Evaluation of toxicity of oxytetracycline on growth of captive nurse sharks, *Ginglymostoma cirratum**

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Validation of age estimates derived from calcified structures is an essential component of all fish age and growth studies (Beamish and McFarlane, 1983). In elasmobranchs, age validation is accomplished through injection of tetracycline antibiotics, particularly oxytetracycline hydrochloride [OTC] (Cailliet, 1990). At a standard dosage level of 25 mg/kg body weight (BW), OTC is deposited at sites of active calcification and produces vivid, enduring marks in dorsal-fin spines and vertebral centra (Holden and Vince, 1973; Smith, 1984; Beamish and McFarlane, 1985; Branstetter, 1987; Brown and Gruber, 1988; Natanson and Cailliet, 1990; Kusher et al., 1992; Natanson, 1993; Walker et al.[1]). The location of these fluorescent marks can then be examined to determine if the structure in question accurately reflects age. Such OTC treatment is generally considered benign, yet few scientists have directly examined the potential toxicity of this compound to elasmobranchs. However, if OTC injection alters the growth or health of treated elasmobranchs, its value as a chemical marker for age validation may be severely compromised. Thus, studies on the toxicity of OTC and other potential chemical markers (Gelsleichter et al., 1997) are essential for the improvement of elasmobranch ageing biotechnology.

Chemical toxicity associated with OTC treatment has been well documented in several teleost ageing studies. For example, injection of, or immersion in, OTC at recommended dosage levels (Beamish and McFarlane, 1983) has been reported to produce lethargy, behavioral abnormalities, and mortality in treated specimens (Schmitt, 1984; Wilson et al., 1987; Marking et al., 1988; Monaghan, 1993; Bumguardner and King, 1996). In contrast, Tanaka (1990) reported that various dosage levels of OTC (20–80 mg/kg BW) did not significantly affect growth rates of injected Japanese wobbegongs (*Orectolobus japonicus*) or swell sharks (*Cephaloscyllium umbratile*). Unfortunately, no other studies have investigated the possible implications of OTC treatment in elasmobranchs and, thus, they remain largely unclear.

The goal of the present study was to evaluate the effect of OTC treatment on the growth rate of captive nurse sharks, *Ginglymostoma cirratum*. The potential effect of OTC injection on nurse shark health is also discussed on the basis of serologic and hematologic observations.

Materials and methods

Nine age-0 and age-1 *G. cirratum* were collected in the Florida Keys and transported to the laboratory, where they were maintained in outdoor circular tanks (capacity ranging from 3,200 to 12,000 L). The experimental tanks can operate as either open or recirculating systems and were subjected to natural photoperiod and temperature

[1] Walker, T. I., R. A. Officer, J. G. Clement, and L. P. Brown. 1995. Southern age validation: Part I—Project overview, vertebral structure and formation of growth increment bands used for age determination. Final Report to the Fisheries Research and Development Corporation, FRDC Project 91/037. Department of Conservation and Natural Resrouces, Queenscliff, Victoria, Australia.

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regimes. After an acclimation period of two weeks, all animals were sexed, measured (stretched total length; STL), weighed, and tagged with rototags for individual identification. Sharks were divided into two groups: an OTC group (n=5; mean STL (±SE)=62.6 ± 3.62 cm; mean weight (±SE)=1.7 ±0.36 kg) and a control (CO) group (n=4; mean STL=51.2 ±2.09 cm; mean weight = 0.9 ± 0.10 kg). All nine animals were injected in the lateral musculature with an elasmobranch Ringer's solution vehicle (Forster et al., 1972) which, for the OTC group, contained OTC at a dosage level of 25 mg/kg body weight (BW). Following the injections, all nurse sharks were returned to the experimental tanks for captive maintenance over a 7-month period.

During the experimental period, all sharks were measured, weighed, and examined for external lesions on a monthly basis. In addition, the general condition of all sharks was evaluated and recorded in terms of behavioral abnormalities or signs of distress. All animals were fed three times per week ad libitum with Atlantic thread herring, *Polydactylus oligodon,* or sardine, *Sardinella aurita*.

Toxicity of OTC on nurse shark growth was evaluated by comparing the mean growth rate (%BW/d) of control and OTC-injected *G. cirratum*. In addition, blood samples were obtained from all specimens at the end of the experimental period and subjected to white blood cell (WBC) and serologic parameter analyses. Afterwards, all remaining specimens were anesthetized with 1 g/L tricaine methanesulfonate (MS-222) and sacrificed by severing the spinal cord. Necropsies were performed for all specimens to assess general animal health.

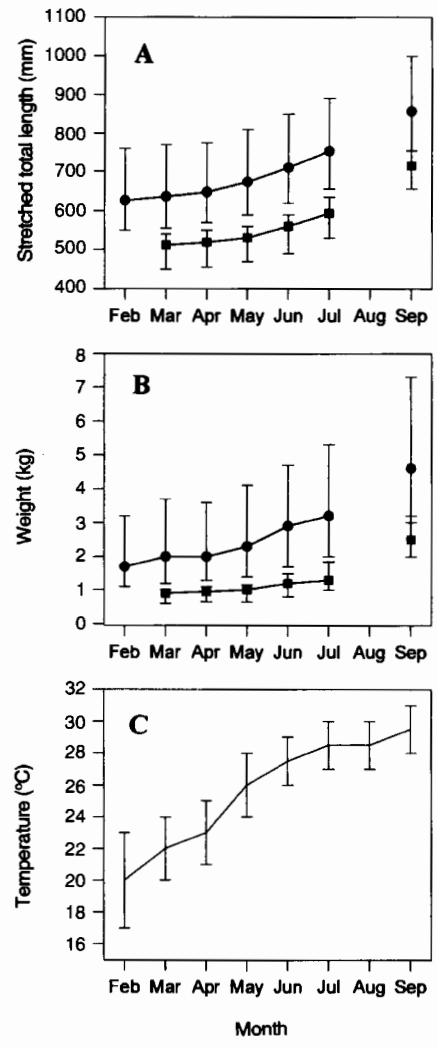

Figure 1

Mean monthly growth (± SE) in **(A)** length and **(B)** weight for nurse sharks in the OTC (●, n=5) and control (■, n=4) groups; **(C)** shows the monthly temperature ranges and midpoints in the experimental tanks.

Results

All nurse sharks survived the experimental period without any apparent behavioral complications. All grew in length (Fig. 1A) and weight (Fig. 1B) while exposed to identical temperature fluctuations (Fig. 1C). No significant differences in growth rates between control and OTC groups were observed (t-test, P<0.05, Table 1).

Observations on serum chemistry also indicated little difference between groups, except that both lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were significantly greater in OTC-injected animals (t-test, P<0.05, Table 2). Differential WBC counts indicated no significant difference between control and OTC groups (t-test, P<0.05; Table 3). Both serologic and hematologic

Table 1

Growth rates (expressed as %BW/d) of nurse sharks in the control and oxytetracycline-treated (OTC) groups. Body weights (BW) are in kg.

Group	n	Initial BW (Mean ± SE)	Final BW (Mean ± SE)	Growth rate (Mean ± SE)
Control	4	0.88 ± 0.10	2.52 ± 0.29	0.91 ± 0.11
OTC	5	1.70 ± 0.38	4.64 ± 0.73	0.79 ± 0.11

parameters were comparable with earlier observations by Stoskopf (1993) for captive *G. cirratum*.

Discussion

Tanaka (1990) determined that OTC injections at dosage levels from 20 to 80 mg/kg BW did not adversely affect growth or cause mortality in Japanese wobbegongs (*O. japonicus*) or swell sharks (*C. umbratile*). In agreement, the present study indicates no adverse effect of OTC treatment on the growth rate of injected *G. cirratum*. Therefore, continued use of OTC injection is still supported as an effective, nontoxic method for determining vertebral growth-band periodicity. In addition, OTC remains the superior chemical marker for elasmobranch age validation in comparison with calcein, which appears highly toxic at similar dosage levels (Gelsleichter et al., 1997).

Although OTC does not appear to affect short-term growth, high serum LDH and AST in OTC-injected *G. cirratum* may suggest that it can be hepatotoxic. High activities of LDH and AST are commonly used in the diagnosis of toxicant stress and hepatocellular injury, when these enzymes are leaked from damaged liver cells (Zimmerman et al., 1965; Racicot et al., 1975; Krajnovic-Ozretic and Ozretic, 1987). Unfortunately, the diagnosis of toxicant stress in elasmobranchs with enzyme activity levels has not been well investigated. In addition, serial blood sampling of injected animals prior to and after injection is necessary for an appropriate evaluation of serologic data. Consequently, interpretation of these data is speculative, yet may still indicate a harmful effect of OTC on elasmobranch physiology and thus require further investigation.

Hepatic dysfunction is a common repercussion of OTC treatment in vertebrates. In several species, OTC administration causes acute hepatocellular injury that is often associated with intracellular fat deposition and cytoplasmic alterations (Weinstein, 1970; Hennigar and Gross, 1977). However, these effects are usually associated with repeated exposure to dosage levels far greater than those used in fish age validation studies (Lepper, 1951; Sborov and Sutherland, 1951). In addition, elasmobranch liver cells regularly contain large deposits of intracellular fat in their normal state. Nevertheless, this standard information may lend some credence to observations on increased LDH and AST in treatment group specimens. Clearly, future studies on OTC toxicity should investigate other characteristics of health that are less conspicuous than animal growth or survival.

In conclusion, the results of the present study indicate that OTC treatment does not produce short-term effects on *G. cirratum* growth that may complicate age validation. However, minor tangential evi-

Table 2

Serologic parameters of nurse sharks at the end of the experimental period after injection with oxytetracycline hydrochloride (OTC) or vehicle at a dosage of 25 mg/kg body weight ; BUN = blood urea nitrogen; ALK P = alkaline phosphatase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase; ALT = alanine aminotransferase. Values are means ± SE. The values marked with an asterisk are significantly different from control at $P < 0.05$.

	Groups	
Serologic parameter	Control (n=4)	OTC (n=5)
Glucose (mg/dL)	16.2 ± 0.86	18.0 ± 0.63
Sodium (mM/L)	272.7 ± 1.93	275.4 ± 1.63
Potassium (mM/L)	5.45 ± 0.10	5.47 ± 0.08
Chloride (mM/L)	260.0 ± 2.71	261.0 ± 1.48
CO_2 (mM /L)	7.7 ± 0.25	7.2 ± 0.20
BUN (mg/dL)	832.0 ± 12.17	811.6 ± 6.22
Creatinine (mg/dL)	0.10 ± 0	0.16 ± 0.02
Uric acid (mg/dL)	0.12 ± 0.03	0.18 ± 0.05
Calcium (mg/dL)	17.3 ± 0.28	17.8 ± 0.24
Phosphorus (mg/dL)	4.12 ± 0.17	4.14 ± 0.12
Total bilirubin (mg/dL)	0.1 ± 0	0.1 ± 0
ALK P (U/L)	33.5 ± 2.60	54.6 ± 9.79
LDH (U/L)	863.5 ± 122.9	1810.8* ± 371.7
AST (U/L)	24.7 ± 3.77	54.6* ± 9.35
ALT (U/L)	19.7 ± 1.25	23.2 ± 1.71

Table 3

Differential white blood cell counts of nurse sharks at the end of the experimental period after injection with OTC or vehicle at a dosage of 25 mg/kg BW; % WBC is the proportion of all blood cells that are white. Values are means ± SE.

Group or individual	n	Granulocytes (%)	Lymphocytes (%)	Monocytes (%)	Thrombocytes (%)	% WBC
Control	4	21.4 ± 2.6	51.1 ± 2.0	1.4 ± 0.3	26.1 ± 1.5	12.0 ± 0.6
OTC	5	22.4 ± 3.0	50.3 ± 3.0	1.9 ± 0.6	25.4 ± 1.9	11.2 ± 0.8

dence suggests that OTC may be hepatotoxic, affecting animal health over longer periods of time. Thus, additional studies on OTC toxicity may have important implications for long-term maintenance of marked elasmobranchs.

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