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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* (Lacepède, 1802)

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Abstract

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 two to nine years old revealed that dorsal spines represent an accurate ageing method (90% agreement with otoliths) that was more precise (APE = 4.1%, CV = 5.8%) than otoliths (APE = 6.2%, CV = 8.7%). Of the three methods for age estimation (otoliths, dorsal spines, teeth), spines were the most time and cost efficient approach. 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 two to nine years old in this tropical species.

Keywords

ageing; age determination; 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 (earbones) that have usually been produced by fluctuations (e.g. daily, seasonal) 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.

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 several fishes, scale loss can significantly limit the success of this approach (Moltschaniwskyj and Cappo, 2009). Fin rays and spines have been successfully used to age many freshwater and marine species (reviewed by Debicella, 2005; Moltschaniwskyj and 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 and 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 and Swearer, 2005) and subtropical reef fishes (Debicella, 2005;

Brusher and Schull, 2009). The suitability of fin spines to age tropical reef fishes has seldom been evaluated (Manooch and Drennon, 1987) and may be difficult because increments are often less well defined in scales and otoliths of fishes from low latitudes (Longhurst and 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 impacts 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 and Myrick, 1980; Hamlin *et al.*, 2000; Childerhouse *et al.*, 2004), but this approach has not yet been tested on fish. 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 must be present at all life stages and provide a continuous record of age, 2. increments must be 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 should have minimal impact 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 focuses on coral trout *Plectropomus leopardus* (Lacepède, 1802) (family Serranidae), because this coral reef fish is an important commercial and recreational fisheries target, and increasing proportions of coral trout 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 to: first determine whether teeth and dorsal spines are present throughout the life of the fish by examining larvae and adults; secondly, 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. Finally, to determine whether the removal of a tooth or dorsal spine affects survivorship, growth or feeding of the study species. Although this study focuses on *P. leopardus*, individuals of barcheek coral trout *P. 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.

Methods and Materials

To determine whether dorsal spines and teeth are present throughout the life of the coral trout, this study first examined 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 detailed methodology see Frisch & Hobbs, 2007). Each day after hatching, three individuals were collected from each species and examined microscopically for spines and teeth. Second, this study examined whether dorsal spines and teeth were missing or damaged across a wide size range of wild-caught individuals. *P. leopardus* and *P. maculatus* were caught by linefishing or spearfishing at various locations (Pelorus Island, Trunk Reef, Bramble Reef, Britomart Reef) in the central section of the Great Barrier Reef, Australia (October – November 2005). This part of the study also included *P. maculatus* because this species and *P. leopardus* cohabit at the study locations and were both caught while linefishing (Frisch and Van Herwerden, 2006). Examination focussed 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 subset of 20 individuals of the above collected *P. leopardus* was 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). The transverse section was ground to approximately 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. However, 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 and 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 grounded 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 coral trout. 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 hrs (then subsequent trials at 6, 12, 24, 48 and 96 hrs during which acid was replaced every 24 hrs). Teeth were then washed and placed in a Shandon Hypercentre where samples go through an automated series of alcohol 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 hrs in a 37°C oven, and then stained using Mayer's Haematoxylin and Young's Eosin-Erythosin (Woods, 1994). The sections were then covered with dibutyl phthalate in xylene and a cover slip, and placed in an oven (37°C for 48 hrs). Sections were then microscopically examined for growth increments.

Transverse sections of sagittal otoliths were processed using standard methods (as per Wilson and McCormick, 1997). Annual increments have been validated previously for *P. leopardus* (Ferreira and Russ, 1995). Three counts were made, each on different days, by the same experienced reader (S. Mutz), and if these counts differed, the median was recorded. The same procedure and reader were 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 and Russ, 1994). Within-reader precision for each technique was estimated using average percent error (APE) and coefficient of variation (CV) (Beamish and Fournier, 1981; Chang, 1982). The cost and time efficiency of each age estimate technique was determined by calculating the total cost and time of handling and processing all samples from the beginning of the otolith/tooth/spine removal to the completion of the readings. Cost includes consumables and does not include equipment and laboratory use.

To determine the impact of removing a tooth or dorsal spine on fish health and survivorship, 19 *P. leopardus* individuals were captured (from Trunk Reef, Bramble Reef, Britomart Reef) by linefishing and transported to aquaria at James Cook University, Townsville. Each individual was then anaesthetised 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 three groups: control (*n* = 7), tooth removed (*n* = 6), or dorsal spine removed (*n* = 6). Whilst anaesthetised, 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 less than 1 min and all fish were placed in a recovery tank. After a few minutes recovering in a small tank, all fish were transferred to a 250,000 L outdoor tank with natural photoperiod and a water exchange rate of ~15% hr^{-1}. No natural habitat was present in the tank, but numerous (> 25) 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* approximately every 5 d. The mean amount eaten by experimental fish in each group was determined by observing which fish ate each pilchard 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 fished were removed and euthanised at the end of the experiment on 17 December 2005.

Results

Microscopic examination of coral trout 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 coral trout (*P. leopardus* and *P. maculatus*), ranging in size from 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 tooth loss in the past.

Increments were visible in the second dorsal spine of all *P. leopardus* individuals examined (n = 20, Figure 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% CI = 0.73 – 1.27) (Figure 2). The age obtained from dorsal spines was identical to estimates obtained from otoliths in 90% (18/20) 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%, CV = 5.8%; otolith APE = 6.2%, CV = 8.7%).

No increments were visible in transverse or longitudinal sections of canine teeth of *P. leopardus*. Despite taking many sections (both transverse and longitudinal) through every part of the tooth, and also trying decalcification and staining, no visible 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 dissections. 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. However, processing teeth in a histology laboratory would have higher additional costs because of the extra chemicals and equipment required.

There was no evidence that removing a tooth or spine had any negative effects on feeding, growth or survival of the study species. All individuals survived to the end of the 36 d trial. There was no significant change in weight between the start and end of the trial for fish in the control (t = 1.26, *d.f.* = 6, P = 0.25) and tooth-removed groups (t = 2.37, *d.f.* = 5 , P = 0.06), but there was a significant increase in weight in the dorsal spine removed group (t = 3.26, *d.f.* = 5 , P = 0.02, Figure 3a). There was no significant difference in fork length between the start and end of the trial for all treatment and control groups (*P* > 0.1 for all groups, Figure 3b). Throughout the trial, treatment and control groups fed on a similar amount of pilchards ($F_{2,18}$ = 0.11, *d.f.* = 2 , *P* = 0.90). At the end of the trial, all individuals from the treatment groups appeared healthy and their minor wound (from tooth/spine removal) had healed completely with no signs of swelling or infection (Figure 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 coral trout meet the criteria for an acceptable method of non-lethal age estimation. Estimating age using dorsal spines was accurate, precise, efficient, and the removal of a spine did not have any detectable impact on the fish. Dorsal spines were present at all life stages and had visible increments that corresponded to the age of the fish (i.e. the number of otolith annuli). Annuli have previously been validated in otoliths of *P. leopardus* (Ferreira and Russ, 1994, and in *P. maculatus*, Ferreira and 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 and 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; Murie and Parkyn 1999; Sipe and Chittenden, 2002; Debicella, 2005). 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 and Russ, 1994). The CV value for dorsal spines in this study (5.8%) was better than the median CV value (7.6%) from 117 ageing studies, and better than the mean CV 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 we 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 less than 10 years (Ferreira and Russ, 1995; Russ *et al.*, 1996). Therefore, the age range (two to nine years old) of fish in this study spans a considerable proportion of the adult population. However, 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 over nine years old because it is difficult to distinguish annuli on the outer edge of spines in older fish (Debicella, 2005; Murie *et al*., 2009; Moltschaniwskyj and Cappo, 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 and Beamish, 1982), this study found dorsal spines to be the most efficient age estimate 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 and Swearer, 2005).

The aquarium-based study found no detectable impact on fish that had a tooth or dorsal spine removed. Removal of fin spines has also been found to be non-lethal in other marine (Metcalf and Swearer, 2005) and freshwater fishes (Beamish and Harvey, 1969; Faragher, 1992; Collins and Smith, 1996). Numerous recaptures of another serranid that had a dorsal spine removed (Brusher and Schull, 2009) are a positive sign that that spine removal may have minimal impact 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 and 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* (Brusher and 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 our aquarium study, spine removal is unlikely to affect the health or survivorship of transiting fishes. However, the impact of spine removal (missing spine, 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 coral trout. We could not see any increments 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 to 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). However, cementum is lacking in fish (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.

This study concludes 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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Tables

Table I. Total time (minutes) and cost (Australian dollars) to dissect, process, and read otoliths, dorsal spines, and teeth from 20 *P. leopardus* individuals. For teeth, processing cost includes 122 minutes for standard processing of all individuals using the same methods as for otoliths and spines, and 580 minutes for the histological approach. Reading of teeth was not possible due to lack of rings. Cost is for consumables only and does not include costs associated with obtaining the necessary equipment (for dissecting, processing and reading) or laboratory access.

Structure	Time (mins)				Cost (AUD)
	Dissection	Processing	Reading	Sum total	
Otoliths	73	88	91	252	21
Dorsal spines	4	73	107	184	21
Teeth	10	702	Not possible	712	72

Figure captions

Figure 1: Transverse section of dorsal fin spine from a 5 year-old *P. leopardus*. Annuli are identified with white dots.

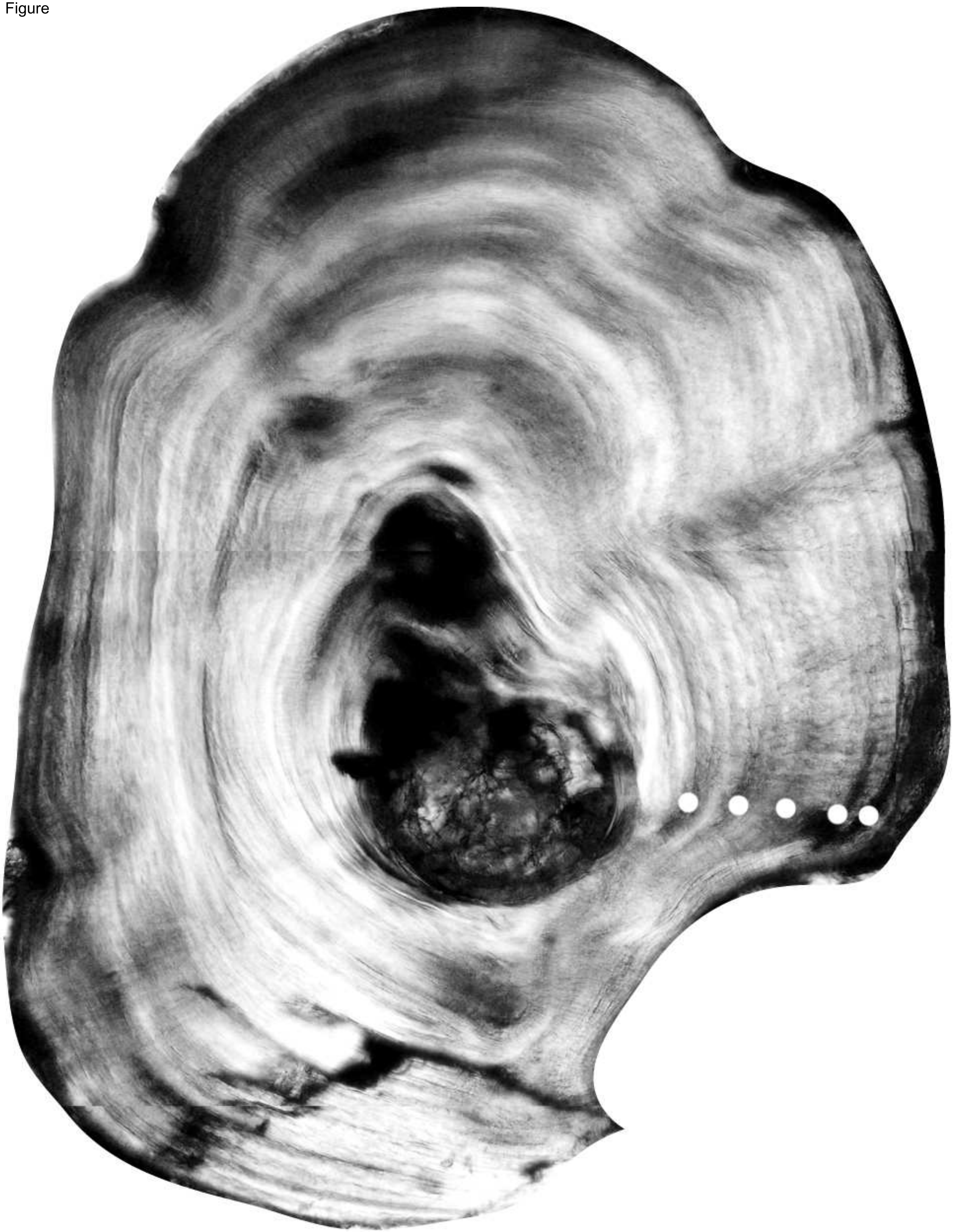
Figure 2: The relationship (y = 1.0024x + 0.2895) between age determined by otolith and dorsal spines for 20 *P. leopardus* individuals. The number of individuals with overlapping ages is indicated with numbers. For comparisons, the solid line represents 100% agreement between age determined by otoliths and dorsal spines.

Figure 3: Mean (± SE) (a) weight and (b) length of *P. leopardus* before (dark bars) and 36 d after (light bars) the removal of a dorsal spine or tooth. The effect of tooth or spine removal on *P. leopardus* growth was tested in a 36 d aquarium-based study where samples 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.

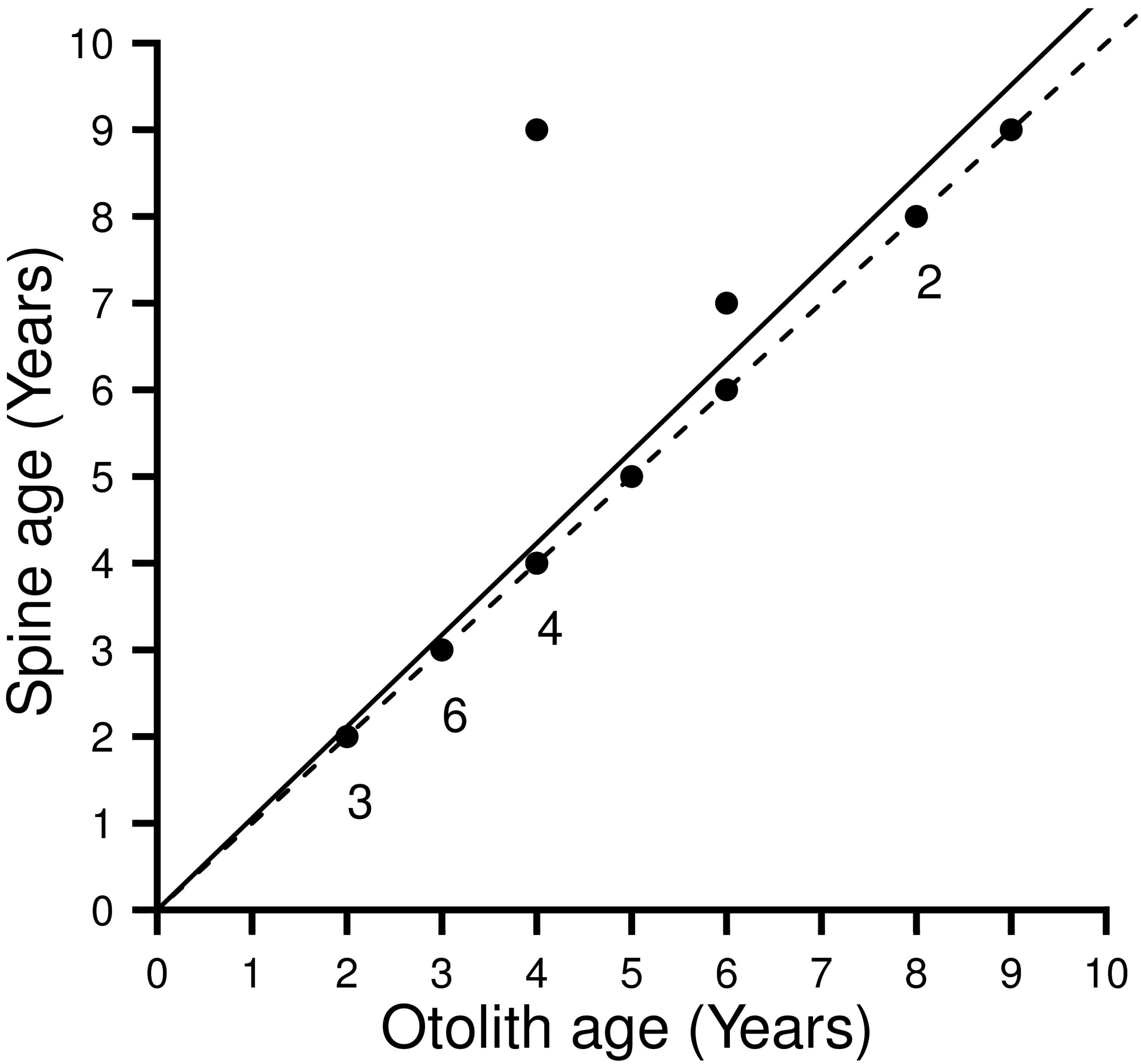
Figure 4: A photograph illustrating the healed and healthy dorsal spine membrane of *P. leopardus* after the 36 d aquarium-based study. An arrow indicates the location of the second dorsal spine that was removed at the beginning of the study. Tweezers are

holding the first dorsal spine, which has not been modified and is naturally shorter than the other dorsal spines.

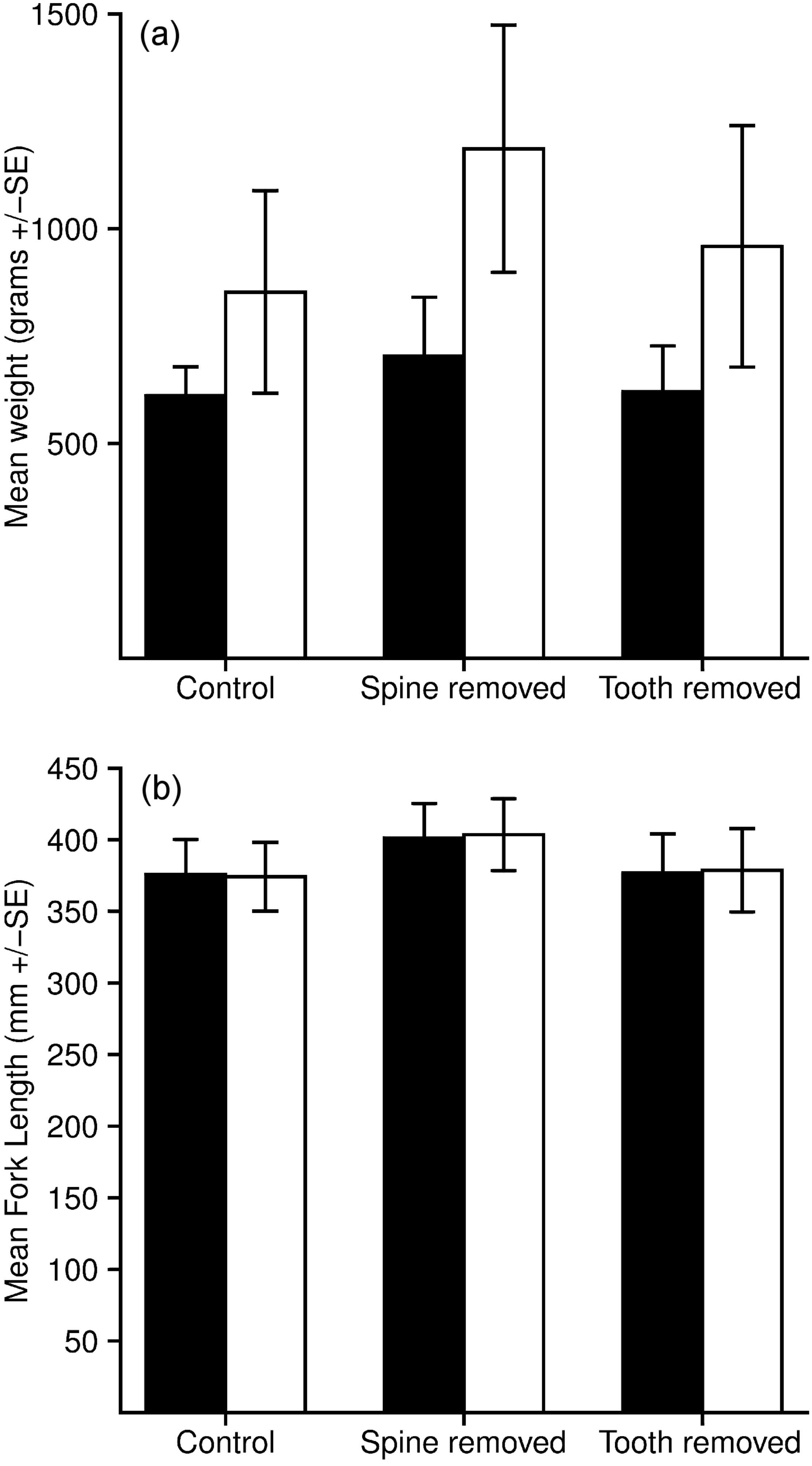
Figure



Figure


10
9
8
7
6
5
4
3
2
1
0
Spine age (Years)
0
1
2
3
4
5
6
7
8
9
10
Otolith age (Years)
3
6
4
2

Figure



(a)
Mean weight (grams +/−SE)
1500
1000
500
Control
Spine removed
Tooth removed
(b)
Mean Fork Length (mm +/−SE)
450
400
350
300
250
200
150
100
50
Control
Spine removed
Tooth removed

Figure