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 Muligan, 1982; Snyder et al., 1992), vertebrae (Behrens-Yamada et al., 1979; Behrens-Yamada and Muligan, 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.). 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) 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 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 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$_2$.6H$_2$O), a relatively cheap, nontoxic and non-radioactive 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$_2$.6H$_2$O; 2) Sr$^{2+}$ ten times ambient = 0.250 g/L of SrCl$_2$.6H$_2$O; and 3) Sr$^{2+}$ forty times ambient = 1.000 g/L of SrCl$_2$.6H$_2$O. Preliminary experiments (Pollard) 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.

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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 (Sr²⁺ 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 × 80 × 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 (C₉H₁₁NO₂) 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 (H₃NO₃) 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 103Rh signal and balance the signals for 24Mg and 208Pb 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 86Sr, 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.

Quality control solutions were analyzed for 86Sr 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 86Sr 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 86Sr 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.

### 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

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### Table 2

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F-ratio</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>2</td>
<td>1005648</td>
<td>18.167</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Exposure period</td>
<td>4</td>
<td>204047.2</td>
<td>163.683</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Conc. × exp.</td>
<td>8</td>
<td>55355.9</td>
<td>44.405</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tanks (conc. × exp.)</td>
<td>15</td>
<td>1246.6</td>
<td>0.575</td>
<td>n.s.</td>
</tr>
<tr>
<td>Control vs. rest</td>
<td>1</td>
<td>237036.4</td>
<td>190.146</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Residual</td>
<td>150</td>
<td>2166.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
had similar concentrations to the wild-caught control fish.

**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.

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.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species investigated</th>
<th>Aquatic medium</th>
<th>Method of administration</th>
<th>Skeletal structure</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>snapper juveniles (<em>Pagrus auratus</em>)</td>
<td>saltwater</td>
<td>immersion in 0.125g/L to 1.0 g/L Sr(^{2+}) (5 to 40× ambient) for 6 h to 5 days</td>
<td>dorsal spines</td>
<td>Strontium levels of greater than 10× ambient for 4–5 days produced marks which could be reliably distinguished from controls and wild snapper.</td>
</tr>
<tr>
<td>Ophel and Judd (1968)</td>
<td>goldfish (<em>Carassius auratus</em>)</td>
<td>freshwater</td>
<td>32 g of strontium lactate per 400 g of wet diet</td>
<td>scales</td>
<td>More than 10 times the levels of Sr in controls after one year.</td>
</tr>
<tr>
<td>Behrens-Yamada et al. (1979)</td>
<td>coho salmon (<em>Oncorhynchus kisutch</em>)</td>
<td>freshwater</td>
<td>10,000 ppm Sr introduced through the diet over 60 days</td>
<td>vertebrae</td>
<td>32 times as much Sr as controls when released; 1.4 times as much in jacks after 5 months; adults could not be distinguished.</td>
</tr>
<tr>
<td>Behrens-Yamada and Mulligan (1982)</td>
<td>coho salmon adults</td>
<td>as above</td>
<td>as above</td>
<td>core samples of scales and vertebrae</td>
<td>Successful identification of adults after 5 months.</td>
</tr>
<tr>
<td>Bogren and Mulligan (1987)</td>
<td>coho salmon fry</td>
<td>freshwater</td>
<td>immersion in 1 µg/mL Sr for 49 days</td>
<td>vertebrae</td>
<td>Sr mark still detectable after 169 days despite dilution.</td>
</tr>
<tr>
<td>Guillou and de la Noue (1987)</td>
<td>brook trout (<em>Salvelinus fontinalis</em>)</td>
<td>freshwater</td>
<td>200–3200 µg/g Sr(^{2+}) introduced through the diet for 4 and 6 weeks</td>
<td>opercular bones</td>
<td>Able to distinguish between marked and unmarked fish at the lowest levels of ingested Sr.</td>
</tr>
<tr>
<td>Snyder et al. (1992)</td>
<td>rainbow trout (<em>Oncorhynchus mykiss</em>)</td>
<td>freshwater</td>
<td>immersion in 1.8, 3.6 and 5.4 mg/L Sr for 30 days and 60 days</td>
<td>scales</td>
<td>A twofold increase in exposure period and a two- or threefold increase in immersion concentration produced corresponding two- and threefold increases in incorporation.</td>
</tr>
<tr>
<td>Brown and Harris (1995)</td>
<td>golden perch and trout cod (<em>Macquaria ambigua</em>)</td>
<td>freshwater</td>
<td>immersion in 1–20 g/L SrCl(_2) for one to four days</td>
<td>otoliths</td>
<td>Still able to distinguish after 12 months. No measurable growth, health, or mortality effects.</td>
</tr>
<tr>
<td>Mugiya and Tanaka (1995)</td>
<td>goldfish (<em>Carassius auratus</em>)</td>
<td>freshwater</td>
<td>immersion in 0.1, 1.0, and 10.0 mg/L Sr for 88 days</td>
<td>otoliths, ribs, and scales</td>
<td>Strontium in otoliths remained after 92 days, whereas that in scales and ribs decreased.</td>
</tr>
<tr>
<td>Schroder et al. (1995)</td>
<td>chum (<em>Oncorhynchus keta</em>) and sockeye salmon (<em>O. nerka</em>)</td>
<td>freshwater</td>
<td>immersion in 120, 1200 and 9000 ppm SrCl(_2) for 24 hours</td>
<td>otoliths, vertebrae and opercula</td>
<td>Able to distinguish after 34 days, and after 21 months in otoliths of fish immersed in 5000 ppm for 24 hours.</td>
</tr>
<tr>
<td>Schroder et al. (1996)</td>
<td>salmon fry</td>
<td>freshwater</td>
<td>immersion in 9000 ppm SrCl(_2) for 12 hours or less</td>
<td>otoliths</td>
<td>Fish held for as little as 30 minutes in 9000 ppm were distinguishable for up to 2 years.</td>
</tr>
<tr>
<td>Gallahar and Kingsford (1996)</td>
<td>rock blackfish (<em>Girella elevata</em>)</td>
<td>saltwater</td>
<td>immersion in SrCl(_2) 2.5× ambient</td>
<td>otoliths</td>
<td>Able to distinguish between marked fish and controls.</td>
</tr>
</tbody>
</table>
incorporation. Their inability to identify strontium-marked adults in following years was attributed entirely to dilution caused by the growth of vertebral, 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 SrCl₂. 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 Sr²⁺ 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 Taggett, 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;
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. Pollard3 investigated iron chloride (FeCl₃·6H₂O) 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 (Pollard3). 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 (Bogoialevskaia, 1959; Anward, 1966), $^{33}$P (Karzinkin et al., 1959), $^{59}$Fe (Scott, 1961), $^{137}$Cs (Scott, 1962), $^{144}$Ce (Hoss, 1967), $^{127}$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 Abe-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 ranned 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 of strontium into the dorsal spines. The 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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