Chemical marking of juvenile snapper, *Pagrus auratus* (Sparidae), by incorporation of strontium into dorsal spines

Morgan J. Pollard
Michael J. Kingsford
School of Biological Sciences A08
University of Sydney
New South Wales 2006, Australia
E-mail address (for M. J. Kingsford, contact author): Mikek@bio.usyd.edu.au

Stephen C. Battaglene
ICLARM, Coastal Aquaculture Centre
P.O. Box 438
Honiara, Solomon Islands

Abstract.–The aim of this project was to investigate the use of strontium as a chemical tag in the dorsal spines of the marine teleost *Pagrus auratus* that would allow the mass tagging of juvenile fish. Previous studies in which the incorporation of strontium has been experimentally manipulated for the purposes of marking have generally concentrated on freshwater and anadromous species. This is the first study to investigate the tagging of spines with strontium, the removal of which is nondestructive. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to measure isotopic concentrations. The dorsal spines of juvenile *P. auratus* that had been immersed in salt water containing 0.125 g/L $SrCl_2 \cdot 6H_2O$ (5× ambient strontium) and 0.250 g/L (10× ambient) for five days incorporated ^{86}Sr at levels greater than those in control fish. The strontium signal was persistent in spines for at least 36 days and showed no sign of decay during the experiment. No effects of the treatments on fish health or growth were detected. Short-term immersion experiments (6 hours to 5 days) indicated that treatments of 10× ambient or greater for 4–5 days were required to tag fish reliably with strontium. Natural levels of strontium in the spines of juveniles varied among locations separated by tens of kilometres along the coast of New South Wales. Natural variations in strontium concentrations were not great enough, however, to obscure the differences between tagged and wild fish. It was concluded that strontium immersion is a useful and relatively environmentally safe method of tagging large numbers of small fish.

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Calcium is incorporated into the calcium carbonate matrix of otoliths and into the calcium phosphate matrix of the skeleton, spines, and scales as fish grow (Francillon-Vieillot et al., 1990). In addition to calcium, trace elements, such as strontium, are also incorporated into the calcified components of fish. The chemical similarities of Ca^{2+} and Sr^{2+} ions (i.e. similar ionic radius and identical valence) allow strontium ions to act as replacements for calcium during the process of calcification. Ca and Sr concentrations in calcified components have been explored for their relationships with variations in environmental conditions (Edmonds et al., 1989; Gallahar and Kingsford, 1992), such as temperature (Kalish, 1989; Radtke et al., 1990; Townsend et al., 1992, 1995) and salinity (Kalish, 1990; Coutant and Chen, 1993; Secor et al., 1995).

The manipulation of ambient levels of chemicals such as strontium can allow fish to be marked or tagged invisibly. The marking of skeletal structures with isotopes offers a potential method for rapid tagging of large numbers of small juvenile fish. Nonradioactive strontium has been investigated, and artificially induced strontium marks have been detected in otoliths (Brown and Harris, 1995; Mugiya and Satoh, 1995; Mugiya and Tanaka, 1995; Schroder et al., 1995, 1996; Gallahar and Kingsford, 1996), scales (Ophel and Judd, 1968; Behrens-Yamada and Mulligan, 1982; Snyder et al., 1992), vertebrae (Behrens-Yamada et al., 1979; Behrens-Yamada and Mulligan, 1982, 1987; Schroder et al., 1995), and opercular bones (Guillou and de la Noue, 1987; Schroder et al., 1995). The majority of this work, however, has been done on freshwater or anadromous species.

The species investigated in this study was the snapper or red sea bream, *Pagrus auratus* (Pisces; Sparidae). The northern hemisphere and southern hemisphere forms (previously *Pagrus major* and *Chrysophrys auratus*, respectively) are now considered to be morphometrically identical (Paulin, 1990). This highly valued commercial species supports a very well established intensive aquaculture industry in Japan (Foscarini, 1988; Davy, 1990, 1991; Fukusho, 1991) and shows good potential for aquaculture in

Australia and New Zealand (Battaglene and Bell, 1991; O'Sullivan, 1992; Treadwell et al.[1]). Commercial catch rates of snapper have been on the decline in many areas (Paul, 1982; Gilbert, 1986), and one means of increasing natural populations is by reseeding the sea with hatchery-reared larvae or juveniles. *Pagrus auratus* is one of the targets for this approach in both the northern (Smith and Hataya, 1982; Ishibasi, 1986; Matsuda, 1992) and southern (Smith and Francis[2]) hemispheres. Tagging techniques are required to measure the success of such stock enhancement measures. Very large numbers of small juveniles must be tagged in these studies, and isotope tagging may be one of the more suitable techniques.

Tagging techniques have been extensively used for research on *Pagrus auratus*. Studies involving tagging have been used to investigate migration (Crossland, 1976; Jones, 1981; Sakamoto, 1984; Kato et al., 1991), the identification of separate stocks (Sanders, 1974; Moran, 1987), growth rates (Sanders and Powell, 1979; Francis and Winstanley, 1989; Tsukamoto et al., 1989; Kato et al., 1991), fishery exploitation and resilience (Crossland, 1980), and the validation of aging with oxytetracycline (Ferrell et al., 1992; Francis et al., 1992). With the exception of fluorochrome markers, such as oxytetracycline, many conventional tagging techniques are, however, unsuitable for tagging small juveniles or are too energy intensive or expensive for the tagging of large numbers of fish.

The objective of our study was to determine whether juvenile snapper (*Pagrus auratus*) could be reliably chemically tagged by the incorporation of strontium into the dorsal spines from immersion in strontium chloride. The specific aims were as follows: 1) to investigate the persistence of chemical tags in the dorsal spines of juvenile snapper following immersion of the fish in strontium chloride; 2) to determine minimum strontium concentrations and immersion periods required for effective strontium tagging; 3) to determine the feasibility of batch tagging with different levels of strontium; and 4) to compare the levels of strontium in tagged snapper with naturally occurring levels in wild-caught snapper from different estuaries. These multiple controls were required to ensure that natural variation did not confound the identification of treated fish.

Methods

Experimental work was carried out at the New South Wales (NSW) Fisheries Brackish Water Fish Culture Research Station (BWFCRS), Port Stephens. The experimental fish were hatched on 13 November 1993 from wild parent snapper caught off Broughton Island, NSW. Rearing procedures and larval characteristics may be found in Fukuhara (1985), Lopez (1986), Pankhurst et al. (1991), Battaglene and Talbot (1992), and Kingsford and Atkinson (1994). Experimental fish were kept in 2000-litre tanks on a constant flowthrough system of biofiltered oceanic water for the first three weeks, followed by biofiltered estuarine water from Salamander Bay, Port Stephens. From 14 January 1994, 770 juvenile snapper were reserved for the experiments.

The strontium salt used was strontium chloride ($SrCl_2 \cdot 6H_2O$), a relatively cheap, nontoxic and nonradioactive chemical. Strontium is found naturally in seawater at an elemental concentration of approximately 8.1 mg/L (Horne, 1969). For simplicity the amounts of strontium chloride added to the saltwater experimental tanks are expressed as multiples of this ambient strontium concentration, as follows: 1) Sr^{2+} five times ambient = 0.125 g/L of $SrCl_2 \cdot 6H_2O$; 2) Sr^{2+} ten times ambient = 0.250 g/L of $SrCl_2 \cdot 6H_2O$; and 3) Sr^{2+} forty times ambient = 1.000 g/L of $SrCl_2 \cdot 6H_2O$. Preliminary experiments (Pollard[3]) showed increased levels of dorsal spine strontium in juvenile snapper that had been immersed in strontium chloride solution.

Experiment 1: persistence of strontium

The aim of this experiment was to determine whether the strontium marks induced in the dorsal spines of juvenile snapper were persistent over at least a 36-day period. The experiment was initiated on 22 March 1994 when the fish were 129 days old and 68 mm mean fork length (SD ±6.7 mm). The experiment consisted of Sr^{2+} five times ambient, Sr^{2+} ten times ambient, and a control, each with four replicate 100-litre tanks containing eight fish each. Two fish were sampled from each tank at zero, 12, 24, and 36 days following the termination of the five-day exposure period. For comparisons between sample times, treatments, and tanks we used a partially hierarchical three-way analysis of variance.

[1] Treadwell, R., L. McKelvie, and G. B. Maguire. 1992. Potential for Australian Aquaculture. Research Report 92.2 of the Aust. Bureau of Agricultural and Resource Economics, Canberra, Australia, 81 p.

[2] Smith, P. J., and M. P. Francis. 1991. Snapper reseeding in the Hauraki Gulf: scientific considerations. N.Z. MAF Fisheries Internal Report 172, Wellington, New Zealand, 22 p.

[3] Pollard, M. J. 1994. Chemical marking of juvenile snapper (*Pagrus auratus;* Sparidae) by the incorporation of iron and strontium in the spines, scales and otoliths. B.Sc. Hons. thesis, School of Biological Sciences, Univ. Sydney, Sydney, Australia, 119 p.

It was possible that Sr could affect fish growth. Thus, the change in mean fish weight per tank between the initial stocking of the tanks and the sampling of the fish remaining after 36 days was compared between treatments by using one-way analysis of variance.

Experiment 2: concentration and exposure period

The aim of this experiment was to investigate a range of strontium treatments and exposure periods in order to determine minimum concentrations and times required for marking the dorsal spines of juvenile snapper. It was also possible that different batches could be identified by different levels of strontium incorporation.

The experiment was initiated on 3 March 1994 when the fish were 110 days old and 64 mm mean fork length (SD ±4.1 mm). The 16 treatments comprised three strontium concentrations (Sr^{2+} five times, ten times, and forty times ambient) matched with each of five exposure periods (6, 12, 24, 36, and 48 hours) and a control treatment. Replication comprised two 100 litre tanks per treatment, each containing six fish. Snapper were sampled seven days after the termination of the chemical treatment. Statistical analysis involved comparisons between treatment concentrations, exposure periods, and tanks using an asymmetrical design analysis of variance and the Student-Newman-Keuls (SNK) multiple range test.

Strontium levels in wild fish

Juvenile *P. auratus* were caught between November 1993 and April 1994 with Opera House fish traps ($100 \times 80 \times 60$ cm, stretched mesh size 11 mm) from each of four estuarine locations around the Sydney region. From each estuary 20 fish of similar size to those used in experiments one and two were selected: those from Botany Bay had a mean fork length of 63 mm (SD ±5.9), from Port Jackson, 80 mm (SD ±9.9), from Middle Harbour, 82 mm (SD ±4.4), and from Port Hacking, 80 mm (SD ±5.6).

The main aim of this experiment was to compare naturally occurring levels of dorsal spine strontium in wild-caught juvenile snapper from multiple locations, of potentially different water chemistries, with 1) snapper immersed in Sr 40× ambient for 48 hours, and 2) snapper immersed in Sr 10× ambient for 5 days. Statistical analysis involved one-way analysis of variance and the SNK multiple range test.

Treatment of experimental fish

The experiments were undertaken in an enclosed room containing thirty-two cylindrical 100-litre fibreglass tanks, each with a 500-µm mesh filter, an aquarium air stone and a clear plastic cover. Twice daily water exchange from Salamander Bay was provided by a 1000-litre supply tank for each row of eight tanks. Photoperiod was ten hours of light per day, and tanks were cleaned weekly. Approximately 0.125 g of feed per fish per day was distributed in three equal portions throughout the day. The specially formulated dry snapper diet was a 50% protein extruded diet based on 64% fish meal, similar in composition to that in Quartararo et al. (1992). To maintain a constant stocking density, fish mortalities were compensated for by replacement with similar-size untreated snapper, fin-clipped for identification.

The water quality parameters measured every second day were pH (which averaged approximately 8.1), electrical conductivity (approx. 52 mS/cm), turbidity (generally 0 or 1 NTU), dissolved oxygen (approx. 8.6 mg/L), water temperature (approx. 19–23°C), and salinity (approx. 34.3 ppt). No significant differences were observed between tanks for any of these parameters, which remained within a range generally acceptable for the maintenance of fish health (Poxton and Allouse, 1982).

Treatments were randomly allocated to tanks. All fish chosen appeared healthy and in good condition. Wet weight and fork length were measured prior to introduction of representative groups to the experimental tanks. Sedation was necessary to allow safe handling and was achieved with 0.5 mL/L of benzocaine solution, which comprised 100 g/L ethyl p-amino benzoate ($C_9H_{11}NO_2$) in 70% ethanol solvent (Summerfelt and Smith, 1990). The fish were acclimatized to the tanks for a period of 5 days for experiment 1 and 8 days for experiment 2. Strontium treatments were initiated by dissolving the appropriate quantity of chemical salt in each tank. For the duration of the treatment there was no water exchange or cleaning activities. There was also no feeding of the fish during treatment to avoid uneven uptake of the chemical marker among individuals as a result of differential feeding (e.g. Gallahar and Kingsford, 1996). Treatment was terminated after the prescribed exposure period by allowing the exchange of new water. Fish were removed by dip net from the tanks and placed into a lethal dose of 1.0 mL/L benzocaine solution at the appropriate time of sampling, then measured, and frozen for storage.

Dorsal spine tissue samples were dissected from the treated and control snapper. Each sample consisted of four sturdy spines from the midfront of the dorsal row. The weaker posterior spines were avoided where possible. These samples were then placed into polypropylene eppendorf tubes and dried overnight in a 75°C oven. The tubes were then cooled for half

an hour prior to weighing. The tubes were sealed during this time to reduce moisture uptake which could have caused an increase in weight. Samples were weighed to the nearest 0.001 mg.

The remaining steps took place in a laminar flow cabinet to avoid contamination. With a pipette we placed 50 µL of 1% nitric acid (H_2NO_3) into the eppendorf tube containing the spines for 10 seconds and then removed the acid. This procedure cleaned possible external contaminants from the surface of the sample. The time and concentration needed for cleaning were determined by microscopic examination of the spines to check for excessive etching and loss of bone. The contents of the eppendorf tube were then dissolved in a proportional amount of 75% nitric acid to produce a standard 1 mg of spine tissue per 10 µL of solution. After allowing an hour or more for the sample to fully dissolve in the sealed tube, 10 µL of solution was removed and added to a sample tube containing 4950 µL of milliQ water and 40 µL of concentrated nitric acid. This resulted in a standardized 0.2 mg/µL sample solution in 1% nitric acid which became the sample concentration used in our experiment after preliminary analyses with ICP-MS.

Measurement of isotopes

Inductively coupled plasma-mass spectrometry (ICP-MS) was used to measure concentrations of strontium in the dorsal spine tissue. The model used was a Perkin-Elmer SCIEX ELAN 5000 with a Gilson 212B autosampler for sample introduction, and procedures were similar to those described in Dove et. al. (1996). The ICP-MS was optimized with a 0.010 mg/L standard solution covering the extremes of the mass range. By using the graphics application, we were able to adjust the nebulizer flow to maximize the ^{103}Rh signal and balance the signals for ^{24}Mg and ^{208}Pb from the standard solution. The quantitative analysis function of the ICP-MS was externally calibrated by using calibration standards with concentrations of 1, 10, 100, and 500 parts per billion, i.e. those covering the range of expected concentrations. The correlation coefficient of the calibration curve was generally 1.000 for ^{86}Sr, the isotope used for analysis. ICP-MS interferences such as isobaric overlaps or plasma-induced polyatomic ions are generally negligible for strontium. A blank solution containing only the 1% nitric acid matrix was also analyzed prior to the sample solutions, so that the isotopic concentration of the acid matrix could be subtracted.

Table 1

Analysis of variance for experiment 1: persistence over 36 days. Three-way partially hierarchical analysis of variance to test for differences in dorsal spine ^{86}Sr among sample times 0, 12, 24, and 36 days (fixed factor=time), treatments (treat) (Sr^{2+} zero, 5× and 10× ambient; fixed factor), and tanks (random factor). Nonsignificance at P=0.05 is indicated by n.s. Cochran's test; df = 1; no. variances (k) = 48; and C = 0.1565; variances homogeneous; no transformation.

Source	df	Mean square	F-ratio	Probability
Time	3	13699.2	6.690	<0.005
Treatment	2	568635.5	105.485	<0.001
Time × treat	6	4536.0	2.215	n.s.
Tanks (treat)	9	5390.7	2.632	<0.050
Time × tanks (treat)	27	2047.8	0.695	n.s.
Residual	48	2946.5		

Quality control solutions were analyzed for ^{86}Sr at the start, middle, and end of each analytical run for each date of analysis, allowing the stability of the analyte signal to be monitored both within and between runs. The quality control readings obtained were not constant over time, owing perhaps to clogged sampling cones. The results for the spine samples, therefore, required adjustment in relation to the quality control results. We assumed that there was no change in the ^{86}Sr concentration of the quality control solution over time. External drift correction can significantly improve both accuracy and precision (Jarvis et al., 1992).

Treatment of data

Data were analyzed by analysis of variance according to the general recommendations of Underwood (1981). Normality of data was tested with Cochran's test. Student-Newman-Keuls (SNK) multiple range tests were used for *a posteriori* comparisons among treatments where appropriate. Where data were missing (<2 per design), the missing values were replaced by the means for their group, and the degrees of freedom of the residual were reduced by the number of missing values.

Results

Persistence of strontium over 36 days

Differences in the ^{86}Sr concentrations of dorsal spines were found among treatments, despite differences among tanks within treatments (Table 1; Fig. 1). Fish exposed to strontium at 10× ambient incorporated more strontium into their spines than fish at 5×

ambient (SNK: Control<Sr 5× <Sr 10×). Slight fluctuations, particularly in the control and Sr 10× ambient treatments (Fig.1), caused significant differences to be found between some sampling times (Table 1), however these were not picked up by the less sensitive SNK test (0 days=36 days=12 days=24 days). The ranks of different treatments remained similar irrespective of sample time (i.e. no sample time × treatment interaction) and there was no indication of a significant decay of the strontium signal over the 36 days of the experiment. There were no significant differences between tanks within each treatment.

There were no significant differences observed among treatments for the growth of snapper as measured by the change in mean wet weight per tank between the initial stocking and sampling at 36 days (ANOVA: df=2, 9; F=3.27; P=0.086, n.s.). Mortality rates were also very low for all treatments. These results suggested that effects on health or growth from the immersion of fish in strontium were minimal.

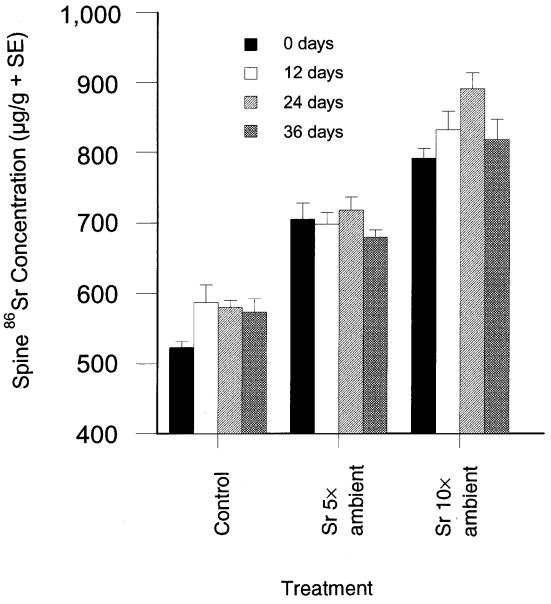

Figure 1

Persistence over 36 days of ^{86}Sr in the spines of juvenile snapper immersed in zero, 5× ambient and 10× ambient strontium for 5 days.

Strontium concentration and exposure period

^{86}Sr was incorporated rapidly into the dorsal spines of treated snapper and on average the concentrations were significantly higher in treated fish than in controls (Table 2; Fig. 2). Significant differences were found among groups of snapper treated with different $SrCl_2$ concentrations, and the magnitude of these differences varied with exposure period, resulting in a significant concentration × exposure period interaction (Table 2). Strontium incorporation increased as the exposure period increased, and the magnitude of this change was also not equal between treatments. The signals from the Sr 40× treatment were generally much greater than either of the Sr 5× or Sr 10× treatments, which were similar to one another, and the Sr 40× signal also increased much more rapidly between exposure periods (Fig. 2).

Strontium in wild fish

The experimental snapper immersed in Sr 10× for 5 days and Sr 40× for 48 hours had spine ^{86}Sr concentrations higher than those of any of the control groups (ANOVA: df=6, 105; F=70.9; P<0.001; Fig. 3), with respective concentrations 1.37 (mean 833 µg/g) and 1.55 (942 µg/g) times greater than the maximum mean concentrations of any wild fish (Port Hacking; 607 µg/g).

There were also significant differences between estuaries of origin of the wild-caught fish (ANOVA: df= 3, 74; F= 5.8; P < 0.01; Fig. 3). Fish from Botany Bay had lower levels of strontium than those from the other estuaries (SNK: Botany Bay<Port Jackson=Middle Harbour=Port Hacking). There was a 102 µg/g difference in mean spine strontium between the highest and the lowest estuary of origin. Fish from Port Jackson and Middle Harbour had intermediate values that were similar to one another. The fish used as controls during the experimental period at Port Stephens also

Table 2

Analysis of variance for experiment 2: asymmetrical design analysis of variance to test for differences in spine ^{86}Sr incorporation among treatment concentrations (conc.) (Sr^{2+} 5×, 10× and 40× ambient; fixed factor), exposure periods (exp.) (6–48 hours; random factor), and tanks (random factor). Nonsignificance at P=0.05 is indicated by n.s. Cochran's test: degrees of freedom (df) = 5; no. of variances (k) = 32; C = 0.1329; variances homogeneous; no transformation.

Source	df	Mean square	F-ratio	Probability
Concentration	2	1005648	18.167	<0.01
Exposure period	4	204047.2	163.683	<0.01
Conc. × exp.	8	55355.9	44.405	<0.01
Tanks (conc. × exp.)	15	1246.6	0.575	n.s.
Control vs. rest	1	237036.4	190.146	<0.01
Residual	150	2166.3		

had similar concentrations to the wild-caught control fish.

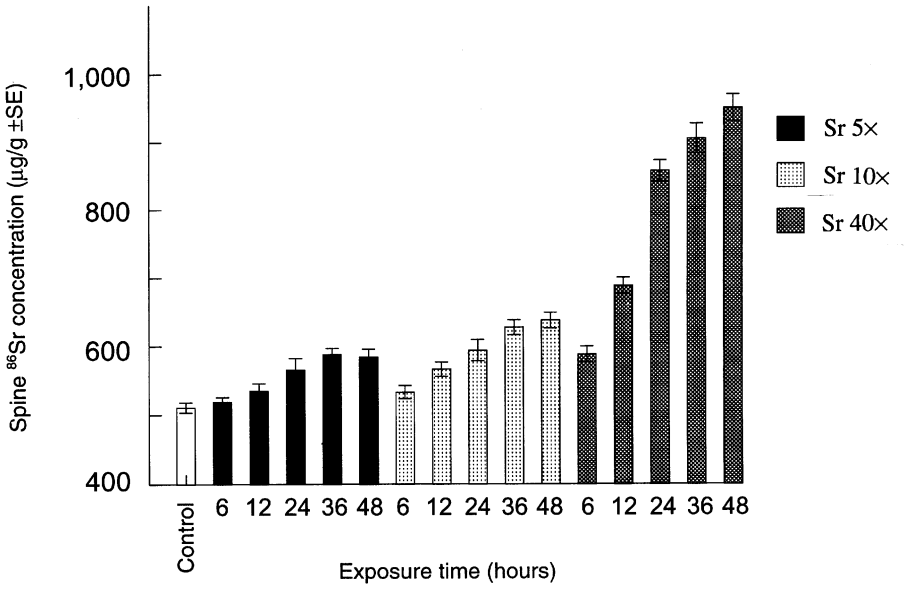

Figure 2

Differences in ^{86}Sr incorporation into the spines of juvenile snapper treated with different immersion concentrations and exposure periods.

Discussion

Persistence of strontium

Fish that were chemically tagged with strontium could be distinguished from control fish, and the chemical marks were persistent for at least 36 days, showing no decrease and no regular pattern of change. Although in practice the length of a tagging experiment would be likely to exceed this time frame, persistence over 36 days may indicate that the strontium signal is also likely to persist over longer periods. This conjecture, however, remains to be validated and may be dependent on factors such as bone metabolism and growth. Artificially induced strontium signals in fish scales (Ophel and Judd, 1968) and otoliths (Brown and Harris, 1995; Schroder et al., 1995, 1996) have been shown to be detectable for periods of greater than a year, and other studies investigating these components have also shown successful strontium incorporation (Table 3).

Scales (Lapi and Mulligan, 1981) and otoliths (Edmonds et al., 1989) are relatively metabolically inert tissues, and there is little biological reworking of trace elements deposited within them. On the other hand, the living cells of bone tissue may be metabolically active during growth and there may be some turnover of material over the long term. This bone remodeling is related to morphogenesis and physiological factors and involves a balance between bone resorption and bone redeposition (Francillon-Veillot et al., 1990). Nevertheless, it is likely that spines are fairly inert components of the skeleton and that active resorption is unlikely to be high, indicated by the ability to age some fish by the growth rings in spines (e.g. McFarlane and Beamish, 1987; Brennan and Cailliet, 1989; Welch et al., 1993). In addition, similar chemical processes of strontium incorporation during calcification exist between scales, otoliths, and bone, and it was expected that the incorporation of strontium into the spines would result in a relatively constant mark.

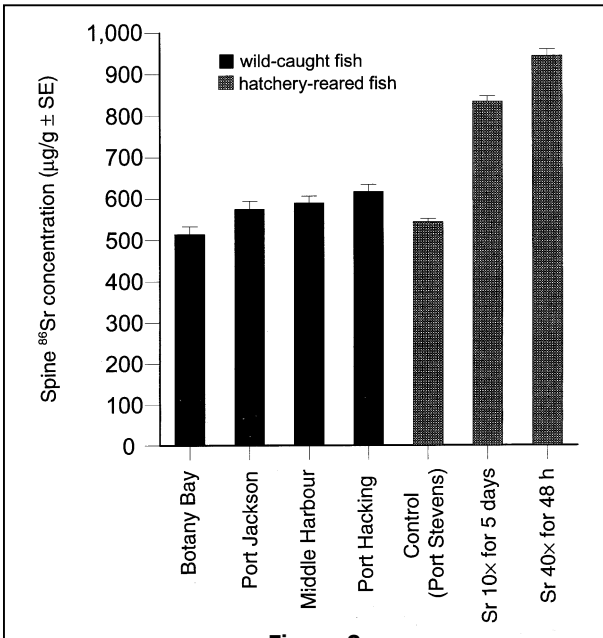

Figure 3

Naturally occurring levels of ^{86}Sr in the spines of wild *Pagrus auratus* from four estuaries in New South Wales, Australia, compared wirh those of hatchery-reared fish (controls from Port Stephens, N.S.W.) and fish treated with strontium.

Behrens-Yamada et al. (1979) found that strontium appears to become metabolically inert in the vertebrae of coho salmon, *Oncorhynchus kisutch,* after

Table 3

Comparison of previous literature in which nonradioactive strontium was incorporated into the tissues of fish for the purposes of chemical marking.

Reference	Species investigated	Aquatic medium	Method of administration	Skeletal structure	Effectiveness
This study	snapper juveniles (*Pagrus auratus*)	saltwater	immersion in 0.125g/L to 1.0 g/L Sr^{2+} (5 to 40× ambient) for 6 h to 5 days	dorsal spines	Strontium levels of greater than 10× ambient for 4–5 days produced marks which could be reliably distinguished from controls and wild snapper.
Ophel and Judd (1968)	goldfish (*Carassius auratus*)	freshwater	32 g of strontium lactate per 400 g of wet diet	scales	More than 10 times the levels of Sr in controls after one year.
Behrens-Yamada et al. (1979)	coho salmon (*Oncorhynchus kisutch*)	freshwater treatment, released into saltwater	10,000 ppm Sr introduced through the diet over 60 days	vertebrae	32 times as much Sr as controls when released; 1.4 times as much in jacks after 5 months; adults could not be distinguished.
Behrens-Yamada and Mulligan (1982)	coho salmon adults	as above	as above	core samples of scales and vertebrae	Successful identification of adults after 5 months.
Behrens-Yamada and Mulligan (1987)	coho salmon fry	freshwater	immersion in 1 µg/mL Sr for 49 days	vertebrae	Sr mark still detectable after 169 days despite dilution.
Guillou and de la Noue (1987)	brook trout (*Salvelinus fontinalis*)	freshwater	200–3200 µg/g Sr^{2+} introduced through the diet for 4 and 6 weeks	opercular bones	Able to distinguish between marked and unmarked fish at the lowest levels of ingested Sr.
Snyder et al. (1992)	rainbow trout (*Oncorhynchus mykiss*)	freshwater	immersion in 1.8, 3.6 and 5.4 mg/L Sr for 30 days and 60 days	scales	A twofold increase in exposure period and a two- or threefold increase in immersion concentration produced corresponding two- and threefold increases in incorporation.
Brown and Harris (1995)	golden perch (*Macquaria ambigua*) and trout cod (*Maccullochella macquariensis*)	freshwater	immersion in 1–20 g/L $SrCl_2$ for one to four days	otoliths	Still able to distinguish after 12 months. No measurable growth, health, or mortality effects.
Mugiya and Tanaka (1995)	goldfish (*Carassius auratus*)	freshwater	immersion in 0.1, 1.0, and 10.0 mg/L Sr for 88 days	otoliths, ribs, and scales	Strontium in otoliths remained after 92 days, whereas that in scales and ribs decreased.
Schroder et al. (1995)	chum (*Oncorhynchus keta*) and sockeye salmon (*O. nerka*) fry	freshwater	immersion in 120, 1200 and 9000 ppm $SrCl_2$ for 24 hours	otoliths, vertebrae and opercula	Able to distinguish after 34 days, and after 21 months in otoliths of fish immersed in 5000 ppm for 24 hours.
Schroder et al. (1996)	salmon fry	freshwater	immersion in 9000 ppm $SrCl_2$ for 12 hours or less	otoliths	Fish held for as little as 30 minutes in 9000 ppm were distinguishable for up to 2 years.
Gallahar and Kingsford (1996)	rock blackfish (*Girella elevata*)	saltwater	immersion in $SrCl_2$ 2.5× ambient	otoliths	Able to distinguish between marked fish and controls.

incorporation. Their inability to identify strontium-marked adults in following years was attributed entirely to dilution caused by the growth of vertebrae, rather than a process of leaching from the tissue. Behrens-Yamada and Mulligan (1982) found that the use of smaller vertebral core samples allowed the identification of strontium-labelled adult salmon. The limiting core diameter was related to the vertebral diameter of the juvenile salmon at the time of treatment.

Similarly, the growth of juvenile snapper spines will cause a dilution of the strontium signal. The ratio of marked to unmarked spine matrix will decrease as the spine enlarges. Spine growth occurs from the base upwards (e.g. McFarlane and Beamish, 1987), and the strontium mark may remain only in the upper regions as spine elongation and enlargement occurs. Only the uppermost section of the spine, corresponding to the length of the spine at the age of incorporation, should be sampled in large fish; this will result in a proportional increase in the amount of strontium in the sample.

Papadopoulou et al. (1980) found that a range of chemical constituents in the otoliths of the mackerel *Scomber japonicus colias* decreased with age. Dilution from growth, dietary changes with age, and otolith compositional changes were mentioned as possible causes. Nevertheless, the duration of most tagging experiments does not generally exceed a period of several years, and growth or age-related problems are unlikely to be a major concern over such time scales. The differences between the strontium-tagged and wild fish are great enough for there to be little confusion between the two groups if samples are taken from the uppermost spine region and tagging experiments are not designed to extend over the very long term.

Strontium in tagged and wild fish

It was a major concern for our study that naturally occurring variation in the levels of strontium in wild fish might obscure the strontium signal in fish that had been tagged by immersion in $SrCl_2$. Adult snapper may undertake movements associated with feeding or spawning (Crossland, 1976), extending the range of water chemistries experienced by the fish. Moreover, juveniles may remain resident in shallow bays and estuaries that are subject to variable inputs of fresh water (Kingsford and Suthers, 1994) and that are likely to have differences in water chemistry.

Despite the observed variation in natural levels of strontium in wild snapper, the treatments with Sr 10× for 5 days (Fig. 3) and Sr 40× for 24 to 48 hours (Figs. 2 and 3) had unequivocally higher readings. Other treatments also had higher readings. They were, however, not considered great enough for effective tagging owing to the increased variation in spine strontium expected to result from growth factors and exposure to the variable marine environment. The minimum concentration recommended is Sr^{2+} 10× ambient, with an exposure period of at least 4–5 days at this concentration. Shorter exposure periods will require concentrations greater than Sr 10×, and snapper significantly larger than those used in our study may also require higher treatment concentrations. Although the comparisons were made with wild snapper captured within a period of six months in the general region of Sydney across a range of less than 40 km of coastline, it is unlikely that fish tagged with strontium at this recommended level would be confused with wild fish from any latitude or time.

It is not surprising to find differences in naturally occurring strontium among fish from different estuaries. For example, Edmonds et al. (1989) investigated eight elements, including strontium and found elemental compositions to be specific to the geographical origin of nonmixing populations of snapper in Western Australia. Similarly, Port Jackson, Botany Bay, and Port Hacking are distinct estuaries which would also be expected to contain nonmixing juvenile snapper populations. On the other hand, Middle Harbour is within Port Jackson, and interestingly no significant difference has been observed between these two groups.

A variety of factors influence natural variation in the Sr content of otoliths. Salinity is perhaps the strongest determinant, and analysis of strontium levels has long been used to interpret the salinity histories of anadromous or catadromous fish species (Castonguay and Fitzgerald, 1982; Coutant and Chen, 1993; Limburg, 1995; Secor et al., 1995; Pender and Griffin, 1996). Decreasing water temperature causes an increase in otolith Sr/Ca ratio, with the greatest effect occurring at low temperatures. This knowledge has been used in attempts to reconstruct temperature histories of wild fish (Radtke and Targett, 1984; Radtke et al., 1990; Townsend et al., 1989, 1992, 1995, Arai et al., 1996). It has been suggested that low temperatures may impair physiological mechanisms that exist in fish and inhibit the uptake of strontium (Kalish 1989; Townsend et al., 1992). Other environmental and biological factors that influence the natural markings of calcified structures include pH (Moreau et al., 1983; Wickins, 1984), fish age and size (Papadopoulou et al., 1980; Gauldie et al., 1995), developmental and reproductive events (Francis, 1994; Kingsford and Atkinson, 1994), physiology (Kalish, 1991), periods of stress (Townsend et al., 1992), dietary differences (Edmonds et al., 1989;

Limburg, 1995; Gallahar and Kingsford, 1996), and anthropogenic pollutants (Kalish, 1995). All of these factors may cause variation in levels of strontium in wild fish. Hence, if strontium tagging is to be successful in a region, the magnitude of natural variation should be investigated at appropriate spatial and temporal scales.

Strontium tagging has been used for freshwater and anadromous species (Table 3), but investigations of saltwater species are limited. Hurley et al. (1985) marked the statoliths of the short-finned squid *Illex illecebrosus* and Gallahar and Kingsford (1996) marked the rock blackfish *Girella elevata*. Because seawater has some 200–400 times the strontium content of freshwater (Guillou and de la Noue, 1987), the range of concentrations available for tagging (i.e. before saturation and precipitation) is reduced for marine fish. This limited our ability to batch mark separate groups of fish with different concentrations of the same element, especially considering the unknown factors affecting long-term persistence, such as dilution due to growth. Multiple immersions in strontium have, however, created multiple, discrete bands on the otoliths of salmon, allowing the production of codes for batch marking (Schroder et al., 1996). Another attractive possibility is to mark batches of fish with different combinations of several elements. A characteristic of ICP-MS analysis is the ease in identification of a number of isotopes with little corresponding increase in preparation time. Schroder et al. (1996) found that rubidium was incorporated into the calcified tissues of juvenile salmon. Caesium, which has a similar ionic radius and the same +2 valence as calcium, may also prove to be a likely candidate for consideration. Pollard[3] investigated iron chloride ($FeCl_3 \cdot 6H_2O$) together with strontium chloride, however the iron was not successfully incorporated into the dorsal spines of snapper.

Advantages, limitations, and applications of the technique

The strontium immersion technique allows hundreds of fish to be tagged in a single batch without the need for individual treatment. Other more labor intensive methods may introduce problems of stress from fish handling and are unsuitable for large numbers, especially the very large numbers involved in reseeding programs. Stock enhancement in Japanese waters exceeds 15 million juvenile snapper each year (Tsukamoto et al., 1989). Strontium tagging may be applied to very small fish and even larval fish (e.g. Behrens-Yamada and Mulligan, 1987). Fast body growth and the difficulties of attachment make the conventional tagging of small fish extremely difficult. Some alternatives to batch tagging with strontium and with comparable advantages include microwire tags (Beukers et al., 1995), thermal marking (Schroder et al., 1996), oxytetracycline (Francis et al., 1992; Lang and Buxton, 1993), alizarin complexone (Lang and Buxton, 1993; Secor and Houde, 1995) and other chemical fingerprints (e.g. Gillanders and Kingsford, 1996). It may be useful for some purposes to use a combination of techniques simultaneously.

The costs associated with the analysis of samples on ICP-MS are relatively high compared with those of many conventional tagging techniques, however they may compare favourably with the costs of some other chemical, electronic, or genetic methods of identification. The ICP-MS has very low detection limits, generally 100 to 1000 times more sensitive than inductively coupled plasma-atomic emission spectroscopy and inductively coupled plasma-atomic fluorescence spectrometry (Horlick and Shao, 1992). Sample preparation for ICP-MS is labor intensive; around 100 fish samples require up to 2 days preparation and a half day for analysis. Strontium marks however may also be detected by using other analytical techniques with lower costs, such as energy-dispersive x-ray spectrometry or wavelength dispersive spectrometry (Schroder et al., 1995). Many other analytical techniques, such as backscattered electron microscopy (e.g. Schroder et al., 1995), or laser ablation ICP-MS (e.g. Fowler et al., 1995), are also designed to measure microconstituents at specific loci across a section of calcified tissue, allowing the application of strontium marking for the validation of aging and batch marking of groups of fish.

Otoliths are commonly used for chemical marking and are the most widely used structures for age determination (Campana and Neilson, 1985). Otoliths comprise discrete, directly comparable samples and are recognized for their elemental stability (Edmonds et al., 1989). However, fish must be sacrificed for otolith sample collection. By contrast, spine and scale removal are nondestructive techniques, allowing catch and release techniques to be used for tagged fish. Spines are not shed or replaced during growth, unlike scales which may be lost, resorbed, or regenerated during fish ontogeny (Coutant and Chen, 1993). The small size of juvenile snapper scales also creates counting, weighing, and manipulation difficulties (Pollard[3]). The dissection of otoliths or other internal skeletal structures from large groups of fish is a labor intensive activity. The ease of removal of spines or scales allows for the routine sampling of these tissues regardless of the original purpose of fish collection. Removal for sampling could take place before the distribution of fish for commercial pur-

poses. Removal of spines or scales causes minimal physical defacement of the product, an aspect of particular relevance to the Japanese market where aesthetic appeal is highly valued.

The immersion technique for incorporation of strontium enables greater control over the degree of exposure of fish to strontium than does introduction through the diet. The latter method may introduce inconsistencies between experimental fish as a result of differential feeding, which often results from size hierarchies within the tanks (Umino et al., 1993). Intraperitoneal or intramuscular injection allows similar control of exposure to that of immersion; however each fish must be dealt with separately. Scott (1961) was unable to mark fish under 8 cm long by using injection techniques. Nevertheless, with large pelagic fish injection provides many advantages, including the ability to tag directly from the capture vessel.

The strontium tag showed no signs of decay due to leaching from the spine tissue, and tag retention times may prove to compare favorably with conventional methods. External physical tags are shed at different rates for different species, fish sizes, tag types, or attachment sites (e.g. Ingram, 1993), and may facilitate disease. Tattoos may be rendered unreadable by fish growth, and vital dyes may be leached from the animal (Laird and Stott, 1978). Clipped fins may regenerate, or there may be confusion between clipped fins and fins that have been naturally excised by predators. Population studies also assume that the proportion of tagged to untagged fish captured is representative of that existing in the population. These assumptions hold for strontium marking, whereas individuals marked with tags may be less fit for survival, more conspicuous to predators, or more likely to become entangled in capture nets.

Radioactive isotopes are effective markers of fish, as evidenced by the permanent marking of many fish by ^{14}C after atomic bomb testing in the 1950s (Kalish, 1995). A number of different radioactive isotopes have been investigated for fish tagging, for example ^{45}Ca (Bogoiavlenskaia, 1959; Anwand, 1966), ^{39}P (Karzinkin et al., 1959), ^{59}Fe (Scott, 1961), ^{137}Cs (Scott, 1962), ^{144}Ce (Hoss, 1967), ^{131}I (Fitzgerald and Keenleyside, 1978), ^{152}Eu and ^{155}Eu (Hansen and Fattah, 1986), ^{85}Sr (Carlson and Shealy, 1972; Lehtonen et al., 1992), ^{89}Sr (Farrell and Campana, 1996), and ^{90}Sr (Zhao et al., 1992). Marking with stable strontium salts however has many advantages over radioactive tagging, which is comparatively expensive, environmentally unattractive, and a potential threat to human health. The latter is an important criticism when radioisotope techniques are used to mark commercially important species, such as radioactive iridium used to tag and release snapper (Kato, 1990; Kato et al., 1991). Conversely, strontium immersion has in fact been suggested as a low-level marking technique for farm-reared brook trout destined for human consumption (Guillou and de la Noue, 1987).

A major disadvantage of the technique is that the existence of the strontium mark cannot be detected in recaptured fish prior to analysis. This means that large batches of snapper of the appropriate size may have to be analyzed when only a small proportion may be marked individuals. Most conventional tagging methods, including most radioactive isotope markers, allow *in vivo* detection of tagged fish. The number of fish requiring analysis should be minimized by the preselection of appropriate-size fish based on growth rate estimates for the appropriate geographic location (e.g. Francis and Winstanley, 1989; Tsukamoto et al., 1989; Paul, 1992). Moreover, other external features may also aid in the preselection of possibly marked fish; for example, a large proportion of hatchery-reared Japanese snapper lack an internostril epidermis (Sobajima et al., 1986). Salmon stocks of different origin have been identified by using naturally occurring differences in scale patterns (Bilton, 1972), otolith patterns (Hindar and Abee-Lund, 1992), vertebral elemental composition (Mulligan et al., 1983), or parasitic fauna (Margolis, 1963).

Stock enhancement or reseeding programmes may be of particular importance when there has been recruitment failure due to natural or anthropogenic environmental change, or as a result of overfishing. *Pagrus auratus* have been ranched and released for many years in Japan. Concerns have been raised about alterations to the gene pool from the introduction of genetically inferior cultured fish (Hindar et al., 1991; Harada, 1992). Smith and Hataya (1982) have however estimated that the release of one million snapper per year would increase annual net returns to the fishery by about 35 metric tons, and the Kagoshima Bay ranching operation appears to have become highly profitable (Matsuda, 1992). There has also been consideration of the establishment of snapper reseeding operations in the southern hemisphere (Smith and Francis[2]). Strontium tagging can assess the economic or conservation value of such stock enhancement operations and lead to refinement of the reseeding techniques, for example selection of the optimal locations and fish sizes for release. Consideration of migratory patterns (e.g. Crossland, 1976; Edmonds et al., 1989) is required to achieve spatial separation of studies, and temporal division of studies may also be achieved by the use of different cohorts separated by sufficient time to allow size separation. The success of the strontium immersion tech-

nique for *Pagrus auratus* indicates that it is probably applicable to many other saltwater fish species, and perhaps also to crustaceans and mollusks, but experimental validation would be required for each species.

Conclusions

The present study has demonstrated that the snapper *Pagrus auratus* can be reliably tagged by the incorporation of strontium into the dorsal spines. The use of strontium as a chemical marker can allow large numbers of small hatchery-bred fish to be tagged before release into the wild. Importantly, fish do not have to be sacrificed to assess whether they have been tagged. Strontium in the spines of snapper persisted for at least 36 days and there was no suggestion that the chemical signal decayed over this time. It is recommended that juvenile snapper require immersion in treatments of strontium chloride greater than 0.25 g/L (10× ambient) for 4 to 5 days for the production of reliable strontium marks in spines. Although natural levels of strontium in spines varied between some locations along the central coast of New South Wales, this variation was not great enough to obscure the differences between tagged and wild fish. It was concluded, therefore, that strontium immersion is a useful and relatively environmentally safe method of tagging large numbers of small snapper.

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Literature cited

Anwand, K.
1966. Die Anreicherung von ^{45}Ca und ^{91}Y in Brut von *Esox lucius* L. und *Anguilla anguilla* L. Verhandlungen der Internationale Vereinigung der Limnologie. 16:1124–1129.

Arai, N., W. Sakamoto, and K. Maeda
1996. Correlation between ambient seawater temperature and strontium-calcium ratios in otoliths of red sea bream *Pagrus major*. Fish. Sci. (Tokyo) 62:652–653.

Battaglene, S., and J. Bell.
1991. Aquaculture prospects for marine fish in New South Wales. Fishnote DF/6, NSW Agriculture and Fisheries, Sydney, Australia, 3 p.

Battaglene, S. C., and R. B. Talbot.
1992. Induced spawning and larval rearing of snapper, *Pagrus auratus* (Pisces: Sparidae), from Australian waters. N.Z. J. Mar. Freshwater Res. 26:179–183.

Behrens-Yamada, S., and T. J. Mulligan.
1982. Strontium marking of hatchery-reared coho salmon, *Oncorhynchus kisutch* Walbaum, identification of adults. J. Fish Biol. 20:5–9.
1987. Marking nonfeeding salmonid fry with dissolved strontium. Can. J. Fish. Aquat. Sci. 44:1502–1506.

Behrens-Yamada, S., T. J. Mulligan, and S. J . Fairchild.
1979. Strontium marking of hatchery-reared coho salmon *Oncorhynchus kisutch,* Walbaum. J. Fish. Biol. 14:267–275.

Beukers, J. S., G. P. Jones, and R. M. Buckey.
1995. Use of implant micro-tags for studies on populations of small reef fish. Mar. Ecol. Prog. Ser. 125:61–66.

Bilton, H. T.
1972. Identification of major British Columbia and Alaska runs of even-year and odd-year pink salmon from scale characters. J. Fish. Res. Board Can. 29:295–301.

Bogoiavlenskaia, M. P.
1959. A study of calcium metabolism with a view to utilising ^{45}Ca as a mark for fish. Vsesoiuznyi N.-I. Institut Morskovo Rybnovo khoziaistva i Okeanografii, 55 p.

Brennan, J. S., and G. M. Cailliet.
1989. Comparative age-determination techniques for white sturgeon in California. Trans. Am. Fish. Soc. 118:296–310.

Brown, P., and J. H. Harris.
1995. Strontium batch marking of golden perch (*Macquaria ambigua* Richardson) (Percichthyidae) and trout cod (*Maccullochella macquariensis)* (Cuvier). *In* D. H. Secor, S. E. Campana, and J. M. Dean (eds.), Recent developments in fish otolith research, p. 693–701. Univ. South Carolina Press, Columbia, SC.

Campana, S. E., and J. D. Neilson.
1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014–1032.

Carlson, C. A., and M. H. Shealy.
1972. Marking larval largemouth bass with radiostrontium. J. Fish. Res. Board Can. 29:455–459.

Castonguay, M., and G. J. Fitzgerald.
1982. Examination of the method of discriminating between anadromous and freshwater fish of the same species by the content of strontium in the scales. Can. J. Fish. Aquat. Sci. 39:1424–1425.

Coutant, C. C., and C. H. Chen.
1993. Strontium microstructure in scales of freshwater and estuarine striped bass (*Morone saxatilis*) detected by laser ablation mass spectrometry. Can. J. Fish. Aquat. Sci. 50:1318–1323.

Crossland, J.
1976. Snapper tagging in north-east New Zealand, 1974: analysis of methods, return rates, and movements. N.Z. J. Mar. Freshwater Res. 10:675–686.
1980. Population size and exploitation rate of snapper, *Chrysophrys auratus*, in the Hauraki Gulf from tagging experiments, 1975–76. N.Z. J. Mar. Freshwater Res. 14:255–261.

Davy, B.
1990. Mariculture in Japan: development of an industry. World Aquacult. 21:36–47.

1991. Mariculture in Japan: current practices. World Aquacult. 22:30–35.

Dove, S. G., B. M. Gillanders, and M. J. Kingsford.
1996. An investigation of chronological differences in the deposition of trace metals in the otoliths of two temperate reef fish. J. Exp. Mar. Biol. Ecol. 205:15–33.

Edmonds, J. S., M. J. Moran, and N. Caputi.
1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. Can. J. Fish. Aquat. Sci. 46:50–54.

Farrell, J., and S. E. Campana.
1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology A 115:103–109.

Ferrell, D. J., G. W. Henry, J. D. Bell, and N. Quartararo.
1992. Validation of annual marks in the otoliths of young snapper, *Pagrus auratus* (Sparidae). Aust. J. Mar. Freshwater Res. 43:1051–1055.

Fitzgerald, J. G., and H. A. Keenleyside.
1978. Technique for tagging small fish with I131 for evaluation of predator-prey relationships. J. Fish. Res. Board Can. 35:143–145.

Foscarini, R.
1988. A review: intensive farming procedure for red sea bream (*Pagrus major*) in Japan. Aquaculture 72:191–246.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold.
1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. 52:1431–1441.

Francillon-Vieillot, H., V. de Buffrenil, J. Castenet, J. Geraudie, F. J. Meunier, J. Y. Sire, L. Zylberberg, and A. de Ricqles.
1990. Microstructure and mineralization of vertebrate skeletal tissues. *In* J. G. Carter (ed.), Skeletal biomineralization: patterns, processes and evolutionary trends, vol. 1, p. 471–530. Van Nostrand Reinhold, New York, NY.

Francis, M. P.
1994. Duration of larval and spawning periods in *Pagrus auratus* (Sparidae) determined from otolith daily increments. Environ. Biol. Fish. 39:137–152.

Francis, R. I., L. J. Paul, and K. P. Mulligan.
1992. Ageing of adult snapper *(Pagrus auratus)* from otolith annual ring counts: validation by tagging and oxytetracycline injection. Aust. J. Mar. Freshwater Res. 43:1069–1089.

Francis, R. I., and R. H. Winstanley.
1989. Differences in growth rates between habitats of southeast Australian snapper (*Chrysophrys auratus*). Aust. J. Mar. Freshwater Res. 40:703–710.

Fukuhara, O.
1985. Functional morphology and behaviour of early life stages of red sea bream. Bull. Jpn. Soc. Sci. Fish. 51:731–743.

Fukusho, K.
1991. Red sea bream culture in Japan. *In* J. P. McVey (ed.), CRC handbook of mariculture, vol, II: finfish mariculture, p. 73–87. CRC Press, Boca Raton, FL.

Gallahar, N. K., and M. J. Kingsford.
1992. Patterns of increment width and Sr/Ca ratios in otoliths of juvenile rock blackfish, *Girella elevata*. J. Fish Biol. 41:749–763.
1996. Factors influencing Sr/Ca ratios in *Girella elevata*: an experimental approach. J. Fish. Biology 48:174–186.

Gauldie, R. W., I. F. West, and G. E. Coote.
1995. Evaluating otolith age estimates for *Hoplostethus atlanticus* by comparing patterns of checks, cycles in microincrement width, and cycles in strontium and calcium composition. Bull. Mar. Sci. 56:76–102

Gilbert, D. J.
1986. A stock reduction analysis of Bay of Plenty snapper. N.Z. J. Mar. Freshwater Res. 20:641–653.

Gillanders, B. M., and M. J. Kingsford.
1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141:13–20.

Guillou, A., and J. de la Noue.
1987. Use of strontium as a nutritional marker for farm-reared brook trout. Prog. Fish Cult. 49:34–39.

Hansen, H. J. M., and A. T. A. Fattah.
1986. Long-term tagging of elvers, *Anguilla anguilla,* with radioactive europium. J. Fish Biol. 29:535–540.

Harada, Y.
1992. Genetic difference between wild and released individuals and the resource enhancement effect of stocking: a theoretical analysis. Nippon Suisan Gakkaishi 58:2269–2275.

Hindar, K., and J. H. Abee-Lund.
1992. Identification of hatchery-reared and naturally produced Atlantic salmon *Salmo salar* L. juveniles based on examination of otoliths. Aquacult. Fish. Manage. 23:235–241.

Hindar, K., N. Ryman, and F. Utter.
1991. Genetic effects of cultured fish on natural fish populations. Can. J. Fish. Aquat. Sci. 48:945–957.

Horlick, G., and Y. Shao.
1992. Inductively coupled plasma-mass spectrometry for elemental analysis. *In* A. Montaser and D. W. Golightly (eds.), Inductively coupled plasmas in analytical atomic spectrometry, p. 551–612. VCH Publishers, New York, NY.

Horne, R. A.
1969. Marine chemistry. Elsevier, Amsterdam, The Netherlands, 568 p.

Hoss, D. E.
1967. Marking of post-larval paralichthid flounders with radioactive elements. Trans. Am. Fish. Soc. 96:151–156.

Hurley, G. V., P. H. Odense, R. K. O'Dor, and E. G. Dawe.
1985. Strontium labelling for verifying daily growth increments in the statolith of the short-finned squid (*Illex illecebrosus*). Can. J. Fish. Aquat. Sci. 42:380–383.

Ingram, B.A.
1993. Evaluation of coded wire tags for marking fingerling golden perch, *Macquaria ambigua* (Percichthyidae), and silver perch, *Bidyanus bidyanus* (Teraponidae). Aust. J. Mar. Freshwater Res. 44:817–824.

Ishibasi, K.
1986. A statistical assessment on the effect of liberation of red sea bream *Pagrus major* larvae in sea-farming. Bull. Tokai Reg. Fish. Res. Lab. 119:93–107.

Jarvis, K. E., A. L. Gray, and R. S. Houk .
1992. Handbook of Inductively Coupled Plasma Mass Spectrometry. Blackie and Son, London, U.K., 380 p.

Jones, G. K.
1981. Biological research on snapper (*Chrysophrys auratus,* syn. *unicolor*) and an analysis of the fishery in Northern Spencer Gulf. SAFIC (South Australian Department of Fisheries, Adelaide) 5(6):5–8.

Kalish, J. M.
1989. Otolith microchemistry: validation of the effects of

physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132:151–178.

1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish. Bull. 88:657–666.

1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. Mar. Ecol. Prog. Ser. 74:137–159.

1995. Application of the bomb radiocarbon chronometer to the validation of redfish *Centroberyx affinis* age. Can. J. Fish. Aquat. Sci. 52:1399–1405

Karzinkin, G. S., E. V. Soldatova, and I. A. Shekhanova.

1959. Some results of mass marking of "non-standard" sturgeon fingerlings by means of radioactive phosphorus. Animal Migrations 1:27–40.

Kato, M.

1990. A study on exploitation of iridium marking for red sea bream. Bull. Nat. Res. Inst. Far Seas Fish. 27:11–28.

Kato, M., H. Sudo, M. Azeta, and Y. Matsumiya.

1991. Field experiments on iridium marking for red sea bream in Shijiki Bay. Bull. Nat. Res. Inst. Far Seas Fish. 28:21–45.

Kingsford, M. J., and M. H. Atkinson.

1994. Increments in otoliths and scales: how they relate to the age and early development of reared and wild larval and juvenile snapper *Pagrus auratus* (Sparidae). Aust. J. Mar. Freshwater Res. 45:1007–21.

Kingsford, M. J., and I. M. Suthers.

1994. Dynamic estuarine plumes and fronts: importance to small fish and plankton in coastal waters of NSW, Australia. Continental Shelf Res. 14:655–672.

Laird, L. M., and B. Stott.

1978. Marking and tagging. *In* T. Bagenal (ed.), Methods for assessment of fish production in fresh waters, 3rd ed., p. 84–100. Blackwell, Oxford, U.K.

Lang, J. B., and C. D. Buxton.

1993. Validation of age estimates in sparid fish using fluorochrome marking. S. Afr. J. Mar. Sci. 13:195–203

Lapi, L. A., and T. J. Mulligan.

1981. Salmon stock identification using a microanalytic technique to measure elements present in the freshwater growth region of scales. Can. J. Fish. Aquat. Sci. 38:744–751.

Lehtonen, H., K. Nyberg, P. J. Vuorinen, and A. Leskela.

1992. Radioactive strontium (^{85}Sr) in marking whitefish [*Coregonus lavaretus* (L.)] larvae and the dispersal of larvae from river to sea. J. Fish Biol. 41:417–423.

Limburg, K. E.

1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. Mar. Ecol. Prog. Ser. 119:25–35.

Lopez, N.A.

1986. Morphohistological study of the early development stages of the red sea bream, *Pagrus major*. *In* J. L. Maclean, L. B. Dizon, and L. V. Hosillos (eds.), First Asian fisheries forum, p. 179–184. Asian Fisheries Society, Manila, Philippines.

Margolis, L.

1963. Parasites as indicators of the geographical origin of sockeye salmon, *Oncorhynchus nerka* (Walbaum), occurring in the North Pacific Ocean and adjacent seas. Int. North Pac. Fish. Comm. Bull. 11.

Matsuda, Y.

1992. Sea bream ranching in Japan: the Kagoshima experience. Infofish Internat. 5:41–44.

McFarlane, G. A., and R. J. Beamish.

1987. Validation of the dorsal spine method of age determination for spiny dogfish. *In* L. C. Summerfelt and G. E. Hall (eds.), Age and growth of fishes, p. 287–300. Iowa State University Press, Ames, IA.

Moran, M.

1987. Tagging confirms separate stocks of snapper in Shark Bay region. FINS (West Australian Department of Fisheries, Perth) 20:3–8.

Moreau, G., C. Barbeau, J. J. Frenette, J. Saint-Onge, and M. Simoneau

1983. Zinc, manganese, and strontium in opercula and scales of brook trout (*Salvelinus fontinalis*) as indicators of lake acidification. Can. J. Fish. Aquat. Sci. 40:1685–91.

Mugiya, Y. and C. Satoh.

1995. Strontium-calcium ratios change corresponding to microincrements in otoliths of the goldfish *Carassius auratus*. Fish. Sci. (Tokyo) 61:361–362.

Mugiya, Y. and S. Tanaka.

1995. Incorporation of water-borne strontium into otoliths and its turnover in the goldfish *Carassius auratus*: effects of strontium concentrations, temperature and 17-beta-estradiol. Fish. Sci. (Tokyo) 61:29–35.

Mulligan, T. J., L. Lapi, R. Kieser, S. Behrens-Yamada, and D. L. Duewer

1983. Salmon stock identification based on elemental composition of vertebrae. Can. J. Fish. Aquat. Sci. 40:215–229.

Ophel, I. L., and J. M. Judd

1968. Marking fish with stable strontium. J. Fish. Res. Board Can. 25:1333–1337.

O'Sullivan, D.

1992. Potential seen for snapper culture. Austasia Aquacult. 6:36–37.

Pankhurst, P. M., J. C. Montgomery, and N. W. Pankhurst.

1991. Growth, development and behaviour of artificially reared larval *Pagrus auratus* (Bloch and Schneider 1801)(Sparidae). Aust. J. Mar. Freshwater Res. 42:391–398.

Papadopoulou, C., G. D. Kanias, and E. Moraitopoulou-Kassimati.

1980. Trace element content in fish otoliths in relation to age and size. Mar. Poll. Bull. 11:68–71.

Paul, L.J.

1982. Snapper decline will hurt. Catch (N.Z.), Oct.:23–24.

1992. Age and growth studies of New Zealand marine fishes, 1921–90: a review and bibliography. Aust. J. Mar. Freshwater Res. 43:879–912.

Paulin, C. D.

1990. *Pagrus auratus*, a new combination for the species known as "snapper" in Australasian waters (Pisces: Sparidae). N.Z. J. Mar. Freshwater Res. 24:259–265.

Pender, P. J., and R. K. Griffin.

1996. Habitat history of barramundi *Lates calcarifer* in a north Australian river system based on barium and strontium levels in scales. Trans. Am. Fish. Soc. 125:679–689.

Poxton, M. G., and S. B. Allouse.

1982. Water quality criteria for marine fisheries. Aquacult. Eng. 1:153–191.

Quartararo, N., G. L. Allan, and J. D. Bell.

1992. Fish meal substitution in a diet for Australian snapper, *Pagrus auratus*. Proceedings of the Aquaculture Nutrition Workshop, Appendix 2:125–126.

Radtke, R. L., and T. E. Targett.

1984. Rhythmic structural and chemical patterns in otoliths of the Antarctic fish *Notothenia larseni*: their application to age determination. Polar Biol. 3:203–210.

Radtke, R. L., D. W. Townsend, S. D. Folsom, and M. A. Morrison.
1990. Strontium:calcium concentration ratios in otoliths of herring larvae as indicators of environmental histories. Environ. Biol. Fish 27:51–61.

Sakamoto, T.
1984. Distribution and movement of the red sea bream in the waters of the southern part of Wakayama Prefecture, adjacent to the Kii Strait, ascertained by tagging experiments in 1981. Bull. Jpn Soc. Sci. Fish. 50:1835–1842.

Sanders, M. J.
1974. Tagging indicates at least two stocks of snapper *Chrysophrys auratus* in south-east Australian waters. N.Z. J. Freshwater Res. 8:371–374.

Sanders, M. J., and D. G. M. Powell
1979. Comparison of growth rates of the two stocks of snapper (*Chrysophrys auratus*) in south-east Australian waters using capture-recapture data. N.Z. J. Mar. Freshwater Res. 13:279–284.

Schroder, S. L., C. M. Knudsen, and E. C. Volk.
1995. Marking salmon fry with strontium chloride solutions. Can. J. Fish Aquat. Sci. 52:1141–1149

Schroder, S. L., E. C. Volk, C. M. Knudsen, and J. J. Grimm.
1996. Marking embryonic and newly emerged salmonids by thermal events and rapid immersion in alkaline earth salts. Bull. Nat. Res. Inst. Aquacult. 0 (suppl. 2):79–83.

Scott, D. P.
1961. Radioactive iron as a fish mark. J. Fish. Res. Board Can. 18:383–391.
1962. Radioactive caesium as a fish and lamprey mark. J. Fish. Res. Board Can. 19:149–157.

Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli.
1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. 192:15–33.

Secor, D. H., and E. D. Houde.
1995. Larval mark-release experiments: potential for research on dynamics and recruitment in fish stocks. *In* D. H. Secor, J. M. Dean, and S. E. Campana (eds), Recent developments in fish otolith research, p. 423–444. Univ. South Carolina Press, Columbia, SC.

Smith, P. J., and M. Hataya.
1982. Larval rearing and reseeding of red sea bream *Chrysophrys major* in Japan. Fisheries Research Division Occasional Publication No. 39. Shizuoka-ken 415, Japan, 19 p.

Snyder, R. J., B. A. McKeown, K. Colbow, and R. Brown.
1992. Use of dissolved strontium in scale marking of juvenile salmonids: effects of concentration and exposure time. Can. J. Fish. Aquat. Sci. 49:780–782.

Sobajima, N., M. Munekiyo, and H. Funata.
1986. Possibility of differentiation between the artificially-released and the wild red sea bream by means of the lack of the inter-nostril epidermis. Kyoto Furitsu Kaiyo Senta. 10:35–40.

Summerfelt, R. C., and L. S. Smith.
1990. Anesthesia, surgery, and related techniques. *In* L. B. Schreck and P. B. Moyle (eds.), Methods for fish biology, p. 213–272. Am. Fish. Soc., Bethesda, MD.

Townsend, D. W., R. L. Radtke, S. Corwin, and D. A. Libby.
1992. Strontium:calcium ratios in juvenile Atlantic herring *Clupea harengus* L. otoliths as a function of water temperature. J. Exp. Mar. Biol. Ecol. 160:131–140.

Townsend, D. W., R. L. Radtke, D. P. Malone, and J. P. Wallinga.
1995. Use of otolith strontium:calcium ratios for hindcasting larval cod *Gadus morhua* distributions relative to water masses on Georges Bank. Mar. Ecol. Progr. Ser. 119:37–44

Townsend, D. W., R. L. Radtke, M. A. Morrison, and S. D. Folsom.
1989. Recruitment implications of larval herring overwintering distributions in the Gulf of Maine, inferred using a new otolith technique. Mar. Ecol. Prog. Ser. 55:1–13.

Tsukamoto, K., H. Kuwada, J. Hirokawa, M. Oya, S. Sekiya, H. Fujimoto, and K. Imaizumi
1989. Size-dependent mortality of red sea bream, *Pagrus major*, juveniles released with fluorescent otolith-tags in News Bay, Japan. J. Fish Biol. 35 (suppl. A):59–69.

Umino, T., M. Otsu, M. Takaba, and H. Nakagawa.
1993. Some characteristics of runty fish appearing in seed production of red sea bream. Nippon Suisan Gakkaishi 59:925–928.

Underwood, A. J.
1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Ann. Rev. 19:513–605.

Welch, T. J., M. J. van den Avyle, R. K. Betsill, and E. M. Driebe.
1993. Precision and relative accuracy of striped bass age estimates from otoliths, scales and anal fin rays and spines. N. Am. J. Fish. Manage. 13:616–620.

Wickins, J. F.
1984. The effect of reduced pH on carapace calcium, strontium and magnesium levels in rapidly growing prawns (*Penaeus monodon* Fabricius). Aquaculture 41:49–60.

Zhao, W., S. Xu, and L. Hou.
1992. Study of absorption and accumulation of super (90) strontium in carp. China Environ. Sci. Zhongguo Huanjing 12:360–364