Selection of Dosages of Oxytetracycline for Age Validation Studies

G. A. McFarlane and R. J. Beamish

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

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Oxytetracycline (OTC), injected to mark the otoliths of sablefish (*Anoplopoma fimbria*), causes mortality directly in proportion to the dosage administered. The selection of an appropriate dosage requires minimizing mortality while maximizing the number of fish that have a usable OTC mark. Laboratory studies could not be used to determine appropriate dosages. Dosages that substantially increased mortality in the ocean did not cause any mortality in the laboratory. For sablefish, we recommend a dosage of 25–35 mg OTC/kg fish.

L'oxytétracycline (OTC), injectée pour le marquage des otolithes de morue charbonnière (*Anoplopoma fimbria*), entraîne une mortalité directement proportionnelle à la dose. La dose appropriée permettrait de minimiser la mortalité tout en maximisant le nombre de poissons porteurs d'une marque à l'OTC. Des études en laboratoire n'ont pu servir à déterminer les doses appropriées. Les doses qui entraînent un accroissement net de la mortalité en mer n'ont pas été la cause de mortalité en laboratoire. Dans le cas de la morue charbonnière, les auteurs recommandent une dose de 25 à 35 mg d'OTC par kilogramme de poisson.

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The possibility that fish may be substantially older than previously thought (Beamish and McFarlane 1983a, 1986) has resulted in renewed interest in age validation. When the otolith is used to age older, slower-growing fish, validation can only be achieved by marking the otolith. Oxytetracycline (OTC) is commonly used for this purpose because it binds with proteins in the blood and is incorporated in newly forming and mineralizing bone and cartilage (Frost et al. 1961). It is known that high dosages of OTC will cause mortality (Weber and Ridgeway 1962; Kobayashi et al. 1964). It is therefore necessary to select a dosage that provides a suitable mark on the otolith while minimizing mortality.

We injected sablefish (*Anoplopoma fimbria*) with dosages of OTC that were recommended in other studies and found that these dosages did not cause mortality in our laboratory studies (Lanzing and Hynd 1966; Weber and Ridgeway 1967; Wild and Foreman 1980; Beamish et al. 1983). However, in field tests the recapture percentage of the injected fish was much lower than for uninjected fish, indicating that additional mortality was occurring in the ocean (Beamish et al. 1983).

As a result of this unexpected mortality, we conducted a series of experiments in the ocean to determine optimal dosages of OTC for marking otoliths of sablefish. A sufficient number of injected and uninjected fish have now been recaptured to determine an optimal dosage for marking otoliths for age validation studies for this species.

Methods

Initially we followed the procedure of Weber and Ridgway (1962, 1967) and Kobayashi et al. (1964) which indicated that intraperitoneal (IP) injections of OTC at a rate of 20–100 mg/kg fish would result in visible marks. The intensity of the mark on otoliths was a function of the dose administered. Kobayashi et al. (1964) recommended the use of 50 mg OTC/kg fish for young goldfish and Weber and Ridgway (1962) recommended 100 mg OTC/kg fish for young salmon. Both reports noted that mortality increased at dosages exceeding 100 mg OTC/kg fish. Mortalities increased from 0–20% at 100 mg OTC/kg fish to 50–75% at 400 mg OTC/kg fish for goldfish and 50% at 400 mg OTC/kg fish for salmon.

OTC was purchased under the brand name Liquamycin. Liquamycin is oxytetracycline hydrochloride dissolved in a stabilizing agent and supplied in 250-mL bottles at a concentration of 100 mg/mL (Jones 1969). A shallow V-shaped tagging board was calibrated using a weight–length relationship so that the amount of injection could be determined directly from the length measurement. All fish receiving an injection were tagged using the procedures described in Beamish and McFarlane (1983b).

Laboratory studies — In 1977, prior to initiating the tagging program, five adult sablefish were caught by bottom trawls, transported to flowing saltwater-holding tanks in the laboratory, measured, tagged, and injected with OTC concentrations of 50 and 100 mg/kg fish. Fish were maintained at ambient water temperature and fed frozen herring. One fish which received 50 mg OTC/kg fish was sacrificed in the first year and the other four fish survived at least 1 more yr. In April 1978, the experiment was repeated and six adult sablefish were injected with a dosage of 100 mg/kg.

In May and June 1979, 104 adult sablefish were captured at normal fishing depths on the west coast of Vancouver Island and transported to a 92 000-L tank at the Pacific Biological

TABLE 1. Numbers of sablefish surviving, by OTC dosage and time, which were held in the laboratory.

	100 mg/kg	25 mg/kg	Control
Adult sablefish, June 1979 – April 1980			
Sample size	34	34	36
No. surviving after 1 wk	16	16	20
No. surviving after 2 wk	12	12	17
No. surviving after 22 wk	11	12	12
No. surviving after 40 wk	11	10	12
Juvenile sablefish, December 1979 – May 1982			
Sample size	20	20	40
No. surviving after 54 wk	19	19	34
No. surviving after 110 wk	13	17	22

TABLE 2. Recovery rates (%), by OTC dosage of sablefish tagged and released at sea during 1981 (recoveries through December 1985).

Dosage	No. released	No. recovered	% recovered	% (arcsine transformed)
No injection	2118	268	12.6	
1% saline	1484	244	16.4	23.89
25 mg/kg	3266	355	11.0	19.37
50 mg/kg	1010	74	7.3	15.68
75 mg/kg	1071	36	3.4	10.63
100 mg/kg	1481	54	3.6	10.94

Station. One third of the fish received an injection of 100 mg OTC/kg fish, one third were given the same volume of OTC diluted with saline to produce one quarter of the concentration (25 mg/kg fish), and one third were not injected. On December 13, 1979, and on April 16, 1980, all adults in the tank were counted and measured.

Eighty juvenile sablefish were captured in Hecate Strait and Queen Charlotte Sound during September, October, and November 1979 using bottom trawls. They were transported to the same 92 000-L tank at the Pacific Biological Station. On December 13, 1979, one quarter of these fish were injected with 100 mg OTC/kg fish, one quarter were injected with the same volume of solution diluted with saline to produce 25 mg OTC/kg fish, and one half received no injections. All juvenile sablefish were measured every 3 mo until May 1982.

Seawater of ambient temperature was piped into the 92 000-L tank. No attempt was made to regulate the light cycle, but one half of the tank was covered with black plastic to provide some dark areas and lights in the room were turned out at 18:00 each day. No treatment of any kind was provided for disease or injury. All fish were fed frozen herring daily.

Ocean studies — Fish tagged and released off Vancouver Island in 1977 and in 1978 off Vancouver Island and the Queen Charlotte Islands were given an IP injection of OTC amounting to 100 mg/kg fish.

To examine the effect of various dosages on sablefish released directly into the ocean, sablefish captured in March and June 1981 off the west coast of the Queen Charlotte Islands were injected and released. Approximately one quarter were injected with 100 mg OTC/kg fish, one quarter were given the same volume diluted with 1% saline to produce one quarter of the concentration (25 mg OTC/kg fish), one quarter were injected with the same volume of 1% saline solution, and one quarter were not injected.

Sablefish were also collected in November 1981 off the west coast of the Queen Charlotte Islands. Volumes were standardized to the March and June dosage of 100 mg OTC/kg fish. One quarter of the sablefish were given an IP injection of 25 mg OTC/kg fish, one quarter received (IP) 50 mg OTC/kg fish, one quarter received (IP) 75 mg OTC/kg fish, one eighth received no injection, and one eighth were given an intramuscular (IM) injection of 25 mg OTC/kg fish.

Oxytetracycline marks in bone were viewed using ultraviolet light as described by Kobayashi et al. (1964). The presence or absence of a usable mark was noted for all otoliths that were recovered from tagged and injected sablefish. If a mark was sufficiently faint to cause doubt that it was an OTC mark, it was considered to be absent.

Results

In 1977 and 1978, 15 183 fish were tagged, injected with 100 mg OTC/kg fish, and released off the west coast of Canada. As of December 31, 1985, 959 (6.3%) of these fish were recaptured. At the same time, 5279 fish were tagged in the same areas but were not injected. As of December 31, 1985, 1347 (25%) of these fish were recovered.

Laboratory studies — There were no mortalities, as of April 1979, in the two initial laboratory studies. In the laboratory experiments that used adult sablefish to examine the effect of 100 and 25 mg/kg dosages, most mortalities occurred in the first week (Table 1). There was no difference (x^2, $p \geq 0.05$) in the number of mortalities among the dosages after 1 wk indicating that mortalities probably resulted from the stress of capture and transport to the laboratory. When the experiment was terminated after 10 mo, there was no significant difference in the number of fish remaining in any of the groups (χ^2, $p \geq 0.05$).

The laboratory experiment to examine the effect of OTC dosages on mortality and growth of juveniles (Beamish et al. 1983) was completed in 1982 (Table 1). There was no immediate mortality associated with either treatment, and while some mortalities occurred later, there was no significant difference among treatments (x^2, $p \geq 0.05$) after 1 yr (54 wk). After approximately 2 yr (110 wk), there was no significant difference between the highest dosage (100 mg/kg) and the control fish. The fish receiving 25 mg OTC/kg fish had significantly (x^2, $p < 0.05$) less mortality than the control.

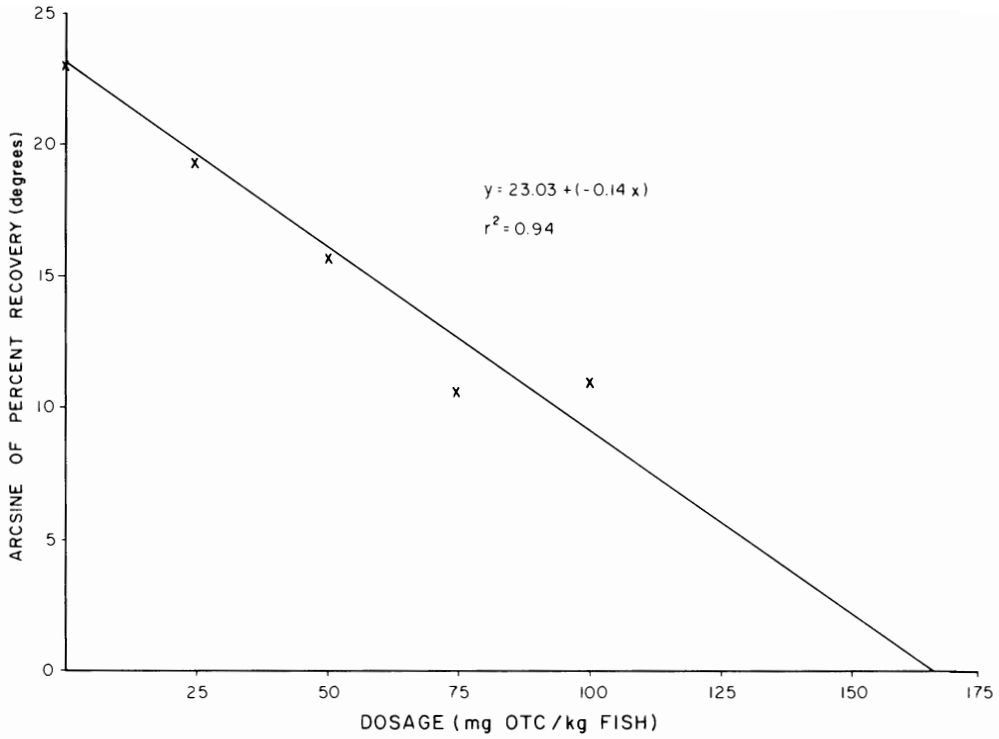

FIG. 1. Relationship between dosage rate of OTC and mortality for tagged and injected sablefish.

TABLE 3. Incidence (%) of OTC marks on otoliths of adult and juvenile sablefish, by OTC dosage and years at liberty, which were tagged, injected, and released at sea in 1981.

Years at liberty	Dosage rate (mg OTC/kg fish)											
	25			50			75			100		
	No. examined	No. with marks	% marked	No. examined	No. with marks	% marked	No. examined	No. with marks	% marked	No. examined	No. with marks	% marked
1	63	37	59	22	18	82	10	9	90	8	6	75
2	87	58	67	24	21	88	17	17	100	21	20	95
3	60	50	83	7	5	71	5	5	100	9	8	89
4	36	27	75	3	2	67	3	2	67	3	3	100
5	8	6	75	—	—	—	—	—	—	1	1	100
Total	254	178	70	56	46	82	35	33	94	42	38	90

Although there was no explanation for the difference between control and 25 mg OTC/kg fish after 2 yr, it is probable that the difference was unrelated to dosage because no significant difference occurred among treatments after 1 yr.

Ocean studies — There was no difference (x^2, $p \geq 0.05$) in the recovery percentages of fish that received IM (9.6%) and IP (11.2%) injections (25 mg OTC/kg fish); therefore, these recoveries were combined (Table 2). The control and 1% saline injections were significantly different (x^2, $p < 0.05$); therefore, their two dosages could not be combined. Because the recovery percentage for fish receiving 1% saline injections was higher, and the treatment procedure for these fish was identical to the procedure for fish receiving OTC injections, this percentage was used in the analysis.

Since percentages are not distributed normally, the values were arcsine transformed (Snedecor and Cochran 1967). A straight line best described the relationship between dosage and mortality (Fig. 1), indicating that mortality is directly related to dosage. An extrapolation of the line in Fig. 1 indicates that a dosage of ~165 mg/kg would appear to cause 100% mortality. There was no indication that a very small dosage would eliminate mortality, that is, no "safe" dosage was found.

The OTC mark occurred less frequently in otoliths from fish that received 25 mg OTC/kg fish than those receiving the higher dosages (Table 3). There was no significant difference (x^2, $p \geq 0.05$) in the percentage of marks (average 92%) resulting from the two highest dosages.

The optimal dosage is that dosage which provides the maximum return of marked otoliths. This dosage was estimated (Fig. 2) and found to be 25–35 mg OTC/kg fish. It is apparent that while smaller dosages resulted in less mortality, they yielded substantially fewer otoliths with an OTC mark. It was only extreme dosages that greatly reduced the probability of recovering otoliths that were usable. These dosages either resulted in no marks or caused high mortality.

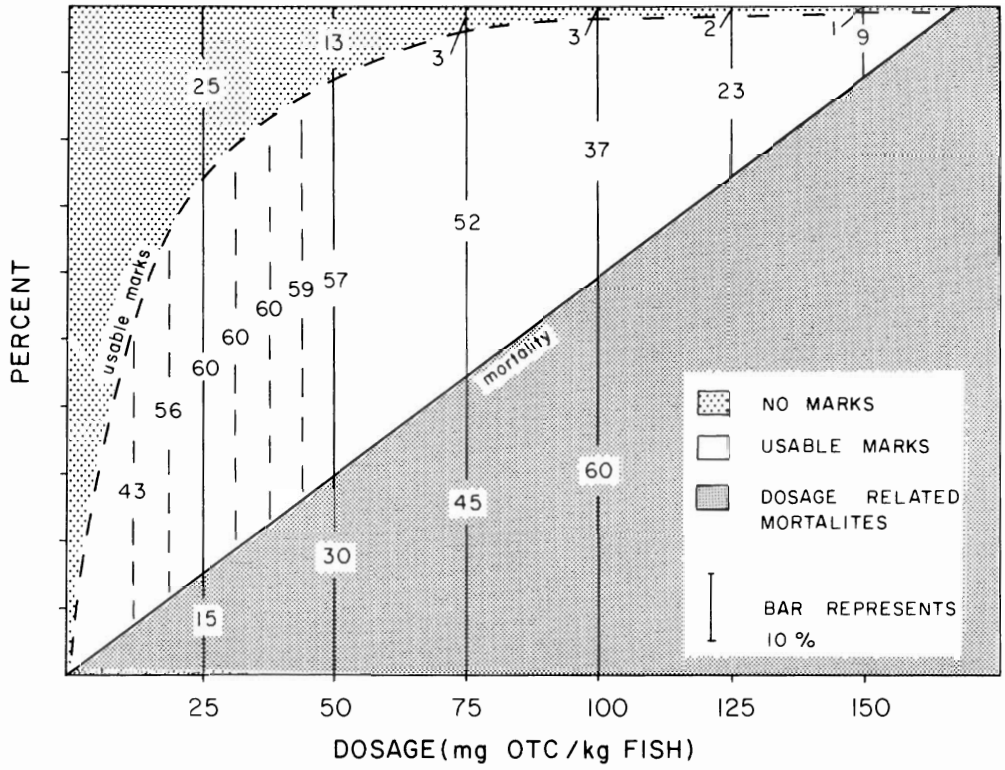

FIG. 2. Nomogram of optimal dosage. Percentage mortality from different dosages was determined from Fig. 1. The percentage of usable marks for each dosage was determined using the percentages in Table 3. For example, a dosage of 25 mg OTC/kg fish causes 15% mortality (Fig. 1), and 70% of the survivers or 60% have usable marks (Table 3). The remainder do not have a mark.

Discussion

The relationship between dosage and survival of injected fish released directly into the ocean was positive and strongly linear. By extrapolation, a dosage of 165 mg OTC/kg fish would likely result in 100% mortality. Because even the smallest dosages cause some mortality, there was no absolutely safe dosage. The relationship between dosage and the percentage of fish that had usable marks, based on recaptures, was not linear. Dosages between 20 and 80 mg OTC/kg fish would produce estimated recoveries up to 75% of those likely to occur with the optimal dosage of 25–35 mg OTC/kg fish. The range of dosages that will produce a usable mark is quite wide and may indicate that dosages close to the optimal for sablefish would be suitable for other species.

The mortality of injected fish observed in the ocean did not occur in any of the laboratory studies. The cause of reduced survival in the ocean, compared with the laboratory, is unknown. However, the fish released in the ocean were tagged and injected immediately after capture, but laboratory fish were allowed to acclimate to the holding tank before injection. Therefore, the two groups may have been stressed differently. In addition, the ambient temperature for fish injected in the laboratory experiments may have been higher than for fish returned directly to the ocean. The reduced metabolic rate of fish returned to the ocean would increase the residence time of OTC and possibly affect survival. Whatever the causes, our study indicates that the laboratory studies did not simulate the conditions affecting injected fish in the ocean and therefore were not controls for the ocean releases. The dosages considered acceptable by other investigators, for other species (Weber and Ridgway 1962, 1967; Kobayashi et al. 1964), were determined in the laboratory. Because our study showed that higher dosages that were safe for laboratory studies caused mortalities in the ocean, it is possible that the higher dosages reported safe for these other species may also cause increased mortality in field experiments. Until the causes of mortality are better understood, the optimum dosage should only be determined from field experiments.

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References

BEAMISH, R. J., AND G. A. MCFARLANE. 1983a. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735–743.

1983b. Summary of results of the Canadian sablefish tagging program, p. 147–183. *In* Proceedings of the International Sablefish Symposium. Alaska Sea Grant Report 83-3.

1986. Current trends in age determination methodology. *In* R. Summerfelt and G. E. Hall [ed.] Age and growth of fish. Iowa State University Press, Ames, IA.

BEAMISH, R. J., G. A. MCFARLANE, AND D. E. CHILTON. 1983. Use of oxytetracycline and other methods to validate a method of age determination for sablefish (*Anoplopoma fimbria*), p. 95–116. *In* Proceedings of the International Sablefish Symposium. Alaska Sea Grant Report 83-3.

FROST, H. M., A. R. VILLANUEVA, H. ROTH, AND S. STANISAVLJEVIC. 1961. Tetracycline bone labelling. J. New Drugs 1(5): 206–216.

JONES, I. W. 1969. Notes on marking salmon fingerlings with tetracycline. Fish. Comm. Oreg. Fish. Cult. Div., Clackamas, OR. 6 p.

KOBAYASHI, S., R. YUKI, T. FURUI, AND T. KOSUGIYAMA. 1964. Calcifica-

tion in fish and shellfish — I. Tetracycline labelling patterns on scale, centrum and otolith in young goldfish. Bull. Jpn. Soc. Sci. Fish. 30 (1): 6–13.

LANZING, W. J. R., AND J. S. HYND. 1966. Tetracycline distribution in body tissues of marine fishes. Aust. J. Sci. 29(6): 177–178.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods. Iowa State University Press, Ames, IA.

WEBER, D. D., AND G. J. RIDGWAY. 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. Prog. Fish-Cult. 24(4): 150–155.

1967. Marking Pacific salmon with tetracycline antibiotics. J. Fish. Res. Board Can. 24: 849–865.

WILD, A., AND T. J. FOREMAN. 1980. The relationship between otolith increments and time for yellowfin and skipjack tuna marked with tetracycline. Inter. Am. Trop. Tuna Comm. Bull. 17(7): 509–560.