scale features form a classification function that can then be used to discriminate between groups in subsequent mixed-stock analyses. Reference scale samples were collected from 100 small and 100 large size-class fish. Scales were taken from a standard area, i.e. the left flank in the region between the spinous dorsal fin and the lateral line. About 10 scales from each fish were removed with a pair of sharp forceps and mounted between glass microscope slides.

Data on circulus spacing were acquired with an Optical Pattern Recognition System (OPRS; BioSonics Inc., Seattle, Washington). The system consists of a microcomputer, closed-circuit video camera and monitor, video frame grabber, digitizing board and extraction software. The video camera was connected to an Olympus BH2 microscope via a MTV-3 parfocalizing adapter.

The video camera converted the optical image of the scale to an analogue electrical signal and transmitted it to the video frame grabber. The frame grabber then converted the analogue signal to a digital image consisting of a 512 × 512 array of pixels or picture elements. Each pixel was given a numerical value that corresponded to the luminance or light intensity of the image at that point. This array of pixels was used for all subsequent image measurements. The digitization of a complete image took 1/30th of a second, but the process was continually repeated to display the image in real time as it was focused and enhanced. To provide feedback to the operator during the digitization process, the digitized image was converted back to analogue signal and displayed on the closed-circuit monitor. Linear distance on the image display was calibrated with a stage micrometer and all measurements were directed by using the digitizing board and its mouse.

Circulus spacing was measured on a clean, non-regenerated scale from each slide. Measurements were taken along a 1·0-mm radial line located just inside the first circulus and extending towards the anterior edge of the scale, incorporating the closely spaced circuli that form in the anterior field (Fig. 1). Once the radial line was established, the luminance values along the line were plotted. The OPRS program automatically recognized circuli as minima in the luminance profile.

Sets of single, double and triple intercirculus measurements were derived along the radial line. For paired circulus measures, the intercirculus distances were combined two at a time beginning at the centre of the scale (e.g. pair 1 = distance between circulus 1 and 3, pair 2 = distance between circulus 3 and 5, ...). Triplet measures were derived by combining intercirculus distances three at a time from the centre.

Differences in circulus spacing between size-classes were examined by a discriminant function analysis developed by Cook (1982) and modified by Cook and Guthrie (1987). Sets of intercirculus distances that showed significant differences between small- and large-size fish were identified using one-way analysis of variance tests. Linear discriminant analysis was then performed on the identified scale parameters. The analysis uses a jack-knifing procedure developed by Lachenbruch (1967) to estimate error-rate. Results of this analysis were displayed as an error-rate matrix which represents estimates of stock separation.

Data Acquisition and Analysis of Unknown Samples

Silver perch were sampled two years after stocking by using a combination of gill-netting (3·75–10·0 cm mesh size) and seine-netting. Three samples were taken from each lake during February 1995. On each sampling occasion, nets were set at approximately 1200 hours and were checked hourly until they were removed at 1900 hours. Seine-netting was conducted in the interim. Captured fish were measured and scale samples were removed from the standard area. The fourth dorsal spine was clipped in order to show that the fish had been sampled, in case of recaptures, and fish were released.

In the laboratory, scale samples were cleaned and mounted prior to analysis. Circulus spacing data were acquired in the same manner as for the reference samples. Linear discriminant analysis, using the classification function developed from the known-stock analysis, was then used to determine the release size of these 'unknown' fish. Assignment of individual unknowns to a category was based on the greatest probability. In the case of imperfect classification of the reference stocks, these natural classification estimates of the unknown fish would be biased. Nearly unbiased classification estimates were obtained by applying an error-rate correction procedure based on the derived error-rate matrix (Cook and Lord 1978). The 95% confidence intervals of these estimates were calculated by the method of Pella and Robertson (1979). Cook (1983) found that these formulae provided statistically valid, albeit conservative, confidence intervals.

Because equal numbers of fish from both size-classes were released, a recapture ratio of 1:1 would indicate that there were no differences in survival as a function of release size, i.e. the sample comes from a population containing equal numbers of small and large size-class fish. χ^2 tests were used to test this hypothesis.

In order to determine whether size at release influenced post-release growth rate of silver perch, Student's *t*-tests were conducted. These tests compared the mean change in length of fish in each size-class over the two-year post-release period. Mean change in length was calculated by subtracting the mean stocking size of fish from final mean lengths. Variances used in the analyses were calculated by adding the variances at stocking size and at final length.

Results

Classification of Known Reference Stocks

Of the intercirculus distances measured, the following combinations of paired measures were identified as the best discriminators for separating the two size-classes: (7–9), (9–11) and (11–13). According to one-way analyses of variance, differences between the groups were highly significant ($P < 0{\cdot}0001$) for all three of these intercirculus measures. The error-rate matrix derived from this function shows numbers of fish in each size-class correctly classified and subsequently misclassified (Table 1).

Table 1. Error-rate matrix derived from circulus measures and discriminant-function analysis showing separation of the two size-classes of silver perch

Actual size-class	Sample size	Calculated classification Small	Large
Small	100	91	9
Large	100	4	96

Evaluation of Stocking Success

In total, 57 silver perch were recaptured in Lake 1, ranging from 335 to 410 mm TL (mean 374 mm). Total recaptures from Lake 2 numbered 42. Fish from this impoundment had a considerably slower growth rate than those from Lake 1 and ranged in size from 195 to 290 mm (mean 235 mm).

Linear discriminant analysis, using the classification function developed from the known size-class reference samples, calculated the origin (size at release) of these 'unknown' fish from each lake. Nearly unbiased classification estimates and the 95% confidence intervals of these estimates are given in Table 2.

Table 2. Classification of unknown silver perch from Lake 1 and Lake 2 into their respective size-at-release classes

Classification was estimated by using the discriminant function derived from analysis of known reference samples

	Size-class	Nearly unbiased estimate	Simultaneous 95% C.I.
Lake 1 (n=57)	Small (33·6 mm)	28	16·0 to 40·8
	Large (50·0 mm)	29	16·1 to 41·0
Lake 2 (n=42)	Small (33·6 mm)	26	17·0 to 34·3
	Large (50·0 mm)	16	7·6 to 25·0

Differences in survival between size classes of silver perch in Lake 1 were not significant ($\chi^2 < 0{\cdot}001$, d.f. = 1, $P > 0{\cdot}975$). Likewise, in Lake 2 the ratio of recaptures for the two size-classes was not significantly different from 1:1, indicating no significant differences in survival (χ^2 = 1·9285, d.f. = 1, $0{\cdot}25 > P > 0{\cdot}10$).

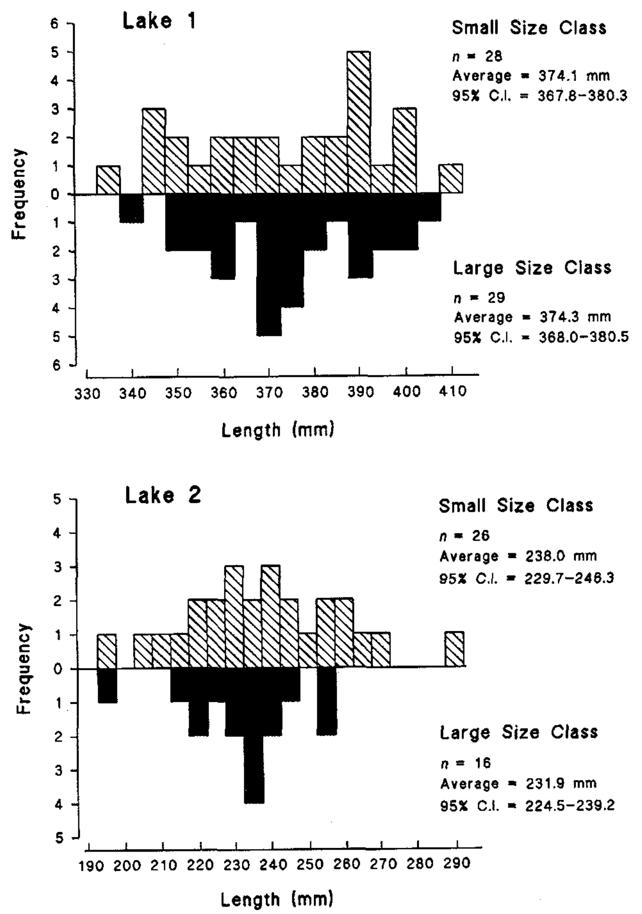

Fig. 2. Length–frequency distribution of silver perch from Lake 1 and Lake 2 according to their size at release. Size at release was determined from scale patterns and by discriminant function analysis.

Table 3. Differences in post-stocking growth of the two size-classes of silver perch in Lake 1 and Lake 2

Size-class	Initial length (mm)	Final length (mm)		Change in length (mm)	
		Lake 1	Lake 2	Lake 1	Lake 2
Small	33·6	374·1^{A}	238·0^{B}	339·5	204·4
Large	50·0	374·3^{A}	231·9^{B}	324·3	181.9

^{A,B}Values with same superscript were not significantly different.

Plots of the length–frequency distribution of unknown silver perch, according to their classified size at release, show growth of the two groups in each lake (Fig. 2). Final lengths of fish from the two groups were not significantly different (Lake 1, $t = 0{\cdot}3345$, $P = 0{\cdot}7392$; Lake 2, $t = 0{\cdot}4313$, $P = 0{\cdot}6686$). However, in both lakes there was a significant difference between the mean growth (change-in-length) of the two groups over the two-year post-stocking period (Lake 1, $t = 3{\cdot}1668$, $P = 0{\cdot}0037$; Lake 2, $t = 4{\cdot}3307$, $P = 0{\cdot}0002$). According to this change in length, the small perch grew 1·05 and 1·12 times faster than the large perch in Lake 1 and Lake 2, respectively (Table 3).

Discussion

In both lakes, relative survival of fish stocked at 35 mm and at 50 mm TL was not significantly different. It has been shown that survival of released fish can be influenced by the size and abundance of food, abundance of predator or competitor species, and quality and condition of fingerlings (Carline *et al.* 1986; Ellison and Franzin 1992). It may be true that silver perch released at 35 mm and at 50 mm target the same food source in an area and are equally at risk from predation. A study by Barlow (1986) similarly found no difference in survival between 25-mm and 40-mm silver perch fingerlings after four months. The different size-class fish in that study, however, were stocked into lakes devoid of predators. In the present study, post-stocking growth rates of the 35-mm fish also were not compromised due to their smaller size at release. In fact, 35-mm fish grew faster than fish released at 50 mm. From a management perspective, this suggests that 35 mm is a more cost-effective release size of silver perch than 50 mm.

Similar results have been found in other species. For instance, Koppelman *et al.* (1992) found that in walleye, *Stizostedium vitreum*, stocking small fingerlings gave consistently better results than stocking more advanced fingerlings. However, Jennings and Philipp (1992) have found in their trials on walleye, that even among similar types of lakes within a single geographic region, physical and biotic conditions can differ enough that the adoption of a single stocking strategy may result in less-than-optimal results.

Although the present study was not designed to monitor the physical and biotic conditions in each lake, each contained two of the most common predator species encountered in Queensland's freshwater enhancement programme, i.e. spangled perch and eels. Lake 1 was visibly the more productive of the two; it was older and contained a large variety of zooplankton, small fish and shrimps suitable as food for silver perch. Lake 2, on the other hand, contained relatively turbid water, which restricted the amount of light penetrating the surface and thus limited plankton and other food production for the silver perch. Fish from this lake were under the added pressure of having to compete with a large population of catfish in addition to the resident spangled perch and eels. These differences in productivity were reflected in the relative growth rates, with fish from Lake 1 being on average 137 mm longer than those from Lake 2. Yet, despite the differences (including availability of food and intensity of predation or competition) between lakes in this trial, relative survival of fish stocked at 35 mm and at 50 mm was not significantly different.

The reason for the differential growth of small and large fish, which resulted in similar final lengths, is unknown, but Jennings and Philipp (1992) have suggested that growth differences in walleye released at different sizes may be attributable to size and abundance of food.

The two size classes of fish were readily distinguishable on the basis of scale circulus spacing. Manipulation of the growing environment of these fishes prior to stocking effectively 'marked' 91% of the small size-class and 96% of the larger test stock. Such results are equal to or better than those obtained from other marking methods. Coded-wire tags, for instance, effectively mark only 71% of stocking-size silver perch after 12 months owing to tag losses and mortality associated with tagging (Ingram 1993). The only deleterious effect of marking scales by manipulating growth rates would be if the method produced lower-quality fish. For example, depriving fish of food to induce a scale check may decrease their strength and ultimately bias survival results. The small size-class fish used in this study, however, were never starved but fed an artificial diet that was restricted to several feeds per day. This resulted in a slower growth rate without causing ill health of the stock.

Other research has also demonstrated that manipulation of environmental parameters can induce specific marks on body parts of different fish species. For instance, scales of brown trout, *Salmo trutta* L., were marked by varying temperature during rearing of the alevins (Skurdal and

Anderson 1985), and manipulation of temperature and feeding rate has induced banding patterns on otoliths of juvenile Pacific salmon (Volk *et al.* 1990).

The use of a scale recognition system to identify the origins of fish holds many advantages over traditional marking methods. For instance, problems inherent in individually tagging large numbers of small fish are avoided. Scale features are permanent and do not affect survival, behaviour or growth. In addition, fish can be released after scale samples are removed, i.e. it is not necessary to kill the fish in order to retrieve the marker. This trial demonstrates that scale circulus patterns can be used effectively to differentially mark multiple groups of fingerlings for controlled experiments.

Acknowledgments

Thanks are extended to Robert Simpson for his assistance in field operations and to Dr Chris Barlow and the Southern Fisheries Editorial Committee for comments on initial drafts of the manuscript. This study was funded by the Queensland Recreational Fishery Enhancement Programme.

References

Anas, R. E., and Murai, S. (1969). Use of scale characteristics and a discriminant function for classifying sockeye salmon, *Oncorhynchus nerka*, by continent of origin. *International North Pacific Fisheries Commission Bulletin* **26**, 157–92.

Barlow, C. G. (1986). Fish in farm dams and implications for extensive aquaculture. In 'Proceedings of the First Freshwater Aquaculture Workshop, February 1983'. pp. 33–43. (Government Printer: Sydney.)

Barlow, C. G., and Bock, K. (1984). Predation of fish in farm dams by cormorants, *Phalacrocorax* spp. *Australian Wildlife Research* **11**, 559–66.

Barlow, C. G., and Gregg, B. A. (1991). Use of circuli spacing on scales to discriminate hatchery and wild barramundi, *Lates calcarifer* (Bloch). *Aquaculture and Fisheries Management* **22**, 491–8.

Cadwallader, P. L., and Backhouse, G. N. (1983). 'A Guide to the Freshwater Fish of Victoria.' (Victorian Government Printing Office: Vic., Australia.)

Carline, R. F., Stein, R. A., and Riley, L. M. (1986). Effects of size at stocking, season, largemouth bass predation, and forage abundance on survival of tiger muskellunge. *American Fisheries Society Special Publication* **15**, 151–67.

Cook, R. C. (1982). Stock identification of sockeye salmon (*Oncorhynchus nerka*) with scale pattern recognition. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 611–17.

Cook, R. C. (1983). Simulation and application of stock composition estimators. *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 2113–18.

Cook, R. C., and Guthrie, I. (1987). In-season stock identification of sockeye salmon (*Oncorhynchus nerka*) using scale pattern recognition. In 'Sockeye Salmon (*Oncorhynchus nerka*) Population Biology and Future Management'. (Eds H. D. Smith, L. Margolis and C. C. Wood.) pp. 327–34. Canadian Special Publication of Fisheries and Aquatic Sciences No. 96.

Cook, R. C., and Lord, G. E. (1978). Identification of stocks of Bristol Bay sockeye salmon, *Oncorhynchus nerka*, by evaluating scale patterns with a polynomial discriminant method. *Fisheries Bulletin* **76**, 415–23.

Ellison, D. G., and Franzin, W. G. (1992). Overview of the symposium on walleye stocks and stocking. *North American Journal of Fisheries Management* **12**, 271–5.

Ingram, B. A. (1993). Evaluation of coded wire tags for marking fingerling golden perch, *Maquaria ambigua* (Percichthyidae), and silver perch, *Bidyanus bidyanus* (Teraponidae). *Australian Journal of Marine and Freshwater Research* **44**, 817–24.

Jennings, M. J., and Philipp, D. P. (1992). Use of allozyme markers to evaluate walleye stocking success. *North American Journal of Fisheries Management* **12**, 285–90.

Koppelman, J. B., Sullivan, K. P., and Jeffries, P. J. (1992). Survival of three sizes of genetically marked walleyes stocked into two Missouri impoundments. *North American Journal of Fisheries Management* **12**, 291–8.

Lachenbruch, P. A. (1967). An almost unbiased method of obtaining confidence intervals for the probability of misclassification in discriminant analysis. *Biometrics* **23**, 639–45.

Lear, W. H., and Misra, R. K. (1978). Clinal variation in scale characters of Atlantic salmon (*Salmo salar*) based on discriminant function analysis. *Journal of the Fisheries Research Board of Canada* **35**, 43–7.

Major, R. L., Mosher, K. H., and Mason, J. E. (1972). Identification of stocks of Pacific salmon by means of scale features. In 'The Stock Concept in Pacific Salmon'. (Eds R. C. Simon and P. A. Larkin.) pp. 209–31. (University of British Columbia: Vancouver, Canada.)

Pella, J. J., and Robertson, T. L. (1979). Assessment of composition of stock mixtures. *Fisheries Bulletin (US)* **77**, 387–98.

Schwartzberg, M., and Fryer, J. K. (1993). Identification of hatchery and naturally spawning stocks of Columbia Basin spring chinook salmon by scale pattern analyses. *North American Journal of Fisheries Management* **13**, 263–7.

Skurdal, J., and Anderson, R. (1985). Influence of temperature on number of circuli of first year scales of brown trout, *Salmo trutta* L. *Journal of Fish Biology* **26**, 363–6.

Volk, E. C., Schroder, S. L., and Fresh, K. L. (1990). Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. *American Fisheries Society Symposium* **7**, 203–15.

Wager, R. (1994). Fish translocation and biodiversity of Queensland freshwater fishes. *Australian Biologist* **7**, 23–32.

Willett, D. J. (1993). Discrimination between hatchery stocks of silver perch, *Bidyanus bidyanus* (Mitchell), using scale growth patterns. *Aquaculture and Fisheries Management* **24**, 423–30.

Willett, D. J. (1994). Use of temperature to manipulate circulus patterns on scales of silver perch, *Bidyanus bidyanus* (Mitchell), for the purpose of stock discrimination. *Fisheries Management and Ecology* **1**, 157–63.

Wydoski, R., and Emery, L. (1983). Tagging and marking. In 'Fisheries Techniques'. (Eds L. A. Neilson and D. L. Johnson.) pp. 215–37. (American Fisheries Society: Bethesda, Maryland.)

Manuscript received 25 August 1995; revised and accepted 1 March 1996