Freshwater Sawfish (*Pristis microdon*) movements and population demographics in the Fitzroy River, Western Australia and genetic analysis of *P. microdon* and *Pristis zijsron*

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

Report to

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

Section I

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Section II

N.M. Phillips, J.A. Chaplin, D.L. Morgan and S.C. Peverell[1] (2009). Microsatellite and mitochondrial DNA assessment of the genetic diversity and population structure of the Freshwater Sawfish, *Pristis microdon*, and the Green Sawfish, *Pristis zijsron,* respectively, in Australian waters: preliminary results. In: Phillips, N.M., Whitty, J.M., Morgan, D.L. Chaplin, J.A. Thorburn D.C. and Peverell[1], S.C. (eds). *Freshwater Sawfish (Pristis microdon) movements and demographics in the Fitzroy River, Western Australia and genetic analysis of P. microdon and Pristis zijsron*. Centre for Fish & Fisheries Research (Murdoch University) report to the Department of the Environment, Water, Heritage and the Arts, Australian Government.

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

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SUMMARY

This report is a continuation of the study that was presented to the DEWHA in 2008 entitled:

Whitty, J.M., Phillips, N.M., Morgan, D.L., Chaplin, J.A., Thorburn, D.C. & Peverell, S.C. (2008). *Habitat associations of Freshwater Sawfish (Pristis microdon) and Northern River Shark (Glyphis sp. C): including genetic analysis of P. microdon across northern Australia*. Centre for Fish & Fisheries Research, Murdoch University report to Australian Government, Department of the Environment, Water, Heritage and the Arts.

Elements of that study have since been published as two journal articles:

Phillips, N., Chaplin, J., Morgan, D. & Peverell, S. (2009). Extraction and amplification of DNA from the dried rostra of sawfishes (Pristidae) for applications in conservation genetics. *Pacific Conservation Biology* **15** (2): 128-134.

Whitty, J.M., Morgan, D.L., Peverell, S.C., Thorburn, D.C. & Beatty, S.J. (2009). Ontogenetic depth partitioning by juvenile freshwater sawfish (*Pristis microdon*: Pristidae) in a riverine environment. *Marine and Freshwater Research* **60**: 306-316.

Section I reports on the habitat use, movements, morphology, population demographics and distribution of the critically endangered Freshwater Sawfish (*Pristis microdon*) and Northern River Sharks (*Glyphis garricki*) in the Fitzroy River and King Sound, Western Australia in 2008 and 2009.

Between 2008 and 2009, 39 *P. microdon* were captured in the Fitzroy River. In October 2008, an additional *P. microdon* (new recruit) was found dead on the banks of Telegraph Pool with a fishing hook and wire trace attached to it, while another (2400 mm TL) was documented to have been pulled from the water and left to die near an upstream freshwater pool. These 41 individuals included 28 *P. microdon* between 812 and 1065 mm TL (i.e. 0+), three between 1423 and 1429 mm TL (i.e. 1+), four between 1883 and 1934 mm TL (2+), four between 2090 and 2210 mm TL (3+) and two between 2400 and 2500 mm TL (4+). One *G. garricki* was also captured in July 2008 near Frazer River in King Sound. Capture of a maturing *G. garricki* on the south-western coast of King Sound led to an extension of their home range throughout turbid waters in King Sound. All captured individuals were measured and tagged with a Rototag, while 16 *P. microdon* were also fitted with an external acoustic transmitter complimented with a temperature and pressure sensor. Transmitters were passively monitored using Vemco VR2W acoustic receivers. Originally installed in 2007 and consisting of six receivers, continued funding of this project allowed for an additional four receivers to be installed. Nine receivers spanning 150 km of the lower Fitzroy River comprised the working 2008/2009 acoustic array.

From catch records between 2002 and 2009, it was observed that the catch per unit effort (CPUE) of *P. microdon* in the lower 150 km of the Fitzroy River has declined, and is attributed to a reduction in our catches of older (> 1+) fish in recent years. In contrast, recruitment was relatively high during the last few years, however, between 2002 and 2005 few new recruits were recorded; subsequently leading to reduced catches of older age classes since 2006. There were also seasonal decreases in CPUE between the early and late dry season and this may represent a period of higher mortality. Stage height (discharge) may influence mortality rates, as has been shown for many other estuarine and freshwater species (Mills & Mann 1985, Drinkwater & Frank 1994), and a significant relationship between the proportion of new recruits in our catches and late wet season (i.e. April) discharge was found.

The range of both male and female teeth counts was expanded and the relationship between total length (TL) and total rostrum length (TRL) of juvenile *P. microdon* was strengthened. This data will assist in the understanding of historical and future *P. microdon* catches from dried rostra that are collected as trophies or discarded on the banks (Thorburn 2006, Thorburn *et al*. 2007, Section II). There remains an inadequate records of mature individuals, which has resulted in a scarcity of morphological data for adult *P. microdon* and a lack of understanding about the morphology and habitats of this species beyond 3 m. It is important to locate and sample adult *P. microdon* populations.

Continuation of the Rototag program has led to recaptures reported by fishers in 2008 and aided in the growth of data for this species, while we recaptured 10 of these individuals, one twice. In 2008 and 2009, publicity of the program was delivered in 15 different talks in the Western Kimberley to catchment groups (FITZCAM), Indigenous communities (Jarlmadangah, Looma, Fitzroy Crossing), schools (all science classes in Broome Senior High School) and different government and public bodies as well as a number of presentations at four different scientific conferences throughout Australia and the US. Involvement of Traditional Owners, including the invaluable assistance by the Yiriman Rangers, continued in 2008 and 2009. National Geographic also recently aired the research in the US.

Acoustic tag data from 2008 and 2009 showed similar patterns to that observed in 2007. Depth sensor data showed *P. microdon* to display a diel vertical movement pattern also observed in 2007. Although this pattern was similar for all tracked *P. microdon*, the timing and rate of ascension was found to be different between *P. microdon* in the turbid estuarine pools and those in the less turbid freshwater pools. As light intensity was shown to be one of the primary cues for this movement, it is hypothesised that the difference in ascensions are a result of the differences in turbidity. *Pristis microdon* were also observed to alter their depth use with changing environment, including water depth, season and pool.

Section II provides the preliminary results of an analysis of spatial patterns of microsatellite variation in *P. microdon*, and of mitochondrial DNA variation in *Pristis zijsron*, in Australian waters. This research is part of an on-going investigation into the genetic diversity and population structure of *Pristis* sawfishes in these waters. The results for *P. microdon* are based upon the analysis of variation at three tetranucleotide microsatellite loci in 22 or more individuals from the Fitzroy River region and 38 or more from the Gulf of Carpentaria region, with at least 60 individuals genotyped for each locus. The results for *P. zijsron* are based upon variation in the nucleotide sequence of a 352-bp portion of the control region in the mitochondrial genome in a total of 42 individuals from three geographic regions, namely the west coast ($N = 22$), the Gulf of Carpentaria ($N = 9$), and the east coast ($N = 11$).

The levels of diversity at each of the three microsatellite loci in *P. microdon* were generally high or moderate (depending on the locus) and were comparable between the samples from the Fitzroy River and Gulf of Carpentaria regions. This finding, together with the mtDNA results of Phillips *et al.* (2008), suggests the levels of genetic diversity in the assemblages of *P. microdon* in these two regions are not unusually low. Since genetic variation is a prerequisite for evolutionary change, this finding is encouraging regarding the prognosis for the long-term survival of these assemblages. However, there is a note of caution in that it can take several generations for a decline in genetic diversity to become apparent in a species like *P. microdon*, which is long-lived with overlapping generations. It is also important to note that it would take a considerable amount of time (if ever) for a species like *P. microdon*, with

a low intrinsic rate of increase, to recover substantial amounts of lost diversity. Therefore, it is crucial to manage the assemblages of *P. microdon* in each of the Fitzroy River and Gulf of Carpentaria regions such that the current levels of abundance, and so genetic diversity, are maintained.

There was no evidence of any genetic differentiation at the microsatellite loci in *P. microdon* between the Fitzroy River and Gulf of Carpentaria regions, as the allelic frequencies in the samples from these two regions were effectively homogeneous. This apparent lack of genetic differentiation in the bi-parentally inherited microsatellite markers is in marked contrast to the situation occurring in a maternally inherited mitochondrial marker, which exhibits a relatively high level of differentiation between these two regions (Phillips *et al.* 2008). In combination, these results raise the possibility that *P. microdon* exhibits sex-biased dispersal in Australian waters, where dispersal in females and males is, respectively, philopatric and relatively widespread. If dispersal in *P. microdon* is male-biased, this would have significant implications for the management plans for this species as they would need to take into account the fact that a decline in the number of females in a particular geographic region would not be replenished by the immigration of females from another region, coupled with the fact that a decline in the abundance of this species in one region could have direct implications on its abundance in another region (via male dispersal). However, information from additional loci and additional analyses are required to properly document spatial patterns of microsatellite variation in *P. microdon* in Australian waters and to confirm (or otherwise) the presence of male-biased dispersal in this species.

The overall level of haplotype diversity in the control region in *P. zijsron* was moderate and generally comparable to that which has been reported for both *P. microdon* and *P. clavata* in Australian waters (Phillips *et al.* 2008). However, the overall amounts of nucleotide diversity and evolutionary divergence in *P. zijsron* appear to be slightly reduced relative to those in other *Pristis* species. More importantly, the levels of both haplotype and nucleotide diversity in *P. zijsron* from the Gulf of Carpentaria were low, both in absolute terms and relative to those in the samples of this species from each of the west and east coasts (and also relative to *P. microdon* in the gulf region) (Phillips *et al.* 2008). If verified (by larger sample sizes), this would suggest that the abundance of *P. zijsron* in the Gulf of Carpentaria has been substantially reduced and the assemblage of this species in this gulf may warrant special protection to prevent any further decline in abundance and genetic diversity.

The control region data also suggest that *P. zijsron* is genetically sub-divided within its Australian range. This research has not fully resolved the pattern and scale of this sub-division, but at least the female component of the assemblages on at least the east and west coasts appear to effectively be isolated from each other and should be managed separately. More samples are needed to properly assess the relationships between the assemblage of *P. zijsron* in the Gulf of Carpentaria and those on the west and east coasts. Future research should also incorporate information from both maternally inherited mitochondrial and bi-parentally inherited nuclear DNA markers as males and females of *P. zijsron* may have very different patterns of gene flow, as is believed to be the case in *P. microdon.*

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SECTION I

Movements and interannual variation in the morphology and demographics of Freshwater Sawfish (*Pristis microdon*) in the Fitzroy River

by

J.M. Whitty, D.L. Morgan and D.C. Thorburn

INTRODUCTION

The Freshwater Sawfish (*Pristis microdon*) is listed as Critically Endangered by the IUCN (2006) and as vulnerable by the *Environment Protection and Biodiversity Conservation Act* (EPBC) *1999* in Commonwealth waters of Australia. Previous research indicates that *P. microdon* is widespread throughout the Fitzroy River, Western Australia, where it is predominantly found in the main channel and within one of its major tributaries, namely the Margaret River (Figure 1) (Morgan *et al.* 2002, 2004, Thorburn *et al.* 2004, 2007).

This part of the study aimed to continue the biological and ecological examination of *P. microdon* in the Fitzroy River, Western Australia via acoustic and conventional tagging and examination and collation (with previous years) of morphological data collected annually between 2002 and 2009 (see Thorburn *et al*. 2007, Whitty *et al*. 2008). Morphological and conventional tagging data have been generated from annual monitoring of the population since 2002 and 2003, respectively, and represents the only long-term data available for *P. microdon*. Acoustic tracking commenced in 2007 (see Whitty *et al*. 2008, 2009) and was continued through 2008 and 2009. This section of the report describes the outcomes of acoustic telemetry from 2008 to 2009 and compares this with data presented from 2007 (see Whitty *et al*. 2008, 2009). Also presented is a continuation of the search for habitats of the Northern River Shark (*Glyphis garricki*) within King Sound, and further information is provided on the distribution, morphology and ecology of the species in addition to that described in Thorburn & Morgan (2004) and Whitty *et al*. (2008).



METHODOLOGY

Study sites and techniques

During the dry season, June to November, of 2008 and in May and September 2009, a total of five different sites (Snag Pool, Telegraph Pool, Myroodah Crossing, Camballin Pool and Lower Barrage Pool) (described in Whitty *et al*. 2008, 2009) in the lower ~160 km of the Fitzroy River, Western Australia were sampled for *P. microdon* (Figure 1 & 2). Milli Milli, Snag Pool, Telegraph Pool and Langi Crossing are located within the tidally influenced estuarine region (lower 16 km) of the Fitzroy River. Having tides > 11 m twice daily stemming from King Sound into this region, the water column is often well mixed and highly turbid. During the dry season, depth of the estuarine pools is often less than 2.0 m with occasionally deeper holes. Langi Crossing is the exception with depths of > 3 m. Myroodah Crossing, Camballin Pool and Lower Barrage Pool as well as the Upper Barrage Pool are located in a freshwater region of the river approximately 120-160 km from the river mouth. These areas are characteristically narrow (50-80 m wide), with depths around 2.5-3 m and occasional deep holes up to ~6 m in

the late dry season. During the dry season these pools are connected by shallow runs with flow declining through the season resulting in relatively low turbidity (c.f. estuarine pools).

In July 2008, a total of 11 sites between Frazer River (17°21'47"S, 123°20'55"E) and Pt Torment (16°59'30"S, 123°36'57"E) in the southern region of King Sound, Western Australia, were sampled for *P. microdon* and *G. garricki* (Figure 1). Three additional sites on the Western side of King Sound between Good Enough Bay (16°46'30"S, 123°07'20E) and Valentine Island (17° 04'50"S, 123°16'49"E) were also sampled for these species in September 2009 (Figure 1). Sites sampled within King Sound included macro-tidal estuarine creeks and near-shore channels. Sites sampled in the southern regions of King Sound were dominated by clay/mud beds populated with mangroves and resultantly highly turbid water. North-western sample sites had a much more sand rich substrate and were less turbid. All sites were < 8 m in depth, with the majority of sites < 3 m in depth; noting that tidal variation causes them to be constantly changing.

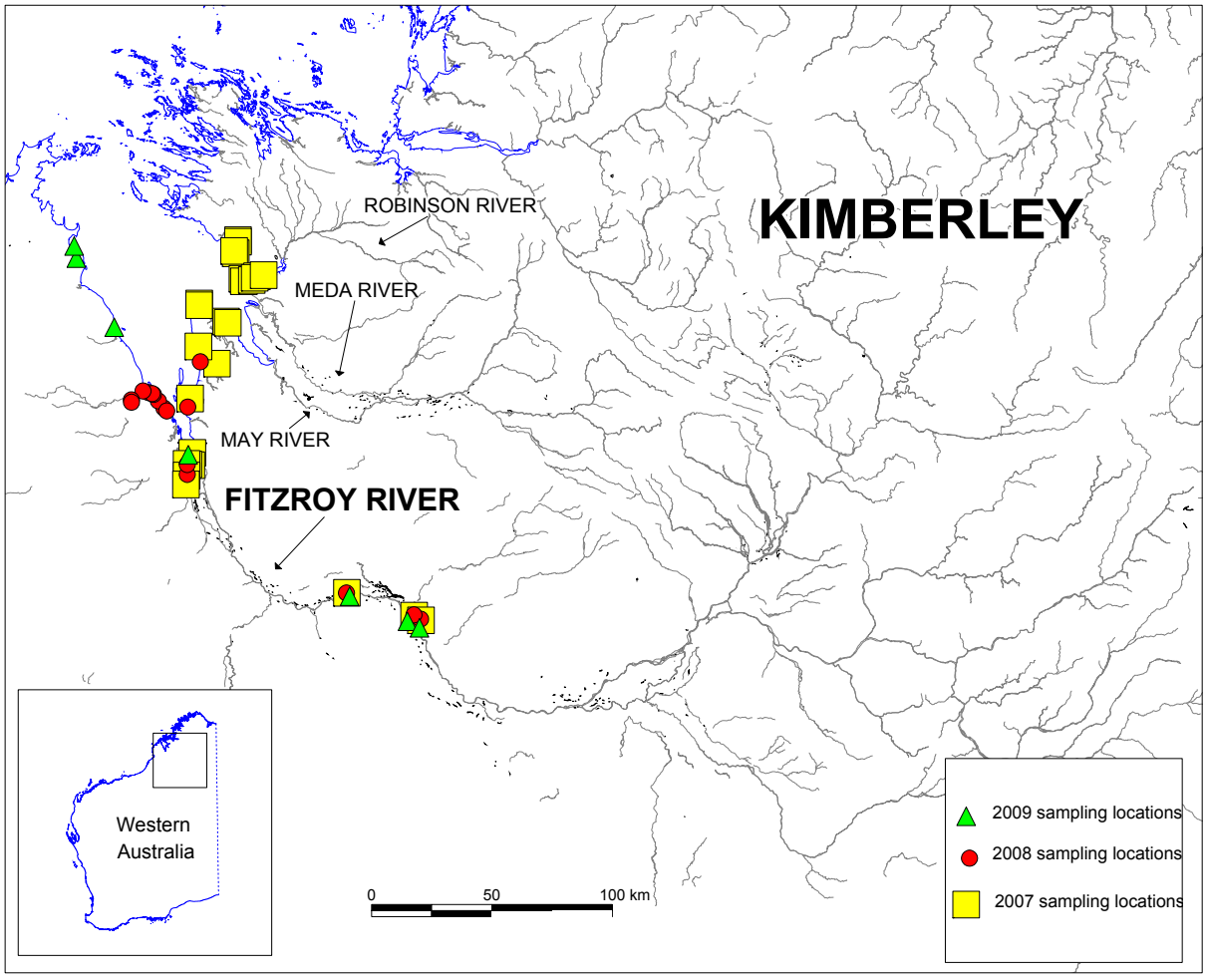


Figure 1 Sampling sites in King Sound and the Fitzroy River in 2007-2009.

Sampling methods in the Fitzroy River consisted of 20 m of 75, 100, 150 and 200 mm stretched monofilament gill nets with soak times of one to two hours depending on the level of by-catch. Two to three nets were often joined to stretch the width of the river. Only during hours between 00:00 and 06:00 were soak times extended to four or five hours if by-catch levels were low. Hand-lines were also employed at sampling sites. Sampling methods in

King Sound consisted of using 50 m of 175 mm stretched monofilament gill nets. Nets were checked at one to two hour intervals or when activity was observed in the nets. All captured fish were identified to the species level and measured for total length (TL) before release. More detailed notes were recorded for all captured elasmobranchs including TL, sex and clasper calcification level (for males). Total rostral length (TRL), which is the distance between rostra tip and base where it flares to join to the head, (previously referred to as rostral length (RL) in Thorburn *et al*. (2004, 2007), Whitty *et al*. (2008)) and teeth counts were also recorded for captured sawfish. Prior to release, sawfishes (including *P. microdon* and Dwarf Sawfish (*Pristis clavata*)), Bull Sharks (*Carcharhinus leucas*) and *G. garricki* were also fitted with Rototags (see "Tagging methodology" below). Tissue samples created as a by-product of tagging were also preserved for genetic analysis (see Section II and Phillips *et al*. (2008)). Data collected during 2008 and 2009 concerning population demographics, morphology, distribution and general biology were used to update records reported in Thorburn *et al*. (2007) and Whitty *et al*. (2008).

Environmental parameters

Environmental parameters including surface and bottom temperature, salinity and turbidity were recorded during sampling efforts when possible. Temperature was also logged at three hour intervals by HOBO Water Temp Pro V2 Data Loggers (Onset Computer Corporation, Bourne, MA, USA) placed on moorings in Milli Milli, Snag Pool, Langi Crossing, Myroodah Crossing and Camballin Pool. HOBO Pendant Loggers (Onset Computer Corporation, Bourne, MA, USA) were also used to monitor temperature and light intensity through the water column at 0.5-m intervals (a surface light logger was also employed in June and October 2008 to explore light attenuation in the studied pools). Pendant Loggers were deployed in Snag Pool, Langi Crossing, Myroodah Crossing and Camballin Pool in June and October 2008 recording in 15 min intervals for a short duration (1-3 days) and again in Myroodah and Telegraph Pool recording in 1 h intervals between November 2008 and April 2009. Timing of sunrise, sunset and twilight were acquired from the United States Naval Observatory (USNO; http://aa.usno.navy.mil/data/). River stage height data was provided by the Department of Water, Government of Western Australia.

Tagging methodology

Rototags

The Fitzroy River Sawfish Conventional Tagging (Rototags) Program, established in 2003, was continued in 2008 and 2009 (see Thorburn *et al*. 2004, 2007, Whitty *et al*. 2008). Gallagher Supertags (aka Rototags) (~20x45-mm polyurethane laser marked sheep tags; Dalton Supplies, Woolgoolga, New South Wales, Australia) were attached to dorsal fins of *P. microdon*, *P. clavata*, *C. leucas* and *G. garricki* in similar fashion to previous studies (e.g. Heupel *et al*. 1998, Thorburn *et al*. 2004, 2007, Whitty *et al*. 2008).

Acoustic Tagging

Nine Vemco (VEMCO Division, AMIRIX Systems Inc., Halifax, Nova Scotia, Canada) VR2W omni-directional acoustic receivers were used to construct the operational 2008 and 2009 acoustic array within the Fitzroy River (Figure 2). This array consisted of five previously installed receivers, placed in Milli Milli, Snag Pool, Langi Crossing, Myroodah Crossing and Camballin Pool (see Whitty *et al*. 2008, 2009). A sixth receiver, previously unobtainable (buried by sand) at the mouth of the river (Lower Pelican Pool in Whitty *et al*. 2008), was recovered and relocated to Telegraph Pool (located between Snag Pool and Langi Crossing) as 2007 data suggested this to be an area of high residence time for *P. microdon*

(Whitty *et al*. 2008). In addition to these receivers, a further three receivers were installed in December 2008, in Myroodah Pool (~1 km upstream to the previously installed receiver), Lower Barrage Pool (pool ~400 m downstream of the Camballin Barrage) and Upper Barrage Pool (installed ~500 m upstream of the Camballin Barrage to determine whether any tagged fish moved over the Barrage during flood events, or via Snake Creek, a perceived bypass channel downstream of Myroodah Crossing to the top of the Barrage, see Morgan *et al*. (2009)).

In June/July 2008, October/November 2008 and May 2009, 18 Vemco acoustic tags were deployed on *P. microdon* in the Fitzroy River. This is in addition to the nine that were deployed in 2007 (see Whitty *et al*. 2008, 2009). Of the 18 tags deployed between 2008 and 2009, 10 V13TP-1L 69 kHz (complimented with temperature and pressure sensors) and three V13-H 69 kHz coded tags (without environmental sensors), were fitted to 12 *P. microdon*. N.B. One *P. microdon* was refitted with a new acoustic (1061753) tag as the previous tag (1061751) was shed during recapture (this *P. microdon* is referred to as 1061751 throughout the report unless otherwise stated) (see Table 1). A number of the V13 tags were donated to the project by external organisations, including The Aquarium Society of New South Wales (three tags), and by the Department of Fisheries, Government of Western Australia (one tag) and by Dr Zeb Hogan (National Geographic, two tags). An additional three V9-H tags were donated by Dr Zeb Hogan (National Geographic) and were deployed on *P. microdon* in May 2009 (Table 1). V16TP-5H 69 kHz coded tags were fitted to two individuals employing methods described in Whitty *et al*. (2009) in June and July 2008 (Table 1). All tags were <1% of the calculated *P. microdon* body weight.

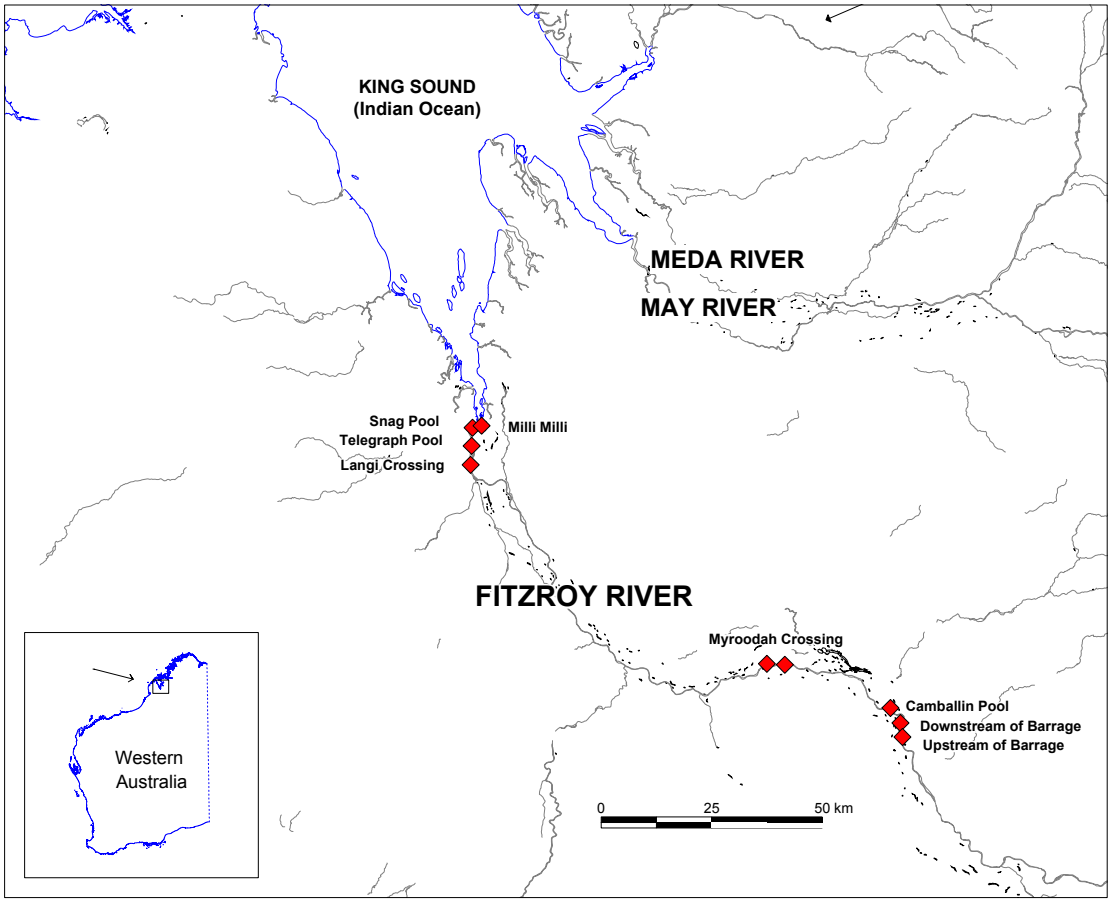


Figure 2 Position of 2008-2009 VR2W acoustic array used in the Fitzroy River and its estuary.

Table 1 *Pristis microdon* captured and tagged with acoustic transmitters in 2007 - 2009. [1]Denotes a fish that was tagged twice with transmitters, as the initial tag was lost on recapture.

Acoustic tag #	Tag model	Rototag	TL (mm)	Capture date	Capture location
1038505	V16	F9835	1555	22-Jun-07	Snag Pool
1038506	V16	F7092	1611	23-Jun-07	Snag Pool
1038503	V16	M1013	1580	18-Jul-07	Snag Pool
1042224	V13	M1007	842	18-Jul-07	Snag Pool
1042226	V13	M1006	990	19-Jul-07	Snag Pool
1042227	V13	M1003	981	19-Jul-07	Snag Pool
1042229	V13	M1005	854	19-Jul-07	Snag Pool
1042225	V13	M1004	1111	1-Nov-07	Camballin Pool
1038502	V16	-	1576	13-Nov-07	Langi Crossing
1042228	V13	M1002	1897	24-Jun-08	Myroodah Crossing
1038504	V16	-	1429	29-Jun-08	Snag Pool
1056892	V13	M1038	1423	29-Jun-08	Snag Pool
1056893	V13	M1022	918	29-Jun-08	Snag Pool
1056894	V13	M1023	959	30-Jun-08	Snag Pool
1038501	V16	-	1934	22-Jul-08	Snag Pool
1061752	V13	M1048	2142	27-Oct-08	Myroodah Crossing
1061751 / 1061753[1]	V13	M1045 / M1050	2210	28-Oct-08 / 20-Nov-08	Camballin Pool
1061748	V13	M1051	1883	31-Oct-08	Snag Pool
1061749	V13	M1049	2090	31-Oct-08	Snag Pool
1061750	V13	M1047	2102	31-Oct-08	Snag Pool
1068357	V9	M1077	965	12-May-09	Snag Pool
1068356	V9	M1076	952	13-May-09	Camballin Pool
1068358	V9	M1078	1027	13-May-09	Camballin Pool
1056896	V13	M1066	1013	14-May-09	Lower Barrage Pool
1068352	V13	M1079	872	14-May-09	Lower Barrage Pool
1068353	V13	M1081	911	14-May-09	Lower Barrage Pool

Data analysis

Statistical analyses were conducted using SPSS version 15 (SPSS, Chicago, IL, USA), Systat V10 (SPSS, Chicago, IL, USA) and SigmaPlot 11.0 (SPSS, Chicago, IL, USA). Analyses conducted on previous years' data were repeated with the updated dataset (through 2009), as the larger sample size increased the power of these tests. Repeating these tests in previous years has indeed changed their outcome as shown in the TL : TRL relationship between male and female *P. microdon* (cf. Thorburn *et al*. 2007, Whitty *et al*. 2008). A linear regression was used to examine the updated relationship between pooled male and female TL to TRL. A Mann-Whitney Rank Sum Test was also used on the updated data to investigate the level of difference between the TL : TRL ratio of males and female *P. microdon* and to look at differences between left and right rostral tooth counts.

Catch per unit effort (CPUE) since 2002 was calculated for the river and sound using 1 h soak of 20 m net as the unit effort (later transformed to 100 h soak of 20 m net to put results into a more meaningful context). CPUE of *P. microdon* between the early (June-August) and late (September-November) dry season within the lower 150 km of the river was compared using

the Friedman's Test. A linear regression was used to determine the significant trend in CPUE throughout the year for the same region. Both analyses involving CPUE were only run with years between 2002 and 2009 when estuarine and freshwater pools were sampled in the early and late dry season (excludes 2004, 2005 and 2009) to decrease possible bias. An inquiry into the possible relationship between CPUE and mean wet season river stage height was run using a simple correlation.

Data from depth sensors was analysed to look at significant differences in depth use of n individuals in varying habitats and seasons. A Mann-Whitney Rank Sum Test was used to compare depths occupied by *P. microdon* in the 2008 dry (prior to 24th Nov.) and wet (starting on 24th Nov.) seasons. Differences between depths occupied by different *P. microdon* were analysed using a Kruskal-Wallis test.

Recorded depth transmissions for each acoustically tagged (with a pressure sensor) *P. microdon* were scored on a scale between 0-3. Results with higher scores were likely to represent a more accurate average daily behaviour than that of a lower score. The scoring scale and its perquisites were as follows:

Score	Score prerequisites
0	Not present for hrs 0-23
1	Present for hrs 0-23 and has an average of < 30 transmissions/h
2	Present for hrs 0-23 and has an average of 30-100 transmissions/h
3	Present for hrs 0-23 and has an average of 100+ transmissions/h

As the number of tracked *P. microdon* was limited, tags with transmissions with scores of zero were used in some analyses to obtain the best overview of *P. microdon* behaviour within different environments. Statistical results produced from analyses using tags with transmissions of zero or one should be treated with caution.

RESULTS

Environments of Fitzroy River and King Sound

Average river stage heights during the 2008 and 2009 wet seasons (January-April) was relatively high in comparison to recent years (Figure 3). In most years, discharge begins to increase in late December, and during 2008 and 2009 stage height of the river peaked in February at 19.4 m and 21.0 m, respectively. River stage height averaged 13.5 m (± 0.09 SE) and 14.3 m (± 0.12 SE) in the 2008 and 2009 wet season, respectively (Figures 3 & 4).

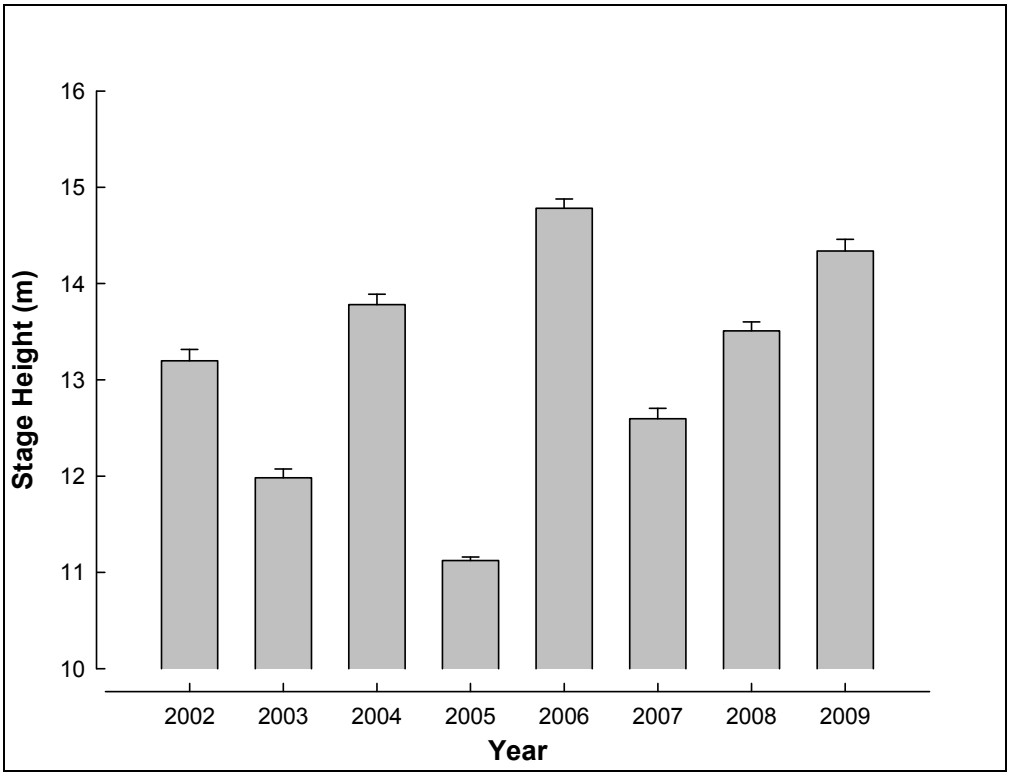


Figure 3 Average stage height of the Fitzroy River during the wet season (January-April) at Noonkanbah (upstream of Camballin Barrage) between 2002 and 2009. Data provided by the Department of Water, Government of Western Australia.

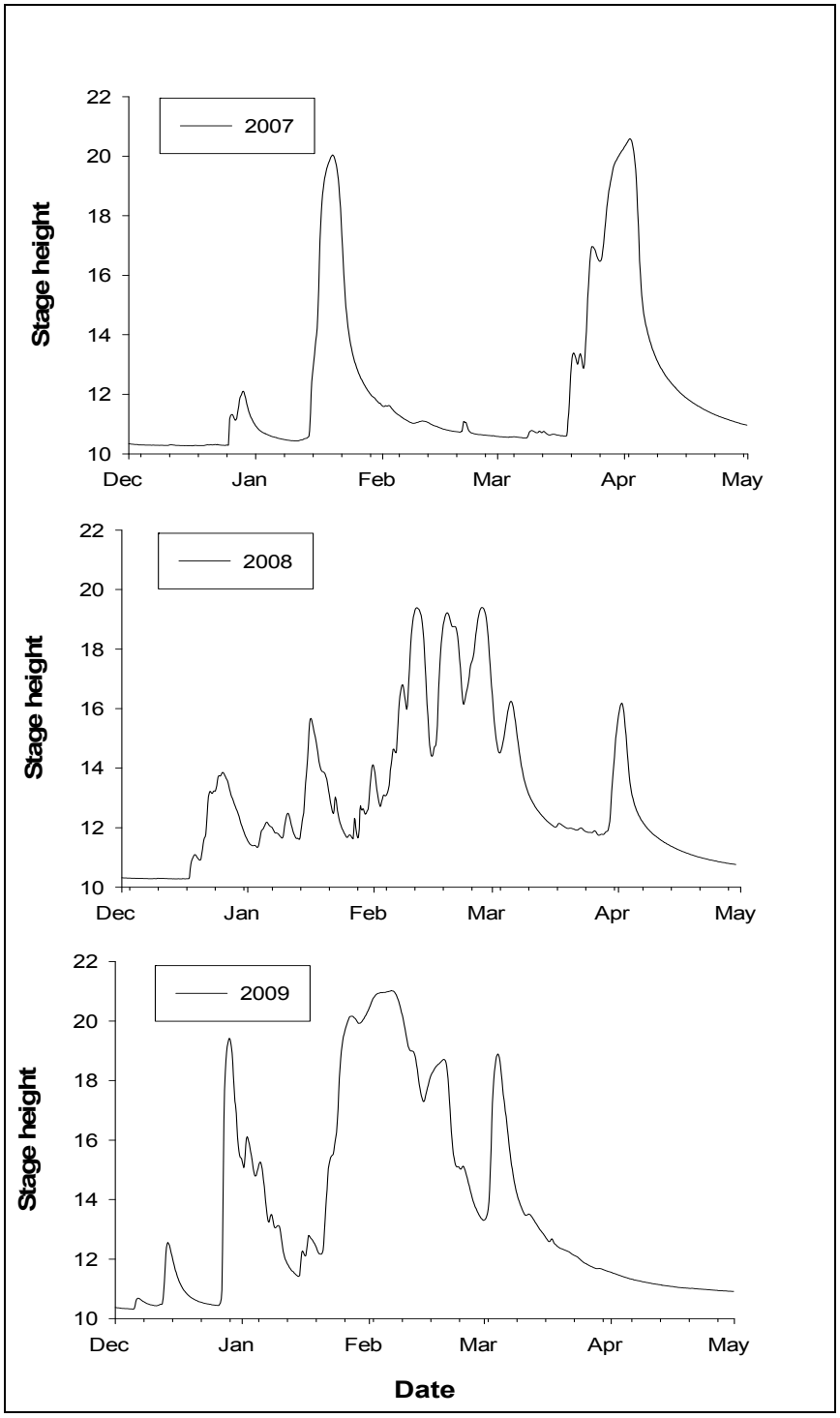


Figure 4 River stage height at Noonkanbah (upstream of Camballin Barrage) between 1st Dec and 30th April in the three years of acoustic telemetry. Stage heights for other years are presented in Morgan *et al*. (2005) and Whitty *et al.* (2008). Data provided by the Department of Water, Government of Western Australia.

Between November 2007 and September 2009, water temperatures in Milli Milli and Myroodah Crossing fluctuated greatly, reaching minimum temperatures of 15-17°C and 18-20°C in July and August and maximum temperatures of 36-38°C in November and December and 33-34°C in late November through March, respectively (Figure 5). Daily water temperature range was found to be significantly greater in tidally influenced pools than the

upper freshwater pools ($p < 0.001$) (Figure 5). Water temperatures between tidal pools and inland freshwater pools were similar during periods of lower river flow except during the late dry season (Figure 5).

Of the eight light loggers deployed in November 2008 (four deployed in Telegraph Pool and four in Myroodah Crossing in 0.5-m intervals on a mooring), only three (one in Telegraph Pool and two in Myroodah Crossing) were recovered. Two in Myroodah Crossing were missing and the remaining three in Telegraph Pool were not accessible as the mooring in which they were attached to, had become immovable. From the recovered loggers, readings of light intensity > 0 lux were recorded on loggers at 0.5 m depth between sunrise and sunset (±10 min.). Direct sunlight (associated with a significant increase in light intensity) penetrated pools at different times due to the pools orientation and tree lines. Loggers at 0.5 m depth were first influenced by the direct sunlight ~1 h post sunrise in Snag and Telegraph Pool, but 3-4 h post sunrise in Myroodah Crossing and Camballin Pool.

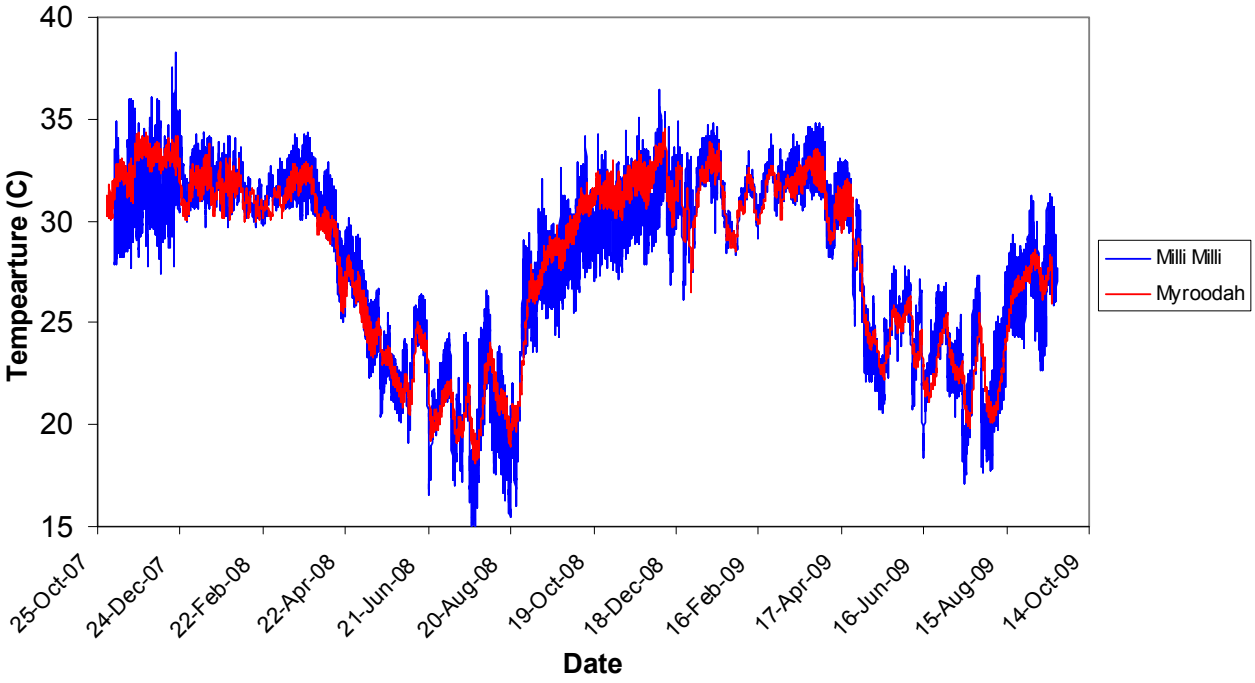


Figure 5 Water temperatures between November 2007 to September 2009 from temperature loggers in Milli Milli and Myroodah Crossing.

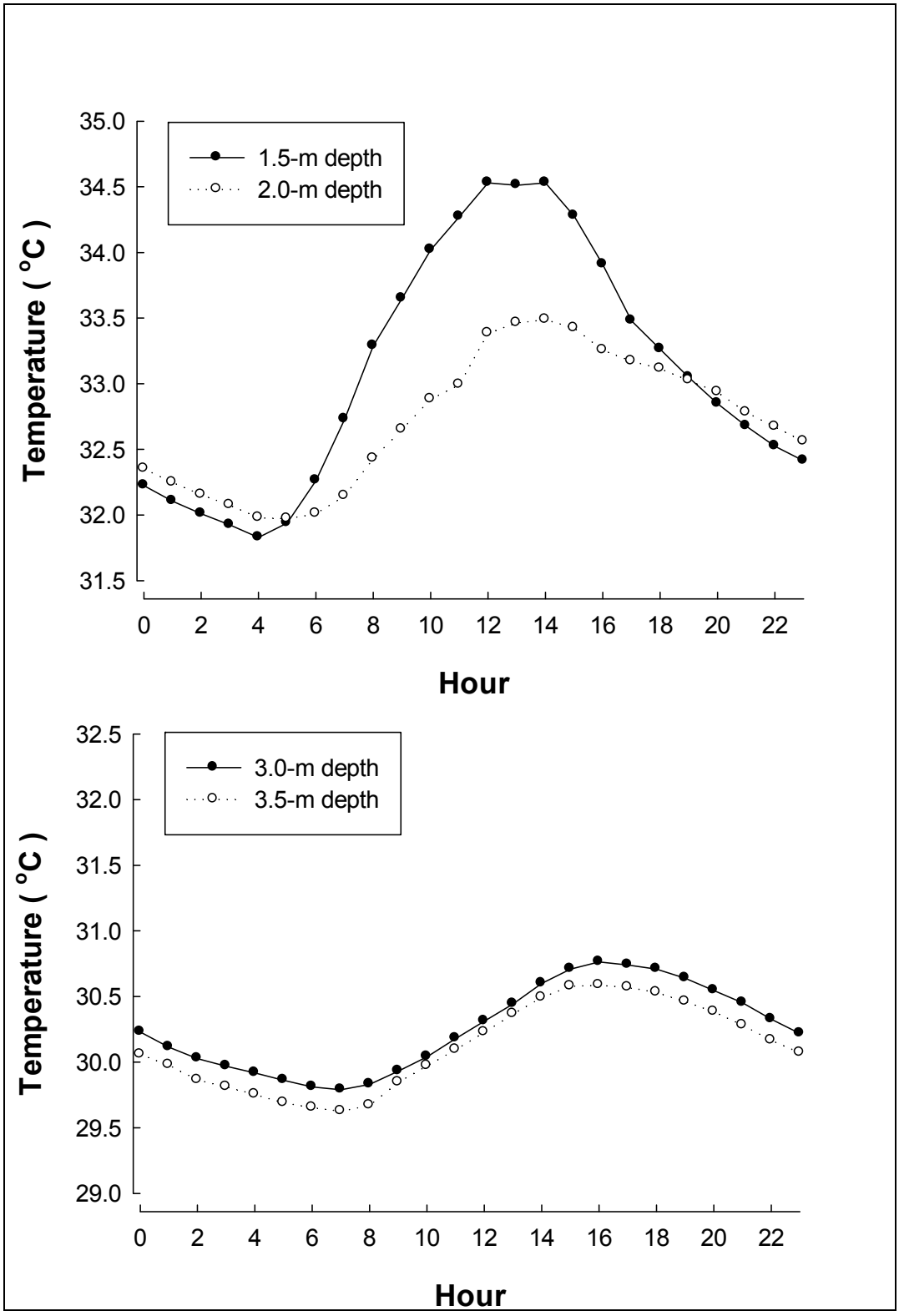


Figure 6 Mean daily vertical temperature profile at Myroodah Crossing in early December 2008 (1.5 and 2.0 m depth) (top) and April 2009 (3.0 and 3.5 m depth) (bottom). Depth of loggers increased due to elevated river levels.

Catch composition, size distribution, sex ratios and morphology

Pristis microdon

A total of 18 and 21 *P. microdon* were captured in 2008 and in 2009 in the Fitzroy River, respectively (see Table 2, Figure 7). *Pristis microdon* was captured in all pools sampled. In October 2008, an additional new recruit (0=) *P. microdon* was discovered dead, high on the banks of Telegraph Pool with a fishing hook and wire trace attached. This fish had presumably been left to die. Photographs of a further dead male sawfish from Camballin (18.1015°S, 124.1786°E), of 2400 mm TL (TRL = 530 mm, 21 left rostral teeth, 20 right) was sent to us; it had been dragged from the water and left to die (see Table 2). This individual was not used in data analyses.

In 2008, 10 of the 19 *P. microdon* (including the deceased *P. microdon*) were between 812 and 1050 mm TL, two were between 1423 and 1429 mm TL, three were between 1883 and 1934 mm TL and four were between 2090 and 2210 mm TL, most likely representing the 0+, 1+, 2+ and 3+ age class, respectively (see Thorburn *et* al. 2007, and Figure 7). In 2009, all captures were between 763 and 2499 mm TL, and consisted of 18 0+, one 1+, one 2+ and one that was likely a 4+ *P. microdon* (Table 2). The large dead male at Camballin would also have likely been in its fourth year of life. Of all captures, only three were caught on hand-line, one in November 2008, and two in May 2009, one being recaptured the day after it was fitted with an acoustic tag.

Overall, there has been a general decline in CPUE of *P. microdon* in the eight years of monitoring the population in the Fitzroy River (Figure 8). Comparisons of CPUE in the early dry seasons of *P. microdon* between the 2003 and 2008 was found to significantly decline between years (p = 0.025). A significant difference between early (median = 5.38 *P. microdon* per 100 h of 20 m net set) and late (median = 1.27 *P. microdon* per 100 h of 20 m net set) dry season in the lower 150 km of the river was also observed (p = 0.046). No significant correlation was found between early and late dry season CPUE and mean wet season river stage height ($p > 0.05$). There was however, a significant relationship (p = 0.001) with the proportion of new recruits in our catches when compared to the mean April discharge (Figure 9). This relationship was not significant over the entire wet season (Jan-April), or in any other month.

Sex ratios of catches in 2008 0+ *P. microdon* were skewed to the female side with seven females and three males. In 2009, this ratio was near parity with nine females, eight males and one unknown 0+ *P. microdon*. The sex ratio of captured *P. microdon* was found to be equal with the 2008 1+ and 3+ age classes, but skewed towards males in 2+ *P. microdon* (all males) (Table 2).

The updated relationship between TL and TRL was found to be TRL = 0.209 (TL) + 37.436. The sexes were pooled as no significant difference was found between this relationship for males and females (p = 0.755) (Figure 10). Left teeth counts for female and male *P. microdon* ranged between 17 and 23 and between 19 and 23, respectively (Figure 11). Total teeth counts for female and male *P. microdon* ranged between 34 and 47 and between 36 and 47, respectively (Figure 12). There was found to be a significant difference between male and female total tooth counts ($p < 0.001$). The range in the difference between left and right

rostral teeth was similar to that previously reported (Figure 13) (cf. Thorburn *et al*. 2007, Whitty *et al*. 2008).

Table 2 Tag number, capture date, capture time, capture location, sex, total length (TL), total rostral length (TRL, formerly RL in Thorburn *et al*. 2007, Whitty *et al*. 2008), and rostral tooth counts for *Pristis microdon* in the Fitzroy River during 2008 and 2009. ***** denotes deceased individuals found on the bank.

Rototag	Capt. date	Capt. time	Capt. location	Sex	TL (mm)	TRL (mm)	R Tooth Count	L Tooth Count
M1002	24-Jun-08	19:35	Myroodah Crossing	M	1897	400	20	20
M1044	25-Jun-08	23:00	Myroodah Crossing	M	879	191	21	20
M1031	27-Jun-08	18:50	Camballin Pool	M	812	198	17	19
M1022	29-Jun-08	21:00	Snag Pool	F	918	234	20	19
M1021	29-Jun-08	23:00	Snag Pool	F	1423	321	20	20
M1038	29-Jun-08	23:00	Snag Pool	F	873	217	21	21
-	29-Jun-08	21:00	Snag Pool	M	1429	346	-	-
M1023	30-Jun-08	6:03	Snag Pool	F	959	236	17	18
M1032	30-Jun-08	19:20	Snag Pool	F	977	246	19	19
M1027	30-Jun-08	17:30	Snag Pool	M	852	221	24	23
M1043	1-Jul-08	6:00	Snag Pool	F	880	214	19	-
M1035	1-Jul-08	6:00	Snag Pool	F	989	248	24	23
-	23-Jul-08	0:00	Snag Pool	M	1934	434	-	-
M1048	27-Oct-08	23:00	Myroodah Crossing	F	2142	474	19	18
M1045	28-Oct-08	20:00	Camballin Pool	M	2210	504	20	21
M1049	31-Oct-08	21:30	Snag Pool	F	2090	466	18	19
M1047	31-Oct-08	21:30	Snag Pool	M	2102	460	21	22
M1051	1-Nov-08	1:40	Snag Pool	M	1883	422	21	21
*	18-Nov-08	7:00	Telegraph Pool	F	925	254	19	20
M1086	12-May-09	15:30	Snag Pool	F	837	205	17	19
M1077	12-May-09	15:30	Snag Pool	M	965	232	19	20
M1083	12-May-09	15:30	Snag Pool	M	763	194	19	20
M1088	12-May-09	17:30	Snag Pool	M	934	225	21	20
-	12-May-09	17:30	Snag Pool	F	796	207	-	-
M1076	13-May-09	20:30	Camballin Pool	F	952	237	19	18
M1090	13-May-09	20:30	Camballin Pool	F	923	229	18	18
M1078	13-May-09	20:30	Camballin Pool	F	1027	245	18	19
M1082	13-May-09	21:45	Camballin Pool	F	867	216	19	18
-	13-May-09	23:31	Camballin Pool	F	940	238	22	20
M1084	14-May-09	0:30	Camballin Pool	M	1016	249	21	22
M1066	14-May-09	21:00	Lower Barrage Pool	F	1013	254	19	18
M1081	14-May-09	23:00	Lower Barrage Pool	-	911	228	20	20
M1079	14-May-09	22:15	Lower Barrage Pool	F	872	216	19	18
M1087	15-May-09	1:00	Lower Barrage Pool	M	901	227	21	20
*	Jul-09	-	Camballin	M	2400	530	21	20
M1098	16-Sep-09	20:30	Myroodah Crossing	M	1003	254	20	21
M1092	16-Sep-09	22:30	Myroodah Crossing	M	968	246	22	22
M1034	17-Sep-09	0:30	Myroodah Crossing	M	1865	400	20	19
M1093	15-Sep-09	21:00	Myroodah Crossing	M	1580	377	20	19
M1094	17-Sep-09	5:00	Myroodah Crossing	F	2499	524	18	18
M1095	22-Sep-09	21:45	Myroodah Crossing	M	1065	254	22	22

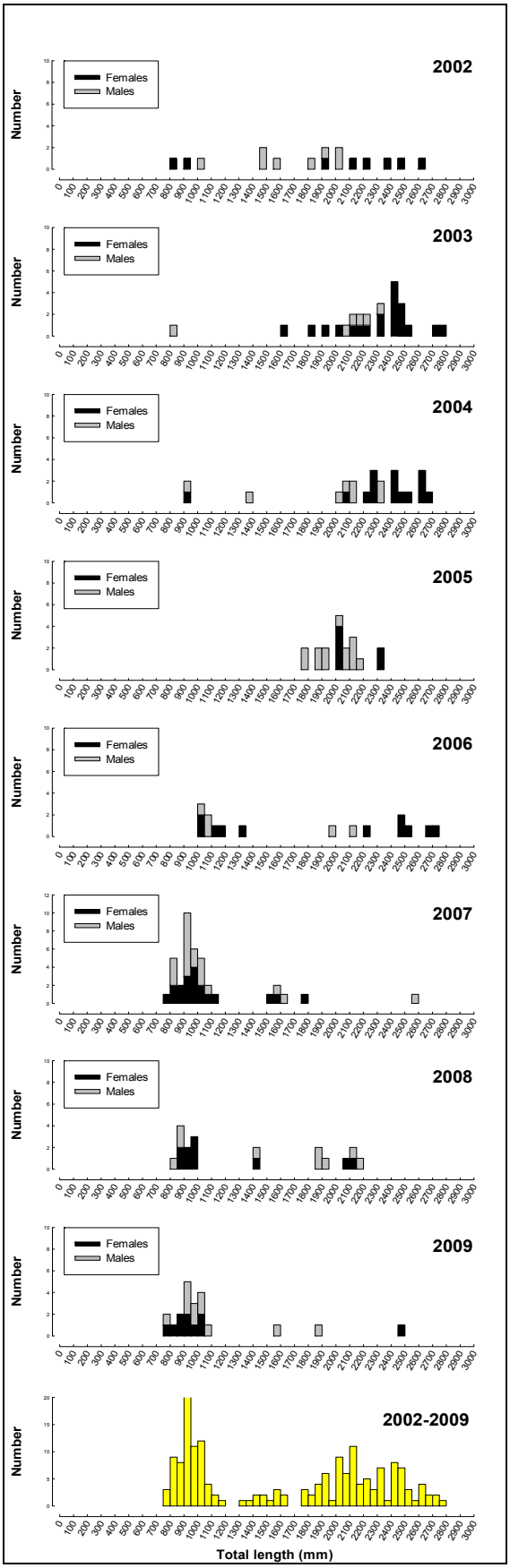

2002
2003
2004
2005
2006
2007
2008
2009
2002-2009
Females
Males
Number
Total length (mm)

Figure 7 Length-frequency histograms of *Pristis microdon* captured in the Fitzroy River and King Sound between 2002 and 2009.

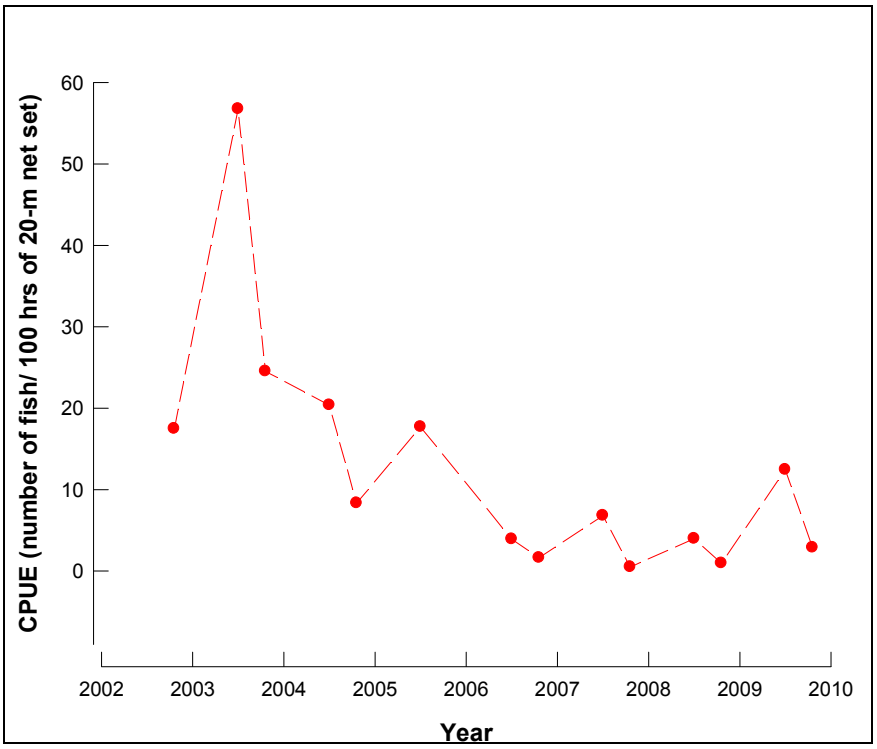


Figure 8 Catch per unit effort (CPUE) of *Pristis microdon* on the different sampling occasions in each year between 2002 and 2009; based on the number of *P. microdon* caught per 100 hour per 20 m of gill net.

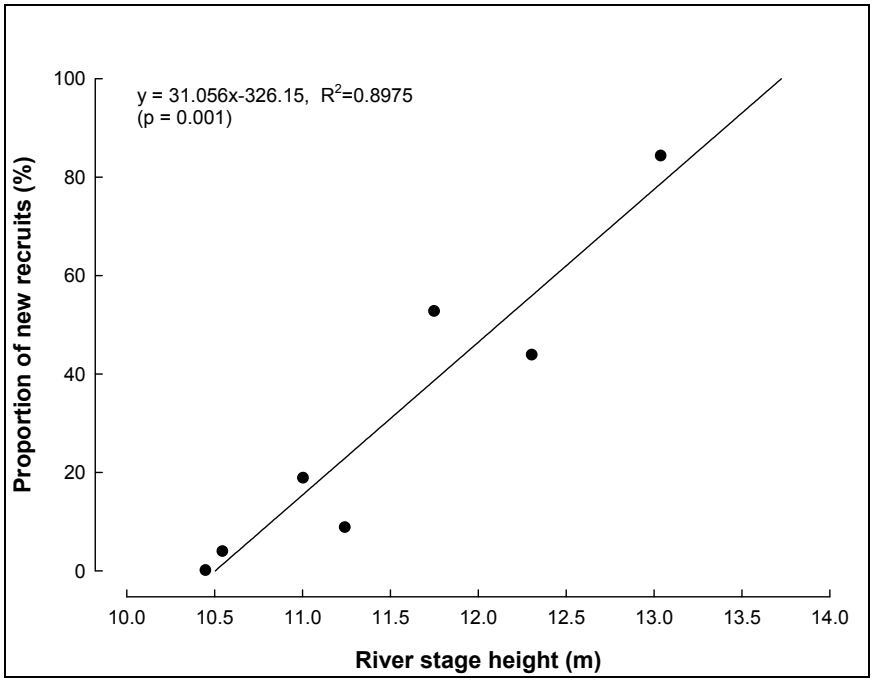


Figure 9 Proportion of new recruits of *Pristis microdon* with mean water levels during April (stage height (m)) for 2002-2008. N.B. A stage height of 10 m is the baseline water level for the river which is attained during low flow periods.

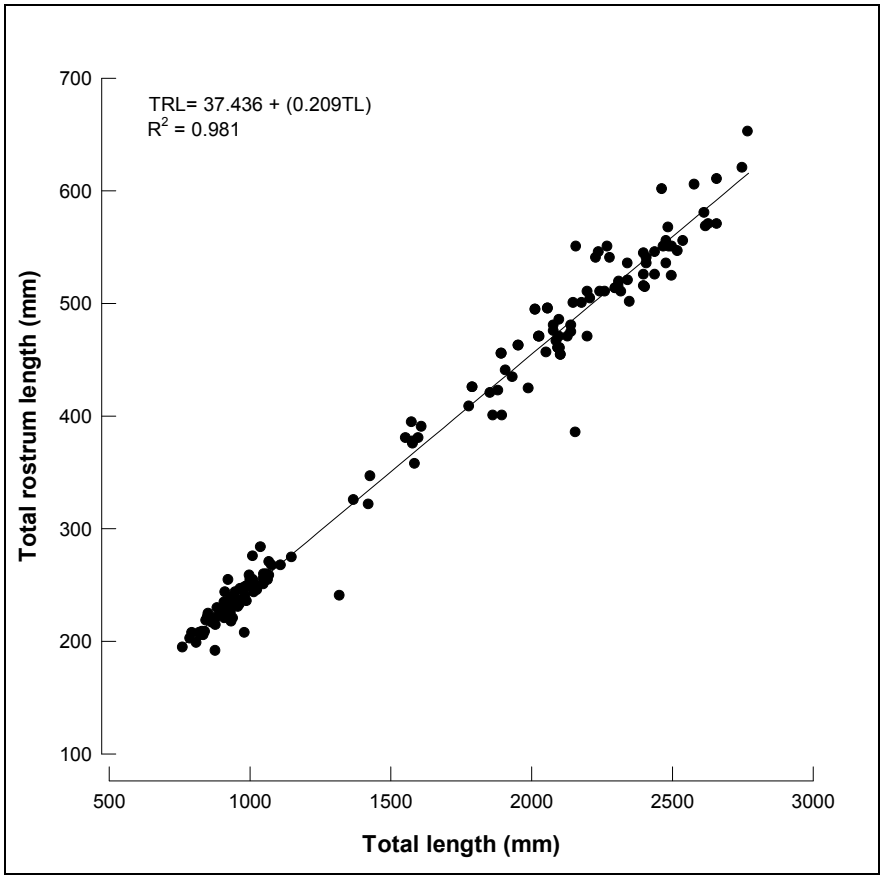


Figure 10 Relationship between total rostrum length and total length of male and female *Pristis microdon* captured in the Fitzroy River.

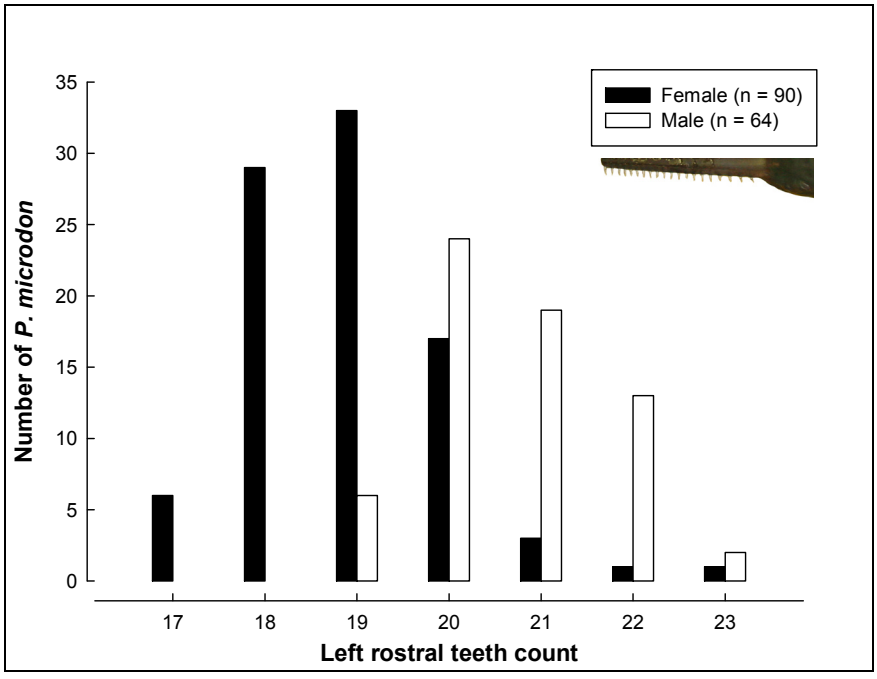


Figure 11 Left rostral teeth counts for male and female *Pristis microdon* captured in the Fitzroy River.

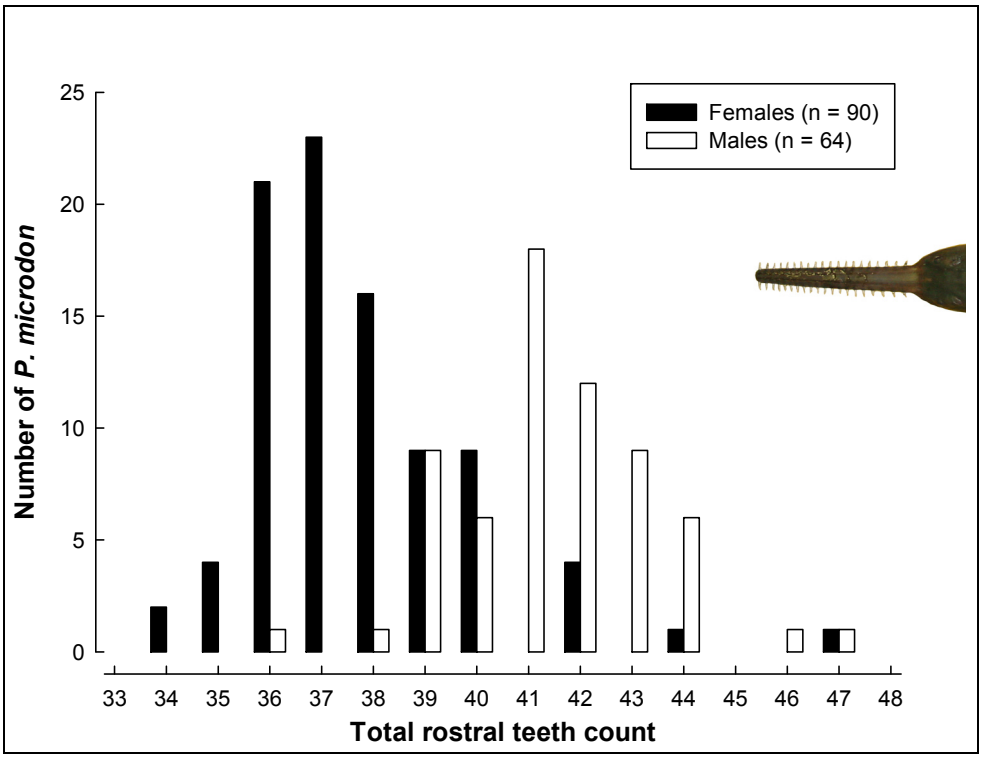


Figure 12 Total number of rostral teeth for male and female *Pristis microdon* captured in the Fitzroy River.

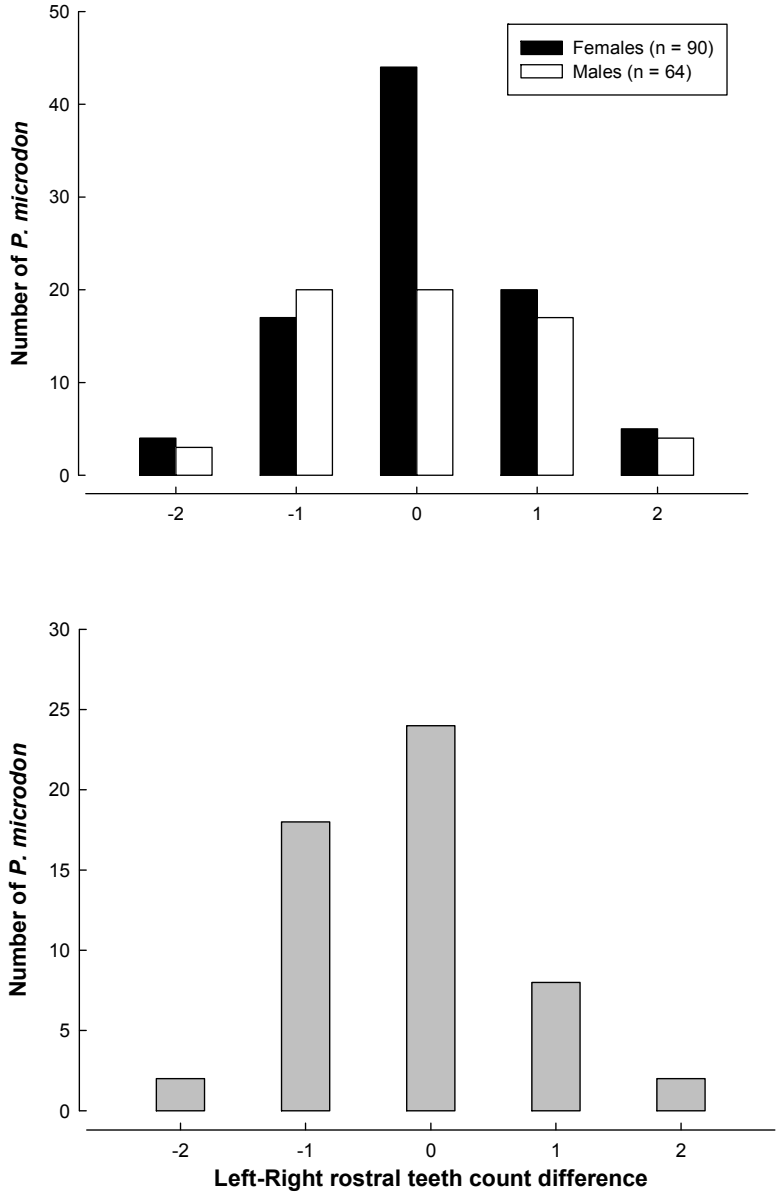


Figure 13 Variation in rostral teeth count from the left side of sexed fish (top) and from rostra donated from private collections from the Fitzroy River (unsexed).

Northern River Shark (Glyphis garricki)

In July 2008 one *G. garricki* was captured in a near-shore channel composed of a mud/clay substrate, several km south of Frazer River (western King Sound) (Figure 14). This individual was a maturing male of 1090 mm TL; indicated by the commencement of clasper calcification. Inner clasper length measured 54 mm while the outer clasper length measured 25 mm. This individual was captured during an incoming tide (~16:00 h) in ~2.8 m depth. Water temperature at time of capture was 20.2°C, salinity was 33.1 ppt, and turbidity was 269

ntu. This represents the first reported record of an individual from the western perimeter of King Sound.

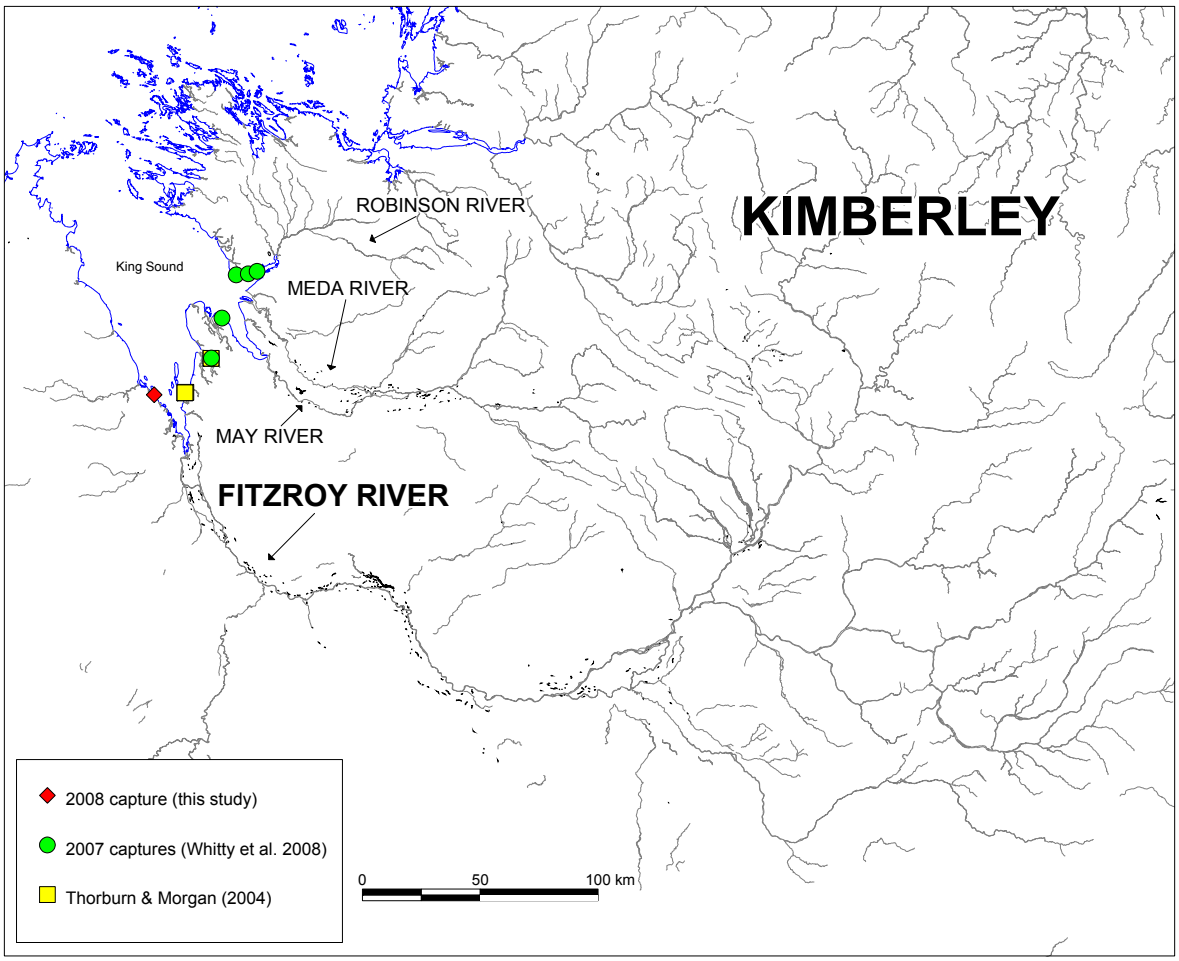


Figure 14 All known *Glyphis garricki* capture locations in King Sound, Western Australia.

Conventional tagging of *Pristis microdon*

In 2008, 16 *P. microdon* were each fitted with a Rototag, of which six were recaptured (Table 2). Of these six, only one was recaptured more than once (M1035) (Table 2). Three of these individuals (M1021, M1027 and M1035) were recaptured within 0-48 h post release. Three other *P. microdon* were at liberty for 14 (M1044), 23 (M1045) and 143 (M1032) days prior to recapture. Two of these three recaptures (M1044 and M1032) were reported by recreational fishers. Two (M1044 and M1045) were recaptured in the same pools that they were initially released in. The third, M1032, was initially released in Snag Pool and was reported to have been recaptured above Langi Crossing, a distance of ~9.5 km upstream and was reported to have grown to 1050 mm TL (from 977 mm TL at initial capture). During 2008 and 2009, a number of anecdotal reports of recaptured sawfish were also relayed to us, some of which were at least 50 km upstream of our most downstream tagging sites.

In 2009, 19 *P. microdon* were fitted with Rototags, of which four were recaptured, three of which were at liberty between captures for < 48 h and the fourth was at liberty for five days.

All recaptures occurred in the same pool the animals were initially released in. One of these individuals was acoustically tagged the night prior to its recapture, and was recaptured on a hand-line.

Acoustic tracking of *Pristis microdon*

Acoustic array

Efforts to download receivers were undertaken in June, September, October and November 2008 as well as May and September 2009. Unfortunately due to mechanical error not all receivers were continuously working between November 2007 and November 2008 (Figure 15). Non-functional receivers were replaced with newly acquired receivers when possible. In May and September 2009, efforts to locate receivers at Snag Pool and Langi Crossing, went unrewarded. Also due to mechanical error, data from the Telegraph Pool receiver was unobtainable for all of 2009.

All located moorings with exception of Camballin Pool, Myroodah Crossing and Upper Barrage Pool (all upstream, non-tidal pools) became immovable; however the side loop created to allow receivers to remain vertical during high flows in which the receivers were attached (see Whitty *et al*. 2008), had enough slack to allow for receivers to still be accessed.

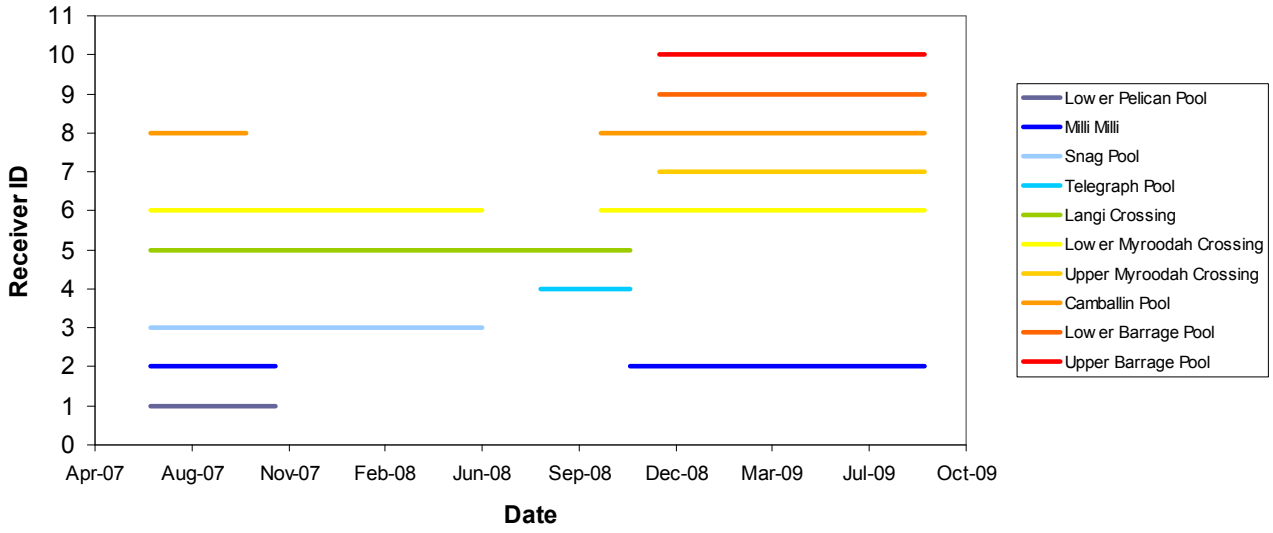


Figure 15 Periods that hydrophones at the corresponding locations were functioning. Receiver ID: 1 = Lower Pelican Pool, 2 = Milli Milli, 3 = Snag Pool, 4 = Telegraph Pool, 5 = Langi Crossing, 6 = Lower Myroodah Crossing, 7 = Upper Myroodah Crossing, 8 = Camballin Pool, 9 = Lower Barrage Pool, 10 = Upper Barrage Pool.

Acoustic tag transmissions

Thirteen of 17 coded transmitters deployed on *P. microdon* in 2008 and 2009 were detected by a minimum of one receiver (Table 3, Figure 16). One tag deployed in November 2007 in Camballin Pool, three additional tags deployed in June/July 2008 in Snag Pool and Myroodah Crossing and one tag deployed in May 2009 at Snag Pool were never detected (Table 3, Figure 16). In addition to the tags deployed and recorded in 2008, tag 1038503, deployed on 13th of November 2007, was also recorded in 2008. No other tags deployed in 2007 were recorded in 2008 or 2009. Time between deployment and last recorded transmission (monitored period) was 139 and 127 days for V16 tags and ranged between 12 and 313 days (mean = 80.9 ± 25.5 days) for V9 and V13 tags (Table 3). The two V16 tagged *P. microdon*

were detected by a receiver at least once per day for 11 and 64 days. This number ranged between three and 61 days (mean = 27.4 ± 5.7 days) for V9 and V13 tagged *P. microdon* (Table 3).

Table 3 Acoustic tag information for 2007-2009. LD: Last detection; MP: Monitored period.

Acoustic tag #	Tag model	LD location	LD date	MP (days)	# days detected
1038502	V16	Langi Crossing	31-Mar-08	139	11
1042228	V13	-	-	-	-
1038504	V16	Langi Crossing	3-Nov-08	127	64
1056892	V13	-	-	-	-
1056893	V13	Telegraph Pool	30-Sep-08	93	47
1056894	V13	Telegraph Pool	30-Aug-08	61	27
1038501	V16	-	-	-	-
1061752	V13	Milli Milli	13-May-09	198	54
1061751	V13	Lower Myroodah	12-Jan-09	76	61
1061748	V13	Milli Milli	9-Sep-09	313	26
1061749	V13	Milli Milli	15-Dec-08	45	13
1061750	V13	Milli Milli	15-Nov-08	15	3
1068357	V9	-	-	-	-
1068356	V9	Camballin Pool	2-Jul-09	50	20
1068358	V9	Camballin Pool	30-Jun-09	48	35
1056896	V13	Lower Barrage Pool	1-Jun-09	18	6
1068352	V13	Lower Barrage Pool	25-Jun-09	42	34
1068353	V13	Lower Barrage Pool	26-May-09	12	3

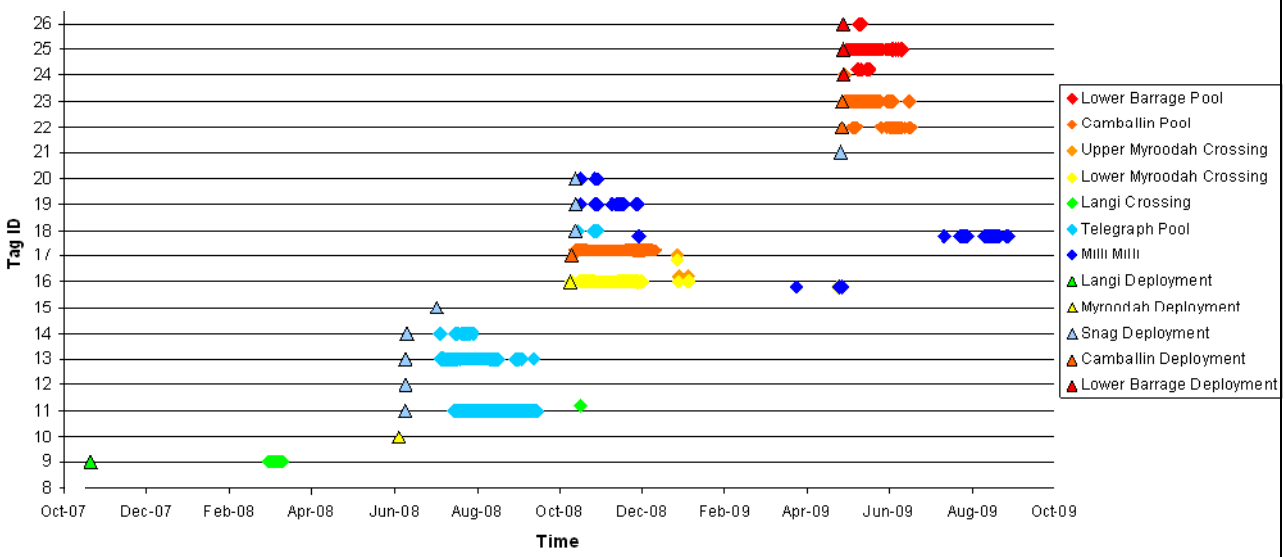


Figure 16 Recorded transmissions from tagged *P. microdon* on different sites. Tag 9 = 1038502, 10 = 1042228, 11 = 1038504, 12 = 1056892, 13 = 1056893, 14 = 1056894, 15 = 1038501, 16 = 1061752, 17 = 1061751/3, 18 = 1061748, 19 = 1061749, 20 = 1061750, 21 = 1068357, 22 = 1068356, 23 = 1068358, 24 = 1056896, 25 = 1068352, and 26 = 1068353.

Eight of the 14 *P. microdon* that were recorded on at least one VR2W receiver tagged after 1st November 2007, were observed moving between pools. Six of these were monitored in the estuarine pools. Two of the three monitored *P. microdon* initially captured in June/July 2008 at Snag Pool (1056893 (918 mm TL) and 1056894 (959 mm TL)) were observed in Telegraph Pool, where they were initially recorded on 25th July when a receiver was installed within the pool, and were last detected on 30th August and September 2008, respectively. The third *P. microdon* (1038504 (1429 mm TL)) arrived on 3rd August of that same year and was last detected at Langi Crossing on 3rd November 2008. *Pristis microdon* tagged in October 2008 at Snag Pool, moved to Telegraph (1061748 (1883 mm TL)) or Milli Milli (1061749 (2090 mm TL) and 1061750 (2102 mm TL)) within a few days of being released. Transmissions ceased for 1061750 and 1061749 on 15th November at Telegraph Pool and 15th December 2008 at Milli Milli, respectively. Tag 1061748 also had a temporary final transmission (last transmission to occur prior to a relatively long absence, after which the animal is again detected) on 15th December 2008 at Milli Milli. Tag 1061748 was later recorded off and on between 25th July and 9th September 2009 at Milli Milli (noting final download occurred 14th September 2009) (Figure 16).

Two *P. microdon* that were observed moving between pools were tagged in the upper freshwater pools. One *P. microdon* tagged at Myroodah Crossing on 27th October 2008, (1061752 (2142 mm TL)) was observed at Myroodah Crossing until 20th January 2009. This animal was later detected over 118 km downstream at Milli Milli on 9th April 2009 and last detected on the 13th April 2009. A *P. microdon* tagged at Camballin Pool in October 2008 (1061751 (2210 mm TL)) was recorded within the pool until 27th December 2008. During this time the animal was recaptured within the same pool. Following its final transmission within Camballin Pool, 1061751 moved over 33 km downstream in 16 days, being detected at the Upper Myroodah Crossing receiver on 12th January 2009 and at the Lower Myroodah Crossing receiver for a few hours only 45 min. later. No further transmissions were recorded for this animal after this period.

Dates of nearly all final transmissions, and in the case of *P. microdon* trapped in the freshwater pools, temporary final transmissions of the five animals tracked in the late 2008 (tag 1038502) or early 2009 wet season (tags 1061748, 1061749, 1061751 and 1061752), occurred during a flood event. N.B. For the purposes of this report, a flood event is defined as a sudden substantial increase in river discharge, resulting in a > 0.5 m rise in stage height (Figure 17a-e). For all but one of these individuals, this occurred on the first day of the flood event. Of the wet season monitored *P. microdon*, only the final transmissions from tag 1061751 and 1061752 occurred during a non-flood event, but did occur after a relatively large flood event when the stage height was ~0.7+ m above the late dry season stage height (Figure 17a-e).

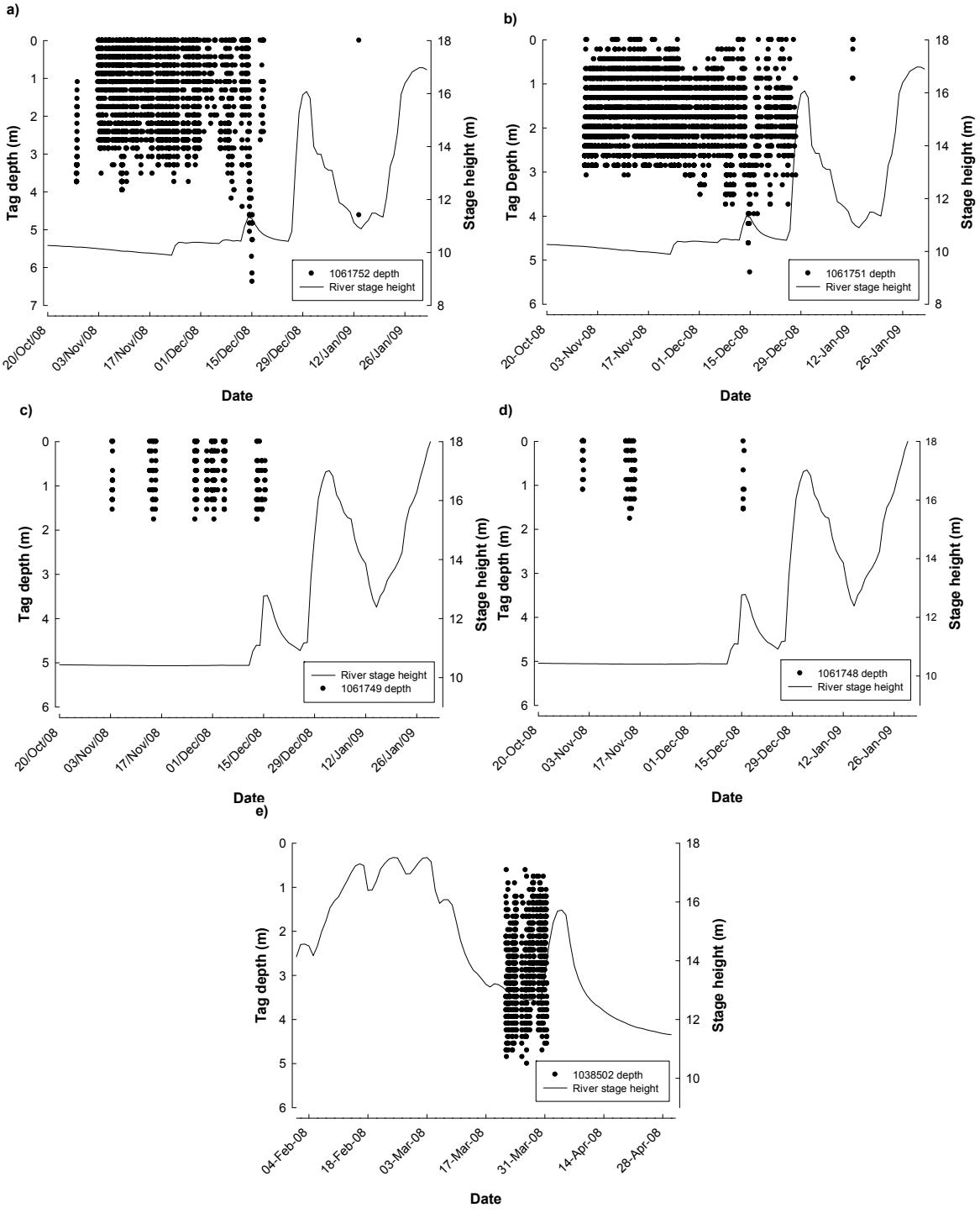


Figure 17 (a-e) Recorded depths of tags a) 1061752 (2142 mm TL; Myroodah Crossing), b) 1061751 (2210 mm TL; Camballin Pool (in Oct.-Dec.) and Myroodah Crossing (in Jan.)), c) 1061749 (2090 mm TL; Milli Milli), d) 1061748 (1883 mm TL; (Telegraph Pool (Nov.) and Milli Milli (Dec.) and e) 1038502 (1576 mm TL; Langi Crossing) in comparison to river stage height (measured at the Camballin Barrage for freshwater pools and at the Willare Bridge for estuarine pools) during the respective wet seasons.

Depth transmissions were recorded from 0+, 1+, 2+ and 3+ year old *P. microdon* between 2008 and 2009; for five different pools and in both wet and dry seasons (Figures 18, 19, 20 and 21). Recorded transmissions for eight *P. microdon* were scored as a one or higher, noting that a higher scores represent a more accurate representation of the animal's behaviour (Table 4). Transmissions from two of these (1061751 and 1061752) were scored as a two or higher for both the wet and dry seasons (Table 4).

Depth profiles of 0+, 1+ and 2+ *P. microdon* tracked in Telegraph Pool (Figures 18-20) were each significantly different ($p < 0.001$) with the exception of tag 1056893 (0+ *P. microdon*) and tag 1038504 (1+ *P. microdon*), which occupied depths between 0.1 and 2.0 m for ~95% of the time they were recorded (median depth = 1.1 (1056893) and 1.0 m (1038504)). These profiles were similar to 1+ *P. microdon* tracked at Snag Pool in 2007 (see Whitty *et al*. 2008, 2009) (Figure 20). The second 0+ (1056894) and the 2+ (1061748) *P. microdon* tracked in Telegraph Pool, spent the greatest percentage of their time in the extreme shallows (similar to 0+ *P. microdon* tracked in Snag Pool in 2007 (Figures 18, 20)). Excluding the time spent at 0 m, tag 1056894 had a similar profile to the other 0+ and the 1+ *P. microdon* tracked in Telegraph Pool, and was observed spending 97.5% of its recorded time between 0 and 1.5 m depth (Figure 18). The 2+ *P. microdon* (1061748), which was also tracked in Milli Milli, was observed to have greater depth range in Milli Milli than it did in Telegraph Pool, spending the majority (97.5%) of its recorded time between 0.2 and 2.6 m (median depth = 1.5 m) c.f. between 0 and 1.3 m (median depth = 0.44 m) (Figure 20).

A 3+ *P. microdon* (1061749) that was also tracked in Milli Milli, inhabited a maximum depth of 1.8 m and spent 93% of its tracked time in water between 0 and 1.5 m (median depth = 0.88 m) (Figure 21). Unlike the 3+ individual tracked in the estuarine Milli Milli, the two 3+ *P. microdon* that were tracked in freshwater pools (i.e. Myroodah Crossing (1061752) and Camballin Pool (1061751)) had a much larger depth range, reaching maximum depths of 6.4 and 5.3 m, respectively and occupied depths of 0-3.1 m (median depth = 2.0 m) and 0.4-3.1 m (median depth = 2.0 m) for 95% of their recorded time, respectively (Figure 21). Depths occupied in the wet season were found to be significantly different ($p < 0.001$) for both *P. microdon* tracked in the freshwater pools, with increases in maximum depths occupied mirroring increases in stage height (Figure 21). This was found to be not significantly different for tags 1061749 ($p = 0.966$) and 1061748 ($p = 0.12$), which were both tracked in the estuarine pools (Figures 20, 21). N.B. Wet season increases in stage height are minimal in tidal areas compared to those in freshwater pools During the wet season, a 1+ individual tracked in Langi Crossing spent over 95% of its recorded time at depths between 2.1 and 3.6 m (median = 2.9 m).

Diel vertical migration patterns similar to that described in Whitty *et al*. (2008, 2009) (moving deep at sunrise and shallow in the afternoon) were observed in all but two individuals whose tags had depth sensors (Figure 22a-e). Of these one had too few transmissions and was not present for all hours of the day to allow for the construction of an average daily vertical profile. The other (1038502), which was only recorded for 11 days, was only present during the wet season (Figure 22c). Statistical analyses of depth utilisation at different hours, suggested that light was found to be positively correlated ($p < 0.001$) with depth occupied. Interestingly, the type of this relationship changed between morning and afternoon hours, as *P. microdon* displayed a different rate of depth change per hour when surfacing than when diving. In estuarine conditions (i.e. turbid waters) surfacing on average was a relatively longer process commonly taking four to seven hours. In freshwater pools timing of this movement was shorter, occurring rapidly over a one to two hour period.

Table 4 Details and scoring of total recorded depth transmissions for corresponding acoustic tags within the different pools and between seasons. Hours present = the hours of the day that the individual *P. microdon* was recorded in that pool in the different season, with 0 representing midnight, 12 representing midday. Mean transmissions per hour = the average number of recorded transmissions detected for all hours it was recorded as being present. Score = numerical score given to rate the accuracy of results of the corresponding tag in the respective pools.

Acoustic tag #	Pool	Season	Hours present	Mean transmissions per hour	Score
1038502	Langi Crossing	Wet	0-23	48.6 (2.2)	2
1038504	Telegraph Pool	Dry	0-23	559.9 (13.4)	3
1056893	Telegraph Pool	Dry	0-23	225.2 (9.3)	3
1056894	Telegraph Pool	Dry	0-23	59 (4.2)	2
1061752	Milli Milli	Dry	3,6-14	2.6 (0.58)	0
1061752	Milli Milli	Wet	0,23	5 (1)	0
1061752	Myroodah Crossing	Dry	0-23	115.3 (8.4)	3
1061752	Myroodah Crossing	Wet	0-23	51.8 (5.0)	2
1061751	Camballin Pool	Dry	0-23	171.8 (13)	3
1061751	Camballin Pool	Wet	0-23	111.5 (12.7)	3
1061751	Myroodah Crossing	Wet	7,9,10	1.7 (.7)	0
1061748	Telegraph Pool	Dry	4-10,12-14,16-19,22	6.9 (.9)	0
1061748	Milli Milli	Dry	0-23	47.0 (1.9)	2
1061748	Milli Milli	Wet	12,13,19	4 (1.2)	0
1061749	Milli Milli	Dry	8-16,18,19,21,23	5.2 (1.3)	0
1061749	Milli Milli	Wet	0-23	14.4 (1.8)	1
1061750	Milli Milli	Dry	1,8,11-14	8 (2.0)	0

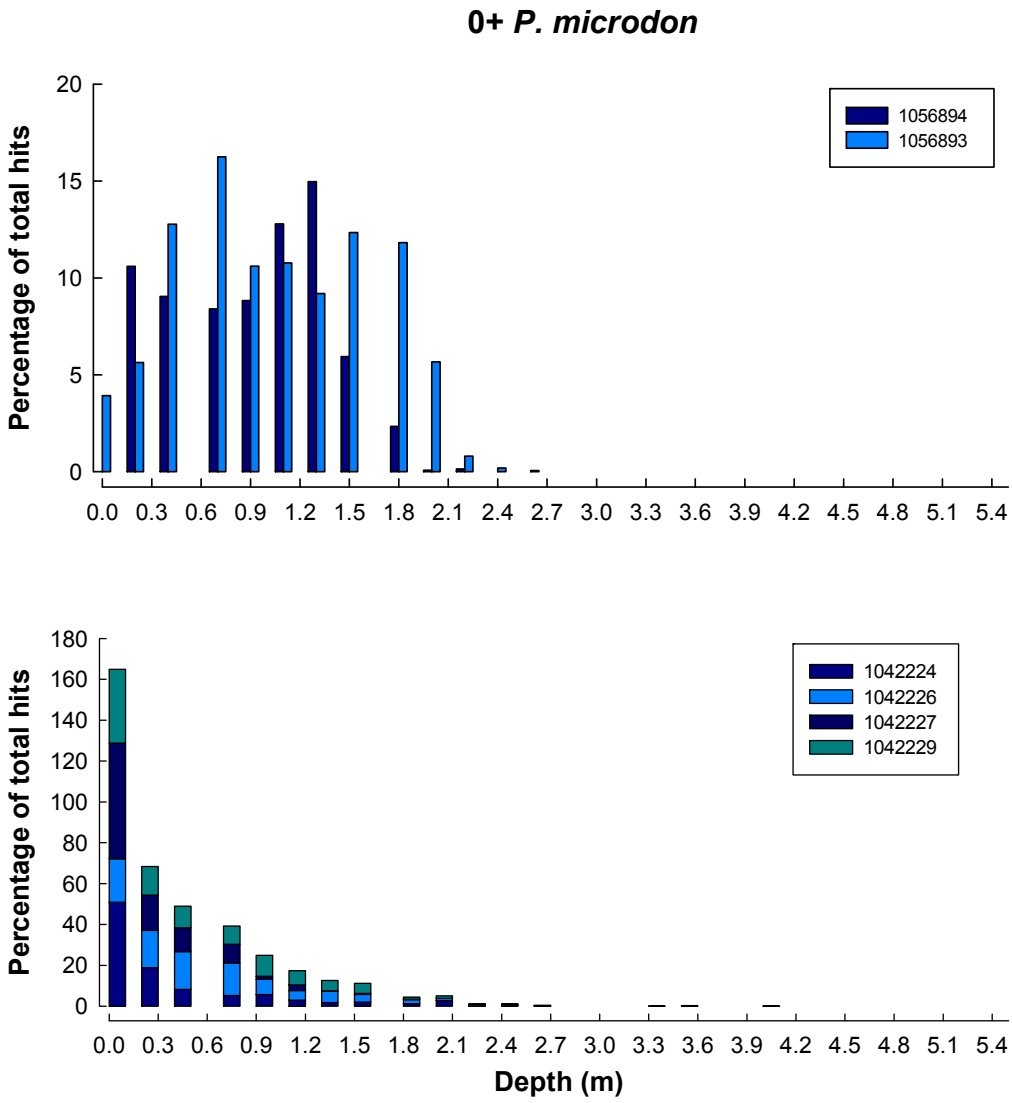


Figure 18 Percentage of time at the different depths during 2008 and 2009 of tracked 0+ *Pristis microdon* in Telegraph Pool (top), c.f. 2007 tracked 0+ *P. microdon* in Milli Milli and Snag Pool (bottom).

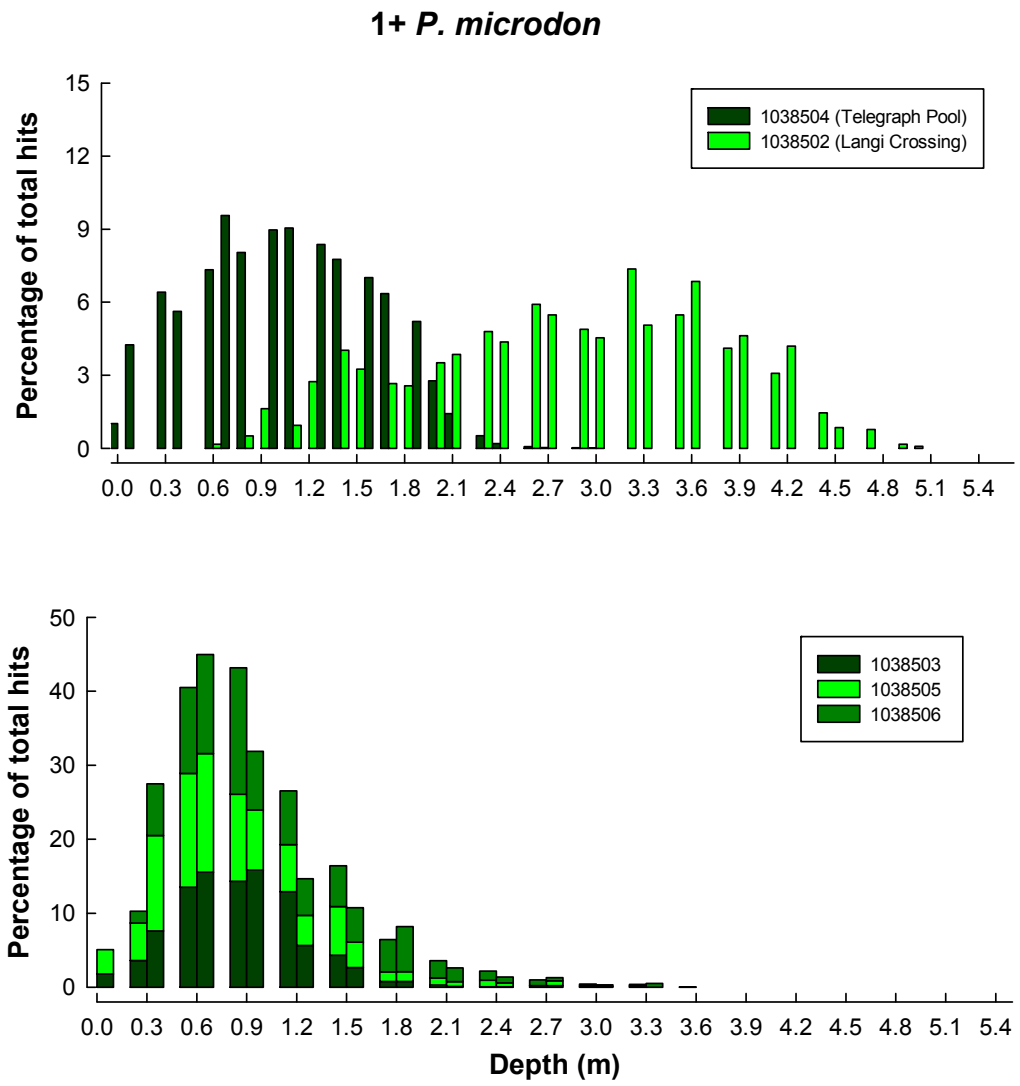


Figure 19 Percentage of time at different depths during 2008 and 2009 for tracked 1+ *Pristis microdon* in Telegraph Pool (dry season) and Langi Crossing (wet season) (top) c.f. 2007 tracked 1+ *P. microdon* in Milli Milli and Snag Pool (bottom).

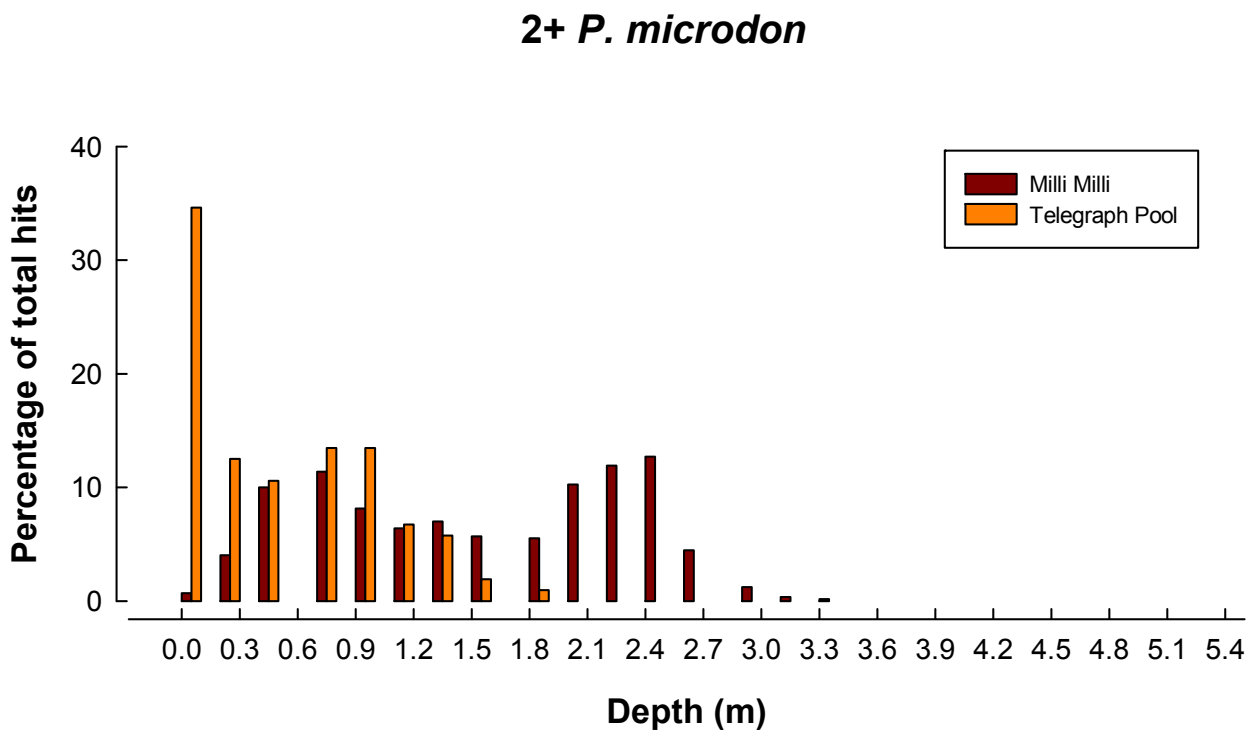


Figure 20 Percentage of time spent at the different depths by a 2+ *Pristis microdon* (1061748) in Milli Milli and Telegraph Pool.

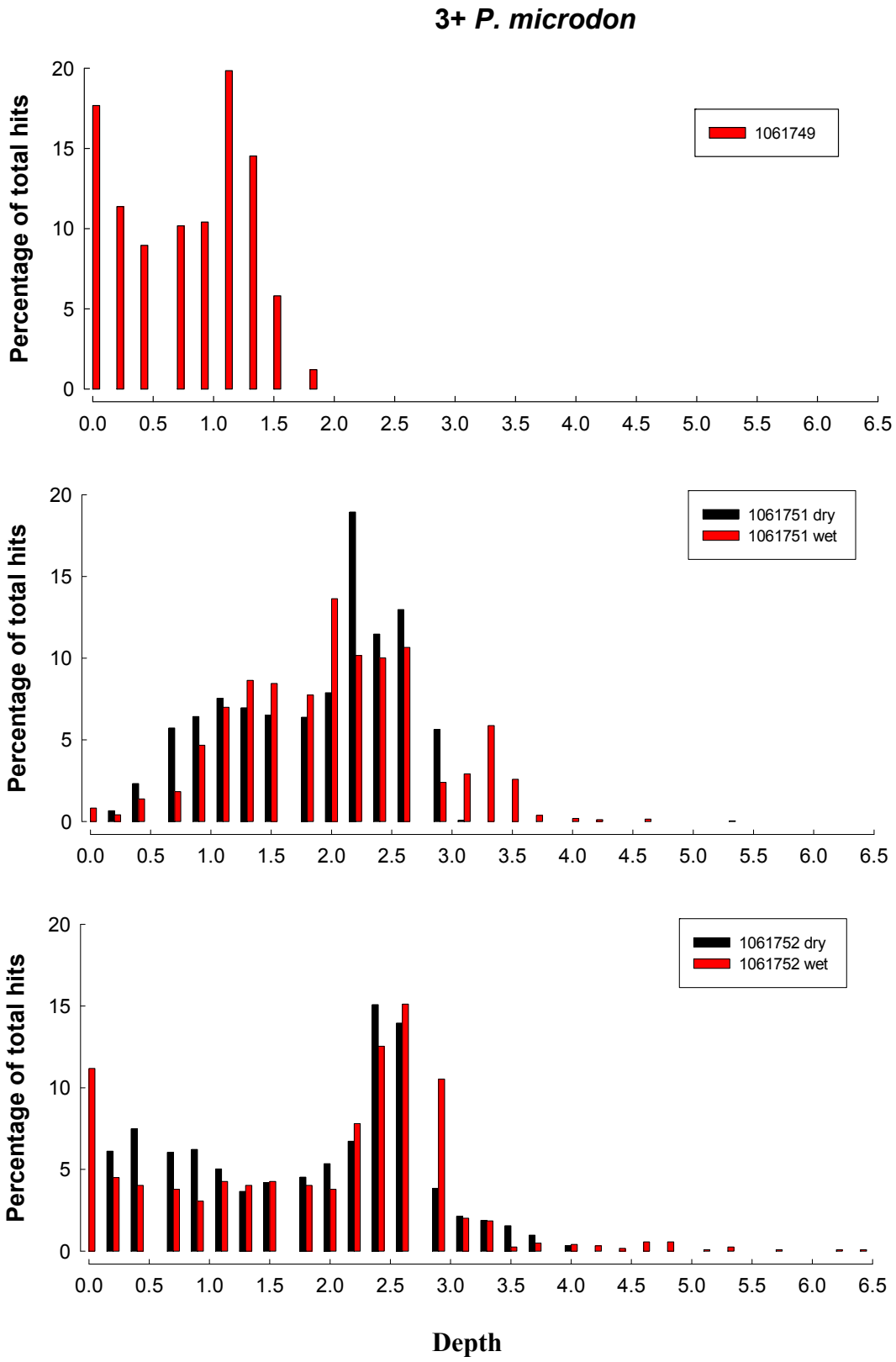
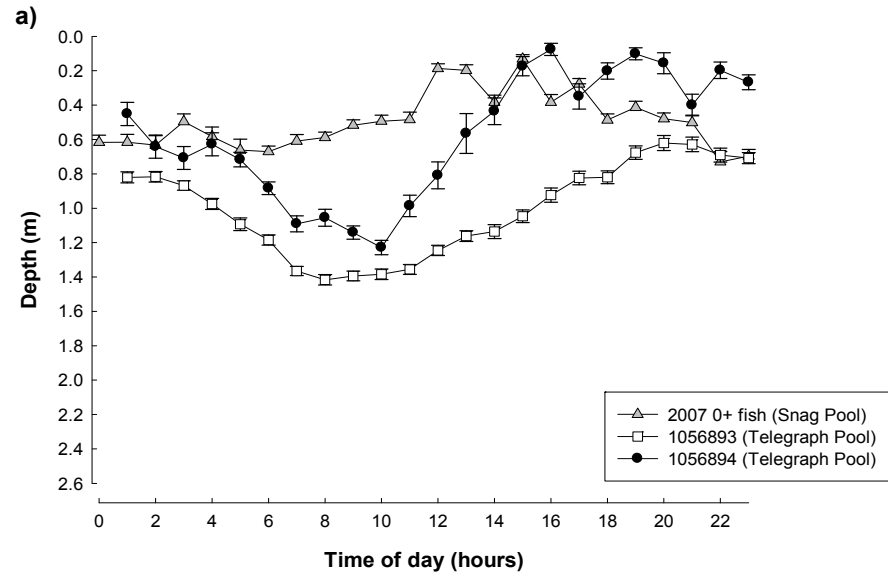
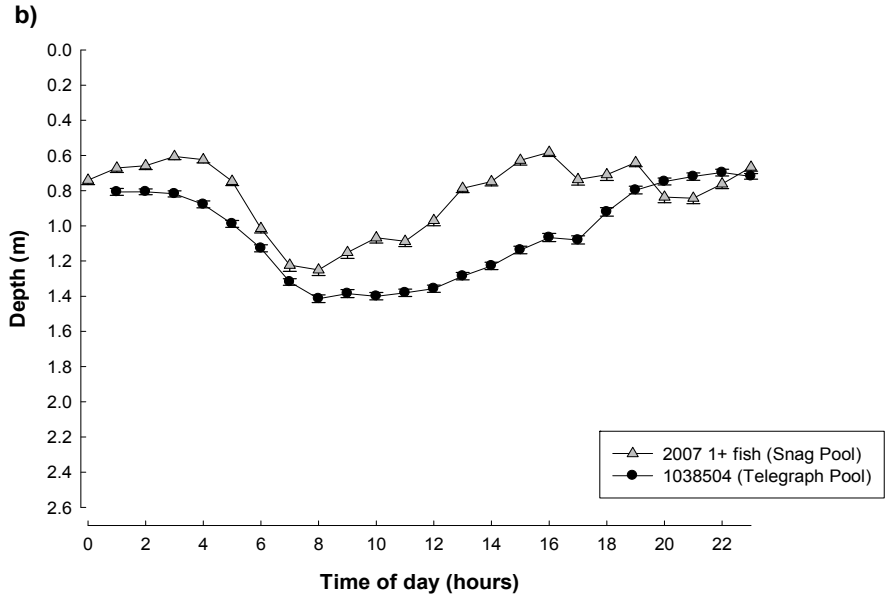
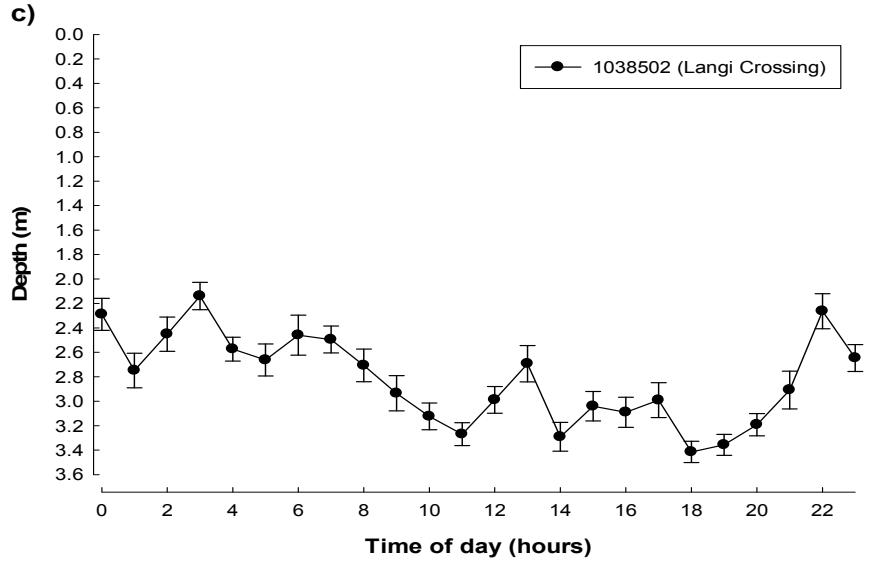
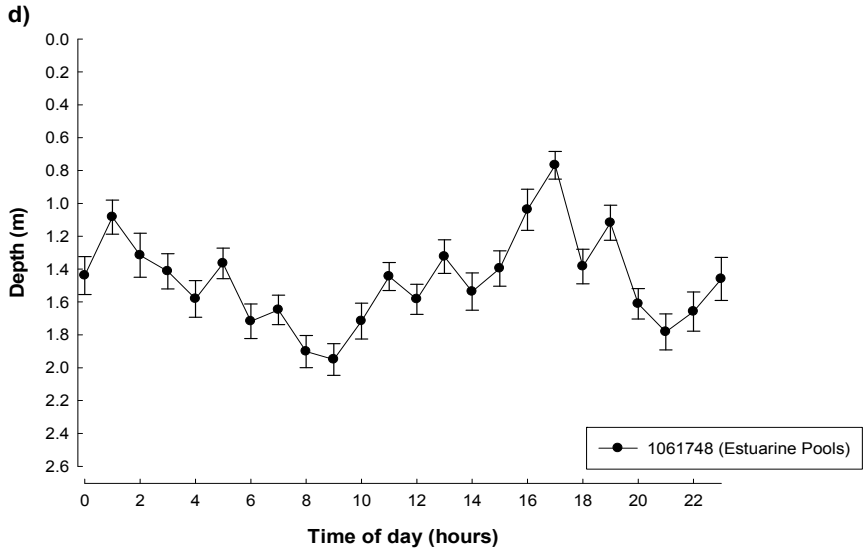


Figure 21 Percentage time at depth of tracked 3+ *Pristis microdon* in the estuarine Milli Milli Pool (top), and the freshwater Camballin Pool (middle) and Myroodah Pool (bottom).


a)
Depth (m)
Time of day (hours)
2007 0+ fish (Snag Pool)
1056893 (Telegraph Pool)
1056894 (Telegraph Pool)


b)
Depth (m)
Time of day (hours)
2007 1+ fish (Snag Pool)
1038504 (Telegraph Pool)


c)
Depth (m)
Time of day (hours)
1038502 (Langi Crossing)



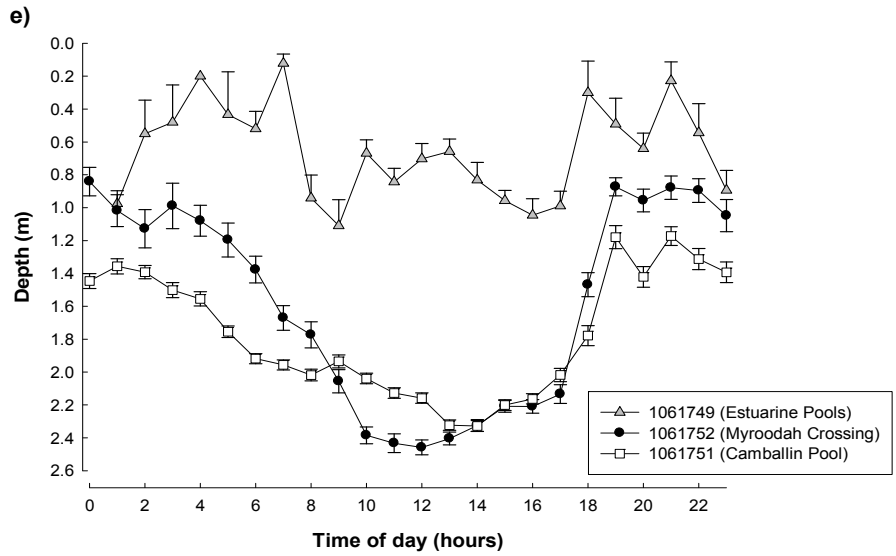


Figure 22 (a-e) Average daily depth profiles of 2008 and 2009 tracked (a) 0+ *Pristis microdon* c.f. pooled (n =4) 2007 tracked 0+ *P. microdon*, (b) 1+ *P. microdon* c.f. pooled (n = 3) 2007 tracked 1+ *P. microdon*, (c) 1+ *P. microdon* tracked in the wet season, (d) 2+ *P. microdon*, and (e) 3+ *P. microdon* (wet and dry season depths are pooled as differences in depth change, while significant, are minimal and don't alter the general pattern of the profiles).

DISCUSSION

Continuation of this study into 2008 and 2009, has substantially enhanced our understanding of the movement patterns, habitat use, morphology, distribution and general biology of *P. microdon* in Western Australia. It has also allowed a more detailed examination of the interannual variation in recruitment of the species and population demographics and is importantly, the only long-term monitoring of a single population of this endangered species. It has also led to a range extension in the distribution of *G. garricki* within Western Australia.

Pristis microdon

CPUE data between 2002 and 2009, for *P. microdon* in the Fitzroy River, suggests that there has been an overall decline in the population during this period. This is despite the capture, during the last four years, of a comparatively large number of new recruits (i.e. 0+ fish), unlike between 2002 and 2005 (see Figure 7 and Whitty *et al*. 2008). Whether this is a continuous overall decline from years past or a decline from an unusually large and temporary recruitment-boom in years just prior to 2002, this decline most likely stemmed from a drop in captures of larger *P. microdon* (> 1+ year old) post 2006. Often, an observed decrease (relative to years prior) in the numbers of an age class can be traced back to a weak year-class/recruitment, like those observed in 2002-2005 (McGlennon *et al*. 2000, Anderson & Rose 2001). A weak year-class/recruitment can be caused by a number of factors including increased predation, reduced health of breeding stocks and reduced river levels, to name a few. River discharge has been shown to be positively correlated with the survivorship of estuarine and freshwater species (Mills & Mann 1985, Drinkwater & Frank 1994), including *P. microdon* (Whitty *et al*. 2008, this study). Although no significant relationship was observed between wet season river stage height and CPUE, the significant difference between early and late dry season CPUE, and the correlation between late wet season discharge and proportion of new recruits in our catches does suggest that water levels influences survivability of *P. microdon* juveniles. It is reasonable to hypothesise that a sustained increase in water levels would increase the survivability of newborn *P. microdon* by increasing productivity and available habitat as well as decreasing predation (Flores-Verdugo *et al*. 1990, Staunton-Smith *et al*. 2004, Whitty *et al*. 2008). It could also be suggested that the drop in CPUE between early and late dry season is due in part to dispersal of the animals through the river. However, as upstream movement of *P. microdon* has been shown to be extremely restricted by low water levels and made impossible beyond the Camballin Barrage during this time (Morgan *et al*. 2004, Whitty *et al*. 2008), dispersal is not as likely to be the cause for this decrease. To better understand the exact influences causing this decrease, continued sampling efforts are needed. As this project is in a unique position having monitored CPUE since 2002, the continuance of sampling would also allow for this project to be able to establish a better understanding of what a current "typical" CPUE is for this system.

Updated morphological data expanded the range of both male and female teeth counts and created a stronger TL : TRL relationship of juvenile *P. microdon*. It is important to understand the morphology of this population as it will allow for more accurate comparisons between different assemblages and in return enhance management of the species. This information will also allow researchers and managers to better understand historical and future *P. microdon* catches from dried rostra, often collected as trophies (Thorburn 2006, Thorburn *et al*. 2007, see Section II, Phillips *et al*. 2008). Unfortunately, few mature individuals have been captured, which has resulted in a scarcity of morphological data for adult *P. microdon* and a lack of understanding about the morphology and habitat associations

of this species beyond 3 m TL. It is therefore important to sample adult *P. microdon*, and additionally locate rostra in private collections from north-western Australia to formulate population demographics in particular regions.

Continuation of the Rototag program has led to 10 recaptures during 2008 and 2009 and recaptures reported by fishers in 2008, the signage also highlights the research and the species rarity. The report in 2008 of a *P. microdon* recaptured just 9.5 km upstream 143 days after it was tagged, aided in confirming previous telemetry findings (Whitty *et a*l. 2008, 2009); that *P. microdon* have high residency times within the estuarine region in the dry season. From this same report a growth rate of 0.49 mm/day was calculated for the recaptured 0+ *P. microdon*. This rate is relatively low compared to the 1.4 mm/day documented for *P. microdon* in Queensland (Peverell 2008) and the Fitzroy River (Thorburn *et al*. 2007) and up to 2.3 mm/day have 0+ Small Tooth Sawfish (*Pristis pectinata*) (Simpfendorfer *et al*. 2008) in the US. Further data is needed to examine growth rates, as increased temperatures and available resources may lead to increases in growth rate (Talbot 1993, Cortes & Gruber 1994). Public reports of recaptures continue to be minimal, with only two confirmed in 2008, although there were many anecdotal reports of tagged sawfish being captured, and we recaptured 10 since 2008. A lack in public reports may be due to a decrease in *P. microdon* with tags, as faster growth of 0+ *P. microdon* may cause early shedding of tags (Simpfendorfer pers. comm.), which would result in fewer marked recaptures than expected, or that mortality is higher than expected in 0+ fish. It is also possible that fishers are not reporting recaptures as has been the case in the past. An increase in public education, on top of what has already been accomplished (posters, signs, public presentations, Science Week display and presentations in Broome August 2009) may help increase the number of reported recaptures. In 2008 and 2009, efforts were made to increase awareness of *P. microdon* and the tagging program through five talks in the Western Kimberley to catchment groups, Indigenous communities and various government and public bodies. Several presentations at three different scientific conferences throughout Australia and one within the United States were also undertaken to expand knowledge of the species through the scientific community. The 2008 and 2009 filming and airing of the study on National Geographic's "Monster Fish" series allowed us to present our findings globally. Involvement of Traditional Owners, including the invaluable assistance by the Yiriman Rangers, has continued in 2008 and 2009, and it is envisaged that this will continue interest in the sawfish project through local communities.

Alongside the Rototag program, the acoustic tracking study involving *P. microdon* continued through 2008 and 2009. Following findings from 2007 (i.e. discovery of habitat partitioning occurring between 0+ and 1+ *P. microdon*), the expansion of this study focused on the comparison of habitat use of individuals occupying different pools that vary in depth, turbidity and salinity throughout the lower 150 km of the Fitzroy River. While temporary mechanical failure of acoustic receivers reduced the amount of possible data to be produced, the increase in receiver numbers and continuation of the project over a two year period aided in construction of a better understanding of *P. microdon* habitat use. To prevent future loss of data, a query into the cause of previous receiver failures has been initiated with the manufacturer. Although the problem was initially thought to be the result of battery failure, as other projects using batteries from the same manufacturer were having similar problems, it is now believed the problem came from a bad batch of receivers, having observed the fifth, of the six original receivers, to fail (noting a different battery manufacturer was used). Besides the original batch of receivers, no problems have been detected with newer and fixed receivers.

Transmissions recorded by the VR2W receivers in 2008 and 2009, from 0+ *P. microdon* tagged in June 2008, lasted between 13 and 93 days whereas those from larger fish (1800+ mm TL) lasted between 15 and 300+ days. Although there are a number of reasons for the termination of recorded transmissions (see Whitty *et al*. 2008), the most likely are that either: the fish moved out of the area (upstream of Langi Crossing or downstream of Milli Milli for estuarine inhabitants), that the tagged fish was removed from the system by a fisher, or died as a result of predation, or that tags were shed early. Although difficult to ascertain the reason behind early termination of tag transmissions, the cessation of transmissions of 0+ *P. microdon* tags in not only 2008 and 2009, but 2007 as well, occurring within a period of less than four months, would suggest that this may be the amount of time required for Rototags (mounting platform of acoustic tags) to be pushed out from the quickly growing young of the year; although this age class is likely to have a higher mortality than older age classes. While the duration of time that transmissions are received does not necessarily equate to the number of days tags were retained, it does demonstrate the possible longevity of the tags on the different individuals/age classes. Longer transmitting tags occurring solely on larger *P. microdon* was not surprising, as Rototags have been observed to last up to at least three years on these age classes. To increase the time that 0+ *P. microdon* can be tracked, it would be advantageous for this study to investigate the use of internal implantation with acoustic tags. Internal implantation of acoustic tags has been shown to be a successful method for long-term monitoring of elasmobranchs and riverine fish (Holland *et al*. 1999, Parkyn 2006). A concomitant study conducted by Stirling Peverell (QLD DPI&F) in Queensland has looked at the effects of internal implantations on *P. microdon* in a riverine environment, with promising results.

Acoustic tracking data from 2008 and 2009, showed similar patterns to that observed in 2007, and supported previous hypotheses. From movement data collected in 2007, it was hypothesised that *P. microdon* in the estuarine area moved into Telegraph Pool around late July or early August when they disappeared from Snag Pool. As Telegraph Pool was not monitored in 2007 this hypothesis was only supported by circumstantial evidence (Whitty *et al*. 2008, 2009). Installation of a receiver into Telegraph Pool in July 2008 allowed for the observation of animals occupying and moving into this pool in late July and early August, with one individual eventually moving to Langi Crossing in November. Telegraph Pool has often been found to host a relatively large number of sawfish, including both *P. microdon* and *P. clavata* (during the end of the dry season) (Thorburn *et al.* 2003, 2007), but for what reason is unknown. A number of 0+ *P. microdon* have been observed over 100 km upstream within a month or two after peak river discharge, which would suggest that there is an "instinct" within in at least a few individuals to move upstream to the more stable environments of the upper pools. As Telegraph Pool and Langi Crossing are the last two most upstream pools that *P. microdon* can access with the aid of tides in the dry season, this requirement to move upstream or a preference to inhabit areas of lower salinity may be a reason for the occurrence of *P. microdon* in these pools in the late dry season. Further investigation is needed to determine the explanation for the inhabitation of Telegraph Pool.

Previous findings (Whitty *et al*. 2008, 2009) demonstrated flow to be highly influential on *P. microdon*, dictating interpool movements of 0+ *P. microdon* and aiding in movement upstream for larger bodied *P. microdon* in the tidally influenced estuarine pools. Increased river flows have also been shown to be a trigger for the migration of various fish species. Gulf Sturgeon (*Acipenser oxyrinchus desotoi)*, which like *P. microdon* migrate from fresh to marine waters, have been reported to make this said migration at a time when temperatures

decrease and river flows increase (Heise *et al*. 2005). During the current study, large (> 2 m) individuals were also documented to leave freshwater pools, where they had previously been confined, and estuarine pools that they could move between with the aid of tides, almost immediately at the onset of a flood event, caused by increased water flows. Three of the four *P. microdon* tracked during these flood events were > 2 m TL and had all moved downstream, one moving over 100 km to the river mouth (Milli Milli), where it was last recorded. A second of these was last recorded at Milli Milli at the initiation of the first flood event. Disappearance of these large fish from the acoustic array at the mouth of the river is potentially evidence of their migration back to the marine environment and could be the completion of the freshwater phase in their lifecycle. This corresponds with the fact that few *P. microdon* greater than 2.5 m TL have been recorded in the freshwater pools, and is likely to be approximately the size at which they leave the river. While a few number of *P. microdon* greater than 2.5 m have been captured, all have been female (Thorburn *et al*. 2007), which further suggests that females may remain in the river longer than males.

Many of the fishes inhabiting the dynamic ecosystems of northern Australia, with large changes in flow and temperature regimes, for example, are able to readily adapt to differing habitats. Changing habitat use to conform to available resources is a valuable and expected ability for a large migratory benthic species such as *P. microdon*. Employing data from the tag depth sensors in this study revealed that *P. microdon* appear to alter their depth use in varying habitats, both in freshwater and the estuary, between individual pools, and seasonally. Although, separating what environmental conditions are 'preferred' vs. accepted is a difficult task and requires continued monitoring of the species. The observed increase in depth occupied by *P. microdon* in the freshwater pools during the wet season suggests that at least the larger (> 2 m) *P. microdon* prefer conditions associated with deeper depths.

Data acquired by the pressure sensors also showed *P. microdon* to display a diel vertical movement pattern, which was also apparent in 2007 (diving to deeper water at sunrise and ascending to shallower water in the afternoon to sunset) (Whitty *et al*. 2008, 2009). Timing and rate of ascent of this pattern was similar for *P. microdon* inhabiting Milli Milli, Snag Pool and Telegraph Pool (gradual ascent in afternoon), but different for *P. microdon* tracked in the freshwater pools (quick ascent at sunset). To explore the reasoning behind these differences it was important to understand what influenced these movements. Vertical migrations such as those previously described, are often triggered by a primary cue or a combination of cues such as light, temperature, physiological state (hunger) and/ or endogenous rhythmicity (internal biological clock) (Gibson 1997). This study investigated two of these cues, light and temperature. Often it can be difficult to separate the influence of these factors as they are typically strongly correlated within one another. An observed delay between light and temperature allowed for statistical evidence to suggest light to be the primary cue (or one of) for *P. microdon* diel vertical movements, either directly or indirectly (i.e. following prey). Light intensity has also been discovered to be used as a primary cue for other elasmobranchs including Manta Rays (*Manta birostris*) (Dewar *et al*. 2008) and Megamouth Sharks (*Megachasma pelagios*) (Nelson *et al*. 1997). It has been a popular belief that the light cue for many aquatic species is the rate of its relative change (Forward 1987, Swift & Forward 1988), which is usually greatest around sunrise and sunset. Contrary to this belief, a study involving *M. pelagios*, which was discovered to follow a particular isolume (depth profile of a particular light intensity), suggested that cues may also be based off the magnitude of light intensity rather than the rate of change (Nelson *et al*. 1997). *Pristis microdon* in the estuarine pools begin their ascent in the afternoon when the change in light levels is relatively minimal, it is likely that like *M. pelagios*, *P. microdon* within at least estuarine pools, follow a

particular light intensity or a combination of the two cues. Reasons for the differences in the rate and timing of ascension may be linked to the differences in turbidity of the two areas. Light attenuation is greater in the less turbid freshwater pools than the highly turbid estuarine pools (Anthony *et al*. 2004). This would result in a desired isolume to be deeper and steeper and reach depths of < 1 m later in the day in the less turbid freshwater pools than the turbid estuarine pools, which is similar to the different surfacing patterns displayed by estuarine and freshwater tracked *P. microdon*. To better understand how light influences *P. microdon*, further work investigating their visual abilities, in particular the sensitivity to various light wavelengths, would be valuable.

Glyphis garricki

Capture of *G. garricki* on the south-western coast of King Sound was important as this new extension of their home range in King Sound demonstrates that they are distributed throughout the turbid waters of both sides of the sound. This *G. garricki* inhabited the same range of conditions of previous captures, with the exception of water temperature which was 0.8°C lower than previously recorded (Thorburn & Morgan 2004, Thorburn & Morgan 2005, Whitty *et al*. 2008). Lack of *G. garricki* captures in September 2009, within the north-western creeks of King Sound was expected as the turbidity within these areas was much lower than that of other regions that *G. garricki* has been previously found to inhabit within King Sound (Thorburn & Morgan 2004, Thorburn & Morgan 2005, Whitty *et al*. 2008).

Sampling of *G. garricki* in previous years has been difficult due to time constraints of researchers and the extreme conditions found in King Sound, including 11+ m tides, harsh weather, minimal access points and dangerous wildlife. To aid in the sampling efforts of this study, a camera was donated to the local commercial barramundi gillnet fisher to document any carcharhinids (whaler sharks) and/or sawfish in the off chance that a *G. garricki* is captured. Trialed in 2008, this project has already been successful with one *G. garricki* being captured on film before its release. Efforts to educate commercial fishers in elasmobranch identification, is also underway to increase the detail of reports to the Department of Fisheries, Western Australian Government.

Management implications and future research

From the culmination of this and previous work, it has become apparent that *P. microdon* is a resourceful species, altering their habitat use with changing resources. However, even with this level of plasticity, a number of threats still challenge the existence of this critically endangered species, including decreased water levels of nursery grounds, destruction of critical habitats and illegal fishing. In previous years sawfish massacres have been documented within the Fitzroy River (e.g. 12 dead sawfish were found on the banks of Telegraph Pool in 2002 (Thorburn *et al*. 2003)). Observations of these massive killings in the Fitzroy River have declined in recent years; however during this study at least one large *P. microdon* (2400 mm TL) was killed and left to die by fishers, as was a smaller juvenile. Despite strong community support for the project and considerable publicity, it is unfortunate that this still occurs, and it is important to continue to publicly inform the local community as to the global rarity of *P. microdon* as well as *G. garricki*.

Information from completed research will greatly aid in the future management of the critically endangered *P. microdon*. Firstly, the data describing decreases in catch rates, which reflects upon density levels of *P. microdon* (likely most heavily with 0+ cohorts) between

early and late dry season, can be used to calculate mortality rates for the species. Calculating mortality rates based on long-term field observations is vital in understanding the recovery rate of a species. Secondly, documenting the increase in depth use by the 3+ and 1+ *P. microdon* in the deeper pools and during the wet season (when river depths are greater), suggest that older age classes show a preference for deeper water. This needs to be taken into account when managing the species. Lastly, high residency time in the upper estuarine pools may cause *P. microdon* to be more susceptible to fishers and increased intra-specific competition for resources during the late dry season. During these times, fishing in this area may need be more restricted, to reduce bycatch levels of *P. microdon* (noting that *P. microdon* are not directly targeted by fishers). Further research will be needed to determine the densities of sawfish in these pools at this time and an approximation of the level of impact that fishers have on *P. microdon* in these pools.

Unlike *P. microdon*, constructing management strategies for *G. garricki* are more difficult as little is known about the species, in part due to its rarity. From the findings of this research, the habitat type of sub-adult and adult *G. garricki* has been fairly well documented. Using this knowledge it is recommended that further sampling be undertaken in similar regions across northern Western Australia and the Northern Territory. Only through increasing sampling efforts will we better understand the range and population status of *G. garricki* and what regions should be considered for protection. Along with increased sampling, educating the public about the existence of *G. garricki* and how to properly identify the species should be one of the key components of any conservation plan to protect the species.

Further work that would aid in the management and conservation of *P. microdon* includes a continuation of acoustic tagging/tracking to look at ontogenetic differences versus pool differences in habitat use. Deployment of tags fitted with depth sensors on 0+ and 1+ *P. microdon* in the upper freshwater pools should be a priority in tracking this species, as it has yet to be accomplished. It is also important to extend the range of sampling to other rivers and open water to better understand the distribution of *P. microdon* in Western Australia as well as to discover the habitat that mature *P. microdon* occupy. It would also be highly beneficial to extend the acoustic array off of One Arm Point, Broome and surrounding areas to commence an adult *P. microdon* tracking program to investigate the locations and timing of mating as well as pupping. Lastly, a continuation and increase of sampling and research of *G. garricki* is strongly needed as very little is known about its biology, population status and range. Although passive tracking would be difficult, due to the conditions these animals are found in (likely to result in the loss of receivers), an active tracking study could prove useful as has been shown in other studies in similar areas.

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SECTION II

Microsatellite and mitochondrial DNA assessment of the genetic diversity and population structure of the Freshwater Sawfish, *Pristis microdon*, and the Green Sawfish, *Pristis zijsron*, respectively, in Australian waters: preliminary results

by

N.M. Phillips, J.A. Chaplin, D.L. Morgan, and S.C. Peverell

INTRODUCTION

The sawfishes (Pristidae) are a group of elasmobranchs comprised of seven species in two genera (*Pristis* and *Anoxypristis*) that are notable because of their large body size and saw-like projection of the upper jaw, termed a rostrum (Bigelow & Schroeder 1953). All sawfish species appear to have undergone dramatic declines in range and abundance in recent times due to anthropogenic activities (*e.g*. Simpfendorfer 2000, Pogonoski *et al.* 2002, Peverell 2005, Thorburn *et al.* 2007). Consequently, these species are all currently listed as critically endangered worldwide by the International Union by the Conservation of Nature (IUCN 2006) and some sources include them amongst the most endangered fishes. However, the group is very poorly studied and the concomitant lack of information on their population biology is hindering attempts to assess their current status and to develop effective plans for their management (Peverell 2005).

Ideally, the management plans for any particular sawfish species should be based upon, *inter alia*, a sound knowledge of the population structure and patterns of dispersal to determine the most appropriate spatial scale(s) at which the species should be managed (Palsbøll *et al.* 2007); the phylogeographic structure as this will reveal the existence of any highly distinctive populations that might warrant special conservation status (Moritz & Faith 1998); and the levels of genetic diversity within populations as this will influence the intrinsic evolutionary potential of the populations (Hedrick 1992, Amos & Balmford 2001). This knowledge can be obtained from population genetic data (*i.e.,* Bergl & Vigilant 2006, Okello *et al.* 2008).

The Freshwater Sawfish, *Pristis microdon*, and the Green Sawfish, *Pristis zijsron,* were once widely distributed in the Indo-west Pacific region, although it seems likely that viable populations of these species are now restricted to northern Australia, making these populations vital in conservation efforts (Pogonoski *et al.* 2002, Last & Stevens 2009). These species of sawfish have a 'typical' elasmobranch life-history, in that they have a large body size, a long life with late maturity, ovoviviparous reproduction, and low fecundity (Tanaka 1991, Thorburn *et al.* 2007, Peverell unpublished data), although the associated details are better known for *P. microdon* than for *P. zijsron*, mainly through very recent work (see Peverell 2005, Thorburn *et al.* 2007; Whitty *et al.* 2008). The life-cycle of *P. zijsron* is fairly typical of sawfishes because it is completed entirely in marine waters (including the lower reaches of estuaries); the juveniles of this species are predominantly found in inshore waters and mangrove areas (Stevens *et al*. 2008). In contrast, *P. microdon* has marine-estuarine adults, but spends the juvenile phase of its life-cycle in the upper reaches of estuaries and freshwater rivers (Taniuchi *et al.* 1991, Peverell 2005, Thorburn *et al.* 2007, Whitty *et al.* 2008, 2009). Hence the separation of juvenile and adult habitat is relatively extreme in *P. microdon*. There is an increasing body of evidence to suggest that female philopatry coupled with male-dispersal is common in elasmobranch species in which adult and juvenile habitat is spatially removed (*e.g.* Springer 1967, Ebert 1996, Feldheim *et al*. 2001, 2004, Keeney *et al*. 2005), but it is not known if this is the case for sawfishes.

The nucleotide sequence of the control region in the mitochondrial DNA (mtDNA) is typically information rich and can be reliably ascertained with a minimum of developmental work, even for species for which there is no prior knowledge of the composition of the mitochondrial genome. Largely for these reasons, the initial phases of the analyses of the population genetic structure of a species are commonly based on information on the distribution of variation in this sequence (see Hoelzel *et al.* 2006, Stow *et al.* 2006). However, such an analysis can only provide information about the amount of genetic diversity

and gene flow in the female component of the species as mtDNA is maternally inherited. Information on the distribution of genetic variation in the nuclear DNA (nDNA), which is bi-parentally inherited, is required to provide information about the amount of diversity and gene flow in both the male and female component of the population. Regardless, in order to test for the presence of sex-biased dispersal in a species via a genetic method (which is effectively the only method available for tests of sex-biased dispersal for most species, including sawfishes), it is necessary to have data on either the distribution of variation in the nDNA separately for each sex or on the distribution of variation in both maternally inherited (mtDNA) and bi-parentally inherited (nDNA) markers (Prugnolle & de Meeus 2002).

Microsatellite loci are non-coding parts of the nuclear genome. They typically comprise of tandemly-arrayed repeats of short sequence (typically 1 – 6 bp) and display alleles with different numbers of repeat units (O'Connell & Wright 1997). Microsatellite loci are very useful for population-level analyses mainly because they tend to exhibit relatively high levels of polymorphism and variation that can readily be ascribed to homozygotes and heterozygotes at particular loci (Ashley & Dow 1994). However, the taxonomic range of individual microsatellite loci is typically narrow and finding suitable loci for a particular species can require a large amount of developmental work (Zane *et al.* 2002).

The genetic component of this report describes some of the results of ongoing research into the conservation genetics of *Pristis* species in Australian waters. The ultimate goal of this research is to use mtDNA and nDNA markers to provide detailed information about the population structure, phylogeographic structure and genetic diversity of each of *P. microdon, P. zijsron* and *P. clavata* and to assess the management implications of the results. The first phase of the genetic research has previously been reported in Phillips *et al.* (2008) and was based upon an analysis of the spatial distribution of nucleotide sequence variation in a portion of the control region of the mtDNA in *P. microdon* in Australian waters. The findings of this first phase indicated the following; (1) the amount of female-mediated gene flow in *P. microdon* between the Fitzroy River on the west coast and the Gulf of Carpentaria region in north-eastern Australia is negligible; (2) *P. microdon* contains 'healthy' levels of genetic diversity in the mtDNA over a range of spatial scales in Australian waters; and (3) *P. microdon* in Australian waters belong to a single evolutionary unit.

The current report describes the second phase of the genetic research. The aims of this second phase are as follows:

(1) Commence an investigation into the levels of genetic diversity and structuring in nDNA (microsatellite) markers in *P. microdon* in Australian waters. This involved assessing the suitability of microsatellite loci in a genomic library of *P. pectinata* for use with *P. microdon* and providing preliminary data on the levels of diversity and broad-scale spatial patterns of variation at three of these loci. N.B. The ultimate goal of the microsatellite analyses is to include data from additional loci and test for evidence of sex-biased dispersal in *P. microdon*.

(2) Examine broad-scale spatial patterns of nucleotide sequence variation in the control region of the mtDNA in *P. zijsron* in Australian waters. This involved providing preliminary information about the levels of control region diversity within selected assemblages and the extent of control region variation between assemblages in different geographic regions. The data were also used to test for the presence of any highly distinctive lineages or populations in this species that might warrant special conservation status. N.B. The results of the research

on *P. zijsron* will ultimately be combined with those for *P. microdon* and used to investigate the relationship between the current conservation status of each of these species and their contrasting patterns of habitat usage.

It was not possible to use control region data to assess the relationships between the assemblage(s) of *P. microdon* on the north coast of Australia outside of the Gulf of Carpentaria and those in the Fitzroy River on the west coast and the Gulf of Carpentaria region, as was initially intended as a part of the second phase of this research. In order to conduct this assessment, it was first necessary to obtain samples, and ultimately DNA, from individuals of *P. microdon* from the north coast outside of the gulf. However, although samples from 11 individuals from this region were obtained, the majority of these were either from dry rostra or poorly preserved. Consequently, adequate DNA was obtained from only three of them. Future trials using different methods of DNA extraction will be implemented in attempt to rectify the problem.

METHODOLOGY

Pristis microdon

Sampling regime

The microsatellite analyses focused on samples of *P. microdon* from the Fitzroy River region on the west coast of Australia and from the Gulf of Carpentaria and associated rivers in the north-east, as the number of available samples of this species from each of these regions was relatively large (compared to other regions) and statistically meaningful results could be obtained. The details of the samples used in these analyses are provided in Table 1; these samples are mainly a sub-set of those of Phillips *et al.* (2008).

Table 1 Details of the samples used for the *Pristis microdon* microsatellite analyses, including the number of individuals sampled at each of the collection sites, the sample types and dates. The samples were from two geographic regions, the west coast (WC) and the Gulf of Carpentaria (GoC). *N* = the total number of individuals from a particular geographic region.

	Site	Number of individuals	Sample type	Date
WC	King Sound	3	Rostra	2002 - 2005
	Fitzroy River	25	Preserved	2006 - 2008
		N = 28		
GoC	Gulf of Carpentaria	1	Preserved	2002
	Kirke River	1	Preserved	2001
	Flinders/Bynoe/Norman rivers	10	Preserved	2002
	Smithburne River	2	Preserved	2001
	Gilbert River	8	Preserved	2004
	Nassau River	3	Preserved	2002
	Mitchell River	11	Preserved	2002 – 2008
	Archer River	6	Preserved	2001 – 2008
		N = 42		

Microsatellite markers

The microsatellite results in this report are based on the analysis of variation at three tetranucleotide loci, *Ppe 5, Ppe 122* and *Ppe 186*. These loci were selected for analysis because preliminary results indicated that each of them could be reliably amplified, was polymorphic in *P. microdon* and did not exhibit scoring artefacts such as null alleles (on the basis that the observed genotype numbers at each locus was effectively the same as the number expected under Hardy-Weinberg Equilibrium conditions). The alleles at the locus *Ppe 122* varied by increments of 4 bp, as expected, and so did the majority of alleles at *Ppe 5* and *Ppe 186.*

Information on the nucleotide sequence composition of the loci *Ppe 5, Ppe 122* and *Ppe 186* and their flanking regions (required to design PCR-primers) was obtained from a partial genomic library constructed for *Pristis pectinata* (found in the western Atlantic Ocean) by Kevin Feldheim and Demian Chapman, who kindly passed on the resultant information. Information about the composition of these loci and their flanking regions will be released once Kevin Feldheim and Demian Chapman have published details of their microsatellite development work for *P. pectinata*. The *P. pectinata* library also contains information on a number of other microsatellite loci, some of which are currently being assessed to determine if they are suitable for use with *P. microdon* (so data on the patterns of variation at six or more microsatellite loci can be included in future research).

Genetic assays

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 the manufacturer's protocol. Skin tissue was used from dry rostra following the methods of Phillips *et al.* (2009), while the ethanol preserved tissue comprised either muscle tissue or fin clips. 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, which was pre-stained with 2.5 µl of SYBR® Safe DNA gel stain (10,000x concentration in DMSO, Invitrogen), subjected to electrophoresis for 15 minutes at 50 mAmps, and illuminated with UV light.

PCR amplification

Polymerase chain reaction (PCR) was used to amplify the alleles at each of the three microsatellite loci. The forward primer for each locus was fluorescently labeled with 6-FAM (*Ppe 5* and *Ppe 122*) or NED (*Ppe 186*) (Applied Biosystems), while the reverse primers were unlabelled. 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 dNTPs (Promega), 0.25 U of *Taq* polymerase (Roche), 1 µmol of each primer, and adjusted to a final volume of 15 µl with PCR-grade water. The alleles were amplified using touchdown PCR with an initial denaturation phase at 94°C for 5 minutes, followed by 50 cycles, with each cycle consisting of: 30 seconds of denaturation at 94°C, 1 minute of annealing at 60 - 40°C (*Ppe 5* and *Ppe 122*) or 63 - 43°C (*Ppe 186*) with a 0.4°C decrease in each cycle, and 30 seconds of extension at 72°C; followed by a final extension at 72°C for 20 minutes.

Screening and scoring of allele sizes

Each PCR product was added to a well in a Fisher Biotech 96-well plate, along with 15 µl of (Hi-Di) formamide and 0.09 µl of 600 LIZ size standard (GeneScan). The PCR products of

the loci *Ppe 5* and *Ppe 186* were multiplexed, meaning they were combined in the same well and analysed in the same capillary tube at the same time. The loading volume for each of *Ppe 5* and *Ppe 122* was 0.5 µl and was 1 µl for *Ppe 186*. The plates were then sealed with a septum (Applied Biosystems) and the PCR products subject to electrophoresis and laser detection in capillary tubes. A raw chromatograph of the results was produced via an Applied Biosystems 3230 DNA Analyser. The size of each allele at each locus was automatically scored using the software GENEMARKER v.1.8 (SoftGenetics Inc.), and was manually checked for error. Three positive controls, samples that had been scored as a part of a previous assay, were included in all assay plates in order to ensure internal consistency in the scoring of alleles. A negative control, a PCR assay without added DNA, was also incorporated into each PCR assay and plate in order to check for contamination.

Data analyses

Levels of genetic diversity

The levels of genetic diversity at each microsatellite locus in selected samples of *P. microdon* was assessed in terms of the amount of expected heterozygosity (H_E), $H_E = 1 - \Sigma(f_i)^2$, where f_i is the frequency of the *i*th allele. Values of expected heterozygosity range from zero (where all individuals have an identical genotype) up to one for highly polymorphic loci.

The levels of genetic diversity at each microsatellite locus were assessed in terms of allelic richness (A), which equals the number of different alleles present. Since the number of alleles observed in a sample strongly depends on the size of the sample, values of A were standardised to facilitate comparisons across samples of different sizes, although raw estimates of A are also provided. Values of A were standardised according to the numbers of individuals present in the smallest sample involved in the comparison, using the standardisation procedure in 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/).

Hardy-Weinberg equilibrium

Exact tests were used to assess the statistical significance of differences between the observed and expected numbers of homozygotes and heterozygotes at each microsatellite locus in selected samples of *P. microdon.* These tests used the Markov chain method to estimate the exact probability of a type I error (Raymond & Rousset 1995), as implemented by GENEPOP, version 1.2 at http://genepop.curtin.edu.au (see Raymond & Roussett 1995). The iteration parameters for these, and all subsequent analyses conducted with GENEPOP, were a dememorisation number of 10,000, 1,000 batches and 10,000 iterations per batch. Exact probability tests were selected for this analysis because they are not biased by small sample sizes or low frequencies of alleles or genotypes (Raymond & Rousset 1995). Departures from Hardy-Weinberg equilibrium conditions might indicate the presence of null alleles (alleles that do not amplify and are therefore not detected) or that the samples comprise an admixture of individuals from different populations.

Population genetic differentiation

Overall

Analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was used to assess how genetic variation at the three microsatellite loci was partitioned within and between samples from the Fitzroy River region and the Gulf of Carpentaria and associated rivers. The statistical significance of the variance estimates were assessed using a nonparametric

permutation approach, in which sequences were randomly permutated among samples (see Excoffier *et al.* 2005). This analysis was conducted using the software ARLEQUIN version 3.0 (Excoffier *et al.* 2005).

Pair-wise comparisons for individual loci
Exact tests were used to assess whether the allele frequency distribution, both at each microsatellite locus and overall, differed between samples of *P. microdon* from the Fitzroy River and the Gulf of Carpentaria regions. The results of these tests are presented in terms of the probability of incorrectly rejecting the null hypothesis (no genetic differentiation). These tests were conducted using the software GENEPOP, version 1.2.

Pristis zijsron

Sampling regime

Overall, samples were obtained from 42 individuals of *P. zijsron* from virtually the entire Australian range of this species (Table 2). The samples were obtained from a total of 16 sites in three major geographic regions, the west coast, the Gulf of Carpentaria and the east coast (Table 2 and Figure 1). Since the number of individuals collected from single sites was typically very small (Table 2), samples from a single region were pooled and most of the analyses conducted on these pooled regional samples.

The *P. zijsron* samples were either tissue biopsies preserved in 100% ethanol or 20% DMSO or from dry rostra. They were from a variety of sources, including the Western Australia Department of Fisheries, private collections, and the research surveys of Peverell (2005).

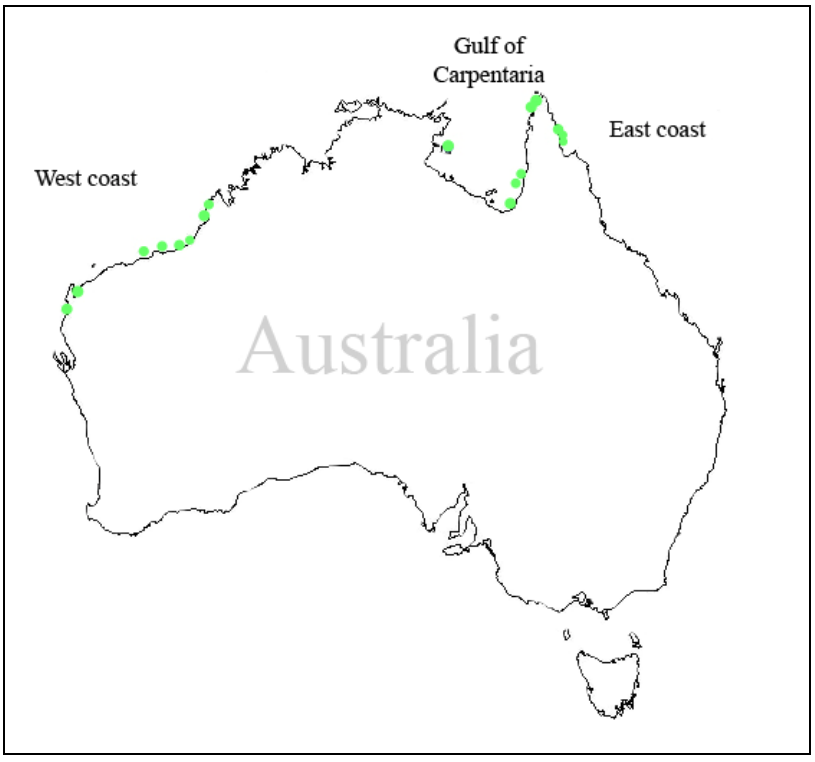

Figure 1 Locations of the sampling sites for *Pristis zijsron.*

Table 2 Details of the samples used for the *Pristis zijsron* mtDNA analyses, including the number of individuals sampled from each of the collection sites, dates and the sample types. The samples were from three geographic regions, the west coast (WC), the Gulf of Carpentaria (GoC), and the east coast (EC). *N* = the total number of individuals collected from a particular geographic region.

	Site	Number of individuals	Sample Type	Date
WC	Coral Bay	1	Preserved	2008
	Exmouth Bay	1	Dry Rostrum	2008
	Port Hedland	1	Preserved	2002
	Cape Keraudren	12	Preserved	2008
	80 Mile Beach	5	Preserved	2003 - 2004
	Roebuck Bay	1	Preserved	2004
	Beagle Bay	1	Dry Rostrum	1990's
		N = 22		
GoC	Gulf of Carpentaria	3	Preserved	2004
	Groote Eylandt	1	Rostrum	Pre- 2000
	Skardon River	2	Preserved	2001 - 2002
	Albert River	1	Preserved	2001 - 2002
	Karumba	1	Rostrum	Pre- 2000
	Mapoon	1	Preserved	2001 - 2006
		N = 9		
EC	Goose Creek	2	Preserved	2001 - 2006
	Lloyd Bay	1	Preserved	2001 - 2006
	Restoration Island	8	Preserved	2001 - 2006
		N = 9		

Genetic assays

DNA extractions

Total genomic DNA was extracted from approximately 5 mg of tissue from individuals of *P. zijsron* using the same method as described for *P. microdon*.

PCR amplification

Polymerase chain reaction (PCR) was used to amplify a 352-bp portion of the control region of the mtDNA of *P. zijsron,* using a forward primer (CRF: 5'-ACGTATCCGTAATACTCAT) and a reverse primer (CRR: 5'-ATGCAAATATTATGTCGAGGGTAG), which were designed during a previous study (Phillips *et al.* 2008). 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 were the same as those described for the *P. microdon* microsatellite assays, except that the annealing temperature ranged from 62 - 42°C. The quality and quantity of the PCR products were visualised prior to sequencing using essentially the same methods as those 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.

Data analyses

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

Unless otherwise stated, the analyses of variation in the partial control region sequences of *P. zijsron* were conducted using the software ARLEQUIN version 3.0 (Excoffier *et al.* 2005).

Neutrality of the mitochondrial control region
Tajima's (1989) D- 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. zijsron* were selectively neutral. The statistical significance of the results of these 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), with values of h ranging 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). 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 the Tamura & Nei (1993) substitution model and a gamma correction of 0.02, 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 other models using the software KAKUSAN2 (Tanabe 2007). The gamma correction was used to correct for variation in the rate of substitution among nucleotide sites (Uzzell & Corbin 1971). The appropriate value of

the correction for the control region haplotypes of *P. zijsron* was empirically determined with the software TREE-PUZZLE 5.2 (Schmidt *et al*. 2002).

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

Analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was used to assess how control region sequence variation was partitioned within and between samples from different geographic regions. 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 comparisons

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 these tests are presented in terms of the probability of incorrectly rejecting the null hypothesis (of no genetic differentiation). The probability values were estimated using the Markov chain method (Raymond & Rousset 1995).

Evolutionary relationships among haplotypes

The evolutionary relationships among the control region haplotypes of *P. zijsron* 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

Pristis microdon

General

The genotypes of individuals of *P. microdon* from the Fitzroy River and Gulf of Carpentaria regions were determined for three microsatellite loci, *Ppe 5*, *Ppe 122* and *Ppe 186*. The exact sample size varied from locus to locus, but was 22 or more for the Fitzroy River region and 38 or more for the Gulf of Carpentaria region, with 60 or more individuals genotyped for each locus (Tables 3 and 4). Some of the general characteristics of the microsatellite loci, such as the type of repeat unit, size range of alleles and underlying levels of polymorphism, are indicated in Table 3. The overall levels of polymorphism (all samples pooled) at two of these loci, *Ppe 5* and *Ppe 186*, were very high, with at least 21 different alleles and an expected

heterozygosity of 0.82 or more (Table 3). The overall level of polymorphism at *Ppe 122* was more moderate, with only six alleles and an expected heterozygosity of 0.68 (Table 3). In conclusion, the amount of microsatellite polymorphism in *P. microdon* in Australian waters appears to be generally high (notwithstanding the small number of loci sampled) and sufficient to test for the presence of genetic subdivision in this species in these waters.

Table 3 Characteristics of three polymorphic microsatellite loci, including the type of repeat unit, allele size range, number of alleles detected (*A*) and expected heterozygosity (H_E) in *Pristis microdon* for all samples pooled. *N* = the number of individuals scored.

Locus	Repeat unit	Allele size range	*N*	*A*	H_E
Ppe 5	Tetranucleotide*	240 - 346	60	30	0.82
Ppe 122	Tetranucleotide	203 – 235	69	6	0.68
Ppe 186	Tetranucleotide*	214 – 296	64	21	0.84

* Most of the alleles varied in increments of 4 bp.

Hardy-Weinberg equilibrium expectations

The observed numbers of each genotype class at each of *Ppe 5*, *Ppe 122* and *Ppe 186* in the sample of *P. microdon* from the Fitzroy River region were not significantly different from the numbers expected under Hardy-Weinberg equilibrium conditions, as was also the case for the sample from the Gulf of Carpentaria (Table 4), and for all samples pooled (Table 3). This finding suggests that there was no preferential amplification of any alleles at any of these loci that could potentially bias the data from these loci. The fact that the patterns of variation at each of the three loci were in accordance with Hardy-Weinberg equilibrium expectations is consistent with the view that at least the males of *P. microdon* in the Gulf of Carpentaria and associated rivers are part of the same breeding population (remembering that the sample from the Gulf of Carpentaria comprised individuals from 11 sites spread throughout this region).

Genetic diversity

The estimates of the standardised number of alleles and observed and expected heterozygosity for any particular locus were generally very similar in the samples of *P. microdon* from the Fitzroy River and Gulf of Carpentaria regions (Table 4). Although the diversity estimates for a particular locus were sometimes slightly different between the two samples, the direction of the differences was not consistent between loci (Table 4). For example, while the amounts of observed and expected heterozygosity at *Ppe 122* were slightly lower in the sample from the Fitzroy River region compared to the gulf region, the reverse was true for *Ppe 186* (Table 4). These results suggest that the amount of microsatellite diversity in *P. microdon* does not vary much, if at all, between the Fitzroy River and Gulf of Carpentaria regions. This is despite the fact that the sample from the Gulf of Carpentaria was composed of individuals from 11 sampling sites from throughout the gulf, while the Fitzroy River sample was from a single river and adjacent marine embayment.

Table 4 The number of individuals scored (*N*), number of alleles detected (*A*), which was then standardised to the smallest sample size (SNA), expected heterozygosity (H_E) and observed heterozygosity (H_O) for each of three microsatellite loci in samples of *P. microdon* from the Fitzroy River and Gulf of Carpentaria regions. *P* is the probability of incorrectly rejecting the null hypothesis (no difference between observed genotype numbers and those expected under Hardy-Weinberg equilibrium conditions). None of the *P* values was statistically significant.

Region		*Ppe 5*	*Ppe 122*	*Ppe 186*
Fitzroy River	*N*	22	27	23
	A	20	6	17
	SNA	20.0	6.0	17.0
	H_E	0.91	0.66	0.94
	H_O	0.86	0.52	0.96
	P	0.17	0.18	0.91
Gulf of Carpentaria	*N*	38	42	41
	A	25	6	16
	SNA	20.5	6.0	13.7
	H_E	0.91	0.71	0.90
	H_O	0.86	0.76	0.90
	P	0.21	0.52	0.07

Population differentiation

The results of the AMOVA indicate that virtually all (99.8%) of the allele frequency variation at the three microsatellite in *P. microdon* was present within samples; the between-sample component of variation was negligible (Table 5). Similarly, the results of the exact tests indicate that the allele frequencies in the samples from the Fitzroy River and Gulf of Carpentaria regions were effectively homogeneous regardless of whether the loci were considered individually or in combination (Table 6). Thus, this research found no evidence of any structuring in the nDNA in *P. microdon* between these two regions. However, since the variance in the degree of differentiation at microsatellite loci within a single species can be large (*e.g.* Burridge & Versace 2007), data from additional loci are required to confirm (or otherwise) this apparent lack of structuring.

Table 5 The results of analysis of molecular variance (AMOVA) based on the allele frequencies at three microsatellite loci in samples of *Pristis microdon* from the Fitzroy River and the Gulf of Carpentaria (GoC) regions. Variance components and estimates of statistical significance (*P*-values) are indicated. Percentage of variation is indicated in parentheses.

Groups	Among samples	Within samples
Fitzroy	0.003	1.280
GoC	*P* = 0.375	***P* = 0.000**
	(0.2)	(99.8)

Table 6 The results (*P*-values) of exact tests, which compared the allele frequencies at three microsatellite loci in samples of *Pristis microdon* from the Fitzroy River and the Gulf of Carpentaria (GoC) regions. None of the *P* values was statistically significant.

	Ppe 5	***Ppe 122***	***Ppe 186***	**All loci**
Fitzroy vs GoC				
***P*-value**	0.120	0.422	0.205	0.165

Pristis zijsron

General

The nucleotide sequence of a 352-bp portion of the left domain of the control region of the mtDNA was determined for a total of 42 individuals of *P. zijsron.* The sequences of these individuals contained a total of six polymorphic sites, which revealed a total of eight different haplotypes (Table 7).

The average base composition of the *P. zijsron* sequences was: A-22.70%, T-31.01%, C-29.59%, and G-16.71%, which is consistent with that of the mtDNA of other sawfishes (Phillips *et al.* 2008) and other elasmobranchs (*e.g.* Keeney *et al.* 2005). The identities of the mitochondrial control region sequences acquired from *P. zijsron* were confirmed by alignment with sequences from *P. microdon* and *P. clavata*. The minimum amount of raw sequence divergence between any control region sequences from different species was 7.4% (between haplotypes of *P. zijsron* and *P. clavata*).

The results of Tajima's (1989) D- and Fu's (1997) Fs-tests, which are based on the patterns of control region variation in pooled samples of *P. zijsron* from each sampling region (west coast, Gulf of Carpentaria, and east coast), conform to the expectations for a selectively neutral sequence in drift-mutation equilibrium, as the values of D and Fs were not significantly different to zero (Table 8).

Table 7 The location and distribution of six polymorphic sites among eight haplotypes for a 352-bp portion of the control region in the mtDNA of the sampled individuals of *Pristis zijsron*. Dots represent matches with nucleotides present in haplotype 1. Numbers refer to position of base pairs from the start of the fragment.

Haplotype number	**5**	**14**	**19**	**204**	**319**	**352**
1	C	A	T	C	A	G
2	.	T	C	.	.	A
3	.	T	.	.	.	.
4	.	.	.	G	.	.
5	.	.	A	.	.	.
6	A	T	.	.	.	.
7	A	T	C	.	.	A
8	.	T	C	.	T	A

Table 8 The results of tests for the neutrality of the control region sequences for pooled samples of *Pristis zijsron* from each of the west coast (WC), the Gulf of Carpentaria (GoC), and the east coast (EC) of Australia. D = Tajima's (1989) neutrality statistic; Fs = Fu's (1997) neutrality statistic. None of the *P* values was statistically significant.

Region	D	Fs
WC	0.9208	0.5407
	$P = 0.8360$	$P = 0.6300$
GoC	-1.0882	-0.2635
	$P = 0.1930$	$P = 0.1720$
EC	-0.5063	-1.0262
	$P = 0.3050$	$P = 0.1210$

Genetic diversity

The overall levels of haplotype and nucleotide diversity in *P. zijsron* in Australian waters were assessed by pooling all samples and found to be moderate and low respectively (Table 9). The levels of haplotype and nucleotide diversity in the samples from the east and the west coasts were similar to each other and approximately the same as in the pooled sample (Table 9). The levels of both haplotype and nucleotide diversity in the sample from Gulf of Carpentaria were noticeably lower than those of the other samples (Table 9).

In conclusion, the results of this research suggest that the amount of haplotype diversity in *P. zijsron* in Australian waters is generally moderate, except in the Gulf of Carpentaria region where it appears to be much lower. The levels of nucleotide diversity in the samples were generally low and especially so in the sample from the Gulf of Carpentaria. Since the samples from the east coast and the Gulf of Carpentaria in particular were relatively small (11 and 9 individuals, respectively), the estimates of the levels of diversity in *P. zijsron* in these regions should be regarded as preliminary.

Table 9 Summary of the levels of diversity in a 352-bp portion of the mtDNA control region in pooled samples of *Pristis zijsron* from each of the west coast (WC), the Gulf of Carpentaria (GoC) and the east coast (EC) of Australia. n/a = not applicable.

Region	Sample size	Number of haplotypes	Standardised number of haplotypes	Number of polymorphic sites	Haplotype diversity (±SE)	Nucleotide diversity (±SE)
WC	22	5	3.21	5	).623 ± 0.080	0.0050 ± 0.003
GoC	9	2	2.00	1	).222 ± 0.166	0.0007 ± 0.0009
EC	11	4	3.63	3	).709 ± 0.099	0.0030 ± 0.002
All samples pooled	42	8	n/a	6	).616 ± 0.075	0.0039 ± 0.0027

Population differentiation

The samples of *P. zijsron* from each of the west coast, the Gulf of Carpentaria and the east coast of Australia all had the same common haplotype (#1) (Table 12 and 13). Nevertheless, the frequency of this haplotype was different in all of these samples, particularly in the sample from the Gulf of Carpentaria compared to the others (Table 12). None of the other four haplotypes in the sample from the west coast were found in the other region, including haplotype 2, which occurred in an appreciable frequency in the west coast sample (Table 12 and 13). Both of the two haplotypes that occurred in the sample from the Gulf of Carpentaria also occurred in the sample from the east coast (which also contained an additional two haplotypes), but in different frequencies (Table 12 and 13).

The results of the AMOVA indicated that most (82.3%) of the control region variation in *P. zijsron* was present within samples (Table 10). Nevertheless, the between-sample component of variation was almost 18% and statistically significant (Table 10), indicating the presence of genetic differentiation between the samples from the different regions. This result reflects some of the above-described variation in the haplotype composition of these samples. The results of the exact tests confirm that the haplotype compositions of the samples from the west and east coast (the two most distant sampling points) were significantly different to each other (Table 11). Although the haplotype composition of the sample from the Gulf of Carpentaria was not significantly different to that in either the east or west coast samples, the power of the analysis to detect differences was very limited due to small sample sizes.

In conclusion, the results of these analyses indicate the *P. zijsron* is genetically sub-divided across its range in Australian waters. The biggest genetic differences appear to be between sites on either side of the continent. Larger sample sizes are required to properly assess the relationships between the assemblage of this species in the Gulf of Carpentaria and those on the east and west coasts. Although they are probably isolated in the contemporary environment, the presence of the same common haplotype in samples from the west coast, Gulf of Carpentaria and east coast, suggests the presence of historic connections among assemblages of *P. zijsron* across northern Australia.

Table 10 Results of analysis of molecular variance (AMOVA) based on the frequency distributions of mtDNA control region sequences in samples of *Pristis zijsron* from the west coast (WC), Gulf of Carpentaria (GoC) and east coast (EC) of Australia. Variance components and estimates of statistical significance (*P*-values) are indicated. Percentage of variation is indicated in parentheses.

Groups	Among samples	Within samples
WC,	0.1866	0.8668
GoC,	***P* = 0.013**	***P* = 0.000**
EC	(17.7)	(82.3)

Table 11 The results (*P*-values) of exact tests, which compared the frequency distribution of mtDNA control region haplotypes between samples of *Pristis zijsron* from the west coast (WC), the Gulf of Carpentaria (GoC), and the east coast. Significant *P* –values are indicated in bold.

Sample locations	WC	GoC	EC
WC			
GoC	$P = 0.133$		
EC	$\mathbf{P = 0.004}$	$P = 0.1715$	

Table 12 Frequencies of the eight mtDNA-control region haplotypes in *N* individuals of *Pristis zijsron* from the west coast (WC), the Gulf of Carpentaria (GoC), and the east coast (EC) of Australia.

Haplotype number	WC	GoC	EC
1	0.55	0.89	0.45
2	0.32	0.00	0.00
3	0.00	0.11	0.36
4	0.00	0.00	0.09
5	0.00	0.00	0.09
6	0.05	0.00	0.00
7	0.05	0.00	0.00
8	0.05	0.00	0.00
N	22	9	11

Evolutionary relationships among haplotypes

The network connecting the eight control region haplotypes in the samples of *P. zijsron* from the west coast, Gulf of Carpentaria and east coast of Australia was shallow, where the maximum number of mutational steps between any two haplotypes was seven (Figure 2). This indicates that the amount of evolutionary divergence in this species in Australian waters is very limited. Other than haplotype 1, the haplotypes from the west coast appeared in one part of the network, suggesting the presence of weak phylogeographic structure (Figure 2).

Table 13 The distribution and abundance of the eight mtDNA-control region haplotypes found in 42 individuals of *Pristis zijsron* from 16 sites in theree sampling regions in Australia. CB = Coral Bay; EX = Exmouth Bay; PH = Port Hedland; CK = Cape Keraudren; 80M = 80 Mile Beach; RO = Roebuck Bay; BB = Beagle Bay; AB = Albert River; SD = Skardon River; KR = Karumba; MP= Mapoon; GY = Groote Eylandt; GoC = Gulf of Carpentaria; GK = Goose Creek; LB = Lloyd Bay; RI = Restoration Island. *N* = the total number of individuals from a particular sample site.

Region	**West coast**							**Gulf of Carpentaria**						**East coast**		
Hap no.	**CB**	**EX**	**PH**	**CK**	**80M**	**RO**	**BB**	**GoC**	**GY**	**KR**	**AB**	**SK**	**MO**	**LY**	**GK**	**RI**
1	1	1	-	6	3	1	-	3	1	1	1	2	-	1	1	3
2	-	-	1	5	1	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	3
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
6	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
7	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
N	1	1	1	12	5	1	1	3	1	1	1	2	1	1	2	8

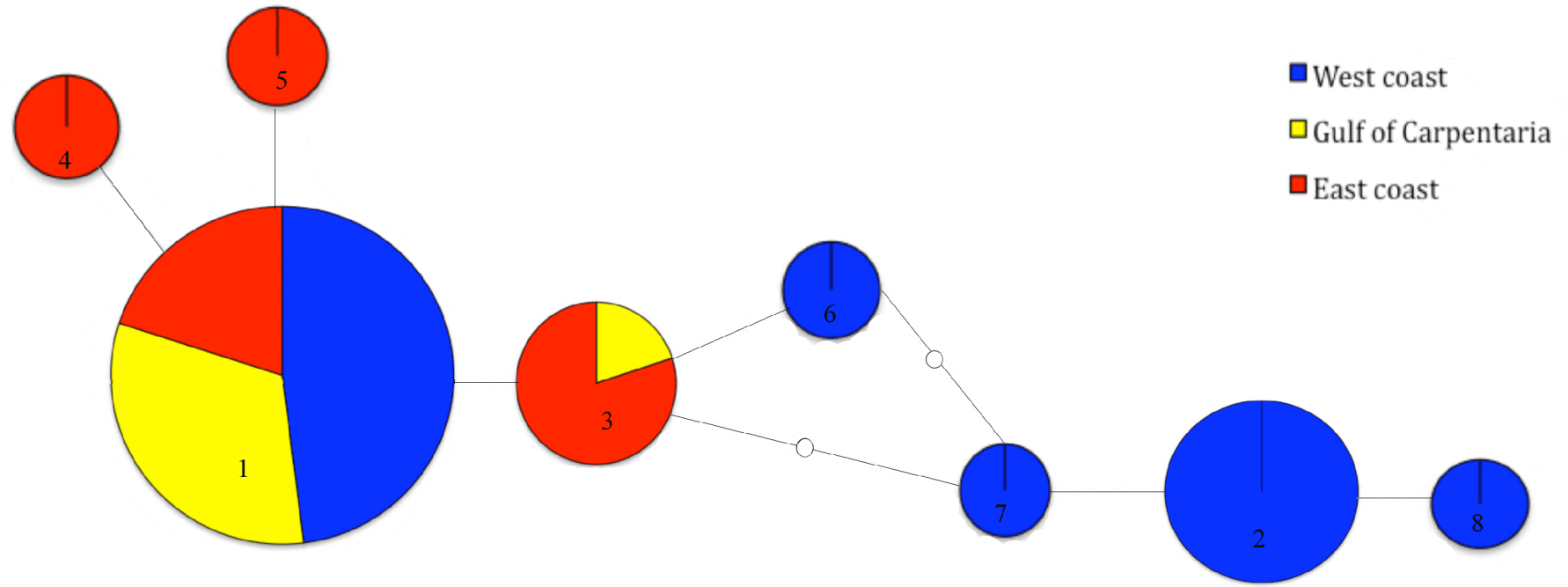

Figure 2 Haplotype network showing the relationships among the eight control region haplotypes of *Pristis zijsron*. Each line in the network shows a single mutational change regardless of the length of the line. A numbered circle is used to represent each haplotype. The surface area of each circle is 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 on the west coast, Gulf of Carpentaria region, and the east coast. Empty circles indicate missing intermediate haplotypes.

DISCUSSION

Pristis microdon

This research provides a preliminary assessment of the distribution of variation at microsatellite loci in *P. microdon* in Australian waters. It is based upon an analysis of the patterns of variation at three tetranucleotide loci in 22 or more individuals from the Fitzroy River and an adjacent marine embayment (King Sound) and 38 or more individuals from the Gulf of Carpentaria and 11 associated river systems (the exact sample size varied from locus to locus, see Table 4). The results complement those of Phillips *et al.* (2008), which are based upon an analysis of variation in the nucleotide sequence of the control region of the mtDNA in *P. microdon* for a comparable set of samples. However, the key difference is that the microsatellite markers provide information about both the male and female component of the population (because they are located in the nuclear genome, which has bi-parental inheritance), while the control region marker provides information only about the female component (because it is in the mtDNA, which is maternally inherited) (see Avise 1994).

The overall level of polymorphism at the three microsatellite loci in the samples of *P. microdon* was high or moderate (depending on the locus). This finding, in combination with the control region data of Phillips *et al.* (2008), indicates that the levels of diversity in both the mtDNA and nDNA in *P. microdon* are not unusually low (as is sometimes the case for endangered species), although it is difficult to know the amount of diversity that was present in populations of this species prior to recent human disturbances and population declines. Since population genetic theory indicates the capacity of a population for evolutionary change depends on the amount of adaptively significant genetic variation therein (Hedrick 1992, Amos & Balmford 2001), and assuming that the results for the sampled loci are broadly reflective of the situation at adaptively significant loci, the above finding is encouraging regarding the prognosis for the long-term survival of the Australian populations of *P. microdon*. However, as indicated by Phillips *et al.* (2008), there is a note of caution in this regard because it can take several generations for reductions in the amount of genetic diversity to become apparent in a long-lived species with overlapping generations, like *P. microdon* (see Amos & Balmford 2001, Lippe *et al.* 2006).

The levels of microsatellite diversity in *P. microdon* appear to be generally similar in the Fitzroy River and Gulf of Carpentaria regions. In contrast, the levels of diversity in the nucleotide sequence of the control region in this species may be slightly lower in the former geographic region compared to the latter (Phillips *et al.* 2008). The lower level of control region diversity in *P. microdon* in the Fitzroy River region, compared to the Gulf of Carpentaria region, could be due to a smaller number of females in association with a smaller number of river systems with suitable nursery areas in this region. The Fitzroy River may be the only river system on the west coast that supports a substantial number of juveniles of *P. microdon*. If dispersal in *P. microdon* is strongly male-biased, then this pattern of spatial variation may not be apparent (or less pronounced) in the nuclear genome. This is because the males may be part of a larger, more wide-ranging breeding group. Regardless, additional loci and individuals are required to properly elucidate spatial patterns of diversity in the mitochondrial and nuclear genomes in *P. microdon*.

There was no evidence of any differentiation at the microsatellite loci in *P. microdon* between the Fitzroy River and Gulf of Carpentaria regions, the amount of variation in allele frequencies at the microsatellite loci between samples from these two regions was negligible. This suggests that the

amount of contemporary or recent gene flow (≡ dispersal) in *P. microdon* between these two regions has been sufficient to prevent structuring of at least the nuclear genome. It is not possible to provide a robust estimate the amount of gene flow, which could be large or small, from these data. Since the variance in the degree of differentiation at microsatellite loci within a single species can be large (*e.g.* Burridge & Versace 2006), data for additional loci are required to confirm (or otherwise) this apparent lack of structuring.

The apparent absence of differentiation in the nuclear genome in *P. microdon* between the Fitzroy River and Gulf of Carpentaria regions, as indicated by the microsatellite markers, is in marked contrast to the situation in the mitochondrial genome (see Phillips *et al*. 2008). For example, about 35% of the total amount of variation in the nucleotide sequence of the control region in samples of *P. microdon* from these two regions is due to differences between samples (Phillips *et al.* 2008); this is a relatively large amount of between-sample variation for a large, mobile marine fish. The apparent discrepancy in the degree of spatial structuring in the nuclear and microsatellite markers in *P. microdon* is consistent with the view that the females of this species are philopatric, while the males are more wide-ranging. Additional data and analyses are required to confirm this suggestion of male-biased dispersal in *P. microdon*. If confirmed, this would be a first for sawfishes, although there is an increasing body of evidence to suggest that this type of dispersal pattern may be common in elasmobranch species in which adult and juvenile habitat is spatially removed (*e.g.* Springer 1967, Ebert 1996, Feldheim *et al*. 2001, 2004, Keeney *et al*. 2005), as is the case for *P. microdon* (see Thorburn *et al.* 2007).

Management Implications

If *P. microdon* exhibits male-biased dispersal, this would have significant implications for the management of this species (*e.g.* Pardini *et al.* 2001). In particular, the management of populations of this species would need to take into account the fact that a decline in the number of females in a particular region would not be replenished by the immigration of females from another region (as females are philopatric), coupled with the fact that a decline in the abundance of this species in one region could have a direct effect on its abundance in another region (as males may disperse between regions). The evolutionary potential of Australian populations of *P. microdon* may be relatively high (in comparison to those of some other endangered species) as the levels of diversity in the mtDNA and nDNA in these populations do not appear to be unusually low. Since the intrinsic rate of increase of a sawfish population is likely to be very low (Simpfendorfer 2000), it would take a sawfish population a considerable number of generations to recover significant amounts of lost diversity. Hence it is important to manage populations of *P. microdon* such that the current levels of abundance, and so genetic diversity, are maintained.

Pristis zijsron

This research provides preliminary data about the levels of genetic diversity and population structure of *P. zijsron* in Australian waters. These are the first population genetic data for this species. The results are based upon an analysis of the patterns of variation in the nucleotide sequence in a 352-bp portion of the control region of the mtDNA in samples of this species from three widely separated geographic regions, namely the west coast ($N = 22$), the Gulf of Carpentaria ($N = 9$) and the east coast of Australia ($N = 11$). They complement the results of a previous analysis, by Phillips *et al.* (2008), of variation in the nucleotide sequence of the control region in: (i)

P. microdon based upon samples of this species from the west coast (N = 40), north coast outside the Gulf of Carpentaria (N = 1), Gulf of Carpentaria and associated rivers (N = 47) and east coast (N = 2); and (ii) *P. clavata* based upon samples of this species from the west coast of Australia (N = 23) and the Gulf of Carpentaria (N = 7). *Pristis zijsron* (and *P. clavata*) differ from *P. microdon* in that they complete their life-cycles in marine waters (including the lower reaches of estuaries), whereas *P. microdon* uses rivers and the upper reaches as a nursery, although the adults are marine-estuarine (Taniuchi *et al.* 1991, Peverell 2005, Thorburn *et al.* 2007, Whitty *et al.* 2008, 2009).

The outcomes of the neutrality tests suggest that the patterns of control region variation in *P. zijsron* 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. zijsron* is interpreted to reflect a balance between the 'effective population size' and the mutation rate, and genetic differences between spatially 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 overall amount of haplotype diversity in the control region in *P. zijsron* was generally comparable to that in each of *P. microdon* and *P. clavata* (Phillips *et al.* 2008) 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). The overall amount of nucleotide diversity in the control region in *P. zijsron* may be slightly lower than that in either *P. microdon* or *P. clavata*. The nucleotide diversity of the pooled sample in *P. zijsron* was 0.0039 compared to 0.0066 and 0.0077 in *P. microdon* and *P. clavata*, respectively (Phillips *et al.* 2008). This is mainly because the number of polymorphic sites and the extent of differences among control region haplotypes in *P. zijsron* were relatively small. Additional data and analyses are required to investigate the basis for, and implications of, the apparently reduced levels of nucleotide diversity in *P. zijsron*.

The control region data suggest that the level of genetic diversity in *P. zijsron* in the Gulf of Carpentaria is reduced compared to the level in this species on each of the west and east coasts of Australia. Since this result is based on data from a single mtDNA locus and a small number of individuals, it should only be considered as preliminary. However, in this regard, it may be relevant that preliminary data indicate that the levels of genetic diversity in *P. clavata* may also be relatively low in the Gulf of Carpentaria (Phillips *et al.* 2008). In contrast, the levels of genetic diversity in *P. microdon* do not appear to be reduced in this region. If the levels of genetic diversity in each of *P. zijsron* and *P. clavata*, but not *P. microdon*, are relatively low in the Gulf of Carpentaria, then this would suggest that each of the two former species has undergone greater declines in abundance in this region compared to *P. microdon*. This could be linked to the fact that the juveniles of these two species typically occur in marine coastal waters (including the lower reaches of estuaries) rather than freshwater rivers, which would imply that the juveniles of *Pristis* sawfishes in marine waters in the Gulf of Carpentaria have been relatively heavily impacted by human disturbance(s).

The control region data indicate that *P. zijsron* is genetically sub-divided within its Australian range, as is also the case for *P. microdon* (Phillips *et al.* 2008). The adults of both of these species are very large and mobile and occur in the marine environment (which has few absolute barriers to gene flow); nevertheless they are not dispersing throughout this entire range. The preliminary results of a comparison of the patterns of mtDNA and nDNA variation in *P. microdon* suggest that female dispersal may be much more restricted than male dispersal in this species. Information on the spatial distribution of nDNA markers in *P. zijsron* is similarly required to investigate whether

dispersal in this species is also male-biased. The patterns and spatial scale of mtDNA differentiation in *P. zijsron* remain unclear, although the results suggest that there are significant mtDNA differences between the assemblages of this species on the east and west coasts of Australia, at the least.

Preliminary evidence indicates that the extent of mtDNA differentiation in *P. zijsron* in Australian waters is, on average, less than that in *P. microdon* in these waters. The amount of between-sample variation in the control region in *P. zijsron* was about 18%, which is about half of that found between samples of *P. microdon* (~37%) from a similar, although not identical, range of sites (see Phillips *et al.* 2008). If confirmed, and given certain assumptions, this difference suggests that female-mediated gene flow is more restricted in *P. microdon* compared to *P. zijsron*. This could be related to *P. microdon*'s reliance on freshwater habitat for nursery areas and the need to find suitable areas of such *i.e.,* the females of *P. microdon* may exhibit strong philopatry to maximise their chances of giving birth in the immediate vicinity of their natal river. Regardless, it is important to realise that the amount of female gene flow in *P. zijsron* between the east and west coasts of Australia, and possibly also between these coasts and intervening regions such as the Gulf of Carpentaria, is severely restricted, even if it is, on average, slightly higher than that in *P. microdon*.

The control region data indicate that the amount of evolutionary divergence in *P. zijsron* in Australian waters is limited. Thus, there do not appear to be any particular subsets of individuals or populations that have distinctive evolutionary histories and thus that might warrant special conservation priorities on this basis. However, there was evidence of weak phylogeographic structure based around the west coast assemblage. Such a structure would suggest that the west coast assemblage is not only isolated from those in the Gulf of Carpentaria and east coast but that this isolation is historic rather than due to recent anthropogenic effects.

Management Implications

The control region data suggest that *P. zijsron* is genetically sub-divided within its Australian range. This research has not fully resolved the pattern and scale of this sub-division, but at least the female component of the assemblages on at least the east and west coasts appear to be effectively isolated from each other and hence should be managed separately. More samples are needed to properly assess the relationships between the assemblage of *P. zijsron* in the Gulf of Carpentaria and those on the west and east coasts, as well as the intervening waters of the north coast. The levels of genetic diversity in *P. zijsron* in the Gulf of Carpentaria region appear to be reduced relative to those in other regions. Such a reduction would indicate that the abundance of this species in this region has been relatively heavily reduced and might warrant special protection of this assemblage to ensure that its abundance and genetic diversity is not further reduced.

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