

A multi-faceted approach for quantifying the estuarine–nearshore transition in the life cycle of the bull shark, *Carcharhinus leucas*

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Abstract. Understanding the ontogenetic habitat linkages of sharks is important for conservation and managing human interactions. We used acoustic telemetry, catch data, elemental and stable isotope signatures and dietary analyses to investigate ontogenetic habitat use in south-east Queensland, Australia, by the bull shark *Carcharhinus leucas*, a IUCN ‘near-threatened’ species that is implicated in many shark attacks on humans in urban estuaries. Sequential analyses for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of vertebrae from five adult *C. leucas* and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) for elemental composition from 23 *C. leucas*, including a pregnant female, were also used to trace ontogenetic habitat dependence. Acoustic telemetry indicated large juvenile and subadult *C. leucas* remained in estuarine habitats. $\delta^{15}\text{N}$ values across shark vertebrae showed an ontogenetic shift in diet with total length (TL), confirmed by stomach contents. LA-ICPMS data reflected the ontogenetic movements of *C. leucas* from natal habitats. Differences among adults were gender related. Shifts in habitat use by subadults were correlated with a sigmoidal $\delta^{13}\text{C}$ relationship with TL. *C. leucas* have a multipartite, stage-specific dependency in their transition between habitats along the freshwater–estuarine–marine continuum, making them particularly susceptible to the habitat alteration that is occurring globally.

Additional keywords: acoustic telemetry, LA-ICPMS elemental analysis, ontogenetic habitat shift, stable isotope analysis.

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Introduction

Linkages between animal populations in freshwater and nearshore coastal waters are most often viewed in terms of larval recruitment processes, but are rarely considered in the context of the life cycle of highly mobile top level predators, especially sharks. Knowledge of habitat usage by sharks is important for understanding the effects of environmental and human disturbances on these species and the potential ecological ramifications for the associated communities (Heithaus *et al.* 2002; Gillanders *et al.* 2003). Sexually mature sharks often occupy habitats in response to abiotic factors including temperature and salinity (Hopkins and Cech 2003; Hight and Lowe 2007) or biological factors such as prey availability and changes in intra- or inter-specific competition (Heithaus 2004). These factors are also likely to be crucial for the juveniles of species that occupy different habitats during their transition to adulthood.

Increasing size confers decreased vulnerability to predation and may drive ontogenetic shifts in habitat use by sharks (Ebert 2002; Heithaus 2004; Wetherbee and Cortes 2004). These changes may result in different ecological roles of a predator in marine communities, mediated by consumption of larger and more varied prey (Ebert 2002; Heithaus *et al.* 2005). Hence, the timing of ontogenetic shifts in habitat use is a critical but poorly understood feature in the life cycle of sharks, probably due to the large spatial scales that are usually required for investigations.

Methods such as catch data analysis (Cliff and Dudley 1991), acoustic monitoring (Heupel and Simpfendorfer 2008; Yeiser *et al.* 2008), tissue chemistry (Estrada *et al.* 2006; Kerr *et al.* 2006; Olsen *et al.* 2011) and stomach content analysis (Cortés 1997) can be used to identify ontogenetic changes in the trophic ecology and habitat use of sharks. These methods are complementary and together provide a multi-faceted approach to understanding ontogenetic habitat transition in sharks. Acoustic

monitoring yields detailed occurrence and movement data of sharks, especially increases in home range by juveniles (Heupel and Hueter 2002; Heupel *et al.* 2004; Knip *et al.* 2011). Chemical analysis via laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) (Hale *et al.* 2006) and stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fry 2006; Lin *et al.* 2007; Kennedy *et al.* 2010) can identify the ontogenetic broadening of diets and habitat (Davenport and Bax 2002; Vanderklift and Ponsard 2003; Olsen *et al.* 2011). Traditional stomach content analysis (SCA) can also demonstrate ontogenetic shifts but requires the examination of numerous individuals (Bass *et al.* 1973; Cortés 1997, 1999). A multi-faceted approach utilising these methods is likely to be particularly amenable with sharks that utilise riverine–estuarine and nearshore marine habitats, including species such as the scalloped hammerhead (*Sphyrna lewini*), pigeye (*Carcharhinus amboinensis*), dusky whaler (*Carcharhinus obscurus*) and the bull shark (*Carcharhinus leucas*).

The bull shark, *Carcharhinus leucas*, occurs circum-globally, inhabits rivers, estuaries and shallow coastal waters and grows to ~4 m total length (TL) (Compagno 2002; Last and Stevens 2009; McCord and Lamberth 2009). The species has been the subject of numerous physiological studies (e.g. Thorson *et al.* 1973; Pillans and Franklin 2004; Pillans *et al.* 2006) because of its ability to occupy habitats in fresh and marine environments. Its life cycle involves movements between both environments (Thorson 1971), with juveniles occupying freshwater–estuarine habitats and adults found in nearshore marine areas (Thorson *et al.* 1973; Brunnschweiler *et al.* 2010; Heupel *et al.* 2010).

There is a well documented ontogenetic transition in the life cycle of the bull shark with neonates and juveniles in riverine through estuarine habitats (Thorson *et al.* 1973; Simpfendorfer *et al.* 2005; Heithaus *et al.* 2009) to subadults and adults in nearshore marine habitats (Cliff and Dudley 1991; Myers *et al.* 2007; Werry 2010). Recent studies (Zeller 1999; Pillans and Franklin 2004) and anecdotal observations by recreational fishers and the public in south-east Queensland (Qld), Australia, suggest that this model may also explain some of the observed patterns of bull shark populations along this coastline. Given this model, we hypothesised that bull sharks would exhibit ontogenetic differences in catch, movements, diet, elemental and isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) signatures in the vertebrae, accompanying their transition across the freshwater–estuarine–marine continuum. A multi-faceted approach involving catch data, acoustic monitoring, tissue chemistry, and stomach content analysis was used to test the ontogenetic changes in habitat use of bull sharks in south-east Qld with a particular emphasis on intermediate life cycle stages.

Materials and methods

Study site

This study was done in subtropical Qld (25°S–28°S) where extensive mangrove forests of *Avicennia marina* occur in estuaries and along rivers. The estuarine environment of southern Moreton Bay comprises two major areas: The Coomera River north to Jumpinpin seaway and the Broadwater and Gold Coast seaway in the south (Fig. 1). The former is fringed by *A. marina* and seagrass, whereas the latter has an urbanised shoreline, with adjoining canals. Substantial water flow occurs through

Jumpinpin and the Gold Coast Seaway, resulting in tidal mixing at the entrance to Coomera River. Flows from Jumpinpin also proceed north into Moreton Bay. Salinity reflects nearshore waters (~35), but declines to 10–20 after substantial rainfall (Werry 2010).

Capture, tagging and movements

To test ontogenetic differences across habitats, *Carcharhinus leucas* in river and estuarine habitats were captured using setlines comprising a 450 kg main line with traces of 2–3 m of 5-mm braided cord attached to a 1 m stainless steel wire leader with two 8/0 (Mustad, Gjøvik, Norway) tuna hooks per line. A 10 kg block was used as a mobile anchor to allow the line to drag if a large adult *C. leucas* was captured. Hooks were baited with standardised pieces of fresh eel (*Anguilla australis*) and mullet (*Mugil cephalus*) and opportunistically set for periods of 30 min to 2 h from January 2007 to March 2009. Samples were also obtained from recreational and commercial fishers. In the nearshore, marine habitats, *C. leucas* were obtained from the Queensland Shark Control Program (QSCP), commercial shark fishers and recreational fishers between 2005 and 2009.

To test movements of intermediate life cycle stages of *C. leucas* across habitats, selected sharks were transferred into a non-abrasive harness and then placed in dorsal recumbency to induce tonic immobility before tagging (Watsky and Gruber 1990). Sex was recorded and TL, precaudal and fork lengths measured to the nearest cm. Large juvenile (1.15–1.6 m TL, $n = 6$) and subadult (1.6–2 m TL, $n = 6$) bull sharks were tagged in early 2009 with Vemco V16, R-coded 69-kHz acoustic tags (Amirix Systems Inc., Nova Scotia, Canada) via surgical implantation in the abdominal cavity (Table 1). *C. leucas* were also externally tagged with a single barb plastic dart tag at the base of the first dorsal fin.

Twelve Vemco VR2W acoustic receivers (Amirix Systems Inc.) were deployed for 9 months in 2009 to document movement among and the occupation of specific habitats. Receivers were deployed by SCUBA divers on existing navigational markers at key locations in river and estuarine habitats and on purpose-built moorings in the nearshore (Fig. 1). Receivers were attached in mid-water and had an approximate detection range of 400–700 m. They were downloaded opportunistically from January to September 2009. Temperature and salinity were also recorded at 10-min intervals using Odyssey conductivity/temperature data recorders (Dataflow Systems Pty Ltd, Christchurch, New Zealand). Daily rainfall records obtained from the Bureau of Meteorology (BOM) aided the interpretation of the movements of *C. leucas*.

Collection and preparation of vertebrae

Vertebrae were obtained from *C. leucas* of different TL and from different habitats to further test their ontogenetic transition across the freshwater–estuarine–marine continuum. Vertebrae were obtained from 23 *C. leucas* caught by commercial and recreational fishers and the QSCP from 2005 to 2008. These sharks comprised 16 juveniles caught in rivers from Noosa to Tweed Heads and three subadults and four adults caught in the nearshore marine environment between Rainbow Beach and Tweed Heads (Fig. 1; Table 1). Vertebrae were excised

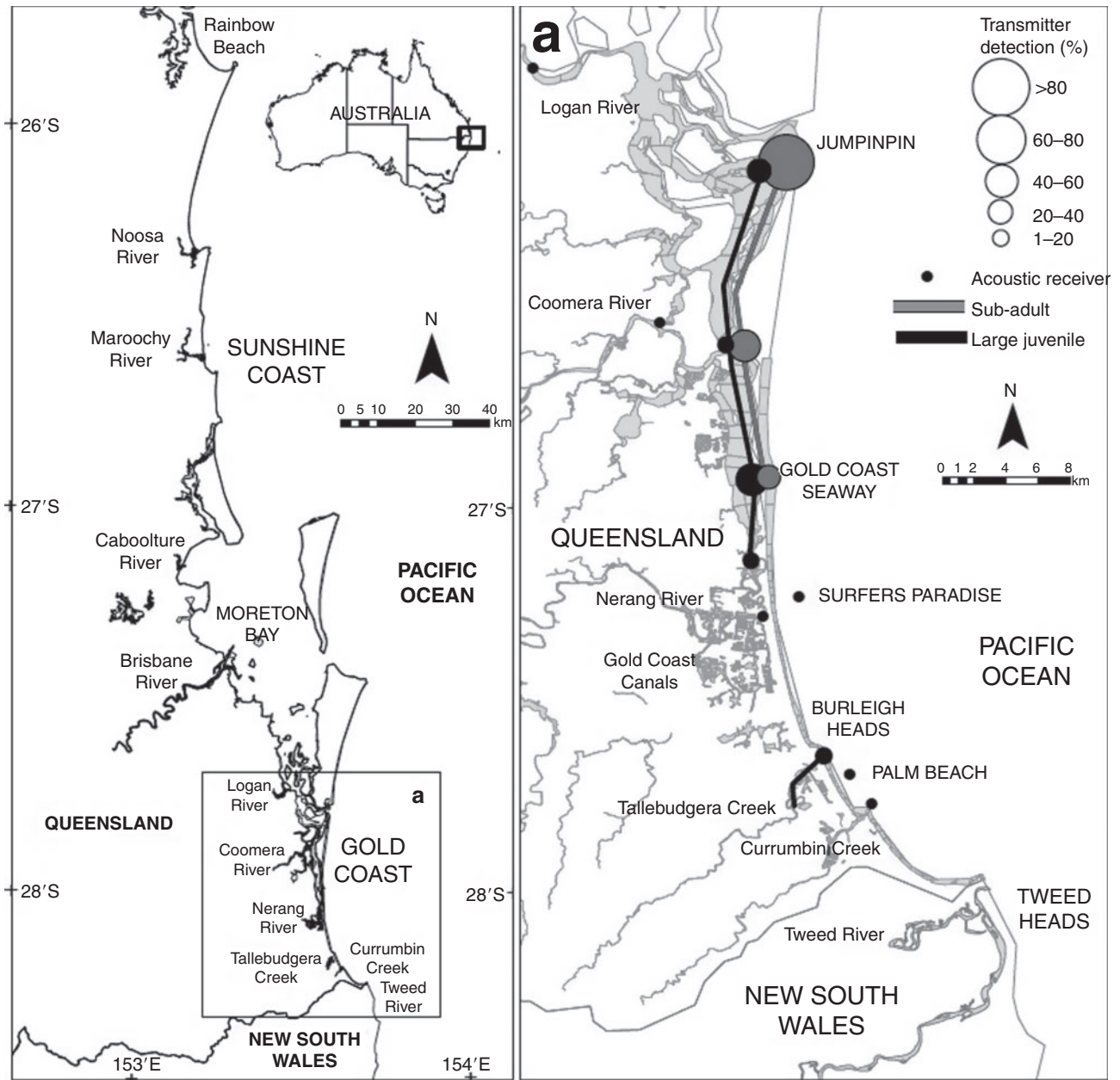


Fig. 1. Study area in south-east Queensland, Australia, showing the locations of the VR2W acoustic receivers in the river (including the Gold Coast canal system that feeds from Nerang River), estuarine, southern Moreton Bay, seaways and nearshore environments. Canals can also be seen connected to the natural waterways. Proportion of detections of large juvenile and subadult bull sharks (Sharks 23–34 in Table 1) at VR2W acoustic receiver locations are shown.

immediately anterior to the first dorsal fin and excess tissue was removed with a scalpel. A thin (1 mm) sagittal section was cut using a IsoMet low speed saw (Buehler, IL, USA) and then mounted on a 25 × 45 mm glass slide.

Laser ablation-inductively coupled plasma mass spectrometry

The *C. leucas* vertebral sections were analysed for seven elements using a Coherent GeolasPro 193 nm laser unit (Gottingen, Germany) coupled to a Varian 820-MS inductively coupled

plasma mass spectrometer (LA-ICPMS) (Melbourne, Vic, Australia) through 2 m of Tygon LEP-lined tubing and a three-way mixing bulb (Akron, OH, USA). Before analysis, vertebral sections were pre-ablated with the laser to remove surface contamination and then the specimen chamber and tubing were purged with high quality helium gas. Sample ablation was done using a ‘step and repeat scanning mode’ with a laser spot size of 32 μm and at a repetition rate of 10 Hz. The ablation distance was set at 32 μm, therefore, each spot was ablated 10 times. The laser energy fluence was at 6 J cm⁻². Measurements were

Table 1. Biological data for different ontogenetic stages of *C. leucas* monitored within the riverine, estuarine and nearshore marine environments and sampled for LA-ICPMS and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

P, pregnant; numbers under experimental technique identify the sharks in Figs 1–4

Location	TL (cm)	Gender	Ontogenetic phase	Experimental technique			
				Acoustic tagging	Laboratory experiment	LA-ICPMS	$\delta^{15}\text{N}/\delta^{13}\text{C}$
<i>Riverine habitat</i>							
Coomera River	55	F	Neonate	–	1	1	–
Coomera River	57	M	Neonate	–	–	2	–
Coomera River	58	F	Neonate	–	–	3	–
Tweed River	74	F	Juvenile	–	4	4	–
Tweed River	79	M	Juvenile	–	–	5	–
Maroochy River	80	M	Juvenile	–	6	6	–
Maroochy River	82	M	Juvenile	–	–	7	–
Gold Coast canals	83	M	Juvenile	–	–	8	–
Noosa River	84	F	Juvenile	–	–	9	–
Noosa River	90	M	Juvenile	–	–	10	–
Maroochy River	91	M	Juvenile	–	–	11	–
Noosa River	93	F	Juvenile	–	–	12	–
Caboolture River	94	M	Juvenile	–	13	–	–
Caboolture River	95	F	Juvenile	–	14	–	–
Caboolture River	95	M	Juvenile	–	15	–	–
Caboolture River	97	F	Juvenile	–	16	–	–
Gold Coast canals	98	F	Juvenile	–	17	–	–
Gold Coast canals	100	M	Juvenile	–	18	–	–
Gold Coast canals	100	F	Juvenile	–	–	19	–
Gold Coast canals	112	F	Juvenile	–	–	20	–
<i>Estuarine habitat</i>							
Tweed River Mouth	125	F	Large juvenile	–	–	21	–
Noosa River	126	M	Large juvenile	–	–	22	–
Tallebudgera Creek	127	M	Large juvenile	23	–	–	–
Gold Coast Seaway	143	M	Large juvenile	24	–	–	–
Coomera River entrance	145	M	Large juvenile	25	–	–	–
Gold Coast Seaway	147	M	Large juvenile	26	–	–	–
Coomera River entrance	151	M	Large juvenile	27	–	–	–
Coomera River entrance	160	M	Large juvenile	28	–	–	–
Jumpinpin Bar	175	F	Subadult	29	–	–	–
Jumpinpin Bar	176	M	Subadult	30	–	–	–
Jumpinpin Bar	177	F	Subadult	31	–	–	–
Jumpinpin Bar	184	F	Subadult	32	–	–	–
Jumpinpin Bar	184	M	Subadult	33	–	–	–
Gold Coast Seaway	192	F	Subadult	34	–	–	–
<i>Nearshore marine habitat</i>							
Surfers Paradise Beach	181	F	Subadult	–	–	35	35
Burleigh Heads	187	M	Subadult	–	–	36	36
Tweed Shelf	195	M	Adult	–	–	37	37
Palm Beach	200	F	Adult	–	–	38	38
Rainbow Beach	260	M	Adult	–	–	39	–
Rainbow Beach	270	M	Adult	–	–	40	–
Tweed Shelf	300	F	Adult (P)	–	–	41	41

carried out along a linear transect from the focus to the outer centrum edge. Elements (including isotopes) monitored were calcium (^{43}Ca and ^{44}Ca), magnesium (^{24}Mg and ^{26}Mg), copper (^{63}Cu), strontium (^{88}Sr), barium (^{137}Ba and ^{138}Ba), phosphorus (^{31}P) and manganese (^{55}Mn). As there is no matrix-matched standard available for shark vertebrae, we utilised two widely used synthetic glass standards (NIST 610 or 612) for instrument calibration and to correct for the matrix effects. An internal standard of ^{43}Ca was used to correct for instrumental drift. The NIST glass standard was ablated immediately before and after

the sample ablation. In each analysis, 80 s of gas blank reading was conducted before the standard and sample ablation. Data arising from the gas blanks were subtracted from the standard and sample reading during data processing and were also used to calculate detection limits. Response from the NIST was typically $<1 \mu\text{g g}^{-1}$. Plasma gas flow was set to 18 L min^{-1} and auxiliary gas flow at 1.8 L min^{-1} ; sheath gas flow operated at 0.24 L min^{-1} with sampler gas flow at 0.95 L min^{-1} . Peak jumping scan ICPMS was used with one point per peak and a dwell time of 10 ms.

A laboratory experiment was done to determine the time taken for the elemental composition of *C. leucas* vertebrae to change from a riverine to a nearshore marine signal. Six juvenile *C. leucas* (0.94–1 m TL) were caught from the Nerang and Caboolture Rivers (salinity ~15) and transported to the laboratory. Three sharks from rivers in south-east Qld were randomly chosen to provide $n=3$ control samples. Each of the six remaining sharks was placed in separate circular (3 m radius, ~40 000 L) tanks, each with a constant flow (1000 L h⁻¹) of fresh filtered sea water (35) pumped directly from the nearshore marine environment. Three *C. leucas* were maintained in the tanks for periods of 3 and 5 weeks, respectively, before euthanasia. Vertebrae were excised and processed for LA-ICPMS as described above.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures

Vertebral samples obtained for LA-ICPMS were also analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures. Five vertebral samples from subadult ($n=3$) and adult ($n=2$) *C. leucas* (Table 1) were prepared for isotope analysis. Vertebral tissue (~4 mg DW) was removed at 1 mm increments across a single vertebra and each sample ground to a powder. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured in each powdered sample using a GV Isoprime continuous-flow, isotope-ratio, mass spectrometer (Cheadle, UK). Standards were introduced at regular intervals to provide quality control as well as to give an indication of the level of precision, which was usually at 0.5‰.

Diet of nearshore sharks

To test for ontogenetic shifts in diet, stomach content data were obtained from 1036 *C. leucas* (0.9–3 m TL) caught from 1996 to 2006 at 10 nearshore locations between Cairns (17°S) and the Gold Coast (28°S) along the Qld coast as part of the QSCP. Stomach contents were examined for major food type and used to quantify ontogenetic shifts in diet with TL. The presence of fish was noted in the SCA, but not included in the analysis to enhance the detection of the broadening of the diet through the inclusion of sharks, reptiles, mammals and birds.

Statistical analyses

Presence of individual tagged *C. leucas* was assessed at each receiver location and occurrence of large juvenile and subadult *C. leucas* determined. These data were also compared with a daily rainfall (BOM) and seawater temperature and salinity. A generalised linear model (GLM) was used to investigate the relationships between *C. leucas* presence and site, rainfall and water temperature as these were likely to influence their presence.

Non-metric multidimensional scaling (nMDS) was used to illustrate ontogenetic partitioning and visually represent the elemental composition pattern across the vertebrae of *C. leucas* of different sizes. Data were square-root transformed and resemblance was determined by the Bray–Curtis similarity index. Results of the stable isotope signatures were analysed by curvilinear regression for $\delta^{15}\text{N}$ and sigmoidal regression for $\delta^{13}\text{C}$, following initial exploratory data analysis of the likely pattern. A possible relationship between the percentage of elasmobranchs, reptiles and birds in the diet of *C. leucas* and

increasing TL was examined using Spearman's non-parametric rank correlation coefficient (r_s). Statistical analyses were carried out using Primer 6.0 (Lutton, Ivybridge, UK), SPSS 17.0 (Armonk, NY, USA) and GENSTAT 13 (Hemel Hempstead, UK) software packages.

Results

Movements, occurrence and catch

Intermediate lifecycle stages of *C. leucas* remained within estuarine habitats. Presence of tagged sharks varied among the receiver locations, but ~80% of the detections of subadult *C. leucas* occurred at the Jumpinpin Bar. Subadult *C. leucas* were consistently present at this site from March to June 2009, periodically detected in the Gold Coast Seaway and occasionally found at the entrance to Coomera River. Large juveniles were detected at the entrance to the Nerang River and in the Gold Coast canal system. No tagged *C. leucas* were detected on the nearshore acoustic receivers. Moreover, the large juvenile *C. leucas* tagged in Tallebudgera Creek remained within this habitat for the period of the study (Fig. 1).

The GLM indicated that the proportion of detections differed significantly according to the salinity and temperature at the receiver location with large juvenile and subadult *C. leucas* (GLM, both $P < 0.001$). Presence of large juveniles peaked between 17.8 and 36 and at 23°C. Subadults were present over a range of salinities (<5–36), but increased proportions of subadults were present at 28–36 and at 21–23.5°C. A significant relationship between previous daily rainfall (1–8 days prior) was evident for large juveniles and subadults (GLM, both $P < 0.001$).

Ontogenetic differences were evident in the catch of *C. leucas* across the freshwater–estuarine–marine continuum. The bull shark population in the riverine habitats comprised individuals <1.4 m TL and a single, pregnant 3.0 m TL shark (Fig. 2a). In estuarine habitats, the TL of bull sharks ranged from >1.3 to <2.0 m (Fig. 2b). Bull sharks caught in the nearshore marine waters ranged from >1.6 to 3.0 m TL (Fig. 2c) and included a single pregnant 3.0 m TL shark with 15 full-term embryos (mean = 0.65 m TL).

Laser ablation-inductively coupled plasma mass spectrometry

Ordination of the LA-ICPMS signatures of *C. leucas* in the laboratory experiment showed a distinct separation between the sharks maintained in nearshore marine waters for 3 and 5 weeks, respectively (Fig. 3a). The control samples taken from the rivers were intermixed with those held in nearshore marine waters for 3 weeks (Fig. 3a).

nMDS ordination of the isotopes of the seven elements examined using LA-ICPMS across the vertebrae of 23 *C. leucas* showed that *C. leucas* from rivers exhibited a relatively close group in the bottom section of the plot (Fig. 3b). Subadult and adult *C. leucas* radiated further from the juveniles and the two adult males and the pregnant female *C. leucas* also appeared to differ. The adult males were more distant from the subadult and juvenile *C. leucas*, whereas the signature of the pregnant female *C. leucas* was close to that of the juveniles caught in the rivers (Fig. 3b; Table 1).

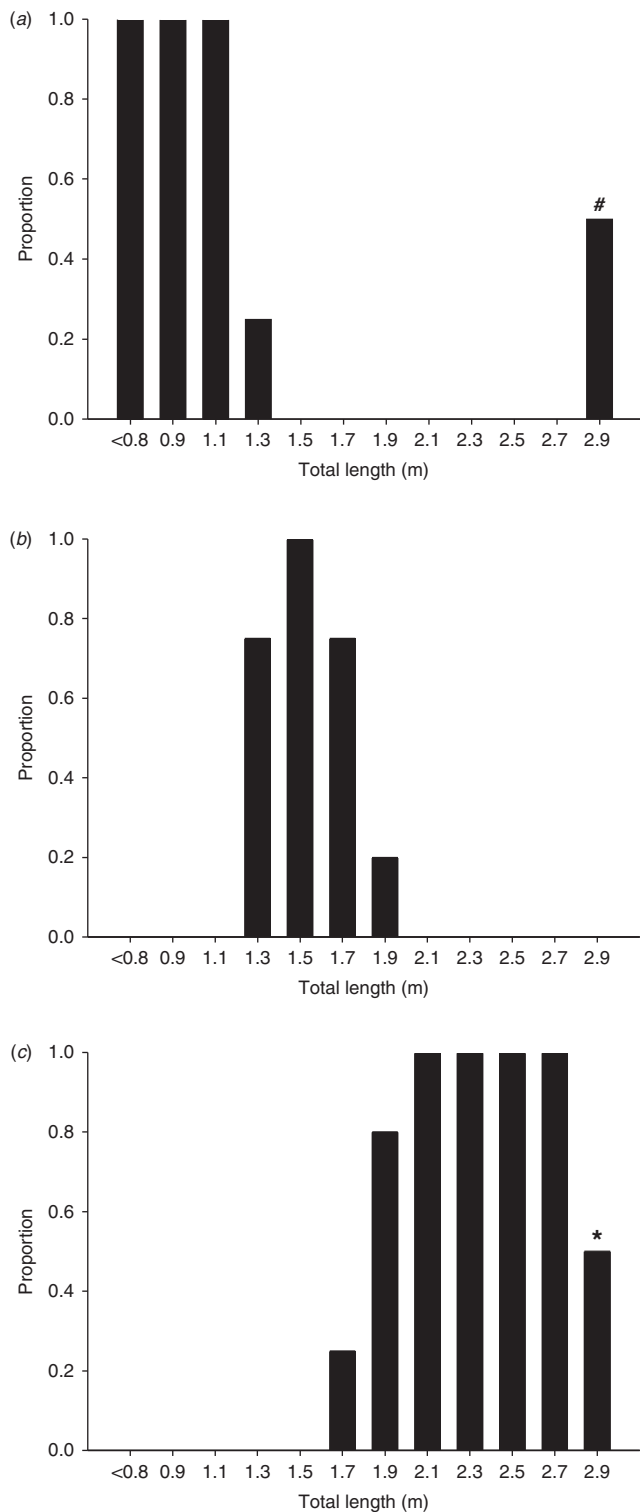


Fig. 2. Proportion of *Carcharhinus leucas* (bull shark) caught in (a) rivers ($n=21$), (b) estuarine waters ($n=14$) and (c) nearshore marine waters ($n=21$) from 2006 to 2009. Symbol # denotes pregnant female caught in river; * denotes pregnant female (Shark 41 in Table 1) caught in the nearshore marine waters.

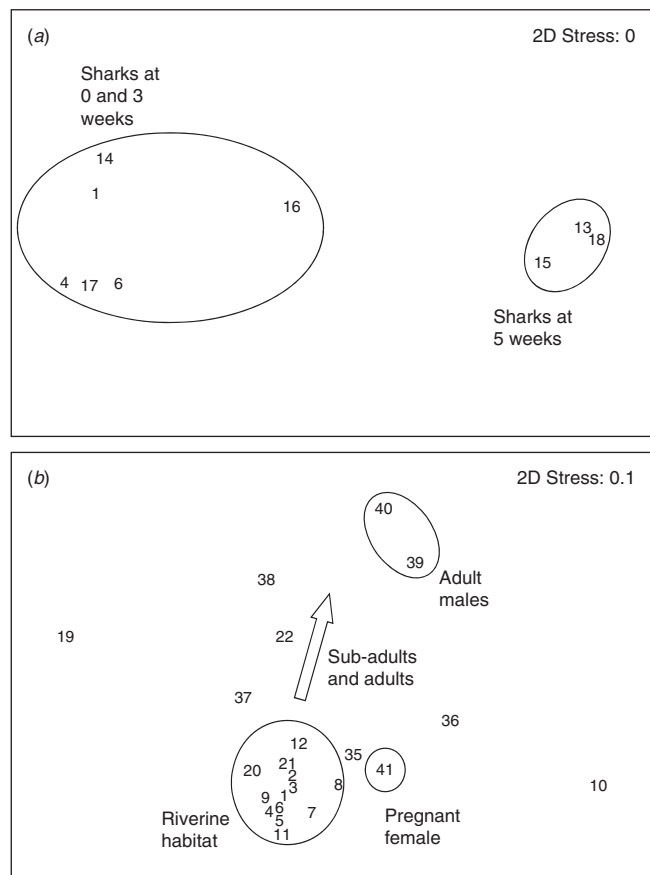


Fig. 3. Non-metric multidimensional scaling of the elemental composition across vertebrae of *Carcharhinus leucas* (bull shark) (a) in a controlled experiment in tanks with sharks at 0 weeks (Sharks 1, 4 and 6 in Table 1) and at 3 weeks (Sharks 14, 16 and 17 in Table 1) and at 5 weeks (Sharks 13, 15, and 18 in Table 1) and of (b) differing total length (TL) (Sharks 1–12, 19–22 and 35–41 in Table 1).

$\delta^{13}C$ and $\delta^{15}N$ isotope signatures

A significant sigmoidal relationship was evident for the mean $\delta^{13}C$ from the five large *C. leucas* sampled across the vertebrae (Fig. 4a, $r=0.97$, $P=0.002$, $n=9$). The mean (\pm s.e.) $\delta^{13}C$ changed from $-12.3 \pm 0.4\text{‰}$ to $-15.5 \pm 1.1\text{‰}$ with increasing distance away from the centrum focus. The $\delta^{13}C$ signature of the individual pregnant female (Fig. 4a) was -12‰ at 1 mm from the centrum focus and indicative of riverine conditions. At 4 mm from the centrum focus, the $\delta^{13}C$ was -16‰ and similar to marine signature. Beyond 10 mm from the centrum focus, the $\delta^{13}C$ was -12.5‰ and again indicative of the riverine signature.

In nearshore *C. leucas*, the mean $\delta^{15}N$ values were described by a significant curvilinear regression ($r=0.97$, $P<0.0001$, $n=9$) with increasing values occurring at a greater distance from the centrum focus (Fig. 4b). The $\delta^{15}N$ signature of the individual pregnant female (Fig. 4b) was 10‰ at 1 mm from the centrum focus and increased to $\sim 13\text{‰}$ at 7 mm from the centrum focus. Thereafter, the $\delta^{15}N$ signature fluctuated between 11 and 13‰.

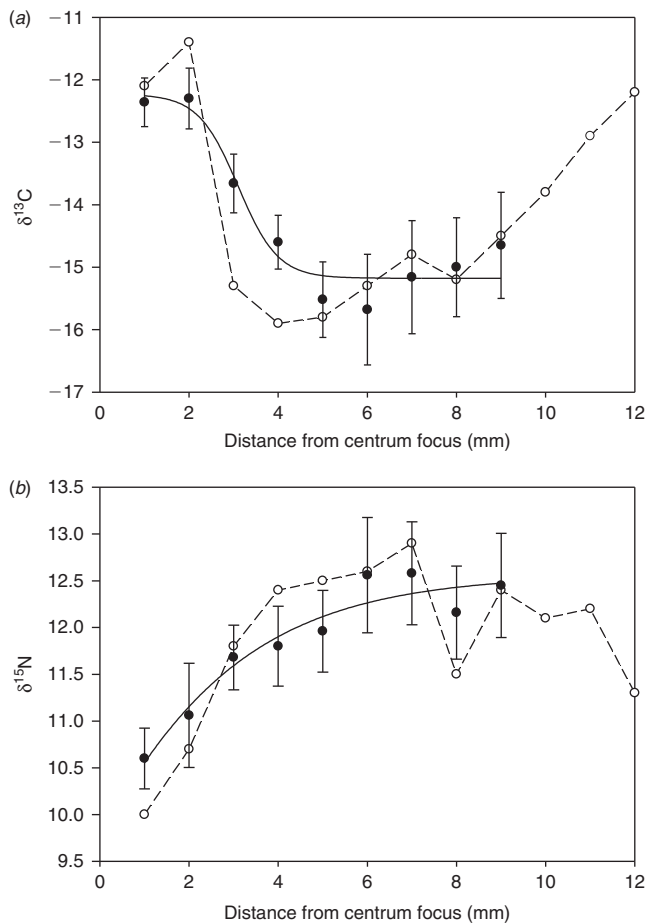


Fig. 4. Change in (a) mean (\pm s.e.) $\delta^{13}\text{C}$ (‰) and (b) $\delta^{15}\text{N}$ (‰) values with distance from the centrum focus of *Carcharhinus leucas* shark vertebrae (●). Means based on $n = 5$ samples. The rapid transition of $\delta^{13}\text{C}$ (‰) at 2–4 mm from the centrum focus correspond to individuals ~ 1.3 m total length (TL). The individual signatures for the pregnant shark (o) in the nearshore marine environment are also shown. Note: samples from Sharks 35–38 and 41 in Table 1.

Diet of nearshore sharks

An ontogenetic shift was evident in the diet of nearshore *C. leucas*. Of the 1036 guts of bull sharks examined, 551 (53.2%) were empty and teleost fish were the most abundant prey (23.09% of stomachs). Clupeids (4.33%), rays (3.48%), unidentified bones (3.02%), turtles (1.76%) and other sharks (1.35%) were the next dominant prey types. The remaining 9.7% of stomachs examined contained crabs, prawns, octopus, squid, sea snakes, turtles and birds. Contents of 404 stomachs with distinguishable dietary items and accurate measurements of shark TL were used to examine the possible broadening of the diet. The combined percentage of sharks and rays, reptiles and birds (Fig. 5) was correlated with an increase in TL of bull sharks (Fig. 5, $r_s = 0.79$, $P < 0.01$, $n = 12$). Larger prey items contributed to 22% of stomachs with prey items for juvenile sharks and up to 100% of stomachs with prey items for adult *C. leucas*.

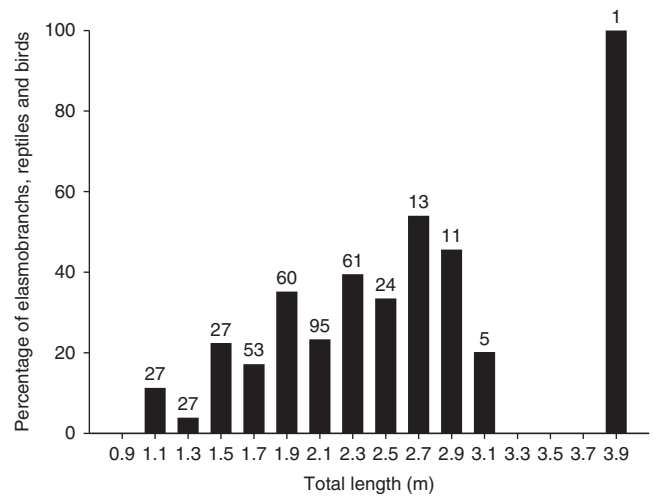


Fig. 5. Percentage of stomachs with rays, sharks, sea snakes, turtles and sea birds ($n = 404$) from individual *Carcharhinus leucas* (bull shark) caught from 1996 to 2006 at 10 nearshore marine locations between Cairns and the Gold Coast in the Qld Shark Control Program. Numbers above columns indicate the number of sharks in each size class.

Discussion

Movements, occurrence and catch

Large juveniles remained in estuarine habitat with occasional movements into canals and rivers. These results are similar to those by Yeiser *et al.* (2008), who showed that large juvenile sharks occupied estuarine coastal lagoons in Florida. In contrast, subadult *C. leucas* were not detected in any of the river or canal systems during the study and their movements were confined to areas at the direct interface between estuarine and nearshore marine habitats, thus confirming the hypothesised ontogenetic transition in habitat use.

Neonates and small juveniles were caught in the riverine habitat with salinity ranging from 6 to 18 and were absent from the catches in the estuarine and nearshore marine environments. These results are consistent with previous studies where neonate and juvenile bull sharks were caught in large numbers in freshwater habitats in Nicaragua (Thorson *et al.* 1973), Florida (Simpfendorfer *et al.* 2005; Heupel and Simpfendorfer 2008; Heithaus *et al.* 2009), Fiji (Rasalato *et al.* 2010) and Australia (Pillans and Franklin 2004; Werry 2010). Large juveniles and subadults were caught in estuarine habitat (salinity 25–35) at the interface between marine and freshwater environments, with a few individuals caught in the nearshore environment. Finally, subadult and adult bull sharks were predominantly caught in nearshore marine habitats, supporting earlier reports off South Africa (Cliff and Dudley 1991), USA (Myers *et al.* 2007) and the wider east coast of Australia (Werry 2010).

Laser ablation-inductively coupled plasma mass spectrometry

Previous studies (e.g. Campana *et al.* 2002) have used shark vertebrae as a record to interpret broadening of the diet, habitat shifts, and thus, reflect the sharks' life history. In this study, LA-ICPMS analysis of elemental signatures indicated habitat

transition with increasing TL of the bull sharks. Further, the laboratory experiment showed that the change in elemental signature occurred in a matter of weeks. The signatures of neonates and small juveniles in riverine habitats formed a distinct cluster, whereas those of the large juveniles, subadult and adult male *C. leucas* were dispersed. This result provides a clear indication of transition across habitats.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures

Estuarine $\delta^{13}\text{C}$ signatures can vary according to the initial carbon source and subtle changes in elemental isotopic signatures can also reveal natal origins of adult fish and movements from estuarine to nearshore habitats (Gillanders and Kingsford 2000; Thorrold *et al.* 2001; Gillanders 2005). The $\delta^{13}\text{C}$ signature of the bull shark vertebrae during the neonate and small juvenile phases was approximately -12‰ . The $\delta^{13}\text{C}$ signature in the nearshore marine environment reflects an oceanic phytoplankton carbon source and ranges -20 to -22‰ (Coffin *et al.* 1994; Fry 2006), whereas riparian/mangrove, seagrass and microalgae have $\delta^{13}\text{C}$ signatures of about -27 , -10 and -15‰ respectively (Kennedy *et al.* 2010; Olsen *et al.* 2011). Moreover, in subtropical Australian estuaries, mullet (*Mugil cephalis*), a common prey to neonate and small juvenile bull sharks, consume benthic microalgae and similar species in Taiwanese estuaries (e.g. *Liza macrolepis*) have a $\delta^{13}\text{C}$ signature of -11 to -16‰ (Lin *et al.* 2007). Hence, the $\delta^{13}\text{C}$ signatures from the bull sharks provided clear evidence of the occupation of riverine habitats during the neonate and small juvenile phases.

The increase in the $\delta^{15}\text{N}$ signature with predator size reflects the broadening of the diet with prey species increasingly at higher trophic levels (Davenport and Bax 2002; Vanderklift and Ponsard 2003; McCutchan *et al.* 2003). The $\delta^{15}\text{N}$ signature of the bull shark vertebrae increased with TL, indicating an ontogenetic shift in the trophic level of prey consumed. Similar results have been found with stable isotope analysis of the white shark (Estrada *et al.* 2006).

Diet of nearshore sharks

Stomach content analysis confirmed the results of the stable isotope analysis, showing a broadening of the diet with prey in higher trophic levels. This was consistent with previous studies showing consumption of mullet by bull sharks in riverine habitats (Bass *et al.* 1973; Zeller 1999) and larger prey in nearshore marine areas (Vorenberg 1962; Cliff and Dudley 1991).

Ontogenetic transition in habitat use

Two competing explanatory models may account for the occurrence of neonates in riverine areas. The first model, attributable to Jensen (1976), suggests that *C. leucas* give birth in the nearshore areas adjacent to river mouths. From this, it can be predicted that, following birth, neonates would move from the nearshore marine and estuarine areas up to riverine habitats. Given that bull sharks are cannibalistic and that the juveniles and subadults occupy the estuarine habitats, neonates moving through these areas would be subjected to cannibalism (Vorenberg 1962). This would be evident in catches across the freshwater–estuarine–marine continuum for a period of time

post parturition. Moreover, neonates would be evident through the analysis of stomach contents of larger conspecifics. The second model suggests that pregnant adult *C. leucas* move from the nearshore marine environment into rivers, swimming into low salinity reaches to give birth. Hence, neonates would be evident only in the catches from the riverine environment and, hence, avoid predation from larger conspecifics. Our data support the latter model as neonates were caught only in the riverine environment and a putatively pregnant female was also caught in the same area.

The two pregnant females caught, one in the immediate nearshore marine environment and the second in riverine habitat 6 km upstream from the mouth of the Nerang River where salinity varies between 15 and 22 (Werry 2010), provided preliminary observations concerning the potential parturition location. The LA-ICPMS signature of the pregnant female (Shark 41 in Fig. 3b) differed from those of the adult males (Sharks 39 and 40), but was similar to that of juveniles occupying riverine conditions, providing evidence that pregnant females move into riverine areas to give birth, a result also suggested by Tillett *et al.* (2011). Furthermore, the later return to a $\delta^{13}\text{C}$ signature of -12‰ indicated a return from marine to riverine habitat, most likely to search for parturition grounds (McCord and Lamberth 2009). Previous studies (e.g. Jensen 1976) have suggested that *C. leucas* give birth in estuarine conditions at river mouths; however, this conclusion was based on the capture of pregnant females and not neonates at these locations.

The results of this study suggest a staged transition from natal grounds to estuarine habitat by bull sharks with increasing TL. This was demonstrated with the distinct shift in the $\delta^{13}\text{C}$ signature during the life history of these sharks (Fig. 4), which reflects a broadening of the diet (Fig. 5) and increasingly larger home ranges. Factors that drive a species to investigate habitats beyond their natal grounds are still poorly understood but ontogenetic shifts in foraging needs are probably important. This study showed that larger *C. leucas* (>1.2 m TL) began to exhibit a broadening of diet that included larger and more varied prey, probably reflecting movement of *C. leucas* from nursery grounds to other habitats. Gradients in prey abundance may structure the distribution of predators as they mature and increase in size (Hart 1997; Sims 2003). Smaller juvenile sharks inhabit shallow areas as a means of avoiding predation, whereas large and older sharks occupy deeper areas (Merson and Pratt 2001). Hence, predator avoidance may be more critical than prey capture for habitat choice amongst small juvenile sharks (Heupel and Hueter 2002). However, reduced predation from conspecifics and other sharks due to the size of subadult and large juvenile *C. leucas* may be a factor contributing to the transition of sharks of this size into estuarine habitats at the interface with the marine environment. The clear dietary broadening with the inclusion of larger prey suggests that this may also drive the transition. In estuarine habitats, large juvenile and subadult *C. leucas* may be the dominant predators – spatially and temporally – and could explain their residency in these habitats.

Estuaries provide important habitats during the late juvenile stages of this species' life history (Yeiser *et al.* 2008). Juvenile and subadult *C. leucas* caught in nearshore marine QSCP gears occurred after substantial rainfall, which may have disrupted the

normal ontogenetic segregation in riverine and estuarine habitat (Werry 2010). With the exception of the Nerang River with the adjoining Gold Coast canal system, no large juvenile *C. leucas* were recorded from accessible sites in the rivers sampled during the study. One of these, the Logan River, is one of the most productive rivers for mud crabs (*Scylla serrata*) in subtropical Queensland (Loneragan and Bunn 1999) and although crabs are prey of juvenile *C. leucas* (Cliff and Dudley 1991), none of the tagged *C. leucas* moved into this river.

All adult *C. leucas* were captured in the nearshore marine environment, other than one pregnant female caught in the Nerang River. These results suggest males may not move back into rivers and estuaries and remain within the nearshore marine environment. This was supported by the $\delta^{13}\text{C}$ values of the post-transition phase, which was more indicative of a marine food chain based on oceanic phytoplankton sources. For some fishes, movement into deeper waters is very often associated with ontogenetic habitat shift, as site fidelity to shallow waters generally diminishes with body size (Macpherson 1998). Further, many marine fishes exhibit directional and long-range migrations (Bruce *et al.* 2006) associated with changing environmental parameters (e.g. rainfall and sea surface temperature) or stimuli such as temporarily abundant food sources and mating requirements (Pittman and McAlpine 2001). Adult mating and breeding grounds may also require migrations to specific locations where sexually mature individuals congregate, driving ontogenetic habitat shift.

Implications for conservation and management

The use of diverse but connected habitats is characteristic of the life history of *C. leucas*. Managing all components across the freshwater–estuarine–marine continuum is, therefore, necessary for the effective conservation of this potentially dangerous shark. A multipartite life cycle with stage-specific occupation of distinct, but connected, habitats by neonate, juvenile, subadult and adult bull sharks is a feature also common in estuarine plants and animals (Pittman and McAlpine 2001; Sheaves 1995). Moreover, *C. leucas* is considered ‘near-threatened’ globally on the IUCN Red List due to human impacts and habitat modification (Cavanagh *et al.* 2003; IUCN 2008). This study has shown that the size, movements, timing and duration of occupation and diet of *C. leucas* differs across the freshwater–estuarine–marine continuum. These biological traits will influence the measures required for effective conservation and achieving this will depend on the area and connectivity of habitats, degree of urbanisation, frequency and extent of human interactions and associated impacts, all of which vary across the freshwater–estuarine–marine continuum. Finally, the resources necessary to drive appropriate conservation measures such as the protection of parturition areas in freshwater habitats, maintenance of natural habitat in urbanised estuaries and the provision of habitat connectivity will differ and necessitate cost-effective prioritisation. This is particularly important given the rapid urbanisation of estuaries that is occurring globally.

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References

- Bass, A. J., D’Aubrey, J. D., and Kitnasamy, N. (1973). Sharks of the east coast of Southern Africa. I. Genus *Carcharhinus* (Carcharhinidae). *Oceanographic Research Institute Investigational Report* **33**, 1–168.
- Bruce, B. D., Stevens, J. D., and Malcolm, H. (2006). Movements and swimming behaviour of white sharks (*Carcharodon carcharias*) in Australian waters. *Marine Biology* **150**, 161–172. doi:10.1007/S00227-006-0325-1
- Brunnschweiler, J. M., Queiroz, N., and Sims, D. W. (2010). Oceans apart? Short-term movements and behaviour of adult bull sharks *Carcharhinus leucas* in Atlantic and Pacific Oceans determined from pop-off satellite archival tagging. *Journal of Fish Biology* **77**, 1343–1358. doi:10.1111/J.1095-8649.2010.02757.X
- Cavanagh, R., Kyne, P., Fowler, S. L., Musick, J. A., and Bennett, M. B. (2003). The conservation status of Australasian chondrichthyans. Report of the IUCN Shark Specialist Group Australia and Oceania Regional Red List workshop, 7–9 March 2003, University of Queensland, Brisbane, Australia. Available at <http://www.uf.edu/fish/organizations/ssg/region8/Austfinal.pdf> [accessed 1 February 2011].
- Cliff, G., and Dudley, S. F. J. (1991). Sharks caught in the protective nets off Natal, South Africa. 4. The bull shark *Carcharhinus leucas* Valenciennes. *South African Journal of Marine Science* **10**, 253–270. doi:10.2989/02577619109504636
- Coffin, R. B., Cifuentes, L. A., and Elderidge, P. M. (1994). The use of stable carbon isotopes to study microbial processes in estuaries. In ‘Stable Isotopes in Ecology and Environmental Science’. (Eds K. Lajtha and R. H. Michener.) pp. 222–240. (Blackwell Scientific: Oxford.)
- Compagno, L. J. (2002). ‘FAO Species Catalogue. Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Vol. 2. Bullhead, Mackerel and Carpet Sharks (Heterodontiformes, Lamniformes and Orectolobiformes).’ (FAO: Rome.)
- Campana, S. E., Natanson, L. J., and Myklevoll, S. (2002). Bomb dating and age determination of large pelagic sharks. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 450–455. doi:10.1139/F02-027
- Cortés, E. (1997). A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 450–455. doi:10.1139/CJFAS-54-3-726
- Cortés, E. (1999). Standardized diet compositions and trophic levels of sharks. *ICES Journal of Marine Science* **56**, 707–717. doi:10.1006/JMSC.1999.0489
- Davenport, S. R., and Bax, N. J. (2002). A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 514–530. doi:10.1139/F02-031
- Ebert, D. A. (2002). Ontogenetic changes in the diet of the sevengill shark (*Notorynchus cepedianus*). *Marine and Freshwater Research* **53**, 517–523. doi:10.1071/MF01143
- Estrada, J. A., Rice, A. N., Natanson, L. J., and Skomal, G. B. (2006). Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* **87**, 829–834. doi:10.1890/0012-9658(2006)87[829:UOIAOV]2.0.CO;2

- Fry, B. (2006). 'Stable Isotope Ecology.' (Springer: New York.)
- Gillanders, B. M. (2005). Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. *Estuarine, Coastal and Shelf Science* **64**, 47–57. doi:10.1016/J.ECSS.2005.02.005
- Gillanders, B. M., and Kingsford, M. J. (2000). Elemental fingerprints of otoliths may distinguish estuarine 'nursery' habitats. *Marine Ecology Progress Series* **201**, 273–286. doi:10.3354/MEPS201273
- Gillanders, B. M., Able, K. W., Brown, J. A., Eggleston, D. B., and Sheridan, P. F. (2003). Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. *Marine Ecology Progress Series* **247**, 281–295. doi:10.3354/MEPS247281
- Hale, L., Dudgeon, J., Mason, A., and Lowe, C. (2006). Elemental signatures in the vertebral cartilage of the round stingray, *Urobatis halleri*, from Seal Beach, California. *Environmental Biology of Fishes* **77**, 317–325. doi:10.1007/S10641-006-9124-2
- Hart, P. J. B. (1997). Foraging tactics. In 'Behavioural Ecology of Teleost Fishes'. (Ed. J. G. J. Godin.) pp. 104–133. (Oxford University Press: Oxford.)
- Heithaus, M. R. (2004). Predator–prey interactions. In 'Biology of Sharks and Their Relatives'. (Eds J.C. Carrier, J.A. Musick and M.R. Heithaus.) pp. 487–522 (CRC Press: Florida.)
- Heithaus, M. R., Dill, L. M., Marshall, G. J., and Buhleier, B. (2002). Habitat use and foraging behaviour of tiger sharks (*Galeocerdo cuvier*) in a seagrass ecosystem. *Marine Biology* **140**, 237–248. doi:10.1007/S00227-001-0711-7
- Heithaus, M. R., Frid, A., Wirsing, A. J., Bejder, L., and Dill, L. (2005). Biology of sea turtles under risk from tiger sharks at a foraging ground. *Marine Ecology Progress Series* **288**, 285–294. doi:10.3354/MEPS288285
- Heithaus, M. R., Delius, B. K., Wirsing, A. J., and Dunphy-Daly, M. M. (2009). Physical factors influencing the distribution of a top predator in a subtropical oligotrophic estuary. *Limnology and Oceanography* **54**, 472–482. doi:10.4319/LO.2009.54.2.0472
- Heupel, M. R., and Hueter, R. E. (2002). Importance of prey density in relation to the movement patterns of juvenile blacktip sharks (*Carcharhinus limbatus*) within a coastal nursery area. *Marine and Freshwater Research* **53**, 543–550. doi:10.1071/MF01132
- Heupel, M. R., and Simpfendorfer, C. A. (2008). Movement and distribution of young bull sharks *Carcharhinus leucas* in a variable estuarine environment. *Aquatic Biology* **1**, 277–289. doi:10.3354/AB00030
- Heupel, M. R., Simpfendorfer, C. A., and Hueter, R. E. (2004). Estimation of shark home ranges using passive monitoring techniques. *Environmental Biology of Fishes* **71**, 135–142. doi:10.1023/B:EBFI.0000045710.18997.F7
- Heupel, M. R., Yeiser, B. G., Collins, A. B., Ortega, L., and Simpfendorfer, C. A. (2010). Long-term presence and movement patterns of juvenile bull sharks, *Carcharhinus leucas*, in an estuarine river system. *Marine and Freshwater Research* **61**, 1–10. doi:10.1071/MF09019
- Hight, B. V., and Lowe, C. G. (2007). Elevated body temperatures of adult female leopard sharks, *Triakis semifasciata*, while aggregating in shallow nearshore embayments: evidence for behavioural thermoregulation? *Journal of Experimental Marine Biology and Ecology* **352**, 114–128. doi:10.1016/J.JEMBE.2007.07.021
- Hopkins, T. E., and Cech, J. J., Jr (2003). The influence of environmental variables on the distribution and abundance of three elasmobranchs in Tomales Bay, California. *Environmental Biology of Fishes* **66**, 279–291. doi:10.1023/A:1023907121605
- IUCN (2008). *Carcharhinus leucas* IUCN (International Union for Conservation of Nature) Red list of threatened species. Available at www.iucnredlist.org [accessed 15 March 2010].
- Jensen, N. H. (1976). Reproduction of the bull shark, *Carcharhinus leucas*, in the Lake Nicaragua Rio San Juan system. In 'Investigation of the Ichthyofauna of Nicaraguan Lakes'. (Ed. T. B. Thorson.) pp. 539–559. (University of Nebraska Press: Lincoln, NE.)
- Kennedy, H., Beggins, J., Duarte, C. M., Fourqurean, J. W., Holmer, M., Marbà, N., and Middelburg, J. J. (2010). Seagrass sediments as a global carbon sink: isotopic constraints. *Global Biogeochemical Cycles* **24**, GB4026. doi:10.1029/2010GB003848
- Kerr, L., Andrews, A., Cailliet, G., Brown, T., and Coale, K. (2006). Investigations of $\Delta^{14}\text{C}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ in vertebrae of white shark (*Carcharodon carcharias*) from the eastern North Pacific Ocean. *Environmental Biology of Fishes* **77**, 337–353. doi:10.1007/S10641-006-9125-1
- Knip, D. M., Heupel, M. R., Simpfendorfer, C. A., Tobin, A. J., and Moloney, J. (2011). Ontogenetic shifts in movement and habitat use of juvenile pigeye sharks *Carcharhinus amboinensis* in a tropical nearshore region. *Marine Ecology Progress Series* **425**, 233–246. doi:10.3354/MEPS09006
- Last, P. R., and Stevens, J. D. (2009). 'Sharks and Rays of Australia.' (CSIRO Publishing: Melbourne.)
- Lin, H. S., Kao, W. Y., and Wang, Y. T. (2007). Analyses of stomach contents and stable isotopes reveal food sources of estuarine detritivorous fish in tropical/subtropical Taiwan. *Estuarine, Coastal and Shelf Science* **73**, 527–537. doi:10.1016/J.ECSS.2007.02.013
- Loneragan, N. R., and Bunn, S. E. (1999). River flows and estuarine ecosystems: implications for coastal fisheries from a review and a case study of the Logan River, southeast Queensland. *Australian Journal of Ecology* **24**, 431–440. doi:10.1046/J.1442-9993.1999.00975.X
- Macpherson, E. (1998). Ontogenetic shifts in habitat-use and aggregation in juvenile spard fishes. *Journal of Experimental Marine Biology and Ecology* **220**, 127–150. doi:10.1016/S0022-0981(97)00086-5
- McCord, M. E., and Lamberth, S. J. (2009). Catching and tracking the world's largest Zambezi (bull) shark *Carcharhinus leucas* in the Breede Estuary, South Africa: the first 43 hours. *African Journal of Marine Science* **31**, 107–111. doi:10.2989/AJMS.2009.31.1.11.782
- McCutchan, J. H., Lewis, W. M., Kendall, C., and McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulphur. *Oikos* **102**, 378–390. doi:10.1034/J.1600-0706.2003.12098.X
- Merson, R. R., and Pratt, H. L. (2001). Distribution, movements and growth of young sandbar, *Carcharhinus plumbeus*, in the nursery grounds of Delaware Bay. *Environmental Biology of Fishes* **61**, 13–24. doi:10.1023/A:1011017109776
- Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S., and Peterson, C. H. (2007). Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science* **315**, 1846–1850. doi:10.1126/SCIENCE.1138657
- Olsen, Y. S., Fox, S. E., Teichberg, M., Otter, M., and Valiela, I. (2011). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ reveal differences in carbon flow through estuarine benthic food webs in response to the relative availability of macroalgae and eelgrass. *Marine Ecology Progress Series* **421**, 83–96. doi:10.3354/MEPS08900
- Pillans, R. D., and Franklin, C. E. (2004). Plasma osmolyte concentrations and rectal gland mass of bull sharks *Carcharhinus leucas*, captured along a salinity gradient. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **138**, 363–371. doi:10.1016/J.CBPA.2004.05.006
- Pillans, R. D., Anderson, G., Goode, J. P., Hyodof, S., Takeif, Y., Hazone, N., and Franklin, C. E. (2006). Plasma and erythrocyte solute properties of juvenile bull sharks, *Carcharhinus leucas*, acutely exposed to increasing environmental salinity. *Journal of Experimental Marine Biology and Ecology* **331**, 145–157. doi:10.1016/J.JEMBE.2005.10.013
- Pittman, S. J., and McAlpine, C. A. (2001). Movements of marine fish and decapod crustaceans: process, theory and application. *Advances in Marine Biology* **44**, 205–294. doi:10.1016/S0065-2881(03)44004-2
- Rasalato, E., Maginnity, V., and Brunnschweiler, J. M. (2010). Using local ecological knowledge to identify shark river habitats in Fiji (South

- Pacific). *Environmental Conservation* **37**, 90–97. doi:10.1017/S0376892910000317
- Sheaves, M. (1995). Large lutjanid and serranid fishes in tropical estuaries: are they adults or juveniles? *Marine Ecology Progress Series* **129**, 31–41. doi:10.3354/MEPS129031
- Simpfendorfer, C., Freitas, G., Wiley, T., and Heupel, M. (2005). Distribution and habitat partitioning of immature bull sharks (*Carcharhinus leucas*) in a Southwest Florida estuary. *Estuaries and Coasts* **28**, 78–85. doi:10.1007/BF02732755
- Sims, D. W. (2003). Tractable models for testing theories about natural strategies: foraging behaviour and habitat selection of free ranging sharks. *Journal of Fish Biology* **63**, 53–73. doi:10.1111/J.1095-8649.2003.00207.X
- Thorrold, S. R., Latkoczy, C., Swart, P. K., and Jones, G. P. (2001). Natal homing in a marine fish metapopulation. *Science* **291**, 297–299. doi:10.1126/SCIENCE.291.5502.297
- Thorson, T. B. (1971). Movement of bull sharks, *Carcharhinus leucas*, between Caribbean Sea and Lake Nicaragua demonstrated by tagging. *Copeia* **1971**, 336–338. doi:10.2307/1442846
- Thorson, T. B., Cowan, C. M., and Watson, D. E. (1973). Body fluid solutes of juveniles and adults of the euryhaline bull shark *Carcharhinus leucas* from freshwater and saline environments. *Physiological Zoology* **46**, 29–42.
- Tillett, B. J., Meekan, M. G., Parry, D., Munksgaard, N., Field, I. C., Thorburn, D., and Bradshaw, C. J. A. (2011). Decoding fingerprints: elemental composition of vertebrae correlates to age-related habitat use in two morphologically similar sharks. *Marine Ecology Progress Series* **434**, 133–142. doi:10.3354/MEPS09222
- Vanderklift, M. A., and Ponsard, S. (2003). Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta analysis. *Oecologia* **136**, 169–182. doi:10.1007/S00442-003-1270-Z
- Vorenberg, M. M. (1962). Cannibalistic tendencies of lemon and bull sharks. *Copeia* **1962**, 455–456. doi:10.2307/1440934
- Watsky, M. A., and Gruber, S. H. (1990). Induction and duration of tonic immobility in the lemon shark, *Negaprion brevirostris*. *Fish Physiology and Biochemistry* **8**(3), 207–210. doi:10.1007/BF00004459
- Werry, J. M. (2010). Habitat ecology of the bull shark, *Carcharhinus leucas*, on urban coasts in eastern Queensland, Australia. PhD Thesis, Griffith University, Australia.
- Wetherbee, B. M., and Cortes, E. (2004). Food consumption and feeding habitats. In 'Biology of Sharks and Their Relatives'. (Eds J. C. Carrier, J. A. Musick and M. R. Heithaus.) pp. 225–246. (CRC Press: Boca Raton, FL.)
- Yeiser, B. G., Heupel, M. R., and Simpfendorfer, C. A. (2008). Occurrence, home range and movement patterns of juvenile bull (*Carcharhinus leucas*) and lemon (*Negaprion brevirostris*) sharks within a Florida estuary. *Marine and Freshwater Research* **59**, 489–501. doi:10.1071/MF07181
- Zeller, B. M. (1999). 'Bull Shark *Carcharhinus leucas* Valenciennes in Estuarine and Nearshore Waters of the Gold Coast, Queensland.' (Department of Primary Industries and Fisheries: Brisbane.)