Habitat associations of Freshwater Sawfish (*Pristis microdon*) and Northern River Sharks (*Glyphis* sp. C): including genetic analysis of *P. microdon* across northern Australia

Report to

Australian Government
Department of the Environment, Water, Heritage and the Arts

May 2008

DRAFT

by
J.M. Whitty, N.M. Phillips,
D.L. Morgan, J.A. Chaplin,
D.C. Thorburn & S.C. Peverell

Centre for Fish and Fisheries Research
Murdoch University

This report is divided into two sections; the suggested referencing style is given below:

Section I

Whitty, J.M., Morgan, D.L., Thorburn, D.C., Fazeldean, T. & Peverell, S.C.[1] (2008). Tracking the movements of Freshwater Sawfish (*Pristis microdon*) and Northern River Sharks (*Glyphis* sp. C) in the Fitzroy River. In J.M. Whitty, N.M. Phillips, D.L. Morgan, J.A. Chaplin, D.C. Thorburn & S.C. Peverell (eds). *Habitat associations of Freshwater Sawfish (Pristis microdon) and Northern River Shark (Glyphis sp. C): including genetic analysis of P. microdon across northern Australia*. Report to Department of the Environment, Water, Heritage and the Arts, Australian Government.

Section II

Phillips, N.M, Chaplin, J.A., Morgan, D.L., Peverell, S.C.[1] & Thorburn, D.C. (2008). Genetic diversity and population structure of the Freshwater Sawfish (*Pristis microdon*) in Australian waters. In J.M. Whitty, N.M. Phillips, D.L. Morgan, J.A. Chaplin, D.C. Thorburn & S.C. Peverell (eds). *Habitat associations of Freshwater Sawfish (Pristis microdon) and Northern River Shark (Glyphis sp. C): including genetic analysis of P. microdon across northern Australia*. Report to Department of the Environment, Water, Heritage and the Arts, Australian Government.

[1] Stirling Peverell is with the Queensland Department of Primary Industries and Fisheries

Acknowledgements
We would like to acknowledge the Department of Environment, Water, Heritage and the Arts for providing the necessary funds for the project. The project would not have been possible without the support of many people. In particular, thanks to the Jarlmadangah Rangers and the Yiriman Project for help with the field work, including Nyaburu Watson, Kimberley Watson, Josh Albert, Angus Butt, William Lennard, Darryl Combs, Mick Apanah and Hugh Wallace-Smith. Also, thanks to other field participants including Simon Visser, Mark Allen, and Ryan Bell and the many people in Broome, Derby, Fitzroy Crossing, Jarlmadangah and Looma that kindly donated tissue samples from old rostra. Thanks also to people from other states that sent in tissue samples and to Justin Chidlow and Rory McAuley (Fisheries WA) for providing their samples. Thanks to Yeeda Station for access through the station and thanks to Mitre 10 Derby for donating consumables to the project. Thanks to Jim and Gerry Kelly for allowing us to store equipment and ourselves at their residence. Thanks to Colin Simpfendorfer for providing invaluable advice on the tracking of sawfish and to Stephen Beatty for help with data analysis. Finally, thanks to Ferdy Bergmann for providing us with his wealth of knowledge on sawfish and *Glyphis* locations.

SUMMARY

This study investigated the ecology, morphology, habitat utilisation and population genetics of the vulnerable (EPBC Act 1999) or critically endangered (IUCN) Freshwater Sawfish (*Pristis microdon*). It also examined the distribution of the Northern River Shark (*Glyphis* sp. C) in the Kimberley region of Western Australia and the utility of satellite tags in tracking the movements of this critically endangered species.

Section I of this project was a tagging-based investigation into the movements and habitat utilisation of the Freshwater Sawfish (*Pristis microdon*) and Northern River Shark (*Glyphis* sp. C) in the Kimberley region of Western Australia. In the case of *P. microdon*, between June and November 2007 (middle to late dry season) 38, nine, and two individuals from the Fitzroy River were tagged using Rototags, acoustic tags and satellite (SPOT) tags, respectively. Satellite tags were also fitted to two individuals of *Glyphis* sp. C from King Sound during this period.

The Fitzroy River is an important nursery area for *P. microdon*, with all individuals recorded from the river being immature. During 2007 there was an extremely high level of recruitment of the species into this river. The presence of a large number of small individuals with umbilical scars, i.e. new recruits, in the lower reaches of the river (estuary) during June suggests that the females may pup in the vicinity of the river mouth. A significant correlation between the strength of recruitment and high water levels during the late wet (i.e. April) between 2002 and 2007 was evident; suggesting that pupping may occur in the late wet or that either releasing their litter at this time may provide offspring with more favourable conditions, or that a longer wet season facilitates higher survivorship of offspring. A later or longer wet season may allow small individuals to migrate upstream relatively unimpeded to the safety of freshwater pools for a protracted period of time. It is predicted that there might be some synchronization in the timing of parturition and/or an initial period of high site fidelity among new recruits prior to their upstream migration. In any case, presumably any propensity of new recruits to travel in groups, would increase an individual's chance of avoiding predation on their journey up river.

The use of acoustic tracking demonstrated a high degree of habitat partitioning between different age classes of *P. microdon*, with the new recruits (0+ fish) clearly spending most of their time in shallow water (<0.6 m) compared to the larger 1+ individuals that mainly occurred deeper than 0.6 m. Following Simpfendorfer (2006) who observed similar behaviour in *P. pectinata*, possible reasons for the prevalence of smaller individuals in shallow water include: (i) predator avoidance, noting that smaller individuals are potentially more susceptible to predation and (ii) maximisation of growth rate due to the relatively warm water temperatures in the shallows. On the hand, the occurrence of the larger individuals in the slightly deeper water allows the animal more space to maneuver while also maintaining a close proximity to potential prey. The +1 individuals also showed a marked tendency to occur at deeper depths by day compared to night. The ontogenetic and diurnal differences in habitat utilisation by different age classes of *P. microdon* are also likely to be reflected in differences in diet and could, in fact, be partly driven by foraging strategies related to prey availability. For example, the prey that is available in the shallows differs significantly between the day and night, with the known prey of larger sawfish being more abundant in the shallows at night compared to the day. The diversity of potential prey in the shallows also increases substantially at night. If the small *P. microdon* are feeding in the shallows during the day then their diet is likely to be different to older individuals that occupy different depths and only venture into the shallow at night.

The acoustic data also demonstrated that small individuals of *P. microdon* in the estuarine reaches of the Fitzroy River readily move between pools, even though most pools at low tide are

separated by very long stretches of shallow waters. Furthermore, on the incoming tides, ~98% of the movements of the 0+ sawfish between pools was in an upstream direction, i.e. they moved with the tide. In contrast, the 1+ sawfish moved to another pool only when tidal waters reached the site and this movement was in both an upstream (i.e. 50% with the tide) and downstream (i.e. 50% against the tide) direction. The ability to swim between pools and utilise the shallow runs and riffle zones between both tidally influenced and riverine pools is potentially beneficial because it allows the 0+ fish to avoid deeper bodied predators and also to forage in areas not being exploited by larger fishes. It also allows the new recruits to continue to migrate upstream relatively unimpeded until late into the dry season; to at least the Barrage, a substantial unnatural barrier, in Fitzroy River.

While there was no obvious change in the behaviour of 0+ sawfish during the various moon phases, 1+ *P. microdon* typically utilised very shallow waters (<20 cm) only during the full moon, and water depths greater than 1.5 m only during the new or half moon. The greater visibility at night during the full moon may make these sawfish more vulnerable to deep water predators, or may make their shallow water prey more visible. Alternatively, the sawfishes may follow prey species that move shallower, perhaps in an effort to avoid visual predators during this time.

Satellite tracking of *P. microdon* using SPOT tags was not successful, possibly due to animals not remaining in the shallows for enough time to allow for the calculation of an accurate location. In this regard, it is relevant that the two individuals with the SPOT tags were both relatively large (TL = 1780-2580 mm), which, on the basis of the acoustic tracking data, may rarely go within 0.5 m of the surface. Nevertheless, satellite tracking, in general, still has the potential to provide important information on the movement patterns of individuals of *P. microdon* that leave the river. Perhaps pop-off archival tags will be a more effective tool to examine this aspect of their life-history because the collection of data via these tags is not dependent on the tagged individuals spending significant amounts of time at the surface.

The capture of large numbers of 0+ individuals of *P. microdon* from the Fitzroy River during 2006 and 2007 allowed for an examination of natal sex ratios. Males and females occurred in equal proportions in these individuals. In contrast, the sex ratio of older (i.e. >1+) fish was skewed towards females. The females also attained a considerably greater size than males while in the river. This suggests that either the males are more heavily predated on, which seems unlikely unless growth rates are slower, or that males mature at a smaller size and younger age and so leave the river earlier. Males appear to leave the river at ~2400 mm TL and females at ~2800 mm TL; presumably prior to attaining maturity.

There were notable differences in the rostral teeth counts between *P. microdon* from Western Australia (Fitzroy River) and the Gulf of Carpentaria in Queensland (Qld), albeit that some of these differences may be attributable to low sample sizes from Qld. Importantly, although the analysis included a considerably smaller number of males from Qld (n = 14) than from Western Australia (n = 47), the former exhibited a greater variability in left rostral teeth (18 to 24) compared to the latter (19 to 23). The range in the number of left rostral teeth in females was narrower in Qld samples (17 to 21) compared to the Western Australian samples (17 to 22).

Six individuals of *Glyphis* sp. C were captured in marine tidal creeks of King Sound, ranging from Doctors Creek to the north-eastern side of the Robinson River estuary. This extends the known distribution of the species in Western Australia by approximately 60 km to the north and east. Although SPOT tags proved slightly more effective with *Glyphis* sp. C than *P. microdon*, too few transmissions were made to calculate a location. Most of the transmissions occurred in the early morning and on an incoming tide, which may indicate either peak foraging times for

the species or that the species moves over shallows during this period to move in with the tide. Some morphometric data were collected and some aspects of these data increased the previously known extent of morphological variation in *Glyphis* sp. C. For example, the ratio of the height of the 2nd dorsal fin to the 1st dorsal fin in each of two individuals (1088 and 1237 mm TL) were determined as 0.57 and 0.67, which are both marginally outside the range of this ratio (0.58-0.66) reported for the species.

Section II of the project investigated the genetic diversity and population structure of *P. microdon* in Australian waters. It was based upon the analysis of variation in the nucleotide sequence of a 353-351-bp portion of the control region of the mitochondrial genome. Samples of *P. microdon* were obtained from a total of 90 individuals from across the entire Australian range of this species, but were mainly from the Fitzroy River on the west coast of Australia (n = 37) and from a number of sites within the Gulf of Carpentaria (n = 47) on the north coast. In order to help place the results for *P. microdon* into perspective, comparable genetic data were produced for a total of 30 individuals of the Dwarf Sawfish (*Pristis clavata*), from several sites along the west coast and in the Gulf of Carpentaria.

The results indicate that *P. microdon* contains a 'healthy' amount of genetic diversity over a range of spatial scales in Australian waters. This was indicated by the fact that the haplotype and nucleotide diversities in the samples of this species were not unusually low either in absolute terms or in comparison with those of other elasmobranchs, including *P. clavata.* Since the evolutionary potential of a population is proportional to the amount of adaptively significant genetic variation therein, and assuming that control region diversity provides a reflection of genome-wide diversity in *P. microdon*, this finding is encouraging regarding the prospects for the long-term survival of *P. microdon* in Australian waters. However, there is a note of caution in that it can take several generations for a decline in the genetic diversity of a long-lived species with overlapping generations, like *P. microdon*, to become evident. Furthermore, most of the control region diversity in *P. microdon* was present in rare haplotype (i.e. in rare alleles), which would be highly susceptible to loss via genetic drift if the abundance of this species should decline in the future. Thus, in order to safeguard current levels genetic diversity, it is vital to develop management plans for *P. microdon* such that its current population sizes are maintained.

The analysis of the population structure of *P. microdon* in Australian waters was focused on the Fitzroy River on the west coast of Australia and the Gulf of Carpentaria. The results indicate that *P. microdon* is genetically sub-divided between these two locations. This finding is significant because it almost certainly means that the demographics of the assemblages of this species in these two locations are independent of each other and can thus be regarded as separate management units. However, the smallest spatial scale at which demographically independent units occur in *P. microdon* could not be resolved. Hence, it is possible that all of the *P. microdon* in the Gulf of Carpentaria belong to a single population, as might all of the individuals of this species on the west coast. Alternatively, this species may typically comprise multiple populations within these and other geographic regions. Since they are demographically independent of each other, the demise of one population will not be offset by immigration from another. Thus, the conservation of each should be a high priority. In addition, data relating to the demographics (e.g. abundance, mortality, recruitment) of *P. microdon* should be collected and interpreted at the level of individual populations.

Finally, analysis of the evolutionary relationships among the control region haplotypes of *P. microdon* indicates that the amount of evolutionary divergence in this species in Australian waters is moderate. There do not appear to be any particular subsets of individuals or population that require special conservation priorities on their basis of their distinctive evolutionary histories.

TABLE OF CONTENTS

GENERAL INTRODUCTION

Only recently has the ichthyological importance of the Fitzroy River and its estuary, in the Kimberley region of northern Western Australia, been revealed. The river and its estuary (King Sound) supports one the most robust populations of the critically endangered (IUCN Red List 2007) or vulnerable (EPBC Act 1999) Freshwater Sawfish (*Pristis microdon*) in the world (Thorburn *et al.* 2003, 2007, Thorburn & Morgan 2005). The Dwarf Sawfish (*Pristis clavata*), is also listed as critically endangered (IUCN Red List 2007) and is known from the lower reaches (tidal) of the river (Morgan *et al.* 2002, 2004, Thorburn *et al.* 2003, in press). Furthermore, the tidal waters of King Sound represents the only known habitat of the critically endangered (IUCN Red List 2007) or endangered (EPBC Act 1999) Northern River Shark (*Glyphis* sp. C) in Western Australia, and one of only four known localities of the species in the world (Thorburn & Morgan 2004). Indeed, most of the *Glyphis* sp. C known to science have come from this region. The river also supports one of the most diverse freshwater fish faunas in Western Australia, with 23 species recorded and a number of additional marine/estuarine species that utilise the river as a nursery (Morgan *et al.* 2002, 2004). As well as diverse fauna, the river houses a number of other cryptic species, many of which are listed as threatened by the IUCN, including the Freshwater Whipray *Himantura chaophraya* which is listed as vulnerable, and three species that are listed as lower risk-near threatened, i.e. Prince Regent Hardyhead *Craterocephalus lentiginosus*, Barnett River Gudgeon *Hypseleotris kimberleyensis* and Greenway's Grunter *Hannia greenwayi* (Morgan *et al.* 2004).

The Fitzroy River is similarly culturally diverse, with over five traditional languages spoken along the river. While the scientific knowledge of the river was minimal prior to the studies listed above, the cultural knowledge surrounding the rivers fishes is extremely high. This was demonstrated in the collaborative study by the Kimberley Land Council, Murdoch University's Centre for Fish & Fisheries Research and the Kimberley Language Resource Centre whereby the river's fishes were documented in five Aboriginal languages (see Morgan *et al.* 2002, 2004). Further collaborative research was used to determine the ecology and cultural significance of Freshwater Sawfish in the river (see Thorburn *et al.* 2004).

Overall aims of the study

When considering two of the critically endangered fishes of the Kimberley, i.e. *P. microdon* and *Glyphis* sp. C, that are the focus of this study, little is known with regard to their habitat utilisation and relationship with other populations, e.g. migration patterns. Within this report we aim to ameliorate some of these deficiencies. The use of conventional (Rototags), acoustic and satellite tags were employed to determine movement patterns of *P. microdon* within the Fitzroy River and in King Sound and Rototags and satellite tags were attached to *Glyphis* sp. C in King Sound. Additionally, both old rostra and contemporary samples, along with the development of new control region (mtDNA) primers, were used to assess the genetic diversity and extent of genetic differences between selected assemblages of *P. microdon* across northern Australia. Throughout most of the field work, the Jarlmadangah Rangers assisted in the capture and tagging of fish.

SECTION I

Tracking the movements of Freshwater Sawfish (*Pristis microdon*) and Northern River Sharks (*Glyphis* sp. C) in the Fitzroy River

by

JM Whitty, DL Morgan, DC Thorburn, T Fazeldean & SC Peverell

INTRODUCTION

The Fitzroy River covers almost 90,000 km^2 of the Kimberley, and the mean annual river flow of 6,150 GL/year at Fitzroy Crossing is the highest recorded for any river system in Western Australia. Unlike most Kimberley rivers, there are few natural barriers in the system and the many large pools within the river act as ideal nurseries for a number of marine and estuarine species (Morgan *et al.* 2002, 2004, Thorburn *et al.* 2007). This applies to the critically endangered Freshwater Sawfish (*Pristis microdon*), which within the Fitzroy River has been recorded ~400 km inland in this system (Figure 1) (Morgan *et al.* 2002, 2004). Our previous research indicates that the species is widespread throughout the river, but is predominantly only found in the main channel of the Fitzroy River and within one of its major tributaries, i.e. Margaret River (Figure 1) (Morgan *et al.* 2002, 2004, Thorburn *et al.* 2004, 2007).

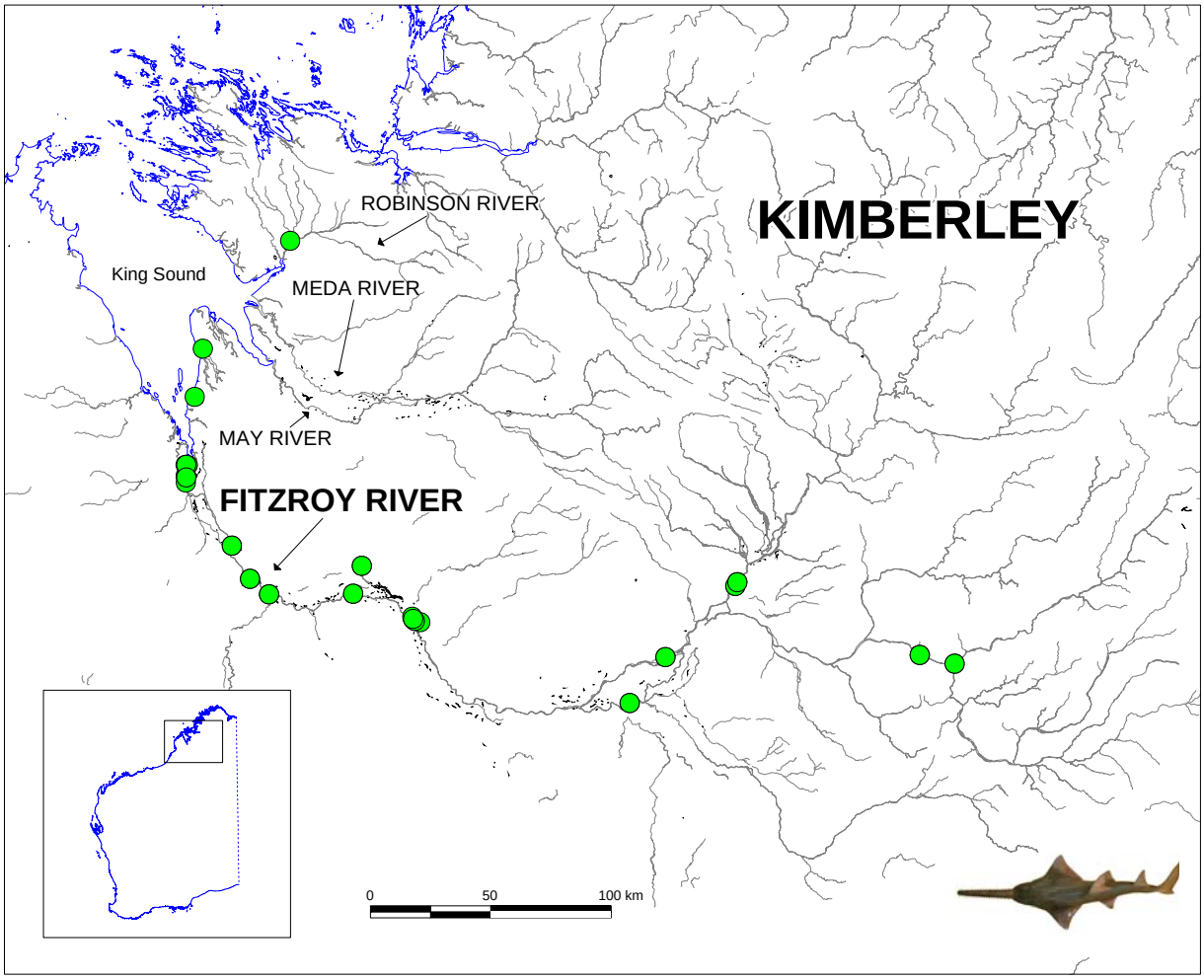


Figure 1 Centre for Fish & Fisheries Research (Murdoch University) capture locations for Freshwater Sawfish (*Pristis microdon*) in the Fitzroy River and King Sound (2001-2006) (Morgan *et al.* 2002, 2004, Thorburn *et al.* 2004, 2007).

The evidence for the Fitzroy River being a nursery for *P. microdon* lies in the fact that all individuals captured thus far in the system have been immature and are comparably small, i.e. <3 m in total length (TL) (Thorburn *et al.* 2007); noting that the species is reported to attain lengths of ~7 m TL (Last & Stevens 1994). Growth rates within the system are rapid, with fish attaining well in excess of 2 m by age four (Thorburn *et al.* 2007). At approximately 2.5 m the

males and females are thought to leave the river and enter King Sound (Thorburn *et al.* 2007), however, with the exception of a few records of fish >3 m TL from the Pilbara coast, and one individual from Cape Naturaliste (south-western Australia) (Chidlow 2007), there is little known with regard to adult habitats and breeding grounds in W.A. In contrast to *P. microdon*, another critically endangered elasmobranch, the Northern River Shark (*Glyphis* sp. C) that is found in the region, has not been observed utilising the Fitzroy River as a nursery, but resides within the marine waters of King Sound (Thorburn & Morgan 2004). However, the lack of records of this species, and the apparent rarity, inhibits inferences on its biology and habitats. Known from only 19 specimens in the world, *Glyphis* sp. C has been found within riverine habitats of the Northern Territory and Papua New Guinea and it is unclear as to why the species has not been recorded from the Fitzroy River proper, considering that 10 of these known specimens were captured in King Sound near the river's mouth (Thorburn & Morgan 2004, 2005, Thorburn 2006). Despite the absence of specimens, the species appears morphologically suited to surviving in both rivers and areas of extreme tidal range and high turbidity, such as that experienced in King Sound. Our current knowledge on the species distribution in Western Australia includes a few sites (over a distance of ~30 km) in or around tidal creeks in the south-eastern corner of King Sound (Figure 2).

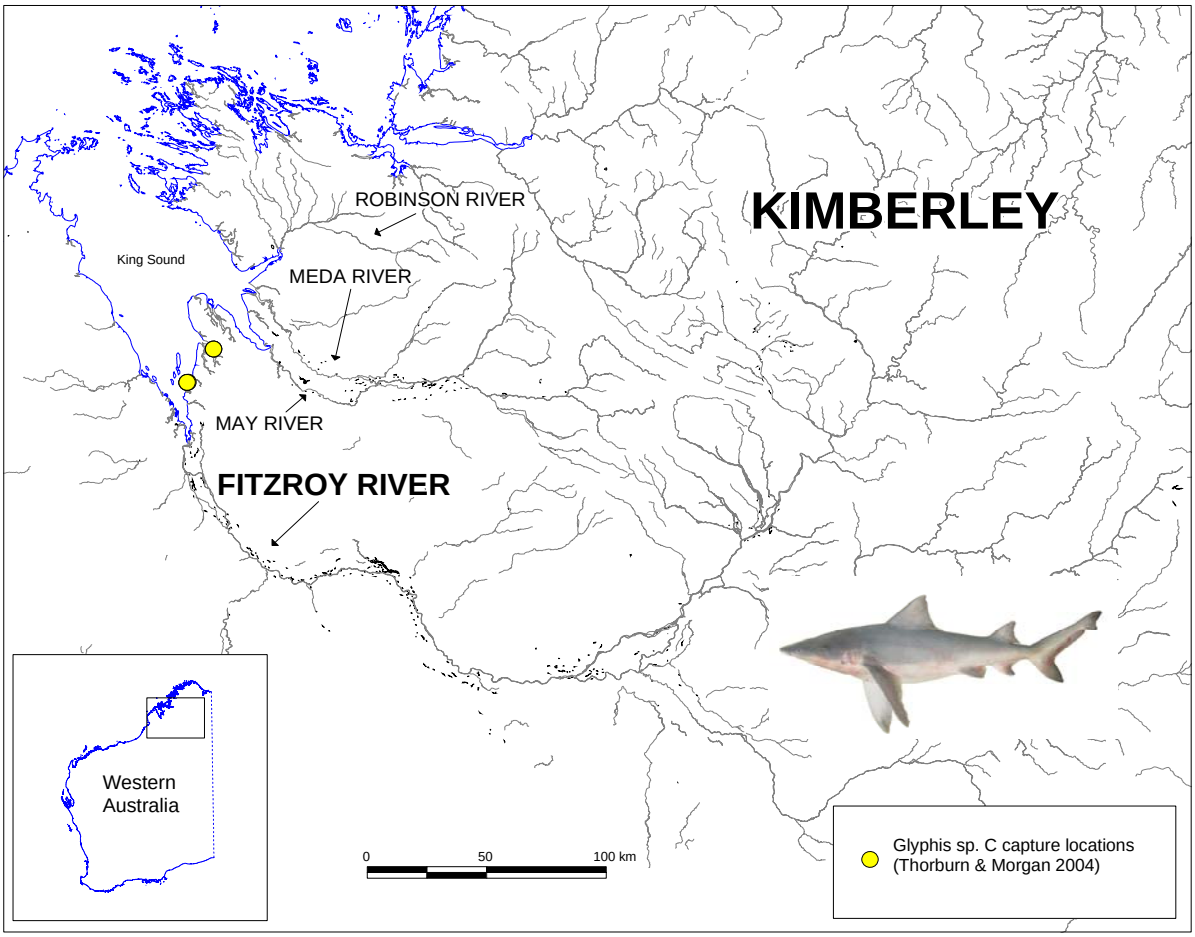


Figure 2 Historical capture locations for Northern River Shark (*Glyphis* sp. C) in Western Australia, i.e. King Sound (see Thorburn & Morgan 2004).

A number of studies have utilised a range of tracking technologies to monitor the movement patterns of elasmobranchs (Holland *et al.*1999, Stevens *et al.* 2000, Dewar *et al.* 2004). The selection of appropriate tracking methods is often dependent upon the target species, habitat and type of study involved. A combination of tracking methods is occasionally employed to provide a variety of long and short-term data (Sibert and Fournier 2001). This is relevant for examining

species such as *P. microdon* and *Glyphis* sp. C in the Fitzroy River and King Sound and is optimal to gain a more complete picture on these apparently wide ranging yet poorly understood species. For example, conventional tagging of these species would allow for a variety of data on growth, movement patterns and population demographics to be determined for these species upon their recapture. Unfortunately movement data using conventional tags is often on the broad scale. For more detailed information on movements of such species, acoustic tracking has been shown to be useful in a wide range of habitats including fresh, estuarine and marine environments (Voegeli *et al*. 2001; Pillans *et al*. 2005; Simpfendorfer 2006). As the utility and reliability of a number of tracking methods differs between fresh or marine environments, acoustic tracking is deemed a more reliable option for tracking animals across various salinities, such as that experienced in the Fitzroy River and estuary. A limitation to passive acoustic tracking is that the area in which an animal can be tracked is dependent upon the number and range of receivers. Certain satellite tags however allow for an animal to be tracked over large expanses, which would prove useful with animals whose habitat range and/or migration pattern are unknown and possibly cover a large area. A combination of these three tags should allow for short and long-term data to be collected for the various age classes of *P. microdon* and *Glyphis* sp. C in the western Kimberley.

The aim of this section of the study was to determine movement patterns and habitat utilisation of *P. microdon* and *Glyphis* sp. C, which is crucial for management plans. This was accomplished via the employment of conventional (Rototags), acoustic and satellite tags, on *P. microdon* and Rototags and satellite tags on *Glyphis* sp. C. The utility of each method is discussed, as are the habitat associations, movement patterns and behaviour of individuals. A second aim was to compile data from historical catches of *P. microdon* captured in the Fitzroy River and King Sound and assess morphological relationships and variations in recruitment patterns.

METHODOLOGY

Study sites and techniques

Water levels in the Fitzroy River are highly variable and are largely influenced by a wet and dry season. During the dry season (typically May-November) little precipitation occurs and as a result the river becomes a series of pools that are connected by long, shallow runs. During the wet season, however, the river experiences severe flooding and is characterised by high but variable discharge. During June, July, October and November 2007, sampling for *P. microdon* and *Glyphis* sp. C was conducted in the southern and eastern regions of the King Sound and in the lower 150 km of the Fitzroy River (Figures 3 and 4). Sampling sites included macrotidal and mangrove dominated areas of King Sound, the lower 16 km of the Fitzroy River which experiences varying degrees of tidal influence, and within a number of large freshwater riverine pools including one directly below the Camballin Barrage (Figure 4). The longitude and latitude of each site sampled was recorded using a GPS. Sampling in King Sound consisted of gill netting at 14 different sites along its southern and eastern regions including bordering creeks as well as the May and Robinson River estuaries, with most sites (12) sampled during October 2007. These sites were all less then 10 m in depth and highly affected by mixed tides reaching up to 11+ m. Sampling on the Fitzroy River took place at six different sites (Milli Milli, Snag Pool, Langi/Langey Crossing, Myroodah, a large pool about one kilometer downriver of the Camballin Barrage and a pool directly below the barrage) along the lower 150 km of the Fitzroy River. River sites were chosen due to their accessibility and on the basis that previous surveys had recorded *P. microdon*.

Sampling Techniques

All individuals were captured with either gill net or hook and line. The majority of fishing effort was conducted with the use of 4", 6", 7" and 8" stretched monofilament gill nets. The 4", 6" and 8"nets were 20 m in length and often joined to create a net sometimes 80 m in length. The 7" nets were 50 m in length. Nets were checked regularly. All fish captured were identified and measured for total length (TL, mm) and released. In the case of sawfish, rostrum length (RL), rostral tooth counts (left and right side), sex, and for males, inner and outer clasper length and degree of clasper calcification (flaccid, semi-calcified or calcified) was recorded. Location, date, approximate time of capture and capture method were also recorded. Also at this time, Rototags, acoustic tags, and/or satellite tags were attached and tissue samples were taken (usually a byproduct of conventional tagging, see below and section II).

Data collated on population demographics, morphology, distribution and general biology from those fish captured during 2007 were compared with our previous catches of these species within the region, i.e. within each year since 2002. Likelihood ratio tests (Cerrato, 1990) were used to determine whether differences existed in the relationship of TL and RL between the sexes.

Seine netting was also used to determine the changes in potential diurnal dry season prey species. A total of six replicate seine net samples were collected both during the day and night on each major sampling period, i.e. June and October at both Myroodah Pool and within the large pool ~1 km downstream from the barrage. The seine net was 26 m in length and consisted of a 10 m pocket of 3 mm woven with two wings comprised of 6 mm woven mesh and on average covered an area of 110 m^2. The fish (and decapod crustacean) species captured were identified, measured (total length (mm)) and released. Based on the number (log transformed) of individuals captured in each seine net, which on average covered ~110 m^2, ANOSIM was employed (Clarke & Goorley 2006) to test the hypothesis that the potential prey fish in the shallows during the day would be different to that at night.

Environmental Parameters

Environmental parameters including bottom and surface temperature and salinity, oxygen, and secchi disc readings were taken during sampling events, during acoustic tag range tests and when data was downloaded from the receivers. Water temperature (~2 m off bottom) was recorded in three hour intervals throughout the study period by the temperature loggers attached to each installed mooring. Loggers were downloaded in October/November 2007. Archived tidal data (for Derby) and river status data was acquired from the Department of Planning and Infrastructure (DPI) and the Department of Water, respectively. Moon illumination data was acquired from the United States Naval Observatory Astronomical Application Department (http://aa.usno.navy.mil/data/docs/MoonPhase). Ambient minimum and maximum temperature data was supplied through the Bureau of Meteorology (http://www.bom.gov.au/climate/dwo/IDCJDW6035.latest.shtml) (See http://www.bom.gov.au/other/copyright.shtml for Bureau of Meteorology Copyright Notice and http://www.bom.gov.au/other/disclaimer.shtml for the Disclaimer).

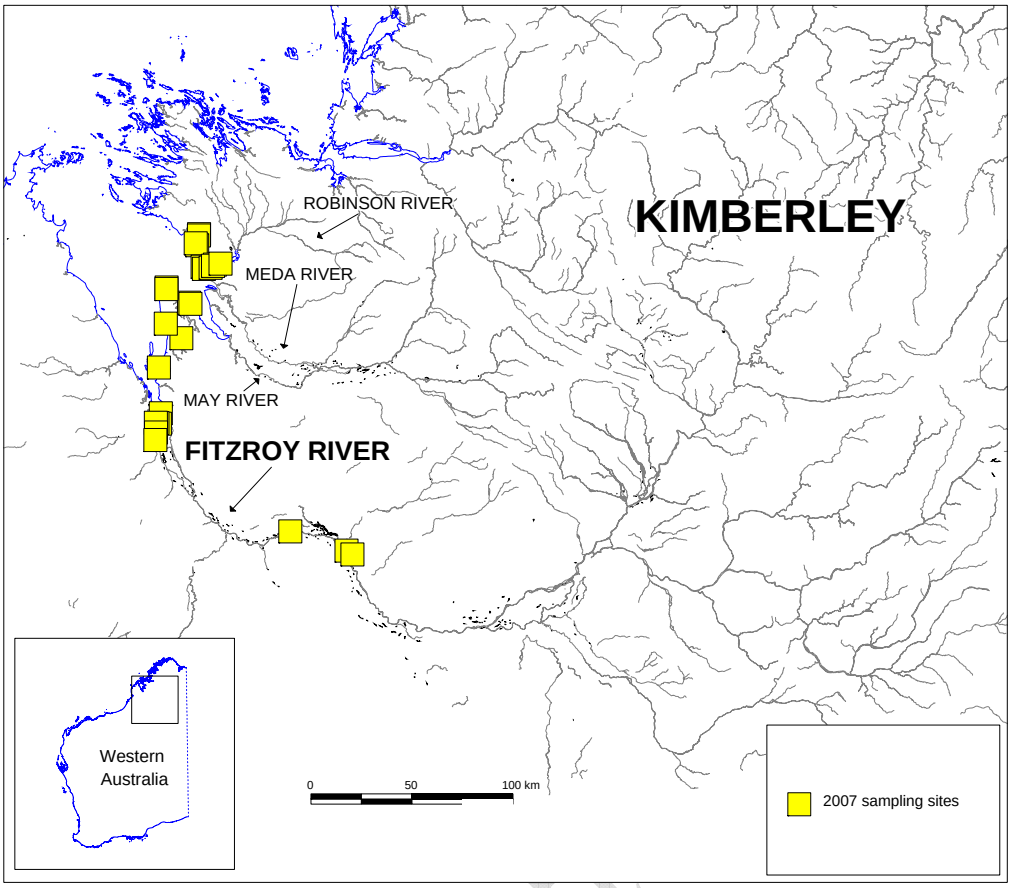


Figure 3 Sampling sites in King Sound and the Fitzroy River during 2007.

Tagging methodology

Rototags

Sawfish surveys were initiated in 2001 and 2002 (see Morgan *et al.* 2002, 2004, Thorburn *et al.* 2003). The following year a conventional tagging (Rototags) programme was developed (Thorburn *et al.* 2004). Much of this research has continued as a collaboration between the Centre for Fish & Fisheries Research (Murdoch University), the Yiriman Project (Jarlmadangah Rangers) and the Kimberley Land Council, which formed during the Fitzroy River Fish Project (see Morgan *et al.* 2002, 2004). Rototags (pictured below) were attached to dorsal fins of *P. microdon* in the Fitzroy River (see Thorburn *et al.* 2004, 2007).

The Rototags used in this study are synonymous with those used by the Department of Fisheries, Government of Western Australia and are ~20 x 45 mm polyurethane laser marked sheep tags. Rototags were attached to the dorsal fin of *P. microdon* (see Thorburn et al 2004, 2007) and the individual number for each fish was recorded. Between 2003 and 2006 a total of 90 *P. microdon* were tagged with these tags in the Fitzroy River (Kimberley, Western Australia). During 2007, Gallagher Supertags Small (~20 x 45 mm polyurethane laser marked sheep tags) were also used on *P. microdon*. A further 21 Dwarf Sawfish (*Pristis clavata*) have also been tagged in the Fitzroy River; none of which have been recaptured (or reported as being recaptured to us).

The Petersen Method was used with recapture data to estimate the population size, in this case of 0+ individuals, in a closed system (King 1995). Although this method assumes that the study area was a closed system, this may or may not have been the case, and results from this method are only be used as a rough estimate.

Figure 4 Some of the sites sampled in the Fitzroy River and King Sound. (Images from Google Earth).

The use of Rototags has lead to a high level of local community involvement and created awareness of the project; also as a result of the positioning of signs (see below) at popular fishing sites on the Fitzroy River, through public seminars, media exposure (e.g. ABC Catalyst http://www.abc.net.au/catalyst/stories/s2043601.htm), community involvement in the tagging programme, an artwork competition and by handing out "Team Sawfish" t-shirts. Importantly, public reports have indicated that sawfish are more likely to be released if they are tagged. See http://www.cffr.murdoch.edu.au/curres/Freshwater.html

Acoustic Tagging

Vemco VR2W omnidirectional acoustic receivers were moored in Lower Pelican Pool, Milli Milli Pool, Snag Pool, the pool at Langi Crossing, Myroodah Pool, and in the large pool approximately one kilometer below the Barrage (Figure 5). Used in association with acoustic transmitters fitted to the fish, receivers recorded the date and time that tagged animals were within in detection range. Depth and temperature data from sensors on tags were also downloaded at this time. Receivers were attached to moorings designed to keep them upright during high flows while also allowing access to the receiver from the surface. The mooring design consisted of a 35 kg concrete block chained (4 m galvanized chain) to a sand anchor ranging between 10-25 kg. The anchor arm was also attached to a 3 m length of rope (25 mm width), which was tied to two aluminum buoys around one meter apart (a surface float and subsurface float were used to compensate for varying water levels). A separate one meter section of the rope was spliced between two points on the main rope about 0.75 m apart to create a hanging loop in which the receiver was attached via five cable ties. When in place the receiver sat about one meter above the substrate. A HOBO Water Temp Pro v2 Data Logger was placed just below the lower buoy. In the three most upstream receivers (see Figure 5) the cement block at the end of the chain was substituted for a large tree/snag or in the case of Langi Crossing, a piling. Anchor sizes were also increased in the upper pools to reduce movement, and because the more solid substrate in this area is more likely to support the weight of the anchor without it sinking and becoming stuck. Data from the receiver and loggers were downloaded in October/November 2007.

Both Vemco V16TP-5H and Vemco V13TP-1L coded acoustic transmitters that emitted a pulse stream at 69 kHz, were used to provide information on the habitat associations and movement patterns of *P. microdon*. It was originally proposed to tag individuals >1.5 m with V16 tags, however, due to the dominance of the smaller 2007 0+ cohort during the initial June tagging field trip, smaller V13 tags were purchased from funds provided by Murdoch University (Research Excellence Grants Scheme); to facilitate the additional tagging of these smaller individuals. Both tag types had temperature and pressure sensors that allowed for receivers to record the temperature and depth of the individual at the time of transmission. V16 tags were

factory set to randomly transmit between every 45 and 75 seconds. These tags were fitted to four *P. microdon* (1555-1611 mm TL), three in Snag Pool, and one in Langi Crossing (Figure 5). These tags were attached to the base of the first dorsal fin of each fish using two nylon bolts and secured via stainless steel nuts. The metal nuts were used so that corrosion would eventually lead to the tag falling off; long after the battery life of the tag. V13 tags were set to transmit randomly every 60 to 180 seconds. Vemco V13 acoustic transmitters were also attached via the dorsal fins to five *P. microdon* (842-1111 mm), four in Snag Pool and one near the most upstream receiver (Figure 5, Table 1). V13 tags were attached to Rototags (see above) via cable ties and marine silicone and the Rototags then fitted to the first dorsal fin of the fish. The weight of the Rototags is <5 g and the combined weight with a V13TP acoustic tag is <12 g. The smallest sawfish tagged (842 mm TL) had a calculated weight of 1312 g; thus the combined Rototag/V13 tag weight is less 1% of the fishes body weight, which is lower than the recommended maximum suggested by Blaylock (1990).

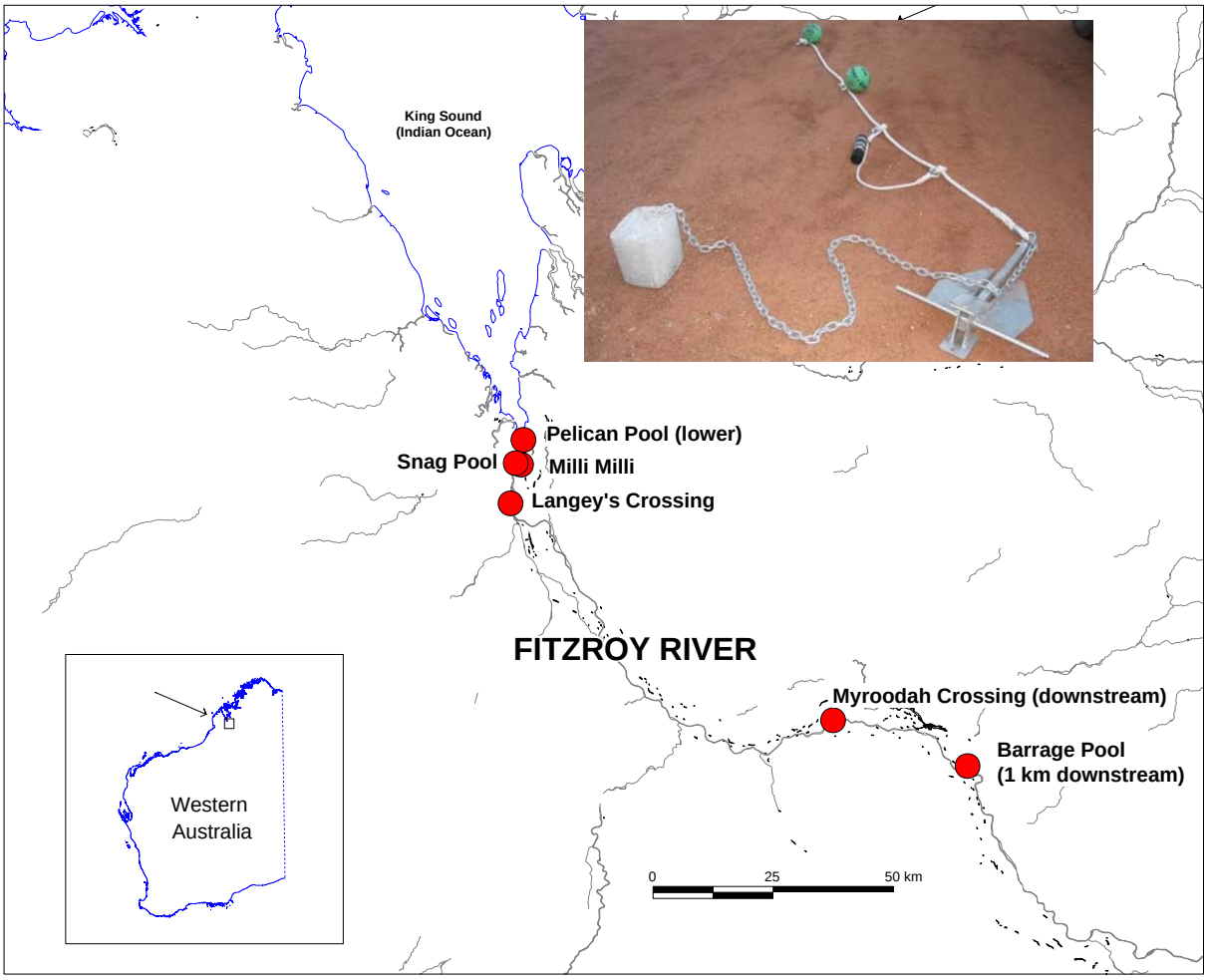


Figure 5 Position of VR2W acoustic array and the mooring design (inset) used in the Fitzroy River and its estuary.

Table 1 Acoustic tag number and model attached to *Pristis microdon* in the Fitzroy River. Also included is the corresponding Rototag number, capture date and location and total length (TL) of each individual.

Acoustic #	Tag Model	Rototag #	Capture Date	Capture Location	TL (mm)
1038505	V16	F9835	22-Jun-07	Snag Pool	1555
1038506	V16	F7092	23-Jun-07	Snag Pool	1611
1038503	V16	M1013	18-Jul-07	Snag Pool	1580
1042224	V13	M1007	18-Jul-07	Snag Pool	842
1042227	V13	M1003	19-Jul-07	Snag Pool	981
1042226	V13	M1006	19-Jul-07	Snag Pool	990
1042229	V13	M1005	19-Jul-07	Snag Pool	854
1042225	V13	M1004	01-Nov-07	Money Pool	1111
1038502	V16	NA	13-Nov-07	Langi Crossing	1576

Range tests were conducted at each site to determine the distance at which the receivers would be able to reliably (>95% of the time) detect the acoustic signal from both the V16 and V13 tags. Range tests were conducted using a "test-tag"; an acoustic tag similar to the tags used to track the fish but with a non-random transmission interval. During the test, test-tags were submersed (one meter off the substrate, or mid water column if the depth was less than one meter) at 50 m intervals starting at the receiver (0 m) and continuing up to between 300 and 1200 m, depending on pool depth and shape, for a minimum of four minute intervals. Bottom and surface temperature, salinity, and depth were measured at each interval. Range tests were conducted both upstream and downstream of receivers for each site using the V16 test tag. For the V13 test tag, range tests were conducted in an upstream and downstream direction at Langi Crossing and at the most upstream receiver (Figure 5). V13 tests were repeated upstream and downstream at Langi Crossing with the addition of a thin layer of silicone coating the tag, to determine any effects that the method of attachment (i.e. silicone) for these tags may have on their signal.

Satellite tagging

Wildlife Computer's SPOT-5 (Smart Position or Temperature Transmitting) satellite tags were fitted to two *P. microdon* and two *Glyphis* sp. C. SPOT tag 41104 was attached to the dorsal fin of a 1780 mm TL female *P. microdon* in June 2007 (Snag Pool), and SPOT tag 41099 was attached to a 2580 mm TL male in October 2007 (Point Torment, King Sound) (Figure 6). SPOT tag 41103 was attached to the first dorsal fin of a 1365 mm TL male *Glyphis* sp. C and SPOT tag 41101 was attached to a 1084 mm TL female *Glyphis* sp. C. SPOT tag 41103 was deployed in June 2007 (Doctor's Creek,) and SPOT tag 41101 was deployed in October 2007 (Robinson River mouth) (Figure 6). All tags were attached to the first dorsal fin of each fish using nylon bolts, nylon lock washers, and stainless steel washers and nuts. Implant-grade drill bits were used to create the holes needed to pass the nylon bolts through. To ensure proper placement and spacing of the holes and decrease tagging time, prefabricated polypropylene drilling templates were used.

Tags were programmed to determine the animals' location which were determined by a series of calculations involving the Doppler effect, previous known locations, and estimated maximum swimming speeds of the fish. An accurate location requires four transmissions during a satellite's single pass, which often lasts 10-15 minutes. Transmissions are possible at previously set transmission times and when the tag antennae are not submerged. To maximise tag life, selected transmission times were programmed according to hours when satellites were most often overhead (determined using SatScape v2.02). If an insufficient number of transmissions were made, locations with a higher error rating were calculated or no location was supplied.

Transmitted data from SPOT tags was downloaded from the ARGOS website (https://www.argos-system.org/). Maps of initial area of capture/tag deployment and subsequent transmissions were created using MapInfo.

Pristis microdon

Glyphis sp. C

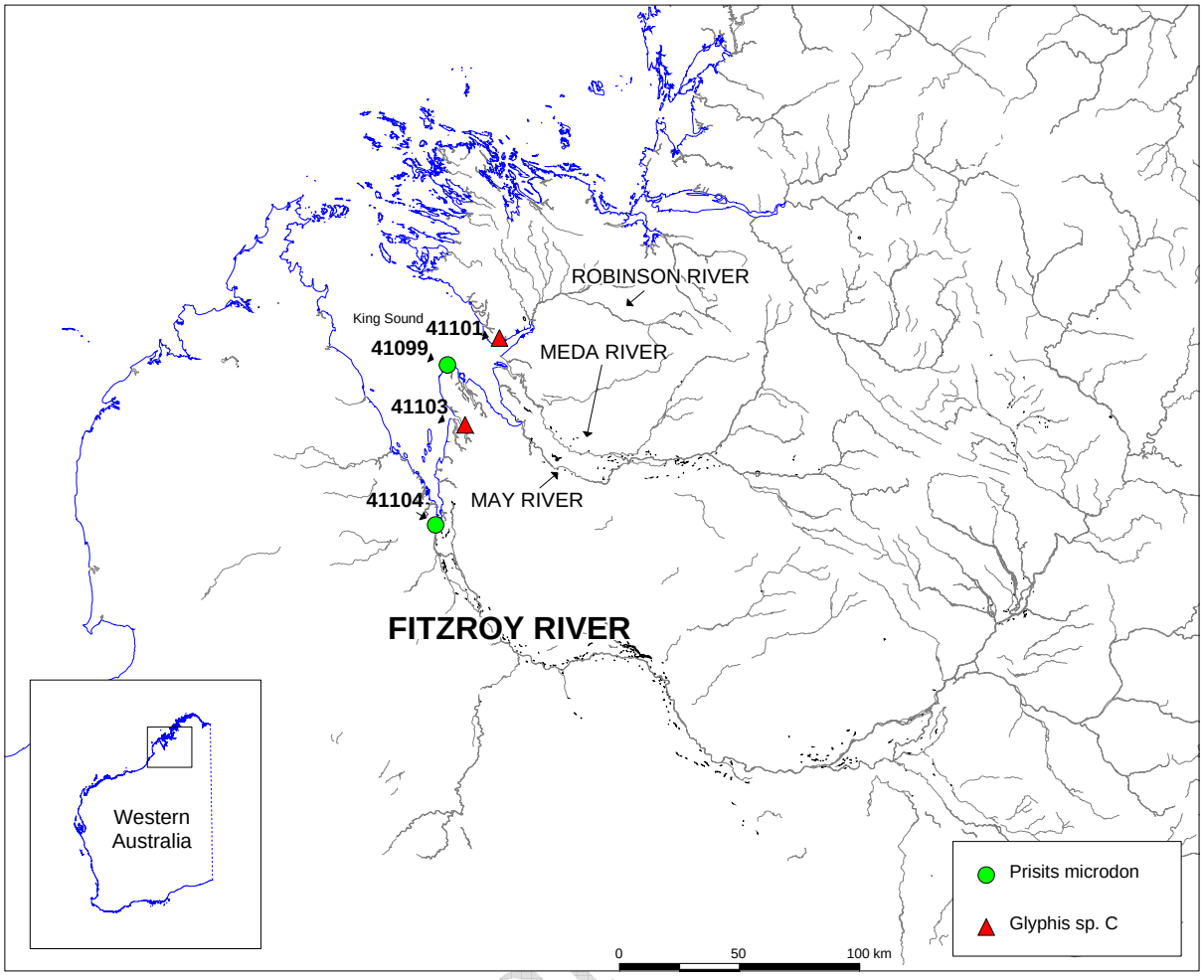


Figure 6 Locations of *P. microdon* and *Glyphis* sp. C that were satellite (SPOT) tagged.

RESULTS

Environments of the Fitzroy River and King Sound

Water levels (and thus discharge) in the Fitzroy River over the last decade have generally peaked between January and April, however there are discrete variations in the length and duration of the wet season between years (Figure 7). For example, in most years discharge was peaking by February, but in some years the peak extended from December until February (December 1998 to February 1999), whereas during some years it lasted until late April. Often the wet season and period of high discharge was relatively short, and in 2003 and 2005 lasted only for between one and three weeks (Figure 7).

During 2007, the peak in discharge and thus high flow period was bimodal, with peaks occurring in late January and early April (Figures 8). On each occasion the period of high discharge was relatively short and lasted for only a few weeks.

Throughout the study period, i.e. June to November, air temperatures and water temperatures within the Fitzroy River generally increased (Figure 9). Differences in water temperatures existed between tidal and non-tidally influenced sites, primarily as those sites that experienced

tides showed greater diurnal variations in water temperatures (Figure 9). Mean water temperatures in King Sound and its associated creeks, over a two day period in June 2007 were 20.1°C (± 0.40), compared to October measurements where the mean was 29.1°C (± 0.11).

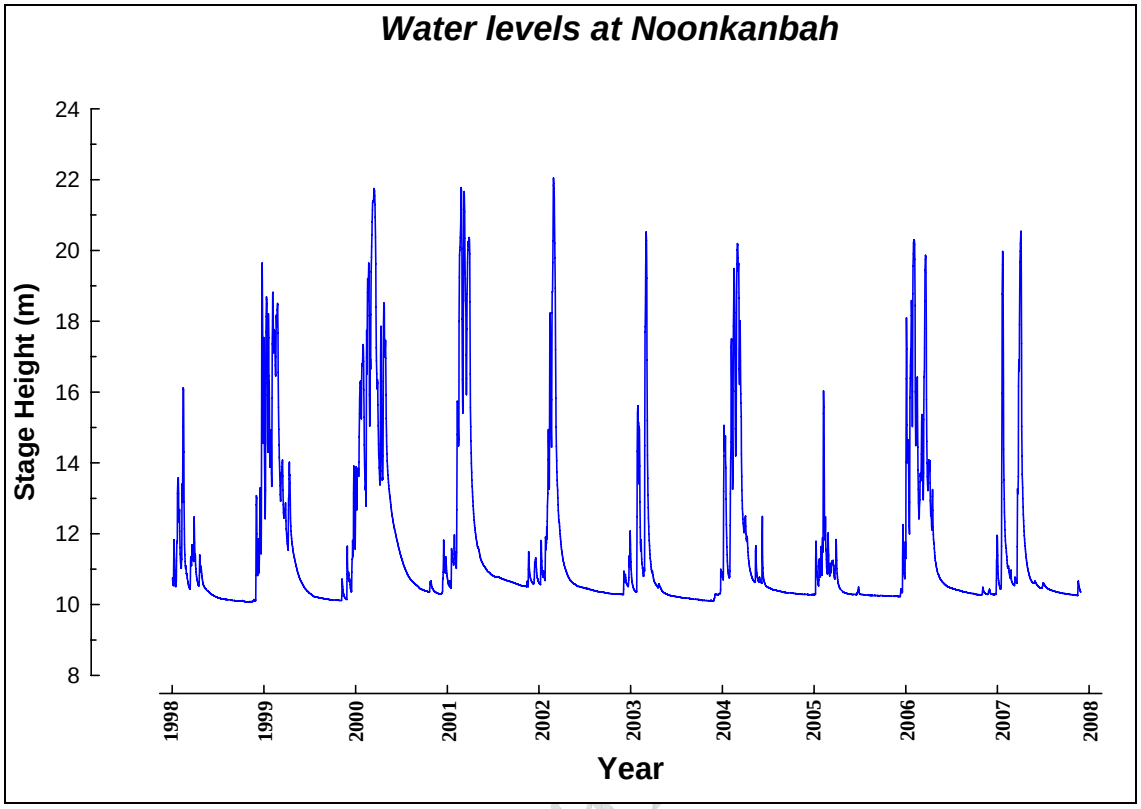


Figure 7 Daily stage height data for the Fitzroy River at Noonkanbah between 1998 and 2008. Data provided by the Department of Water, Government of Western Australia.

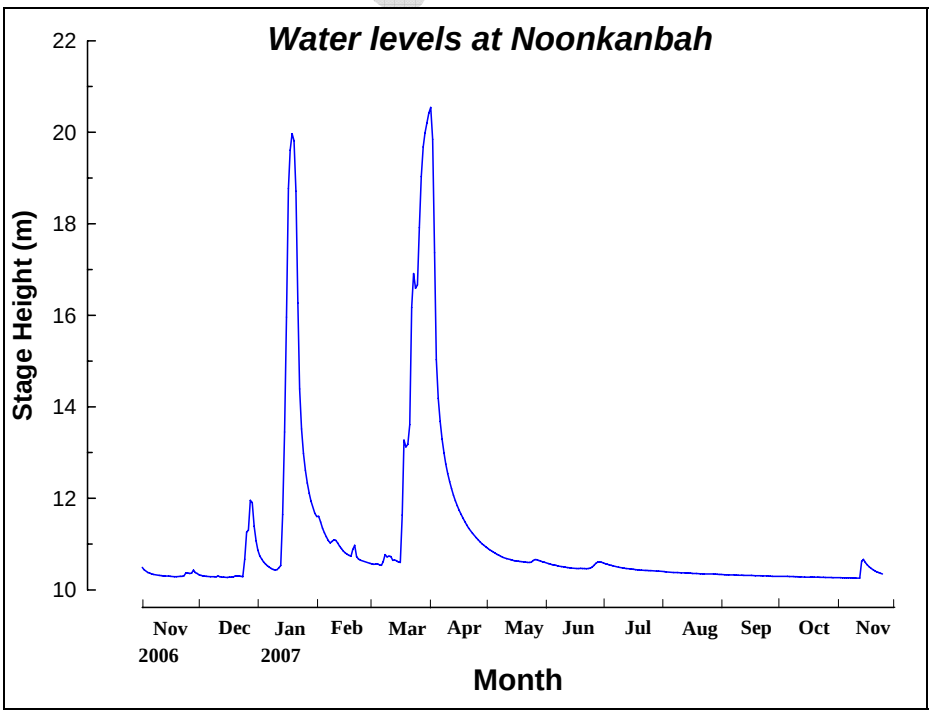


Figure 8 Daily stage height data for the Fitzroy River at Noonkanbah during 2007. Data provided by the Department of Water, Government of Western Australia.

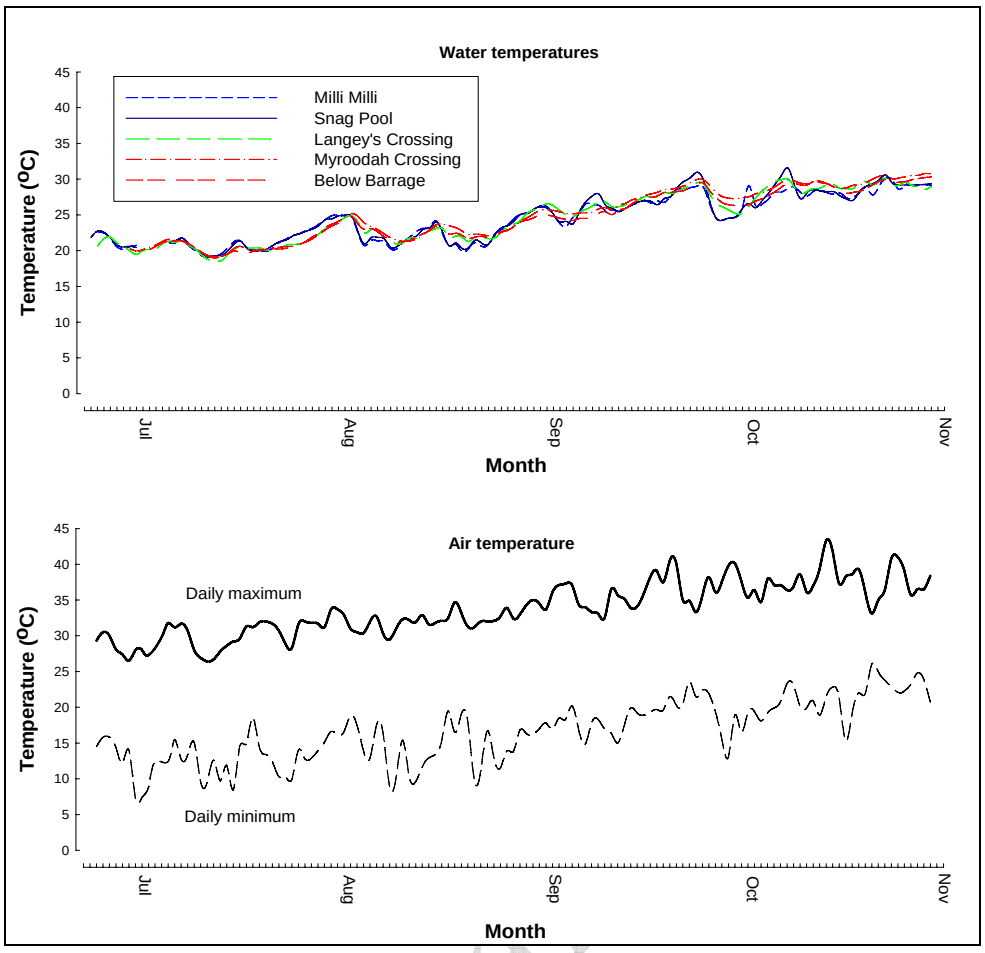


Figure 9 Logged water temperatures for five sites on the Fitzroy River and air temperatures for Derby between late June and November 2007. Air temperature data provided by the Bureau of Meteorology.

Salinity levels in King Sound and the most downstream sites increased between June and October 2007 (Figure 10). In June 2007 an average salinity of 30.2 (± 0.77) ppt was recorded in King Sound sites compared to 35.9 (± 0.32) ppt recorded in October 2007. The sites that experience varying degrees of tidal influence (i.e. Pelican Pool, Milli Milli, Snag Pool and Langi Pool) increased considerably in salinity between June and October (Figure 10). Within these pools the bottom salinities (>1 m depth) were generally higher than surface salinities. Interestingly, at Langi Pool which was fresh in June (0.2 ppt), the surface and bottom salinities changed markedly, with mean surface and bottom salinities increasing by 55 and 75 fold, respectively. In contrast, the most upstream riverine sites remained very fresh throughout the year.

Water levels, as a result of huge tidal movements, in King Sound are constantly changing with generally two tides per day. During the study period, high tides reached almost 12 m, while low tides were occasionally less than 1 m (Figure 11). The timing of high tide time between Derby and Snag Pool was estimated to be 3.3 hours. High tides were found to reach Snag Pool when the tidal height was over approximately 9 m ± 0.5 m. Only occasional tides reach Langi Pool.

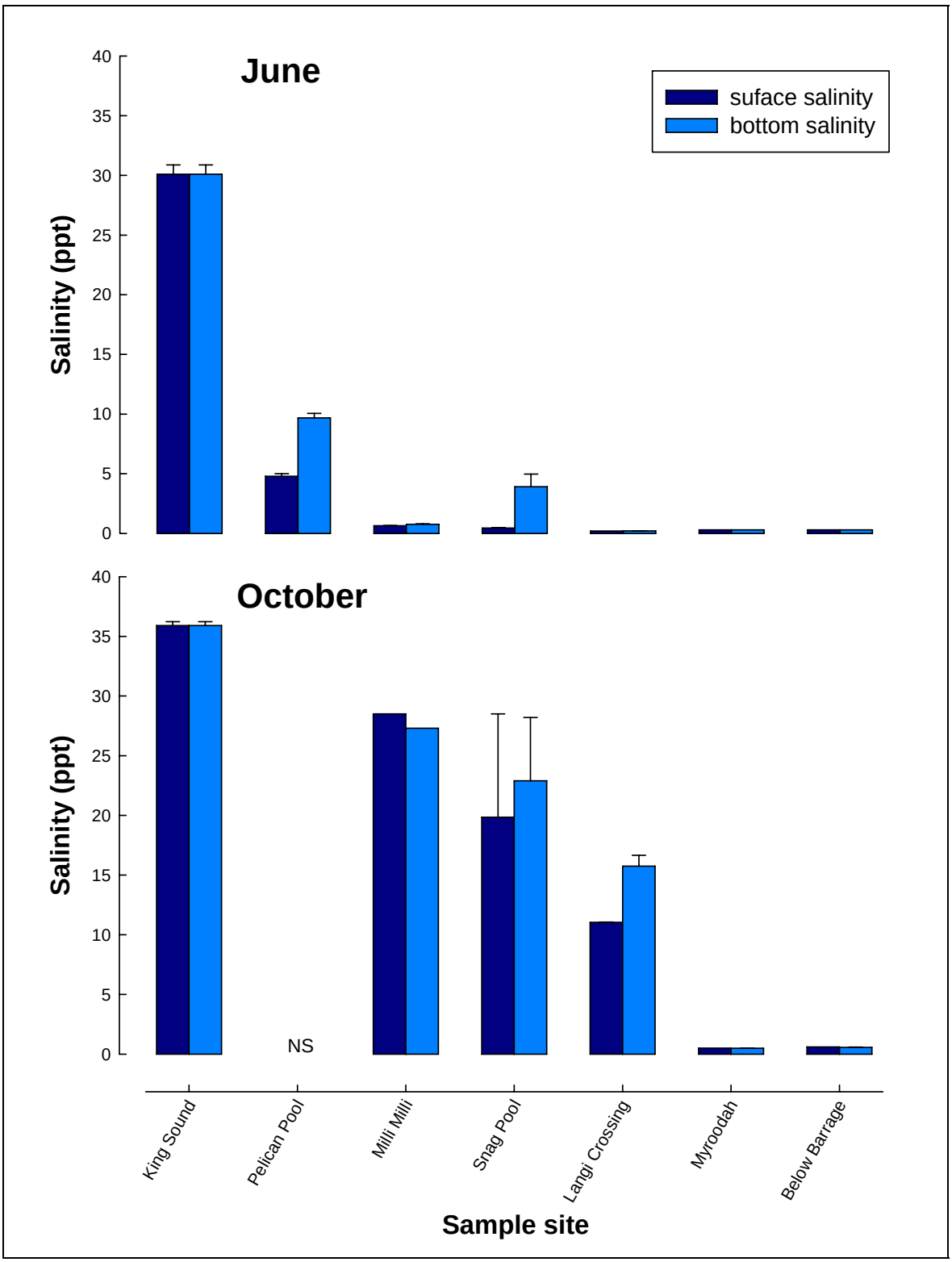


Figure 10 Salinity levels for the sites sampled in King Sound and the Fitzroy River during June and October 2007.

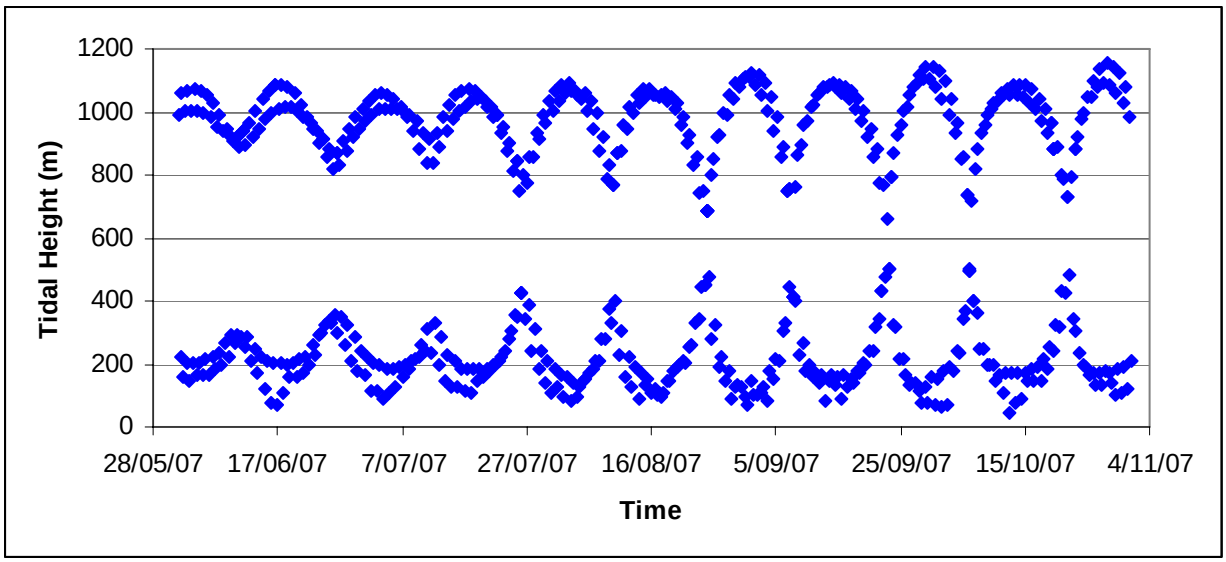


Figure 11 Derby tide data from June to November 2007. Data provided by the Department of Planning and Infrastructure.

Catch composition, size distribution, sex ratios and morphology

Pristis microdon

A total of 38 different *P. microdon* were captured between June and November 2007 in the Fitzroy River and King Sound, with all but three caught in late June/early July (see Table 2). All were captured in the Fitzroy River between Snag Pool and the Camballin Barrage, with the exception of one individual that was captured off of Pt Torment in King Sound in October (Table 2, Figure 12). 32 of the *P. microdon* ranged between 789 and 1111 mm TL and consisted of 16 males and 16 females (Table 2, Figure 13), and are likely to represent the 2007 0+ age class; umbilical scars were observed of many of these. The remaining six ranged between 1555 and 2580 mm TL (three males, three females), with the largest being the individual captured in King Sound. All males had flaccid claspers (indicating immaturity (see Thorburn *et al.* 2007)) with the exception of the largest male found in King Sound whose claspers were semi-calcified. The number of 0+ (i.e. new recruits) *P. microdon* captured during 2007 (see Figure 13), exceeded the combined total of new recruits captured in all years between 2002 and 2006 (Figure 13). However, in contrast to previous years, there were few older fish (i.e. >1 year old) captured in 2007. Linear regression demonstrated that the number of new recruits was highly correlated to discharge in the late wet (April) ($p = 0.002$) (Figures 14 and 15).

While the sex ratio of 0+ individuals was equal, there is a dominance of females in older age classes (~2:1) (Figure 13). Furthermore, all larger individuals greater than 2500 mm TL are almost exclusively female. There are also considerable differences in number of rostral teeth of females compared to males, with females having between 17 and 22 left rostral teeth (mode of 19), compared to males which have 19 to 23 teeth (mode of 20) (Figure 17). There are also differences in rostral teeth counts between *P. microdon* in the Fitzroy River compared to those in the Gulf of Carpentaria (Queensland), where males have up to 24 rostral teeth with a mode of 21 (Figure 17). In terms of overall counts of rostral teeth, females captured in the Fitzroy River since 2002 possessed between 34 and 44 (mode of 36), compared to males which possessed between 38 and 46 (mode of 41) rostral teeth (Figure 18). From rostra donated from private collections however, this range was extended to 47 teeth and was likely from a male (Figure 18).

In Queensland, however, the range of teeth for males was 36 to 47 (mode of 42) and for females it was 34 to 42 (bimodals of 36 and 41) (Figure 19). Variation between left and right teeth counts was similar for both sexes in the Fitzroy River, but differed from Queensland fish in that females in the Fitzroy River demonstrated greater variation (Figures 20 and 21).

The relationships between the TL and RL of female and male *P. microdon* are presented in Figure 16. There was, however, no significant difference between the sexes for this relationship (likelihood-ratio test, $P < 0.05$). The relationship between the TL and RL, combined for the sexes was thus: $RL = 0.2088TL+39.112$, $r^2=0.9757$.

Table 2 Individual *Pristis microdon* captured in the Fitzroy River and King Sound during 2007; including Rototag number, capture date, time and location, sex, total length (TL), rostrum length (RL) and right and left rostral tooth counts.

Rototag	Capture Date	Capture Time	Capture Location	Sex	TL (mm)	RL (mm)	R Tooth Count	L Tooth Count
F9086	22/6/2007	17:15	Snag Pool	F	960	230	18	19
F9836	22/6/2007	17:20	Snag Pool	F	1000	250	18	20
F9835	22/6/2007	19:13	Snag Pool	F	1555	380	18	18
F9838	22/6/2007	19:13	Snag Pool	M	985	240	20	21
F7094	23/6/2007	12:20	Snag Pool	F	983	207	19	20
F8086	23/6/2007	12:20	Snag Pool	M	950	243	22	20
F9810	23/6/2007	12:20	Snag Pool	M	822	207	20	20
F9837	23/6/2007	12:20	Snag Pool	M	1050	250	21	21
F7092	23/6/2007	13:40	Snag Pool	M	1611	390	19	20
F9839	23/6/2007	14:25	Snag Pool	M	950	235	-	-
F9807	23/6/2007	14:45	Snag Pool	M	941	220	19	20
F9808	23/6/2007	15:15	Snag Pool	M	989	248	19	21
F9809	23/6/2007	15:15	Snag Pool	M	825	205	19	20
F9840	23/6/2007	17:30	Snag Pool	F	1060	259	18	18
F9847	23/6/2007	18:30	Snag Pool	M	911	234	22	21
F9849	23/6/2007	18:30	Snag Pool	M	1016	243	21	20
F9848	23/6/2007	20:50	Snag Pool	F	1024	249	19	18
F9850	23/6/2007	20:50	Snag Pool	M	1000	258	22	21
F9841	23/6/2007	22:30	Snag Pool	F	846	218	17	18
F9826	24/6/2007	21:47	Snag Pool	F	933	233	19	17
F9842	24/6/2007	14:00	Snag Pool	M	941	239	20	19
F7096	29/6/2007	9:00	Barrage	M	935	233	21	21
F9843	30/6/2007	16:40	Barrage	F	900	225	18	18
F9834	3/7/2007	6:10	Snag Pool	F	886	229	20	20
F9844	3/7/2007	6:10	Snag Pool	F	914	243	18	19
F4830	3/7/2007	17:22	Snag Pool	F	1780	408	19	17
F9831	3/7/2007	19:20	Snag Pool	F	885	221	19	19
F9832	3/7/2007	19:20	Snag Pool	F	789	202	18	18
F7093	3/7/2007	19:20	Snag Pool	M	936	217	21	20
F9833	3/7/2007	19:20	Snag Pool	M	1070	258	21	21
M1013	18/7/2007	21:00	Snag Pool	M	1580	375	21	20
M1007	18/7/2007	23:40	Snag Pool	F	842	268	18	19
M1003	19/7/2007	5:50	Snag Pool	F	981	247	19	19
M1006	19/7/2007	5:50	Snag Pool	F	990	235	17	17
M1005	19/7/2007	5:50	Snag Pool	M	854	224	21	20
NA	24/10/2007	20:30	Pt Torment	M	2580	605	-	-
M1004	1/11/2007	20:00	Barrage	F	1111	267	18	19
NA	13/11/2007	21:00	Langi Crossing	F	1576	394	18	19

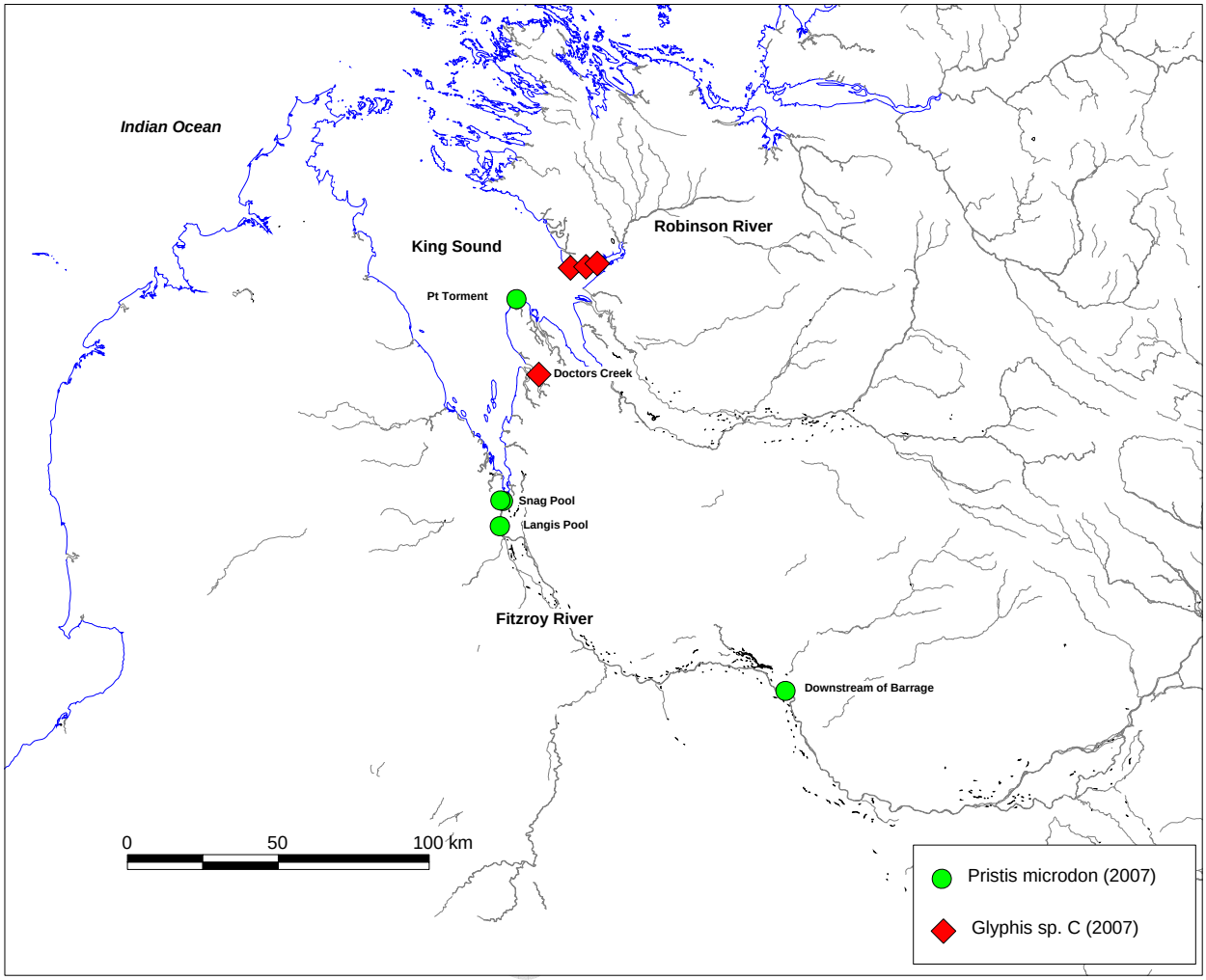


Figure 12 Capture locations of *Pristis microdon* and *Glyphis* sp. C during 2007.

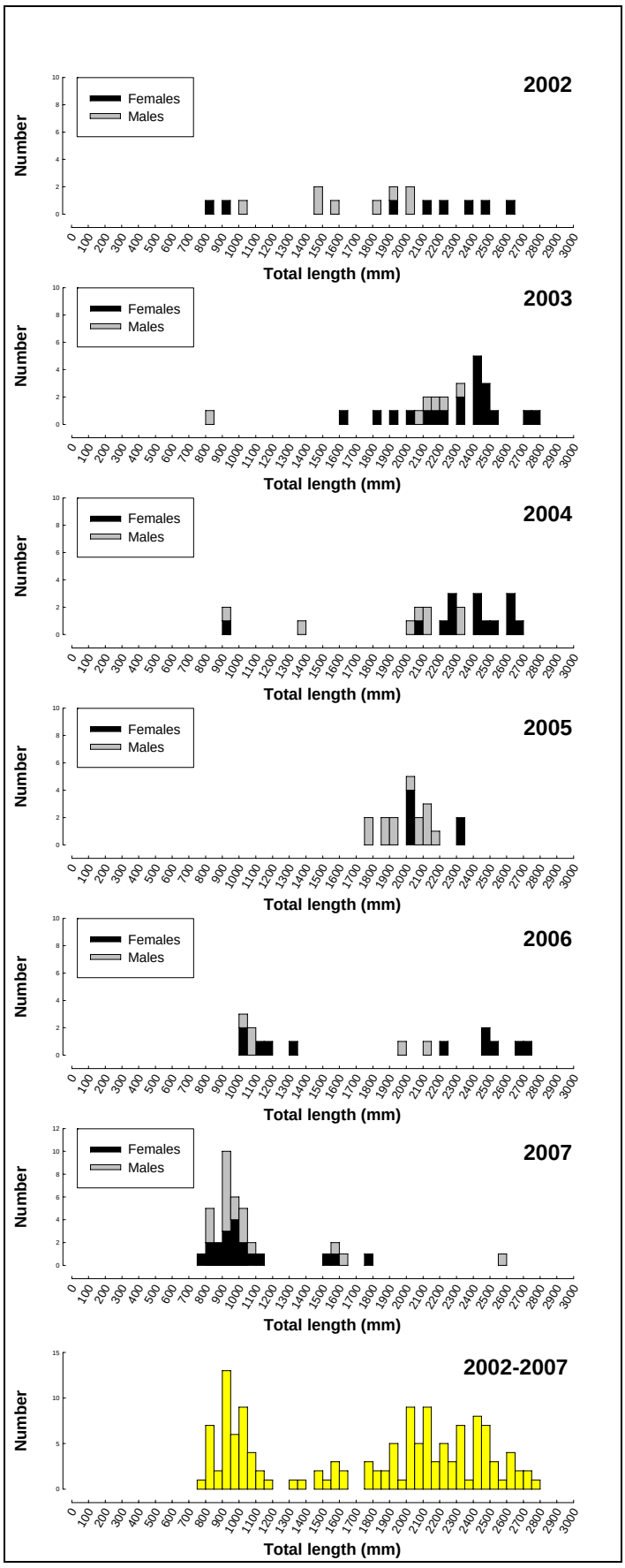


Figure 13 Length-frequency histograms of *Pristis microdon* captured in the Fitzroy River and King Sound between 2002 and 2007. N.B. Most captured since 2003 have been rototagged.

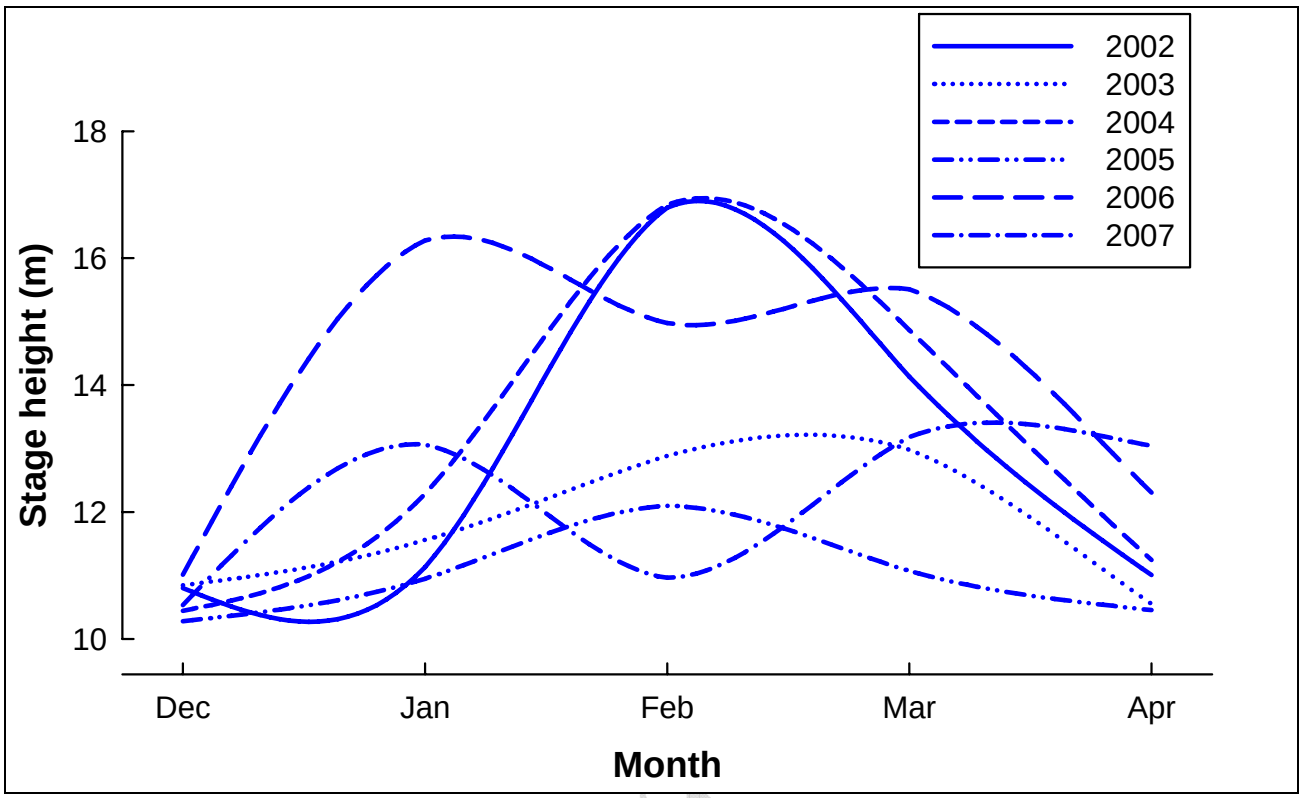


Figure 14 Mean monthly stage heights (m) for the lower Fitzroy River during the wet season (December to April) between 2002 and 2007.

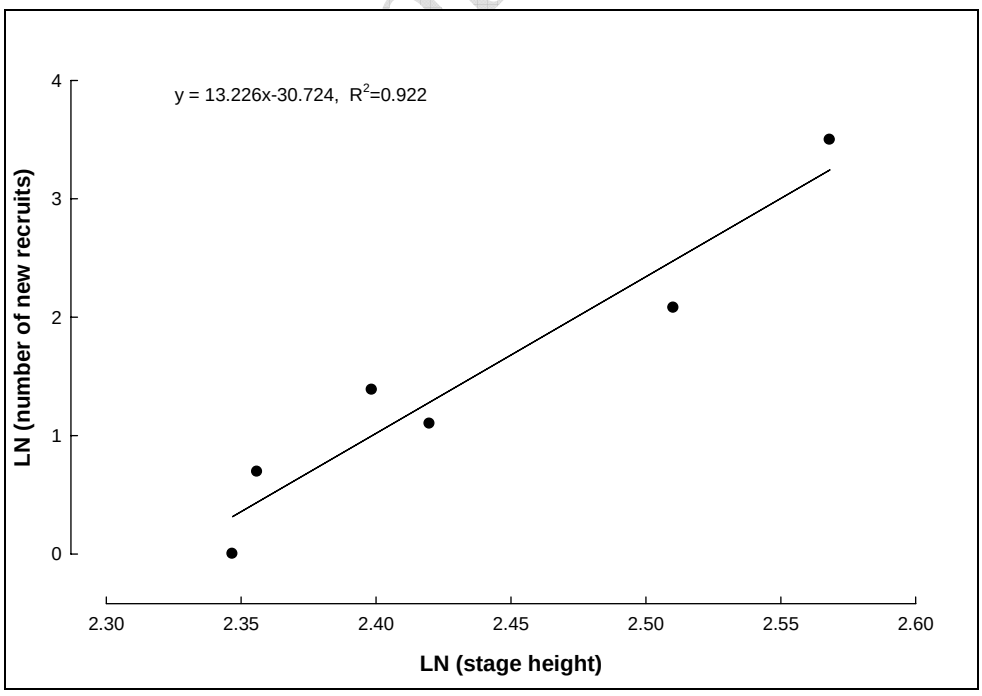


Figure 15 Natural log of late wet mean (April) discharge compared to natural log of the number of new recruits in each year between 2002 and 2007.

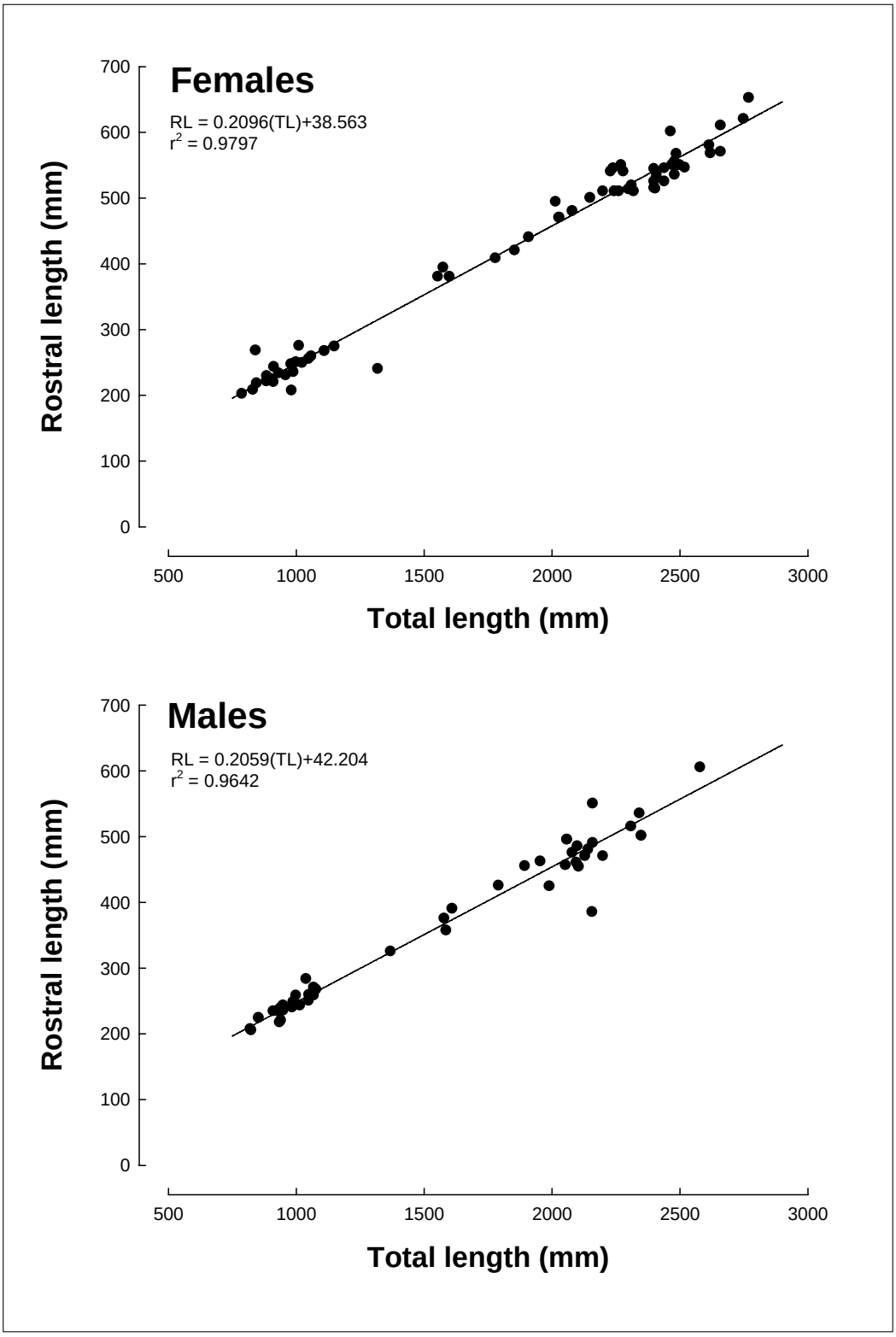


Figure 16 Relationship between rostrum length and total length of male and female *Pristis microdon* captured in the Fitzroy River.

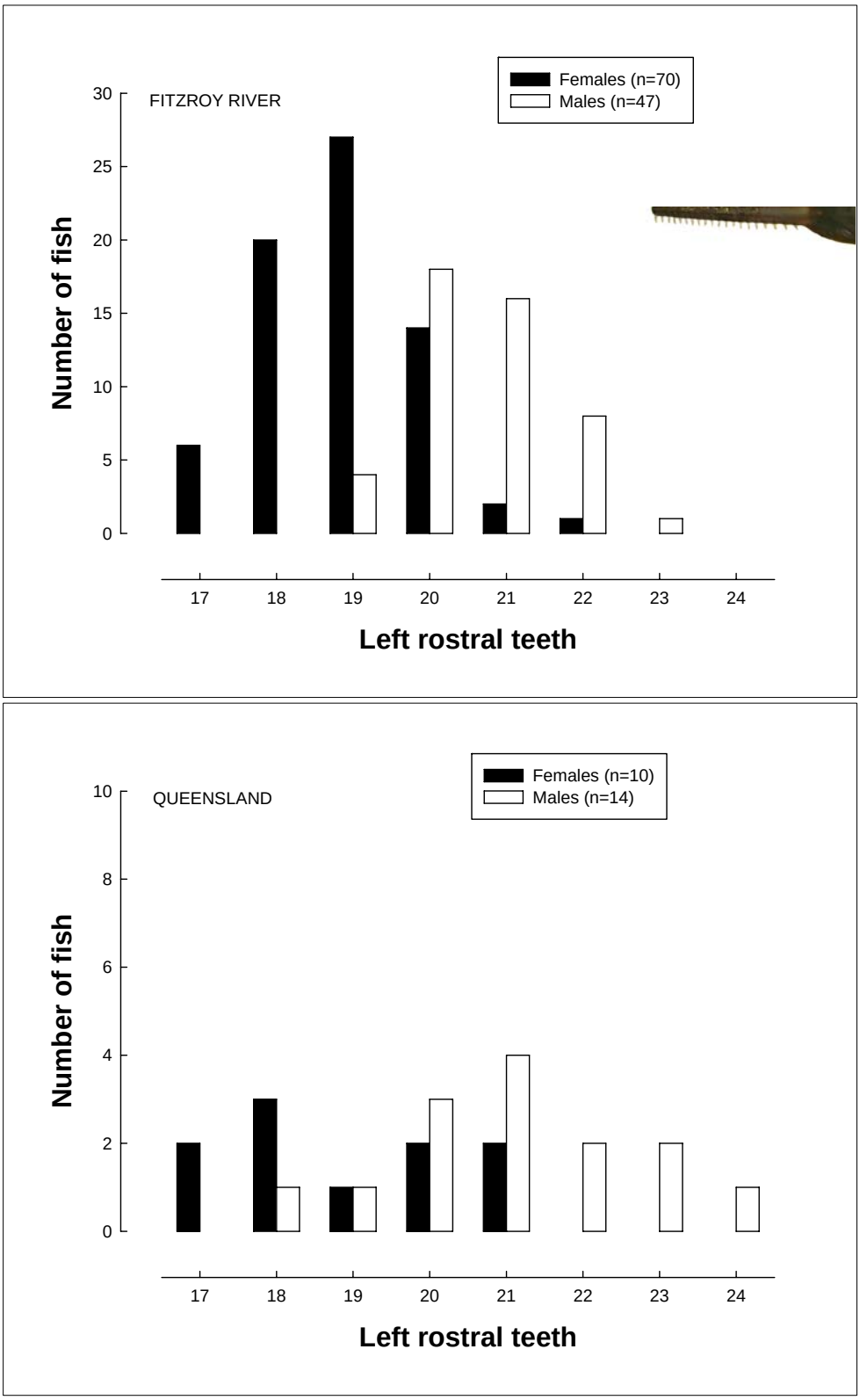


Figure 17 Left rostral teeth counts for male and female *Pristis microdon* captured in the Fitzroy River (top), compared to those from Queensland (bottom).

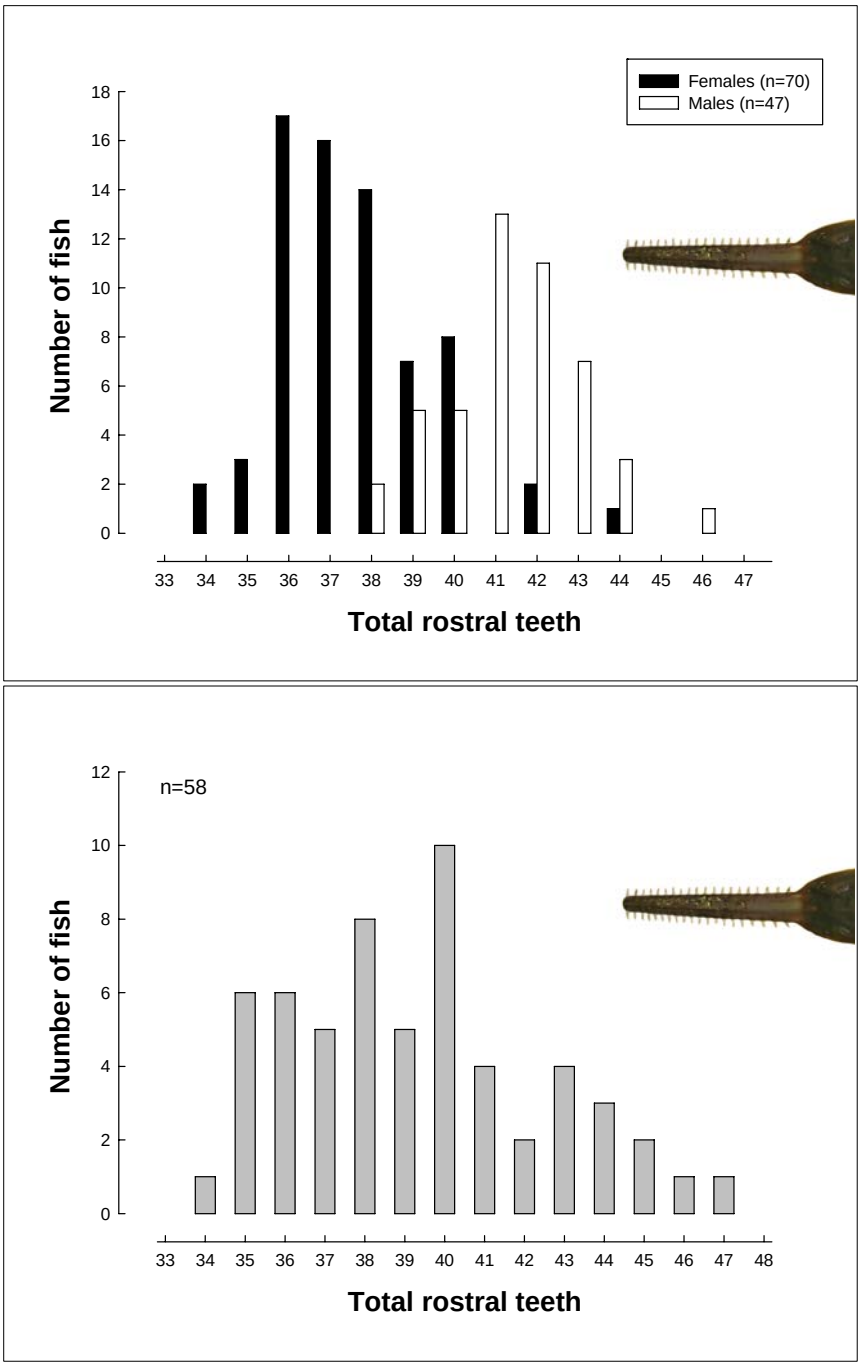


Figure 18 Total number of rostral teeth for male and female *Pristis microdon* captured in the Fitzroy River (top), compared to those from private collections that were donated during the study and were thus not able to be accurately sexed.

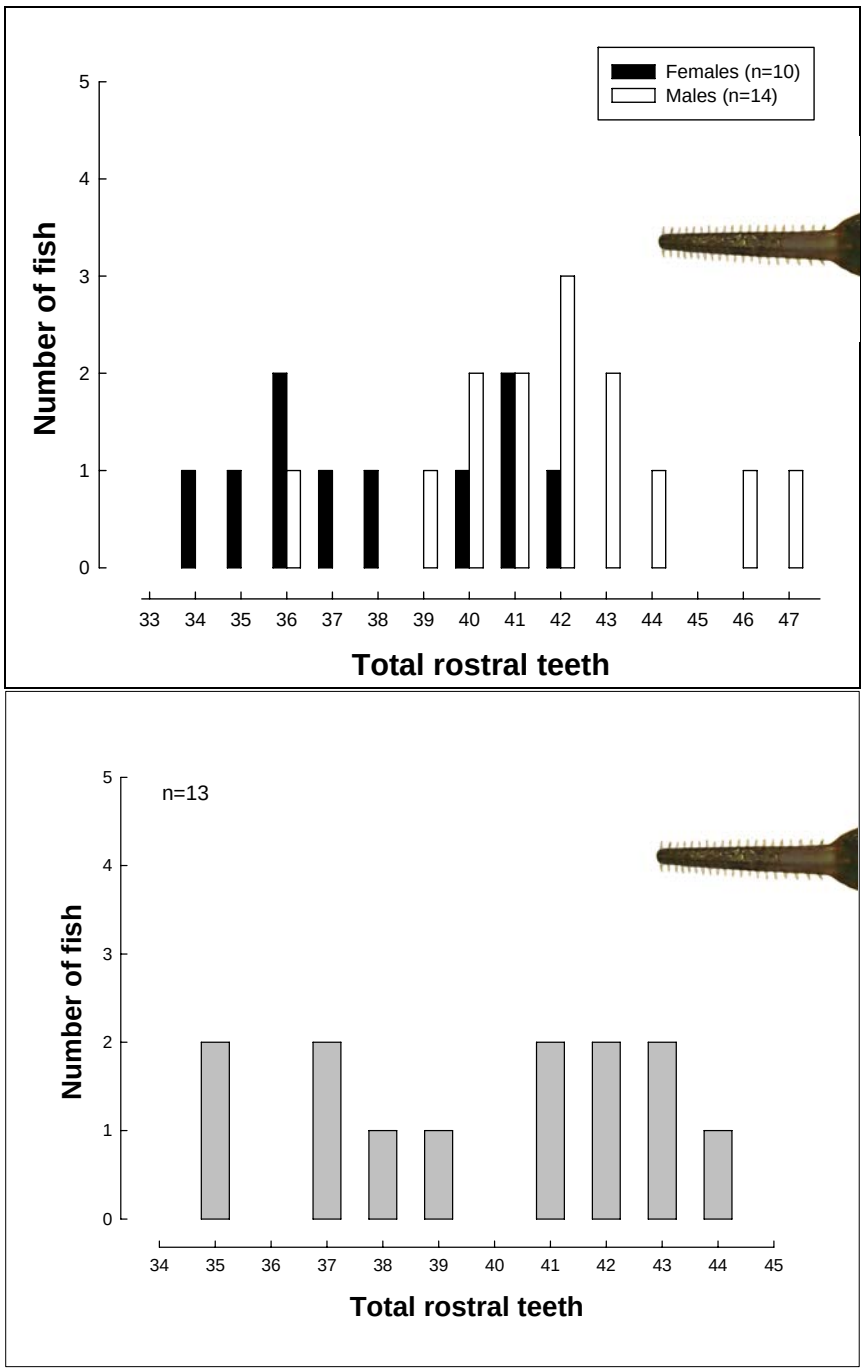
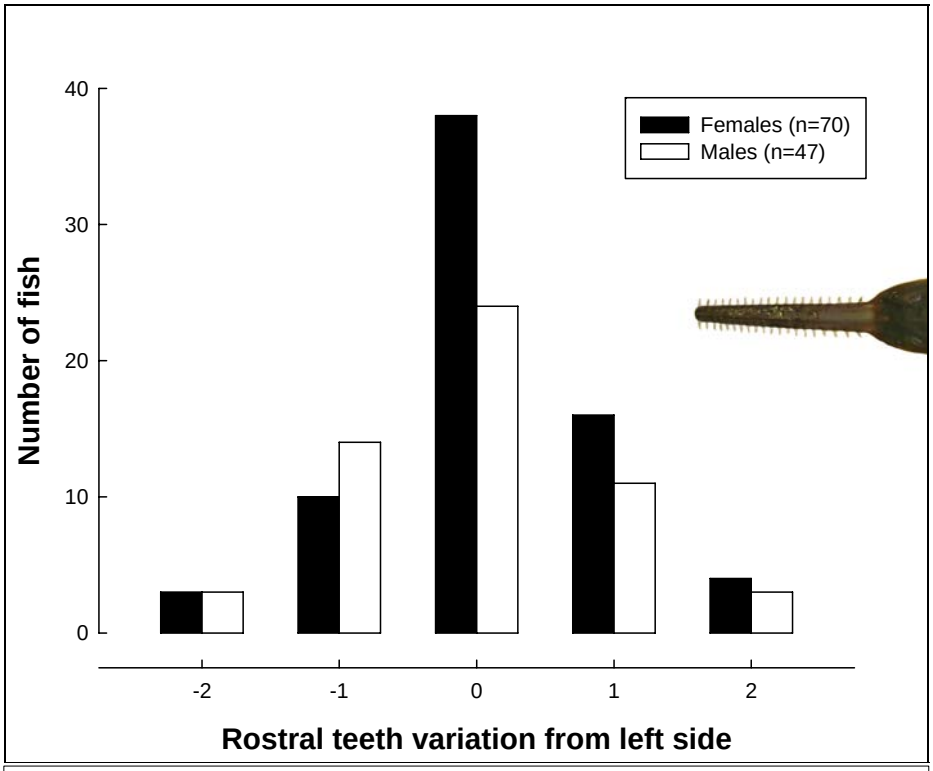


Figure 19 Total number of rostral teeth for male and female *Pristis microdon* captured in Queensland (top), compared to those of unsexed fish.



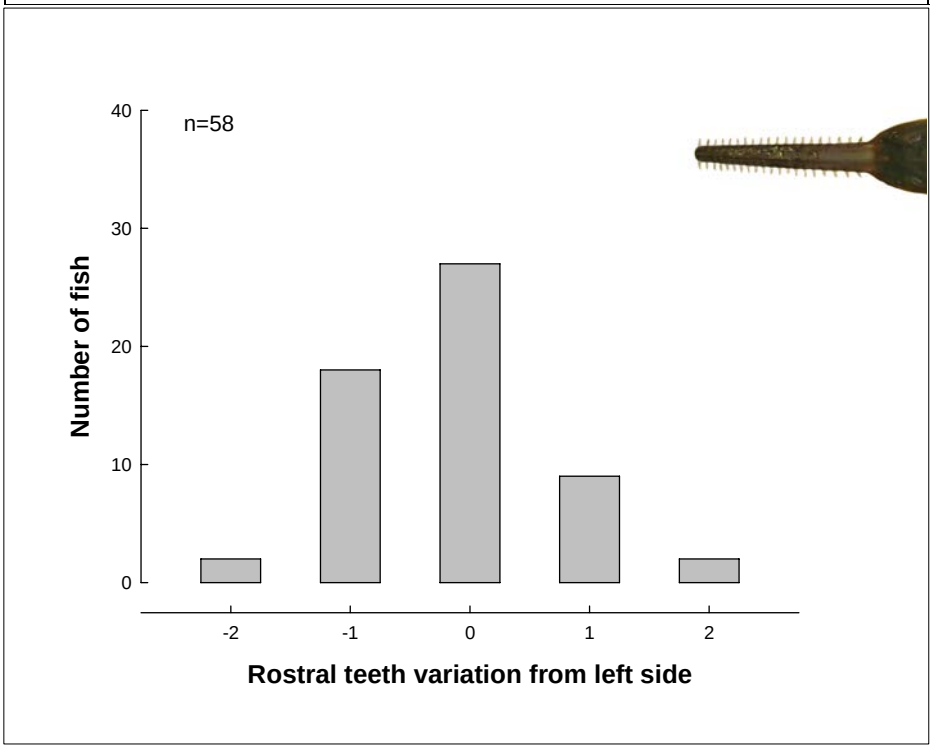


Figure 20 Variation in rostral teeth count from left side of sexed fish (top) and from rostra donated from private collections from the Fitzroy River.

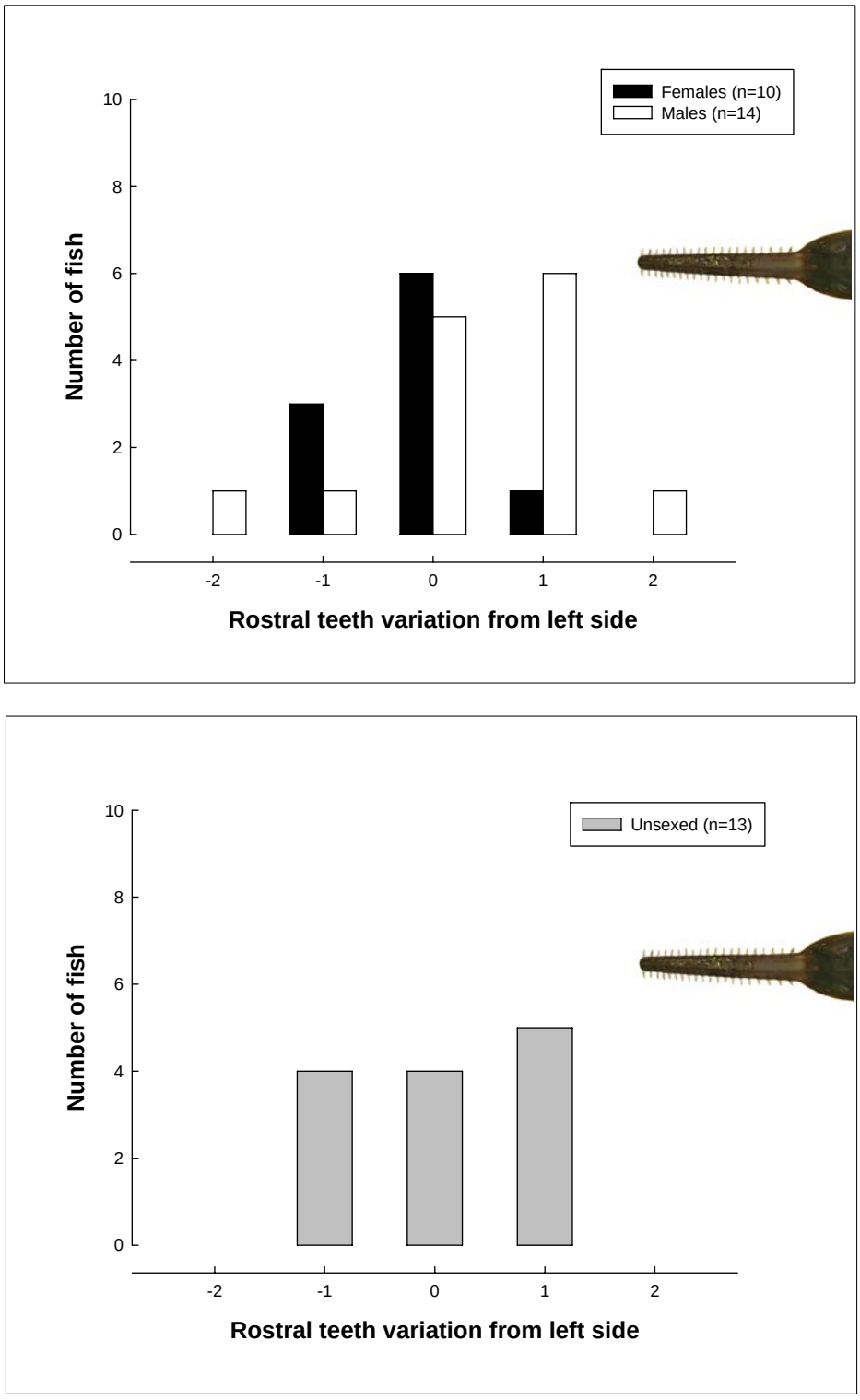


Figure 21 Variation in rostral teeth count from left side of sexed fish (top) and from rostra donated from unsexed fish from Queensland.

Glyphis sp. C

Six *Glyphis* sp. C were captured in creeks and estuaries of King Sound from Doctors Creek to the north-eastern side of the Robinson River estuary (approximately 100 km of coastline in King sound) between June and October 2007 (see Table 3, (see Figures 4, 6 and 12)). The capture times were generally early morning (07:00 to10:00, 4 individuals) or late at night (22:00 to

00:00). The depths at which these fish were caught ranged from 1.1 to 6 m (mean = 3 m; SE ± 1.10); water temperature ranged from 21.0 to 29.5°C (mean = 25.6°C; SE ± 1.47); and, salinity ranged from 32 to 36.8 ppt (mean = 35.8 ppt; SE ± 0.77). Two *Glyphis* sp. C were captured on the outgoing tide while four were captured on the incoming tide.

Lengths of these individuals ranged from 1000 to 1365 mm TL. The 1365 mm individual was male with fully calcified claspers, indicating that this species had attained sexual maturity (see Thorburn & Morgan 2004).

The ratio of 2nd dorsal fin height to 1st dorsal height of two individuals (1088 and 1237 mm TL) was 0.57-0.67.

Table 3 Capture details, TL, and sex of the *Glyphis* sp. C captured in King Sound during 2007.

Date	Time	Location	TL (mm)	Sex
19/6/07	9:45	Doctors Creek	1365	M
19/10/07	7:00	Robinson River mouth	1084	F
20/10/07	22:30	Stokes Bay Creek	~1000	U
21/10/07	23:20	Robinson River mouth	1088	M
22/10/07	9:45	Robinson River mouth	1298	F
23/10/07	23:30	lower May River	1237	F

Rototagging *Pristis microdon*

Between 2003 and 2007 24 individuals of the 91 *P. microdon* tagged with Rototags were recaptured, two in 2003, four in 2004 and 2005, five in 2006, and 26 in 2007. Of 39 recaptures, which does not including two individual whose tags were shed soon after attachment (captured in 2007), one individual has been recaptured four times, three captured three times, six others twice, and the remaining fourteen once. Of these 25 were recaptured less than 48 hours after previous capture, and 32 were recaptured within a month. The remaining six recaptures were at liberty (between initial capture and final recapture) for 83, 105, 140, 308, 481 and 1,010 days. All animals were recaptured near the site of initial capture with exception of the individual at liberty for 1,010 days; which was initially captured in the pool ~1 km downstream of the Barrage and was recaptured at Snag Pool ~150 km downstream. This individual was a female of 2330 mm TL when first captured and almost three years later had grown only by 220 mm TL. The individual at liberty for 481 days grew by 82 mm TL (2262 mm TL v 2344 mm TL), while the individual at liberty for 140 days grew by 30 mm TL (2150 mm TL vs 2180 mm TL).

Based on 25 tagged individual 0+ *P. microdon* and their recapture in Snag Pool over five sampling days/nights between 22nd June and 4th July 2007, a population estimate using the Peterson method indicated that the pool contained ~51 0+ individuals. This information is based on a total of 47 captures, including 21 recaptures.

Acoustic tracking of *Pristis microdon*

Acoustic array

Acoustic tag transmission data was able to be successfully retrieved from all receivers with the exception of the receiver at lower Pelican Pool which shifted and became buried. The Lower

Pelican receiver and data logger was inaccessible for downloading in October/November as it had become buried by 2 m of sand. Receivers appeared to have been left undisturbed with the exception of bullet holes in the surface floats at Myroodah and the large pool downstream of the Barrage. Due to the holes surface floats sank, however the subsurface floats remained intact and kept the moorings upright. Surface floats were replaced with foam floats when possible to reduce the chance of gun shots sinking floats. Biofouling was minimal and consisted of only of a thin film of algae that was easily removed.

Range testing

Range test results during the dry season in the Fitzroy demonstrated, on average, a detection radius of 246 m (SE ± 33) and 183 m (SE ± 88), based on a 95% detection limit (furthest distance that the receiver detects 95% of the test-tags transmissions) for the V16 and V13 acoustic tags, respectively. Range tests comparing V13 transmitters with and without silicone, showed that the silicone had no significant effect on their signal strength (t-test p =0.07). Both recorded up to 350 m (83 and 100% of transmissions received with and without silicone, respectively) in Langi Crossing, but neither transmitted a signal that was received beyond this point.

Acoustic tag transmissions

All animals tagged with either V16 or V13 acoustic tags prior to October 2007 successfully transmitted data to the deployed receivers (Table 4, Figures 22 and 23). The number of transmissions for the larger fish (1+) that were fitted with V16 tags ranged from 5842 to 14366 (mean = 9449), while the number of transmissions of the 0+ fish fitted with V13 tags ranged from 958 to 4294 (mean = 2047) (Table 4). The duration of transmissions varied from 25-75 days. Final transmissions for all seven animals were between 30th July and 2 October, with four of the seven between 13th and 21st of August. Several animals moved between Milli Milli Pool and Snag Pool, though only one individual was detected moving upstream to Langi Crossing (Figure 22). Individuals also moved out of detection range for short periods of time (days), although a few individuals had one or two periods that they were gone for two to four weeks. The six transmissions at Langi Crossing were the last detected transmissions for that animal. No animal was detected at Myroodah or the large pool below the Barrage.

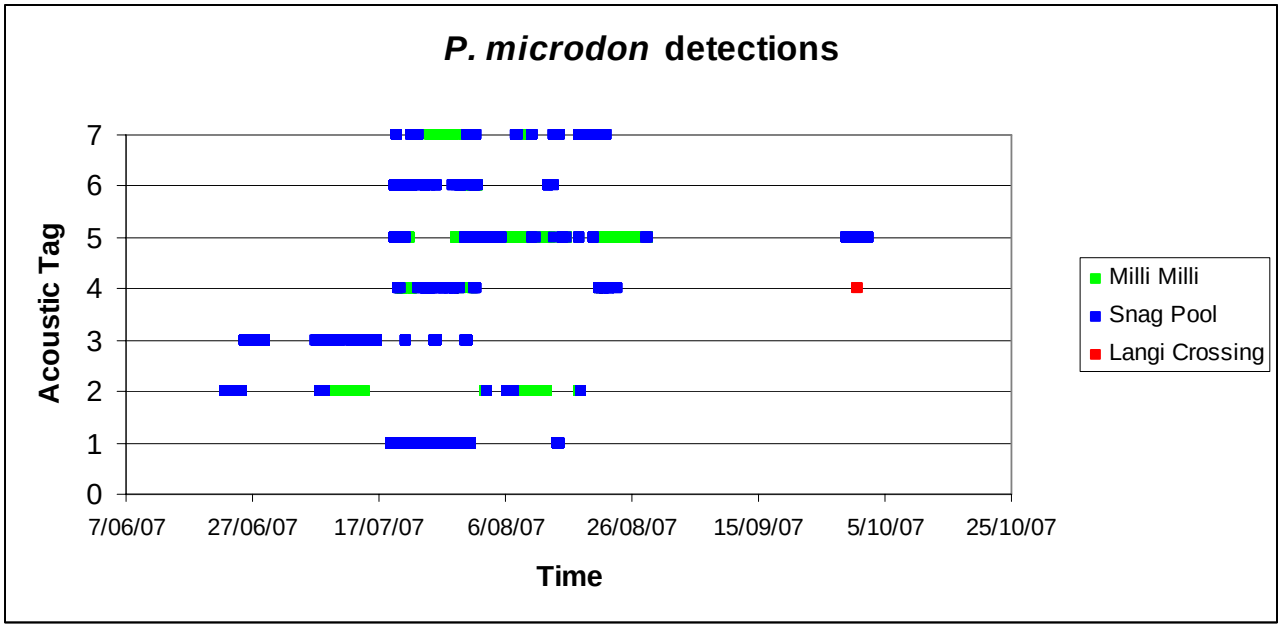


Figure 22 *Pristis microdon* detections at monitored sites. Acoustic tag 1 = 1038503, 2 =1038505, 3 = 1038506, 4 = 1042224, 5 = 1042226, 6 = 1042227, 7 = 1042229.

Table 4 Acoustic tracking details of *Pristis microdon* in Fitzroy River; transmissions day^{-1} are calculated only from days that at least one transmission was received. * denotes that these individuals were tagged after last receiver download event.

Acoustic #	Last Transmission Location	Last Transmission Date	Total Transmissions	Transmissions day^{-1}
1038505 (V16)	Snag Pool	17-Aug-2007	14366	1197
1038506 (V16)	Snag Pool	30-Jul-2007	5482	261
1038503 (V16)	Snag Pool	14-Aug-2007	8498	566
1042224 (V13)	Langi	30-Sep-2007	1081	60
1042227 (V13)	Snag Pool	13-Aug-2007	958	60
1042226 (V13)	Snag Pool	2-Oct-2007	4294	126
1042229 (V13)	Snag Pool	21-Aug-2007	1854	81
1042225 (V13) *	NA	NA	0	0
1038502* (V13) *	NA	NA	0	0

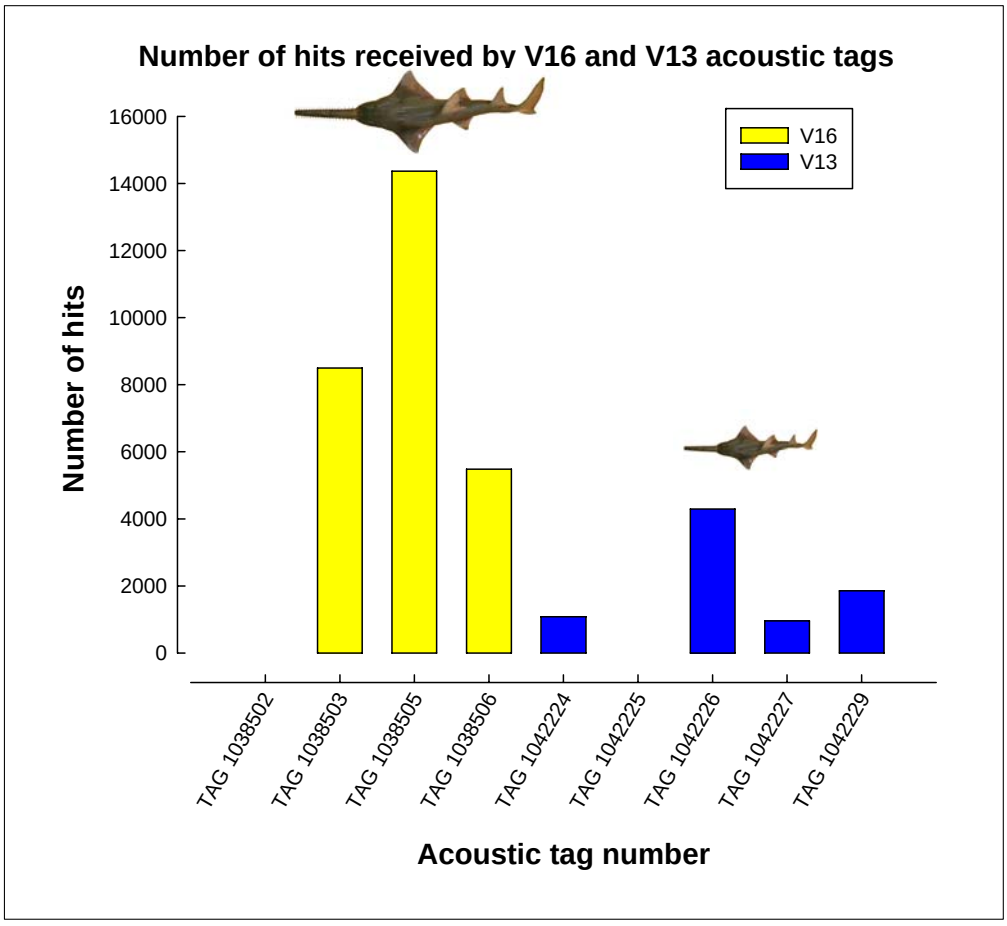


Figure 23 Total number of transmissions received from tagged *Pristis microdon* in the Fitzroy River. N.B. V16 tags were set to transmit twice as often as V13.

Movement patterns and habitat associations

Movements of the 1+ year old individuals between Milli Milli and Snag Pool were observed to occur only during large tides (8.9+ m). These movements were multidirectional with 50% of individuals moving upstream with the tide and 50% moving downstream into the tide. In contrast to the 1+ *P. microdon*, the 0+ fish moved between pools regardless of tidal height. However, during incoming tides the migration of the 0+ fish was in an upstream direction on all (97.9%) but one occasion, i.e. they moved with the tide.

There were considerable differences in the habitat (depth) associations of the 0+ versus 1+ individuals (Figures 24, 25 and 26), with the younger (and thus smaller) *P. microdon* being almost entirely, on average, restricted to depths of <1m, and generally moved into very shallow waters at noon and during the early afternoon. In contrast, the 1+ individuals generally maintained a deeper depth than the 0+ individuals during the day, before moving in waters of 0.6 to 0.8 m during the afternoon, where they remained until dawn.

The movement patterns of the 1+ and 0+ *P. microdon* were also demonstrated to respond differently to each other depending of the phase of the lunar cycle (Figure 27). For example, the 0+ fish did not appear to alter their behaviour (depth) during the different phases of the lunar cycle, where they spent ~30-40% of their time in shallow waters <0.2 m. In contrast, the larger 1+ fish only generally utilised these very shallow waters (<0.2 m) during the full moon. The depth range of the 1+ fish during the full moon was also more restricted, with fish generally remaining in depths above 1.6 m. However, these fish never ventured into the shallows during the new moon and rarely did so during the half moon. During the new and half moons the depth range of 1+ fish was also considerably greater than during the full moon. Interesting to note these patterns occurred during daylight hours as well a night.

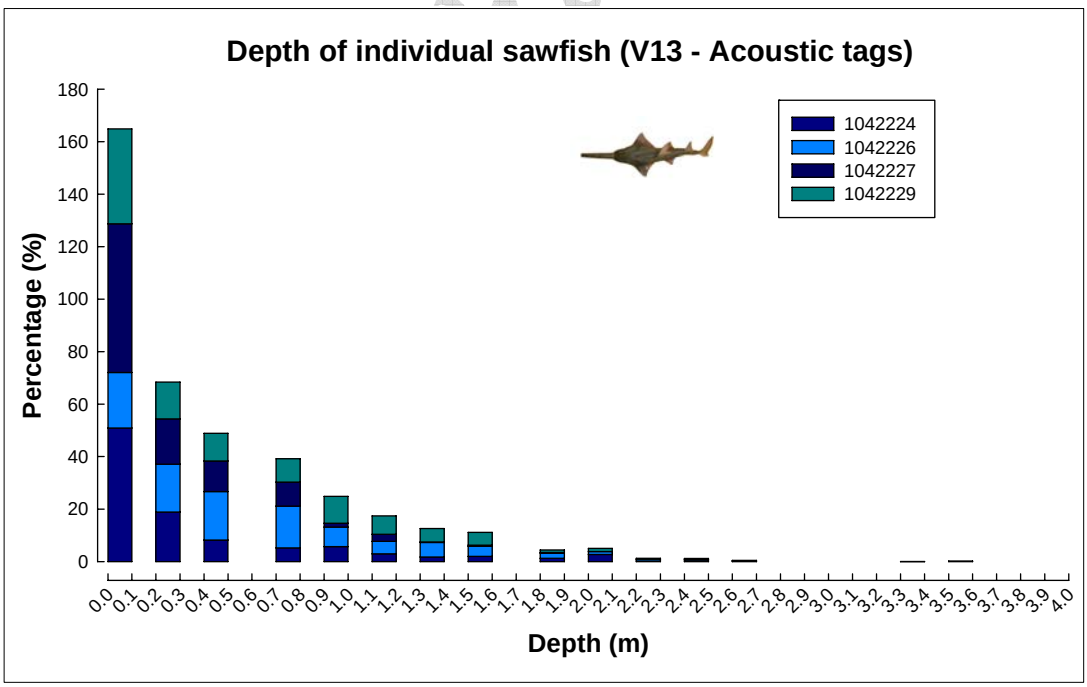


Figure 24 Percentage of time spent at the different depths of individual 0+ *Pristis microdon* tagged with V13s in the Fitzroy River.

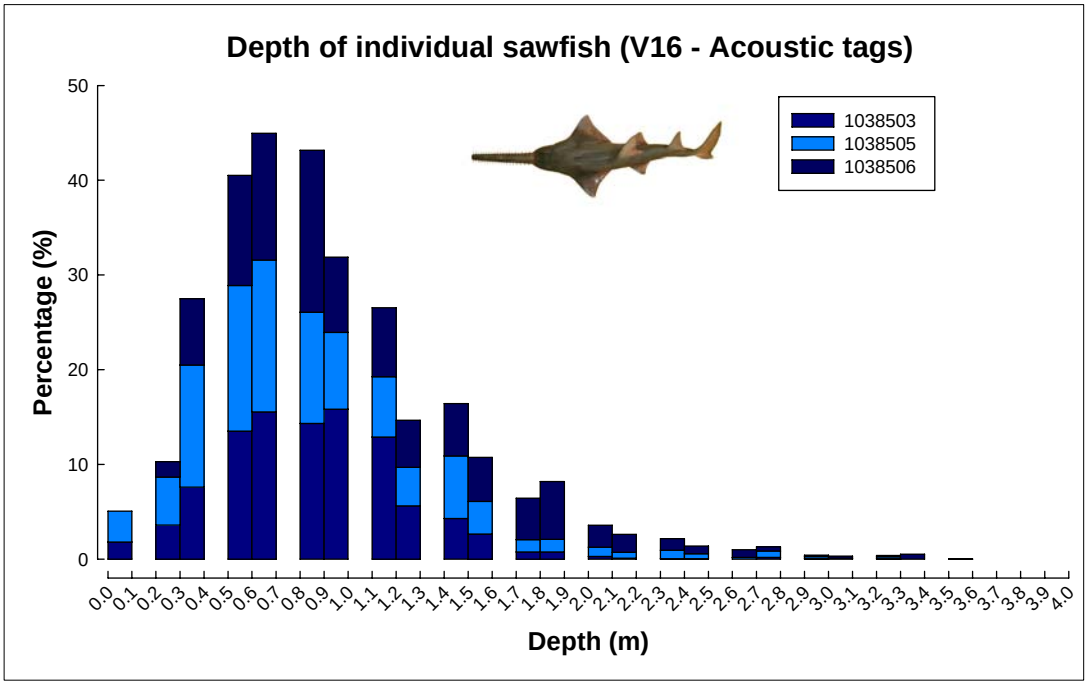


Figure 25 Percentage of time spent at the different depths of individual 1+ *Pristis microdon* tagged with V16s in the Fitzroy River.

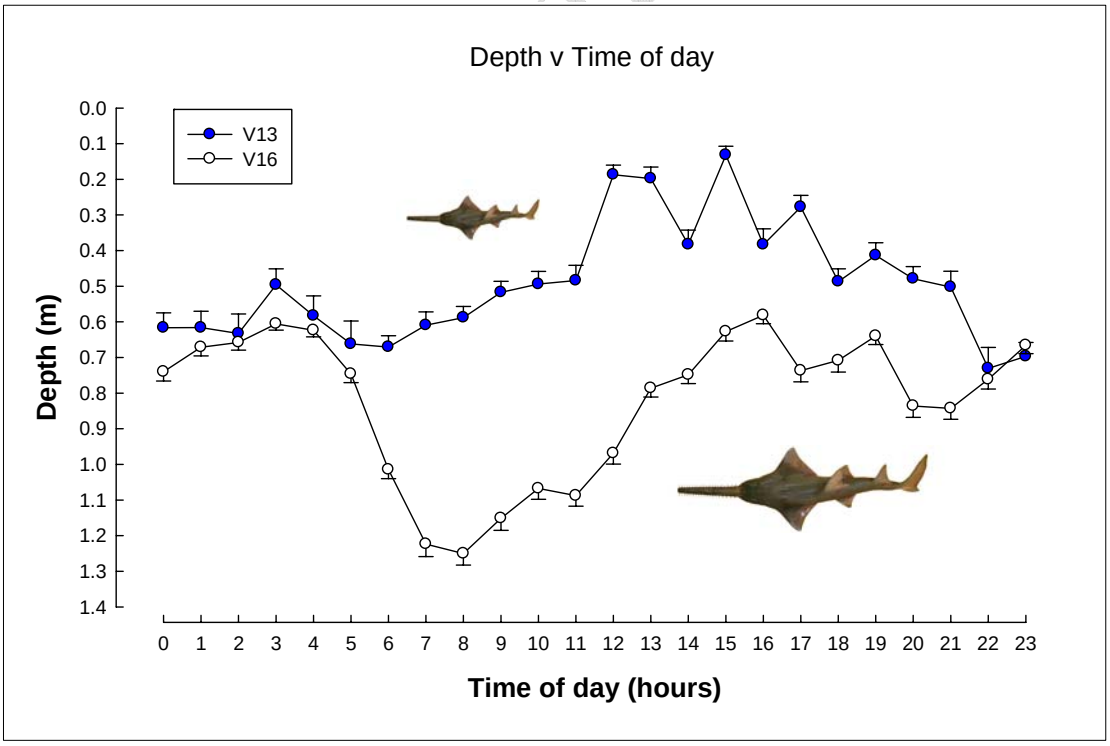


Figure 26 Diurnal habitat utilisation (depths) of the 0+ (V13) and 1+ (V16) *Pristis microdon* at in the Fitzroy River.

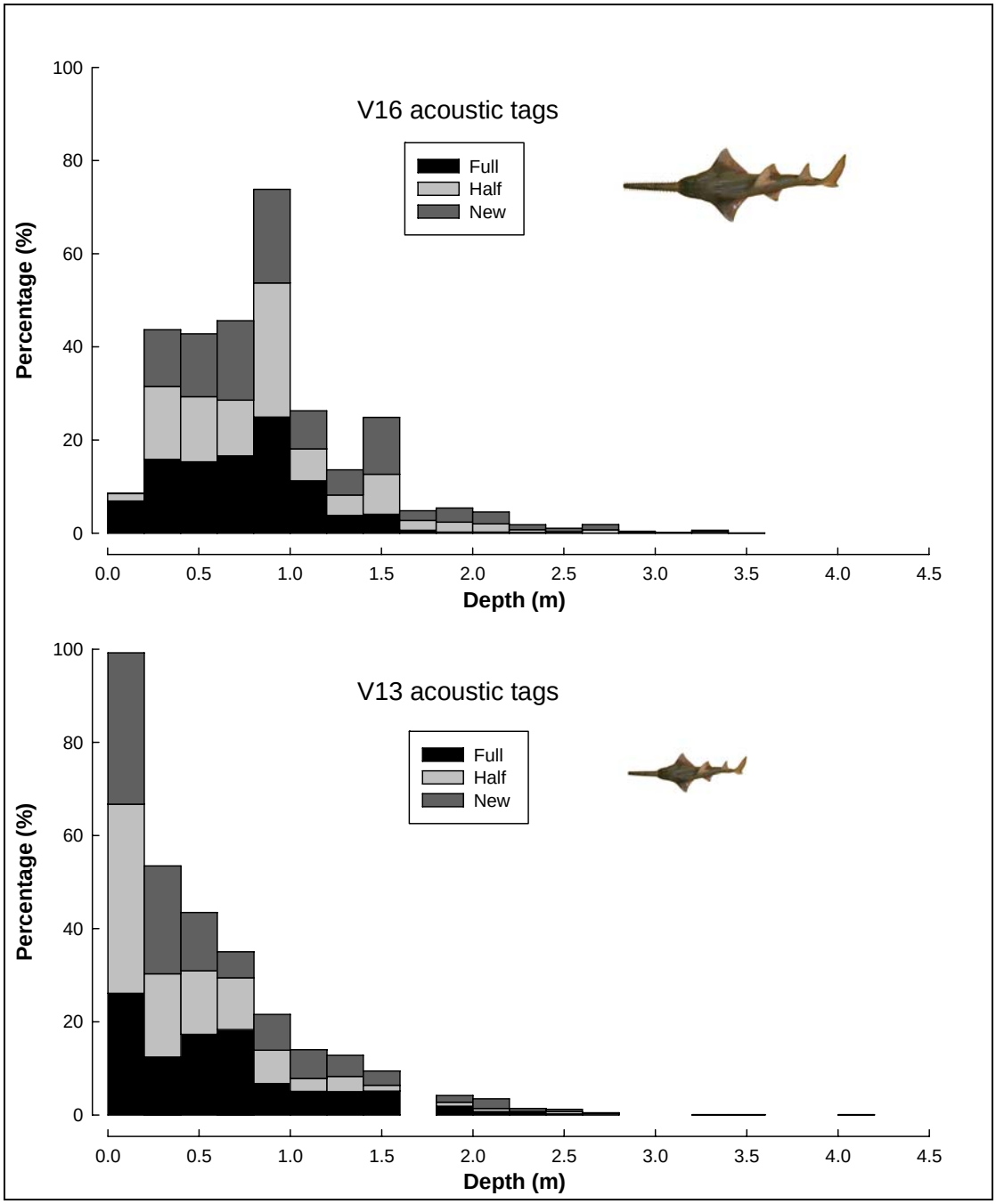


Figure 27 Habitat (depth) utilisation for *Pristis microdon* in relation to the phase of the lunar cycle.

Satellite Tracking

Pristis microdon

SPOT tag #41104 was deployed on a *P. microdon* in Snag Pool in the Fitzroy River (Figure 6). Since the time of release only two transmissions (2/9/07 and 12/11/07) have been received. Insufficient transmissions were collected to calculate a position for this animal. SPOT tag #41109 was deployed on a *P. microdon* off Point Torment in King Sound (Figures 6 and 28). Since the time of release 10 transmissions have been received and have provided three approximate locations. Each transmission was on the day of release.

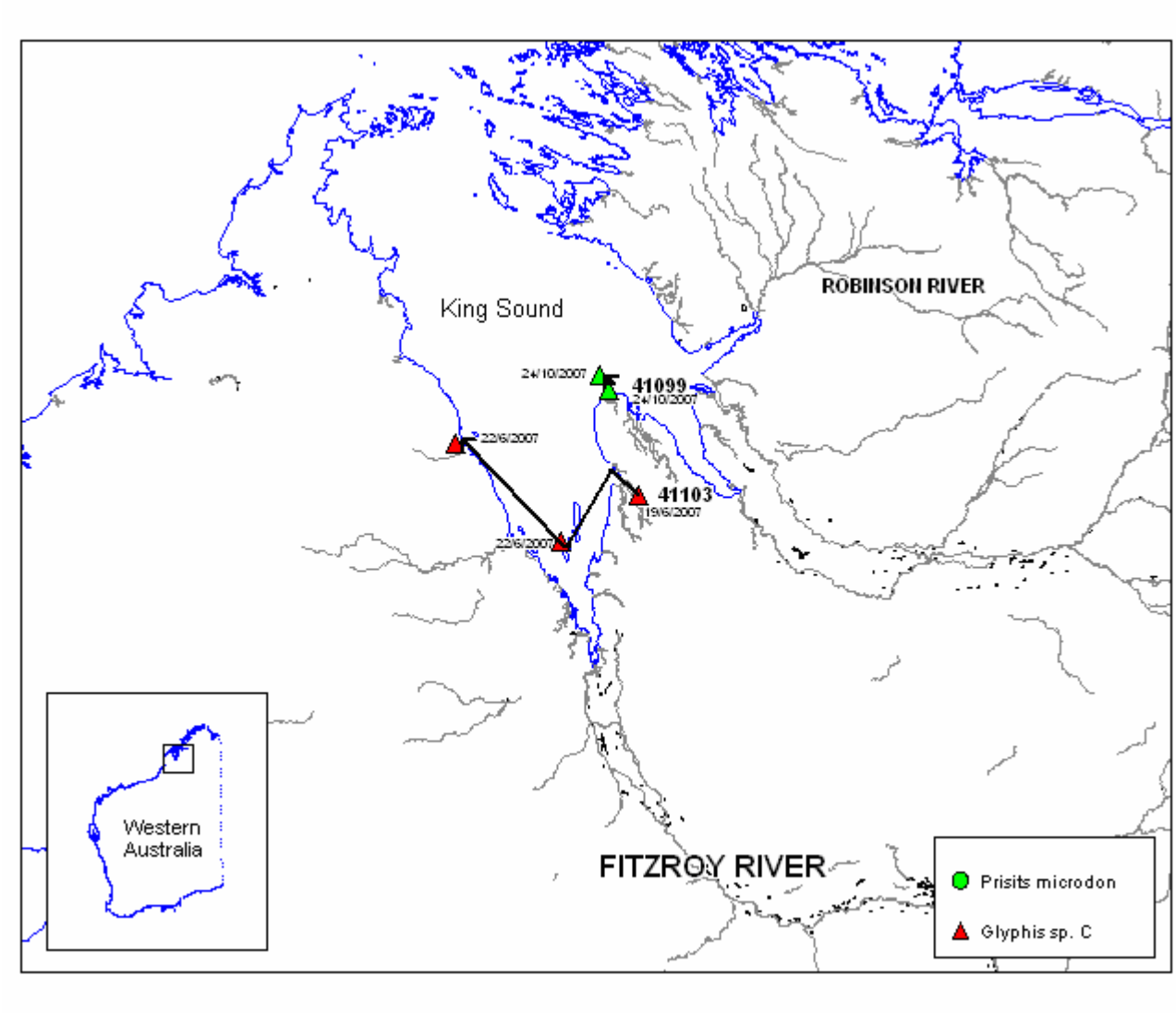


Figure 28 SPOT tag transmissions of *Pristis microdon* and *Glyphis* sp. C.

Glyphis sp. C

SPOT tag #41103 was the only SPOT tag deployed on *Glyphis* sp. C that successfully transmitted (Figure 28). Within the first week after deployment the location of the fish was calculated twice. As these transmissions were limited (two transmissions per satellite pass) only a location with a large possible error was given. A further nine transmissions were also received between 4/08/07 and 26/10/07. None has been received since. Of the 13 transmissions, nine were between 01:00 and 04:00, two were between 13:00 and 15:00 and two were between 19:00 and 21:00. Eleven of the transmissions were on the incoming tide.

Prey species in the riverine pools

Seine netting during the night and day to determine the potential prey species that utilise the shallows of the riverine pools in the Fitzroy River revealed that the diversity of potential prey in the shallows at night is considerably higher than during the day, i.e. 16 versus 10 species recorded (Figure 29). The cherabin, *Macrobrachium rosenbergii* was only captured during the night, as were the three plotosids (*Anodontiglanis dahlia*, *Neosilurus ater* and *Neosilurus hyrtlii*), the rainbowfish (*Melanotaenia australis*), Mouth Almighty (*Glossamia aprion*), Greenway's Grunter (*Hannia greenwayi*), the mugilid (*Liza alata*) and the Oxeye Herring

(*Megalops cyprinoides*). The abundances of other species, e.g. *Nematalosa erebi*, *Arius graeffei*, *Leiopotherapon unicolor* and *Glossogobius giuris* were also greater during the night compared to the day. ANOSIM suggested that there were significant differences ($p = 0.013$) between the fish fauna captured in the shallows at night compared to the day.

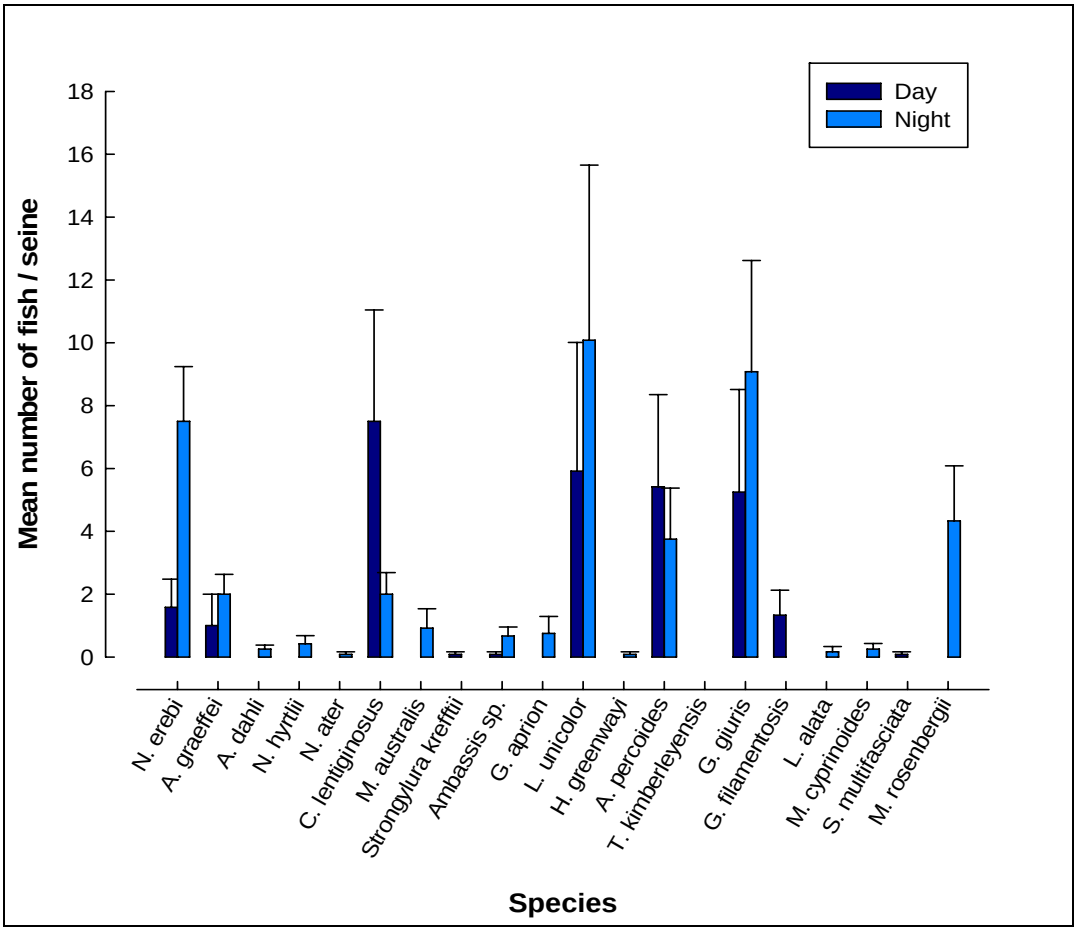


Figure 29 Abundance of potential prey species captured during the day and night in the riverine pools of the Fitzroy River during the dry season.

DISCUSSION

The ongoing monitoring and addition of passive tracking techniques (i.e. acoustic tracking) of *P. microdon* and *Glyphis* sp. C in the Fitzroy River and King Sound is providing important information on the morphology, biology, habitat associations and migration patterns of these critically endangered species.

Pristis microdon

The Fitzroy River is an important nursery for *P. microdon*, with all individuals recorded from the river being immature. There are few records of mature individuals within Western Australia, and there is also an absence of information on pupping grounds and maturity data for the species. However, the presence of a large number of small new recruits with umbilical scars that were captured in the estuary during June 2007 suggests that the mature females may pup in the vicinity of the river mouth in the late wet. During this study it was demonstrated that the

number of new recruits captured in the dry season of each year is significantly correlated to higher water levels during the late wet (i.e. April). Pupping during the late wet would be a strategy that ensures the larger adults have sufficient water levels to move into a river system that, for most of the year (even during the early wet season in many years) may consist of shallow expanses between pools. It would reduce the potential for adults becoming stranded, and the higher water levels would reduce the degree of predation on their offspring. Thus, a later or longer wet season would not only increase the available habitat and reduce the levels of predation, but would also allow small individuals to migrate upstream into freshwater for a protracted period. The level of freshwater flowing into estuaries has previously been correlated with an increase in recruitment of other northern Australia species, including Barramundi (*Lates calcarifer*) (Staunton-Smith 2004).

Based on mark-recapture using Rototags in Snag Pool in 2007, it is estimated that this pool contained approximately 50 juvenile (0+) *P. microdon* at the time of sampling. Since *P. microdon* are thought to have only very small litters (1-12 pups) (Wilson 1999), the captures of large numbers of new recruits suggest that there may have been some synchronization in timing of parturition or that there is an instinct of newborns to travel in groups, which would increase an individual's chance of avoiding predation on their journey up river (Heupel & Simpfendorfer 2005). It is also plausible that their may be an initial period of high site fidelity among new recruits prior to migrating upstream, such as is seen in *Pristis pectinata* (Simpfendorfer 2006).

Rototag data from previously tagged individuals (2003-2006) recaptured repeatedly below Camballin Barrage has demonstrated that during years when wet season discharge is low, *P. microdon* movements upriver can be impeded by this unnatural barrier. A female *P. microdon* of 2320 mm TL that was recaptured approximately 150 km down river of its initial tag site that had grown to 2540 mm TL provides further insight into the movement patterns and timing of the species. This individual would have been expected to be in its fourth year of life when first tagged (see Thorburn *et al.* 2007) and it was still in the river at seven years of age at 2540 mm TL. Noting that very few individuals larger than this have been recorded in the river, and its close approximation to the mouth, it is probable that this individual was on its way out of the river.

The use of acoustic tracking of *P. microdon* in the Fitzroy River demonstrated a high degree of habitat partitioning between different age classes, with the new recruits (0+ fish) clearly remaining in the shallows for much of the day compared to the larger 1+ individuals that rarely moved into the extreme shallows. Furthermore, these larger individuals moved to deeper water at dawn, before moving shallower in the afternoon. Thus, the 1+ fish displayed predictable movements, exhibiting diel vertical migration patterns and similar diel movement patterns have been observed in a number of other predatory elasmobranchs (Nakano *et al*. 2003, Skomal & Benz 2004). Depth preference of the species appears to be positively correlated to depth utilisation, which may explain why the satellite tagging of the large *P. microdon* was unsuccessful. This ontogenetic habitat stratification may be related to foraging activities and/or predator avoidance, noting that these environments are also inhabited by Estuarine Crocodiles (*Crocodylus porosus*) and Bull Sharks (*Carcharhinus leucas*). The smaller individuals are potentially more susceptible to predation by these species, and it is particularly relevant that *C. leucas* has been shown to predate on *P. microdon* in the Fitzroy River (Thorburn *et al*. 2004, Thorburn 2006). Simpfendorfer (2006) reported similar behaviour for *P. pectinata* and suggested that along with decreasing predation, the occurrence of the larger individuals in the slightly deeper water allows the animal more space to maneuver while also maintaining a close proximity to potential prey. Simpfendorfer (2006) also suggested that the smaller individuals (< 1 m) of *P. pectinata* may reside with the shallows to take advantage of the warmers temperatures to maximise growth rates.

In terms of prey utilised by *P. microdon*, Thorburn *et al*. (2007) demonstrated that in the Fitzroy River the ariid catfish *A. graeffei* comprised ~60% of the gut contents of nine *P. microdon*, while detrital matter and Cherabin *Macrobrachiun rosenbergii* were also important prey. Another important food source, as well as *A. graeffei* and *M. rosenbergii* that is assimilated into the tissue of *P. microdon* is *N. erebi* (Thorburn 2006). The ontogenetic and diurnal differences in habitat utilisation by different age classes of *P. microdon* are also likely to be reflected in differences in diet. For example, the prey available in the shallows differs significantly between the day and night, with *M. rosenbergii* and *N. erebi* being more abundant in the shallows at night compared to the day. The diversity of small fishes in the shallows at night also increases substantially with 16 potential prey (fish and decapod) species caught during the night compared to 10 during the day. If the small *P. microdon* are feeding in the shallows during the day then their diet is likely to be comprised of the most abundant small fishes that occupy these habitats at this time, i.e. *L. unicolor*, *C. lentiginosus*, *A. percoides*, *G. giurus* and *A. graeffei*.

Importantly, the acoustic data demonstrated that the small *P. microdon* in the estuarine reaches of the river moved between pools at will, even though most pools at low tide are separated by very long stretches of shallow waters. Furthermore, on the incoming tides, ~98% of movements of the 0+ fish between pools was in an upstream direction, i.e. they moved with the tide. This contrasts the 1+ fish, which moved to another pool only when tidal waters reached the sites and this movement was in both an upstream (i.e. 50% with the tide) and downstream (i.e. 50% against the tide) direction. The ability to swim between pools and utilise the shallow runs and riffle zones between both tidally influenced and riverine pools is a beneficial adaptation. It allows the 0+ fish to not only avoid deeper bodied predators but to also forage in areas not being exploited by larger fishes, such as older *P. microdon*, *L. calcarifer*, *C. leucas* or crocodiles. Moreover, it allows the new recruits to continue to migrate upstream relatively unimpeded until the late dry; to at least the Barrage (pictured below, inset), a substantial unnatural barrier on the system (Morgan *et al*. 2006).

While there was no obvious change in the behaviour of 0+ fish during the various moon phases, the utilisation of very shallow waters (<20 cm) by the 1+ *P. microdon* generally only occurred during the full moon, while water depths greater than 1.5 m were only utilised during the new or half moon. The greater visibility at night during the full moon may make these fish more vulnerable to predators, or may make prey more visible. Alternatively, the prey species may move shallower in an effort to avoid predators. Similar patterns were also observed in daylight hours, suggesting that light levels may not be the only contributing factor to these movements.

Although satellite tracking using SPOT tags was not successful, possibly due to animals not remaining on the surface for a long enough duration to allow for calculation of an accurate location, satellite tracking in general still has the potential to provide important information on the movement patterns of individuals that leave the river. Perhaps pop-off archival tags may be a more effective tool to examine this aspect of their life-history.

From the captures of sawfish during the current study, and with comparisons during our previous work on the river we have been able to provide more robust data in terms of sexual dimorphism, sex ratios and for the first time can provide comparisons with populations in north-eastern Australia (i.e. Gulf of Carpentaria). For example, the sex ratios in Thorburn *et al*. (2007) which utilised fish from 2002 to 2004 in the Fitzroy River was skewed towards the females (1.4 females: 1 male). The capture of large numbers of 0+ individuals during 2006 and 2007 allowed the examination of natal sex ratios, where the proportions of the different sexes were equal. In contrast, the older (i.e. >1+) fish were still skewed towards the females, even though females attain a considerably greater size than males in the river. This suggests that either males are

more heavily predated on, which seems unlikely unless growth rates are slower, or that males mature at a smaller size and younger age and leave the river earlier. In comparing the presence of the different size classes of males and females throughout the years, the lack of males greater than 2400 mm TL and females greater than 2800 mm TL suggests that emigration from the river occurs around these sizes for the different sexes. The capture of a 2580 mm TL, semi-mature male in Kind Sound during 2007, as well as a mature male of ~ 3000 mm TL from Eighty Mile Beach to the south of King Sound (Thorburn *et al.* 2007) supports this theory. Thorburn *et al.* (2007) suggested that emigration from the river is possibly cued by the onset of maturation and due to the needs of such a large bodied animal. A similar size at maturity is reported for the congener *P. pectinata* in Florida (Simpfendorfer 2000).

While Thorburn *et al*. (2007) found significant differences in the relationship between RL and TL of female and male *P. microdon* in the Fitzroy River, these relationships are no longer considered to be different. The relationships in Thorburn *et al*. (2007) utilise data from between 2002 and 2004 in the Fitzroy River (37 females and 22 males) whereas we utilised an additional 33 females (n=70) and 25 males (n=47), and included a higher proportion of 0+ fish. In terms of comparisons of left rotral teeth counts with that of Thorburn *et al*. (2007), the noticeable differences by including these additional animals are: one female had 23 left teeth (previously reported to have 17-22 left rostral teeth); modal number of left rostral teeth for males now 20 instead of bimodal at 20 and 21. For total teeth counts a number of updates include: range of females now 34 to 44 (cf. 34 to 42); modal total teeth count for females now 36 cf. 38; one donated rostrum, that is presumably male, had 47 teeth in total, cf. previous maximum of 46. In terms of symmetry of left and right rostral teeth, a lower proportion had the same number of teeth on the left and right side, i.e. ~38 and 25% of females and males, respectively had equal number of rostral teeth on the left and right side.

A few notable differences exist in the rostral teeth counts between Western Australian and Gulf of Carpentaria (Qld) populations, albeit some of these differences may be attributable to low sample sizes in Qld. Importantly, although the sample size of 14 male fish in Qld is considerably less than the 47 males examined in WA, the Qld males exhibit a greater variability in left rostral teeth (18 to 24) compared to the Fitzroy River (19 to 23). The range in number of left rostral teeth in females is narrower in Qld (17 to 21) compared to WA (17 to 22). The mode of total rostral teeth in Qld male *P. microdon* is 42 cf. 41 in WA; while the number of total rostral teeth in Qld is bimodal at 36 and 41, cf. 36 in WA. In contrast to males in WA, the Qld males were also more likely to exhibit variation in rostral teeth between the left and right side, and are more likely to have higher teeth counts on the right side.

Glyphis sp. C

The six *Glyphis* sp. C were captured in marine tidal creeks of King Sound from Doctors Creek to the north-eastern side of the Robinson River estuary during this study, and were not recorded within the freshwaters of the Fitzroy River, a finding consistent with previous work (e.g. Morgan *et al*. 2004, Thorburn & Morgan 2004). However, the few previous captures of the species elsewhere are reported from freshwaters (Taniuchi *et al*. 1991, Compagno & Niem 1998). The collection of these individuals during 2007 represents ~25% of all *Glyphis* sp. C known to science, and the proportion of all known specimens of the species to be captured in King Sound is now ~64%. Importantly, their range within King Sound (and Western Australia) was also extended by approximately 60 km to north and east.

SPOT tags proved slightly more effective with *Glyphis* sp. C than *P. microdon*, sending a number of successful transmissions, although after a four day period, too few of transmissions were made to calculate a location. Although a few locations with high error ratings (> 1000 m) were given during the first four days, these locations were likely fairly accurate as they were calculated to be at near-shore areas within King Sound near to where *Glyphis* sp. C have been previously captured by the authors. Only the last location appeared to be erroneous as the distance traveled between it and the prior transmission was too great for the species to travel in the five minutes between reported transmissions. Fortunately, information on the timing of transmissions was gathered from the SPOT tags. It was found that most of the transmissions occurred in the early morning and on an incoming tide, which may either indicate peak foraging times for the species or that the species moves over shallows during this period to move in with the tide. Regardless, both would be aided by the morphological adaptations that the species possess, including reduced high, complex sensory ampullae and large dorsal and pectoral fins (Thorburn *et al.* 2004). Due to the lack of tracking data it was not possible to comment on their movement patterns at this time.

Some minor morphological variation was recorded for the species, and is consistent with an increase in sample size. For example, the ratio of 2nd dorsal fin height to 1st dorsal height of two individuals (1088 and 1237 mm TL) was 0.57 and 0.67 which is marginally outside the range of 0.58-0.66 reported for the species by Thorburn & Morgan (2004). The maturity status of the males also corresponded to that described in Thorburn & Morgan (2004).

Management implications and future research

Juvenile *P. microdon* exhibit a high degree of ontogenetic changes in habitat utilisation, at least during their first two years of life. However, this is based on acoustic tracking of a few individuals for a few months during the dry season. The continuation of the tracking of currently tagged fish as well as additional individuals (both small and large) is crucial in

understanding habitat utilisation throughout their life. An expansion of the existing acoustic array in the Fitzroy River beyond the Camballin Barrage will allow an understanding of the impact of this artificial barrier on the movements of juvenile *P. microdon*.

The addition of new tracking technologies to track the movements of *Glyphis* sp. C and mature *P. microdon*, as well as microchemical analysis of teeth/vertebrae in comparison to environments occupied by these species would compliment existing data. The continuation of conventional and acoustic tracking data and morphological studies aids in the management of the species through the involvement of the local, scientific and managerial community; and will provide the much needed information that can be used to construct management strategies to help conserve these species.

SECTION II

Genetic diversity and population structure of the Freshwater Sawfish (*Pristis microdon*) in Australian waters

by

NM Phillips, JA Chaplin, DL Morgan, SC Peverell & DC Thorburn

INTRODUCTION

Sawfishes are large, iconic species of elasmobranchs (Family Pristidae), which are characterised by a saw-like projection of the upper jaw, termed a rostrum (Bigelow & Schroeder 1953) that is used to hunt and stun prey (Compagno 1977, Last & Stevens 1994). The Freshwater Sawfish, *Pristis microdon,* is one of several sawfish species that occur in the Indo-west Pacific bioregion. Specifically, its distribution extends from New Guinea, through southeast Asia and northern Australia, to the east coast of Africa (Last & Stevens 1994, Compagno & Last 1998), notwithstanding the chaotic nature of the taxonomy of the genus *Pristis* (Ishihara *et al.* 1991, Deynat 2005). *Pristis microdon* is believed to have habitat partitioning in different life stages, with juveniles utilising freshwater rivers as nursery grounds while adults use marine-estuarine waters (Section I, Taniuchi *et al.* 1991, Thorburn *et al.* 2003, 2007, Peverell 2005). *Pristis microdon* is reported to grow to a large size (e.g. up to 7 m), be long lived (e.g. up to 44 years), slow to mature (at ~ 7 years) and have low fecundity (e.g. to have a litter size of 1-12 young) (Tanaka 1991, Thorburn *et al.* 2007, Peverell unpublished data). However, when assessing any aspect of the biology of *P. microdon*, it is important to remember that the amount of relevant information is very limited. For example, only a handful of published studies contain primary biological data on this species (e.g. Thorson 1973, Ishihara *et al.* 1991, Tanaka 1991, Watabe 1991, Peverell 2005, Thorburn *et al.* 2007) and the quantity of the data therein is sometimes scant (e.g. Watabe 1991).

Pristis microdon is listed as vulnerable under the *Environmental Protection and Biodiversity Conservation (EPBC) Act 1999* and as critically endangered on the International Union on the Conservation of Nature (IUCN) Red List in recognition of the fact that it is "characterised by extreme and continued vulnerability to fisheries (evidenced by serious declines in virtually all known populations), compounded by habitat loss and degradation over most of its range" (IUCN 2006). A range of factors contributes to this vulnerability, including that *P. microdon*: (i) has a relatively slow rate of population growth (e.g. see Simpfendorfer 2000); (ii) is actively exploited for a range of reasons, including as trophies and for 'shark' product (Peverell 2005, Thorburn *et al.* 2007); and (iii) features in the by-catch of several fisheries and as a consequence suffers significant amounts of mortality (Simpfendorfer 2000, Pogonoski *et al.* 2002, Stobutzki *et al.* 2002). In addition, since the juveniles likely depend on rivers for their survival, *P. microdon* is vulnerable to the effects of the (sometimes severe) degradation of freshwater systems, as well as to the effects of coastal influences, and individuals may also be more susceptible to capture when in rivers (e.g. Saunders *et al.* 2002). Although the number of *P. microdon* in Australian waters has declined in recent times, these declines are probably not as extreme as those experienced in other regions (Thorson 1982, Simpfendorfer 2000). In fact, northern Australia may be the only location where viable populations of this species remain (Pogonoski *et al.* 2002). If so, these populations are central to the development of conservation plans of this species. However, the development of such is currently hindered by a lack of information about the biology of these (and other) populations of this species (Peverell 2005).

Information on the population genetics of an endangered species can be used to estimate a variety of demographic parameters, such as population structure, size and history, that are essential for the development of effective plans for the management of the species and which are often unattainable by other means (Avise & Hamerick 2004, DeSalle & Amato 2004). The quality and quantity of the population genetic information that is produced by a particular study will depend on a range of factors, including the type(s) of genetic data employed by the study (Kohn *et al.* 2006). The control region of the mitochondrial genome (mtDNA) is generally relatively information rich and can be utilised via relatively inexpensive means, even without specific knowledge of the genome of the species under investigation (Hoelzel *et al.* 1991, Avise

1994, Zhang & Hewitt 1997). Hence, first studies of the population genetics of a particular species often rely on this type of data (see Hoelzel *et al.* 2006, Stow *et al.* 2006). Some specific applications of this type of data include estimation of the amount genetic diversity in a population (Hoelzel *et al.* 2006), which can be used as a proxy for its genetic health (Lacy 1997, Bjilsma *et al*. 2000, Coulon *et al*. 2004). In addition, since the demographically independents units of a species (true populations) are each typically genetically unique, such units can sometimes be identified from the spatial distribution of control region variation (Ward 2000). Furthermore, mtDNA variation is particularly useful for revealing the presence of divergent lineages, which may warrant special conservation status, within single taxa (Moritz 2002).

Aims:

The specific aims of this project concern the Freshwater Sawfish, *Pristis microdon*, in Australian waters and use variation in the nucleotide sequence of the control of the mtDNA to:

(1) assess the extent of genetic diversity within selected assemblages;

(2) assess the extent of genetic differences between selected assemblages in different geographic regions;

(3) identify any highly distinctive lineages/populations.

In order to help place the results for *P. microdon* in perspective, comparable genetic data were also produced for selected Australian assemblages of the Dwarf Sawfish, *Pristis clavata.*
The resultant information was used to provide information on the genetic health of assemblages of *P. microdon* in Australian waters, the extent to which assemblages of this species in different geographic regions in these waters are independent of each other, and the presence of distinctive populations/lineages (if any) that might warrant special conservation status.

METHODOLOGY

Sampling regime

Overall, samples were obtained from 90 individuals of *P. microdon* from a total of 16 sampling sites, ranging from Cape Naturaliste in south-western Australia, through northern Australia, to the Normanby River on the east coast of Australia (see Figure 1 and Table 1). Most samples were obtained from the Fitzroy River on the west coast and the Gulf of Carpentaria in the north-east (see Table 1). Samples were in the form of tissue biopsies preserved in 100% ethanol or, from dry rostra (see Figure 2, Table 1). The samples were obtained from a number of sources, including from captures taken during past surveys (e.g. Morgan *et al.* 2002, 2004, Thorburn *et al.* 2003, Peverell 2005), the accompanying tracking study (Section 1 of this report) and in the case of the dried rostra, private collections. (N.B. An additional 12 specimens of *P. microdon* were obtained from the Gulf of Carpentaria but, since attempts to obtain DNA from these specimens were unsuccessful, they have not been considered in this report).

Samples were obtained from a total of 30 individuals of *P. clavata* from eight sites. These comprised 23 individuals from four sites on the north-west coast of Australia and another seven individuals from four sites in the Gulf of Carpentaria (see Figure 3 and Table 2). As was the case for *P. microdon,* the samples of *P. clavata* were either in the form of ethanol preserved tissue or dry rostra (see Table 1) and from a variety of sources, including WA Department of Fisheries, past surveys and tracking studies (e.g. Morgan *et al.* 2002, 2004, Thorburn *et al.* 2003, Peverell 2005), and the accompanying tracking study (Section 1 of this report).

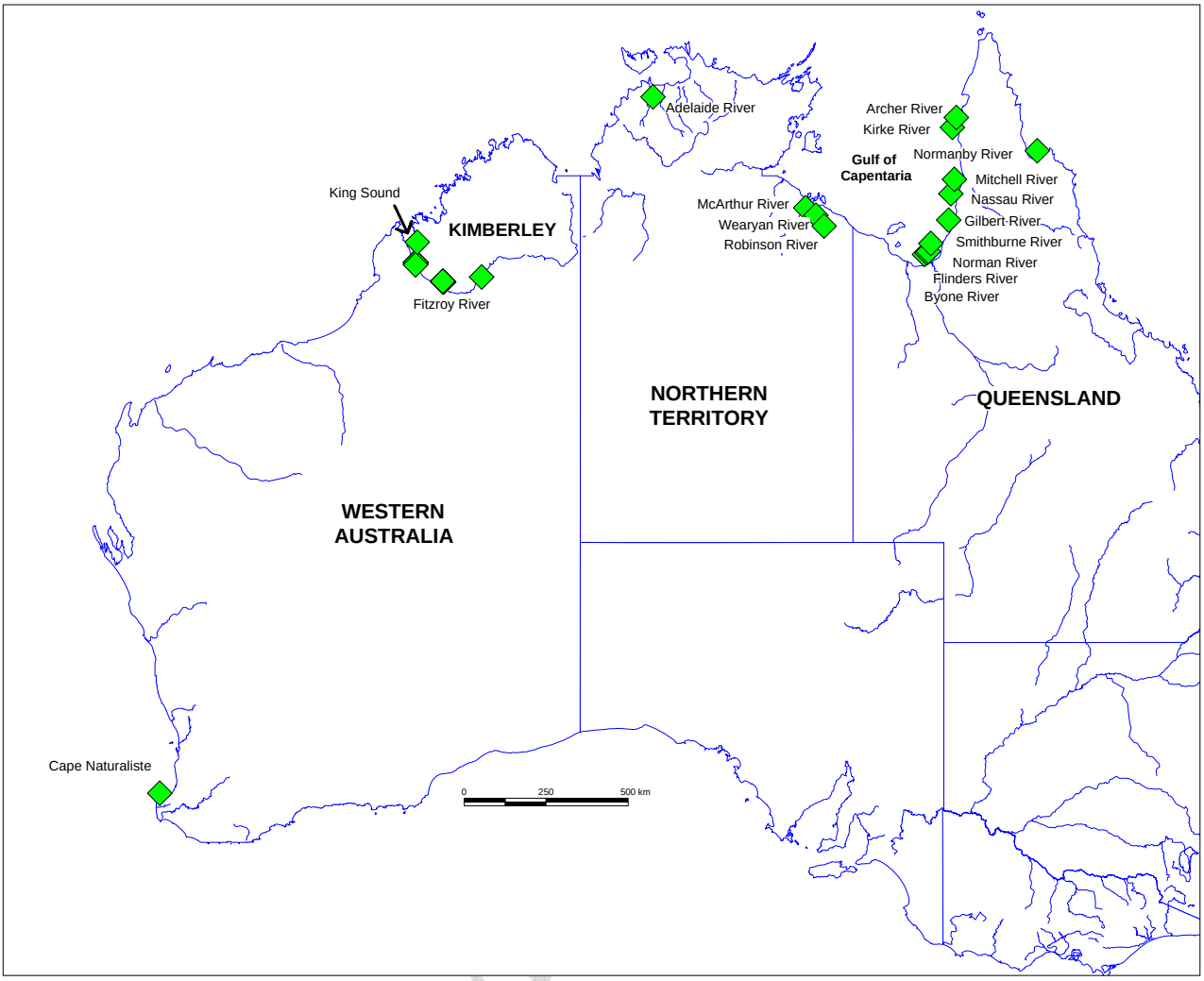


Figure 1 Locations of the sampling sites for the Freshwater Sawfish, *Pristis microdon.*

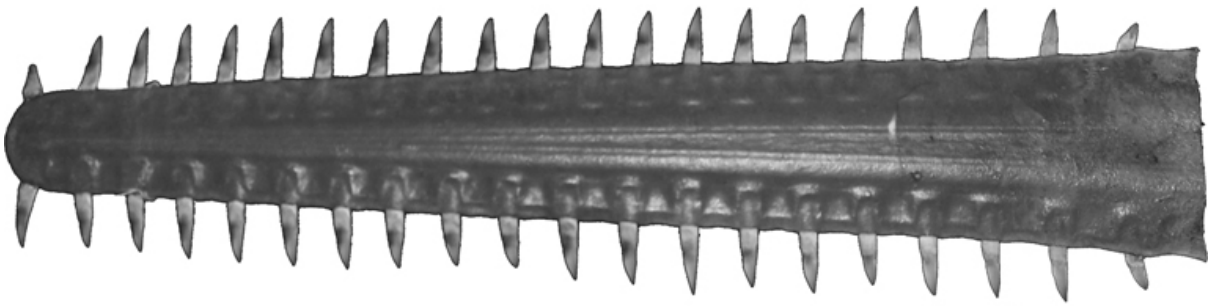


Figure 2 A dried rostrum of the Freshwater Sawfish, *Pristis microdon.* This study extracted DNA from skin tissue on the dry rostra of both the *P. microdon* and *Pristis clavata*.

Table 1 The number of individuals of the Freshwater Sawfish, *Pristis microdon*, collected from each sampling site, the approximate collection dates and the type(s) of sample obtained. WC= West Coast; NC=North Coast, outside of the Gulf of Carpentaria; GoC= Gulf of Carpentaria; EC= East Coast.

	Site	No. of individuals	Sample Type	Date
WC	King Sound	2	Dried rostra	2002-2005
	Fitzroy River	37	Preserved & Dried rostra	2006-2007
	Cape Naturaliste	1	Preserved	2003
NC	Adelaide River	1	Preserved	2002
	McArthur River (NT)	1	Preserved	2001-2002
	Wearyan River (NT)	1	Preserved	2001-2002
	Robinson River (NT)	1	Preserved	2001
GoC	Gulf of Carpentaria	2	Preserved	2002
	Kirke River	3	Preserved	2001
	Flinders/Byone/Norman Rivers	11	Preserved	2002
	Smithburne River	2	Preserved	2001
	Gilbert River	9	Preserved	2004
	Nassau River	4	Preserved	2002
	Mitchell River	8	Preserved	2002
	Archer River	5	Preserved	2001
EC	Normanby River	2	Preserved	2004

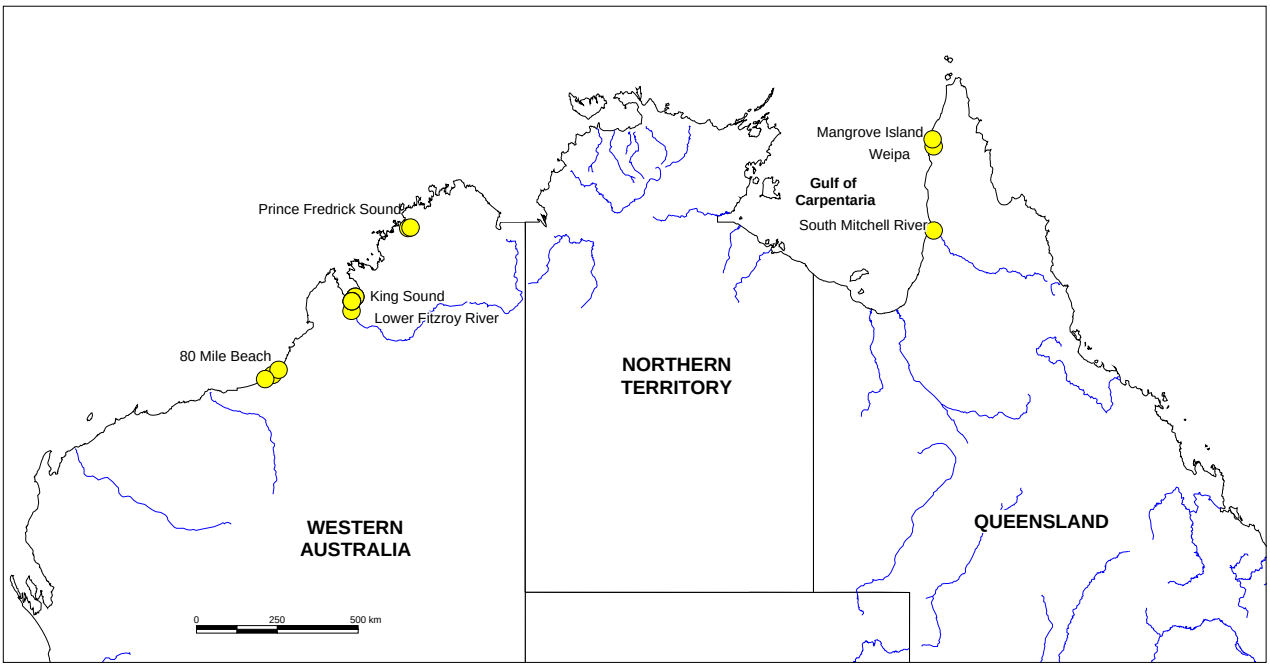


Figure 3 Approximate catch locations of the specimens of the Dwarf Sawfish, *Pristis clavata*.

Table 2 The number of individuals of the Dwarf Sawfish, *Pristis clavata,* collected from each sampling site, the collection dates and the type(s) of sample obtained.

	Site	Number of individuals	Sample type	Date
WA	80 Mile beach	5	Preserved	2003-2004
	King Sound	15	Dried Rostra	2002-2005
	Fitzroy River (Telegraph Pool)	1	Dried Rostra	2002
	Prince Fredrick Sound	2	Preserved	2003
Gulf of Carpentaria	Weipa River	3	Preserved	2003
	Mangrove Island	1	Preserved	2003
	South Mitchell River	2	Preserved	2002
	Gulf of Carpentaria	1	Preserved	2002

Genetic methods

Overview

The genetic work is based upon the analysis of variation in the nucleotide sequence of the control region of the mtDNA. The control region was targeted for analysis because in most vertebrates it consists of a central conserved region, which facilitates the development of suitable primers, surrounded by two adenine-rich and highly variable end domains, which are characterised by substitution rates five- to 10-fold higher than any other portion of the mtDNA molecule and are thus likely to reveal relatively high levels of intraspecfic variation (Kocher *et al*. 1989, Hoelzel *et al.* 1991, Lopez *et al*. 1997).

DNA extractions

Total genomic DNA was extracted from approximately 5 mg of tissue from individual sawfish using a Masterpure™ (Epicentre Technologies, Sydney) DNA extraction kit, according to manufacturers' protocol. In the case of the ethanol preserved samples, skin tissue from dorsal fin clips or muscle tissue was used, while skin tissue was used from the dry rostra, following the findings of Phillips (2006), Phillips *et al.* (unpublished data). The quality and quantity of the extracted DNA was assessed via the appearance of a 2 µl aliquot of the extract on a 2% agarose gel that was subject to electrophoresis for 25 minutes at 46 mAmps, stained with ethidium bromide, and illuminated with UV light.

PCR amplification

Polymerase chain reaction (PCR) was used to amplify a 353–351-bp and a 351-350-bp portion of the control region of the mtDNA of *P. microdon* and *P. clavata,* respectively, using the forward primer (CRF: 5'-ACGTATCCGTAATACTCAT) and reverse primer (CRR: 5'-ATGCAAATATTATGTCGAGGGTAG). These primers were designed during the present study from conserved regions in the nucleotide sequences of the entire control region of 1 - 9 individuals of each of *P. clavata, Pristis zijsron,* and *Anoxyprisitis cuspidata.* The primers of Pardini *et al.* (2001) were used to amplify the entire control region from these individuals, but amplified multiple products in *P. microdon* rather than a single locus, hence the need to design new primers.

PCR amplification was performed in a reaction mixture containing approximately 10 ng of DNA template, 10 mM TAQ buffer with 1.5 mM $MgCl_2$ (Roche), 0.1 mM of each of the dNTPs (Promega), 0.5 U of *Taq* polymerase (Roche), 20 µmol of each primer, and adjusted to a final volume of 50 µl with PCR-grade water. The amplification conditions consisted of a 5 minute

initial denaturation phase at 94°C, followed by 35 cycles, with each cycle consisting of; 30 seconds denaturation at 94°C, 30 seconds of annealing at 59°C (*P. microdon*) or 58°C (*P. clavata*), and 30 seconds of extension at 72°C; followed by a final 7 minute extension phase at 72°C. The quality and quantity of the PCR product was visualised prior to sequencing using essentially the same above-described methods that were used to screen the DNA extracts.

Sequencing
Prior to sequencing, the PCR products were cleaned using Qiaquick columns (Qiagen), according to the manufacturer's protocol.

The sequencing was carried out using the dye terminator cycle sequencing method. Each sequencing reaction was prepared using approximately 30 ng of clean PCR product, 3.2 pmol of the forward or reverse primer and a Big Dye 3.1 terminator cycle sequencing ready reaction kit following the manufacturer's protocol (Applied Biosystems Inc. 2001), except that all sequencing was done using 'half' reactions. The sequencing products were electrophoresed, and the raw data chromatograms generated using an Applied Biosystems 3230 DNA Analyzer automated sequencer.

Protocols for dry rostra
Since the dry rostra used in this study had not been handled and stored specifically for genetic analyses, and hence the DNA that they contain is likely to be partly degraded Phillips (2006), Phillips *et al.* (unpublished data); there was a relatively high risk that assays involving these samples might become contaminated with non-target DNA. In order to minimise the risk of such contamination, all genetic work involving dry rostra samples was carried out in accordance with the protocols for working with ancient DNA (aDNA) set out by Mulligan (2005), including that this work was conducted in laboratory that had not previously held contemporary pristid tissue. The findings of Phillips (2006), Phillips *et al.* (unpublished data) suggest that these protocols are adequate in this regard.

Data analyses
The forward and reverse sequences of a portion of the mitochondrial control region were determined for each individual of *P. microdon* and *P. clavata*. The forward and reverse sequences of each individual were aligned using GeneTool™ Lite 1.0 (Wishart *et al*. 2000), the primer sequences removed from both ends and a forward reading consensus sequence generated. A multiple alignment of the forward consensus sequences of all individuals of each species was performed with GeneTool™ Lite 1.0 (Wishart *et al*. 2000). The partial control region sequence of an individual is termed a 'haplotype'.

The haplotypes of both *P. microdon* and *P. clavata* included indels (insertions or deletions of nucleotides at particular positions in the sequence). Since recent studies indicate that indels potentially yield valuable information about the distribution of genetic variation in a species (Pearce, 2006), they were included in all analyses, except for the estimation of gamma correction because they could not be accommodated in the associated software. Unless otherwise stated, the data analyses were conducted using the software ARLEQUIN version 3.0 (Excoffier *et al.* 2005). When multiple tests were conducted as a part of an analysis, a sequential Bonferroni procedure, which controls for group-wide Type I error rates (Rice 1989), was used to assess the statistical significance of the probability values.

Neutrality of the mitochondrial control region
Tajima's (1989) D-test and Fu's (1997) Fs-tests were applied to test whether the patterns of variation in the control region sequences in selected samples of *P. microdon* were selectively

neutral. The statistical significance of the tests was assessed by randomly sampling the data under the assumptions of selective neutrality and population equilibrium.

Genetic diversity

The level of genetic diversity within selected samples was estimated in terms of haplotype diversity (*h*), nucleotide diversity (π) and the standardised number of haplotypes (SNH). Haplotype diversity (*h*) represents the probability that two randomly selected individuals exhibit different haplotypes (Nei 1987). Thus, values of *h* range from 0 (all individuals have the same haplotype) to 1 (all individuals have different haplotypes). The estimates of haplotype diversity, and associated standard errors, were calculated, according to equation 8.5 in Nei (1987) and the variance formula presented in the ARLEQUIN manual, respectively.

Nucleotide diversity (π) is the probability that two randomly selected homologous nucleotides are different (Nei 1987). Thus, it provides a measure of the extent of genetic differences between individuals in a sample; the greater the genetic differences, the higher the value. Nucleotide diversities were calculated according to equation 10.5 in Nei (1987) using Tamura & Nei's (1993) substitution model and a gamma correction of 0.05 for *P. microdon* and 0.13 for *P. clavata*, while the standard errors of these estimates were calculated using the variance formula presented in the ARLEQUIN manual. The Tamura & Nei (1993) substitution model, which takes into account excess transitions, unequal nucleotide frequencies, and variation in substitution rates among sites, was determined to be the best model via comparisons with others using the software MrAIC.pl 1.4.2 (Nylander 2004). The gamma correction is used to correct for variation in the rate of substitution among nucleotide sites (Uzzell & Corbin 1971). The appropriate values of the correction for the control region haplotypes of each of *P. microdon* and *P. clavata* were empirically determined with the software GZ gamma (Gu & Zhang 1997).

The number of haplotypes observed in a sample strongly depends on the size of the sample. Thus, in order to facilitate the comparison of haplotype numbers between samples of different sizes, the number of haplotypes present in selected samples was standardised according to the numbers of individuals present in the smallest sample involved the comparison. The standardisation procedure was carried out using POPTOOLS (add-in for Microsoft Excel, written by Greg Wood, Commonwealth Scientific and Industrial Research Organization, Australia, available at www.cse.csiro.au/CDG/poptools/).

Population genetic differentiation

Overall levels of genetic subdivision

Analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was used to assess how genetic variation was partitioned within and between samples from individual sites. The statistical significance of the variance estimates was assessed using a nonparametric permutation approach, in which sequences were randomly permutated among samples (see Excoffier *et al.* 2005).

Pair-wise comparison of assemblages

Exact tests were used to ascertain whether the haplotype frequency distributions in selected pairs of samples were significantly different to each other. The results of this analysis are presented in terms of the exact probability of a Type I error (*P* values), *i.e.,* the probability of rejecting the null hypothesis (no significant genetic difference between a particular pair of samples) when it is true. The probability values were estimated using the Markov chain method (Raymond & Rousset 1995), with 10,000 steps in the Markov chain. Exact probability tests were used because they are not biased by small sample sizes or low haplotype frequencies (see Raymond & Rousset 1995).

Relationships among assemblages
In order to resolve the relationships among samples, the multi-dimensional scaling method with the software Primer v 6 (Clark & Gorley 2006) was used to map the 'genetic distance' between selected pairs of samples (where $N \geq 8$) in two-dimensional space. The 'genetic distance' between pairs of samples was estimated in terms of values of the genetic variance, *i.e.,* F_{ST} (Weir & Cockerham 1984).

Evolutionary relationships among haplotypes
The evolutionary relationships among haplotypes of each of *P. microdon* and *P. clavata* were estimated by constructing a haplotype network using the parsimony method of Templeton *et al*. (1992). This method estimates the maximum number of substitutions required to connect any two haplotypes parsimoniously (with 95% confidence) and builds the network by firstly linking sequences with the smallest number of differences. This analysis was performed using the software TCS version 1.21 (Clement *et al*. 2000).

RESULTS

General
The nucleotide sequence of a 353-351-bp portion of the left domain of the control region of the mtDNA was determined for a total of 90 individuals of the *P. microdon.* The sequences of these individuals contained a total of 22 polymorphic sites, included indels at 2 sites, and revealed a total of 17 different haplotypes (Table 3). In the case of *P. clavata,* the nucleotide sequence of 351-350-bp of the same portion of the control region was determined for a total of 30 individuals. The resultant sequences contained a total of 19 polymorphic sites, included indels at 1 site, and revealed a total of 10 haplotypes (Table 4). Thus, the underlying level of polymorphism in the control region in both species is broadly similar and adequate to inform about aspects of their population genetic structures.

The average base composition of the *P. microdon* sequences was: A-22.84%, T-29.94%, C-30.47%, and G-16.74%; while that of the *P. clavata* sequences was A-25.22%, T-30.40%, C-28.91% and G-15.47%. Thus, the base composition in both species was slightly biased towards adenine and thymine, which is consistent with that of the mtDNA of other elasmobranchs (*e.g.* Keeney *et al.* 2005) and also of teleosts (*e.g.* Manchado *et al.* 2007, Ponce *et al.* 2008).

The identities of the mitochondrial control region sequences acquired from *P. microdon* and *P. clavata* were confirmed by BLAST searches of the GENBANK database (http://www.ncbi.nih.gov/BLAST/). The most similar sequences on the BLAST database were those of two other elasmobranchs, namely the Bonnethead Shark, *Sphyrna tiburo* and the Deepwater Stingray, *Plesiobatis daviesi.* Sequences obtained for both *P. microdon* and *P. clavata* from this study will be deposited in GENBANK for future reference.
According to the results of Tajima's D and Fu's Fs-tests, the patterns of control region variation in pooled samples of *P. microdon* from each of the west coast and the Gulf of Carpentaria conformed to expectations for a selectively neutral sequence in drift-mutation equilibrium as the values of D and Fs obtained for these samples were not significantly different from zero after the Bonferroni correction was applied (Table 5). These statistics were not calculated for other samples because of low sample sizes.

Genetic Diversity
Pristis microdon
The analysis of the amount of genetic diversity in *P. microdon* was done at three levels: (i)

overall, for all samples pooled; (ii) regional, for all samples from single geographic regions pooled and (iii) individual sites, providing that the sample size was $n \geq 8$. The overall levels of haplotype and nucleotide diversity in *P. microdon* were moderate and low respectively (Table 8). This was also the case for the pooled samples from each of the Gulf of Carpentaria and the west coast of Australia (Table 6). The numbers of individuals obtained from the east coast and the north coast outside of the Gulf of Carpentaria were too small to be useful in this regard (see Table 7). The amounts of haplotype and nucleotide diversity in samples from single sites were variable (Table 7). Nevertheless, multiple haplotypes were usually present at single sites, even though the number of individuals sampled from each site, other than the Fitzroy River, was small (Table 7). Furthermore, the levels of haplotype and nucleotide diversity in samples from a few sites such as the Norman/Flinders/Bynoe and Archer rivers in the Gulf of Carpentaria, were relatively high (see Table 7).

The control region data indicate that the amount of genetic diversity in *P. microdon* may vary within and/or between geographic regions. Thus, for example, all measures of control region diversity in the pooled samples from the Gulf of Carpentaria were higher than those in the pooled west coast samples (Table 6). However, meaningful comparisons of the regional and site diversity were confounded by the fact that all but three of the 40 individuals from the west coast were collected from a single site (the Fitzroy River), whereas the 47 individuals from the Gulf of Carpentaria were scattered over a much larger number of sites and the maximum number of individuals collected from any one site was 11 (see Tables 1 and 7).

In conclusion, the results of this research indicate that the amount of haplotype diversity in *P. microdon* in Australian waters is generally moderate, even at small spatial scales. The level of nucleotide diversity was generally low, which can probably be attributed more to the relatively slow rate of evolution of the mitochondrial DNA in elasmobranchs (Martin *et al.* 1992, Martin 1995) rather than to any recent declines in the abundance of *P. microdon.* Regardless, more intensive and more even sampling is required to be able to properly compare the amounts of genetic diversity within and between geographic regions in this species.

Comparison with Pristis clavata

In order to help place the results for *P. microdon* into perspective, data on the amount of control region diversity in samples of *P. clavata* were also produced. The overall levels of genetic diversity in *P. microdon* and *P. clavata* were similar (see Table 8). However, some differences in the levels of diversity between selected subsets of samples of the two species were evident. For example, in marked contrast to the situation for *P. microdon* (described above), no genetic variation was detected in *P. clavata* in the entire Gulf of Carpentaria, although admittedly only seven individuals of this species were sampled from this location (see Table 8). In contrast, all measures of genetic diversity for samples of *P. clavata* from the Fitzroy River and adjacent King Sound on the west coast of Australia were noticeably higher than those for the samples of *P. microdon* from this same location (see Table 8). No other comparisons of the diversity in *P. microdon* and *P. clavata* were performed due to the small sample sizes for *P. clavata*.

Table 3 The location and distribution of 22 polymorphic sites among 17 haplotypes for a 353-351-bp portion of the control region in the mtDNA of the sampled individuals of *Pristis microdon*. Dots represent matches with nucleotides present in haplotype 1. Numbers refer to position of base pairs from the start of the fragment. Dashes indicate indels.

Haplotype no.	7	20 35	43 171	79	85	86	89	129	200	227	239	241	259	263	268	269	270	272	300	325	342	343
1	A	C	--	G	A	A	A	A	A	T	G	A	T	T	C	T	T	T	C	T	T	T
2	.	.	--	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
3	.	.	--	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
4	.	.	--	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	--	C	.	.	.	.	.	G	A	C	.	.	.	C	.	G	.	--	G	G
6	.	.	--	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	--	.	.
7	.	.	--	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	G	G
8	.	.	--	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	--	G	G
9	.	.	C	C	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	--	.	.
10	.	.	--	C	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11	.	.	--	C	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.
12	.	.	--	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	G	G
13	.	.	--	.	.	C	.	.	.	.	.	C	.	.	.	.	.	G	.	.	G	G
14	.	.	--	.	.	.	.	.	.	.	.	.	.	G	.	.	.	G	G	.	.	.
15	.	.	--	.	.	.	.	C	C	.	A	.	C	.	.	.	.	.	.	.	.	.
16	C	G	C	C	.	.	.	.	.	.	A	.	.	.	.	.	.	A	.	G	.	.
17	.	.	--	.	.	.	.	.	.	.	.	.	.	.	A	G	A	G	.	.	G	G

Table 4 The location and distribution of 19 polymorphic sites among 10 haplotypes for a 351-350-bp portion of the control region in the mtDNA of the sampled individuals of dwarf sawfish, *Pristis clavata*. Dots represent matches with nucleotides present in haplotype 1. Numbers refer to position of base pairs from the start of the fragment. Dashes indicate indels.

Haplotype number	57	7935	95	110	112	127	128	129	136	165	202	234	238	240	249	273	288	295	323
1	T	--	A	C	T	T	A	C	A	A	G	A	A	A	T	C	G	G	T
2	.	--	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.
3	.	G	.	.	.	G	G	G	.	.	.	.	.	.	.	.	.	.	.
4	.	--	.	.	.	.	.	.	.	.	C	C	C	.	.	.	C	.	G
5	.	--	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
6	.	--	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	.	--	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	C.	G
8	.	--	.	.	.	.	.	.	.	.	C	C	.	.	.	.	C	.	G
9	.	--	C	.	C	.	.	.	C	C	C	.	C	C	.	G	.	.	G
10	G	G	.	.	.	G	G	G	.	.	.	C	.	.	.	.	.	.	.

Table 5 Tests of neutrality of the control region sequences for pooled samples of *Pristis microdon* from each of the west coast of Australia and Gulf of Carpentaria. D=Tajima's (1989) neutrality statistic; Fs= Fu's (1997) neutrality statistic. None of the *P* values was statistically significant once the level of significance was corrected for multiple tests.

Sample locations	D	Fs
West Coast	-0.4576 *P*=0.3680	-1.3975 *P*=0.2190
Gulf of Carpentaria	-1.5027 *P*=0.0430	-2.2718 *P*=0.0430

Table 6 A summary of the levels of diversity in a 353-351-bp portion of the mtDNA control region in pooled samples of freshwater sawfish, *Pristis microdon* from each of the west coast of Australia and the Gulf of Carpentaria. Data for other geographic regions are not included because of the small number of sampling sites and individuals (see Table 7).

Sample locations	Sample size	Number of haplotypes	Standardised number of haplotypes	Number of polymorphic sites	Haplotype diversity (±SE)	Nucleotide diversity (±SE)
West coast	40	6	6	5	0.5282 ± 0.0876	0.002739 ± 0.002117
Gulf of Carpentaria	47	12	10.804	21	0.6232 ± 0.0775	0.007723 ± 0.004632

Table 7 A summary of levels of diversity in a 353-351-bp portion of the mtDNA control region in samples of freshwater sawfish, *Pristis microdon*, from individual sites on the west coast (WC), north coast outside of the Gulf of Carpentaria (NC), Gulf of Carpentaria (GoC) and east coast of Australia (EC).

Sample locations	Sample size	Number of haplotypes	Number of polymorphic sites	Haplotype diversity (±SE)	Nucleotide diversity (±SE)
Fitzroy River (WC)	37	6	5	0.4970 ± 0.0950	0.0027 ± 0.0021
King Sound (WC)	2	1	0	-	-
Cape Naturaliste (WC)	1	1	NA	NA	NA
Adelaide River (NC)	1	1	NA	NA	NA
McArthur River (GoC)	1	1	NA	NA	NA
Wearyan River (GoC)	1	1	NA	NA	NA
Robinson River (GoC)	1	1	NA	NA	NA
Flinders/Norman/Bynoe Rivers (GoC)	11	6	14	0.8727 ± 0.0707	0.0165 ± 0.0096
Smithburne River (GoC)	2	2	6	-	-
Gilbert River (GoC)	9	2	3	0.3889 ± 0.1644	0.0033 ± 0.0026
Nassau River (GoC)	4	1	0	-	-
Mitchell River (GoC)	8	2	1	0.4286 ± 0.1687	0.0012 ± 0.0013
Kirke River (GoC)	3	2	1	-	-
Archer River (GoC)	5	3	8	0.7000 ± 0.0165	0.0097 ± 0.0069
Gulf of Carpentaria	2	2	4	-	-
Normanby River (EC)	2	1	0	-	-

NA = not applicable because only a single individual was sampled. - = not estimated because of inadequate sample size.

Table 8 A comparison of the levels of diversity in a portion of the mtDNA control region between samples of *Pristis clavata* and *Pristis microdon*. Three sets of sample comparisons are made –the total sample for each species (*i.e.*, all samples pooled), pooled samples from Fitzroy River and adjacent Kind Sound on the west coast, and pooled samples from the Gulf of Carpentaria. No other comparisons were made due to inadequate sample sizes.

Sample locations	Species	Sample size	Number of haplotypes	Standardised number of haplotypes	Number of polymorphic sites	Haplotype diversity ±SE	Nucleotide diversity ±SE
Total pooled samples	***P. microdon***	90	17	9.498	22	0.7738 ±0.0315	0.0066 ±0.0041
	P. clavata	30	10	10	19	0.6023 ±0.1044	0.0072 ±0.0044
King Sound & Fitzroy River (WC)	***P. microdon***	39	6	4.317	5	0.5385 ±0.0877	0.0028 ±0.0022
	P. clavata	16	8	8	12	0.8083 ±0.0926	0.0092 ±0.0056
Gulf of Carpentaria	***P. microdon***	47	12	3.537	21	0.6232 ± ±0.0775	0.0077 ±0.0046
	P. clavata	7	1	1	0	0.0000 ±0.0000	0.0000 ±0.0000

Population differentiation

Pristis microdon

The results of the AMOVA indicate that the amount of control region variation in *P. microdon*, both between individuals within samples and between samples from different sites, is large and statistically significant (Table 9). More detailed analysis of the between-sample variation was focussed on relatively large samples; in particular on that from the Fitzroy River on the west coast ($n = 37$) and those from sites in the Gulf of Carpentaria with $n \geq 8$ individuals. The results of the exact tests indicate that the haplotype composition of each of these gulf samples was significantly different to that of the Fitzroy River sample, but not to other gulf samples once the Bonferroni correction was applied (Table 10). Similarly, the gulf samples were positioned relatively close to each other, but away from the sample from the Fitzroy River, in the MDS ordination, which depicts the relationships among samples based on the 'genetic distance' between them (Figure 4).

Two control region haplotypes (# 1 and 2) were common to samples from both the west coast of Australia and the Gulf of Carpentaria; haplotype 1 was also found in the Adelaide River on the north coast outside of the Gulf of Carpentaria (Table 12). However, while haplotype 1 was relatively common in the gulf samples, haplotype 2 was relatively common in the west coast samples (Table 12). The remaining 15 control region haplotypes were much more rare and spatially restricted (Table 12), although additional sampling is likely to extend the known distribution of at least some of them. In this regard, it is worth noting that one haplotype (#8) only was found on the east coast (in two individuals from the Normanby River), and this haplotype was not found in any other sample (Table 12). This raises the possibility that the assemblages of *P. microdon* on the east coast are relatively distinctive compared to those on the north and west coasts, but more data are required to confirm this (or otherwise)

In conclusion, the above findings indicate that the assemblages of *P. microdon* in the Gulf of Carpentaria are genetically differentiated from, and thus demographically independent of, that in the Fitzroy River on the west coast of Australia. However, it was not possible to determine the smallest spatial scale at which the demographically independent units occur. Although isolated in the contemporary environment, the presence of shared haplotypes between the west coast and the north coast, both inside and outside of the Gulf of Carpentaria, provides evidence of the existence of historic connections between the assemblages of *P. microdon* in these regions.

Table 9 Results of analysis of molecular variance (AMOVA) based on the frequency distributions of mtDNA control region sequences in samples of *Pristis microdon*. Variance components and estimates of statistical significance (*P*-values) are indicated. Percentage of variation is indicated in parentheses. See Table 7 for a key to the sample codes.

Groups	Among samples	Within samples
WC (FR, CN, KS); **GoC** (MA, WY, RB, GN, K FBN, SM, G, NS, M, A, GQ, NM); **NC** (AD); **EC** (NM)	0.1690 ***P*= 0.000** (36.9)	0.2890 ***P*=0.000** (63.1)

Table 10 Comparisons of the frequency distributions of control region haplotypes between pairs of samples of freshwater sawfish, *Pristis microdon* from individual sites but only including those samples where N ≥ 8. The outcomes of these comparisons are expressed in terms of the exact probability of rejecting the null hypothesis (no genetic difference) when it is true. *P* values that were significant after a sequential Bonferroni correction was applied are indicated in bold. The sample from the Fitzroy River was from the west coast of Australia; the remainder of the samples were from the Gulf of Carpentaria.

Sample locations	Fitzroy River	Flinders/Norman/ Bynoe rivers	Gilbert River	Mitchell River
Fitzroy River Flinders/Norman/ Bynoe Rivers	***P*= 0.0000**			
Gilbert River	***P*= 0.0000**	*P*= 0.0234		
Mitchell River	***P*= 0.0000**	*P*= 0.3015	*P*= 0.2208	

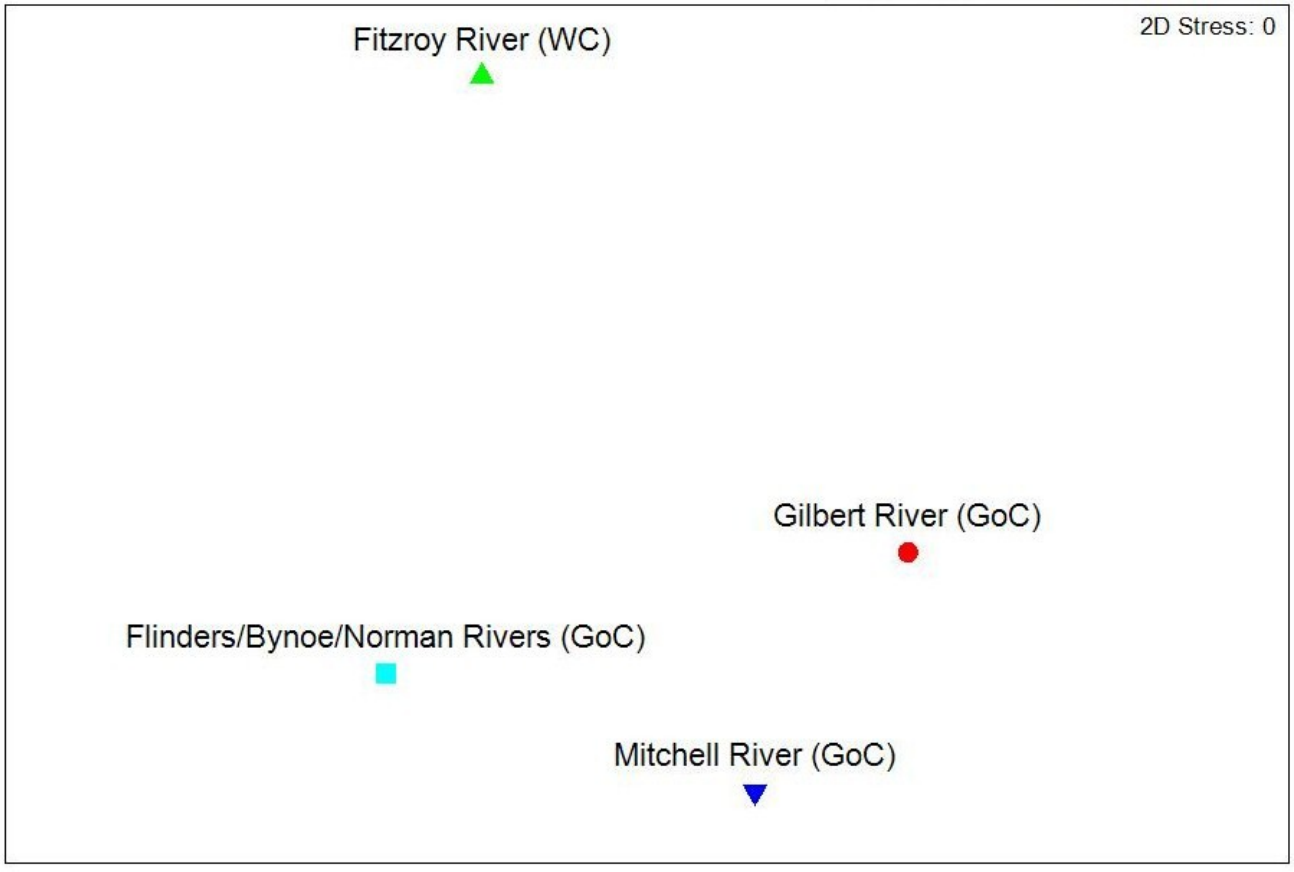


Figure 4 A two-dimensional ordination of standardised values of F_{ST}, based on variation in a 351-350-bp portion of the control region of the mtDNA, between pairs of samples of *Pristis microdon*. The stress value provides an indication of how accurately the variation in the underlying data set is portrayed in the MDS. A stress value of 0 indicates that the ordination provides a reliable representation of the relationships among the samples (see Clarke & Gorley 2006). Only samples with N ≥ 8 were included in this analysis.

Table 11 The distribution and abundance of the 17 mtDNA-control region haplotypes found in 90 individuals of *Pristis microdon*, from 16 sites across its distribution in Australia. KS = King Sound; FR = Fitzroy River; CN = Cape Naturaliste; AD = Adelaide River; MA = McArthur River; WY = Wearyan River; RB = Robinson River; K = Kirke River; FBN = Flinders/Bynoe/Norman Rivers; SM = Smithburne River; G = Gilbert River; NS = Nassau River; M = Mitchell River; A = Archer River; G = Gulf of Carpentaria; NM = Normanby River.

Region	**West Coast**			**NC***	**Gulf of Carpentaria**										**East Coast**
					Western Gulf			**Eastern Gulf**							
Hap No.	**KS**	**FR**	**CN**	**AD**	**MA**	**WY**	**RB**	**K**	**FBN**	**SM**	**G**	**NS**	**M**	**A**	**G**	**NM**
1	2	3	-	1	1	-	1	2	3	-	7	4	6	3	1	-
2	-	26	1	-	-	1	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	1	2	1	-	-	2	-	-	-
4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
7	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
9	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
11	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
16	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Total	2	37	1	1	1	1	1	3	11	2	9	4	8	5	2	2

*NC = north coast outside of the Gulf of Carpentaria.

Comparison with <u>Pristis clavata</u>

The results of the AMOVA indicate that the proportion of control region variation between samples of *P. microdon* was much higher (Table 9) than that between samples of *P. clavata* (Table 12). This raises the possibility that the level of population subdivision in *P. microdon* is higher than that in *P. clavata*. Similarly, the haplotype composition of the pooled west coast samples was significantly different to that of the pooled Gulf of Carpentaria samples for *P. microdon* (*P*=0.000), but not for *P. clavata* (*P*=0.9488). Indeed, the same haplotype (#1) essentially dominated all of the samples of *P. clavata* from both the west coast and Gulf of Carpentaria (Table 13). Nevertheless, since the remaining nine haplotypes were restricted to samples from the west coast (Table 13), it is possible that *P. clavata* is subtly subdivided between the Gulf of Carpentaria and the west coast, but that this was not detectable in the present study because of the relatively small number of individuals that were sampled from the gulf (n = 7).

Table 12 Results of analysis of molecular variance (AMOVA) based on the frequency distributions of mtDNA control region sequences of *Pristis clavata*. Variance components and estimates of statistical significance (*P*-values) are indicated. Percentage of variation is indicated in parentheses. Individuals from single sites within the Gulf of Carpentaria were pooled into a single sample for this analysis because of the small number obtained from each site.

Groups	Among samples	Within samples
WA: [80M, KS, FR, PFH] QLD: [WP, MI, STM, G]	0.0154 *P*= 0.2506 (5.16)	0.2832 *P*=0.0024 (94.84)

Table 13 The distribution and abundance of the 10 mtDNA control region haplotypes found in 30 individuals of *Pristis clavata* from 4 sites on the west coast of Australian and 4 sites in the Gulf of Carpentaria. 80M = 80 Mile Beach; KS = King Sound; FR = Fitzroy River; PF = Prince Fredrick Harbour; WP = Weipa; MI = Mangrove Island; SMR = South Mitchell River; G = Gulf of Carpentaria.

Region	**West Coast**				**Gulf of Carpentaria**			
Haplotype Number	**80 M**	**KS**	**FR**	**PFH**	**WP**	**MI**	**SMR**	**G**
1	4	6	1	1	3	1	2	1
2	-	2	-	-	-	-	-	-
3	-	2	-	-	-	-	-	-
4	-	1	-	-	-	-	-	-
5	-	1	-	-	-	-	-	-
6	1	-	-	-	-	-	-	-
7	-	1	-	-	-	-	-	-
8	-	1	-	-	-	-	-	-
9	-	-	-	1	-	-	-	-
10	-	1	-	-	-	-	-	-
Total	5	15	1	2	3	1	2	1

Evolutionary relationships among haplotypes

Pristis microdon

Most of the haplotype divergence in *P. microdon* was centred on haplotypes 1 and 2 (the two abundant and relatively widespread haplotypes), which were separated from each other by only a single mutational step (Figure 5). The maximum number of mutational steps (in the shortest route) between these central (putatively ancestral) haplotypes and the more distal (putatively derived) haplotypes was nine, while the maximum number of mutational steps between any two haplotypes (in the shortest route) in the network was 17 (Figure 5). As mentioned haplotypes 1 and 2 were relatively widespread; similarly there was no strong geographic pattern to the distribution of closely-related 'derived' haplotypes (Figure 5). The combination of the above information indicates that the amount of evolutionary divergence in *P. microdon* in Australian waters is moderate and suggests that the individuals of this species in these waters share a relatively recent evolutionary history. Since this analysis included 90 individuals of *P. microdon* from across its entire Australian distribution (west, north and east coast), the results are likely to be relatively robust, although additional sampling may extend the known geographic distribution of some haplotypes, reveal missing haplotypes, and resolve ambiguities in the network.

Comparison with P. clavata

The haplotype network for *P. microdon* is fundamentally similar to that for *P. clavata* (Figure 6). The only noteworthy difference is that divergence in *P. clavata* was centred on a single (relatively common and widespread) haplotype (Figure 6), rather than two as in *P. microdon*. In conclusion, the fundamental similarities in the haplotype networks of *P. microdon* and *P. clavata* suggest that the evolutionary histories of these two species in Australian waters are similar.

DISCUSSION

This study provides some of the first information about the population genetic structure of *Pristis microdon* in Australian waters or indeed about any species of sawfish. For example, published genetic studies of sawfishes are limited to Watabe (1991), which provides only a comparison of the lactate dehydrogenase isozyme patterns of a total of 12 individuals of *P. microdon* from northern Australian and New Guinea. The present study was motivated by a need to generate information about the biology of populations of *P. microdon* in Australian waters in order to facilitate the assessment of their conservation status and the development of plans for their management. The results are based on samples of this species from across its entire Australian range (west, east and north coasts), but are driven mainly via relatively large samples from the Gulf of Carpentaria and the Fitzroy River on the west coast. The genetic information was derived from a single locus (control region of the mtDNA), which is maternally inherited and therefore strongly influenced by female-mediated gene flow (Avise 1986, Zhang & Hewitt 1997, Reyes & Ochando 2004). The results suggest that *P. microdon* contains 'healthy' levels of genetic diversity over a range of spatial scales in Australian waters. They also demonstrate that this species is subdivided into independent demographic units (populations), rather than comprising a single panmictic population in Australian waters. Nevertheless, the *P. microdon* in these waters appear to belong to a single evolutionary unit.

The outcomes of the neutrality tests suggest that it is appropriate to conclude that the patterns of control region variation in *P. microdon* are selectively neutral, which is not unexpected given that the control region is a non-genic (non-coding) part of the genome (Non *et al.* 2007). Thus, the amount of genetic diversity in *P. microdon* is interpreted to reflect a balance between the 'effective population size' and the mutation rate, and genetic differences between spatially

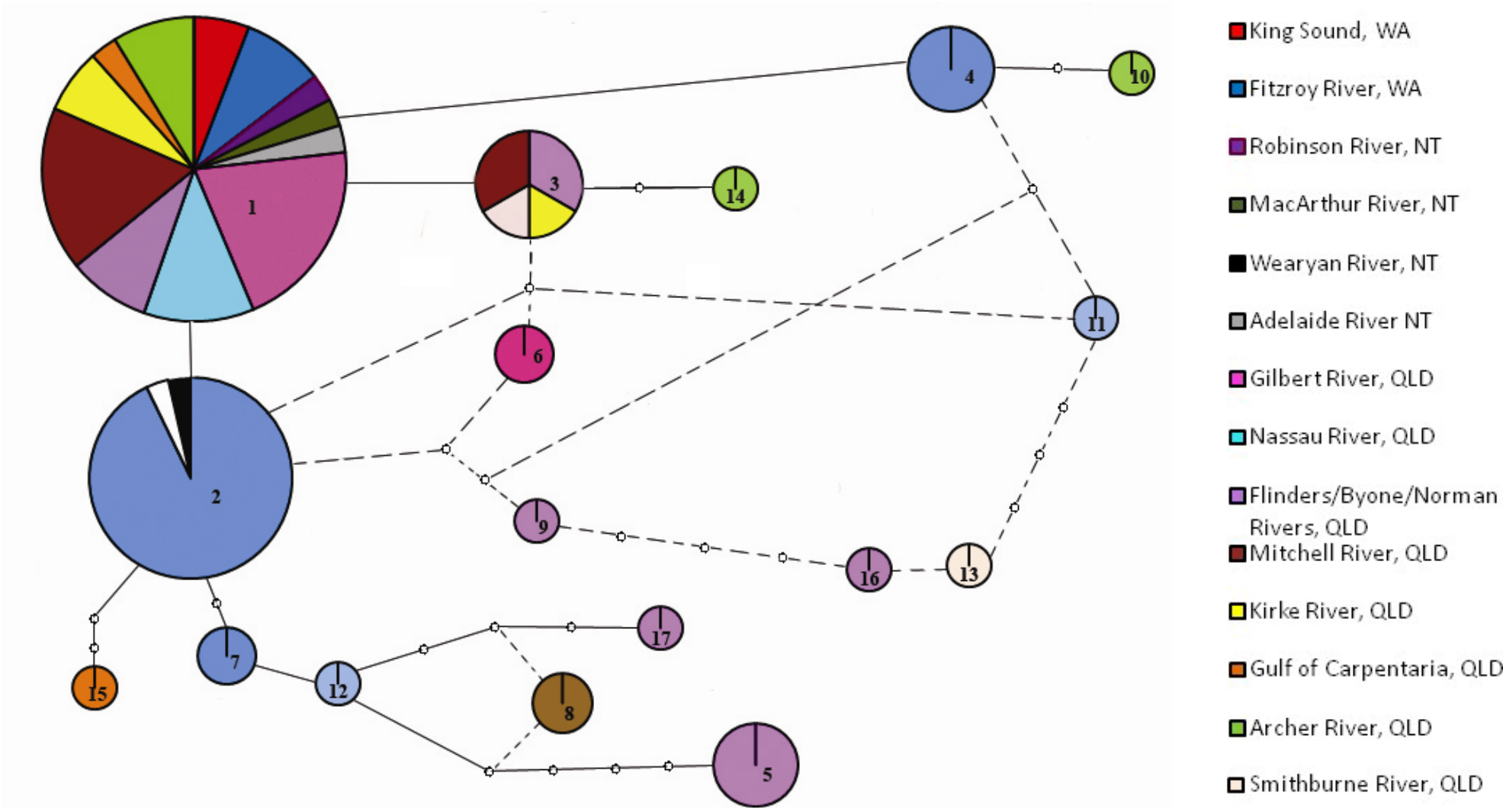


Figure 5 Haplotype network showing the relationships among the 17 control region haplotypes of freshwater sawfish, *Pristis microdon.* Each line in the network shows a single mutational change regardless of the length of the line. A numbered circle is used to represent each haplotypes. The surface area of each circle is proportional to the total number of individuals with that haplotype. The coloured regions of the circles are used to represent the abundance of individuals with each haplotype at each sampling site (see key). Empty circles indicate missing intermediate haplotypes. These missing haplotypes are necessary to link all observed haplotypes present in the network.

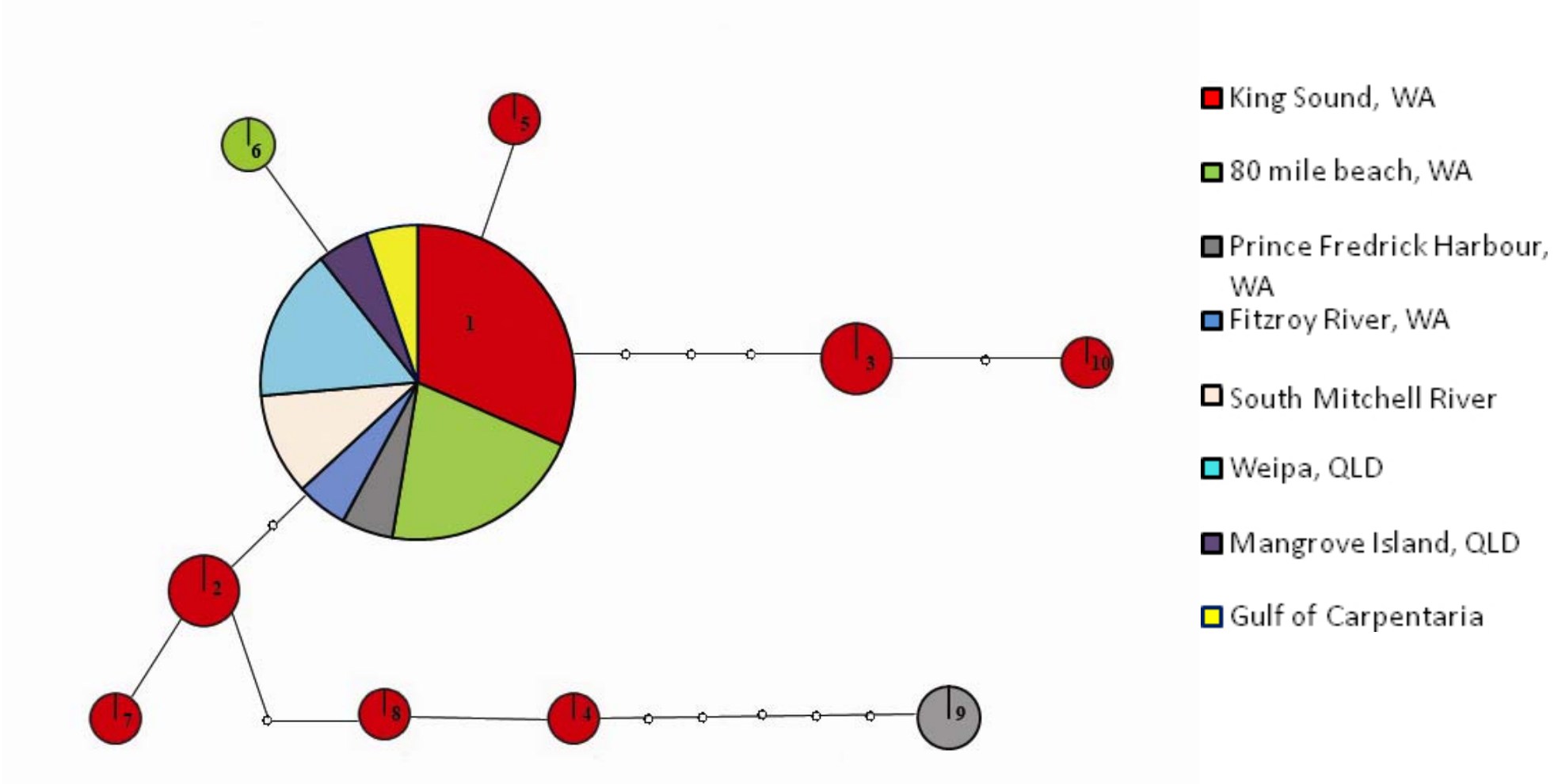


Figure 6 Haplotype network showing the relationships among the 10 control region haplotypes of dwarf sawfish, *Pristis clavata.* Each line in the network shows a single mutational change regardless of the length of the line. A numbered circle is used to represent each haplotypes. The surface area of each circle is proportional to the total number of individuals with that haplotype. The coloured regions of the circles are used to represent the abundance of individuals with each haplotype at each sampling site (see key). Empty circles indicate missing intermediate haplotypes. These missing haplotypes are necessary to link all observed haplotypes present in the network.

isolated assemblages are assumed to be due to reduced rates of gene exchange, rather than to any direct effects of selection on the genetic marker (Carvalho 1998, Bos *et al.* 2008).

The amount of control region diversity present in the samples of *P. microdon* was comparable to that found in *P. clavata* and within the range reported for other species of elasmobranch (Sandoval-Castillo *et al.* 2004, Keeney *et al.* 2005, Duncan *et al.* 2006, Hoelzel *et al.* 2006, Stow *et al.* 2006). This indicates that the present levels of genetic diversity in *P. microdon* are not unusually low, although the amount of diversity to be expected in pristine populations of coastal species of elasmobranch remains elusive because all populations investigated to date have suffered some degree of decline (e.g. Sandoval-Castillo *et al.* 2004, Keeney *et al.* 2005, Hoelzel *et al.* 2006, Stow *et al.* 2006, Lewallen *et al.* 2007). Since population genetics theory indicates that the capacity of a population for evolutionary change depends on the amount of adaptively significant genetic variation therein (Hedrick 1992, Amos & Balmford 2001, Keller & Waller 2002), and assuming that the amount of control region diversity provides a reflection of genome-wide diversity in *P. microdon,* the above finding is encouraging regarding the prognosis for the long-term survival of Australian populations of this species. However, there is a note of caution in that it can take several generations for reductions in the amount of genetic diversity in a long-lived species with overlapping generations, like *P. microdon,* to become apparent (Amos & Balmford 2001, Kuo & Janzen 2004, Goossens *et al*. 2005, Lippe *et al.* 2006). Furthermore, additional work is required to document how the amount of genetic diversity in *P. microdon* varies within and between geographic regions and to investigate the relationship between past and present population sizes and levels of genetic diversity in this species. In addition, most of the control region diversity in *P. microdon* was present in rare haplotypes (i.e. in rare alleles), which are the ones most likely to be lost during population bottlenecks or as abundance declines (Hedrick 1992, Amos & Balmford 2001). Thus, it seems that this species is at a high risk of losing most of its genetic diversity should its abundance decline in the future. Furthermore, since the mutation rate of the mtDNA in elasmobranchs is generally relatively slow (Martin *et al.* 1992, Martin 1995) and the abundance of *P. microdon* relatively low (e.g. in comparison with some teleost fishes)**,** this species could only recover substantial amounts of lost genetic diversity on an evolutionary time-scale, if at all.

The analysis of the population structure of *P. microdon* in Australian waters was necessarily focused on those sites that were sampled relatively intensively, namely the Fitzroy River on the west coast of Australia and the Gulf of Carpentaria. The results indicate that *P. microdon* is genetically sub-divided between these two locations. This finding is significant in terms of the management of this species because it almost certainly means that the demographics of the assemblages of this species in these two locations are independent of each other. These assemblages should thus be regarded as separate management units (populations). However, the smallest spatial scale at which demographically independent units occur in *P. microdon* could not be determined from the results of this study. Thus, it is possible that all of the *P. microdon* in the Gulf of Carpentaria belong to a single population, as might all of the individuals of this species on the west coast. Alternatively, this species may typically comprise multiple populations within these and other geographic regions.

Since the adults of *P. microdon* are relatively large and potentially quite mobile (Simpfendorfer 2002, Peverell 2005, Thorburn *et al*. 2007), and there are no obvious major impediments to the dispersal of such individuals in tropical coastal waters in Australia, the subdivision of *P. microdon* in these waters is most likely associated with some aspect of adult behaviour. In this regard, it may be relevant that there is an increasing body of evidence indicating that sex-biased dispersal, in the form of female philopatry and male dispersal, is relatively common in elasmobranchs (Heist *et al.* 1996, Pardini *et al.* 2001, Feldheim *et al.* 2001, Keeney *et al.* 2003, 2005, Schrey & Heist 2003),

especially in those species that (like *P. microdon*) remain in coastal areas as juveniles but are more wide ranging as adults (Springer 1967, Ebert 1996, Feldheim *et al.* 2001, Keeney *et al.* 2005). If the females of *P. microdon,* in fact, home to their birth place to give birth, then at least the female component of the population structure of this species will be sub-divided over relatively fine spatial scales, possibly at the level of single embayments or even single rivers. Ideally, future studies of the population structure of *P. microdon* should incorporate information from both (maternally-inherited) mitochondrial and (bi-parentally inherited) nuclear DNA markers so that the effects of any sex-biased dispersal on this structure can be disentangled (e.g. Pardini *et al.* 2001, Keeney *et al.* 2005).

The extent and distribution of control region variation indicates that the *P. microdon* in Australian waters share a relatively recent evolutionary history. Thus, there do not appear to be any particular subsets of individuals or populations that warrant special conservation priorities on the basis of their relatively unique evolutionary histories. A more limited data set indicates that this is also the case for the Australian *P. clavata*. This suggests that these two species have similar evolutionary histories in this region, although the details of these histories remain to be elucidated.

Management implications

Two findings of this study are critical with regards to the development of plans for the conservation of Australia populations of *P. microdon,* as follows. Firstly, while the levels of genetic diversity in assemblages of this species in these waters were not unusually low in the context of contemporary elasmobranch populations, much of this diversity may be present in rare alleles, which are highly susceptible to loss via genetic drift. This suggests that the prognosis for the genetic health and long-term survival of Australian populations of this species is good, providing that measures are put in place to curb any further declines in its abundance and genetic diversity of these populations. Secondly, the assemblages of *P. microdon* in the Gulf of Carpentaria and the Fitzroy River on the west coast, and probably also in other locations as well, represent different biological populations, although it is not currently possible to elucidate the fine-scale spatial boundaries of these populations. Since they are demographically independent of each other, the demise of one population will not be offset by immigration from another. Thus, the conservation of each population should be a high priority. In addition, data relating to the demographics (e.g. abundance, mortality, recruitment) of *P. microdon* should be collected and interpreted at the level of individual populations. Finally, the results of this research have highlighted the need for additional information about the population genetic structure of *P. microdon* in Australian waters.

REFERENCES

Amos, W. & Balmford, A. (2001). When does conservation genetics matter? *Heredity* **87**: 257-265. Applied Biosystems, Incorporated (2001). Seq Ed version 1.0.3.

Avise, J. C. (1986). Mitochondrial DNA and the evolutionary genetics of higher animals. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **312**: 325–342.

Avise, J. C. (1994). *Molecular Markers, Natural History and Evolution.* Chapman & Hall, New York, N.Y.

Avise, J. & Hamrick, P. (eds). (2004). *Conservation genetics: case studies from nature.* Chapman & Hall, New York, N.Y.

Bigelow, H. B. & Schroeder, W. C. (1953). Sawfishes, guitarfishes, skates and rays Fisheries of the Western North Atlantic. *Memoir Sears Memorial Foundation for Marine Research* **1**: 1-514.

Bijlsma, R., Bundgaard, J., & Boerema, A. C. (2000). Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila. Journal of Evolutionary Biology* **13**: 502-514.

Blaylock, R.A. (1990). Effects of external biotelemetry transmitters on behavior of the cownose ray *Rhinoptera bonasus* (Mitchill 1815). *Journal of Experimental Marine Biology and Ecology.* **141***:* 213-220.

Bos, D. H., Gopurenko, D., Williams, R. N., DeWoody, J. A. (2008). Inferring population history and demography using microsatellites, mitochondrial DNA and major histocompatibility complex (MHC) genes. *Evolution :* doi:10.1111/j.1558-5646.2008.00364.x

Carvalho, G. R. (1998). *Advances in molecular ecology.* IOS Press, Netherlands.

Cerrato, R. M. (1990). Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1416-1426.

Chidlow, J.A. (2007). First record of the freshwater sawfish, *Pristis microdon*, from southwestern Australia. *Records of the Western Australian* **23**: 307-308.

Clarke, K.R. & Gorley, R.N. (2006). *PRIMER v6.* Primer-E Ltd., Plymouth, UK.

Clement, M., Posada, D., & Crandall, K.A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9** (10): 1657-1659.

Compagno, L. J. V. (1977). Phyletic relationships of living sharks and rays. *American Zoologist* **17:** 303-322.

Compagno, L.J.V. & Last, P.R. (1998). Order Pristiformes Pristidae sawfishes. *In*: K.E. Carpenter & Niem, V.H. (Eds) *FAO Species Identification Guide for Fisheries Purposes. The Living Marine Resources of the Western Central Pacific*. Volume 3. Batoid fishes, chimaeras and bony fishes part 1 (Elopidae to Linophrynidae). FAO, Rome, 1410–1417.

Compagno, L.J.V. & Niem, V.H. (1998). Order Carcharhinidae. In: Carpenter, K.E. and Niem, V.H. (Eds), *FAO Species Identification Guide for Fisheries Purposes. The Living Marine Resources of the Western Central Pacific*. Volume 2. Cephalopods, crustaceans, holothurians and sharks. FAO, Rome, 1312-1360.

Coulon, A., Cosson, J. F., & Angibault, J. M. (2004). Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, **13**: 2841–2850.

DeSalle, R. & Amato, G. (2004). Expansion of conservation genetics. *Nature Reviews Genetics* **5**: 702-711.

Dewar, H., Domeier, M. & Nasby-Lucas, N. (2004). Insights into young of the year white shark, *Carcharodon carcharias*, behavior in the Southern California Bight. *Environmental Biology of Fishes* **70**: 133-143.

Deynat, P. P. (2005). New data on the systematics and interrelationships of sawfishes (Elasmobranchii, Batoidea, Pristiformes). *Journal of Fish Biology* **66**: 1447-1458.

Duncan, K. M., Martin, A. P., Bowen, B. W., & de Couet, H. G. (2006). Global phylogeography of the scalloped hammerhead *Sphyrna lewini. Molecular Ecology* **15**: 2239–2251.

Ebert, D. A. (1996). Biology of the sevengill shark, *Notorynchus cepedianus* (Peron, 1807), in the temperate coastal waters of southern Africa. *South African Journal of Marine Science* **17**, 93–103.

EPBC Act of 1999. *EPBC act Lists*. [Internet]: http://www.environment.gov.au/epbc/index.html. Accessed May 2008.

Excoffier, L., Laval, G., & Schneider, S. (2005). Arelquin version 3.0: An integrated software package for populations genetics data analysis. *Evolutionary Bioinformatics Online* **1**: 47-50.

Excoffier, L., Smouse, P.E., & Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics* **131**: 479-491.

Feldheim, K. A., Gruber, S. H., Ashley, M. V. (2001). Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology* **10**: 295–303.

Fu, Y.X. (1997). Statistical tests of neutrality against population growth, hitchhiking and background selection. *Genetics* **147**: 915-925.

Goossens, B., Chikhi, L., Jalil, M. F., Ancrenaz, M., Lackman-Ancrenaz, I., Mohamed, M., Andau, P., & Bruford, M. W. (2005). Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. *Molecular Ecology* **14** (2): 441-456.

Gu, X., & Zhang, J. (1997). A simple method for estimating the parameter of substitution rate variation among sites. *Molecular Biology and Evolution* **14**:1106-1113.

Hather, S. (2008). *SatScape v2.02 Pro*. Freeware [Internet]: www. satscape.com. Downloaded June 2007.
Hedrick, P. W. and Miller, P. S. (1992). Conservation genetics: techniques and fundamentals. *Ecological Applications* **2 (1)**: 30-46.
Heist, E. J., Musick, J. A., & Graves, J. E. (1996). Genetic population structure of the shortfin mako (*Isurus oxyrinchus*) inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 583-588.
Heupel, M.R. & Simpfendorfer, C.A. (2005). Quantitative analysis of aggregation behavior in juvenile blacktip sharks. *Marine Biology* **147**: 1239-1249.
Hoelzel, A. R., Hancock, J. M., & Dover, G. A. (1991). Evolution of the cetacean mitochondrial D-loop region. *Molecular Biology and Evolution* **8**: 475-493.
Hoelzel, A. R., Shivji, M. S., Magnussen, J., & Francis, M. P., (2006). Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology Letters* **2**: 639-642.
Holland, K.N., Wetherbee, B.M., Lowe, C.G. & Meyer, C.G. (1999). Movements of tiger sharks (*Galeocerdo cuvier*) in coastal Hawaiian waters. *Marine Biology* **134**: 665-673.
Ishihara, H., Taniuchi, T., & Shimizu, M. (1991). Sexual dimorphism in number of rostral teeth in the sawfish, *Pristis microdon* collected from Australia and Papua New Guinea. *University Museum, University of Tokyo, Nature and Culture* **3**: 83-89.
IUCN 2006. *2006 IUCN Red List of Threatened Species.* [Internet]: www.iucnredlist.org. Accessed April 2008.
IUCN 2007. *2007 IUCN Red List of Threatened Species*. [Internet]: www.iucnredlist.org. Accessed May 2008.
Keeney, D. B., Heupel, M., Hueter, R. E., & Heist, E. J. (2003). Genetic heterogeneity among blacktip reef shark, *Carcharhinis limbatus,* continental nurseries along the U. S. Atlantic and Gulf of Mexico. *Marine Biology* **143**: 1039-1046.
Keeney, D. B., Heupel, M. R., Hueter, R. C., & Heist, E. J. (2005). Microsatellite and mitochondrial DNA analyses of genetic structure of blacktipshark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Molecular Ecology* **14**: 1911-1923.
Keller, L. F. & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**: 230–241.
King, M. (1995). *Fisheries Biology, Assessment and Management.* Fishing News Books, London 94-95.
Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X. & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA* **86**: 6196-6200.
Kohn, M. H., Murphy, W. J., Ostrander, E. A., & Wayne, R. K. (2006). Genomics and conservation genetics. *Trends in Ecology & Evolution* **21** (11): 629-637.
Kuo, C. H. & Janzen, F. J. (2004). Genetic effects of a persistent bottleneck on a natural population of ornate box turtles (*Terrapene ornate). Conservation Genetics* **5** (4): 425-437.
Lacy, R. C. (1997). Importance of genetic variation in the viability of mammalian populations. *Journal of Mammology* **78**: 320-335.
Last, P. R. & Stevens, J. D. (1994). *Sharks and Rays of Australia.* CSIRO Division of Fisheries, Melbourne, Australia.
Lewallen, E. A., Anderson, T. W., & Bohonak, A. J. (2007). Genetic structure of leopard shark (*Triakis semifasciata*) populations in California waters. *Marine Biology* **152**: 599-609.
Lippe, C., Dumont, P., & Bernatchez, L. (2006). High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. *Molecular Ecology* **15** (7): 1769-1780.
Lopez, J. V., Culver, M., Stephens, J. C., Johnson, W. E., & O'Brien, S. J. (1997). Rates of nuclear and cytoplasmic mitochondrial DNA sequence divergence in mammals. *Molecular Biology and Evolution* **14**: 277-286.
Manchado, M., Catanese, G., Ponce, M., Funes, V., & Infante, C. (2007). The complete mitochondrial genome of the Senegal sole, Solea senegalensis Kaup. Comparative analysis of tandem repeats in the control region among soles. *DNA Sequencing* **18**: 169–175.
Martin, A. P., Naylor, G. J. P. & Palumbi, S. R. (1992). Rates of mtDNA evolution in sharks are slow compared to humans. *Nature* **357**: 153–155.
Marin, A. P. (1995). Mitochondrial DNA sequence evolution in sharks-rates, patterns, and phlogenetic inferences. *Molecular Biology and Evolution* **12**: 1114-1123.
Morgan, D., Allen, M., Bedford, P. & Horstman, M. (2002). *Inland Fish Fauna of the Fitzroy River Western Australia, including the Bunuba, Gooniyandi, Ngarinyin, Nyikina, and Walmajarri Aboriginal names.* Report to the Natural Hertiage Trust: 56 pp.
Morgan, D., Allen, M., Bedford, P., & Horstman, M. (2004). Fish Fauna of the Fitzroy River in the Kimberley region of Western Australia-including the Bunuba, Gooniyandi, Ngarinyin, Nyikina, and Walmajarri Aboriginal names. *Records of the Western Australian Museum* **22**: 147-161.

Morgan, D., Thorburn, D., Fenton, J., Wallace-smith, H. & Goodson, S. (2005). *Influence of the Camballin Barrage on fish communities in the Fitzroy River, Western Australia*. Murdoch University/Kimberley Land Council/Department of Environment report to Land and Water Australia.

Moritz, C. (2002). Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* **51** (2): 238-354.

Mulligan, C. J., (2005). Isolation and analysis of DNA from archaeological, clinical, and natural history specimens. *Methods of Enzymology* **395**: 87-102.

Nakano, H., Matsunaga, H., Okamoto, H. & Okazaki, M. (2003). Acoustic tracking of bigeye thresher shark Alopias superciliosus in the eastern Pacific Ocean. *Marine Ecology Progress Series* **265**: 255-261.

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York, USA.

Non, A. L., Kitchen, A. & Mulligan, C. J. (2007). Identification of the most informative regions of the mitochondrial genome for phylogenetic and coalescent analyses. *Molecular Phylogenetics and Evolution* **44**: 1164-1171.

Nylander, J. A. A. (2004). MrAIC.pl. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pardini, A. T., Jones, C. S., Noble, L. R., Kreiser, B., Malcom, H., Bruce, B. D., Stevens, J. D., Cliff, G., Scholl, M. C., Francis, M., Duffy, C. A. J., & Martin, A. P. (2001). Sex-biased dispersal in great white sharks. *Nature* **412**: 139-140.

Pearce, J. M. (2006). Minding the gap: frequency of indels in mtDNA control region sequence data and influence on population genetic analyses. *Molecular Ecology* **15**: 333-341.

Peverell, S. C. (2005). Distribution of sawfishes (Pristidae) in the Queensland Gulf of Carpentaria, Australia, with notes on sawfish ecology. *Environmental Biology of Fishes* **73**: 391-402.

Phillips, N. (2006). *Development of genetic and morphological methods for the study of sawfish (Pristidae) populations via analysis of old rostra*. Honours Thesis, Murdoch University, Perth, Western Australia.

Pillans, R.D., Stevens, J.D., Kyne, P.M. & Salini, J. (2005). Acoustic tracking of *Glyphis* sp. A in the Adelaide River, Northern Territory, Australia. Report to the Department of the Environment and Heritage: 22 pp.

Pogonoski, J. J., Pollard, D. A., & Paxon, J. R. (2002). *Conservation overview and action plan for Australian threatened and potentially threatened marine and estuarine fishes*. Environment Australia. Department of the Environment and Hertitage.

Ponce, M., Infante, C., Jimenez-Cantizano, R. M., Perez, L., Manchado, M. (2008). Complete mitochondrial genome of the blackspot seabream, *Pagellus bogaraveo* (Perciformes: Sparidae), with high levels of length heteroplasmy in the WANCY region. *Gene* **409**: 44-52.

Qiagen, 2000. Qiaquick® Handbook.

Raymond, M. & Rousset, F. (1995). An exact test for population differentiation. *Evolution* **49**: 1280-1283.

Reyes, A. & Ochando, M. D. (2004). Mitochondrial DNA variation in Spanish populations of *Ceratitis capitata* (Wiedemann) (Tephritidae) and the colonization process. *Journal of Applied Entomology* **128** (5): 358-364

Rice, W.R. (1989). Analysing tables of statistical significance. *Evolution* **43**: 223-225.

Sandoval-Castillo, J., Rocha-Olivares, A., Villavicencio-Garayzar, C., & Balart, E. (2004). Cryptic isolation of Gulf of California shovelnose guitarfish evidenced by mitochondrial DNA. *Marine Biology* **145**: 983–988.

Saunders, D. L., Meeuwig, J. J., & Vincent, A. C. J. (2002). Freshwater protected areas: strategies for conservation. *Conservation Biology* **16** (1): 30-41.

Schrey, A. W. & Heist, E. J. (2003). Microsatellite analysis of population structure in the shortfin mako and cross-species amplification in Lamniformes. *Conservation Genetics* **3**: 459-461.

Sibert, J. and Fournier, D. (2001). Possible models for combining tracking data with conventional tagging data. In: Sibert J.R., Nielsen, J.L. (Eds) *Electronic Tagging and Tracking in Marine Fisheries*. Kluwer Academic Publishers, Dordrecht, p 443-456.Simpfendorfer, C. A. (2000). Predicting population recovery rates for endangered western Atlantic sawfishes using demographic analysis. *Environmental Biology of Fishes* **58**: 371-377.

Simfendorfer, C. A. (2000). Predicting recovery rates for endangered western Atlantic sawfishes using demographic analysis. *Environmental Biology of Fishes* **58**: 371-377.

Simfendorfer, C. A. (2002). Smalltooth Sawfish: The USA's First Endangered Elasmobranch? *Marine Matters* **19**: 45-49.

Simpfendorfer, C. A. (2006). *Movement and Habitat use of Smalltooth Sawfish*. Mote Marine Laboratory Technical Report **1070**: 26 pp.

Skomal, G.B. & Benz, G.W. (2004). Ultrasonic tracking of Greenland sharks, *Somniosus microcephalus*, under Arctic ice. *Marine Biology* **145**: 489-498.

Springer, S. (1967). Social organisation of shark populations, In: *Sharks, Skates, and Rays,* Gilbert, P.W., Mathewson, R. F. & Rall, D. P., Eds, Johns Hopkins University Press, Baltimore, 149-174.

Staunton-Smith, J., Robins, J.B., Mayer, D.G., Sellin, M.J., & Halliday, I.A. (2004). Does the quality and timing of fresh water flowing into a dry tropical estuary affect year-class strength of barramundi (*Lates calcarifer*)? *Marine and Freshwater Research* **55**: 787-797.

Stevens, J.D., West, G.J. & McLoughlin, K.J. (2000). 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.

Stobutzki, I. C., Stevens, J., Gribble, N., Buchworth, R., Lenanton, R., McAuley, R., & McLoughlin, K. (2002). Sustainability of elasmobranchs caught as bycatch in a tropical prawn (shrimp) trawl fishery. *Fishery Bulletin* **100**: 800-822.

Stow, A., Zenger, K., Briscoe, D., Gillings, M., Peddemors, V., Otway, N., & Harcourt, R. (2006). Isolation and genetic diversity of endangered grey nurse shark (*Carcharias taurus*) populations. *Biology Letters* **2**: 308-311.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512-526.

Tanaka, S. (1991). Age estimation of freshwater sawfish and sharks in northern Australia and Papua New Guinea. *University Museum, University of Tokyo, Nature and Culture* **3**: 71-82.

Taniuchi, T., Kan, T. T., Tanaka, S., & Otake, T. (1991). Collection and measurement data and Diagnostic characters of elasmobranchs collected from three river systems in Papua New Guinea. *University Museum, University of Tokyo, Nature and Culture* **3**: 27-41.

Templeton, A.R., Crandall, K.A., & Sing, C.F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.

Thorburn, D.C. (2006). *Biology, ecology and trophic interactions of elasmobranchs and other fishes in riverine waters of northern Australia*. PhD Thesis, Murdoch University, Perth, Western Australia.

Thorburn, D.C. & Morgan, D.L. (2005). Threatened fishes of the world: *Glyphis* sp. C (Carcharhinidae). *Environmental Biology of Fishes* **73**: 140.

Thorburn, D.C. & Morgan D.L. (2004). The northern river shark *Glyphis* sp. C (Carcharhinidae) discovered in Western Australia. *Zootaxa* **685**: 1-8.

Thorburn, D., Morgan, D., Gill, H., Johnson, M., Wallace-Smith, H., Vigilante, T., Croft, I. & Fenton, J. (2004). *Biology and cultural significance of the freshwater sawfish (Pristis microdon) in the Fitzroy River Kimberley, Western Australia*. Report to the Threatened Species Network.

Thorburn, D. C., Morgan, D. L., Rowland, A. J., & Gill, H. S. (2007). Freshwater Sawfish *Pristis microdon* Latham, 1794 (Chondrichthyes : Pristidae) in the Kimberley region of Western Australia. *Zootaxa* **1471**: 27-41.

Thorburn, D.C., Morgan, D.L., Rowland, A.J., Gill, H.S. & Paling, E. (in press). Life history notes of the critically endangered Dwarf Sawfish Pristis clavata Garman 1906 from the Kimberley region of Western Australia. *Environmental Biology of Fishes*.

Thorburn, D. C., Peverell, S., Stevens, J. D., Last, P. R., & Rowland, A. J. (2003). *Elasmobranchs in the Fitzroy River, Western Australia.* Report to the Natural Heritage Trust.

Thorson, T. B. (1973). Sexual dimorphism in number of rostral teeth of the sawfish, *Pristis perotteti* Muller and Henle, 184 1. *Transactions of the American Fisheries Society* **102**: 612-614.

Thorson, T.B. (1982). Life history implications of a tagging study of the largetooth sawfish, *Pristis perotteti*, in Lake Nicaragua-Rio San Juan System. *Environmental Biology of Fishes* **7**: 207–228.

Voegeli, F.A., Smale, M.J., Webber, D.M., Andrade, Y. & O'Dor, R.K. (2001). Ultrasonic telemetry, tracking and automated monitoring technology for sharks. *Environmental Biology of Fishes* **60**: 267-281.

Ward, R. D. (2000). Genetics in fishery management. *Hydrobiologia* **420**: 191-201.

Watabe, S. (1991). Electrophoretic analyses of freshwater elasmobranchs from northern Australia and Papua New Guinea. *The University Museum, the University of Tokyo, Nature and Culture* **3**: 103-109.

Weir, B. S. and Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

Wilson, D. (1999). Freshwater sawfish *Pristis microdon*. In: *Australia New Guinea Fishes Association' A-Z Notebook of Native Freshwater Fish (Australia New Guinea Fishes Association Bulletin)*. 41 pp.

Wishart, D. S., Stothard, P., & Van Domselaar, G. H. (2000). PepTool and GeneTool: platform-independent tools for biological sequence analysis. *Methods of Molecular Biology* **132**: 93–113.

Wolanski, E. & Spagnol, S. (2003). Dynamics of the turbidity maximum in King Sound, tropical Western Australia. *Estuarine, Coastal and Shelf* Science **56**: 877-890.

Uzzell, T. & Corbin, K.W. (1971). Fitting discrete probability distributions to evolutionary events. *Science* **172**: 1089-1096.

Zhang, D.X., Hewitt, G. M. (1997): Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. *Biochemical Systematics and Ecology* **25**: 99–120.