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# A TECHNIQUE FOR TETRACYCLINE INJECTING AND TAGGING BILLFISH

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ABSTRACT

Various techniques are available for validation of aging techniques for fishes. Modifications are usually required to suit the immediate needs. While promising results have been presented for the determination of age in species of billfish, the periodicity of incremental marks in hardparts has not been established. I describe the equipment developed and applied to achieve such validation through the injection of oxytetracycline into Pacific sailfish and black marlin in east coast Australian waters. I have outlined the practical constraints of the exercise and the utility of the tagging system highlighted by the results. Over 400 sailfish and black marlin were tagged and simultaneously injected by the crews of gamefishing vessels undertaking normal charter operations. Five black marlin were recaptured of which three were available for study. Each of these fish displayed easily discernible oxytetracycline marks in the otoliths and fin-spines. There has been no rigorous validation of aging techniques in any species of billfish and the success of this project and the utility of the technique offers a means to achieve this necessary proof of age.

Several approaches have been used in attempts to estimate the ages and growth rates of billfishes. These include modal progression and length frequencies (DeSylva, 1957; Skillman and Yong, 1976; Pepperell, 1990), the examination of periodic marks in fin-spine sections (Jolley, 1977; Hill et al., 1989), vertebral central (Hill et al., 1989) and otoliths (Wilson, 1984; Prince et al., 1986; Hill et al., 1989; Prince et al., 1991).

Periodic marks in the otoliths and fin-spines have provided the best estimates of age for these fish. The recapture of an Atlantic sailfish, *Istiophorus platypterus,* at large for almost 11 years, enabled critical evaluation of aging techniques based on these structures (Prince et al., 1986). Counts of presumed annuli from fin-spines grossly under-estimated the known minimum age of this fish, apparently due to the resorption of annuli through the proliferation of the core matrix. Counts of external ridges on the sagitta did, however, suggest an annual periodicity in the formation of these morphological structures.

Prince et al. (1991) examined the microstructure of otoliths from larvae, juvenile and adult Atlantic blue marlin and, on the basis of back-calculated spawning dates, produced evidence for daily increment deposition. Increments, presumed daily, have also been observed in larval and juvenile black marlin (Speare, unpubl. data).

Wilson (1984) concluded that otoliths were useful in estimating ages in white marlin and Atlantic and Pacific blue marlin. In a detailed comparison of hardpart aging techniques for Pacific blue marlin, Hill et al. (1989) found that both spines (anal and dorsal) and otoliths were potentially useful in aging studies, provided that resorbed increments in the spines were estimated by interpolation (Hill, 1986).

Despite the apparent utility of increments on hardparts as estimators of age and growth in billfish (Radtke and Shepherd, 1991), the periodicity of formation of these increments has not been determined for any species of billfish. The incorporation of an artificial mark, by chemical means such as a tetracycline injection, into the structure of the hardpart at a known time is a particularly effective strategy of determining increment periodicity (Wild and Foreman, 1980; Fowler, 1989).

Foreman (1987) described a method of simultaneously tagging and injecting large pelagic fish such as yellowfin tuna, *Thunnus albacares,* with tetracycline. The equipment and procedures described below acknowledge the utility of his technique which has been modified to take advantage of recreational gamefishing practices in Australian waters.

Black marlin, *Makaira indica,* and sailfish are prevalent in east coast Australian waters. Marlin are regularly encountered by hook and line fishermen as juveniles (<10–60 kg) and as mature fish (200–>450 kg) (Pepperell, 1990). Sailfish are also prevalent in all size classes, particularly between 10 and 45 kg. Tagging and release of recreationally caught billfish is well established. This tagging effort is quite extensive with, for example, over 1,200 fish liberated in 1989. Recapture rates are about 0.5% for black marlin and about 2.5–3.0% for sailfish. The majority of recaptures (68% of black marlin and 98% of sailfish) are by recreational fishermen (Pepperell, 1990). The balance is predominantly by foreign, mostly Japanese, longliners.

Substantial material (otoliths, fin-spines and morphometrics) has been collected over the past 4 years to provide estimates of age for both black marlin and sailfish. Because of the well supported tag and release program in Australian waters, an excellent opportunity exists to undertake tetracycline injection and tagging of black marlin and sailfish with the aim of establishing the periodicity of increment formation in the hardparts of these fish.

## Materials and Methods

*Design Criteria.*—Several criteria had to be met for the tetracycline tagging to be effective and compatible with existing game fishing practices. These were: 1) Delivery of the tetracycline must provide an effective dose to a range of fish sizes. The 5–60 kg size range was targetted because it includes most sailfish and immature black marlin in nearshore coastal waters. 2) The tetracycline must be fast acting and produce a clear mark to facilitate the identification and quantification of daily as well as annual growth increments. 3) The injection and tagging must be simultaneous so that all tagged fish are injected. As a reward of $400 is offered for recaptures, payments on tagged but non-injected fish would be undesirable. 4) The tagging system must be similar to existing equipment, especially in weight and balance, thereby minimizing resistance by fishermen to its use. 5) The tagging pole must meet Australian Game Fishing Association tag and release equipment regulations so that it might be used as the primary tagging system during competitive angling. 6) Its preparation and use must be simple so as to minimize dependence on the skill of the operator. 7) The tag must be conspicuous and readily distinguished from existing game fish tags and endoparasitic copepods, *Pennella instructa,* which can be prevalent and abundant along the dorso-lateral body of the fish (Speare, 1990). 8) The tagging program must be well publicized as success rests solely with the return of recaptured fish.

*Design and Operation.*—The tag pole is a nominal 2 m long by 20 mm diameter light-gauge anodized aluminum tube (Fig. 1). The ends are plugged to provide buoyancy should it be lost overboard. The applicator is rivetted to one end and consists of a body fabricated from 25 mm × 3.2 mm anodized aluminum tube.

The syringe holder, fabricated from material of the same specifications as the pole, slides inside the applicator body. It is locked in place by a spring loaded catch which locates in a slot machined in the syringe holder.

Inside the applicator body and behind the syringe holder, a stainless steel spring places tension on the syringe holder against the catch through pressure on the body of the syringe. The 10 ml syringe has been cut down by removing the finger grips and the end of the plunger when set at the maximum dose mark. In this way the dimensions of the applicator and its weight are substantially reduced.

The spring surrounds a plastic rod which acts on the syringe plunger as spring pressure is overcome. Free travel of 10 mm, set by a plastic bushing (marked PVC collar, Fig. 1), allows penetration of the fish before the plastic rod contracts the syringe plunger and initiates discharge.

A collar fixed to the syringe holder carries the tag applicator which is offset from the 14 gauge needle by 25 mm. This offset eliminates leakage of the dose through the tag puncture wound. The collar also acts to limit the travel of the syringe holder and thereby the dose delivered as it comes against another "dose control" collar which rotates about the distal end of the applicator body. The latter collar travels axially to allow rapid selection of dosage dependent on the estimated fish weight. Four doses can be

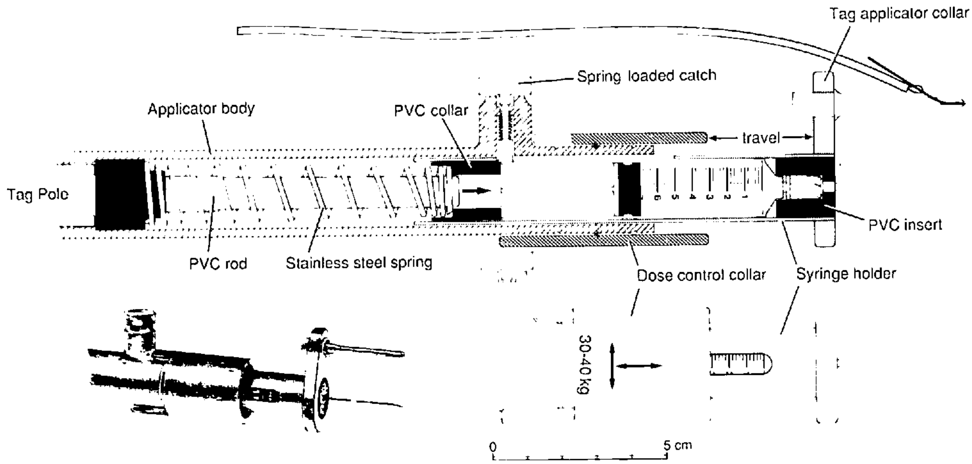


Figure 1. Structural drawing of the tagging pole used to inject oxytetracycline and simultaneously tag juvenile black marlin and sailfish.

selected to deliver tetracycline to fish up to 15 kg (3 ml), 15–30 (6 ml), 30–40 (7 ml) and 40–60 kg (8 ml) weight, respectively. By this means, the effective dose delivered to fish below 40 kg ranged between 20 and 50 mg·kg$^{-1}$ and 10–20 mg·kg$^{-1}$ for fish over 40 kg. All machined aluminium parts were anodized.

Terramycin/MA (Pfizer), veterinary, injectable oxytetracycline hydrochloride (OTC) rated at 100 mg·ml$^{-1}$ was used. This solution was readily available and does not carry the slow release agent, 2-pyrrolidone, as found in the more concentrated form, Terramycin/LA (200 mg·ml$^{-1}$).

Tetracycline injections into skipjack, *Katsuwonas pelamis*, and yellowfin, *Thunnus albacares*, tuna (Foreman, 1987; Wild and Foreman, 1980) indicated dosages of 27 mg·kg$^{-1}$ produced a clearly discernible mark in fish to 45 kg. Larger fish received half this dose rate. A nominal dosage of 30 mg·kg$^{-1}$ was used in this tagging program for fish to 40 kg and 15 mg·kg$^{-1}$ for heavier fish (see dose selection above).

## Results and Discussion

The tag pole was tested on the billfish grounds off Cape Bowling Green, south of Townsville, in July–August, 1989, aboard two professional gamefishing charter boats. Forty-one black marlin were tagged by the crews of these boats over this period. The tagging gear was then made available to each of seven boats competing in two, 5-day tournaments in late August and early September of the same year. In this period, 242 billfish, predominantly black marlin, were tagged or caught. Of these fish, 139 black marlin and 2 sailfish (60% of all fish) were released with an orange 20 cm filament tag indicating that they had been injected with OTC. Only five of these fish carried the tag without the injection due to malfunction of the equipment.

Similarly, in 1990, 177 black marlin and 18 sailfish were successfully tagged, injected and released. The majority of these tags were placed during the two major annual tournaments and the balance during normal charter operations.

The tagged fish were estimated to weigh between 4 and 93 kg, with the majority between 10 and 30 kg (Fig. 2). The dosages delivered varied considerably but the majority of fish received between 15 and 30 mg·kg$^{-1}$ OTC. High and low doses were usually attributable to incorrect setting of the dose control collar or the needle folding on striking the fish (low dose).

During tournament fishing, the primary goal of the angler and crew is to tag and release the fish as quickly and cleanly as possible, thereby scoring angling

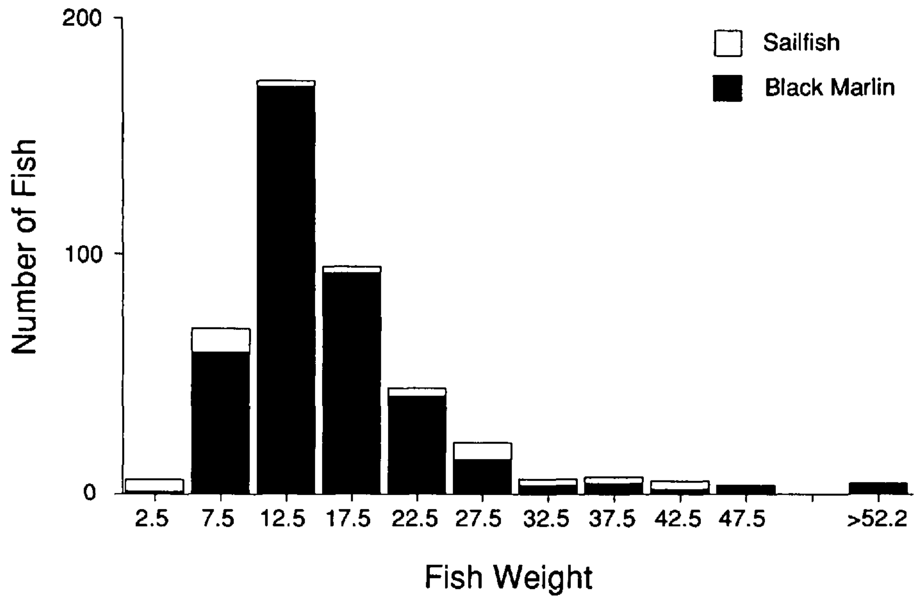


Figure 2. Size distribution of sailfish and juvenile black marlin injected with oxytetracycline and tagged off Cape Bowling Green in 1989 and 1990. X axis values designate 5 kg weight-class midpoints.

points. In these instances, most crews preferred to use their familiar tagging pole, carrying the standard yellow gamefish tag, and then proceed to simultaneously tetracycline inject and tag (orange "tetracycline" tag) the fish. This procedure, while adding additional stress to the fish, was advantageous in that the injection and second tag was administered under more controlled conditions. Double tagging also improved the chances of recognizing a tetracycline injected fish (see below).

Failure to inject or partial delivery of the intended dose was mainly a consequence of the needle folding upon striking the fish. Four factors influence the selection of the needle: the bore which limits the rate of delivery, the wall thickness which provides strength to the needle, the consequent puncture diameter and the length of the needle. Amongst single use disposable needles, there is no commercially available compromise between bore and wall thickness where rapid delivery is the major criterion. The relatively heavy 14 gauge needle (Monoject), as used, facilitated rapid delivery and leakage from the relatively large puncture was minimized by the depth of penetration (40 mm).

The effective delivery of the OTC to the fish was largely dependent on the care taken to direct the tag shot perpendicular to the body of the fish. Billfish can be very agile close to the boat and successful tagging, incorporating a minimum of stress to the fish, is dependent on the skill and attention of the operator.

To date, five OTC black marlin have been recaptured. This represents a recapture rate of 1.1%, which compares favorably with that achieved through normal tagging practices, despite the perceived additional stress inflicted during tagging.

The first recapture was re-released, after 6 months at large, as only the yellow N.S.W. Fisheries tag was detected. Unfortunately, re-release of tagged fish appears to be increasing amongst Australian billfishermen. The second fish recaptured was at large for only 7 days. It weighed 17.2 kg and received a 23 $mg \cdot kg^{-1}$ dose. A clear tetracycline mark was visible on the margins of otolith and fin-spine

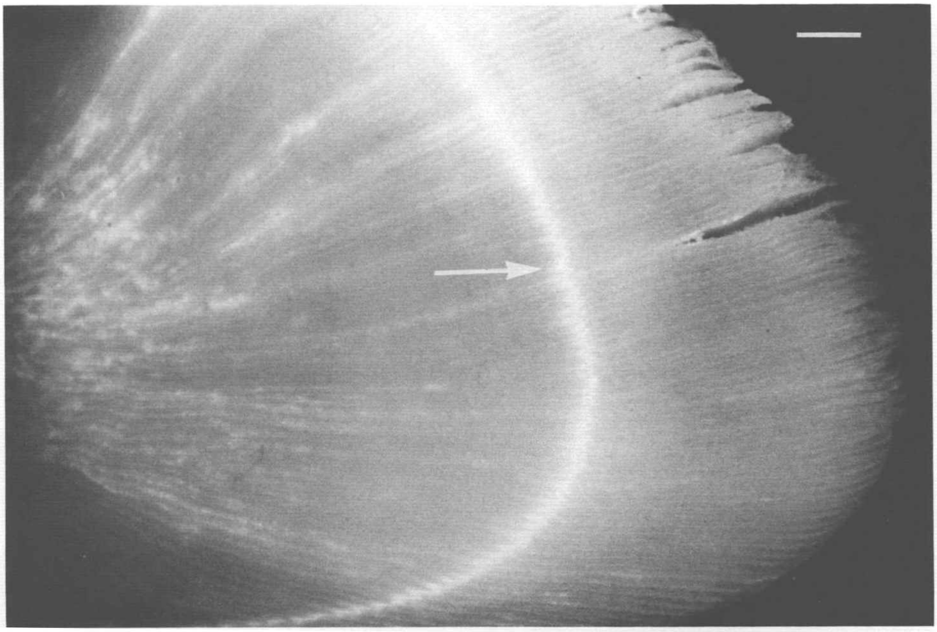

Figure 3. Photomicrograph of a fin-spine section from a recaptured 27 kg (estimated 20 kg on release) juvenile black marlin showing the oxytetracycline mark (white arrow). Scale bar = 0.5 mm.

sections. The third fish was recaptured 6 months later by a Papua New Guinea fisherman in a dugout canoe. The orange tag was returned as it was inedible. The fourth fish, as with the first, was not recognized by an orange tag (missing) but, was boated as a normal recapture. It was subsequently identified from the relevant yellow tag card which also carried the OTC tag number.

This fourth recapture was at large for 198 days and had travelled south a distance of 2,200 km from Cape Bowling Green, off Townsville, northern Queensland, to Montague Island, off Bermagui, southern N.S.W. The fish was recorded on the tag card as receiving 3 ml of OTC. Based on the estimated weight of 20 kg, this fish received a maximum dosage of 15 $mg \cdot kg^{-1}$ OTC. An excellent fluorescent mark was discernible on the otolith and fin-spines (Figs. 3, 4).

The fifth recapture, a fish of 8 kg, was at large for 9 days at Cape Bowling Green. This double tagged black marlin received 38 $mg \cdot kg^{-1}$ of OTC and, again, a clear tetracycline mark was incorporated into the margins of otoliths and fin-spines.

The lowest dosage delivered to these three fish (15 $mg \cdot kg^{-1}$) probably overestimates the effective dose by a few milligrams as, while the puncture wound remains patent, some leakage does occur. Given the excellent visibility of the tetracycline mark in this fish, an effective dose of 10 $mg \cdot kg^{-1}$ will suffice to produce a readily discernible mark in black marlin.

It is now evident that a successful OTC tagging program can be managed for billfish. Clearly, the system works within the limits imposed by a sometimes lively boat, agile fish and variably proficient users of the equipment. As the orange tag identifies the fish as having received a tetracycline injection, the loss of this tag (2 of 5) is a major concern. Given the relatively short periods at large of these two fish, it is most probable that the tag was not anchored in the flesh and fell into the water. The stainless head of this tag is the same as that used on the proven

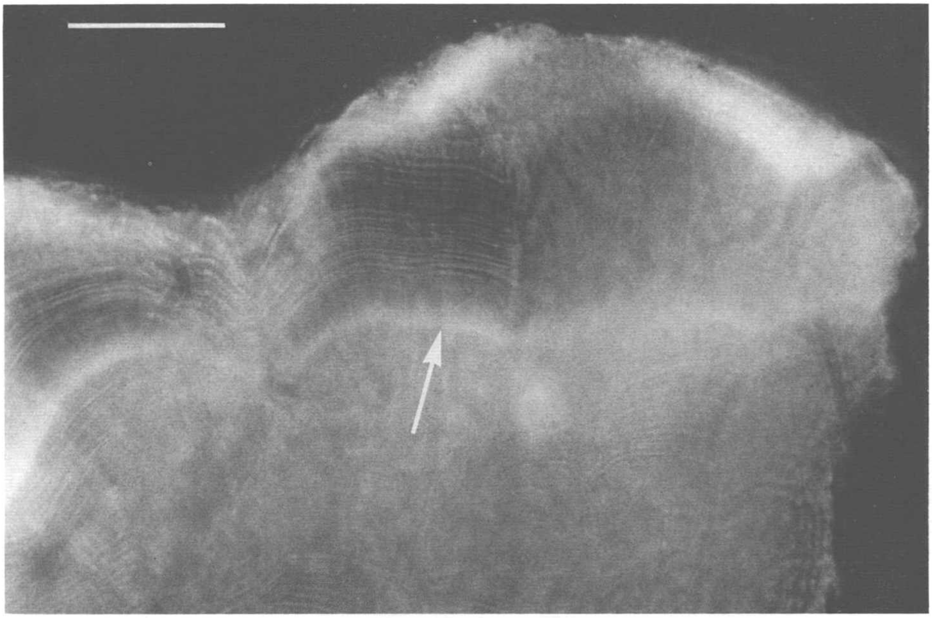

Figure 4. Photomicrograph of the ground right sagitta from the same black marlin indicated in Figure 3 showing the tetracycline mark (white arrow). Scale bar = 0.1 mm.

yellow tag which, even when poorly set, remains well anchored (pers. obs.—23 recaptured billfish).

While recapture rates remain low, they exceed current mean recapture rates of yellow gamefish tags and, normal, therapeutic dosages of tetracycline are sufficient to produce a clearly discernible mark. Given the support of the gamefishing fraternity, such a tagging program can be very cost effective.

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