

Development of non-lethal methods for determining age and habitat use of sawfishes from northern Australia



Field IC^{1,2}, Meekan MG³ and Bradshaw CJA^{4,5}

- ¹ School for Environmental Research, Institute of Advanced Studies, Charles Darwin University, Darwin, NT 0909, Australia
- ² Australian Institute of Marine Science, P.O. Box 40197, Casuarina MC, NT 0811, Australia
- ³ Australian Institute of Marine Science The University of Western Australia (MO96) 35 Stirling Highway Crawley WA 6009
- ⁴ Research Institute for Climate Change and Sustainability, School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia
- ⁵ South Australian Research and Development Institute, P.O. Box 120, Henley Beach, SA 5022, Australia

Final Report April 2009

Summary

The measurement of fish life history traits provides important information to estimate rates of population change that are essential for species conservation and fisheries management. Such traits ultimately determine a population's rate of recovery, sustainable harvest and risk of extinction. Of the various traits, age is a key variable that must be estimated to calculate parameters such as population growth rates, mortality, and productivity. Furthermore, life history information should be combined with habitat use patterns and movement data because these determine the degree to which sub-populations are connected. Low connectivity (genetic exchange among sub-populations) increases the risk of extinction. In the past decade it has become clear that marine biodiversity worldwide is under ever-increasing threat, primarily as a result of over-harvesting, pollution and the direct and indirect impacts of climate change. One taxonomic group of marine fishes that has come under increasing scrutiny in terms of extinction risk from these processes is chondrichthyans (sharks and rays and chimaeras), especially those species that live in coastal environments and have critical habitat requirements, such as sawfishes.

There are four species of sawfish in Australia: freshwater (*Pristis microdon*), green (*P. zijsron*), dwarf (*P. clavata*), and narrow (*Anoxypristis cuspidata*). *P. microdon* and *P. zijsron* are listed as *Vulnerable* under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). Although globally endangered, Australian populations may be relatively abundant and the least threatened compared to populations elsewhere and therefore, they provide a unique opportunity to learn and understand the dynamics of arguably the most intact population anywhere in the world. We aimed to develop a non-lethal aging technique for sawfishes and determine if stable isotope analyses could be used as a tool for identifying population sources and movement patterns.

To date, most methods to estimate age have required lethal sampling. We provide evidence that the bands on the rostral teeth of narrow sawfish can be used to estimate age in a non-destructive manner. In this preliminary study, we compared growth rings from vertebrae and rostral teeth for 16 *Anoxypristis cuspidata*. Teeth were stained with silver nitrate and increments counted. There was a positive relationship between size and estimated age of individuals. However, the estimated age of the individual determined from the rostral teeth became more uncertain with increasing age and size. Relative estimates of age from teeth were similar to ages determined from counts of vertebral growth bands, but there was some evidence to suggest that ages were underestimated for older sawfishes relative to those obtained from vertebrae. More research is needed to reduce variation in counts and to verify if this technique can be applied to other species.

We also examined the stable isotope composition of sawfish rostral teeth to determine whether chemical fingerprints could be used as tags to identify populations. We used a solution-based inductively coupled plasma-mass spectrometer (ICP-MS) to obtain chemical signatures. We found regional differences in the stable isotope composition of Anoxypristis cuspidata for individuals from the Gulf of Joseph Bonaparte in the Northern Territory and from the Gulf of Carpentaria in Queensland. We found some evidence to suggest that local differences in stable isotope composition exist for Pristis microdon and P. clavata caught along the Queensland coast, but results were uncertain due to small sample sizes. When individuals of all species were combined (assuming that stable isotope physiological integration processes were the same), we found a difference in chemical signatures that might indicate that freshwater sawfish (Pristis microdon) and dwarf sawfish (P. *clavata*) have overlapping habitats or diet and that these differ to those of Anoxypristis cuspidata. Further investigation of fine-scale spatial differences is required to refine and verify our findings along with new studies to understand the processes that influence the integration of stable isotopes into body tissues.

Table of Contents

Background information	4
Australian sawfishes	4
Aims	6
Developing non-lethal methods for Aging Sawfishes	7
Introduction	7
Specific aims	8
Methods	9
Sample collection	9
Vertrbrae and teeth preparation and aging	. 10
Vertebral and tooth age comparisons and growth	. 12
Results	. 13
Sawfish age and length relationship	. 13
Sawfish length and age variation	. 14
Sawfish vertebral and tooth age comparison	. 15
Discussion and conclusions	. 16
Stable isotope analyses for determination of spatial distribution of sawfishes	. 19
Introduction	. 19
Specific aims	. 21
Methods	. 21
Sample collection	. 21
Determining elemental composition of rostral teeth.	. 22
Analysis	. 23
Results	. 24
Inter-regional differences in stable isotope composition	. 24
Intra-regional differences in stable isotope composition	. 24
Environmental differences in species stable isotope composition	. 25
Discussion and conclusions	. 25
Summary and direction for future research	. 29
References	. 31

Background information

Northern Australia has a relatively pristine marine environment (Halpern et al. 2008) compared to the other regions where sawfishes (Pristidae) can be found (Martin 2005). While these species are thought to have large potential ranges, there is relatively little detailed data available describing their life history and distribution patterns. This lack of information is of concern for conservation of these species, as sawfishes in Australia are caught as by-catch by recreational and commercial fisheries; however, the implications of by-catch for long-term population persistence are unknown.

Sawfishes populations have declined (Martin 2005), and all seven species are classified as *Critically Endangered* by the IUCN (IUCN 2007). The greatest threats are thought to be from target and by-catch fisheries because of the organism's tendency to become ensnared by its elongated rostrum in commercial and recreational fishing nets (Simpfendorfer 2000). These species are also assumed to be susceptible to and deterioration and degradation of habitats with increasing use and alteration of coastal and estuarine environments (IUCN 2007).

In Australia there are four species: freshwater (*Pristis microdon*), green (*P. zijsron*), dwarf (*P. clavata*), and narrow (*Anoxypristis cuspitata*). All species are protected under the Commonwealth Environment Protection and Biodiversity Conservation (EPBC) Act 1999, and *P. microdon* and *P. zijsron* and listed as *Vulnerable* (Pogonoski et al. 2002). Although globally endangered, Australian populations may be relatively abundant and the least threatened compared to populations elsewhere (Martin 2005) and therefore, provide a unique opportunity to measure the basic ecological and life history attributes of a relatively intact population.

Australian sawfishes

Despite their poor conservation status, little is known about the ecology of these species primarily due to their rarity (Pogonoski et al. 2002, Thorburn et al. 2003, Martin 2005). However, biological information about their life history

is a high priority as there are some interactions with the inshore and offshore fisheries for all four species, especially in the Gulf of Carpentaria (Peverell 2005, Field et al. 2008), for which little information on population impacts is available.

Effective conservation management requires knowledge of status and trends in population size. To estimate these parameters, the size at age and growth rate of individuals must be determined. In fishes, age can be obtained by analysis of growth bands in calcified tissues such as otoliths, scales and vertebrae (Campana 2001, Campana & Thorrold 2001). Age estimates, length data and other demographic rates such as maturity and litter size can be incorporated into age-structured demographic models to estimate population recovery potential and extinction risk (Sinclair et al. 2006). For the majority of elasmobranch species the only method to collect the age data is analysis of the vertebrae, which is, or course, lethal to the animal. This has obvious conservation and ethical implications for these endangered sawfishes.

Appropriate management strategies also require knowledge about the connectivity of populations because extinction risk varies among species depending on their degree of permanent (genetic) connectivity. It is a truism in conservation ecology that fragmented, isolated and small populations have higher rates of extinction (Brook et al. 2008). The degree of connectivity among populations thus defines the appropriate spatial scale for management (Jennings 2000). A recent innovation in this area has been the analysis of isotopic signatures in the calcified structures such as otoliths and vertebrae to track movements among habitats and locations. This is possible because chemical signatures within otoliths and vertebrae are often unique to the environment that the fish inhabits. Thus, the origin of individuals and movement among habitats is recorded in the chemical fingerprints within the calcified structure (Campana & Thorrold 2001, Rooker et al. 2001). For sawfishes, chemical fingerprints within vertebrae can be used to determine the natal rivers of some species and to track migration between river systems (S. Peverell, unpubl data). However, like age analysis, examination of the chemical fingerprints within vertebrae requires lethal sampling of the animal.

We aimed to determine whether age and chemical fingerprint information can be reliably extracted from rostral teeth of sawfishes. Unlike vertebrae, these can be removed and analysed without harming the animal. If rostral teeth age estimates adequately reflect those derived from lethally sampled vertebrae, it would allow routine non-lethal sampling of these species so that sufficiently large databases of age, growth and movement patterns can be acquired.

Aims

Due to conservation and management concerns over sawfishes the specific aims of this preliminary study were to:

1) Develop a non-lethal method to accurately predict the ages of sawfishes using the rostral teeth, and

2) Determine stable isotope analyses could create chemical fingerprints from sawfish rostral teeth to provide estimates of the population source and movement patterns of sawfishes.

Developing non-lethal methods for aging sawfishes

Introduction

Elasmobranchs populations are declining worldwide (Stevens et al. 2000a, Coelho et al. 2003, Myers et al. 2007, Dulvy et al. 2008). The primary cause for these declines are increased fishing pressure to provide shark and ray products (mainly meat and fins), unsustainable by-catch rates and the cascading effects from the overfishing of prey species (Stevens et al. 2000a). Effective management of elasmobranch fisheries and by-catch and other potential drivers of population decline require biological information from which population models can built to estimate demographic rates (Sinclair et al. 2006). However, many of these parameters have not been measured (or have been measured imprecisely) for most elasmobranch species (Cortés 2002).

Demographic models typically require age data to determine growth, survival and age-specific fertility rates. In elasmobranchs, age is most often estimated from counts of annuli, consisting of a translucent and opaque band, within vertebrae and referred to as 'paired growth bands'. This process requires sacrificing individuals so that vertebrae can be removed (Cailliet et al. 2006). This can be problematic, especially when studying threatened species or those where populations have been greatly reduced. Therefore, the need to avoid destructive sampling has led to recent investigations of alternative techniques of live aging using calcified structures that do not require the death of individuals.

Recently, there have been studies that have used alternative verified (confirmed age using known age markers or proxies) and relative aging techniques. Gallagher et al. (2006) demonstrated that growth bands observed in sections of the caudal thorns of the thorny skate (*Amblyraja radiate*) could be used for determining relative ages non-lethally. Campana et al. (2006) used bomb radiocarbon dating to show that counts of enamel growth bands in the dorsal spines of spiny dogfish (*Squalus acanthias*) provided accurate age

estimates. Similarly, Irvine et al. (2006a, b) showed that growth bands within the dorsal spines of giant lantern sharks (*Etmopterus baxteri*) and golden dogfish (*Centroselachus crepidater*) were reliable indicators of relative age. Growth rates have been estimated for *P. microdon, A. cuspidata, P. clavata,* and *P. zijsron* (Tanaka 1991, Peverell 2000, Thorburn et al. 2007) using the traditional method of removing the vertebrae and counting the number of paired growth bands.

Narrow sawfish are relatively abundant in the Northern Territory compared to populations elsewhere (Field et al. 2008) and frequently caught in commercial fisheries. However, the effects of commercial fishing are not currently known for this species and age and growth information will be important for determining potential impact. Due to the commercial catch and number of rostra in private collections, this species provides an ideal opportunity to develop new, non-lethal aging techniques that may be also applied to other sawfishes.

Rostral teeth are permanent structures not replaced once lost. If damaged, the tooth will not grow to the maximum size (Slaughter & Springer 1968). Therefore, rostral teeth may provide markers by which the individuals can be aged. In this study, we compared the age estimates made from growth bands within rostral teeth to those made from vertebrae to assess the potential for using the former non-lethal technique as an effective ageing technique for these species. There are currently no verified age estimates for this species of sawfish so we consider all ages estimates to be relative.

Specific aims

We used the banding found on the rostral teeth of narrow sawfish (*Anoxypristis cuspidata*) to provide a relative age estimate of individuals. These age estimates were compared with those calculated using the traditional analysis of vertebral growth bands for the same individuals by Peverell et al. (2000). We also investigated whether the location of the tooth influenced the estimates. Finally, we determined if there was a general

8

relationship between estimated age and a relative rostral length to support the use of tooth-based age estimates.

Methods

Sample collection

Vertebrae and rostra were collected from 16 *A. cuspidata* caught by commercial and recreational fishers (10 in the Gulf of Carpenteria, Queensland; 14° 20' S, 138°29'E) and 6 in Shoal Bay, Northern Territory; 12° 27' S, 130° 50' E) from March 2001 to June 2007. In June 2007, 61 rostra (without associated vertebrae) were purchased from private collections that had been caught around Fog Bay, 150 km west of Darwin, Northern Territory (12° 51' S, 130° 16' E). The dates of capture of these specimens were not known, but were generally taken some time between 2004 and 2007.



Figure 1. The broad sampling locations of narrow sawfish collected from the Northern Territory (orange ellipse) and Queensland (maroon ellipse). Only broad sampling areas can be shown due to the commercial nature of fisheries associated data.

Morphometric measurements were taken for each of the individual as described by Peverell (2005): total length (TL), fork length, lower-jaw total length, and lower-jaw fork length. We also recorded rostrum morphometrics for all samples, and included total rostral length, relative rostral length and rostral width (Figure 2). Total rostral length (TRL) is defined as the length measured dorsally from the tip of the rostrum to the point at the base where the head begins to broaden. The relative rostral length (RRL) was measured dorsally from the tip of the rostrum to the point at the base where the head begins to broaden. The relative rostral length (RRL) was measured dorsally from the tip of the rostrum to the point where left-side teeth stop protruding, and rostral width (RW) was the distance across the rostrum at the 10th tooth from the tip on the left hand side.



Figure 2. The rostral measurements taken for all narrow sawfish rostra.

Vertebrae and rostral teeth preparation and aging

A total of 5 vertebrae from beneath the first dorsal fin were removed from each individual and cleaned of excess flesh and connective tissue by soaking in 5 % bleach for 40 minutes. Vertebrae were then rinsed under warm water, dried for 24 hours and placed in a square Teflon®-coated cooking dish and set in epoxy resin. Vertebrae were thin-sectioned along the vertical axis at a thickness of 500 µm using a Buehler® diamond wafering blade.

The vertebral thin sections were mounted on a slide and observed under a dissecting microscope under reflected light. Growth bands (also known as 'annuli') were defined as the narrow translucent bands representing reduced

growth periods, as opposed to the adjacent wider opaque bands that represent faster growth. The relative vertebral age (RVA) as measured by the number of growth bands were counted for each individual, commencing after the birth mark which identified by a change of angle on the outer edge of the corpus calcerium (Goldman 2004).

The teeth from fresh rostra were removed by cutting down the side of the teeth, severing any connecting tissue and lifting the tooth away using padded forceps. The tooth was then cleaned of any remaining flesh. Whole teeth were removed from the dried rostrum using a fine tooth Dremel tool (Dremel, South Clayton, Victoria, Australia). Teeth were boiled in water and scraped with a scalpel to remove all remaining cartilage.

All teeth were then immersed in a 1 % solution of AgNO₃ for 30–60 minutes depending on tooth size. Smaller teeth required approximately 20–30 minutes and the larger teeth required 30–60 minutes. When immersed the teeth were covered by aluminium foil to prevent the reaction of the AgNO₃ stain with light. The stained teeth were then placed on the bench to expose the AgNO₃ to light and begin the reaction. Teeth were then exposed to light for 5 minutes to darken the bands, thus allowing for better differentiation between light and dark bands. Once the bands in the teeth were darkened, the reaction was fixed by washing the teeth with distilled water for 30 seconds followed by immersion in a 10 % solution of sodium thiosulphate for 10 minutes.

From the tip of the tooth to the base, wide translucent and narrow opaque bands were observed for stained and unstained teeth as lighter and darker bands. The stained teeth gave greater contrast and were viewed under a dissecting microscope using reflected light. The opaque bands were much thinner than the dark/calcified bands but were deemed to be an age band if the band was visible around the whole tooth. Furthermore, when viewed with directional light under the microscope, areas where light bands were present were slightly raised and cast a shadow across the tooth that further aided in the identification of band pairs. No birth band could be identified. The relative tooth age (RTA) was determined from counts of the bands on each tooth made by two observers. Counts were repeated twice by each observer and counts were done 'blind' so that readers had no knowledge of any characteristics of the individual from which the tooth was derived or previous counts of the tooth being aged. Where there were different ages recorded by observers we used the mean age for analysis and comparison.

Vertebral and tooth age comparisons and growth

We used only teeth located at positions 10 to 11 from the tip of the rostrum to provide a standard measure of relative age to compare between differentsized individuals. To test whether age estimates increase as a function of individual size, we regressed RRL and age. To determine if there was any influence of the age/size of an individual, we grouped individuals into three broad age/size classes based on the distribution of the RRL (Figure 3; small: RRL < 35 cm, medium: 35 < RRL < 50 cm, and large: RRL > 50 cm) and compared the standard deviation in age estimates from all age readings combined. Finally, for the 16 individuals where both vertebrae and rostral teeth were available, we compared the ages from both techniques to determine if the ages from teeth and vertebrae were correlated. All comparisons were done using linear least-squares regression (Goldman 2004) in R (R Development Core Team 2008). Akaike information criteria weights (corrected for small sample sizes, AIC_{cwt}) are used to provide a measure of the strength of evidence for each model (evidence ratio, ER), that represents the ratio of AIC_{cwt} values for each model relative to the whole set of candidate models along with the % of deviance explained (%DE). To investigate whether there was an increase in the variance of age with RRL we use a test of non-constant variance, in R (ncv.test), which hypotheses constant error variance against the alternative that the error variance changes with the level of the response, using chi-square scores (Breusch & Pagan 1979).





Results

External banding was seen on 98 % of the rostral teeth of narrow sawfish individuals (n = 77) that were used in the relative aging analysis.

Sawfish age and length relationship

Before analysis we tested for evidence of a relationship between the relative age of an individual estimated from rostral teeth (RTA) and size of the rostrum (RRL) (Figure 4). There was a strong positive relationship between RTA and RRL (ER=1.674e+22; %DE=66.3 %).



Figure 4. Relationship between relative age from rostral teeth (RTA) and size of individuals (RRL) (ER=1.674e+22; %DE=66.3 %).

Sawfish length and age variation

The positive relationship between RTA and RRL indicates that RTA does indeed reflect relative age; however, variance in RTA was non-uniform. The variation is estimated by its standard deviation increased with age/size. Small sawfish had lower standard deviations compared to the other size classes (Figure 5). We tested this using a test of non-covariance and found only weak support (p=0.5) rejecting constant variance in our data. However, this does indicates greater possibility of uncertainty in the ages of larger individuals.



Figure 5. Relationship between the size of individuals (RRL) and the variation in RTA (standard deviation) (test of non-constant variance; p=0.05) for the three size classes of narrow sawfish.

Sawfish vertebral and tooth age comparison

We compared the ages from vertebral and tooth growth band counts for sixteen individual sawfish. The ages from the different techniques were highly correlated (Figure 6; ER=1.575e+7; %DE=89.3%). Although the relative ages were close to a 1:1 relationship, the figure below indicates that the ages from rostral teeth (slope=0.738; SE=0.06) tended to become underestimates as age increased, and ages obtained from the vertebrae and the teeth tend to differ by more than one year after age six.



Figure 6. Relationship between narrow sawfish ages determined from growth bands observed form vertebrae (RVA) and rostral teeth (RTA) (ER=1.575e+7; %DE=89.3%). Solid line represents the line of best fit for the data and the dotted line a 1:1 relationship.

Discussion and conclusions

Non-lethal techniques for determining ages have rarely been investigated for elasmobranchs, principally due to the lack of calcified structures in these animals (Campana 2001, Gallagher et al. 2006, Irvine et al. 2006a, b). However, the rostral teeth of sawfishes can be used in this manner and have the advantage of being relatively easy to remove, clean and process. The removal of a tooth is unlikely to affect an individual's foraging success or longterm survival because most sawfish are missing or have damaged up to 35 % of their rostral teeth (I. Jupp, unpublished data). We have shown that growth bands observed on the rostral teeth of narrow sawfish can provide a relative age for individuals; however, this preliminary investigation is limited and needs further investigation to be developed and validated so that it can be trialled for other sawfish species. Limitations include the inability to identify a birth mark in a tooth's structure and the underestimation and variability of relative age with an increase in animal size. The lack of a birth mark means that it is almost impossible to determine the true age (Goldman 2004); however, the strong relationship between the counts of growth rings from vertebrae, where birth marks were identifiable, and from rostral teeth, supports the use of teeth growth bands for ageing.

We also identified underestimation in relative ages determined from teeth relative to vertebrae. Discrepancies in age estimates from different calcified structures such as scales and otoliths within an individual are common in teleost fishes (Barnes & Powers 1984, Hammers & Miranda 1991). In our study, the underestimation of ages for older or larger individuals may arise because sawfishes use the rostrum to hunt and investigate the coastal environment in which they live (Last & Stevens 1994). Rostral teeth are permanent structures for sawfishes (Slaughter & Springer 1968), so they wear as the animal ages, particularly as growth slows once adult size is attained.

The teeth of young animals (RRL < 35 cm) are sharp and possess a cusp, but as the animal ages, wear of the tooth changes its shape so that it becomes blunter and more rounded. Slower growth during the adult phase may also result in narrower growth bands, making them difficult to discern at the base of the tooth. In general for older narrow sawfish, we have shown that the tooth-aging technique decreases in precision and accuracy with size and age; however, the shape and size of rostral teeth in relation to other body morphometrics might prove a useful proxy of age if a consistent relationship could be demonstrated and validated using conventional methods.

Another source of potential error was the assumption that growth bands were laid down annually. This can be tested by mark-recapture studies using chemical tags, such as tetracycline to deposit permanent marks within hard parts, including the rostral teeth. Once tagged, the time at liberty between marking and recapture can be compared to the number of growth increments within the most recently deposited section of the body part used for aging (Campana 2001). The difficulties associated with validating ages of elasmobranchs are well-recognised (Campana 2001, Carrier et al. 2004, Cailliet et al. 2006), and have generated recent novel approaches to aging, such as the use of bomb radiocarbon dating (Campana et al. 2006). Current recapture rates for narrow sawfish are relatively low in tagging studies in northern Australia (~ 2 %, Field, unpublished data), making validation difficult and slow. However, populations of Australian sawfishes tend to be in remote and difficult-to-access regions where there has been relative low fishing pressure, and fisheries interactions can be used as an effective way of collecting data and releasing by-catch sawfish as part of mark-recapture studies. Such studies have committed to validate the frequency and timing of deposition of growth bands in teeth and vertebrae for some Australian sawfishes (Northern Territory Fisheries, unpublished data; S. Peverell, unpublished data), but they are likely to take many years to complete.

In summary, there is strong evidence to support the use of rostral teeth to estimate relative ages of narrow sawfish. There is a strong, positive relationship between age and individual size, but non-constant variance in age estimates increases uncertainty for larger, older animals. The ages of these older sawfish obtained from teeth were also underestimated when compared to those derived from the vertebrae. Other techniques, such as ultrasound and fine-scale surface roughness indices, might provide future alternatives. We have, however, shown the possibility of deriving ages from rostral teeth. Combined with more expansive sampling, archived specimens of rostra in museums and private collections could be used to describe historical patterns of age and growth for narrow sawfish populations. Future work should include age validation using bomb radiocarbon, chemical tagging and laser-ablating inductively coupled plasma mass spectrometry approaches.

Stable isotope analyses for determination of spatial distribution of sawfishes

Introduction

Determining structure and connectivity of populations can provide information on a species' long-term extinction risk (Brook et al. 1997, Brook et al. 2000). Generally, isolated populations are more susceptible to local extinction than those connected by dispersing individuals (Fahrig & Merriam 1985, Root 1998) because low external recruitment or genetic effects such as inbreeding can impede population recovery (Fahrig & Merriam 1985, Hanski 1998, Jennings 2000). Thus, a thorough understanding of the connectivity of managed populations is required to decide whether local or regional strategies are needed for each population or meta-population (Palumbi 2003, Cowen et al. 2006). For example, local management can be tailored in situations of low connectivity where each population is isolated from the others (Roberts 1997). Alternatively, regional management strategies are suited to species with high connectivity between sub-populations (Palumbi 2003) because changes in one population can affect neighbouring populations (Cowen et al. 2006).

One of the most commonly used methods for determining the connectivity of populations is measurement of gene flow (Palumbi 2003). Similarities in the genetic structure of neighbouring populations can indicate individual movements and population mixing. If there is little variation in the genetic structure among populations, strong mixing and high connectivity are likely; however, genetically isolated populations have a higher probability of local extinction events after population decline (Mora & Sale 2002). Such approaches are unfortunately limited by the assumption that genetic diversity is high within species (Frankham et al. 2002). Genetic diversity within shark species has been found to be low and can therefore lead to false interpretation, the requirement of large sample sizes, and time-consuming and expensive development of appropriate genetic markers. Another method to measure connectivity is capture-mark-recapture (CMR) where marked individuals can be tracked among sub-populations (Sinclair et al. 2006). However, CMR can be expensive and logistically difficult in challenging

environments such as the turbid and remote tropical estuaries of northern Australia (Pollock 2000, Stevens et al. 2000b, Wood et al. 2007).

An alternative method has been developed using heavy metal isotopes through inductively coupled plasma-mass spectrometry (ICP-MS) analysis (Gillanders 2005). These isotopes are incorporated from ingested food and water into the growing calcified structures of fish and have been used to determine stream occupancy and connectivity for a number of species (Thorrold et al. 1998, Kennedy et al. 2002, McCulloch et al. 2005). There are many isotope ratios which can be used to distinguish populations originating from different rivers, such as ⁸⁷Sr/⁸⁶Sr (Kennedy et al. 2000, Kennedy et al. 2002, McCulloch et al. 2005) in otoliths (Campana et al. 1995, Kennedy et al. 1997, Kennedy et al. 2002), vertebrae (Kennedy et al. 1997), scales (Wells et al. 2003), and fin rays (Clarke et al. 2007). Despite the growing literature on this subject, there have been few intra-structure comparisons to determine ontogenetic changes in habitat use (Kennedy et al. 1997, Wells et al. 2000, Wells et al. 2003, Clarke et al. 2007). Otoliths are generally the most appropriate structures for analysis because other calcified structures do not often have enough variation to distinguish between closely located habitats (Wells et al. 2000, Wells et al. 2003, Clarke et al. 2007). Another problem is that sampling calcified structures normally requires killing the organism, which is clearly not desirable for threatened species.

There have been no studies using isotopic concentrations in calcified structures for chondrichthyans to determine habitat use and broad movement patterns. Chemical signatures have only been measured from the vertebrae of the round stingray *Urobatis halleri* to verify annual deposition of the growth bands (Hale et al. 2006). Isotopic signatures have also been derived from the scales of fish (Wells et al. 2000, Wells et al. 2003) and from sawfish rostral teeth, which are modified scales that are not expected to be replaced once lost (Bigelow & Schroeder 1953, Slaughter & Springer 1968)

Specific aims

We aimed to determine whether the rostral teeth three Australian sawfishes (*Anoxypristis cuspidata, Pristis microdon and P. clavata*) can be used as reliable structures to determine chemical signatures and discriminate (1) between regional populations, 2) within regional sampling locations and 3) broad habitats.

Methods

Sample collection

Samples were collected from commercial fishing boats from the Northern Territory (NT) and Queensland (QLD) coasts from March 2001 to June 2006. Rostral teeth were collected from 11 narrow sawfish (*Anoxypristis cuspidata*; n = 6, Gulf of Carpentaria, NT and n = 5, Gulf of Carpentaria, QLD), and four freshwater sawfish (*Pristis microdon*) and four dwarf sawfish (*P. clavata*) were collected from various locations in Queensland. The specific locations of the samples collected with known capture sites are shown in Figure 7.



Figure 7. The sawfishes sampling location across northern Australia. Species are denoted as \blacksquare = narrow sawfish (*Anoxypristis cuspidata*), \bullet = freshwater sawfish (*Pristis microdon*), and \blacktriangle = dwarf sawfish (*P. clavata*). Also shown in adjacent to the species symbols are the sample sizes for each species and location.

Determining elemental composition of rostral teeth

The elemental composition of the rostral teeth was determined by energy dispersive microscopy elemental analysis using a scanning electron microscope. The teeth were removed from the rostrums using a sharp knife if the samples were fresh or by a rotary saw if dry. Excess flesh was removed by a scraping the surface of the tooth lightly with a scalpel blade. Rostral teeth were placed in labelled 60-ml acid-rinsed plastic falcon tubes with lid, then ultrasonically cleaned in high-purity water (HPH₂O) for 10 minutes and rinsed under HPH₂O between sonications. This process was repeated three times. Teeth were dried in a convection oven at 60 °C overnight and then weighed on acid-washed tared scales. The samples were dissolved in 30 % ultra pure HNO₃ at a ratio of 13.3 ml of acid solution to 1 g of the analyte. Samples were left to dissolve for three days in 60-ml acid-washed falcon tubes. Once dissolved, the falcon tubes containing the dissolved analyte were not moved. This allowed all undissolved material to settle at the bottom or float on the surface. Five ml of analyte were transferred into labelled 15-ml acid-washed ICP-MS tubes and diluted to a 15 % acid mix with 5 ml of HPH₂O. The analyte was taken from the centre of the solution to avoid any floating or sunken matter. Transfers were done using acid-washed pipette tips rinsed once in 30 % ultra pure acid and twice in HPH₂O between samples. If there was < 5 ml of analyte, the sample was placed in 30% acid at 1:1 dilution, with HPH₂O to ensure standard amounts of acid concentration in each ICP-MS vial.

Elemental analysis of the rostral teeth was done using a quadruple ICP-MS (Agilent 7500 Series). Rostral teeth from each individual species were analysed for the isotopic ratios Mg:Ca, Ba:Ca, and Cd:Ca. To account for instrument drift (Eggins et al. 1998), 5 standard solutions were made up from the reference sample and the standard itself. The standard solution contained 10 ml of Sr (1000 ppm), 1 ml of Mg (1000 ppm), 2 ml of ICP 2 standard (10 ppm), and 0.2 ml of concentrated ultra-pure HNO₃. The standard solution was then diluted up to 25 ml. The first standard contained only 5 ml of the standard. The following standards all contained the 5 ml of standard, but one contained 50 μ l of a reference sample, 1 contained 250 μ l of reference sample was a randomly

22

selected digested sawfish sample from the species being analysed. All standard solutions were then diluted to 10 ml with HPH₂O measured with an acid-washed 10 ml volumetric flask. The standards were run after every fourth sample to account for instrument drift and machine variation.

Analysis

The limits of detection (LOD) for each of the elements were calculated as 3 σ of the concentration of the blank solutions to determine the minimum viable values at which analysis of the elements could be done. The concentrations of the isotopic ratios of the solutions where analysed by principal components analysis (PCA) in R (R Development Core Team 2008). Prior to PCA, a Spearman's rank correlation matrix was constructed to determine if any elements were strongly correlated. If so, these elements were removed from analysis. We used a one-way analysis of variance, on the coordinates of the individuals on the axis explained by the qualitative variable, to test there were differences between categories. We then used a student T-test to compare the average of the category with the general average producing an associate p-value to support whether there were differences between categories. To determine if there were inter- and intra-regional differences, we analysed each species separately because they are thought to use different habitats. For inter-regional differences, we tested only narrow sawfish individuals sampled from the Gulfs of Joseph Bonaparte (NT) and Carpentaria (QLD). For intraregional differences, we tested freshwater sawfish samples from around the Archer and Smithburne Rivers, and for dwarf sawfish from the Staaten and Leichardt Rivers although these data are only described and not subject to analysis due to low sample sizes. Finally, assuming that all sawfishes ingest and retain chemical components according to similar physiological processes, we tested whether the species could be differentiated based on broad differences in their environment or foraging behaviour. All descriptions of changes in stable isotope ratios are expressed in relation to Calcium (Ca) unless otherwise noted.

Results

The chemical composition of rostral teeth for narrow, freshwater and dwarf sawfishes show that the teeth are primarily composed of calcium, phosphate and oxygen, with these three elements comprising approximately 96 % of the total elemental composition of the tooth (Table 1). The LOD (ppm) for each of the elements were: Mg = 360.8, Ca = 3884.2, Ba = 217.42, and Cd = 21.82.

Element	A. cuspidata	P. microdon	P. clavata
0	41.42	39.11	39.56
Са	35.42	37.97	38.21
Р	19.83	19.67	19.10
Na	1.66	1.56	1.61
Mg	0.82	0.90	0.78
CI	0.41	0.39	0.39
S	0.22	0.18	0.15
Si	0.20	0.20	0.17
AI	0.02	0.01	0.02
К	0.01	0.01	0.01

Table 1. The Elemental composition (%) of the rostral teeth of Anoxypristis cuspidata (n = 11), Pristis microdon (n = 4), and Pristis clavata (n = 4).

Inter-regional differences in stable isotope composition

The principal component analysis of narrow sawfish tooth samples from the Gulf of Joseph Bonaparte and the Gulf of Carpentaria found that was evidence for a difference from their chemical compositions (p=0.04; Figure 8). There was almost a complete separation of the samples from the two regions. The first two dimensions of the analysis explained over 62 % of the variance and were characterised by increases in ratios of Pb, Ba, Cu, and a decrease in Mn in the first dimension and an increase in Fe and decrease in Mg in the second dimension (Figure 8).

Intra-regional differences in stable isotope composition

We used samples of freshwater and dwarf sawfish to test for differences within the Gulf of Carpentaria. No differences were found for either freshwater sawfish or dwarf sawfish (Figure 7), although these analyses were based on two samples from different locations for each species.

Environmental differences in species stable isotope composition

Combining all samples regardless of their locations, species could be separated by their isotope ratio components (Figure 9; *p*=0.002. The first two dimensions of the PCA accounted for over 70 % of the variance and were characterised by increases in ratios of Mn, Fe, Pb, Ba and Cu with a decrease in Mg and Cd in the first dimension, and an increase in Mg, Cd, Mn, Ba, Fe, and Ba with decrease in Pb and Cu in the second dimension (Figure 8).

Discussion and conclusions

The persistence of populations of many fish species depends on of the interchange of individuals among sub-populations (Westemeier et al. 1998). Thus, human-induced reductions in connectivity, through excessive fishing-related mortality or habitat loss and degradation, can reduce the contribution of external recruitment and subsequently increase the chance of a local extinction, especially for already depleted populations (Westemeier et al. 1998). This is compounded for species that have a limited range size (Brook et al. 2008). Therefore, methods that can measure the potential degradation of connectivity in threatened fish populations, especially if they are non-lethal, provide a major tool to ascertain a species' conservation status and restoration prospects.





Figure 8. Principal components analysis (left plot) and the separation of the stable isotope ratios across the fist two dimensions (right plot) for narrow sawfish (*Anoxypristis cuspidata*; top panel), freshwater sawfish (*Pristis microdon*; middle panel) and dwarf sawfish (*P. clavata*; bottom panel). Individuals are shown as dots with eclipses showing the 95 % confidence grouping of individuals. Open squares indicate the centre of their composition distribution. Locations are denoted as: GB=Gulf of Joseph Bonaparte (black), GC= Gulf of Carpentaria (red), AR=Archer River (black), SM=Smithburne River (red), LR=Leichardt River (black) and SR=Staaten River (red).



Figure 9. Principal components analysis (left plot) and the separation of the stable isotope ratios across the fist two dimensions (right plot) for all sawfishes. Narrow sawfish (Ac=*Anoxypristis cuspidata*, black dots), freshwater sawfish (Pm=*Pristis microdon, green dots*) and dwarf sawfish (Pc=*P. clavata,* red dots). Species are shown by coloured dots with eclipses showing the 95 % confidence grouping of individuals. Open squares indicate the centre of the species chemical composition distribution.

We found that chemical signatures obtained from the rostral teeth of sawfish can separate individual between capture regions, thus providing essential information on movement and connectivity which can be used to determine population vulnerability.

Our research shows that stable isotope concentrations in the rostral teeth of globally threatened sawfish can be used to distinguish individuals between capture regions in northern Australia. These results indicate that the elemental compositions of the teeth depend on chemical conditions on a regional scale and provide some evidence at a local extent to which individuals are exposed.

Rostral teeth of sawfish are thought to be modified denticles or scales (Bigelow & Schroeder 1953, Slaughter & Springer 1968), so our results are comparable to studies of fish scales. Scales are chemically less stable than otoliths and vertebrae (Campana 1999, Wells et al. 2003), and Wells et al. (2003) found that chemical fingerprints obtained from scales were less able to distinguish individual trout among streams that those based on otoliths. Our results confirm that chemical fingerprints from rostral teeth produced strong separation for narrow sawfish, but small sample sizes for the other species prevented analysis. For other fish species, isotopic variation has been observed in the scales when compared to other structures that may cease growing at some point early in the life cycle (Bilton & Robins 1971), and some chemicals may be reabsorbed during times of physiological stress and scale replacement (Bilton 1975). This may explain some of the variation among sampling regions.

Although the isotopic composition the rostral teeth of sawfishes can be used to discriminate between regions, more information is required to examine how physical conditions alter isotopic signatures. Wells et al. (2000) found that under controlled laboratory conditions, the ratios of Mg:Ca, Ba:Ca, and Cd:Ca in scales can differ from those within the surrounding water matrix due to differences in substitution rates. Fish scales also have high protein content which may elevate Ba concentrations relative to other structures (Degens et al. 1969). Furthermore, individuals exposed to the same environments at different stages of their life cycle incorporate elements at different rates (Thresher et al. 1994, Campana et al. 1995).

Despite these limitations, we conclude that the chemical signatures from the rostral teeth of sawfish appear to be reliable indicators of capture site in narrow sawfish, and might be used to indicate fine-scale distribution of freshwater and dwarf sawfishes, although greater samples are required. Further application of this technique will depend partially on how tooth wear affects chemical signatures over time. Regardless, the technique shows promise for using rostral teeth from previously harvested rostrums to reconstruct past environmental histories of individual fish. Unpublished capture-mark-recapture data by the Queensland and the Northern Territory government fisheries agencies suggest that sawfish do not disperse far from capture location (perhaps < 50 km), so finer-scale separation through chemical signatures may be possible. Further development of the technique could also lead to chronologies of individual movements if the relevant isotopic signature libraries were available for the gradient of water chemistries

available within an individual's home range. Currently there are databases of stream chemistry being constructed for freshwater systems in north-eastern Australia (McCulloch et al. 2005), providing an additional tool to determine connectivity of sawfish populations for conservation management.

Summary and direction for future research

Determining age of individuals and connectivity patterns of populations are vital for determining a species' response to human-induced environmental change and ultimately, its risk of extinction. Northern Australia has some of most pristine waters in the world and relatively healthy populations of sawfishes, which are Critically Endangered elsewhere. Research is therefore required to understand population structures for conservation and fisheries management. Our preliminary studies show that rostral teeth of globally threatened sawfishes can be used to estimate the age of individuals and their regional source populations. For the use of teeth for aging from growth bands, more investigation is required to validate our results and refine these methods for other sawfish species and across a broader range of ages and sizes. Other data could also be collected and analysed to provide additional support and evidence for the use of non-lethal aging including 1) tooth shape and morphometric data, 2) the use of chemical composition of the teeth to determine the chemical structure of growth bands, and 3) the trialling of alternative imaging techniques to identify growth bands better and to understand their development and wearing processes.

Stable isotope composition of the teeth has provided another tool to determine the regional source population of individuals. Greater sample sizes are required to confirm and improve this technique for all sawfish species and to refine spatial resolution. Our study suggests that it might be possible to identify specific stable isotope signatures that could be used to determine if there are both intra- and inter-specific differences in environment or habitat use among different age and size classes of sawfishes. This information will be important in identifying critical habitat and conservation value as well as

29

understanding the movements and connectivity of populations in their environment, and will be an alternative to conventional tracking studies.

The two techniques described here also have the advantage that they can be used on historical sawfish rostrum collections that may allow retrospective studies of population structure and distribution, in conjunction with other tools such as population genetics, to understand better the current status of sawfish populations in northern Australia and for their conservation across their global ranges.

References

- Barnes MA, Powers G (1984) A comparison of otolith and scale ages for western Labrador lake whitefish, *Coregonus clupeaformis*. Environmental Biology of Fishes 10:297-299
- Bigelow H, Schroeder W (1953) Fishes of the Northwest crn Atlantic. Part II. Sawfishes, Guitarfishes. Skates, Rays and Chimaeroids. Memoirs of the Sears Foundation for Marine Research 1:1-514
- Bilton HT (1975) Factors influencing the formation of scale characters. International North Pacific Fisheries Commission Bulletin 32:102-108
- Bilton HT, Robins GL (1971) Effects of starvation, feeding, and light period on circulus formation on scales of young stock eye salmon (*Oncorhynchus nerka*). Journal of Fisheries Research Board Canada 28:149-155
- Breusch TS, Pagan AR (1979) A simple test for heteroscedasticity and random coefficient variation. Econometrica 47:1287-1294
- Brook BW, Lim L, Harden R, Frankham R (1997) Does population viability analysis software predict the behaviour of real populations? A retrospective study on the Lord Howe Island woodhen Tricholimnas sylvestris (Sclater). Biological Conservation 82:119-128
- Brook BW, O'Grady JJ, Chapman AP, Burgman MA, Akcakaya HR, Frankham R (2000) Predictive accuracy of population viability analysis in conservation biology. Nature 404:385-387
- Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. Trends in Ecology & Evolution 23:453-460
- Cailliet GM, Smith WD, Mollet HF, Goldman KJ (2006) Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. Environmental Biology of Fishes 77:211-228
- Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology-Progress Series 188:263-297
- Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol 59:197-242
- Campana SE, Gagne JA, Mclaren JW (1995) Elemental Fingerprinting of Fish Otoliths Using Id-Icpms. Marine Ecology-Progress Series 122:115-120
- Campana SE, Jones C, McFarlane GA, Myklevoll S (2006) Bomb dating and age validation using the spines of spiny dogfish (Squalus acanthias). Environmental Biology of Fishes 77:327-336
- Campana SE, Thorrold SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences 58:30-38
- Carrier JC, Pratt HL, Castro JI (2004) Reproductive biology of elasmobranchs. In: Carrier JC, Musick JA, Heithaus MR (eds) Biology of sharks and their relatives. CRC Press LLC, Boca Raton, p 269-286
- Clarke AD, Telmer KH, Shrimpton JM (2007) Elemental analysis of otoliths, fin rays and scales: a comparison of bony structures to provide population and life-history information for the Arctic grayling (Thymallus arcticus). Ecology of Freshwater Fish 16:354-361

- Coelho R, Bentes L, Goncalves JM, Lino PG, Ribeiro J, Erzini K (2003) Reduction of elasmobranch by-catch in the hake semipelagicnearbottom longline fishery in the Algarve (Southern Portugal). Fisheries Science 69:293-299
- Cortés E (2002) Incorporating uncertainty into demographic modeling: application to shark populations and their conservation. Conserv Biol 16:1048-1062
- Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522-527
- Degens ET, Deuser WG, Haedrich RL (1969) Molecular structure and composition of fish otoliths. Marine Biology 2:105-113
- Dulvy NK, Baum JK, Clarke S, Compagno LJV, Cortes E, Domingo A, Fordham S, Fowler SL, Francis MP, Gibson C, Martinez J, Musick JA, Soldo A, Valenti S (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquatic Conservation: Marine and Freshwater Ecosystems
- Eggins SM, Kinsley LPJ, Shelley JMG (1998) Deposition and element fractionation processes during atmospheric pressure laser sampling for analysis by ICP-MS. Applied Surface Science 127:278-286
- Fahrig L, Merriam G (1985) Habitat patch connectivity and population survival. Ecology:1762-1768
- Field IC, Charters R, Buckworth RC, Meekan MG, Bradshaw CJA (2008) Distribution and abundance if Glyphis and sawfishes in northern Australia and their potential interactions with commercial fisheries, Charles Darwin University, Darwin, Australia
- Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, p 617
- Gallagher MJ, Green MJ, Nolan CP (2006) The potential use of caudal thorns as a non-invasive ageing structure in the thorny skate (Amblyraja radiata Donovan, 1808). Environmental Biology of Fishes 77:265-272
- Gillanders BM (2005) Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuarine Coastal and Shelf Science 64:47-57
- Goldman KJ (2004) Age and growth of elasmobranch fishes, FAO, Rome, Italy
- Hale LF, Dudgeon JV, Mason AZ, Lowe CG (2006) Elemental signatures in the vertebral cartilage of the round stingray, Urobatis halleri, from Seal Beach, California. Environmental Biology of Fishes 77:317-325
- Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. Science 319:948-952
- Hammers BE, Miranda LE (1991) Comparison of methods for estimating age, growth, and related population characteristics of white crappies. North American Journal of Fisheries Management 11:492-498
- Hanski I (1998) Metapopulation dynamics. Nature 396:41-49
- Irvine SB, Stevens JD, Laurenson LJB (2006a) Comparing external and internal dorsal-spine bands to interpret the age and growth of the giant

lantern shark, Etmopterus baxteri (Squaliformes : Etmopteridae). Environmental Biology of Fishes 77:253-264

- Irvine SB, Stevens JD, Laurenson LJB (2006b) Surface bands on deepwater squalid dorsal-fin spines: an alternative method for ageing Centroselachus crepidater. Canadian Journal of Fisheries and Aquatic Sciences 63:617-627
- IUCN (2007) 2007 IUCN Red List of Threatened Species. International Union Conservation of Nature and Natural Resources, Gland, Switzerland
- Jennings S (2000) Patterns and prediction of population recovery in marine reserves. Reviews in Fish Biology and Fisheries 10:209-231
- Kennedy BP, Blum JD, Folt CL, Nislow KH (2000) Using natural strontium isotopic signatures as fish markers: methodology and application. Canadian Journal of Fisheries and Aquatic Sciences 57:2280-2292
- Kennedy BP, Folt CL, Blum JD, Chamberlain CP (1997) Natural isotope markers in salmon. Nature 387:766-767
- Kennedy BP, Klaue A, Blum JD, Folt CL, Nislow KH (2002) Reconstructing the lives of fish using Sr isotopes in otoliths. Canadian Journal of Fisheries and Aquatic Sciences 59:925-929
- Last PR, Stevens JD (1994) Sharks and Rays of Australia. CSIRO Division of Fisheries, Australia, Melbourne
- Martin RA (2005) Conservation of freshwater and euryhaline elasmobranchs: a review. J Mar Biol Assoc Uk 85:1049-1073
- McCulloch MA, Cappo MB, Aumend JB, Müller WA (2005) Tracing the life history of individual barramundi using laser ablation MC-ICP-MS Srisotopic and Sr/Ba ratios in otoliths. Marine and Freshwater Research 56:637-644
- Mora C, Sale PF (2002) Are populations of coral reef fish open or closed? Trends in Ecology & Evolution 17:422-428
- Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315:1846-1850
- Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications 13:S146-S158
- Peverell SC (2000) Growth rates of Australian sawfishes. James Cook University
- Peverell SC (2005) Distribution of sawfishes (Pristidae) in the Queensland Gulf of Carpentaria, Australia, with notes on sawfish ecology. Environmental Biology of Fishes 73:391-402
- Pogonoski J, Pollard DA, Paxton JR (2002) Conservation overview and action plan for Australian threatened and potentially threatened marine and estuarine fishes, Environment Australia, Canberra
- Pollock KH (2000) Capture-recapture models. Journal of the American Statistical Association 95:293-296
- R Development Core Team (2008) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria
- Roberts CM (1997) Ecological advice for the global fisheries crisis. Trends in Ecology & Evolution 12:35-38

- Rooker JR, Secor DH, Zdanowicz VS, Itoh T (2001) Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. Marine Ecology-Progress Series 218:275-282
- Root KV (1998) Evaluating the effects of habitat quality, connectivity, and catastrophes on a threatened species. Ecological Applications 8:854-865
- Sinclair ARE, Fryxell JM, Caughley G (2006) Wildlife Ecology, Conservation and Management. Blackwell Publishing Limited, Carlton, Australia
- Slaughter BH, Springer S (1968) Replacement of rostral teeth in Sawfishes and Sawsharks. Copeia 3:499-506
- Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000a) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. Ices J Mar Sci 57:476-494
- Stevens JD, West GJ, McLoughlin KJ (2000b) Movements, recapture patterns, and factors affecting the return rate of carcharhinid and other sharks tagged off northern Australia. Marine and Freshwater Research 51:127-141
- Tanaka S (1991) Age estimation of freshwater sawfish and sharks in Northern Australia and Papua New Guinea, University Museum, University of Tokyo, Tokyo, Japan
- Thorburn DC, Morgan DL, Rowland AJ, Gill HS (2007) Freshwater sawfish Pristis microdon Latham, 1794 (Chondrichthyes : Pristidae) in the Kimberley region of Western Australia. Zootaxa:27-41
- Thorburn DC, Peverell SC, Stevens JD, Last PR, Rowland AJ (2003) Status of freshwater and estuarine elasmobranchs in Northern Australia, Natural Heritage Trust, Canberra
- Thorrold SR, Jones CM, Campana SE, McLaren JW (1998) Trace element signatures in otoliths record natal river of juvenile American shad (Alosa sapidissima). Limnology and Oceanography:1826-1835
- Thresher RE, Proctor CH, Gunn JS, Harrowfield IR (1994) An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). Fish B-Noaa 92:817-840
- Wells BK, Bath GE, Thorrold SR, Jones CM (2000) Incorporation of strontium, cadmium, and barium in juvenile spot (Leiostomus xanthurus) scales reflects water chemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2122-2129
- Wells BK, Rieman BE, Clayton JL, Horan DL, Jones CM (2003) Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Trans Am Fish Soc 132:409-424
- Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. Science 282:1695-1698
- Wood AD, Collie JS, Kohler NE (2007) Estimating survival of the shortfin mako Isurus oxyrinchus (Rafinesque) in the north-west Atlantic from tag-recapture data. J Fish Biol 71:1679-1695

© Commonwealth of Australia 2009

This work is copyright. Apart from any use as permitted under the Copyright Act 1968, no part may be reproduced by any process without prior written permission from the Commonwealth. Requests and inquiries concerning reproduction and rights should be addressed to the Commonwealth Copyright Administration, Attorney General's Department, Robert Garran Offices, National Circuit, Barton ACT 2600 or posted at http://www.ag.gov.au/cca

The contents of this document have been compiled using a range of source materials and is valid as at April 2009. The Commonwealth Government is not liable for any loss or damage that may be occasioned directly or indirectly through the use of or reliance on the contents of the document.

Acknowledgements

This work would not be possible without funding from the Australian Government's Department of the Environment, Water, Heritage and the Arts and support from Charles Darwin University.

We thank Stirling Peverell, Queens land Department of Primary Industries, for the donation of samples collected from Queensland fisheries, and for providing information, teaching and guidance for aging sawfishes. David Parry and technical staff at the School for Environment and Life Sciences, Charles Darwin University, who provided invaluable assistance and advice for the inductively coupled plasma mass spectrometry. We thank Rik Buckworth, Northern Territory Fisheries, and David Parry, Charles Darwin University, for comments on earlier drafts of the manuscript.

Finally, we thank the skippers and crews of all the fishing vessels that agreed to have the observers on board and making them welcome.