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Evaluating the effectiveness of teeth and dorsal fin spines for non-lethal age estimation of a tropical reef fish, coral trout *Plectropomus leopardus*

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This study investigated whether teeth and dorsal fin spines could be used as non-lethal methods of age estimation for a vulnerable and highly valued tropical fisheries species, coral trout *Plectropomus leopardus*. Age estimation of individuals from 2 to 9 years old revealed that dorsal spines represent an accurate ageing method (90% agreement with otoliths) that was more precise [average per cent error (APE) = 4·1, coefficient of variation (c.v.) = 5·8%] than otoliths (APE = 6·2, c.v. = 8·7%). Of the three methods for age estimation (otoliths, dorsal spines and teeth), spines were the most time and cost efficient. An aquarium-based study also found that removing a dorsal spine or tooth did not affect survivorship or growth of *P. leopardus*. No annuli were visible in teeth despite taking transverse and longitudinal sections throughout the tooth and trialling several different laboratory methods. Although teeth may not be suitable for estimating age of *P. leopardus*, dorsal spines appear to be an acceptably accurate, precise and efficient method for non-lethal ageing of individuals from 2 to 9 years old in this tropical species.

Key words: age determination; ageing; fisheries management; non-destructive; Serranidae; vulnerable species.

INTRODUCTION

Age estimation is important for calculating population parameters that are required for managing fisheries and conserving vulnerable species. Estimating the age of a fish typically involves counting increments (rings) on otoliths that have been usually produced by fluctuations in environmental conditions (Green *et al.*, 2009). Obtaining otoliths requires collection and sacrifice of many individuals, which is undesirable for species of conservation, economic or recreational importance. Therefore, use of non-lethal techniques would be highly beneficial for estimating the age of vulnerable species or valuable fisheries targets.

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Scales, fin rays and fin spines have been proposed as useful structures for non-lethal age estimation of fish. Although scales have been used to age many fishes, scale loss can significantly limit the success of this approach (Moltschaniwskyj & Cappo, 2009). Fin rays and spines have been successfully used to age many freshwater and marine species (Debicella, 2005; Moltschaniwskyj & Cappo, 2009); however, in some species, it can be difficult to determine the first annulus (due to occlusion and resorption) and to distinguish annuli on the outer edge of spines of older fish (Beamish & Chilton, 1977; Graynoth, 1996; Debicella, 2005). In the marine environment, fin spines have been used to successfully age pelagic species (Franks *et al.*, 2000; Kopf *et al.*, 2010), as well as temperate (Metcalf & Swearer, 2005) and sub-tropical reef fishes (Debicella, 2005; Brusher & Schull, 2009). The suitability of fin spines to age tropical reef fishes has seldom been evaluated (Manooch & Drennon, 1987) and may be difficult because increments are often less well defined in scales and otoliths of fishes from low latitudes (Longhurst & Pauly, 1987; Fowler, 2009). Development of non-lethal age estimation techniques for tropical species would be beneficial given the high diversity of fishes on coral reefs and the increasing effects from habitat loss and overfishing.

The presence of annuli in teeth has been used successfully for non-lethal age estimation of several terrestrial and marine mammals (Perrin & Myrick, 1980; Hamlin *et al.*, 2000; Childerhouse *et al.*, 2004), but this approach has not yet been tested on fishes. For any structure (teeth, fin rays, fin spines or scales) to be deemed an effective non-lethal method of age estimation, several criteria must be met: (1) the structure is present at all life stages and provide a continuous record of age, (2) increments are visible, (3) age estimation is accurate (*i.e.* number of increments correspond to the age of the fish), (4) age estimation is precise, (5) processing and handling of samples is time and cost efficient and (6) the removal of a structure has minimal effect on the health and survival of a fish.

The overall aim of this study was to determine if fin spines and teeth are effective for non-lethal age estimation of a tropical marine fish. This study focused on coral trout *Plectropomus leopardus* (Lacépède 1802) (family Serranidae), because this coral reef fish is an important commercial and recreational fisheries target, and increasing proportions of *P. leopardus* populations occur in marine reserves where destructive sampling is prohibited or undesired. Furthermore, the majority of species in this genus (including *P. leopardus*) are listed as Near Threatened or Vulnerable due to reductions in population sizes (IUCN, 2012), which heightens the need for non-lethal sampling methods.

The specific aims of this study were firstly, to determine whether teeth and dorsal spines are present throughout the life of the fish by examining larvae and adults; secondly, to determine whether teeth and dorsal spines have visible increments and whether these increments are formed at the same frequency as otolith increments and thirdly, to determine whether teeth and dorsal spines represent an acceptably accurate, precise and efficient method for non-lethal age estimation. The final aim was to determine whether the removal of a tooth or dorsal spine affects survivorship, growth or feeding of the study species. Although this study focused on *P. leopardus*, individuals of barcheek coral trout *Plectropomus maculatus* (Bloch 1790) were also used to address the first aim and determine whether teeth and dorsal spines are present throughout the lives of these two species.

MATERIALS AND METHODS

To determine whether dorsal spines and teeth are present throughout the life of the coral trout, this study first examined at what age these structures develop. Larvae of *P. leopardus* and *P. maculatus* were raised in the larval-rearing aquarium facility at James Cook University, Townsville [for methodology see Frisch & Hobbs (2007)]. Each day after hatching, three individuals were collected from each species and examined microscopically for spines and teeth. Secondly, this study examined whether dorsal spines and teeth were missing or damaged across a wide size range of wild-caught individuals. *Plectropomus leopardus* and *P. maculatus* were caught by linefishing or spearfishing at various locations (Pelorus Island, Trunk Reef, Bramble Reef and Britomart Reef) in the central section of the Great Barrier Reef, Australia (October to November 2005). Examination focused on the second dorsal spine and the four prominent canine teeth (two on the upper jaw and two on the lower jaw) because these structures are relatively large and easy to locate in adult fish.

A sub-set of 20 individuals of *P. leopardus* were kept for age estimation. Whole canine teeth (the two on the upper jaw), second dorsal spine and sagittal otoliths were removed from each individual by dissection. The dorsal spine was removed by cutting the spine where it emerged from the flesh. A 2 mm section at the base of the spine was cut, placed on a microscope slide and set in Crystal Bond thermoplastic cement (Aremco; http://aremco.com/). The transverse section was ground to *c.* 0·5 mm thickness and the cement was melted so that the section could be inverted. The section was then reset and ground further until it was sufficiently thin that increments could be observed microscopically.

The same method was initially used for canine teeth, but due to the lack of visible increments at the tooth base, additional transverse sections were taken throughout the tooth. Transverse sections (1–2 mm thick) were cut, ground and continually checked for increments until there was no tooth left. Following this, the second canine tooth of each fish was sectioned longitudinally throughout and ground whilst continually checking for increments. Because no increments were visible in transverse and longitudinal sections, additional canine teeth from the lower jaw were kept for an alternative histological approach (decalcification). The histological approach developed by Fox (2006) for age estimation of bats (Chiroptera) was used because the size and shape of the teeth were similar to those of *Plectropomus* spp. This method was used first and then systematically modified to search for visible increments. The process involved decalcifying teeth in 10% formic acid initially for 2 h (then subsequent trials at 6, 12, 24, 48 and 96 h during which acid was replaced every 24 h). Teeth were then washed and placed in a Shandon Hypercentre (www.gmi-inc.com) where samples go through an automated series of ethanol and xylene washes and are eventually embedded with paraffin wax. Samples were embedded in a wax block and sectioned at 5–10 µm using a manual rotary microtome. Many transverse and longitudinal sections were taken throughout each tooth. Sections were placed on microscope slides, dried for 48 h at 37° C and then stained using Mayer's haematoxylin and Young's eosin-erythrosin (Woods, 1994). The sections were covered with dibutyl phthalate in xylene and a cover slip, and placed in an oven (37° C for 48 h). Sections were microscopically examined for growth increments.

Transverse sections of sagittal otoliths were processed using standard methods (Wilson & McCormick, 1997). Annual increments have been validated for *P. leopardus* (Ferreira & Russ, 1995). Three counts were made, each on different days, by the same experienced reader, and if these counts differed, the median was recorded. The same procedure and reader was used to estimate age from spines. Otoliths and spines were coded independently to prevent any observer bias (*i.e.* spines and otoliths were read blind).

The accuracy of non-lethal techniques was judged by comparing the number of increments on spines and teeth to the number of increments on the corresponding otolith. The increments observed in otoliths were deemed to represent the true age of the fish because they have been validated as annuli for this species (Ferreira & Russ, 1994). Within-reader precision for each technique was estimated using average per cent error (APE) and coefficient of variation (C.V.) (Beamish & Fournier, 1981; Chang, 1982). The cost and time efficiency of each age estimation technique was determined by calculating the total cost and time of handling and processing all samples from the beginning of the otolith, tooth and spine removal to the completion of the readings. Cost included consumables but did not include equipment and laboratory use.

To determine the effect of removing a tooth or dorsal spine on fish health and survivorship, 19 *P. leopardus* individuals were captured (from Trunk Reef, Bramble Reef and Britomart Reef) by linefishing and transported to aquaria at James Cook University, Townsville. Each individual was then anaesthetized using a 10% clove oil solution and weighed, measured (fork length, L_F) and tagged with a uniquely numbered T-bar anchor tag. Each tagged fish was randomly allocated to one of the three groups: control ($n = 7$), tooth removed ($n = 6$) or dorsal spine removed ($n = 6$). Whilst anaesthetized, treatment fish either had a canine tooth (upper left) removed with pliers or the second dorsal spine was cut (using scissors) where it emerges from the flesh of the fish. The removal of a tooth or spine took <1 min and all fish were placed in a recovery tank. After a few minutes of recovering in a small tank, all fish were transferred to a 250 000 l outdoor tank with natural photoperiod (19·25° S) and a water exchange rate of *c*. 15% h^{-1}. No natural habitat was present in the tank, but numerous (>25) polyvinyl chloride (PVC) pipe sections (7·5–15 cm diameter, 40–75 cm long) were placed at the bottom of the tank and were used by fish as shelter. Fish were kept undisturbed except for feeding with whole pilchards *Sardinops* spp. *ad libitum c*. every 5 days. The mean amount eaten by experimental fish in each group was determined by observing which fish ate each *Sardinops* spp. and identifying if it was a tooth-removed, spine-removed or control fish by the position of the tag. Fish entered the tank on 12 November 2005 and all fish were removed and euthanized at the end of the experiment on 17 December 2005.

RESULTS

Microscopic examination of *P. leopardus* larvae revealed that dorsal spines and canine teeth emerged 6–7 days after hatching. In the same larvae, otoliths were visible 2–3 days after hatching. Eighty-seven wild-caught *P. leopardus* and *P. maculatus*, 28 to 59 cm L_F, were examined and all had undamaged second dorsal spines. All four of the prominent canine teeth were present in all individuals; however, 14 individuals had different sized canines, which may indicate earlier tooth loss.

Increments were visible in the second dorsal spine of all *P. leopardus* individuals examined ($n = 20$; Fig. 1). The age estimates obtained from dorsal spines were closely aligned to estimates obtained from otoliths, with the slope of this relationship not differing significantly from 1 ($F_{1,19}$, $P < 0.001$, adjusted $r^2 = 0.76$, 95% C.I. = 0·73–1·27) (Fig. 2). The age obtained from dorsal spines was identical to estimates obtained from otoliths in 90% of cases. In two cases, the spine-based age was higher than the otolith-based age. Age estimates from dorsal spines were more precise than those obtained from otoliths (spine APE = 4·1, C.V. = 5·8%; otolith APE = 6·2, C.V. = 8·7%).

No increments were visible in transverse or longitudinal sections of canine teeth of *P. leopardus*. Despite taking many transverse and longitudinal sections through every part of the tooth, and also trying decalcification and staining, no increments were observed.

Of the three different approaches (otoliths, spines and teeth), spines were the most efficient way to age *P. leopardus* (Table I). Otoliths and spines had similar low costs for consumables, and spines took the least time, largely due to the ease and speed of removal. Additional costs associated with equipment and laboratory use were not examined, but would be similar for otolith and spines because the same approach is used to process samples. Processing teeth in a histology laboratory, however, would incur additional costs because of the extra chemicals and equipment required.

There was no evidence that removing a tooth or spine had negative effects on feeding, growth or survival of the study species. All individuals survived to the end

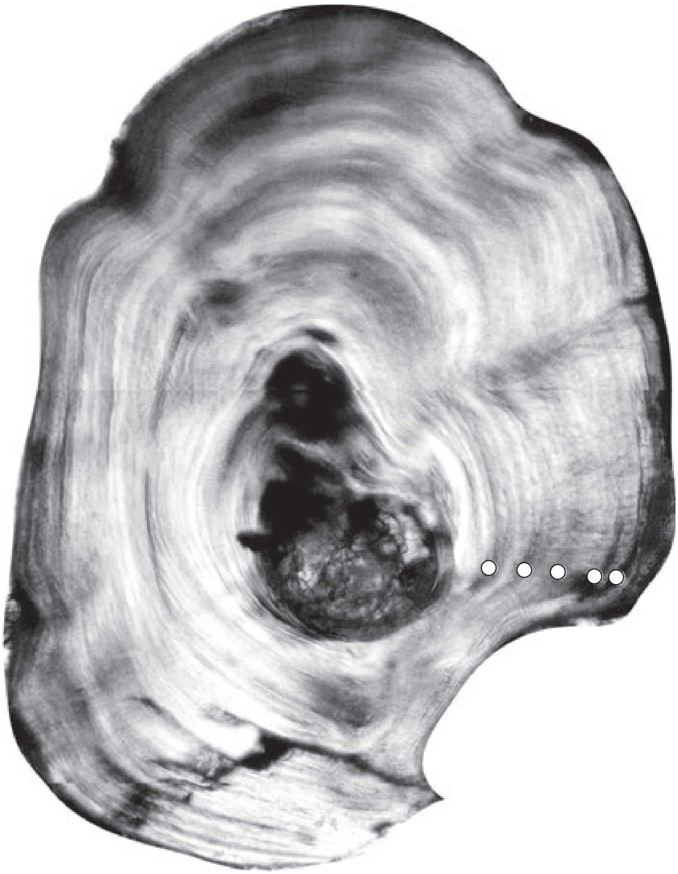

FIG. 1. Transverse section of dorsal fin spine from a 5 year-old *Plectropomus leopardus*. Annuli are indicated (○).

of the 36 day trial. There was no significant change in mass between the start and end of the trial for fish in the control ($t = 1{\cdot}26$, d.f. = 6, $P > 0{\cdot}05$) and tooth-removed groups ($t = 2{\cdot}37$, d.f. = 5, $P > 0{\cdot}05$), but there was a significant increase in mass in the dorsal spine removed group [$t = 3{\cdot}26$, d.f. = 5, $P < 0{\cdot}05$; Fig. 3(a)]. There was no significant difference in L_F between the start and end of the trial for all treatment and control groups [$P > 0{\cdot}05$ for all groups; Fig. 3(b)]. Throughout the trial, treatment and control groups fed on a similar amount of *Sardinops* spp. ($F_{2,18} = 0{\cdot}11$, d.f. = 2, $P > 0{\cdot}05$). At the end of the trial, all individuals from the treatment groups appeared healthy and their minor wound (from tooth and spine removal) had healed completely with no swelling or infection (Fig. 4).

DISCUSSION

Although annuli tend be less well defined in structures from tropical fishes (*e.g.* otoliths; Fowler, 2009), this study has shown that dorsal spines of *Plectropomus*

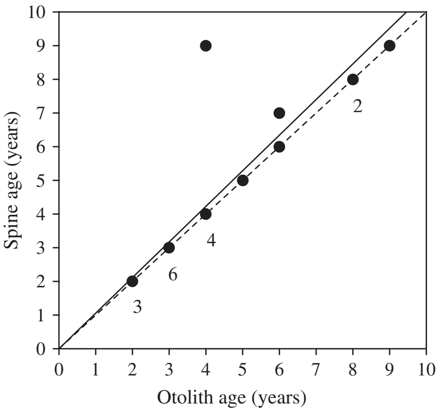

FIG. 2. The relationship ($y = 1{\cdot}0024x + 0{\cdot}2895$; - - - -) between age determined by otolith and dorsal spines for 20 *Plectropomus leopardus* individuals. The number of individuals with overlapping ages is indicated with numbers. ——, 100% agreement between age determined by otoliths and dorsal spines.

leopardus meet the criteria for an acceptable method of non-lethal age estimation. Estimating age using dorsal spines was accurate, precise and efficient, and the removal of a spine did not have a detectable effect on the fish. Dorsal spines were present at all life stages and had visible increments that corresponded to the number of otolith annuli. Annuli have previously been validated in otoliths of *P. leopardus* (Ferreira & Russ, 1994) and in *P. maculatus* (Ferreira & Russ, 1992), and the close agreement between ages determined from dorsal spines and otoliths indicates that increments observed in dorsal spines are annuli. Furthermore, annuli have been validated in dorsal spines of another serranid species (Brusher & Schull, 2009).

The relationship between spine and otolith age did not differ significantly from complete agreement, indicating that the accuracy of age estimates obtained from spines is similar to that of otoliths. There was 90% agreement between dorsal spine and otolith age estimates, which is equal to or higher than previous studies between dorsal rays or spines and otoliths (agreement of 49–90%; Sikstrom, 1983; Sipe & Chittenden, 2002; Debicella, 2005; D. J. Murie & D. C. Parkyn, unpubl. data). The precision of age estimates for *P. leopardus* dorsal spines (APE = 4·1) was better than for otoliths (6·2) and better than the 6·7 and 12·1 recorded for whole and sectioned otoliths of this species in a previous study (Ferreira & Russ, 1994). The C.V. value for dorsal spines in this study (5·8%) was better than the median C.V. value (7·6%)

TABLE I. Total time to dissect, process and read otoliths, dorsal spines and teeth from 20 *Plectropomus leopardus* individuals. Reading of teeth was not possible due to lack of rings

Structure	Time (min) Dissection	Processing	Reading	Sum total
Otoliths	73	88	91	252
Dorsal spines	4	73	107	184
Teeth	10	702	Not possible	712

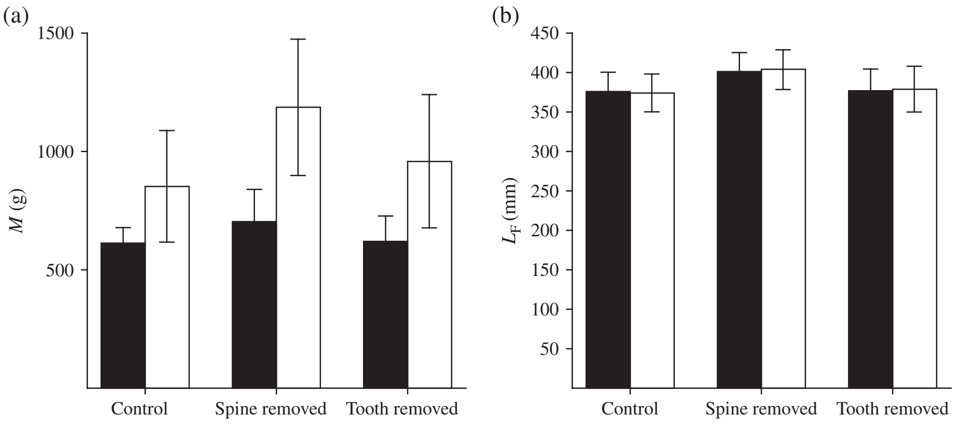

FIG. 3. Mean ± S.E. (a) mass (M) and (b) fork length (L_F) of *Plectropomus leopardus* before (■) and 36 days after (□) the removal of a dorsal spine or tooth. The effect of tooth or spine removal on *P. leopardus* growth was tested in a 36 day aquarium-based study where sample sizes for each group were: control (n = 7), dorsal spine removed (n = 6) and tooth removed (n = 6). All fish survived for the duration of the aquarium study.

from 117 ageing studies, and better than the mean C.V. values calculated for otoliths, spines, scales and vertebrae from those studies (Campana, 2001). Therefore, dorsal spines appear to be a suitably accurate and precise approach for age estimation of *P. leopardus*, within the ages that were examined.

In the study region (central section of the Great Barrier Reef), *P. leopardus* can live to 14 years with the majority of adult fish being <10 years (Ferreira & Russ,

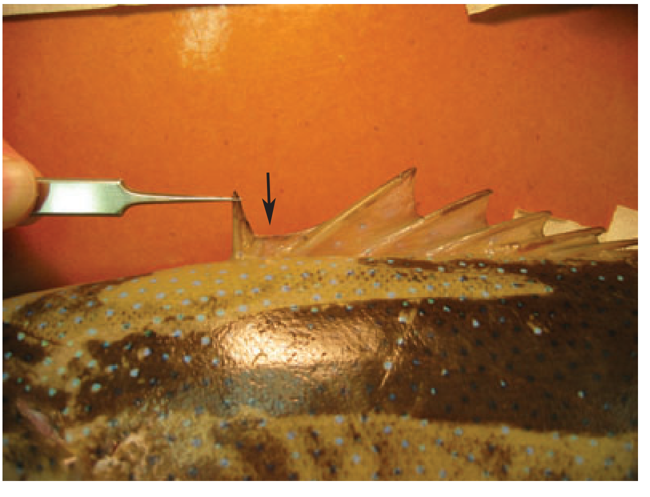

FIG. 4. A photograph illustrating the healed and healthy dorsal spine membrane of *Plectropomus leopardus* after the 36 day aquarium-based study. →, the location of the second dorsal spine that was removed at the beginning of the study. Forceps are holding the first dorsal spine, which has not been modified and is naturally shorter than the other dorsal spines.

1995; Russ *et al.*, 1996). Therefore, the age range (2–9 years old) of fish in this study spans a considerable proportion of the adult population. Additional comparisons between spines and otoliths outside this age range are required to determine if spines are suitable for estimating all ages. This is particularly important for fish >9 years old because it is difficult to distinguish annuli on the outer edge of spines in older fish (Debicella, 2005; Moltschaniwskyj & Cappo, 2009; Murie *et al.*, 2009). The potential for spines to underestimate the age of older fish (due to the above limitation) is critically important for managing vulnerable and fisheries species because biased estimates of growth and mortality rates can result in overexploitation and population collapses (Campana, 2001).

Although previous studies have found dorsal fin rays to be more time consuming to handle and process than otoliths (Beamish, 1981; Chilton & Beamish, 1982), this study found dorsal spines to be the most efficient age estimation method. Although dorsal spines and otoliths had the same low costs, dorsal spines took considerably less time to dissect. This would be advantageous in the field because it will take only seconds to measure the length of a fish and clip its dorsal spine before releasing it live. Furthermore, unlike the otolith approach, there are no time and space requirements associated with storage, transportation and dissection of fish. Because dorsal spine age estimation is non-lethal, easy and cost efficient, larger sample sizes could be obtained more efficiently, which increases the ability to accurately estimate critical demographic parameters (Metcalf & Swearer, 2005).

The aquarium-based study found no detectable effect on fish that had a tooth or dorsal spine removed. Removal of fin spines was also non-lethal in other marine (Metcalf & Swearer, 2005) and freshwater fishes (Beamish & Harvey, 1969; Faragher, 1992; Collins & Smith, 1996). Numerous recaptures of another serranid that had a dorsal spine removed (Brusher & Schull, 2009) are a positive sign that spine removal may have minimal effect on survivorship in wild populations, but further studies are required to confirm this.

The family Serranidae includes numerous valuable fisheries species and recent population declines from overfishing have resulted in the IUCN (2012) listing many serranids as vulnerable and endangered. For example, species in the genus *Plectropomus* are among the most highly valued commercial and recreational target species on coral reefs (Heemstra & Randall, 1993; Sadovy *et al.*, 2003; Frisch *et al.*, 2008) and the majority are listed as Near Threatened or Vulnerable (IUCN, 2012). The success of dorsal spines and fin rays for estimating age in another serranid, the critically endangered goliath grouper *Epinephelus itajara* (Lichtenstein 1822) (Brusher & Schull, 2009; Murie *et al.*, 2009), indicates that this non-lethal approach may be effective for a wide range of serranids. Given that serranids (particularly *Plectropomus*) are major targets for the live fish trade (Sadovy *et al.*, 2003), it would be relatively easy and beneficial to monitor age structure of exploited stocks by removing the dorsal spine of live fish during transit. Given the results of the present aquarium study, spine removal is unlikely to affect the health or survivorship of transiting fishes, but the effect of spine removal (missing spine and potential discolouration) on the high market prices of these target fishes requires investigation.

Teeth do not appear to be suitable for non-lethal age estimation of *Plectropomus*. Increments could not be seen in the teeth, despite taking numerous transverse and longitudinal sections and trialling decalcification. The composition of fish teeth (dentine and enameloid; Lund *et al.*, 1992) differs from otoliths and spines and it is possible that teeth may be too dense to see increments. In mammal teeth, annuli are present in the cementum (Fox, 2006), but cementum is lacking in fishes (Lund *et al.*, 1992) and therefore it is possible that increments are not present in the tooth structure, or processes such as resorption or secondary infilling may make annuli too difficult to distinguish. Further exploratory studies involving other fish species and a variety of histological methods are required before concluding that teeth are unsuitable for estimating fish age.

It can be concluded from this study that dorsal spines can be used as a viable non-lethal alternative to age a tropical reef fish. Although this study focused on one species (*P. leopardus*), it adds to a growing body of evidence that dorsal spines can be used to age a wide range of marine and freshwater fishes across different habitats and ecosystems. While otoliths have been the traditional and most common approach to estimate fish age, an increase in research evaluating and using dorsal spines is warranted given the rising number of overfished and threatened species. The development of non-lethal age estimation techniques will enable fisheries and conservation management to obtain critical demographic data with minimal impact on wild populations.

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References

Beamish, R. J. (1981). Use of fin ray sections to age walleye pollock, Pacific cod, and Albacore, and the importance of this method. *Transactions of the American Fisheries Society* **110,** 287–299.

Beamish, R. J. & Chilton, D. E. (1977). Age determination of lingcod (*Ophiodon elongates*) using dorsal fin rays and scales. *Journal of the Fisheries Research Board of Canada* **34,** 1305–1313.

Beamish, R. J. & Fournier, D. A. (1981). A method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 982–983.

Beamish, R. J. & Harvey, H. H. (1969). Age determination in the white sucker. *Journal of the Fisheries Research Board of Canada* **26,** 633–638.

Brusher, J. H. & Schull, J. (2009). Non-lethal age determination for juvenile goliath grouper *Epinephelus itajara* from southwest Florida. *Endangered Species Research* **7,** 205–212.

Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59,** 197–242.

Chang, W. Y. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences* **39,** 1208–1210.

Childerhouse, S., Dickie, G. & Hessel, G. (2004). Ageing live New Zealand sea lions (*Phocarctos hookeri*) using the first post-canine tooth. *Wildlife Research* **31,** 177–181.

Chilton, D.E. & Beamish, R.J. (1982). Age determination methods of fishes studied by the Pacific Biological Station. *Canadian Special Publications on Fisheries and Aquatic Sciences* **60**, 1–102.

Collins, M. R. & Smith, T. I. (1996). Sturgeon fin ray removal is nondeleterious. *North American Journal of Fisheries Management* **16,** 939–941.

Debicella, J. M. (2005). Accuracy and precision of fin-ray aging for gag (*Mycteroperca microlepis*). MSc Thesis, University of Florida, Gainesville, FL, USA. Available at http://etd.fcla.edu/UF/UFE0010802/debicella_j.pdf/

Faragher, R. A. (1992). Growth and age validation of rainbow trout, *Oncorhynchus mykiss* (Walbaum)' in Lake Eucumbene, NSW. *Marine and Freshwater Research* **43,** 1033–1042.

Ferreira, B. P. & Russ, G. R. (1992). Age, growth and mortality of the inshore coral trout *Plectropomus maculatus* (Pisces: Serranidae) from the central Great Barrier Reef, Australia. *Australian Journal of Marine and Freshwater Research* **43,** 1301–1312.

Ferreira, B. P. & Russ, G. R. (1994). Age validation and estimation of growth rate of the coral trout, *Plectropomus leopardus*, (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef. *Fishery Bulletin* **92,** 46–57.

Ferreira, B. P. & Russ, G. R. (1995). Population structure of the leopard coralgrouper, *Plectropomus leopardus*, on fished and unfished reefs off Townsville, Central Great Barrier Reef, Australia. *Fishery Bulletin* **93,** 629–642.

Fowler, A. J. (2009). Age in years from otoliths of adult tropical fish. In *Tropical Fish Otoliths: Information for Assessment, Management and Ecology, Reviews: Methods and Technologies in Fish Biology and Fisheries* (Green, B. S., Mapstone, B. D., Carlos, G. & Begg, G. A., eds), pp. 55–92. New York, NY: Springer.

Fox, S. (2006). Population structure in the spectacled flying fox, *Pteropus conspicillatus*: a study of genetic and demographic factors. PhD Thesis, James Cook University, Townsville, Australia.

Franks, J. S., Brown-Peterson, N. J., Griggs, M., Garber, N. M., Warren, J. R., Larsen, K. M. & Creswell, R. (2000). Potential of the first dorsal fin spine for estimating the age of wahoo, *Acanthocybium solandri*, from the northern Gulf of Mexico, with comments on specimens from Bimini, Bahamas. *Proceedings of the Gulf and Caribbean Fisheries Institute* **51,** 428–440.

Frisch, A. J. & Hobbs, J.-P. A. (2007). In vitro hybridization of coral trouts, *Plectropomus leopardus* (Lacepède, 1802) and *Plectropomus maculatus* (Bloch, 1790): a preliminary investigation. *Aquaculture Research* **38,** 215–218.

Frisch, A. J. & Van Herwerden, L. (2006). Field and experimental studies of hybridization between coral trouts, *Plectropomus leopardus* and *Plectropomus maculatus* (Serranidae), on the Great Barrier Reef, Australia. *Journal of Fish Biology* **68,** 1013–1025.

Frisch, A. J., Baker, R., Hobbs, J.-P. A. & Nankervis, L. (2008). A quantitative comparison of recreational spearfishing and linefishing on the Great Barrier Reef: implications for management of multi-sector coral reef fisheries. *Coral Reefs* **27,** 85–95.

Graynoth, E. (1996). Determination of the age of brown and rainbow trout in a range of New Zealand lakes. *Marine and Freshwater Research* **47,** 749–756.

Green, B. S., Mapstone, B. D., Carlos, G. & Begg, G. A. (2009). Introduction to otoliths and fisheries in the tropics. In *Tropical Fish Otoliths: Information for Assessment, Management and Ecology, Reviews: Methods and Technologies in Fish Biology and Fisheries* (Green, B. S., Mapstone, B. D., Carlos, G. & Begg, G. A., eds), pp. 1–22. New York, NY: Springer.

Hamlin, K. L., Pac, D. F., Sime, C. A., DeSimone, R. M. & Dusek, G. L. (2000). Evaluating the accuracy of ages obtained by two methods for Montana ungulates. *Journal of Wildlife Management* **64**, 441–449.

Heemstra, P. C. & Randall, J. E. (1993). FAO Species Catalogue, Vol. 16. Groupers of the World (Family Serranidae, subfamily Epinephelinae). An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper and Lyretail Species Known to Date. *FAO Fisheries Synopsis* **125**.

Kopf, R. K., Drew, K. & Humphreys, R. L. Jr. (2010). Age estimation of billfishes (*Kajikia* spp.) using fin spine cross-sections: the need for an international code of practice. *Aquatic Living Resources* **23,** 13–23.

Longhurst, A. R. & Pauly, D. (1987). *Ecology of Tropical Oceans*. San Diego, CA: Academic Press.

Lund, R., Bartholomew, P. & Kemp, A. (1992). The composition of the dental hard tissues of fishes. In *Structure, Function and Evolution of Teeth* (Smith, P. & Tchernov, E., eds), pp. 35–72. London: Freund Publishing House Ltd.

Manooch, C. S. & Drennon, C. L. (1987). Age and growth of yellowtail snapper and queen triggerfish collected from the US Virgin Islands and Puerto Rico. *Fisheries Research* **6,** 53–68.

Metcalf, S. J. & Swearer, S. E. (2005). Non-destructive ageing in *Notolabrus tetricus* using dorsal spines with an emphasis on the benefits for protected, endangered and fished species. *Journal of Fish Biology* **66,** 1740–1747.

Moltschaniwskyj, N. A. & Cappo, M. C. (2009). Alternatives to sectioned otoliths: the use of other structures and chemical techniques to estimate age and growth for marine vertebrates and invertebrates. In *Tropical Fish Otoliths: Information for Assessment, Management and Ecology, Reviews: Methods and Technologies in Fish Biology and Fisheries* (Green, B. S., Mapstone, B. D., Carlos, G. & Begg, G. A., eds), pp. 133–173. New York, NY: Springer.

Murie, D. J., Parkyn, D. C., Koenig, C. C., Coleman, F. C., Schull, J. & Frias-Torres, S. (2009). Evaluation of finrays as a non-lethal ageing method for protected goliath grouper *Epinephelus itajara*. *Endangered Species Research* **7,** 213–220.

Perrin, W. F. & Myrick, A. (1980). *Age Determination of Toothed Whales and Sirenians*: *Reports of the International Whaling Commission* (*Special Issue 3*). Cambridge: International Whaling Commission.

Russ, G. R., Lou, D. C. & Ferreira, B. P. (1996). Temporal tracking of a strong cohort in the population of a coral reef fish, the coral trout, (*Plectropomus leopardus*) Serranidae: Epinephelinae, in the central Great Barrier Reef, Australia. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 2745–2751.

Sadovy, Y. V., Donaldson, T. J., Graham, T. R., McGilvray, F., Muldoon, G. F., Phillips, M. J., Rimmer, M. A., Smith, A. & Yeeting, B. (2003). *While Stocks Last: The Live Food Reef Fish Trade*. Manila: Asian Development Bank.

Sikstrom, C. B. (1983). Otolith, pectoral fin ray, and scale age determination for arctic grayling. *Progressive Fish-Culturist* **45,** 220–223.

Sipe, A. M. & Chittenden, M. E. Jr. (2002). A comparison of calcified structures for aging bluefish in the Chesapeake Bay region. *Transactions of the American Fisheries Society* **131,** 783–790.

Wilson, D. T. & McCormick, M. I. (1997). Spatial and temporal validation of settlement-marks in the otoliths of tropical reef fishes. *Marine Ecology Progress Series* **153,** 259–271.

Woods, A. E. (1994). Haematoxylin and counterstains. In *Laboratory Histopathology: A Complete Reference* (Woods, A. E. & Ellis, R. C., eds), pp. 1–20. London: Churchill Livingstone.

Electronic Reference

IUCN (2012). *IUCN Red List of Threatened Species*. *Version 2012.2*. Available at http://www.iucnredlist.org/ (last accessed 18 October 2012).

