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## Advancing the ecological knowledge base of the dusky shark (Carcharhinus obscurus) off Southern Africa

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## Advancing the ecological knowledge base of the dusky shark (*Carcharhinus obscurus*) off Southern Africa.

A thesis presented for the degree of Doctor of Philosophy



By

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### Advancing the ecology knowledge base of the dusky shark (Carcharhinus obscurus) off Southern Africa

Nigel E. Hussey<sup>1</sup>

#### ABSTRACT

The dusky shark (Carcharhinus obscurus) is listed 'vulnerable' on the IUCN red list. Here, archived data sets and the application of modern biogeochemical tags are combined to advance the ecological knowledge base of this species off Southern Africa. Tag-recapture data identified that juveniles undertake small scale movements (<100 km) throughout the year within a core nursery region in KwaZulu-Natal (KZN) and defined a seasonal migration between KZN and the Cape region. Calculated growth rates of small dusky sharks ranged from 10.3 – 11.5 cmyr<sup>-1</sup>. A comparative analysis of condition indices [hepatosomatic (HSI), condition factor, relative condition and residual condition] identified that HSI was a more rapid and sensitive measure of condition. Accurate inferences on condition, however, require the careful division of data by size, sex, reproductive state and the isolation of neonatal and juvenile sharks. The first empirical evidence for maternal investment in newborn sharks in the form of an enlarged liver, constituting ~20% of body mass, is presented. Additionally, through a quantitative modelling approach, it was found that the reproductive output of the dusky and spinner shark (C. brevipinna) increased with maternal size with evidence for a moderate decline in the largest mothers. Near-term pup mass increased with month identifying either variable parturition strategies and/or regulation of the length of the gestation period. Through a semi-controlled experiment, diet-tissue discrimination factors for shark muscle tissue of 2.29‰  $\pm$  0.22 ( $\delta^{15}$ N) and 0.90‰  $\pm$  0.33 ( $\delta^{13}$ C) were derived. The application of these values to wild sampled animals found that stables isotopes do provide a reasonable estimate of trophic position (TP) when compared to standardised stomach content data (SC). Ontogenetic  $\delta^{13}$ C data provided reasonable coarse resolution movement profiles. Data presented provide new techniques to study sharks and will assist regional management plans.

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## **CHAPTER ONE**

General Introduction

### Marine predator exploitation, population decline and ecological consequences

Our global oceans have undergone considerable change since human industrialisation and our insatiable desire to over-exploit marine systems. With the advent of industrial fishing and initial absence of structured multi-species management frameworks, stocks of marine predators have undergone drastic declines (Myers & Worm 2003; Hutchings & Baum 2005; Myers & Worm 2005) with the rate of biodiversity loss several orders of magnitude higher than the historic extinction rate (Mace *et al.* 2005). While there are few reported marine predator extinctions (Carlton *et al.* 1999), marine predator populations have been reduced by 50 - 90% of historical biomass (Hilborn *et al.* 2003; Myers & Worm 2005) with the estimated time to quasi extinction of the Western Australian sand tiger (*Carcharias taurus*) population in 45-53 years (Otway *et al.* 2004).

The large scale removal of marine predators has raised questions over their roles and the consequent effects of their removal on ecosystem structure, function, stability and resilience (Stevens et al. 2000; Myers et al. 2007; Heithaus et al. 2008; Baum & Worm 2009). This is based on the concept of predators exerting top-down control in marine systems, whereby strong predator effects, i.e. population depletion through overfishing, cascade through the food web impacting consecutive trophic levels (Pace et al. 1999). Evidence to date, suggests that top down controls include the release of mesopredators, i.e. an increase in the abundance of medium size vertebrate predator populations after removing their key predators (Carscadden et al. 2001; Blanchard et al. 2005; Daan et al. 2005); invertebrate release (Caddy & Rodhouse 1998; Myers & Worm 2003) and; trophic cascades of mesopredator and invertebrate population explosions on lower trophic levels (Möllmann et al. 2008). Quantitative examination of the concept of 'top down' control, however, is a relatively recent phenomenon that has received limited scientific investigation. The reason for this most likely relates to the requirement for large-scale biological and environmental data sets (both in temporal and spatial domains), the logistical difficulties of modelling trophic processes in oceanic environments and the complex nature of linkages between coastal and pelagic systems.

For a recent comprehensive review on 'cascading effects' of predator removal and 'top down' control, please refer to Baum & Worm (2009).

#### Status of global shark stocks

Over the past half century, large predatory sharks have been exploited both as bycatch in pelagic longline fisheries (Bonfil 1994) and as target species in directed fisheries that have rapidly expanded since the 1980s (Rose 1996). Additionally, global artisanal fisheries, estimated at 12 million fishers compared to 0.5 million in industrialised fisheries (Pauly 2006), are generally unaccounted for due to a lack of quantitative assessments or survey data available. From data available, the FAO estimated that global reported landings of chondrichthyans grew from 271,800 tonnes in 1950 to 828, 364 tonnes in 2000. More recently, Clarke *et al.* (2006) developed models using trade records from commercial markets and estimated a median of 32-50 million sharks traded per year. This equates to total shark median biomass of 1.70 million tonnes per year (Clarke *et al.* 2006). It is important to note, however, that these values may be underestimated due to the general under reporting of chondrichthyan by-catch (Bonfil 1994; Walsh *et al.* 2002).

Recent studies have documented global declines of large coastal and pelagic sharks by over 50% (Baum *et al.* 2003; Ferretti *et al.* 2008) with reported stocks of certain species, for example the scalloped hammerhead shark (*Sphyrna lewini*), reduced by ~90% since the mid 1980s (Baum *et al.* 2003). To date, systematic declines in coastal and pelagic sharks have been documented for the Northwest Atlantic (Campana *et al.* 2002; Baum *et al.* 2003), Northwest Atlantic and Gulf of Mexico (Baum *et al.* 2005), the Eastern U.S. coast (Myers *et al.* 2007), the northern Gulf of Mexico (Baum & Myers 2004; Shepherd & Myers 2005); the Pacific Ocean (Ward & Myers 2005), the Mediterranean Sea (Ferretti *et al.* 2008) and the Great Barrier Reef (Robbins *et al.* 2006). It is possible that over exploitation of large predatory sharks stocks will result in a reduction in the maximum size of species. For the world's largest fish, the whale shark (*Rhincodon typus*), there has been a reported decline in abundance and size of individuals in Australia (Bradshaw *et al.* 2008), similar to previous findings for blue sharks (*Prionace glauca*) reported by Ward & Myers (2005) in the Pacific.

Additionally, Sminkey & Musick (1995) reported an increase in growth rate of small sandbar sharks (*Carcharhinus plumbeus*) after population depletion and Holden (1972, 1977) provided evidence of density-dependent changes in litter size of spiny dogfish (*Squalus acanthias*). Alternatively, Frisk *et al.* (2005) and McAuley *et al.* (2007) suggest that sharks may have limited capacity to alter fecundity, somatic growth and longevity in response to density-dependent mechanisms and conclude that management intervention is critical to prevent stock depletion.

#### Why are sharks vulnerable?

Life-history traits of sharks, such as late age at maturity coupled with slow growth, low fecundity and long inter-birth interval, result in a low resilience to fishing mortality when compared to most teleost and invertebrate species (Hoenig & Gruber 1990; Musick et al. 2000a; Frisk et al. 2001; Garcia et al. 2008) and are consequently more vulnerable to extinction (Myers & Worm 2005). Furthermore, chondrichthyes are generally constrained to 30% of our global oceans which consist of coastal areas, surface layers, ocean margin regions, around oceanic islands, mid-ocean ridges and seamounts which are typically the most heavily exploited by both commercial and artisanal fisheries (Priede et al. 2006). Species which demonstrate high site attachment, such as the reef sharks [e.g., whitetip reef (Triaenodon obesus), grey reef (Carcharhinus amblyrhynchos) and blacktip reef shark (Carcharhinus melanopterus)] may be depleted relatively quickly by targeted fisheries (Robbins et al. 2006; Heupel et al. 2009). These localised stock depletions in conjunction with low levels of population connectiveness can limit the regeneration capacity of these sub populations. These type of species are particularly vulnerable in the developing world where artisanal fisheries commonly are restricted to certain fishing grounds, i.e. coral reefs or seamounts, by the boats and fishing gear available (McVean et al. 2006; Henderson et al. 2007; Bizarro et al. 2009)

Larger more pelagic species may demonstrate wide roaming movement patterns with ontogenetic shifts in habitat use, such as trans-oceanic and trans-equatorial migrations of the white shark (*Carcharodon carcharias*) and the basking shark (*Cetorhinus maximus*), (Bonfil *et al.* 2005, Skomal *et al.* 2009, respectively). These species may be afforded protection in certain regions of their range, but may be vulnerable to

exploitation or bycatch by open ocean fisheries or in countries where protection is not in place and/or enforced. Trans-boundary movement of many large shark species therefore requires international collaborative efforts to implement management plans, which are often complex and impractical to enforce (Dulvy et al. 2008). Furthermore ontogeneticdriven shifts in habitat selection can result in large numbers of individual species aggregating at specific regions on a seasonal basis. Concentrated fishing on a site can thus deplete large segments of a particular size class and/or sex which are commonly the reproductively active components of the population (Klimley 1987; Weber & Fordham 1997). The scalloped hammerhead shark provides a classic case study. This species forms large schooling aggregations at seamounts and demonstrates high seasonal site fidelity (Klimley 1985,1987) and is considered one of the most exploited large shark species in recent stock assessment from commercial fisheries (Baum et al. 2003) and forms the bulk of catch in artisanal fisheries (McVean et al. 2006). Recent evidence for sexual segregation of large sharks in open water environments, for example the shortfin mako (Isurus oxyrinchus) in the southern Pacific Ocean also has implications for differential exploitation by spatially focused fisheries (Mucientes et al. 2009).

Over the past decade there has been an increase in the awareness of delineating and protecting shark nursery habitat. Many large sharks birth in coastal waters where the young reside for extended periods of time (Morrisey & Gruber 1993; Merson & Pratt 2001; Heupel & Heuter 2002; Dicken *et al.* 2007). Considering the low intrinsic rate of population increase of most sharks species coupled with the anthropogenic impacts in the coastal environment (coastal development, human recreational activities and commercial, artisanal and recreational fisheries), it is therefore critical that these regions are appropriately managed to ensure sustainability of viable mature populations (Bonfil 1997; Beck *et al.* 2001; Heupel *et al.* 2007). The occurrence of philopatric behaviour in sharks (Feldheim *et al.* 2002; Heuter *et al.* 2005) and long-term natal site fidelity of sub-adult sharks to their nursery grounds (Chapman *et al.* 2009) further highlights the importance of appropriate management, due to the predictability of easily accessible sites for exploitation.

#### Management of sharks: providing relevant data

The low economic value of sharks, when considering both flesh and fins, has up until recently resulted in their low priority designation in terms of conservation, research and management (Bonfil 1994; Walker 1998; Shotton 1999; Fowler *et al.* 2005; Lack & Sant 2006).

With the reported global decline of many shark species relative to pre-exploitation biomass (see Status of global sharks), concern has now been raised over their conservation, which has led to a call for the development of effective species-specific regional management plans (Barker & Schluessel 2005; Dulvy et al. 2008; Kinney & Simpfendorfer 2009). When considering the general lack of species-specific biological data, this has necessarily resulted in the adoption of teleost-based management approaches. This form of management is based on the protection of younger age classes. principally neonate and juvenile sharks, to increase recruit survival and therefore boost adult populations (Bonfil 1997; Heupel et al. 2007; Kinney & Simpfendorfer 2009). In terms of sharks stocks, this approach has been plausible due to the ease of generating reliable data for juvenile animals when considering the logistical difficulties of studying larger individuals. With increasing interest focused on the biology and ecology of juvenile life-stages, this has led to defining 'essential fish habitat' in the United States (NOAA 1996), and has resulted in large scale research efforts to define and accurately delineate shark nursery regions with a view to implementing a framework of marine protected areas (Bonfil 1999; Heupel et al. 2007; McCandless et al. 2008). Additionally, recent work has focused on clarifying what constitutes a shark nursery area (Heupel et al. 2007) to render the process of designating marine protected areas and enacting legislation feasible. This is required as historically shark nursery areas have been considered to encompass large areas of coastline which are impractical to protect and likely result in under protection, or diluted protection of core activity regions (Kinney & Simpfendorfer 2009). Furthermore, by providing a clear definition of shark nursery regions, scientists and managers are now able to standardise methods (Heupel et al. 2007). The approach of delineating shark nursery areas has been widely adopted on an international scale (Garla et al. 2006; Dicken et al. 2007; DeAngelis et al. 2008), but in many global locations there remains limited data on nursery ground

selection and species composition (Bass *et al.* 1973; Dudley *et al.* 2005; Heupel *et al.* 2007). Furthermore, advancing the knowledge of coastal movement patterns of juvenile sharks (for example, Dicken *et al.* 2007; Yeiser *et al.* 2008) is required for species such as the dusky shark (*Carcharhinus obscurus*) which have undergone systematic population declines across their global range (Musick *et al.* 1993; McAuley *et al.* 2007)

With an increased focus on the management of neonatal/juvenile sharks (McCandless *et al.* 2008), initial work has investigated the energetic condition of sharks (Rossouw 1983; Duncan & Holland 2006; Hoffmayer *et al.* 2006) and applied condition indices to examine survival and selection on life-history traits (DiBattista *et al.* 2007). Measures of health or energetic state of juvenile sharks in their nursery habitat facilitate a more detailed assessment of the effects of anthropogenic perturbations, nursery population density-dependent mechanisms and climate change in the coastal region and may also provide insights into both life-history strategies and ecological interactions (reviewed in Stevenson & Woods 2006). For teleost fish, traditional measures of condition [for example, condition factor (CF) and relative condition (Kn) and hepatosomatic index (HSI)] are widely applied as a management tool (Brown & Murphy 1991; Rätz & Lloret 2003; Rennie & Verdon 2008), but an assessment of their applicability to elasmobranchs has yet to be undertaken.

With the understanding that neonatal/juvenile sharks inhabiting coastal nursery areas are prone to high levels of exploitation and habitat degradation, it is also important that management recognises that nursery systems comprise only one segment of a species' life-history (Kinney & Simpfendorfer 2009). This is of particular importance for sharks when considering stock-recruitment curves (the relationship between the number of recruits and the number of breeders). For sharks, stock-recruitment curves generally have limited curvature, i.e. recruitment is strongly correlated to the size of the breeding population (Hoenig & Gruber 1990; Kinney & Simpfendorfer 2009). This is in contrast to teleost fish, which generally have high curvature, in other words high recruitment, even when breeding population size is low. Consequently for sharks, the effects of a depleted adult population will have direct consequences for the demographics of nursery bound animals (Simpfendorfer 1999; Kinney & Simpfendorfer 2009).

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Current knowledge of the effects of adult depletion on elasmobranch stock-recruitment curves remains limited. Demographic models are commonly applied to study elasmobranchs due to limited long-term data series available on catch, effort and abundance estimates of sharks, i.e. detailed fishery records which are required for quantitative stock assessments (Cortés 1998; Beerkircher et al. 2003; Romine et al. 2009). These types of models therefore provide the best available descriptors of the population being studied (Cailliet et al. 1992; Simpfendorfer 2005) and are critical for estimating the productivity of a species and understanding its susceptibility to exploitation (Cortés 2007). Life-history data required for accurate species-specific demographic analysis of shark populations includes accurate biological information on age at maturity, growth rate, longevity, reproduction and natural and age-related mortality rates (Cortés 1998). Additionally, for the female component of the population, there is a requirement for data on percent of mature females at age, the percentage of reproductively active females within a given year, birth frequency, sex ratio at birth and age-specific litter size (Cortés 1998).

Although demographic analysis continues to develop through incorporating more reliable data, and including more complex parameter estimates related to life-stage (Xiao 2002; Romine et al. 2009), areas of uncertainty remain. One area of particular interest is the reproductive output of the mature female component of the population (Cortés 1998). For teleost fish it is well established that age-dependent patterns of reproductive performance can influence population dynamics (Carr & Kaufman 2009) and consequently, neglecting the age structure of a population may overestimate viable larval production (Trippel et al. 1997; Scott et al. 1999). For sharks, demographic analysis generally accounts for mother size-litter size interactions, i.e. increasing litter size with maternal size (Allen & Cliff 2000; Dudley et al. 2005), but assumes that the rate of productivity of reproductively active females with size is constant, in other words, that sharks do not exhibit a decline in reproductive performance with age (size) (Cortés 1998). More detailed information on the reproductive potential of mature female sharks and the maternal and environmental phenotypic controls which exert control are required. For teleost fish there has also been considerable work undertaken to examine factors affecting survival rate of young, for example size at birth and/or size of maternal investment (Berkeley et al. 2004; Green & McCormick 2005; Gagliano &

McCormick 2007; Skibiel *et al.* 2009). To date there has been limited work investigating maternal investment in sharks.

Sharks, as apex predators, are predicted to play an important role as high order consumers in marine food webs (Cortés 1999). An accurate understanding of the trophic position (TP) of sharks is therefore required to assist in documenting species, gender and life-stage specific roles within both coastal and pelagic marine systems. Furthermore, with accurate knowledge of the TP of top marine predators, ecologists will be better equipped to refine food web models to examine; (i) multi-level trophic interactions (Hobson & Welch 1992; Revill *et al.* 2009), (ii) temporal and spatial variations in food web dynamics (Deegan & Garritt 1997; O'Reilly *et al.* 2002) and; (iii) to predict the effects of changes in predator abundance and/or removal (Lepak *et al.* 2006). For organisms which undergo a large change in body size with maturation, understanding species-specific ontogenetic variation in TP will also assist in improving community based stable isotope structuring (Layman *et al.* 2005)

Trophic position of marine predators has traditionally been calculated using standardised diet compositions (Cortés 1999), whereby diet-trophic position (herein referred to as  $TP_r$ ) is calculated using the relative contribution of prey types identified from stomach content analyses and the retrospective prey trophic levels (Pauly et al. 1998). This method has provided detailed insights into community structure (Zhang et al. 2007; Chassot et al. 2008), but is limited and may even be biased by multiple interacting factors including: high levels of stomach evacuation on capture, the inability to comprehensively sample animals across their complete geographic range (both spatially and temporally) and differences in digestibility of prey items. Accepting these limitations, the stable isotope ratios of carbon  $({}^{13}C/{}^{12}C)$  and nitrogen  $({}^{15}N/{}^{14}N)$  have been employed and widely accepted as a tool to elucidate aspects of the feeding, trophic and movement ecology of both marine and terrestrial organisms (Koch et al. 1995; Lewis et al. 2006; Menard et al. 2007; Caut et al. 2008a; Cherel et al. 2009a & b). This is based on the fact that the stable isotopes of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) in the tissues of a study animal are directly related through predictable transfer mechanisms to those of their prey and the environment they inhabit (Peterson & Fry 1987). Specifically, for carbon,  $\delta^{13}$ C values are generally considered to remain fairly constant. with enrichment values (referred to as fractionation, diet-tissue shift or discrimination factor) ranging from 0.1‰ to 1‰ between predator and prey tissue (DeNiro & Epstein 1978; Fry *et al.* 1984). This conservative enrichment is less reliable for trophic level studies, but has provided a valuable tool to track the movement and migration patterns of animals between regions with isotopically distinct baseline  $\delta^{13}$ C values (Burton & Koch 1999; Rubenstein *et al.* 2004; Ramos *et al.* 2009). In the case of nitrogen, the  $\delta^{15}$ N enrichment between predator and prey is more marked, typically ranging from 2‰ - 3.4‰ (Mingawa & Wada 1984; Caut *et al.* 2009) and it has therefore been commonly used as a predictor of trophic position (Pinnegar *et al.* 2002; Post 2002; Jennings & Mackinson 2003; herein referred to as TP<sub>SIA</sub>). The ability to undertake minor invasive sampling of animals for stable isotope analysis (i.e. through sampling feathers, blood, muscle biopsy) has therefore provided new insights in to the ecology of animals which were previously little understood (Cherel & Hobson 2005; Estrada *et al.* 2006).

With the observed growth in the application of stable isotopes in ecological studies over recent years, there have been questions raised over some of the basic assumptions (Gannes *et al.* 1997; Martinez del Rio *et al.* 2009). A principal concern has been the application of standard discrimination factors (typically 1‰ for  $\delta^{13}$ C and 3.4‰ for  $\delta^{15}$ N) to the study of a broad range of species (Caut *et al.* 2008b, 2009). A large body of experimental work now recognises that diet discrimination factors may be species-specific (Vanderklift & Ponsard 2003; Caut *et al.* 2009), tissue-specific (Pinnegar & Polunin 1999; MacNeil *et al.* 2006) and influenced by diet quality and environment (McCutchan *et al.* 2003; Barnes *et al.* 2007), growth rate (Trueman *et al.* 2005) and sample preparation (Sotiropoulos *et al.* 2004; Murray *et al.* 2006; Logan *et al.* 2008). Consequently it is now considered necessary to determine species-specific diet discrimination factors to provide confidence when interpreting stable isotope data (Martinez del Rio *et al.* 2009). This is also required if stable isotopes are to be viewed as an informative metric for management and conservation.

The management of shark populations is a developing science. Management approaches require species-specific data for all life-stages, but in many cases this is complicated by the logistical difficulties associated with studying large marine vertebrates and the ability to generate the required data. Archived data sets, such as those recorded from sharks caught in beach protection nets in South Africa, may provide a valuable tool to answer specific ecological and biological questions pertinent to management. New

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techniques for examining the ecology of sharks, for example, the application of stable isotope of carbon and nitrogen, may also provide an additional tool to advance the current knowledge base and improve management recommendations.

# Advancing the ecological knowledge base of the dusky shark (*Carcharhinus obscurus*) off Southern Africa.

With specific regard to dusky sharks off southern Africa, there is minimal knowledge regarding size-specific movement and feeding patterns and trophic ecology. Davies & Joubert (1967) and Bass et al. (1973) undertook preliminary analysis of tag-recapture data, but conclusions drawn were limited by the time period of data collection. The movement of dusky sharks, however, is of importance considering concerns over the level of recreational fishing mortality (Govender & Brinie 1997) and the developing commercial fishery for juvenile animals in KwaZulu-Natal (Dudley & Simpfendorfer 2006; Dudley pers. comm.). A better understanding of dusky shark seasonal movement patterns and core activity zones within South African coastal waters will therefore assist (i) regional management planning for defining sustainable fishing quotas and (ii) the current designation of marine protected areas. Initial work on growth rates of dusky sharks, a prerequisite for demographic analysis, were undertaken from vertebral samples (Natanson & Kohler 1996). This work in conjunction with studies in Australia (Simpfendorfer 2000; Simpfendorfer et al. 2002) demonstrated that this species has one of the lowest growth rates of all the elasmobranchs, further raising concern over the level of exploitation a population can sustain. Currently, there has been no continuation of this work in South African waters, but a rigorous analysis of a comprehensive tagrecapture data set following the techniques detailed by Simpfendorfer (2000) may provide important insights.

To date, maternal investment and maternal reproductive strategies in sharks in general have received limited attention. Considering the low fecundity of the dusky shark (3-16 pups; Clarke & Von Schmidt 1965; Dudley *et al.* 2005), late maturation (~20 years old; Natanson *et al.* 1995) and estimated gestation period of two years (Musick *et al.* 1993; Dudley *et al.* 2005), quantification of maternal investment/output is required for effective management of the mature component of the population. This is of particular

importance when considering the philopatric behaviour of mature females (Heuter *et al.* 2005) and possible exploitation of this mature population in other geographic localities outside of South Africa.

The dusky shark is one of the larger carcharhinids reaching a maximum total length of approximately 4 metres (Dudley *et al.* 2005). Cortés (1999) provided initial data on the trophic position of sharks using standardised stomach content analysis, but this approach is complicated by numerous biasing factors. More recently the stable isotopes of carbon and nitrogen ( $\delta^{13}$ C and  $\delta^{15}$ N) have provided a reliable tool to determine trophic interactions and to compliment traditional stomach content analysis (Hobson *et al.* 1995; Cherel *et al.* 2009b). For the dusky shark, which is typically a difficult to study animal in their natural environment, stable isotopes may afford a new technique to examine their ontogenetic movement, feeding and trophic ecology.

The overall objective of this thesis was to elucidate the above defined aspects of the ecology of the dusky shark (*Carcharhinus obscurus*) from South African waters through a combination of traditional techniques, using large archived data sets, and to examine the applicability of modern chemical tracer techniques (the stable isotopes of carbon,  $\delta^{13}$ C and nitrogen,  $\delta^{15}$ N). It is envisioned that the data and analysis provided will assist regional management planning, will contribute to general shark ecology and will provide direction for future research initiatives.

In writing this thesis, I intended each chapter to constitute a stand alone component suitable for publication.

The data/methods used and specific aims were therefore as follows:

#### 1. Long-term archived tag and release data set (1983 - 2006)

a) To identify core nursery habitat and to determine both spatial and temporal movements of dusky sharks, *C. obscurus*, in coastal waters of South Africa and;

**b**) To calculate growth rates of juvenile *C. obscurus* for comparison with preliminary estimates from vertebral counts.

#### 2. Morphometric archived data set (1982 - 2007)

a) To calculate multiple condition indices (morphometric and somatic) to examine seasonal trends in condition by life-stage and sex of *C. obscurus*;

**b)** To facilitate a comparison of the four calculated condition indices by life-stage/sex and examine the effect of increasing animal size and;

c) To examine the sensitivity of the condition indices and discuss why differences between condition indices occur.

#### 3. Reproductive archived data set (1978 -2008)

a) To determine if the reported enlarged liver of near-term shark pups represents a form of maternal investment for free swimming, neonatal *C. obscurus* and;

**b)** To examine the variability in maternal investment in near-term carcharhinid pups (*C. obscurus* and the spinner shark, *C. brevipinna*) to determine (i) what maternal and environmental effects might be driving this variability and (ii) if there is evidence of size-specific reproductive investment.

### 4. Validation of stable isotopes ( $\delta^{15}N$ and $\delta^{13}C$ ) in large sharks under semicontrolled conditions

a) To estimate diet-tissue discrimination factors ( $\Delta^{15}$ N and  $\Delta^{13}$ C) for white muscle tissue, liver, vertebral cartilage and various organs of large sharks (the sand tiger shark, *Carcharias taurus* and the lemon shark, *Negaprion brevirostris*) held under semi-controlled conditions.

## 5. Stomach content archived data set and stable isotope ( $\delta^{15}N$ and $\delta^{13}C$ ) analysis of muscle tissue sampled from wild caught sharks

a) To examine size- and gender-specific variations in diet and estimated trophic position of the scalloped hammerhead shark (*Sphyrna lewini*) and *C. obscurus* by combining stomach content analysis and stable isotope analysis ( $\delta^{15}$ N and  $\delta^{13}$ C) of muscle tissue.

**b)** To examine the utility of carbon isotopes ( $\delta^{13}$ C) as an indicator of coarse resolution ontogenetic movement profiles of *S. lewini* and *C. obscurus*.

### **CHAPTER TWO**

3

Nursery grounds, movement patterns and growth rates of dusky sharks, *Carcharhinus obscurus*: a long-term tag and release study in South African waters.

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#### ABSTRACT

Knowledge of movement patterns of sharks in coastal waters is critical for the structuring of regional management plans. Through a long-term tag-recapture programme, 9716 dusky sharks (Carcharhinus obscurus) were tagged and released along the east coast of South Africa. A total of 648 C. obscurus, principally small sharks (<100 cm PCL), were recaptured. Most recaptures were within 100-km of the tagging location in the nursery habitat in KwaZulu-Natal (KZN) with small scale movements occurring throughout the year. Forty-eight C. obscurus undertook movements >200 km that indicated a southerly migration between KZN and Eastern/Southern Cape (E/SC) between June and November. Seasonal northerly migrations were less well defined. The largest southerly and northerly movements were 1323 km and 1374 km, respectively. For sharks moving 1-100 km south from their tagging locality in KZN, an increase in displacement occurred between June and September identifying animals beginning their migration to the E/SC. With increasing displacement, there was also an increase in minimum swimming speed. Calculated growth rates of small sharks of 10.3 - 11.5 cm y<sup>-1</sup> were in agreement with current literature values. Established tag-recapture programmes provide an important tool in understanding the ecology of early life-stages of coastal shark species.

#### INTRODUCTION

Effective management of marine species in coastal waters requires a comprehensive understanding of how their habitat use facilitates future recruitment of individuals into exploited stocks (Beck *et al.* 2001). Nursery habitat identification, through knowledge of defined spatial and temporal movement patterns, in conjunction with life history data provide the principal information needed to elucidate habitat importance. Determination of this information for large predators is often dependent on long-term, large sample size data sets (Heupel *et al.* 2007).

Sharks commonly occupy the upper trophic positions within coastal food webs with neonates (young-of-year) and juvenile (>1y old) life-stages remaining resident in a core nursery region with a restricted home range for an extended period of time (Morrissey & Gruber 1993; Dicken *et al.* 2007). Certain species utilise multiple nursery habitats with movement between regions defined by season (Merson & Pratt 2001). Typically, philopatric behaviour of this type in the coastal zone renders species vulnerable to increased mortality rates from anthropogenic pressures associated with coastal development.

The dusky shark *Carcharhinus obscurus* (Lesueur, 1818) is a large member of the family Carcharhinidae with a global distribution in tropical, sub-tropical and temperate oceans (Compagno 1984). It has been described as one of the most K-selected of all elasmobranchs (Simpfendorfer *et al.* 2002) as a result of its slow growth rate (Natanson *et al.* 1995; Natanson & Kohler 1996; Simpfendorfer *et al.* 2002), late maturation (at *ca.* 20 years old; Natanson *et al.* 1995), prolonged gestation period (estimated to be 2 years; Musick *et al.* 1993; Dudley *et al.* 2005) and limited number of pups per litter (3-16; Clarke & Von Schmidt 1965; Dudley *et al.* 2005). A species with such conservative life history parameters has limited capacity to absorb systematic anthropogenic pressure. The rapid decline in the *C. obscurus* population in the Western Atlantic has been well documented (Musick *et al.* 1993). Off south-western Australia, neonate and juvenile *C. obscurus* have been targeted in coastal waters by a demersal gillnet fishery since the 1970s (McAuley *et al.* 2007). Although preliminary analysis deemed exploitation rates to be sustainable (Simpfendorfer 1999), a declining trend in catch rates has been

recently reported despite a 22% effort reduction in the targeted fishery (McAuley *et al.* 2007). Off South Africa there is a well-developed recreational shore fishery and commercial line fishery for small *C. obscurus* (Dudley & Simpfendorfer 2006). Previously, Govender & Birnie (1997) expressed concern over the level of recreational fishing mortality in KwaZulu-Natal (KZN). More recently, Pradervand *et al.* (2007) found a decreasing trend in catch per unit effort (CPUE) of *C. obscurus* in the KZN competitive shore fishery. In April 2005, the recreational fishery bag limit in KZN was reduced from 10 individuals per day to one due to an increased illegal trade in shark flesh.

A number of techniques are available to monitor the spatial and temporal movements of marine animals, including direct observation, the application of tags in mark-recapture programmes and the use of electronic/satellite tracking technology (reviewed in Block *et al.* 1998; Voegeli *et al.* 2001). Of these, conventional tag-recapture programmes for elasmobranchs are the most well-established and provide comprehensive long-term data sets with large numbers of recaptured individuals. This enables detailed quantitative analyses of movement and residency patterns of a species at the population level (Francis 1988a; Dicken *et al.* 2007). Conclusions drawn from long-term datasets also provide greater confidence in observations derived from short-term field studies. Furthermore, tag-recapture data enable the calculation/estimation of growth rates which are a key requisite for fisheries management and stock assessment.

The first tag-recapture study in South Africa was initiated in 1960 by the Oceanographic Research Institute (ORI) and targeted elasmobranch species inhabiting coastal and continental shelf waters. Preliminary qualitative data on the movement patterns of young *C. obscurus* were presented by Davies & Joubert (1967) and Bass *et al.* (1973). These studies suggested that KZN constituted the core nursery area for *C. obscurus* and that small animals undertook seasonal migrations to the Eastern Cape. These long distance movements were thought to be both coastal and driven by area-specific temperature profiles (Bass *et al.* 1973). Both recreational fisheries and catches in beach protection nets also indicate seasonal peaks in abundance of *C. obscurus* (Dudley *et al.* 2005).

Here we analyse tag-recapture data for *C. obscurus* collected between 1983 and 2006 by the ORI/World Wildlife Fund–South Africa (WWF-SA) tagging programme. The aims of the study are (1) to describe the selection of nursery habitat and provide information on both small and large scale spatial and temporal movements of *C. obscurus* within coastal waters of South Africa and (2) to determine growth estimates based on tag recapture data for juvenile animals from the region for comparison with published preliminary estimates from South Africa and with findings from other geographic localities.

#### **MATERIALS AND METHODS**

#### Location of study region

The study area extends from the Mozambique/South Africa border to Cape Point, comprising 2085-km of coastline bordering three geopolitical provinces (from north to south): KwaZulu-Natal, Eastern Cape and Western Cape (32°49'-18°29'E, 26°56'-33°56'S; Fig. 1). KwaZulu-Natal has a sub-tropical marine environment, Western Cape a warm temperate environment and Eastern Cape one that is intermediate between the other two. The study area is characterised by a narrow continental shelf and steep continental slope that is bordered at its eastern edge by the warm south-westward flowing Agulhas Current (Hutchings *et al.* 2002). To better define sea surface temperature regimes in coastal waters the study area was divided into five geographical regions: KwaZulu-Natal (KZN), Transkei (Trans), Eastern Cape (EC), Southern Cape (SC) and Western Cape (WC) (Fig. 1). The locations of initial tagging and subsequent recapture were reported using a 1-km interval grid section and recorded as the distance from the Mozambique/Tanzania border (Bullen & Mann 2006). To examine spatial movements along the South African coastline, the study area was divided into twenty-one 100-km coastal sections according to Dicken *et al.* (2007) (Fig. 1).

#### Tagging

The tag-recapture programme was initiated by ORI and WWF-SA in 1983 (Bullen & Mann 2006). The principal aim of this ongoing programme is to elucidate information on migration routes, nursery areas, growth rate, stock identity and population dynamics of important linefish species. Throughout the ORI/WWF-SA tagging programme, four

different tags have been applied to *C. obscurus*. A- and B-tags, (Hallprint, Victor Harbour, South Australia), consist of a plastic barb or stainless steel dart anchor, respectively, connected to a monofilament vinyl streamer. The tags are inserted into the dorsal musculature at the base of the first dorsal fin. The two tags



**Fig. 1.** Map of South Africa showing the 3 geopolitical provinces that encompass the study area, KwaZulu-Natal, Eastern Cape and Western Cape. The twenty-one 100-km coastal sections and 5 geographic regions (KwaZulu-Natal - KZN, Transkei - Trans, Eastern Cape - EC, Southern Cape - SC and Western Cape - WC) used to analyse the spatial and temporal movement patterns of *Carcharhinus obscurus* from tag-recapture data are illustrated. Temperature ranges for each region are presented according to Van der Elst (1981), Bolton & Stengenga (1987) and Beckley & Van Ballegooyen (1992).

differ in size (length x diameter; A- tag, 114 mm x 1.6 mm; B- tag, 161 mm x 2.3 mm) with A-tags being used for sharks >5 kg (the approximate weight of a newborn *C. obscurus*) and B-tags for large sharks in excess of 25kg. D-tags, which are similar in design to A-tags but have smaller streamers (85 mm x 1.6 mm), were also applied to

small sharks in this study. In the case of A-, B- and D-tags, individual tag codes were marked inside a transparent sheath. A fourth type, the C-tag, was locally designed (Durban, South Africa) and consists of two plastic discs which clip together *via* a small hole punctured through the base of the first dorsal fin. The tag was used for sharks of between 5 and 25 kg and tag information was printed on the outside surface of the disc. The use of this tag was discontinued in 2001 due to fin damage and excessive biofouling (Dicken *et al.* 2007).

On initial tagging or subsequent recapture of individual sharks the following information was recorded: date of capture/recapture, unique tag code, location (identified in the database by the distance codes from the Mozambique/Tanzania border), precaudal length (PCL) and total length (TL) with the caudal fin in its natural position. Sex was not recorded for any tagged or recaught *C. obscurus*. The ORI/WWF-SA tagging programme was ongoing throughout each year of the study period.

#### Analysis of spatial and temporal movement patterns

The tag-recapture data enabled the calculation of: number of days at liberty (t), minimum displacement distance ( $d_{min}$ , km) and minimum rate of movement (calculated as  $d_{min}/t$ , km day<sup>-1</sup>) between tag and recapture localities. The principal length measurement requested by the ORI tagging programme is PCL. Where\_fork length (FL) and TL measurements of sharks were provided, these were converted to PCL using the length equations given in Dudley *et al.* (2005).

To investigate spatial trends within the data set, *C. obscurus* were divided into three size classes: small sharks (<100 cm PCL), medium sharks (100 - 209 cm PCL) and large sharks ( $\geq$ 210 cm PCL). These size categories approximate to juvenile (reproductive organs undeveloped), adolescent (reproductive organs developing) and adult (sexually mature) sharks, respectively, accepting a degree of overlap at the lower end of the medium size category (Dudley *et al.* 2005). Since the orientation of the study region is in a roughly north-south direction, the data were divided into sharks undertaking northerly or southerly movements. To examine seasonal and spatial movement patterns, the data were subdivided into displacement categories defined as movements of 1 - 100 km, 101 - 200 km and >200 km. Movement patterns of individual *C. obscurus* were

then plotted by location (y-axis) and month of both capture and recapture (x-axis) constrained within a two-year period to account for animals that were tagged at the end of a year and recaught the following year. Sharks at liberty >24 months extend off the right side of the plot frame and hence these data were not appropriate for inferring seasonal movement patterns.

For statistical analyses the data were divided into two seasons, autumn/winter (season 1) and spring/summer (season 2), encompassing April to September and October to March, respectively. Each shark was assigned to either autumn/winter or spring/summer season according to the month in which the individual was tagged. Displacement data (km) were not normally distributed and were heteroscedastic. Therefore, the non-parametric Kruskal-Wallis H test (ANOVA on ranks) was used to examine trends in the direction of movement in relation to the size of displacement and season. Dunn's method was used to undertake post hoc pairwise comparisons of the data to identify significant factors (Zar 1999). To test for differences in rate of movement between months for sharks undertaking large southerly migrations, a one way analysis of variance (ANOVA) was performed on  $\log_{10}$ -transformed data. The transformed data were both normal and homoscedastic. Prior to conducting this analysis, C. obscurus recaught more than 365 days after tagging were discarded as these animals may have undertaken two migrations during their time at liberty. A Kruskal-Wallis H test (with Dunn's post hoc pairwise comparisons) was used to examine whether the rate of movement of sharks increased with increasing displacement category, which would imply that sharks moving greater distances were in the process of migrating.

#### **Growth models**

In many cases, length data for *C. obscurus* were not provided on recapture and thus these animals were omitted from growth analyses. Two independent growth models, those of Fabens (1965) and Francis (1988b) were applied to the data. The Fabens (1965) model involves the fitting of a non linear growth function to the data to calculate  $L_{\infty}$  and K. The value of  $t_0$  is then calculated by solving the function for T as detailed in Fabens (1965). The mean size at birth used for this calculation was 65.5 cm PCL (Dudley, unpubl. data). The Francis (1988b) model was fitted using GROTAG, a linear growth model adapted from Francis (1988b) using a solver-based spreadsheet in Excel

(Simpfendorfer 2000). GROTAG uses a maximum likelihood approach to fitting the growth model using one selected length. Since the bulk of the data were from juvenile sharks, growth can be considered to be a linear function. Four other parameters are encompassed within the model; the coefficient of growth variability (v), the mean (m) and standard deviation (s) of measurement error and outlier probability (p). The first step of the model uses only two parameters, the pre-selected length and s. Additional parameters are consecutively added to increase model complexity and a significant improvement in the model is determined using log likelihood ratio tests (Francis 1988b). Maximum longevity of the dusky shark was calculated using both Fabens (1965) and Ricker (1979) methods.

#### RESULTS

#### Annual and monthly tagging summary

Reliable data were available for a total of 9716 *C. obscurus*, tagged and released over the 24-year study period. KZN was the dominant tagging region with a mean ( $\pm$  1 SE) of 312.5  $\pm$  42.1 small sharks tagged per annum (Fig. 2a). A trend of decreasing number of animals tagged with increasing distance in a southerly direction along the South African coastline was observed except Trans and EC where the average number of tags applied per annum was similar (Fig. 2a). Corresponding temporal peaks in the numbers of animals tagged were evident across regions (Fig. 2a): for example KZN, Trans and EC in 1986/87, KZN, Trans and SC in 1992/93 and EC and SC in 2006. The majority of *C. obscurus* tagged in KZN and EC were small individuals whilst a larger proportion of medium-sized individuals were tagged and released in Trans, SC and WC (Fig. 2a).

The number of tags applied monthly peaked in November/December in all geographical areas except SC (Fig. 2b). In KZN the peak tagging period extended to January, with minimal variation in the numbers tagged throughout the rest of the year, a similar trend to Trans (Fig. 2b). By contrast, in SC and WC there were peaks in the number of tags applied in March and January-March, respectively, with no animals tagged between June and August (Fig. 2b). Similarly, in EC, few *C. obscurus* were tagged in the late autumn/winter period between June and August (Fig. 2b).



Fig. 2. Total number of *Carcharhinus obscurus* tagged and released (a) per year and (b) per month, between Kosi Bay and Cape Point as part of the ORI/WWF-SA shark tagging programme (1983 - 2006). Data are presented for the five geographical regions, KwaZulu-Natal (KZN), Transkei (Trans), Eastern Cape (EC), Southern Cape (SC) and Western Cape (WC).  $\blacksquare$  = small sharks (<100cm PCL),  $\blacksquare$  = medium-sized sharks (100-209cm PCL),  $\blacksquare$  = large sharks

 $(\geq 210 \text{ cm PCL})$ ,  $\Box$  = sharks with no length recorded on capture. The mean ( $\pm$  SE) number of tags applied to small (S) and medium (M) sharks (a) per year and (b) per month (over the 24 year period) for each region are shown.

#### Spatial and temporal analysis

#### **Tag-recapture summary**

Of the 9716 *C. obscurus* tagged and released, 8627 were classified as small, 879 as medium and 63 as large. A total of 591 small (6.9%), 32 medium (3.6%) and 1 large (1.6%) *C. obscurus* were recaptured. In addition, a further 147 individuals for which no length data were recorded on capture were tagged and released and 24 (16.3%) of these were subsequently recaptured.

Small *C. obscurus* were tagged throughout the study region with coastal section 4 being the dominant tagging region (48.7%) (Table 1). Of 591 small *C. obscurus* recaptured, 210 individuals showed zero movement between tagging and recapture with an average time at liberty of 69.7 days (95% CI = 39.4 - 100.0 days). The majority of these (n = 197) were tagged and recaptured in central-southern KZN between Richards Bay and Palm Beach (sections 3 - 6; Fig. 1). A further 12 small individuals that showed no movement were tagged between the Coega River mouth and Port Elizabeth (section 12 in EC) and one was tagged at Struis Bay (section 18 in SC; Fig. 1). Of the remaining 381 small *C. obscurus* that were recaptured, 314 moved 1 - 100 km and remained approximately within the coastal section of tagging, 24 moved 1 - 2 coastal sections (101 - 200 km) and 43 moved a distance larger than 2 coastal sections (>200 km). The average time at liberty for all small sharks that moved from their tagging location was 86.8 days (95% CI = 64.4 - 97.2 days, range 2 days to 7.7 years).

Medium-sized *C. obscurus* were also tagged throughout the study area with the exception of coastal section 19 (Table 1). Coastal sections 4 - 7 formed the dominant tagging area (68.7%). Of the total of 32 medium-sized *C. obscurus* recaptured, 14 did not move between tagging and recapture and spent an average of 85.9 days (95% CI = -5.4 - 177.2 days) at liberty. The majority of these (n = 10) were tagged and recaptured between Richards Bay and Mbizana in KZN (sections 3-6; Fig. 1) with 2 individuals tagged/recaptured in EC (sections 10 and 12; Fig. 1). Eleven of the medium-sized *C. obscurus* moved 1 - 100 km between tagging and recapture, 2 individuals moved 101 -

## Table 1. Carcharhinus obscurus tagged and recaptured between 1983 and 2006 for each 100-km coastal section.

Data are divided into small, medium and large sharks (PCL - cm) and those where no length data were recorded on capture.

	Number of C. obscurus tagged				Number of C. obscurus recaptured					
Coastal section	No length data	Small (<100)	Medium (100-209)	Large (≥210)	Total	No length data	Small (<100)	Medium (100-209)	Large (≥210)	Total
1	0	4	2	0	6	0	0	0	0	0
2	2	56	10	0	68	0	1	0	0	1
3	11	588	43	5	647	2	32	4	0	38
4	68	4202	139	8	4417	15	324	11	1	351
5	38	1763	122	10	1933	3	131	5	0	139
6	12	905	225	37	1179	3	60	5	0	68
7	3	375	118	0	496	0	6	1	0	7
8	1	31	61	0	93	0	1	0	0	1
9	1	24	7	0	32	0	2	0	0	2
10	1	17	4	0	22	0	2	1	0	3
11	1	7	5	0	13	0	0	1	0	1
12	4	314	49	0	367	0	20	3	0	23
13	1	122	31	0	154	0	4	0	0	4
14	1	23	11	0	35	0	0	0	0	0
15	1	26	4	0	31	0	0	0	0	0
16	2	108	25	0	135	1	4	1	0	6
17	0	21	10	0	31	0	1	0	0	1
18	0	32	7	3	42	0	2	0	0	2
19	0	2	0	0	2	0	0	0	0	0
20	0	1	1	0	2	0	0	0	0	0
21	0	6	5	0	11	0	1	0	0	1
Total	147	8627	879	63	9716	24	591	32	1	648

200 km and 5 individuals moved >200 km. The average time at liberty for all mediumsized sharks exhibiting movement was 203.4 days (95% CI = 88.8 - 318.0 days, range 1 day to 2.3 years).

A single large *C. obscurus* was tagged at Umdloti beach (section 4) in KZN and recaptured 7 days later 54 km to the north at Zinkwazi (section 4; Fig. 1). The large *C.* 

*obscurus* was excluded from subsequent analyses of movement patterns. The data on small and medium-sized *C. obscurus* were combined to examine a combination of spatial (1 - 100, 101 - 200 and > 200 km movement), seasonal [autumn/winter (season 1) *vs.* spring/summer (season 2)] and directional (north *vs.* south) patterns of displacement.

#### **Movement patterns**

The majority (n = 296; 91.1%) of the small and medium-sized *C. obscurus* moving 1 - 100 km to the north or south were tagged and recaptured along an approximate 200-km stretch of coastline in central-southern KZN between Sheffield Beach and Leisure Bay (sections 4 - 6; Figs. 1, 3a and 3b). Small-scale movements were recorded throughout the year (Figs. 3a and 3b). Over an annual cycle, a significant difference in the median displacement of sharks moving 1 - 100 km south was found [H<sub>11</sub> = 33.65, p < 0.001]. The largest movements occurred between July (season 1) and October (season 2) (Fig. 4). Eleven sharks exhibiting movements of 1 - 100 km north or south were tagged/recaptured in EC/SC regions, with nine (81.8%) of these sharks tagged during the spring/summer (season 2; Figs. 3a and 3b).

Small and medium-sized *C. obscurus* moving 101 - 200 km in a southerly direction were tagged principally in the central-southern KZN region between June (season 1) and November (season 2; Fig. 3c). Northerly movements of sharks within the 101 - 200 km displacement category were primarily undertaken in KZN and Trans with six sharks (75%) tagged in autumn/winter (season 1; Fig 3d).

The region between Mtunzini and Port Edward (sections 3 - 6; Fig. 3e) constituted the core tagging area for *C. obscurus* moving >200 km in a southerly direction (n = 29; 80.6%). Most of these sharks were tagged between June and October, from the middle of season 1 to the beginning of season 2. September and October (i.e. the boundary between season 1 and 2) were the most active months in terms of the numbers of *C. obscurus* moving >200 km north were more dispersed with the application of tags occurring over a 1594 km range between Macassar in WC and Umgababa in KZN (sections 21 - 5; Figs. 1 and 3f). Northward migrations of >200 km tended to occur in spring/summer (season 2) with 9 of the 12 sharks (75%) moving at this time (Fig. 3f).



Fig. 3. Displacement (km) by location and month of capture and recapture for small and medium-sized *Carcharhinus obscurus* moving (a) 1-100 km south, (b) 1-100 km north, (c) 101 – 200 km south, (d) 101 – 200 km north, (e) >200 km south and (f) >200 km north from their tagging location. — = *C. obscurus* starting movement in season 1; — = *C. obscurus* starting movement in season 2. The angle of the displacement line between tag and recapture months indicates the minimum speed of travel (km day<sup>-1</sup>). The shaded area demarcates season 1

(autumn/winter); the unshaded area demarcates season 2 (spring/summer). Horizontal lines indicate the borders between the 5 geographical regions (KZN – KwaZulu-Natal, Trans – Transkei, EC – Eastern Cape, SC – Southern Cape and WC – Western Cape).  $\times$  = marks the tag and recapture location and months for sharks migrating the furthest distance in both north and south directions.



Fig. 4. Box plots of the distance (km) travelled each month between tagging and recapture for small and medium-sized *Carcharhinus obscurus* combined moving 1-100 km south. The numbers represent the sample sizes for each month.

The largest recorded southerly displacement in this study was 1323 km for an individual measuring 103 cm PCL, tagged in September (towards the end of season 1) and recaptured the following February (season 2). The shark was at liberty for 143 days and travelled at a minimum speed of 8 km day<sup>-1</sup> between its tagging and recapture locations (Mtunzini, north of Durban in section 3 and Gouritz River, west of Mossel Bay in section 15, respectively) (Fig. 3e). The largest northerly displacement was 1374 km from Macassar, just east of Seal Island in April (section 21; season 1) to Port St Johns in July (section 7; season 1) (Fig. 3f). This shark, which measured 80 cm PCL, was at liberty for 82 days and travelled at a minimum speed of 16.8 km day<sup>-1</sup>, the highest speed reported for a northerly movement in this study.

The maximum rate of travel recorded during the tagging study was for an individual measuring 72 cm PCL, tagged at Orange Rocks (south of Durban; section 6) at the end of November (season 2) and recaptured in Jeffreys Bay (east of Seal Point; section 13) in mid December. The shark travelled a total of 713 km in 22 days at a minimum speed
of 32.4 km day<sup>-1</sup> (Figs. 1 and 3e). A second shark (75 cm PCL), tagged at St Georges Beach (north of Port Elizabeth; section 12) was recaptured 124 km further south at Cape St Francis (section 13) and travelled at a minimum speed of 31 km day<sup>-1</sup>. This movement took place in October (the beginning of season 2) over a liberty period of 4 days (Figs. 1 and 3c).

When the season of movement was defined by the month in which an individual shark was tagged, the median displacement distances for *C. obscurus* travelling north and south between seasons was significant [H<sub>3</sub> = 33.54, p < 0.0001]. The median distance travelled south by *C. obscurus* was significantly larger in the autumn/winter (season 1) than sharks moving south in the spring/summer (season 2). (Dunn's pairwise comparisons, p < 0.05).

To test for differences in the rate of movement of migrating sharks between the latter section of season 1 and the beginning of season 2, a one way analysis of variance was undertaken on *C. obscurus* rate of movement >200 km south between the months of June/July, August/September (season 1) and October/November (season 2). Two-month periods were selected to provide sufficient data for analysis. Accepting that swimming speed calculated from the tag-recapture data is a minimum as the shark may remain in a region before undertaking movement, the rates of movement during these three time periods were similar ( $F_{2,25} = 0.09$ , p = 0.91).

A trend of increasing minimum swimming speed with increasing displacement from tagging location was observed (Fig. 5). The rate of movement (km day<sup>-1</sup>) of *C. obscurus* for each of the displacement categories and for both directions of movement was significant [H<sub>5</sub> = 131.11 p < 0.0001]. The median speed (km day<sup>-1</sup>) of sharks travelling 101 - 200 km north and south was significantly higher than sharks travelling 1 - 100 km north and south (p < 0.05). The median speed of sharks moving 101 - 200 km and >200 km in either direction of movement was similar (p > 0.05).

# **Growth models**

Length was recorded for a total of 242 *C. obscurus* on capture and recapture. These sharks were at liberty for periods ranging between 1 and 1716 days (4.7 years); 11



Fig. 5. Box plots of calculated minimum speed (km day<sup>-1</sup>) of small and medium-sized *Carcharhinus obscurus* combined for the three displacement categories (1-100 km, 101-200 km and >200 km movement) showing (a) southerly and (b) northerly movements from their tagging location. The numbers represent the sample sizes for each month.

individuals were at liberty for more than 1 year, of which one animal was at liberty for 2 years and two animals for more than 3 years. Based on the criteria of Simpfendorfer (2000), only dusky sharks at liberty >60 days were used for the Fabens (1965) growth model. This reduced the data set from 242 sharks to 59 individuals with useable data. For the Francis (1988b) model, 187 sharks with reliable data were included in the analysis.

The Fabens (1965) method to estimate the von Bertalanffy parameters yielded values of K = 0.054 year <sup>-1</sup>,  $L_{\infty} = 310$  cm and  $t_0 = -4.4$  years ( $r^2 = 0.8$ ) based on  $L_{PCL}$ . Back-calculated maximum longevity for *C. obscurus* was 89.9 years based on Ricker (1979) and 64.2 years according to Fabens (1965). Log-likelihood ratio tests using the GROTAG growth model of Francis (1988b) found that a significant result was obtained at a pre-selected length of 75 cm and the inclusion of three model parameters; growth variability (v), and the mean (m) and standard deviation (s) of measurement error (Table 2). The mean annual growth rate of *C. obscurus* estimated by the method of Francis (1988b) was 10.3 cm yr<sup>-1</sup>, a value comparable to that produced by the Fabens (1965) model for mean growth ( $\pm 1$  SD) over the first 5 years (11.5  $\pm 0.4$  cm yr<sup>-1</sup>).

 Table 2. Growth parameter estimates and log likelihood values for four growth models

 fitted to Carcharhinus obscurus tag-recapture data using GROTAG (Francis 1988).

For a significant improvement in fit (p<0.05), the addition of one extra growth parameter must increase  $\lambda$  by 1.92 (Francis, 1988). Bold text indicates the best fit model.

		Model					
Model parameter	Unit	1	2	3	4		
Log Likelihood	λ	-460.72	-459.83	-457.26	-456.34		
Mean growth rate (cm y <sup>-1</sup> )	<b>g</b> 75	12.66	12.32	10.28	10.13		
Growth variability	v		0.86	0.87	0.99		
Measurement error	S	0.94	0.86	0.99	0.85		
	m			0.37	0.36		
Outliers	р				0.14		

# DISCUSSION

#### **Tag recapture rates**

When comparing the three tag-recapture studies undertaken on *C. obscurus* in South Africa, the total recapture rate of 6.7% in this study is higher than that reported by Bass *et al.* (1973) (4.5%) but far less than that of Davies & Joubert (1967) (44.3%). In the latter study however, a reward system for shark captures likely encouraged the repeated recapture of sharks thus elevating the recapture rate (Davies & Joubert 1967). One characteristic of all three studies is that catches consist of small/juvenile sharks with minimal catch of larger (sub-adult/adult) individuals in nearshore waters.

#### Annual and monthly tagging variation

The variation in the numbers of small animals tagged annually in KZN resembled the trend of catch rates of small *C. obscurus* in beach protection nets. For the period 1978 - 2004, NSB data showed peaks in catch rates for 1986 and 2004 (NSB unpubl. data). A major peak in the number of animals tagged in 1993, however, was not replicated in net catches. Given constant effort, peak periods in catch may reflect an abundance of neonatal sharks. Beach protection nets, accepting limited changes in net installations,

reflect standardised fishing effort per year. Tag-recapture data, in comparison, depend on recreational fishing effort and consequently are not standardised between years (Kohler & Turner 2001). The seasonal distribution of tags applied in KZN, with peak catch rates in December and January, was consistent with seasonal catch rates of small sharks in beach protection nets (Dudley *et al.* 2005), recreational fishing catches (Van der Elst 1979) and a previous mark-recapture study (Bass *et al.* 1973). The seasonal distribution of tags applied in EC corresponded with catch data for *C. obscurus* in the same region (Smale 1991).

#### Spatial and temporal movement

#### Testing the nursery concept

Heupel *et al.* (2007) defined a shark nursery area based on 3 criteria: (1) juvenile sharks are more commonly encountered in *the area* than in other areas, (2) sharks have a tendency to remain in *the area* or to return for extended periods and (3) *the area* is used repeatedly across years, whereas others are not. Small *C. obscurus* are present in KZN in large numbers relative to the four other geographical regions (criterion 1). *C. obscurus* have been present in the region on an annual basis for at least the last 24 years, fulfilling criterion 3 (tag-recapture data; Dudley *et al.* 2005). The movement data indicate that most of the small sharks in this region undertake small movements throughout the year (criterion no. 2). Furthermore, Dudley *et al.* (2005) reported catches of pregnant *C. obscurus* with near-term pups, postpartum *C. obscurus* and small *C. obscurus* (with varying levels of umbilical scar closure) in beach protection nets in KZN. Using the concept defined by Heupel *et al.* (2007) it can be confidently stated that KZN is the principal pupping and nursery area for *C. obscurus* on the South African east coast as proposed by previous work (Bass *et al.* 1973; Dudley *et al.* 2005).

# Defining the C. obscurus nursery

Our study found that a 400-km stretch of coastline from Long Beach in Zululand (section 3) to Port Grosvenor in the Transkei (the boundary of coastal sections 6 and 7) encompassed the largest number of small *C. obscurus* tagged. Coastal section 4, from the Tugela River on the KZN/Zululand border to Ansteys Beach, which encompasses the Durban area, was the principal tagging region, in agreement with Davies & Joubert (1967) and Bass *et al.* (1973) and the highest catch rate of small *C. obscurus* in NSB

nets (Dudley *et al.* 2005). This coastal area is likely the core activity zone for small *C. obscurus* within the KZN nursery. It is important to note, however, that inferences on movement and space utilisation of sharks by tag-recapture data may be biased by the actual fishing effort undertaken in a region (Kohler & Turner 2001; Dicken *et al.* 2006).

Branstetter (1990) and Heupel *et al.* (2007) stated that the selection of a shark nursery is a complex trade-off between the characteristics defining the nursery region (exposure/food availability/predation risk etc) and the life history traits of the species in question. By selecting an exposed coastal nursery, *C. obscurus* may be exerting a tradeoff between its large size at birth and its slow growth rate, small litter size, time to maturity and frequency of breeding. It is also possible that newborn *C. obscurus*, the largest shark of three sympatric species which overlap in nursery usage in KZN (Bass *et al.* 1973; Dudley *et al.* 2005), may use its increased size and thus manoeuvrability to rapidly expand its home range/nursery habitat to different spatial scales to reduce interspecific competition. A similar observation on four species of juvenile sharks overlapping in nursery habitat in Apalachicola Bay, Florida, was made by Bethea *et al.* (2004).

The occurrence of small sharks in all 5 geographical regions indicates that C. obscurus are capable of extensive movements in their early years. Furthermore, Smale (1991) reported that a few C. obscurus caught in EC had partially open umbilical scars suggesting the movement of neonatal animals born in KZN to EC in their first few weeks of life. The residency time of some neonatal C. obscurus in KZN may be very short and the expansion of home range rapid. However, whether all neonatal animals undertake such movement in their first year is unknown. Small C. obscurus demonstrated small-scale movements in KZN throughout the year. Consequently there may be two alternative life history strategies present in the population, with some animals, the 'nomads', undertaking large scale movement while others, the 'residents', remain in KZN. Heupel et al. (2006), examining the residency and movement patterns of bonnethead sharks (Sphyrna tiburo), found that most animals undertook small-scale movements but a few individuals migrated larger distances, suggesting similar alternative migratory behaviour. The apparent residency of small C. obscurus in KZN coastal waters throughout the year may, however, reflect a prolonged pupping period (Dudley et al. 2005).

#### Seasonal regulated movements

Seasonal changes in water temperature have been proposed as the possible factor driving the distribution and movement of *C. obscurus* along the South African coast. Davies & Joubert (1967) and Bass *et al.* (1973) reported that few small *C. obscurus* were tagged in Durban between February and April. The current study and that of Dudley *et al.* (2005) found that *C. obscurus* are present in KZN throughout the year. This indicates the sharks are able to tolerate seasonal temperatures ranges in that geographical region. Smale (1991) reported that *C. obscurus* were only present in the EC region between September and March - with a seasonal peak between December and February - which is consistent with this study. The low numbers of sharks tagged in EC and the absence of sharks in WC and SC during June, July and August do suggest that cooler winter temperatures may determine northeastward obligate movement.

#### The southerly migration

C. obscurus migrations from KZN occur between June and December, with migrants arriving in EC between October and March. In agreement with Davies & Joubert (1967) and Bass et al. (1973), most sharks start to migrate in September and October. Connell (1997) suggested that the South African sardine (Sardinops sagax) undertakes a return southerly migration in shallow coastal waters to EC past the Park Rynie area (KZN section 5) from October to December. Once the large predators feeding on the northerly moving sardine run have dispersed and predation risk is reduced, it is possible that small C. obscurus exploit this remnant food resource. In stomach content analysis of C. obscurus in EC, Smale (1991) identified Loligo squid as the most important food item. The EC/SC region is an important spawning area for chokka squid. Loligo vulgaris reynaudii, between September and December (Sauer et al. 1992, 2000). The southerly migration of C. obscurus may therefore be linked to this abundant food resource. Consequently, temperature may indirectly drive the southerly migration of C. obscurus from KZN through regulating the seasonality of both the return migration of S. sagax in coastal waters and squid spawning aggregations in EC. The region between KZN and the EC/SC border forms an important migration corridor for southward moving C. obscurus. In accordance with Davies & Joubert (1967) and Bass et al. (1973), there is some evidence of a return migration from EC to KZN but the data are insufficient to be conclusive.

The highest recorded speed of movement in this study of 32.4 km day<sup>-1</sup> is lower than that reported by Davies & Joubert (1967) of 58.9 km day<sup>-1</sup>. Kohler *et al.* (1998) reported a maximum speed for *C. obscurus* of 41.3 km day<sup>-1</sup>, although no indication of the size of animal is given. *C. obscurus* are clearly capable of moving large distances in a relatively short period of time. The increase in median swimming speed with increasing displacement suggests that animals in the displacement categories 101 - 200 km and >200 km were migrating and that movement is relatively fast and continuous. The largest displacement of *C. obscurus* moving 1 - 100 km south between July and October coincides with the timing of the migration to EC and suggests these *C. obscurus* may have been caught as they were beginning this movement.

#### Occurrence of medium-sized sharks

Medium-sized sharks were tagged and recaptured in all 5 geographical regions and exhibited a similar seasonal distribution to small *C. obscurus*. This suggests that both size classes of shark exhibit similar patterns of coastal movement and overlap in area usage on a seasonal basis along the entire study region.

The number of medium-sized sharks tagged relative to small sharks tagged was higher in Trans, EC, SC and WC than in KZN. Dudley *et al.* (2005) found that a low number of intermediate size animals were caught (100 - 139 cm; a similar size range to most of the medium-sized sharks in this study) in beach protection nets in KZN. The lower frequency of occurrence of medium-sized sharks in KZN may be indicative of a high mortality rate for *C. obscurus* in the region as previously described by Govender & Birnie (1997), or, as suggested by Dudley *et al.* (2005), it may mean that most of the medium-sized sharks move out of the study area.

#### **Occurrence** of large sharks

A single large C. obscurus was tagged and recaptured in KZN throughout the study period. Dudley *et al.* (2005) reported that most large C. obscurus captures in beach protection nets were in June/July directly associated with the annual sardine run. With the exception of large C. obscurus coastal movement influenced by the annual sardine run and near-term pregnant sharks which enter KZN costal waters for parturition

(Dudley *et al.* 2005), it would appear that large *C. obscurus* are not commonly present in KZN coastal waters. Spear fishermen report the occurrence of large *C. obscurus* throughout the year on the outer continental shelf/shelf edge in KZN (Heydorn, pers. comm.) in agreement with Compagno (1984).

#### **Growth models**

The Fabens (1965) model produced a reasonable estimate of  $L_{\infty}$  (310 cm PCL), when compared to the largest *C. obscurus* caught in the NSB nets (284 cm PCL; Dudley *et al.* 2005) and the preliminary value calculated from vertebral ring counts (309 cm PCL; Natanson & Kohler 1996). The calculated K value (0.054) for this study was within the range of values reported for *C. obscurus* in the South West Indian Ocean (0.047; Natanson & Kohler 1996), North East Atlantic (0.034 - 0.062; Natanson *et al.* 1995) and the South East Indian Ocean (0.031 - non-injected animals; Simpfendorfer 2000; 0.043 - 0.045; Simpfendorfer *et al.* 2002). The annual growth rates for small sharks, 10.3 - 11.5 cm y<sup>-1</sup>, calculated in this study using Fabens (1965) and Francis (1988b) models, are in agreement with estimates for juvenile *C. obscurus* by Simpfendorfer (2000, 2002), and with estimates calculated for all size classes by Natanson *et al.* (1995) and Natanson & Kohler (1996).

Within the Francis (1988b) model, the value for growth variability, calculated using GROTAG, was the highest recorded in comparison with eight other studies (Table 3). It is possible that in South Africa the migration to EC may lead to annual growth variation. Growth variability may also be a result of variable life strategies exhibited by juvenile *C. obscurus*, whereby some animals remain in KZN waters while others migrate southwards to EC. Simpfendorfer (2000) suggested that juvenile *C. obscurus* off south-western Australia may exhibit seasonal growth variation regulated by water temperature. Growth variability may be more apparent in the model when examining only one size class of shark, for example, juveniles. Mean measurement error (m) and the standard deviation of measurement error (s) were low when compared to other growth studies (Table 3). This provides confidence that the high growth variability is a true response and not a combined effect of measurement error and growth variation as

# Table 3. Growth model parameter estimates for 7 species of shark, from models fitted to tag-recapture data using GROTAG (Francis 1988b).

All models included were best fit models and deemed significant through log likelihood ratio tests.

		This study	Simpfendorfer (2000) <sup>a</sup>	Francis & Francis (1992) <sup>b</sup>	Francis & Mulligan	Simpfendorfer et al. (2000)	Natanson et al. (2002)	Skomal <i>et</i> <i>al.</i> (2003)	Natanson <i>et al.</i> (2006)
Study region		SE Africa	SW Australia	New Zealand	New Zealand	SW Australia	NW Atlantic	N Atlantic	N Atlantic
	Model parameter								
Species name		Carcharhinus obscurus	Carcharhinus obscurus	Mustelus lenticulatus	Galeorhinus galeus	Furgaleus macki	Lamna nasus	Prionace glauca	Isurus oxyrinchus
Common		dusky	dusky	rig	school	whiskery	porbeagle	blue	shortfin mako
name									
Sample size	n	187	241	-	77	29	76	43	22
Mean growth	$\mathbf{g}_1$	10.28	9.23	5.87/3.67	12.46	7.05	19.21	44.18	47.5
rate	$\mathbf{g}_2$			2.52/1.73	0.91	2.04	9.52	25.46	29.5
Growth variability	v	0.87	0.34	0,58	0.00	0.13		0.27	
Measurement error	S	0.99	2.35	1.57	7.19	1.18	9.43	5.39	11.01
Outliers	m P	0.37	0.69		-	0.14	1.0 0.07	-2.03	

<sup>a</sup> For Simpfendorfer (2000) the GROTAG results presented are for all sharks. <sup>b</sup> For Francis & Francis (1992) mean growth rate at 70cm and 100 cm for both female/male sharks are present.

suggested by Francis & Mulligan (1998). The back-calculated maximum age for *C. obscurus* of 64 years is within the range of Simpfendorfer *et al.* (2002) but lower than that of Natanson & Kohler (1996). Simpfendorfer *et al.* (2002) pointed out that an estimated theoretical maximum age of 60-64 years may be too high because calculated  $L_{\infty}$  was much higher than observed maximum length of sharks sampled. In the current study the  $L_{\infty}$  value is only slightly higher than the maximum length reported, as would be expected (Hoenig 1979). This may suggest that the maximum age of 64 years is a reasonable estimation and supports the evidence that *C. obscurus* is a slow-growing, long-lived species.

# CONCLUSIONS

The results of an extensive tagging study show that small C. obscurus utilise the centralsouthern region of KZN as their core nursery area and undertake large southerly migrations, using a defined migration corridor. This migration may be linked to migrating S. sagax and the mass spawning of Loligo sp. in the EC/SC region. As previously recognised, seasonal sea surface temperature may stimulate the movement of C. obscurus off the east coast of South Africa, but it appears that only obligate movement may be northeastward away from Cape waters prior to winter. Small C. obscurus may remain in KZN waters throughout the year. Whether there is a division in the life history strategies adopted by neonatal/juvenile C. obscurus in the region, with some migrating 'nomadic' sharks and other 'resident' individuals, or if apparent residency in KZN is an artefact of the prolonged pupping period, remains unknown. Small C. obscurus utilise large coastal areas and are clearly capable of expanding their home range very quickly after birth. By utilising an open water nursery and taking advantage of their large size at birth, C. obscurus have developed strategies to enhance survival through coastal migrations likely to benefit the growth of the animal through exploitation of abundant food resources and to offset interspecific competition within the core nursery. The low numbers of medium-sized sharks tagged relative to small individuals may suggest these animals occur in regions not comprehensively sampled in the current study or that mortality rates are high in the region. Given the K-selected life history characteristics of this species and population declines in certain parts of the

world, the movements of these animals require further investigation, possibly using satellite/archival telemetry.

# **CHAPTER THREE**

The condition conundrum: application of multiple condition

indices to the dusky shark, Carcharhinus obscurus

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40

# ABSTRACT

Measuring fish condition has become a standard practice in the management of fishes. both at the individual and population level. The comparative application of several condition indices to sharks, however, has not yet received a rigorous evaluation. Data for a total of 2120 dusky sharks, Carcharhinus obscurus (Lesueur, 1818), ranging in size from newborns [ $\leq$  79 cm precaudal length, (PCL)] to mature adults ( $\geq$  210 cm PCL) were used to calculate seasonal trends in condition and to facilitate index comparisons. Four commonly used condition measures were selected, including a somatic measure, hepatosomatic index (HSI) and three morphometric measures, condition factor (CF), relative condition (Kn) and residual condition (Rr PCL). The effect of month was significant for most condition analyses by size class, sex and reproductive state. HSI was found to be the most sensitive index and rapid indicator of condition, but its appropriate use requires the disaggregation of data by clearly defined life stages and reproductive states. The relatively large liver size of neonates  $(0.58 \pm 0.01 \text{ kg}; \text{ range};$ 0.10 - max. 1.70 kg) and the relatively small liver size of pregnant and postpartum females  $(21.20 \pm 0.55 \text{ and } 25.90 \pm 1.00, \text{ respectively; range: } 5.70 - 70.00 \text{ kg})$  may otherwise bias interpretations of seasonal variations in condition. HSI was also affected by increasing size of animal, which confounds inter-size-class comparisons and may require the further division of life-stage data into additional size classes. The results of the three morphometric measures were comparable but were not correlated with HSI. CF, Kn and Rr PCL lagged behind HSI, were unable to differentiate between neonate and juvenile animals and were insensitive to short term variations in condition. The effect of increasing animal size did not affect calculated CF and Rr PCL but Kn demonstrated a negative correlation. The fact that large predators ( $\geq 210$  cm PCL) may consume large volumes of food in a single feeding event (max. stomach content mass recorded = 36.19kg) was identified as a possible complicating factor in interpreting condition indices.

# INTRODUCTION

The use of condition indices to assess 'health' or 'energetic state' has been applied widely to both marine and terrestrial animals. In vertebrates, these condition indices have been used to assess the effects of environmental threats (pollution and climate change), life history strategies (migration and reproduction) and ecological interactions (diet, competitive interactions and parasite load) on animal condition (reviewed in Stevenson & Wood 2006). Condition indices typically are calculated from morphological and somatic measures of the animal (e.g. Fulton's condition factor, relative condition and relative weight, and hepatosomatic and gonadosomatic index, respectively) and the biochemistry of body fluids and tissues or total body composition (e.g. protein and lipid content). Modern thought is that the biochemical measures should correlate and complement the more traditional morphological and somatic approaches. Within the spectrum of traditional condition indices, particularly for fishes, debate exists over the appropriate condition index to use and the type of analysis to be undertaken (Bolger & Connolly 1989; Springer *et al.* 1990; Jakob *et al.* 1996; Pope & Kruse 2007).

Sharks, members of the class Chondrichthyes, have received limited attention with regard to the application of condition indices. Under most circumstances the required data for large and/or highly migratory species are difficult to obtain restricting comparative condition analyses. Considering the volume of published literature on the use of condition indices for the management of teleost fishes, coupled with the important biological and ecological information that can be elucidated through this approach, it is appropriate to address the subject for this large group of fishes.

The most widely applied measure of condition in sharks is the hepatosomatic index (HSI), which expresses liver weight relative to body weight, and is often used as an estimate of the energy status of a fish (Pope & Kruse 2007). The livers of sharks can constitute up to 25% of body mass and are principally formed of lipids (Baldridge 1970, 1972; Watson & Dickson 2001). Springer (1967) first suggested that they act as a food store or energy resource; however, research has primarily focused on its function in buoyancy control and in providing hydrostatic lift (Bone & Roberts 1969; Baldridge,

1970, 1972). More recently, Hoffmayer *et al.* (2006) examined the energy storage capability of the liver of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, using HSI and found that there was a seasonal pattern in the energetic condition of the species. Consequently it would appear that the liver may act as an indicator of the state of balance between food consumed and food required for body metabolism (King 1984). In sharks, HSI has been applied in the study of shark migration ecology (Rossouw 1983; King 1984) and, most frequently, in studies examining the reproductive development/state of sharks (Allen & Cliff 2000; de Bruyn *et al.* 2005; Dudley *et al.* 2005; Lucifora *et al.* 2005).

Morphometric measures of condition, such as Fulton's condition factor (Ricker 1975; Nash *et al.* 2006) and relative condition (Le Cren 1951), measure individual variation from an expected weight at a given length. Fulton's condition index has been used to examine seasonal condition patterns in sharks (Atlantic sharpnose shark, Parsons & Hoffmayer 2005; scalloped hammerhead shark, *Sphyrna lewini*, Duncan & Holland 2006) and relative condition has been correlated with survival rates of newborn/juvenile lemon sharks, *Negaprion brevirostris* (Dibattista *et al.* 2007). The application of morphometric condition indices to sharks, however, has been limited.

A rigorous comparative approach is required to determine the suitability of the various traditional condition indices when applied to sharks. For teleost fishes this has been resolved by calculating multiple condition indices and performing inter-condition index comparisons (Bolger & Connolly 1989; Jakob *et al.* 1996). This paper reports a similar comparison of condition indices for sharks by making use of a comprehensive long-term data set on the dusky shark, *Carcharhinus obscurus*, caught in beach protection nets in KwaZulu-Natal (KZN), South Africa. The objectives of the study were to calculate three standard morphometric indices and a somatic index for dusky sharks to (1) examine seasonal trends in condition by life-stage, (2) to facilitate a comparison of the four calculated condition indices by life-stage and examine the effect of increasing animal size, (3) to examine the sensitivity of the condition indices and (4) to discuss why differences between condition indices occur.

# **MATERIALS AND METHODS**

#### **Study location**

Data for the dusky shark were accessed from the KwaZulu-Natal Sharks Board (KZNSB) archived database (1982-2007). All sharks were caught in beach protection nets set at popular bathing localities along the coastline of KwaZulu-Natal. Only sharks in good condition were returned to the KZNSB laboratory for dissection and included in the analyses. For specific details of the net locations, net types, KZNSB service operations, and modifications in net installations for the study period, refer to Cliff *et al.* (1988) and Dudley *et al.* (2005). On an annual basis, sea surface temperatures in KZN range from 21 - 27°C (Van der Elst 1981). The cooler autumn/winter season and warmer spring/summer season are defined by the months of April to September and October to March, respectively.

The dusky shark was selected as the model species for this study due to its high catch rate (20% of total annual shark catch) and the sampling of all size classes (Dudley *et al.* 2005). Sufficient data were available to allow detailed statistical analyses to be conducted in relation to changes in condition indices by season, life-stage (size), sex and reproductive state.

The KZN coast is a nursery ground for dusky sharks (Bass *et al.* 1973; Dudley *et al.* 2005) with juveniles remaining in the region until they attain a size of approximately 100cm precaudal length (PCL). As a result, juveniles (including neonates) are caught in the nets throughout the year. Larger dusky sharks – sub-adults and adults of both sexes - are caught less frequently, however, both pregnant and postpartum animals are sampled. June and July form the peak catch period for both sub-adult and adult animals, corresponding with the migration of sardines, *Sardinops sagax*, along the KZN coastline (Armstrong *et al.* 1991). For details on the geographic and spatial distribution of dusky shark catches in KZN waters refer to Dudley *et al.* (2005).

The data required for the condition analyses included sex, date of capture, capture location, precaudal length (PCL, cm), total body mass (kg), total liver mass (kg), stomach mass (kg), total pup mass (kg) (for pregnant sharks), reproductive state and the

seasonal catch of postpartum dusky sharks. PCL was measured as the straight line distance from the tip of the snout to the precaudal notch as defined by Dudley *et al.* (2005). Visual assessment of maturity and reproductive state were based on the criteria defined by Bass *et al.* (1973).

#### Data division by size class

Prior to analyses, the data were divided into size classes as recommended by Pope & Kruse (2007). Three size classes were selected: small < 100 cm PCL, medium 100-209 cm PCL and large  $\geq 210$  cm PCL, approximating maturity states. The small sharks were further subdivided into  $\leq$  79.0 cm PCL (assumed to be neonates after Dudley et al. (2005)) and 79.1 - 99.9 cm PCL (assumed to be > 1 yr old), hereafter referred to as neonates and juveniles, respectively. Small male and female sharks were combined since they inhabit the same nursery region. Because larger animals may pursue distinct sex-specific life strategies, medium and large sharks were divided by sex and large sharks further subdivided by reproductive state. Large males ( $\geq 210$  cm PCL) included immature (stage 2), and mature (stage 3) individuals, as well as individuals with regressed testes (stage 6). No males considered to be in mating condition were recorded in the data. All large males were therefore amalgamated into one data set. Large females included immature (stage 2), mature but inactive or non-gravid (stage 3), pregnant (stage 5) and postpartum (stage 6) animals. Given the large energetic demand on pregnant sharks during the gestation phase, pregnant and postpartum animals were isolated from the large female data set  $(\geq 210 \text{ cm PCL})$  and presented independently.

#### **Condition indices**

For each size class, four commonly used condition indices were calculated. Hepatosomatic index (HSI), the most widely used somatic measure (Stevenson & Woods 2006; Pope & Kruse 2007), was calculated using:

$$HSI = [liver mass (kg) / total body mass (kg)] \times 100$$
(1)

The morphometric indices of condition assessed in this study included condition factor (Ricker 1975; Nash *et al.* 2006), relative condition (Le Cren 1951) and residual condition (Fechhelm *et al.* 1995). Fulton's condition factor (CF), was calculated using:

$$CF = [total body mass (kg) / precaudal length (cm)3] x 105$$
 (2)

whereby the linear measure of length is raised to the power of three, assuming that mass and the linear dimension increase isometrically. Relative condition (Kn) was calculated using:

$$Kn = [total body mass (kg) x precaudal length (cm)^{-b} x 10^{5}$$
(3)

where b = slope of a regression plot of  $log_{10}$  mass vs.  $log_{10}$  PCL. In this study a value for b of 3.04 was used (n = 2627, p < 0.001: Dudley *et al.* 2005). To determine the residual morphometric condition index, a linear least squares regression model was applied to the  $log_{10}$  transformed length and mass data (Fechhelm *et al.* 1995; Schulte-Hostedde *et al.* 2005). Individual sharks were then assigned their residual value of predicted body mass (Rr <sub>PCL</sub>) from the fitted model for all subsequent analyses. Rr <sub>PCL</sub> measures the variability in the data after variation tied to increasing size/mass of an animal has been removed. To standardize pregnant female data prior to the calculation of all condition indices, actual body mass was calculated by subtracting the total mass of the near term pups. For the selected condition indices, total body mass included viscera and stomach contents, in line with standard practices (Bolger & Connolly 1989). Monthly mean somatic and morphometric condition index values ( $\pm 1$  SE) were then plotted for each size class and sex/reproductive state.

#### Statistical analysis

HSI, CF, Kn and Rr  $_{PCL}$  were each tested using a two factor ANOVA in which each condition index was entered as the response variable and month and size class (for small - neonates and juveniles), month and sex (for medium and large sharks) and month and reproductive state (for large reproductively active pregnant and postpartum sharks) entered as treatments. Respective interaction terms were included in all models. Because the sample sizes were unbalanced, adjusted Bonferroni pairwise comparisons were undertaken to test for monthly variations in condition indices within and between defined treatments. To examine for possible length related bias in HSI, CF, Kn and Rr  $_{PCL}$  within the medium and large size classes, a two factor ANOVA was performed with

PCL as the response variable and the above prescribed treatments per size class. For neonates and juveniles, a one factor ANOVA tested each size class with PCL as the response variable and month as the treatment. The testing for the assumptions of the analysis of variance was undertaken as recommended by Underwood (1997). For data that did not meet the requirement of normality and homogeneity of variance, the graphical approach recommended by McGuiness (2002) was adopted prior to accepting non-parametric tests. Observation of residual vs. fitted plots/variance vs. mean plots indicated adequate conformity to homoscedasticity. To test for differences in condition between reproductively inactive large sharks and pregnant/postpartum animals a two sample t-test was undertaken for each condition index. To examine the influence of increasing size on the condition indices and possible complications in interpreting and comparing the results between size classes, each shark's calculated value for each condition index was plotted as a function of PCL (Colautti et al. 2006). Each condition index was then correlated with PCL using Pearson's r (Jakob et al. 1996). To investigate the relationship between the condition indices, Pearson correlation coefficients were calculated for all pairwise combinations of the four condition indices. The level of statistical significance for all the above tests was set at  $\dot{\alpha} = 0.05$ .

# RESULTS

A total of 2120 dusky sharks (1384 females and 736 males) were used to calculate the condition indices. Sample sizes, mean ( $\pm$  1 SE) and range of PCL, total body mass, liver mass and stomach mass for each size class are presented in Table 1. On average stomach mass accounted for 0.75% of the total body mass (Table 1). A least squares regression of log<sub>10</sub> PCL vs. log<sub>10</sub> total body mass for all data resulted in a significant relationship from which residual values for Rr <sub>PCL</sub> for each shark were calculated (Fig. 1). The sample sizes of sharks per month for each size class/sex and reproductive state are detailed in Figures 2b-5b.

#### Comparison of condition indices by size class

#### Small neonate and juvenile sharks

The seasonal trend of HSI was markedly different for neonates and juveniles (Fig. 2a, Table 2). HSI of the neonates increased from April to a peak in June-August followed

**Table 1.** Carcharhinus obscurus. Precaudal length (PCL), body mass, liver mass and stomach mass for each predetermined size classification (Mean  $\pm$  SE). Values in brackets indicate min. and max. range. Codes determining maturity/reproductive state: (1) immature, (2) adolescent, (3) mature but inactive; for male (6) regressed testis; for female (5) pregnant and (6) postpartum (after Bass et al. 1973). The mass of pregnant sharks is that minus total pup mass.

Size class PCL (cm)	Sex	Maturity/ reproductive state	n	Length PCL (cm)	Body mass (kg)	Liver mass (kg)	Stomach mass (kg)
Small neonate		1	469	$71.8 \pm 0.2$ (59.0 - 79.0)	$5.3 \pm < 0.1$ (2.7 - 10.0)	$0.58 \pm 0.01$ (0.10 - 1.70)	$0.03 \pm 0.04$ (0 - 0.60)
Small juvenile		1	129	87.9 ± 0.5 (79.4 – 99.4)	$9.6 \pm 0.2$ (6.0 - 16.0)	$0.58 \pm 0.02$ (0.15 - 1.60)	$0.13 \pm 0.02$ (0 - 1.09)
Medium 100-129	Male	1 & 2	374	$159.7 \pm 1.2$ (101.2 - 209.0)	$63.5 \pm 1.5$ (10.7 - 214.0)	$6.17 \pm 0.21$ (0.42 - 24.00)	$0.31 \pm 0.05$ (0 - 8.14)
	Female	1 & 2	584	$161.4 \pm 1.1$ (101.0 - 209.1)	$67.3 \pm 1.3$ (12.0 - 182.0)	$6.77 \pm 0.19$ (0.50 - 25.00)	$0.31 \pm 0.05$ (0 - 17.12)
Large inactive $\geq 210$	Male	2,3 & 6	106	$238.7 \pm 0.9$ (210.0 - 256.0	$198.7 \pm 2.6 (104.0 - 365.0)$	$27.54 \pm 0.86$ (5.90 - 46.00)	$2.48 \pm 0.61$ (0 - 29.80)
	Female	2 & 3	104	$238.2 \pm 1.6$ (210.0 - 275.0)	$195.3 \pm 4.8$ (104.0 - 365.0)	25.41 ± 1.25 (7.30 – 66.00)	$1.79 \pm 0.49$ (0 - 30.00)
Large active ≥210	Female	5	208	$253.4 \pm 0.8$ (220.0 - 279.0)	$226.2 \pm 3.2$ (122.2 - 349.2)	$21.14 \pm 0.55$ (5.70 - 56.00)	$0.31 \pm 0.10$ (0 - 11.10)
	Female	6	146	$255.2 \pm 1.0$ (224.0 - 276.0)	$228.8 \pm 3.4$ (108 - 350.0)	$25.90 \pm 1.00$ (6.40 - 70.00)	$1.96 \pm 0.46$ (0 - 36.19)
Total			2120				

by a gradual decline throughout the rest of the year. A significant effect of month was detected (Table 2, Fig 2a). The peak in neonate HSI correlated with the highest catch rate of postpartum sharks (Fig. 2a). For the juveniles, HSI remained constant (Fig 2a). Direct month to month comparisons between the two size classes found significant differences in HSI for 7 of the 12 months (Table 3, grey section). The trends for all three morphometric indices, CF, Kn and Rr <sub>PCL</sub> were similar on an annual cycle for neonates and juveniles (Fig. 2c-2e). In contrast to HSI, no significant difference was detected in direct month to month comparisons of CF, Kn and Rr <sub>PCL</sub> between the two size classes. The monthly mean PCL of neonates was variable throughout the year in contrast to juveniles where no significant effect of PCL was observed (F = 2.62, df = 11, p < 0.003 and F = 1.81, df = 11, p = 0.06 respectively, Fig. 2b).



**Fig. 1.** *Carcharhinus obscurus*. Least squares linear regression of  $log_{10}$  total body mass against  $log_{10}$  precaudal length for all sharks sampled between 1982 and 2007.

#### **Medium sharks**

The mean HSI value of male medium sized sharks was significantly different from females (Table 2). HSI of both sexes peaked in June and July but was relatively constant throughout the rest of the year (Fig. 3a). There was an effect of month for both sexes with significantly higher HSI values in June and July than all other months (Table 2, Fig. 3a). In the two periods, February/March and September/October, males showed a



Fig. 2. Carcharhinus obscurus. Monthly measures of condition of small neonate and juvenile sharks calculated using a) Hepatosomatic index (HSI) including the seasonal catch of postpartum females (bars), c) Condition factor (CF), d) Relative condition (Kn) and e) Residuals from the regression of log<sub>10</sub> total body mass vs. log<sub>10</sub> precaudal length (Rr <sub>PCL</sub>). Monthly values of precaudal length (PCL) are shown in (b). Data points represent mean values ( $\pm 1$  SE) for each month for males and females combined into two size classes ( $---= \pm 79.0$  cm juvenile). Values displayed above a given data point indicate the month(s) for which pairwise tests revealed significant differences with the given month. Number of observations (n) for each month is shown in (b) for both size classes of shark.

**Table 2.** *Carcharhinus obscurus*. Results of a two factor ANOVA to test for effects of month and size class<sup>1</sup> ( $\leq 79/\leq 100$  cm PCL) for small neonate and juvenile sharks; month and sex<sup>2</sup> for medium (100-209 cm PCL) and large reproductively inactive sharks ( $\geq 210$  cm PCL); and month and reproductive state<sup>3</sup> for reproductively active pregnant and postpartum sharks ( $\geq 210$  cm PCL) for each of the condition indices. All model interactions are included. HSI – Hepatosomatic index, CF – Condition factor, Kn – Relative condition and Rr <sub>PCL</sub> – Residuals from the regression of  $log_{10}$  body mass *vs.*  $log_{10}$  precaudal length.

							Large					
	Small			Medium		Rep	Reproductively inactive		Reproductively active females			
Source	df	F	р	df	F	р	df	F	р	df	F	р
HSI												
Month	11	5.38	<0.001	11	27.14	<0.001	2	1.80	0.169	4	4.92	<0.001
Treatment	1	194.26	<0.001	1	6.72	0.010	1	3.18	0.076	1	0.21	0.651
<b>Month-Treatment</b>	11	2.33	0.008	11	1.56	0.107	2	0.68	0.508	4	6.92	<0.001
CF												
Month	11	2.27	0.010	11	8.46	<0.001	2	5.13	0.007	4	3.22	0.013
Treatment	1	3.32	0.069	1	4.24	0.040	1	0.35	0.557	1	3.58	0.060
<b>Month-Treatment</b>	11	0.50	0.906	11	0.92	0.523	2	0.92	0.401	4	3.95	0.004
Kn												
Month	11	2.28	0.010	11	7.65	<0.001	2	5.12	0.007	4	3.22	0.014
Treatment	1	5.55	0.019	1	3.73	0.054	1	0.34	0.560	1	3.49	0.063
<b>Month-Treatment</b>	11	0.49	0.912	11	0.91	0.527	2	0.93	0.397	4	3.97	0.004
RrPCL												
Month	11	2.44	0.006	11	7.73	<0.001	2	5.57	0.005	4	2.57	0.039
Treatment	1	2.87	0.09	1	5.05	0.025	1	0.79	0.375	1	3.94	0.048
<b>Month-Treatment</b>	11	0.58	0.848	11	1.03	0.415	2	1.13	0.325	4	3.20	0.014



**Fig. 3.** *Carcharhinus obscurus*. Monthly measures of condition of medium sized sharks calculated using a) Hepatosomatic index (HSI), c) Condition factor (CF), d) Relative condition (Kn) and e) Residuals from the regression of  $log_{10}$  total body mass *vs.*  $log_{10}$  precaudal length (Rr <sub>PCL</sub>). Monthly values of precaudal length (PCL) are shown in (b). Data points represent mean values (± 1 SE) for each month for female () and male () sharks between 100 and 209 cm. Values displayed above a given data point indicate the month(s) for which pairwise tests revealed significant differences with the given month. Number of observations (n) for each month is shown in (b) for both sexes.

trend for lower HSI values than females (Fig. 3a). CF, Kn and Rr <sub>PCL</sub>, for both sexes followed a similar monthly trend to HSI with 'slump' periods in condition for males in March and October. All morphometric indices, for both sexes, peaked in July but, unlike HSI, not in June (Fig. 3c-e, Table 2). Mean monthly PCL values of males and females increased in June and July (Fig. 3b) following a similar annual trend to all condition indices (Fig. 3a-e). Both sexes were significantly larger in size in June and July than all other months (F = 8.00, df = 11, p < 0.0001, Fig. 3b).

#### Large reproductively inactive sharks

Sample sizes restricted data analysis of large sharks to the months of June, July and August (Fig. 4b). No significant effects of month or sex were detected for HSI (Table 2; Fig. 4a). For all three morphometric condition indices (CF, Kn and Rr <sub>PCL</sub>), month had a significant effect (Table 2). Female reproductively inactive sharks had significantly higher CF, Kn and Rr <sub>PCL</sub> values in June than in August (Fig. 4c-e). The interaction term month-sex was not significant for any somatic or morphometric condition index, indicating large sharks of both sexes exhibited similar trends in condition over the three months. The mean size of both sexes was similar across the three month period (F = 0.47, df = 2, p = 0.63, Fig. 4b).

#### Large pregnant and postpartum sharks

Sample size restricted data analysis for pregnant and postpartum sharks to the months of May to September (Fig. 5b). HSI of pregnant sharks increased to a peak in July with a gradual reduction throughout the rest of the year, before a second peak in December. Monthly mean HSI values of postpartum sharks were more variable (Fig. 5a). A significant effect of month was detected (Table 2, Fig 5a). The trend of morphometric condition of pregnant and postpartum animals was dissimilar between August and November (Fig. 5c-e). For pregnant sharks, all three morphometric condition indices remained constant between February and July, increasing to a peak in September (Fig. 5c-e). For the months of May, August and October, postpartum sharks showed small peaks in CF, Kn and Rr <sub>PCL</sub> similar to HSI (Fig. 5c-e). The mean size of both pregnant and postpartum sharks was similar across the five month period (F = 2.31, df = 4, p = 0.058, Fig. 5b). Large reproductively inactive males and females were in





Fig. 5. Carcharhinus obscurus. Monthly measures of condition of large reproductively active female sharks calculated using a) Hepatosomatic index (HSI), c) Condition factor (CF), d) Relative condition (Kn) and e) Residuals from the regression of  $log_{10}$  total body mass vs.  $log_{10}$  precaudal length (Rr <sub>PCL</sub>). Monthly values of precaudal length (PCL) are shown in (b). Data points represent mean values (± 1 SE) for each month for pregnant (——) and postpartum (——) sharks  $\geq$  210cm. Shaded area indicates months tested by ANOVA. Values displayed above a given data point indicate the month(s) for which pairwise tests revealed significant differences with the given month. Number of observations (n) for each month is shown in (b) for both reproductive states.



**Fig. 6.** *Carcharhinus obscurus*. The effect of precaudal length (PCL) on all calculated indices used to assess the condition of sharks. a) Hepatosomatic index (HSI); b) Condition factor (CF); c) Relative condition (Kn); d) Residuals from the regression of  $log_{10}$  total body mass *vs.*  $log_{10}$  precaudal length (Rr <sub>PCL</sub>).  $\bigcirc$  = neonates,  $\spadesuit$  = juveniles,  $\blacktriangle$  = medium female,  $\bigtriangleup$  = medium male,  $\diamondsuit$  = large reproductively inactive female,  $\blacklozenge$  = large reproductively inactive female.

**Table 3.** Carcharhinus obscurus. All monthly hepatosomatic index (HSI) pairwise comparisons between neonate ( $\leq$  79.0 cm PCL) and juvenile sharks (79.1-99.9 cm PCL). The level of significance for each pairwise test is denoted by  $\bullet \bullet \bullet$ , p<0.001,  $\bullet \bullet$ , p<0.01,  $\bullet \bullet$  p<0.05. The grey shading indicates direct month to month comparisons between the two size classes.



**Table 4.** *Carcharhinus obscurus.* Mean condition values ( $\pm 1$  SE) for all pregnant/postpartum and reproductively inactive large male/female sharks for all calculated condition indices: HSI = hepatosomatic index, CF = condition factor, Kn = relative condition and Rr<sub>PCL</sub> = regression of log<sub>10</sub> body mass vs. log<sub>10</sub> precaudal length. Associated *t*-test statistics (with associated degrees of freedom) and p-values are detailed.

Condition Index	Mean condition	value (± 1 S.E.)	<i>t</i> -test statistic / p-value
	Pregnant/Post- partum female	Non active large female	
HSI	$10.01 \pm 0.18$	13.26±0.30	(356) 9.25 p<0.0001
CF	$1.38 \pm 0.01$	$1.45 \pm 0.01$	(383) 4.17 p<0.0001
Kn	$1.10\pm0.01$	1.16±0.01	(382) 4.38 p<0.0001
Rr PCL	-0.019±0.003	$0.002 \pm 0.004$	(393) 3.80 p<0.0001

significantly higher condition than pregnant/postpartum females for all calculated condition indices (Table 4).

#### Effect of PCL on condition indices

Plots of calculated HSI, CF, Kn and Rr <sub>PCL</sub> value *vs.* PCL for each individual shark for all size classes and reproductive states are shown in Figures 6a-6d. Neonates had HSI values ranging from 2.4 to 26.2 (Fig. 6a). Between juvenile and mature adults, HSI followed an asymmetric distribution, with maximum HSI values increasing with size of animal (Fig. 6a). HSI of pregnant females was lower than that of large reproductively inactive male and female sharks (Fig. 6a; Table 4). The peak HSI values of the reproductively inactive sharks were equivalent to that of the neonate sharks. HSI was positively correlated with increasing body length ( $r_{2118} = 0.164$ , p < 0.001). CF, Kn and Rr <sub>PCL</sub> values plotted against PCL revealed little effect of increasing PCL on the morphometric condition indices (Figs. 6b-d). Pearson's r correlation found that CF and Rr <sub>PCL</sub> were not significantly correlated with shark length ( $r_{2118} = -0.025$  and  $r_{2118} = -0.030$  respectively, p > 0.05) while for Kn there was a significant negative correlation ( $r_{2118} = -0.166$ , p < 0.001). Pearson correlation coefficients for all pairwise comparisons of the four condition indices are presented in Table 5. CF, Kn and Rr <sub>PCL</sub> were highly correlated but the three morphometric condition indices were not correlated with HSI.

**Table 5.** *Carcharhinus obscurus*. Pearson correlation coefficients for all pairwise comparisons of the 4 condition indices calculated in the present study for the complete data set. HSI = hepatosomatic index, CF = condition factor, Kn = relative condition and  $Rr_{PCL}$  = regression of log<sub>10</sub> body mass vs. log<sub>10</sub> precaudal length. All correlations marked with a \* were significant at the level of p < 0.001.

Index	HSI	CF	Kn	
CF	0.19			
Kn	0.17	0.99*		
RrPCL	0.19	0.99*	0.98*	

## DISCUSSION

The data of this study are unique in that 2120 dusky sharks were available to enable a comprehensive comparison of multiple condition indices for a large predator. Generally, such data are both scarce and difficult to obtain considering the necessity for mortalities coupled with the global declines observed for many large sharks. By applying several widely-used condition indices, this study enabled a detailed comparison of their sensitivity, their seasonal variability within each life-stage and for sex and reproductive state and, the effect of increasing animal size. For teleosts, differences between condition indices proposed. This study on an elasmobranch found that differences between somatic and morphometric condition indices occur and that several confounding factors require attention when examining the condition of sharks. The data underscore the value of condition indices for elucidating seasonal condition patterns for various life-stages of a large shark.

# Comparison of condition by size class, sex and reproductive state Small neonate and juvenile sharks

Marked differences in the seasonal trends in HSI between neonate and juvenile sharks were evident. The observed HSI peak for neonates corresponded with the highest catch rate of postpartum sharks and the reported peak pupping period (Bass *et al.* 1973; Dudley *et al.* 2005). Our data is in agreement with Francis & Stevens (2000) who reported that near term porbeagle shark (*Lamna nasus*) embryos had higher HSI levels than juveniles. Dudley *et al.* (2005) amalgamated the small and medium dusky sharks included in this analysis to examine HSI and detected peak values for the months of June and July which they attributed to the abundant food resource of the sardine run. While this conclusion is consistent with the high incidence of sardines in the stomachs of medium sharks, sardines were infrequently recorded in the stomachs of small sharks (Dudley *et al.* 2005). The lack of a June/July HSI peak for juveniles in this study supports this latter observation. The peak observed by Dudley *et al.* (2005) in June/July was influenced, in part, by the inclusion of neonates in their dataset. The seasonal HSI cycles observed for neonate and juvenile dusky sharks confirm the importance of separating condition data into ecologically-relevant size classes prior to analysis (Pope

& Kruse 2007). Differentiating between neonate and juvenile sharks can be achieved through either umbilical scar presence/healing or by determining approximate size-at-age classes. If these data are unavailable, simple plots of liver mass vs. PCL for the species in question will identify the inclusion of neonates in the data. No seasonal fluctuations in HSI of the juvenile sharks were observed in this study indicating a tolerance of the summer-winter temperature regime in contrast to the findings of Hoffmayer *et al.* (2006) for the Atlantic sharpnose shark.

The morphometric condition indices did not clearly distinguish between the juveniles and neonates. Dusky sharks are born at variable sizes in KZN (Bass *et al.* 1973; Dudley *et al.* 2005) and it is likely that our proposed neonates included some >1-year old animals. This dilution effect combined with the low sensitivity of the morphometric indices may explain why CF, Kn and Rr <sub>PCL</sub> were not able to clearly separate the two life-stages. The peak in morphometric condition of juveniles in October correlated with a slight increase in HSI in the same month. Dudley *et al.* (2005) reported that larger near term embryos were found in pregnant sharks caught towards the end of the year, and hence it is possible that some of the animals categorized as juveniles were actually large neonates.

#### **Medium sharks**

The peak in HSI for medium size dusky sharks during June and July correlates with the presence of the sardine run and the reported high incidence of sardines, *Sardinops sagax* in the stomach contents of this size class (Dudley *et al.* 2005). The increase in HSI in June is dramatic (from *ca.* 8 to 12%), suggesting an immediate response by the liver to the increased feeding regime. The lipid content, and hence the energetic value, of sardines is extremely high (Van der Elst 1979) which may enable rapid storage as lipid in shark livers. Mature spinner sharks, *Carcharhinus brevipinna*, exhibited a similar trend in HSI (from *ca.* 10 to 15%) in the study region (Allen & Cliff 2000). Craik (1978) identified an increase in HSI (from *ca.* 5 to 8%) for female lesser spotted catsharks, *Scyliorhinus canicula*, in the Irish Sea. For medium size sharks in the present study, larger individuals of both sexes were caught in June and July. It is likely when assessing HSI of a large size range of animals grouped within one class (100 - 209cm PCL), that interpretation of HSI is confounded by PCL. The notable 'slump' periods in

condition of the males are months when the larger animals within the medium size category are not normally present in the study region (Dudley *et al.* 2005). Rossouw (1983) found that the lesser guitarfish, *Rhinobatos annulatus*, had lower liver lipid content after migration. These slump periods may indicate male dusky sharks that have undertaken migrations. Alternatively, the 'slump' periods may reflect the smaller sharks in the medium size category that are present in KZN waters year round (Dudley *et al.* 2005). CF, Kn and Rr <sub>PCL</sub> demonstrated similar seasonal patterns. The marked distinction between the morphological indices and HSI was the gradual increase in condition from May to a peak in July. This may suggest that the morphometric indices lag behind HSI in reflecting improved condition. Alternatively, it may further highlight the effect of PCL on HSI. The general similarity in trend of somatic and morphometric indices for the medium dusky sharks corresponds to the findings of Parsons & Hoffmayer (2005) and Hoffmayer *et al.* (2006) for CF and HSI of Atlantic sharpnose sharks.

#### Large reproductively inactive sharks

Accepting the limited sample size, there was no apparent influence of the sardine run on HSI of the large sharks, in contrast to medium sharks. Large dusky sharks, particularly males, feed primarily on sardines in June and July (Dudley et al. 2005; NSB unpublished data). This suggests that the absence of an HSI peak may be an artefact of the small sample size, an issue previously raised by Bolger & Connolly (1989). HSI remained relatively constant throughout the year for both sexes (ignoring single datum points). Large reproductively inactive sharks may therefore be in good condition prior to the sardine run, indicating an optimum HSI value or a maximum size the liver can attain for a given life-stage. The existence of the latter would be expected as the abdominal cavity size limits liver size. The peak values of HSI for neonates are equivalent to those of the reproductively inactive sharks, further indicating an optimal HSI value. All three morphometric indices followed identical seasonal trends but indicated a more pronounced peak in condition in June than HSI. Large sharks can consume up to 26.6 kg of sardines or 11.3 % of total body mass in one feeding event (NSB unpublished data). The inclusion of gutted mass could therefore result in lower HSI and higher morphometric condition values than expected. Within our data, it is unlikely that the inclusion of gutted mass had a significant overall influence on our

results considering most individuals had empty stomachs and only a few sharks had a high stomach mass. Although observed annual trends of both somatic and morphometric measures of condition were similar, the inclusion of gutted mass for a few individuals during June/July, i.e. the sardine run, may have resulted in this discrepancy between HSI and CF, Kn and Rr <sub>PCL</sub>. Measuring morphometric condition of fish is traditionally undertaken on live animals and therefore the inclusion of gutmass is viewed as a contributor to the measure of fitness (Weatherley 1972). Considering global declines in shark populations worldwide, the future application of condition measures will typically be non-invasive (Duncan & Holland 2006; Dibattista *et al.* 2007), which will preclude the removal of gutted mass. Gutted mass data were unavailable in the present study; however future work could address this point through the calculation of Clarke's isometric and allometric condition factors (Ricker 1975) using archived datasets.

## Large pregnant and postpartum sharks

The condition of pregnant sharks was similar to that of postpartum sharks, but both were lower than that of reproductively inactive sharks for all condition indices. King (1984) found that pregnant and postpartum female rig, Mustelus lenticulatus, had the lowest HSI values of all mature reproductive stages. Dudley & Cliff (1993) and Allen & Cliff (2000) also found that the HSI of blacktip sharks, Carcharhinus limbatus, and spinner sharks peaked during mating and then decreased during the pregnant and postpartum phases. The energetic cost of gestation and pupping is likely to account for this decrease in condition (Ranzi 1933 cited in Bone & Roberts 1969). For postpartum dusky sharks there was no clear evidence of an increase in condition relative to pregnant females, except for the month of May. King (1984) reported that the HSI of postpartum rig rapidly increased after pregnancy and pupping ceased. This suggests that most postpartum dusky sharks are caught directly after pupping and that their residency in coastal waters is limited. For both postpartum and pregnant sharks, the minor improvement in HSI in July may be correlated with the sardine run. It would be expected that postpartum sharks would take advantage of the sardine run but there was no immediate peak in condition in June. This supports dietary data (Dudley et al. 2005; NSB unpublished data) showing that pregnant and postpartum sharks do not actively feed on the sardine run which may be a result of their weakened condition. The reduced

liver size of pregnant sharks coupled with the additional near term pup mass (ca. 50 kg) would suggest these sharks were approaching negative buoyancy. Baldridge (1970) reported that pregnant sandbar sharks, Carcharhinus plumbeus, had relatively low average densities in sea water in comparison with non pregnant females due to the embryos' lower densities. The reduction in parental liver mass is therefore likely a twofold process providing nourishment to developing young while offsetting the increasing pup HSI throughout the gestation phase to regulate buoyancy. Differences between the morphometric indices and the somatic index for pregnant sharks occurred in the latter part of the year. Accepting a prolonged pupping season, neonates are born at a larger size towards the end of the year (Dudley et al. 2005), consequently, liver size of the mother may be smaller and calculated HSI would be lower than expected, while CF, KN and Rr PCL would be higher. A further explanation may be the result of the high incidence of empty stomachs reported for pregnant dusky sharks (Dudley et al. 2005). Baldridge (1972) reported a significant increase in tissue water in starved sharks and calculated that to increase the water content of a 100 kg shark from the expected tissue water value of ca. 80 % to the starved value of ca. 85 % would require the addition of 33.3 kg of water. Baldridge (1972) further stated that the increase in water content of muscle tissue resulted in a decrease in observed weight loss rate. Thus the trend of increasing morphometric condition of pregnant sharks later in the year, which are carrying larger pups, may be a result of an increase in water load in muscle tissue, hence reflecting sharks in extremely poor condition.

#### Comparison of somatic and morphometric condition indices

For dusky sharks, HSI was clearly a more sensitive measure of condition than the three morphometric indices. Adams & McLean (1985) reported similar findings for largemouth bass, *Micropterus salmoides*. The authors concluded that HSI was a relatively rapid response indicator whereas CF was a long term response indicator and insensitive to short term variation. When examining seasonal condition trends in the Atlantic sharpnose shark, Parsons & Hoffmayer (2005) and Hoffmayer *et al.* (2006) found that CF and HSI followed a similar trend across months but the increase in HSI was more pronounced. The marked increase in HSI with increasing size of shark enforces the need for selecting appropriate size, maturity and reproductive state categories for analysis and comparison, as previously stated by Pope & Kruse (2007).

HSI is also complicated on a seasonal basis because it reflects short term energetic and reproductive states, combined with metabolic demands regulated by temperature and other environmental factors (Adams & McLean 1985; Hoffmayer *et al.* 2006). Consequently, when using HSI to assess condition, it may be necessary to consider a more complex range of indices including reproductive measures and environmental parameters to fully understand trends in the data. There was minimal variation between the morphometric condition indices, CF, Kn and Rr <sub>PCL</sub>, for each life-stage, sex and reproductive state. This suggests any of these condition indices would be suitable for intra-population comparisons. The negative correlation between Kn and PCL of shark was significant. This is surprising because Kn, based on the weight-length for the whole population, was developed to overcome biases of length with increasing size of fish identified in Fulton's condition factor (Le Cren 1951).

Green (2001) questioned the use of residuals for understanding the condition of animals in ecological studies. Our findings suggest no observed difference in the ecological information provided when compared to standard morphometric condition measures, as found by Schulte-Hostedde *et al.* (2005).

Although the morphometric condition indices may have limited sensitivity, they provide a rapid, non-invasive measure of the physiological status of the fish (Brown & Murphy 1991; Neumann & Murphy 1991). This is an increasingly important consideration when studying large predators undergoing population declines and future work could investigate the utility of validating ultrasound techniques to devise a non-invasive method of estimating HSI. In order to advance the use of morphometric indices for elasmobranchs, it would be useful to calculate and test the reliability of relative weight indices to enable accurate inter-population comparisons. This is a requirement as comparative population analyses using CF, Kn and Rr <sub>PCL</sub> are not possible as they violate the basic assumptions of the indices (Bolger & Connolly 1989).

# CONCLUSION

Condition indices are widely used as an ecological and management tool for monitoring the health or fitness of fishes. Their appropriate use may provide valuable insights into
ecological and physiological processes (migration, lipid storage, growth and maturation) and the effect of components in the environment that the fish inhabits (prey availability, habitat and temperature). To date, a comprehensive comparative analysis of multiple condition indices for sharks has been limited by the scarcity of available data. This study demonstrates that condition indices are a useful tool for studying the seasonal health or fitness of sharks but that the appropriate condition index and size range of animals must be selected. The significant effect of month for most size class, sex and reproductive state tests underlines that seasonal variations in both morphometric and somatic condition occur. Our data found that the application of CF, Kn and Rr PCL would provide comparable results but that HSI is a more sensitive measure of condition responding more rapidly to environmental/physiological cues. HSI was also more able to determine seasonal trends in condition of juvenile and neonate sharks. The effect of increasing animal size was most apparent for HSI which confounds inter-size class comparisons. Considering the large liver size of neonates, the reduced liver size of pregnant and postpartum sharks and, the notable effect of PCL on HSI, it is important to disaggregate data sets by clearly defined size class, sex and reproductive states for accurate interpretation of the data. Sample sizes and preliminary examination of the data to determine the suitability of statistical tests are also important considerations. Although, gutted mass is unlikely to have significant effects on overall seasonal patterns, our results raise possible concerns over the inclusion of gutted mass when examining both somatic and morphometric condition of large predators. Considering that condition measures are usually undertaken on live animals, subsequent work should determine the possible effects of gutted mass for large individuals through the calculation of Clarke's isometric and allometric condition factors (Ricker 1975). Noninvasive ultrasound methods may provide future alternative strategies to examine HSI eliminating the requirement for animal mortalities.

# **CHAPTER FOUR**

Maternal investment and size-specific reproductive output in carcharhinid sharks.

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The results of this chapter have been accepted for publication and are currently in press. Hussey, N.E., Wintner, S.P., Dudley, S.F.J., Cliff, G., Cocks, D.T. & MacNeil, M.A. (2009) Maternal investment and size-specific reproductive output in carcharhinid sharks. *Journal of Animal Ecology*. doi: 10.1111/j.1365-2656.2009.01623.x

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# ABSTRACT

Life history theory predicts that organisms will provide an optimal level of parental investment for offspring survival balanced against the effects on their own survival and future reproductive potential. Optimal resource allocation models also predict an increase in reproductive output with age as expected future reproductive effort decreases. To date, maternal investment in sharks has received limited attention. We found that neonatal dusky sharks (Carcharhinus obscurus) are not independent from maternal resource allocation at the point of parturition but instead are provisioned with energy reserves in the form of an enlarged liver that constitutes approximately 20% of total body mass. Analysis of long-term archived data sets showed that a large proportion of this enlarged liver (~50%) is utilised during the first weeks or months of life indicating that the reported weight loss of newborn sharks signifies a natural orientation process and is not necessarily related to prev abundance and/or indicative of high mortality rates as previously suggested. Interrogation of near-term pup mass in two carcharhinids, the dusky and spinner shark (Carcharhinus brevipinna), further revealed an increase in reproductive output with maternal size, with evidence for a moderate decline in the largest mothers [≥270 cm precaudal length (PCL) and >210 PCL, dusky and spinner shark, respectively]. For the dusky shark, there was a trade-off between increasing litter size and near-term pup mass in support of optimal offspring size theory (decrease in individual pup mass of 0.79 kg between litter sizes of 3 - 16 pups). For both the dusky and spinner shark, there was a linear increase in near-term pup mass with month [1.69 kg (Feb. - Dec.) and 0.26 kg (Feb. - Aug.), dusky and spinner shark, respectively], which may indicate variable parturition strategies and/or that carcharhinids are able to adjust the length of the gestation period. The identification of optimal size-specific reproductive output has direct implications for improving the reproductive potential of exploited shark populations and for structuring future management strategies.

# **INTRODUCTION**

Life history theory predicts that organisms should provide an optimal level of parental investment or energy expenditure to increase both offspring survival and future reproductive success, balanced against components of their own maintenance, survival and future reproductive potential (Williams 1966; Clutton-Brock 1991; Stearns 1992; Roff 1992). For many organisms, including sharks, males contribute no parental care; consequently the number, size and growth of young are determined by the energy allocated by the mother, i.e. the level of maternal investment (Evans 1990).

In terms of reproductive mode, carcharhinid sharks are analogous to marine mammals in that they bear live young (viviparity). A principal difference between the reproductive strategies of these two groups, however, is the allocation of postnatal maternal resources. For most mammals, postnatal costs are incurred through the direct supply of food to the young from the mother's reserves (Bowen *et al.* 2001) and through maternal-young behavioural interactions (Mann & Smutts 1999; Szabo & Duffus 2008).

In the case of sharks, evidence suggests that at the point of parturition, offspring independence is distinct and terminal, similar to most spawning fish (Wourms 1977; Jönsson *et al.* 1998) and marine reptiles (Broderick *et al.* 2003). For these later taxa, however, the independent young and emerging hatchlings are provided with a component of prenatal reproductive resources, or a maternal head-start, through the provisioning of yolk reserves (Congdon 1989; Berkeley *et al.* 2004; Donelson *et al.* 2008). The young ultimately do not become independent from maternal resource allocation until they have exhausted this endogenous energy store. Importantly, strong positive relationships between the quantity (size) and quality of these reserves and factors such as time to exogenous feeding, growth rate, and early survival have been widely reported (Wallace & Aasjord 1984; Springate & Bromage 1985; Hare & Cowen 1997; Marteinsdottir & Steinarsson 1998; Nagle *et al.* 1998; Berkeley *et al.* 2004; Gagliano & McCormick 2007; Donelson *et al.* 2008). Prenatal maternal investment in most oviparous ectothermic vertebrates, which exhibit no direct maternal care or postnatal costs, can therefore be considered to include the allocation of reproductive

resources for postnatal use or the provisioning of a maternal head-start (Congdon 1989; Berkeley *et al.* 2004; Wallace *et al.* 2007).

In contrast to teleost fish, newborn carcharhinid sharks are equipped with fully functional jaws and teeth (Wourms 1977; Carrier *et al.* 2004) and have therefore been considered independent of maternal resource allocation at the point of birth. Gilmore *et al.* (1983a), Gilmore (1983b) and Francis & Stevens (2000), however, noted that near-term pups of sand tiger (*Carcharias taurus*), longfin mako (*Isurus paucus*), and porbeagle sharks (*Lamna nasus*) possessed a larger liver than both small prenatal and neonatal sharks. For large adult sharks, it has long been recognised that the liver acts as a storage site for lipid reserves which are used in reproduction and migration and during periods of prey limitation (Springer 1967; Hoffmayer *et al.* 2006; Hussey *et al.* 2009b). Gilmore *et al.* (1983a), Gilmore (1983b) and Francis & Stevens (2000) proposed that the enlarged liver of neonatal sharks may act as an energy reserve to be utilised after birth. Consequently, if neonatal sharks are provisioned with an enlarged liver they may be reliant on a maternal head-start to enable the transition to independent foraging and to increase their likelihood of survival.

Comparative analysis of life-history traits predicts that a mother's phenotype will impact the phenotype of her young, independent of genotype (Bernardo 1996; Mousseau & Fox 1998). Maternal phenotypic effects are typically mediated by the age (size) and experience of the female, commonly resulting in a positive correlation between maternal size and offspring size (Chambers & Leggett 1996; Coté & Festa-Bianchet 2001; Green & McCormick 2005; Skibiel et al. 2009) and/or the number of offspring produced (Wootton 1990; Morris 1996; Sogard et al. 2008), i.e. an increase in overall reproductive output. These effects, which are common across taxa, likely reflect available space for developing offspring and the acquired experience of larger individuals to obtain/store food for offspring development (Mattingly & McCure 1982). Optimality models further predict that reproductive effort will increase with age, as expected future reproductive success decreases (Pianka & Parker 1975; Charlesworth & Leon 1976). At the species level, heterogeneity in near-term young mass/length and litter size vs. mother mass/length relationship is common due to multiple competing functions exerting influence. Aside from maternal size, environment-induced phenotypic effects include the mother's ability to secure high-quality habitats (prior to

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breeding and throughout gestation; Mappes *et al.* 1995; Räsänen *et al.* 2008) and temporal variability, over scales of months and years, in environmental conditions and resource availability within selected habitats (Sikes & Ylönen 1998; Castro *et al.* 2009; Wilson *et al.* 2009). Flexibility in a species' reproductive strategy, such as open breeding seasons (Hutchings & Myers 1993) and flexible gestation periods (Asher 2007), will also impact on the quality and survival of young. For many organisms, physiological changes in maternal condition cued by a general decrease in body condition with age may also occur (Roff 1992). As a result of these trade-offs, a specific age or reproductive size may be the most reproductively efficient strategy in terms of breeding resource allocation and production of healthy offspring (Williams 1966; Boltnev & York 2001; Carr & Kaufman 2009).

Considering the estimated decline in many shark populations (e.g. Baum *et al.* 2003) and the tendency of fisheries to target larger individuals within the population (Myers & Worm 2005), understanding the allocation of maternal investment by mothers of different sizes has important implications for potential conservation and management strategies.

The aim of this study was to determine if the reported enlarged liver of near term shark pups represents a form of maternal investment for neonatal, free swimming sharks. A carcharhinid, the dusky shark (*Carcharhinus obscurus*), was selected as a test species. With data for two species, the dusky and the spinner shark (*Carcharhinus brevipinna*), an additional aim was to examine the variability in maternal investment in near term carcharhinid sharks to determine (i) what maternal and environmental effects might be driving this variability and (ii) if there is evidence of optimal size-specific reproductive investment.

## MATERIALS AND METHODS

### Study location and sampling

Data for all sharks included in this study were recorded from animals incidentally caught in beach protection nets in KwaZulu-Natal (KZN), South Africa, between 1978 and 2008. Nets were set approximately 300-500m from beaches, parallel to the

shoreline in 10-14m of water (Dudley et al. 2005). All dead sharks retrieved from the nets in good condition were transported to the KwaZulu-Natal Sharks Board (KZNSB) main laboratory for dissection. For specific details regarding changes to net installations over time and net servicing operations refer to Cliff et al. (1988) and Dudley et al. (2005). On arrival at the laboratory, data on species, sex, capture date and location, as well as basic morphometric measurements including precaudal length (PCL (cm)), and total body mass (kg) were recorded. PCL was measured as the straight line distance from the tip of the snout to the precaudal notch as defined by Dudley et al. (2005). Sharks were then stored frozen (-20°C) prior to dissection. During the main dissection, the complete stomach was removed and total stomach mass and total liver mass of combined lobes of each shark were recorded to the nearest gramme. For netcaught neonatal/juvenile free swimming sharks, the stage of umbilical scar healing or umbilical scar stage (USS) was also recorded. A qualitative five point USS scale was devised, where (1) the wound was completely open, (2) wound open but muscle tissue was closing, (3) skin was closing, (4) a faint scar remained and (5) no scar was present. The estimated time between USS1 and USS3 is 2-4 weeks (Bass et al. 1973; Duncan & Holland 2006). For pregnant sharks, all pups were extracted from both uteri and the number (litter size), sex, PCL (cm) and total body mass (kg) were recorded. Accepting that net-caught pregnant sharks may abort pups, the total litter size of each individual female was confirmed by counting the total number of placental scars. Total liver mass of unborn pups was not recorded throughout the archived data period.

### Maternal investment in free swimming neonatal/juvenile sharks

To examine if an enlarged liver represents a maternal investment in free swimming neonatal dusky sharks, we isolated data on total body mass, total liver mass, PCL and stomach mass for all small sharks with reported USS data. We then extracted total body mass and PCL data for all near-term and mid-term dusky pups. Near-term pregnant dusky sharks were defined as having pups with a PCL of  $\geq 60$  cm (Dudley *et al.* 2005). Liver mass data for near-term pups were recorded for eight individuals from three separate mothers between 2007 and 2008. The two most commonly applied condition indices; a somatic measure, hepatosomatic index (HSI) and a morphometric measure, condition factor (CF), were then calculated for mid-term/near-term pups and neonatal/juvenile sharks with USS data using the following equations:

$$HSI = [M_{TL} (kg) / M_{TB} (kg)] \times 100; \text{ and}$$
(1)

$$CF = [M_{TB} (kg) / PCL (cm)^{3}] \times 10^{5}$$
(2)

where M<sub>TL</sub> equals the total mass of both liver lobes combined per individual shark and M<sub>TB</sub> is the total body mass of each individual shark. Linear models were applied to the data to examine the trends in total liver mass, total body mass, and condition (HSI and CF) of sharks by the defined life-stages. Because the sample sizes were unbalanced, significance of pairwise comparisons was tested using adjusted Bonferroni tests. To visually interpret changes in total stomach mass for each USS, i.e. an indication of the level of exogenous feeding, the data were plotted in boxplot format.

### Maternal investment in near-term pups

To examine what maternal and environmental factors may influence investment in nearterm shark pups and if there is evidence for optimal size-specific reproductive output, we accessed all mother-pup data for near-term pregnant dusky sharks, and a second carcharhinid, the spinner shark. Near-term spinner sharks were defined as having pups with a PCL of  $\geq$  50cm (Allen & Cliff 2000). Both species utilise the same reproductive strategy, viviparity, with developing embryos receiving nourishment through a pseudoplacental sac. The population status of both species is considered to be stable in the study region (Dudley & Simpfendorfer 2006), and therefore we could rule out any influence of complicating factors, for example, compensatory density-dependent mechanisms related to overfishing (Rose *et al.* 2001). We examined pup mass as a response variable representative of pup fitness.

The structure of the data (multiple pups from within the same mother) necessitated the use of hierarchical linear models to properly structure the statistical dependence among pups from the same mother and the reduced number of observations for mothers, relative to pups. The pup-level model was of the form:

$$y_{ik} \sim N(\beta_{0k} + \beta_1 x_1 + ... + \beta_n x_n, \sigma_k^2),$$
 (3)

with pup-level variables, x, and a mother-dependent intercept,  $\beta_{0k}$  and standard deviation  $\sigma_{k}^{2}$ . The mother-level model for average pup mass within mother was:

$$\beta_{0k} \sim N(\gamma_0 + \gamma_1 z_1 + \dots + \gamma_n z_n, \sigma^2), \tag{4}$$

with mother-level variables, z. We adopted an a priori model-building strategy and developed nine candidate models of increasing complexity, factoring in the above defined mother-pup variance structure and fixed effects for mother-pup and seasonal relationships (i.e. maternal and environmental phenotypic controls) that were reasonably suspected of operating during the sampling period (Table 1). This included a pup-level sex covariate, maternal covariates of mother PCL and litter size, environmental covariates of month and year and associated interactions. For the maternal covariates of mother PCL, we formally tested both linear and quadratic functions, the latter via pairwise interactions, as we may expect an initial increase in maternal reproductive performance with size followed by a senescent decline in later life. Of the suspected model interactions, we included litter size-mother size as previously a weak relationship between these effects has been reported for both the spinner and dusky shark (Allen & Cliff 2000; Dudley et al. 2005). The candidate model set for both dusky and spinner sharks were implemented using the nonlinear mixed effects package (nlme; Pinheiro & Bates 2000) in the statsistical program R (R Development Core Team, 2009). Given our large sample sizes. Akaiki's Information Criterion (AIC) were used to assign relative strengths of evidence to each candidate model in the set and parameter estimates were solved for using the likelihood-ratio tests provided in R.

## RESULTS

#### Maternal investment in free swimming neonatal/juvenile sharks

A marked reduction in total liver mass was found between near-term pups and USS5 dusky sharks with mean liver mass values ranging from  $1072.8 \pm 78.4g$  (mean  $\pm$  SE) to  $517.3 \pm 47.4g$  (Fig. 1a). When considering total body mass, a declining trend was observed between near-term pups and USS2 sharks, followed by an increase between USS2 and USS5 sharks (Fig. 1a). In agreement with total liver mass, HSI decreased from  $19.5 \pm 0.96$  (mean  $\pm$  SE) to  $6.4 \pm 0.50$ , between NTP and USS5 sharks (Fig. 1b).

**Table 1.** Candidate model set for quantifying near-term pup mass in dusky (*Carcharhinus obscurus*) and spinner (*Carcharhinus brevipinna*) sharks. Variance structure provided in equations 2 & 3 in the Methods section of the text.

Model	Equation
<b>M</b> 0	$pMASS_{ik} = \gamma_0 + \beta_{0k}$
<b>M</b> 1	$pMASS_{ik} = \gamma_0 + \beta_{0k} + \beta_1 pSEX_i$
M2	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \beta_{0k} + \beta_1 pSEX_i$
M3	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \beta_{0k} + \beta_1 pSEX_i$
M4	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \gamma_3 mPCL^2_k + \beta_{0k} + \beta_1 pSEX_i$
M5	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \gamma_3 mPCL^2_k + \gamma_4 MONTH_k + \beta_{0k} + \beta_1 pSEX_i$
M6	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \gamma_3 mPCL^2_k + \gamma_4 MONTH_k + \gamma_5 YEAR_k + \beta_{0k} + \beta_1 pSEX_i$
<b>M</b> 7	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \gamma_3 mPCL^2_k + \gamma_4 MONTH_k + \gamma_5 mPCL_k * LS_k + \beta_{0k} + \beta_1 pSEX_i$
M8	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \gamma_3 mPCL^2_k + \gamma_4 MONTH_k + \gamma_5 MONTH_k * LS_k + \beta_{0k} + \beta_1 pSEX_i$
M9	$pMASS_{ik} = \gamma_0 + \gamma_1 mPCL_k + \gamma_2 LS_k + \gamma_3 mPCL_k^2 + \gamma_4 MONTH_k + \gamma_5 mPCL_k * MONTH_k + \beta_{0k} + \beta_1 pSEX$

pMASS - mass (g) for pup *i*; pSEX - pup sex (male=1/female=0); mPCL - group-centered precaudal length (cm) for mother *k*; LS - litter size; MONTH - month of capture (Feb=2, ..., Dec=12); YEAR - median-year centred year of capture.

Data for HSI/liver mass of mid-term pups were unavailable. For CF, there was a large increase in condition from mid-term pups to near-term pups, but then a noticeable reduction in condition with increasing USS. CF values ranged from  $1.55 \pm 0.001$  for near-term pups to  $1.33 \pm 0.02$  for USS5 sharks, an identical value to mid-term pups ( $1.33 \pm 0.01$ ) (Fig. 1c). Linear model results were significant for liver mass, total body mass, HSI and CF (F<sub>5, 134</sub> = 14.16, p < 0.0001; F<sub>5, 134</sub> = 17.93, p < 0.0001; F<sub>5, 134</sub> = 31.24, p < 0.0001, F<sub>6, 2226</sub> = 84.15, p < 0.0001, respectively). For total liver mass and HSI, pairwise comparisons were highly significant indicating a consistent graduated decrease with USS (Fig. 1a & 1b). For CF, pairwise comparisons found that NTP and USS1 sharks were in significantly better condition than all other stages. No difference in CF was detected between mid-term pups and USS2-5 sharks (Fig. 1c). Total stomach content mass of USS1-2 sharks was negligible while an increase in total stomach content mass was observed for USS3-5 sharks (Fig. 1d).

#### Maternal investment in near-term pups

A total of 228 and 136 pregnant dusky and spinner sharks and 2230 and 1277 near-term dusky and spinner pups, respectively, were included in the analyses. Model fits were adequate for all candidate models except M1 (Table 2) and quantile-quantile plots showed the data to be well-described by normally-distributed errors for both species. Candidate-model results for the relationship between pup mass and mother-environment phenotypic effects favoured models M7 and M6 for the dusky and spinner shark, respectively (Table 2). For both species there was a clear relationship between pup mass and both linear and quadratic functions of mother PCL (Table 3; Fig. 2a & b). Mean pup mass increased with mother size to an asymptote, with evidence for a decline at the largest mother lengths of both species (Fig. 2a & b). For the dusky shark, there was an effect of litter size, with mean pup mass decreasing with increasing litter size (Table 3; Fig. 2c). A model-fit regression found an overall decrease in dusky shark pup mass of 0.79 kg between litters composed of 3 and 16 pups. A similar trend was observed for spinner sharks (Fig. 2d), but the negative effect of litter size was of a much smaller magnitude within the best-fit model (Table 3). For both species, pup mass showed a strong linear increase with month (Fig. 2e & f), with model-fit regression values ranging from 4.14 kg (February) to 5.83 kg (December) and 2.01 kg (February) to 2.27 kg



Figure 1. Transition in (a) actual liver mass and total shark mass, (b) hepatosomatic index (HSI), (c) condition factor (CF) and (d) total stomach mass for mid-term dusky shark (*Carcharhinus obscurus*) pups to free swimming sharks with a healed umbilical scar (USS5). Data is the mean  $\pm$  SE. For total stomach mass, boxes illustrate the interquartile range, whiskers identify the largest non-outlier observations, solid circles are outliers and open circles are the mean values. Values displayed above a given life-stage indicate the life-stage(s) for which pairwise tests revealed significant differences with the given life-stage. Numbers in each plot represent the sample size of sharks per prenatal and umbilical scar stage (USS) class. For total stomach mass, samples sizes are the same as for condition factor.

(August) for the dusky and spinner shark, respectively (Table 3; Fig. 2e & f). The peak catch period of postpartum dusky and spinner sharks in beach protection nets was July and June, respectively (Fig 2e & f). For spinner sharks, there was evidence for heavier female pups and a trend of increasing pup mass since the late 1970s (Table 3).

**Table 2.** Model selection results for top-ranked models of dusky (*Carcharhinus obscurus*) and spinner (*Carcharhinus brevipinna*) shark near-term pup mass. GOF - likelihood-ratio test goodness of fit; K - number of model parameters; logLik - model log-likelihood;  $\Delta AIC$  - relative AIC differences;  $w_i$  - Aikaike weights.

Model	GOF	K	logLik	AIC	ΔΑΙC	Wi
Dusky						
<b>M7</b>	< 0.001	7	-16548.34	33114.68	0.00	0.35
M5	< 0.001	6	-16549.86	33113.71	0.17	0.33
M6	< 0.001	7	-16549.58	33115.17	1.62	0.16
<b>M8</b>	< 0.001	7	-16548.86	33117.73	3.04	0.07
M9	< 0.001	7	-16549.04	33118.08	3.40	0.06
<b>M</b> 4	< 0.001	5	-16553.60	33119.19	5.70	0.03
M3	< 0.001	4	-16573.23	33156.47	43.0	0.00
M2	0.067	3	-16578.18	33194.92	51.7	0.00
<b>M</b> 1	1.00	2	-16580.00	33169.76	55.1	0.00
<b>M0</b>	1.00	1	-16581.27	33168.5	53.9	0.00
Spinner						
<b>M</b> 6	< 0.001	7	-8246.38	16510.8	0.00	0.52
<b>M8</b>	< 0.001	7	-8246.29	16512.6	1.83	0.21
M9	< 0.001	7	-8246.37	16512.7	1.98	0.19
M5	< 0.001	6	-8250.13	16516.3	5.52	0.03
<b>M</b> 7	< 0.001	7	-8249.40	16516.9	6.10	0.03
<b>M</b> 4	< 0.001	5	-8252.15	16518.3	7.54	0.02
M3	< 0.001	4	-8255.45	16520.9	10.15	0.00
M2	< 0.001	3	-8254.47	16520.9	10.19	0.00
<b>M</b> 1	1.00	2	-8265.32	16538.7	27.89	0.00
MO	1.00	1	-8267.57	16541.1	30.39	0.00

Parameter	Estimate	SE	95% CI
Dusky (M7)			
Intercept	4061.2	131.4	[3804, 4319]
pSEX	-14.2	15.1	[-43.9, 15.4]
LS	-48.1	18.3	[-84.2, -12.0]
mPCL	9.3	4.5	[0.59, 18.1]
mPCL <sup>2</sup>	-0.97	0.32	[-1.60, -0.35]
LS*mPCL	2.35	1.60	[-0.79, 5.49]
MONTH	149.6	22.5	[105.2, 193.9]
Spinner (M6)			······································
Intercept	2032.7	119.1	[1800, 2266]
pSEX	-16.7	7.8	[-31.9, -1.4]
LS	-7.5	8.0	[-23.2, 8.3]
mPCL	7.2	2.2	[2.8, 11.6]
mPCL <sup>2</sup>	-0.31	0.15	[-0.60, -0.02]
MONTH	52.7	18.1	[17.1, 88.4]
YEAR	7.5	2.7	[2.1, 12.9]

**Table 3.** Parameter-estimate results for best-fit models of dusky (*Carcharhinus obscurus*) and spinner (*Carcharhinus brevipinna*) shark near-term pup mass. Parameters not overlapping zero are given in bold; abbreviations provided in Table 1.

# DISCUSSION

To the best of our knowledge these data provide the first characterisation of a maternal head-start for newborn sharks, in the form of an enlarged liver and provide evidence for both size-specific reproductive output (or investment) and environmental phenotypic control on pup fitness for two carcharhinid sharks.

The clear decline in total liver mass, HSI and CF of neonatal dusky sharks with umbilical scar stage provide empirical evidence that liver reserves, provisioned by the mother, are used in the first few weeks (or even months) of life. In agreement with our



Fig. 2. The relationship between near-term pup mass and maternal precaudal length (a,b), litter size (c.d) and the seasonal effect of month (e,f) for dusky (*Carcharhinus obscurus*; model M7) and spinner sharks (*Carcharhinus brevipinna*; model M6), respectively. Small grey dots are raw data (a-f), large grey dots with black outline (a,b) and large black dots (c-f) are mean data ( $\pm$  SD) with best-fit model regressions where significant. Grey dotted lines indicate the minimum and maximum values of the best-fit regression lines. For (e,f), the grey bar indicates the peak catch period of postpartum females for both species in beach protection nets.

observed decline in CF and neonatal dusky shark mass, Duncan & Holland (2006) reported that captive neonatal scalloped hammerhead sharks (Sphyrna lewini) lost weight with umbilical scar healing stage and that the CF of free ranging animals in Kāne'ohe Bay, Hawaii, was lower in the months following the birthing peak. Similar findings were reported in previous studies by Lowe (2002) and Bush & Holland (2002). Lowe (2002) and Duncan & Holland (2006) concluded that newborn scalloped hammerhead sharks were in a poor nutritional state and attributed this to population size and poorly developed foraging skills and/or reduced prey availability, suggesting this was indicative of high mortality rates within the nursery habitat. It is likely that the weight loss of newborn sharks is a combined result of the utilisation of the provisioned maternal head-start and both density dependent mechanisms and possible reduced prey availability within the nursery (as reported for scalloped hammerheads in Hawaii; Duncan & Holland 2006). Certainly the rate of total body mass loss of newborn sharks will be dependent on individual foraging development and success. Possible variation in the allocation of maternal resources may also be a contributing factor to pup survival in high density nursery regions.

The limited stomach content mass of early USS dusky sharks support the role of the liver in maternal investment in agreement with observations on neonatal scalloped hammerhead sharks (Bush 2003). The enlarged liver acts as a food reserve to maintain the young sharks while they orientate themselves in their environment and develop their foraging skills. This point is further supported by observations of a newborn sand tiger shark (*Carcharias taurus*) born in captivity that did not feed for the first 25 days (Gilmore *et al.* 1983a). Furthermore, Hussey *et al.* (2009b) reported a clear difference in HSI values of suspected neonatal and juvenile dusky sharks over a seasonal cycle. Current concerns over the weight loss of newborn sharks in nursery areas and associated high mortality rates (Heupel *et al.* 2007 and references therein) require further detailed investigation. The existence of an enlarged liver in neonatal sharks and associated natural weight loss may also be a complicating factor when studying selection on life history traits of newborn sharks (DiBattista *et al* 2007).

Although the provisioning of a maternal head-start in sharks is not surprising considering documented maternal provisioning of teleost fish and marine reptiles (Berkeley *et al.* 2004; Gagliano & McCormick 2007; Donelson *et al.* 2008), the

allocation of excess liver reserves appears to be a novel maternal investment strategy among viviparous marine vertebrates and may in part explain the evolutionary success of sharks. For viviparous sharks, the decoupling of postnatal care may result in considerably higher reproductive output per individual female's lifespan, while the birthing of multiple, large, well-provisioned live young increases the likelihood of pup survival, in the absence of maternal care.

In the case of many oviparous fishes, predation and starvation during larval development frequently govern survival and subsequent recruitment (Houde 1987). Once endogenous yolk reserves have been absorbed, larvae must begin to feed themselves immediately, without time for environment acclimatisation coupled with limited ability to disperse. This may lead to high mortality rates when larvae find themselves in food-deprived environments (*sensu* Hjort's critical period hypothesis; Hjort 1914). For sharks, the mother is able to provision her young with a level of endogenous prenatal resource allocation irrespective of exogenous resource availability at parturition and these reserves can be directly utilised by the pups in conjunction with independent foraging. Near-terms sharks can therefore maximise their survival potential and mitigate the effects of the critical period hypothesis.

How the level of maternal investment in near-term sharks and rays varies between species or families adopting different reproductive strategies (i.e. viviparous, ovoviviparous) is unknown. Reported data for the oophagous white shark (*Carcharodon carcharias*) suggests lower levels of provisioning than observed in this carcharhinid study (NTP/newborn HSI = 16.5 [n=3], free swimming juvenile HSI = 12.5 [n=6], Cliff *et al.* 1996; Francis 1996). For sandtiger sharks, which are also oophagous but exhibit uterine cannibalism, the reported enlarged liver of a newborn animal had a HSI value of 9.9% (Gilmore *et al.* 1983a), suggesting placental species demand a larger liver reserve than oophagous species prior to parturition. Indeed, direct maternal-young nutrient placental transfer may enhance the fitness of viviparous newborn sharks, but may also increase the potential for parent-offspring conflicts (Crespi & Semeniuk 2004), a situation further exacerbated in populations with polyandrous mating systems (Zeh & Zeh 2000). It is likely that inter-specific variation in the level of maternal investment is related to not only reproductive mode but also the life history strategies (region of parturition, size at birth, growth rate etc.) of the species in question.



**Fig. 3.** Percentage frequency of litter sizes for the dusky = (*Carcharhinus obscurus*) and spinner shark = (*Carcharhinus brevipinna*).

The occurrence of heavier pups in the mid-size class of mature carcharhinid sharks provides evidence for optimal size-specific reproductive output. It is expected that mothers possess phenotypic plasticity and can adjust offspring phenotype in a way that enhances offspring fitness (Mousseau & Dingle 1991) and therefore variation in pup mass relative to maternal size may be influenced by several alternative factors, such as genotypic variation in reproductive traits, litter size, paternal genetic input, population demography, timing of parturition and environmental-resource heterogeneity (Clutton-Brock 1991; Roff 1992; Jordan & Snell 2002; Roff 2002). For the dusky shark, we found evidence for a trade-off between pup mass and increasing litter size in support of optimal offspring size theory (Smith & Fretwell 1974; Stearns 1992). Allen & Cliff (2000) and Dudley et al. (2005) previously reported a significant relationship between litter size and maternal size for both study species, with litter size increasing with maternal PCL. The inclusion of the mother size-litter size interaction within our candidate models, however, had limited effect on best-fit model selection, indicating that the observed pup mass-litter size relationship is unlikely to be regulated by maternal size. For leatherback turtles, Wallace et al. (2007) found that females maximise the number, but not necessarily the size of the young per breeding event and that mother size exerted minimal influence on this relationship. Similar to the

suggestion of Wallace *et al.* (2007), it seems likely that physical constraint of body size in carcharhinid sharks may have limited impact on observed litter size patterns. Therefore, differential resource availability may better explain observed variation in litter size (Reznick & Yang 1993; Reznick *et al.* 1996) although we cannot rule out a combination of genotype-environmental interactions driving the observed pattern (Reznick *et al.* 2000; Roff 2002). If we consider that litter size will approach an optimal value through evolutionary time (Lack 1947), it was apparent that dusky and spinner sharks preferentially selected for litter sizes of 8-12 and 6-12 pups, respectively (Fig. 3).

The consistent linear increase in pup mass with month for both species may also be a result of phenotypic plasticity. In the case of the dusky shark, near-term pregnant and postpartum females and newborn sharks with early-stage umbilical scars are caught in beach protection nets throughout the year (Dudley et al. 2005; KZNSB unpubl. data). This suggests that either mating occurs year round and/or reported sperm storage (Pratt 1993) allows fertilisation plasticity enabling females to opt for variable parturition strategies to optimise offspring survival. This would entail pupping smaller offspring earlier in the year when environmental conditions are optimal and during a period of abundant prey availability in the nursery habitat (i.e. the annual sardine run, Armstrong et al. (1991)), and pupping larger animals in the warmer, summer months when the density of large predators in coastal waters is reduced and pups are conferred the advantage of the larger size at birth. Birthing later in the year may also enable mothers to increase the size of their pups by exploiting this seasonal prey base, although there has been limited evidence to date of sardines, Sardinops sagax, in the diet of juvenile or pregnant female sharks (Van der Elst 1979; Dudley et al. 2005; Hussey et al. 2009b). Additionally, the effect of month and the linear increase in near-term pup mass may suggest that carcharhinid sharks are able to regulate the length of the gestation period dependent on both maternal condition and resource availability. Similar observations have been reported for large terrestrial mammals (Kiltie 1982; Asher 2007; Mysterud et al. 2009).

For the spinner shark, our best-fit model result found that female near-term pups were slightly heavier than males. Sex-biased pre- and postnatal maternal investment in young is widely recognised, and is thought to be adaptive dependent on species-specific life-



Fig. 4. The relationship between pregnant female size (precaudal length) and year (1978-2006) for the dusky (*Carcharhinus obscurus*) and spinner (*Carcharhinus brevipinna*) shark. Black dots are raw data with fitted linear regression lines (for the dusky: y = 0.04Year +183;  $F_{1,2228} = 1.44$ , p = 0.23; and for the spinner: y = 0.35Year - 495;  $F_{1,1274} = 79.23$ , p < 0.0001).

history strategies (Charnov 1982; Cockburn *et al.* 2002). For some carcharhinid sharks, including the spinner shark, sexual-size dimorphism occurs with females attaining a larger overall size than males (Cliff *et al.* 1988; Allen & Cliff 2000). This is in contrast to most polygynous mammals, where sexual-size dimorphism is male biased (Weckerly 1998), and selection for larger size is thought to be related to male-male competition for females (Clutton-Brock 1989), and higher variation in male reproductive success

(Cockburn *et al.* 2002). Our result, in conjunction with the observed increase in reproductive output with size of mature female, indicate that maternal investment is more important for the reproductive value (body size) and success (survival) of female offspring (Leimar 1996; Schulte-Hostedde *et al.* 2002; Koskela *et al.* 2009). However, when we consider the difference in mean body mass between sexes is minimal (35g, difference of 1.6% of female body weight) and that individual litter ratios can be sex biased (NSB unpubl. data), it is equally likely that this is a result of a litter ratio-pup mass-mother size relationship and future models examining pup mass as an indicator of fitness, should incorporate an associated interaction. Similarly the observed increase in spinner shark pup mass since the late 1970s, which was not seen for the dusky shark, was influenced by an increase in the length (PCL) of net-caught pregnant spinner sharks over time (Fig. 4). This increase in mean pup mass therefore reflects the higher reproductive output of larger females rather than possible density-dependent compensatory mechanisms reported for exploited fish species (Rose *et al.* 2001).

Aside from environmental phenotypic and predicted genotypic effects, this study affirms the prediction that reproductive effort will increase with age (Williams 1966) but with some evidence of a cost to the condition of individual newborn animals at larger maternal sizes due to an eventual decline in reproductive performance (Reznick et al. 2004; Tedesco et al. 2008). It is well-established that age-dependent patterns of reproductive performance can influence population dynamics of teleosts (Carr & Kaufman 2009) and consequently, neglecting the age structure of a population, may overestimate viable larvae production (Trippel et al. 1997; Scott et al. 1999); a similar case may exist for viviparous carcharhinids and the survival of their pups. Considering the large knowledge gap in our understanding of the reproductive success of female sharks and the associated fitness of their near-term pups in conjunction with the limited curvature of shark stock-recruitment curves (Kinney & Simpfendorfer 2009), the implications of a peak reproductive size in commercially harvested species are substantial. Small increases in fishing mortality can have a disproportionally large effect on population viability (Walters & Martell 2004), especially at key life-history stages. If depleted shark populations are to be efficiently restored there is considerable work to do in quantifying their life-histories and setting appropriate management conditions.

# **CHAPTER FIVE**

 $\delta^{15}$ N and  $\delta^{13}$ C diet-tissue discrimination factors for large sharks under semi-controlled conditions

Aspects of this work were presented at the IsoEcol VI Conference, University of Hawai'i at Manoa, Honolulu, U.S.A. 25<sup>th</sup> – 29<sup>th</sup> August, 2009.

The results of this chapter have been accepted for publication and are currently in press. Hussey, N.E., Brush, J., McCarthy, I.D. & Fisk, A.T. (2009)  $\delta^{15}N$  and  $\delta^{13}C$  diet-tissue discrimination factors for large sharks under controlled conditions. *Comparative Biochemistry* and Physiology – Part A.

## ABSTRACT

Stable isotopes ( $\delta^{15}$ N and  $\delta^{13}$ C) are being widely applied in ecological research but there has been a call for ecologists to determine species- and tissue-specific diet discrimination factors ( $\Delta^{13}$ C and  $\Delta^{15}$ N) for their study animals. For large sharks stable isotopes may provide an important tool to elucidate aspects of their ecological roles in marine systems, but laboratory based controlled feeding experiments are impractical. By utilizing commercial aquaria, we estimated  $\Delta^{15}N$  and  $\Delta^{13}C$  of muscle, liver, vertebral cartilage and a number of organs of three large sand tiger (Carcharias taurus) and one large lemon shark (Negaprion brevirostris) under a controlled feeding regime. For all sharks mean  $\pm$  SD for  $\Delta^{15}$ N and  $\Delta^{13}$ C in lipid extracted muscle using lipid extracted prev data were  $2.29\% \pm 0.22$  and  $0.90\% \pm 0.33$ , respectively. The use of non-lipid extracted muscle and prev resulted in very similar  $\Delta^{15}N$  and  $\Delta^{13}C$  values but mixing of lipid and non-lipid extracted data produced variable estimates. Values of  $\Delta^{15}N$  and  $\Delta^{13}C$  in lipid extracted liver and prey were  $1.50\% \pm 0.54$  and  $0.22\% \pm 1.18$ , respectively. Non-lipid extracted diet discrimination factors in liver were highly influenced by lipid content and studies that examine stable isotopes in shark liver, and likely any high lipid tissue, should strive to remove lipid effects through standardising C:N ratios, prior to isotope analysis. Mean vertebral cartilage  $\Delta^{15}$ N and  $\Delta^{13}$ C values were 1.45‰ ± 0.61 and 3.75‰  $\pm$  0.44, respectively. Organ  $\Delta^{15}$ N and  $\Delta^{13}$ C values were more variable among individual sharks but heart tissue was consistently enriched by ~1-2.5‰. Minimal variability in muscle and liver  $\delta^{15}N$  and  $\delta^{13}C$  sampled at different intervals along the length of individual sharks and between liver lobes suggests that stable isotope values are consistent within tissues of individual animals. To our knowledge, these are the first reported diet-tissue discrimination factors for large sharks under semi-controlled conditions, and are lower than those reported for teleost fish.

# INTRODUCTION

The application of naturally occurring isotopes of nitrogen ( $^{15}$ N) and carbon ( $^{13}$ C) to address ecological questions has grown exponentially over the last 20 years (Martinez del Rio & Wolf 2005; Martinez del Rio *et al.* 2009; Wolf *et al.* 2009). An important reason for this advancement is the ability to undertake minor invasive sampling of animals, such as blood, feathers and muscle biopsy (Hobson *et al.* 1993; Kurle & Worthy 2001) to study endangered or difficult-to-study species (Hobson 1999). This has led to ground breaking insights into the diet, trophic and movement ecology of organisms which were previously not well understood (e.g. Koch *et al.* 1995; Cherel & Hobson 2005; Caut *et al.* 2008a).

More recently, our understanding of species' isotopic diet profiles has improved through the development of stable isotope mixing models such as IsoSource (Phillips & Greg 2003) and MixSIR (Semmens & Moore 2008). These multi-source models have enabled ecologists to quantify complex diets of species, in many cases without sacrificing the animal and without the need for laborious stomach content analysis. Isotope mixing models are based on potential contributions of different isotopic sources (i.e., prey) to an isotopic mixture (i.e., the predator), but require species- and tissuespecific knowledge of the diet discrimination factor ( $\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{prev}$  and  $\Delta^{13}C = \delta^{13}C_{\text{consumer}} - \delta^{13}C_{\text{prey}}$ ) of the study species. Mean  $\Delta^{15}N$  and  $\Delta^{13}C$  of ~3.4‰ and ~1.0‰, respectively, have been determined to be appropriate for general use in ecological isotopic frameworks (DeNiro & Epstein 1978; Fry et al. 1984; Minagawa & Wada 1984; Post 2002). These values have been adopted and widely applied in the literature (see review by Caut et al. 2009; Martinez del Rio et al. 2009). Several authors, however, have questioned both trophic level calculations and the precision of mixing models, based on concerns over the inclusion of suitable diet discrimination factors for the species and/or tissue type in question (Gannes et al. 1997; Robbins et al. 2005; Caut et al. 2008b, 2009, Martinez del Rio et al. 2009). This is in light of multiple studies that have identified large differences in diet-tissue discrimination factors between taxa (Vanderklift & Ponsard 2003; Caut et al. 2009), between tissues (Pinnegar & Polunin 1999; MacNeil et al. 2006), with diet quality (McCutchan et al. 2003; Robbins et al. 2005) and with environment and feeding rate (Barnes et al. 2007). To refine the accuracy and precision of conclusions drawn from stable isotopes, there have been repeated calls for ecologists to determine diet-tissue discrimination factors for their study species through controlled laboratory experiments (Gannes *et al.* 1997; Caut *et al.* 2008b; Wolf *et al.* 2009). Moreover, if stable isotopes are to be considered as informative metrics for management and conservation, confidence in their interpretation is required.

Sharks are generally large, highly migratory predators that are typically difficult to study in their natural environment. Correctly applied, stable isotope methods may provide a versatile ecological tool to complement and further our understanding of this important (Heithaus et al. 2008) and threatened (Baum et al. 2003) group of marine vertebrates. To date, only a few studies have applied stable isotopes to sharks to investigate trophic level (Fisk et al. 2002; Estrada et al. 2003, 2006), diet and diet switching (Domi et al. 2005; MacNeil et al. 2005) and isotope turnover rates (MacNeil et al. 2006). In studies where a diet discrimination factor was used, however, the reported values of ~3.4‰ and ~1.0‰ ( $\Delta^{15}$ N and  $\Delta^{13}$ C respectively) were assumed. Using this surrogate value, Fisk et al. (2002) reported inconsistencies between trophic level estimated by stable isotopes and contaminant tracers in the Greenland shark (Somniosus microcephalus). In contrast, Estrada et al. (2003) found that trophic level estimates for the blue (Prionace glauca), shortfin mako (Isurus oxyrinchus), thresher (Alopias vulpinus) and basking shark (Cetorhinus maximus) were in agreement with those calculated using standardised diet compositions (Cortés 1999). Observed discrepancies, in conjunction with the call from the literature, accentuate the need to establish baseline diet-tissue discrimination factors for large sharks.

For many organisms, controlled rigorous experimental designs enable researchers to estimate  $\Delta^{15}$ N and  $\Delta^{13}$ C values in multiple tissues (e.g. Pinnegar & Polunin 1999; Caut *et al.* 2008c). For sharks, the complications of maintaining large individuals in captivity under laboratory conditions make this approach impractical and for some species impossible. Here we opportunistically sampled large sharks (sand tiger<sup>1</sup>, *Carcharias taurus* and lemon shark, *Negaprion brevirostris*) held in commercial aquaria to estimate diet-tissue discrimination factors for white muscle and liver tissue, vertebral cartilage

<sup>&</sup>lt;sup>1</sup> Carcharias taurus are referred to as 'sand tiger shark' in Europe and North America, 'raggies' in South Africa and 'grey nurse shark' in Australia.

and a selection of internal organs. Detailed feeding records and samples of diet items for stable isotope analysis were maintained by the aquaria allowing us to generate an accurate estimate of the feeding history of the individual sharks.

# **MATERIALS AND METHODS**

### **Experimental sharks**

Monitoring of four large sharks (three sand tiger and one lemon shark) held at three public aquaria [The Deep, Hull (TD); The Blue Planet, Ellesmere Port (BP) and Deep Sea World, North Queensferry (DSW)] was undertaken. All sharks were obtained from the wild as juvenile animals, maintained in captivity for extended periods of time and were euthanized due to medical conditions; details of the sharks sampled are included in Table 1.

### Feeding history and environmental conditions

The feeding history of the three sharks, including the (i) feeding dates, (ii) prey species, and (iii) mass of each prey item fed to individual sharks were recorded at all aquaria for the 12 month period prior to euthanasia. For each shark, the percent gravimetric weights of each prey item to total diet (for the 12 month period) were then calculated. Feeding records demonstrated that sharks fed normally until the point of euthanasia. Archived data on feeding regimes were also accessed to determine that all four sharks had been held on constant diets for a minimum of 2 years prior to this point. To determine mean annual tank temperatures, data loggers were installed in each tank (Tinytag, Gemini Data Loggers Ltd, Chichester, UK) and programmed to log data at 45 minute intervals.

### Tissue sampling, preparation and analysis

Various tissue types were sampled from each shark: (1) 5g white muscle tissue samples were excised from the dorsal muscle block at 20 cm intervals between the posterior section of the gills and anterior to the caudal fin; (2) 5g liver samples were excised from

	Table 1.	Details of the four	large sharks sample	ed from Dee	p Sea World (	(DSW). The	Deep (TD)	and the Blue	Planet (BP) aq	Juaria
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Aquaria	Species	Common name	Dissection date	Reason for euthanasia	Total length	Sex	Maturity status	Estimated age (yr) <sup>a</sup>
					(cm)			
DSW	Carcharias taurus	sand tiger	27/06/2006	Spinal disorder <sup>b</sup>	198.0	М	mature- adult	7-8
TD	Carcharias taurus	sand tiger	05/06/2008	Spinal disorder <sup>b</sup>	241.6	F	mature- adult	10-11
BP	Carcharias taurus	sand tiger	07/05/2009	Spinal disorder <sup>b</sup>	261.0	М	mature adult	12-13
BP	Negaprion brevirostris	lemon shark	01/03/2007	Neurological condition	199.0	М	sub-adult	9-10

<sup>a</sup> Sharks were introduced to aquaria at age 1-2 yrs. Age is estimated on the period of time maintained in captivity prior to euthanasia plus 1-2 yrs.

<sup>b</sup> Spinal deformities are reported in wild sharks (Hoenig & Walsh 1983; Bansemer & Bennett 2009) and are not known to affect feeding regime or overall animal condition (Heupel *et al.* 1999).

the upper/mid and lower region of both liver lobes; (3) 5g of other organs were sampled where practical and: (4) vertebral centra were excised from anterior to the dorsal fin<sup>2</sup>.

Muscle tissue of prey items was sampled from each bulk food order acquired by individual aquaria, between March 2006 and March 2007 (for TD and BP, prey samples were collected until May 2008 and May 2009, respectively). For teleost prey, muscle was excised from the dorsal/flank muscle section anterior to the first dorsal fin; for cephalopods, muscle tissue from both mantle and tentacles were sampled. Total mass (g) and fork length (FL)/ mantle length (ML) were recorded for all sampled prey. All shark and prey samples were immediately frozen and stored at -20°C prior to analysis. For certain occasional prey items (e.g. hake, *Merluccius merluccius*), samples were not available and stable isotope values were sourced from the literature.

Tissues sampled from both shark and prey were freeze-dried and homogenised in an SPEX CertiPrep 8000-D ball milling unit (SPEX CertiPrep; Metuchen, NJ, USA). Vertebral centra were cleaned to remove connective tissue and then oven dried at 40°C for 24 hours. The outer edges of the corpus calcareum of each centra, i.e. the most recent growth band, were drilled using a 0.4mm diameter steel carbide burr (Minerva Dental Ltd) attached to a hand-held dental drill.

For shark and prey muscle tissue and shark internal organs, lipid extraction was undertaken by twice agitating the dried powdered tissue in a 2:1 chloroform-methanol solution for 24 hrs according to MacNeil *et al.* (2005). The tissue and solvent were then filtered through 25mm GF/F filters and the resulting residue/filter paper dried at  $60^{\circ}$ C for 48 hours to evaporate off remaining solvent. For shark liver tissue, which has a high lipid content (Jayasinghe *et al.* 2003), lipid extraction was undertaken using a 2:1 chloroform-methanol soxhlet extraction for a period of 16 hours and samples dried as above. Between 400-600 µg of both non-lipid extracted (BULK) and lipid extracted (LE) dried tissue per sample were weighed into tin capsules and both stable carbon and nitrogen isotope ratios and total percent carbon (C) and percent nitrogen (N) were determined by a continuous flow isotope ratio mass spectrometer (IRMS, Finnigan

 $<sup>^2</sup>$  Vertebral centra were excised anterior to the deformity in the spine, where this condition occurred – see Table 1.

MAT Delta V, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valenica, CA, USA).

Stable isotope abundances are expressed in delta ( $\delta$ ) values as the deviation from standards in parts per thousand (‰) from the following equation:

$$\delta^{15}$$
N or  $\delta^{13}$ C =  $\left[ \left( \begin{array}{c} -R_{sample} \\ R_{standard} \end{array} \right) - 1 \right] \ge 1000$  (1)

where R is the ratio  ${}^{15}$ N/ ${}^{14}$ N or  ${}^{13}$ C/ ${}^{12}$ C. The standard reference material was Pee Dee Belemnite carbonate for CO<sub>2</sub> and atmospheric nitrogen N<sub>2</sub>. The analytical precision based on the standard deviation of three standards (82 standards analysed) for  $\delta^{15}$ N ranged from 0.13‰ to 0.15‰ and for  $\delta^{13}$ C ranged from 0.03‰ to 0.07‰ during the analysis of these samples. The analysis of NIST standards (sucrose and ammonia sulphate; n = 3 for each) during the analysis of samples generated values that were within 0.01‰ and 0.07‰ of certified values for  $\delta^{15}$ N and  $\delta^{13}$ C, respectively.

### Lipid extraction effects

Paired t-tests were performed to examine the effects of lipid extraction on  $\delta^{13}$ C, C,  $\delta^{15}$ N and N of the muscle tissue of prey items fed to sharks. The mean difference between BULK and LE  $\delta^{13}$ C, C,  $\delta^{15}$ N, N and C:N ratio of (i) shark muscle, (ii) shark liver and (iii) muscle tissue of prey items fed to sharks was then calculated to examine directional trends of the lipid extraction process on the above defined parameters of the three sets of tissues.

### Diet-tissue discrimination factor calculation

To enable the calculation of diet-tissue discrimination factors, the isotopic composition of the total diet of each shark over the 12 month period prior to euthanasia were calculated. Accepting that prey items vary in proximate composition, i.e. the proportion of BULK N and BULK C, it was first necessary to constrain the fractional contributions of N, C and biomass (i.e. the % gravimetric contribution; B) to 1. If  $f_{X,B}$ ,  $f_{Y,B}$  and  $f_{Z,B}$ equal the fractions of assimilated biomass of prey items X, Y and Z and  $f_{X,N}$ ,  $f_{Y,N}$ ,  $f_{Z,N}$ ,  $f_{X,C}$ ,  $f_{Y,C}$  and  $f_{Z,C}$  represent the fractions of assimilated N and C of individual prey items, then:

$$f_{XB} + f_{YB} + f_{ZB} = 1$$
 (2)

$$f_{X,N} + f_{Y,N} + f_{Z,N} = 1$$
(3)

$$f_{\rm X,C} + f_{\rm Y,C} + f_{\rm Z,C} = 1 \tag{4}$$

According to Phillips & Koch (2002), the contribution of a prey item to the consumer was assumed to be proportional to the assimilated biomass multiplied by the elemental (N and C) concentration of that prey. So if  $[N]_X$ ,  $[N]_Y$ ,  $[N]_Z$ ,  $[C]_X$ ,  $[C]_Y$  and  $[C]_Z$  are equal to the contributions of N and C in prey items X,Y and Z, then for nitrogen:

$$f_{X,N} = \frac{f_{X,B}[N]_X}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z}$$
(5)

$$f_{\rm Y,N} = \frac{f_{\rm Y,B}[N]_{\rm Y}}{f_{\rm X,B}[N]_{\rm X} + f_{\rm Y,B}[N]_{\rm Y} + f_{\rm Z,B}[N]_{\rm Z}}$$
(6)

$$f_{Z,N} = \frac{f_{Z,B}[N]_Z}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z}$$
(7)

The above calculations were repeated for carbon,  $f_{X,C}$ ,  $f_{Y,C}$ ,  $f_{Z,C}$  by substituting [C]<sub>X</sub>, [C]<sub>Y</sub> and [C]<sub>Z</sub> for [N]<sub>X</sub>, [N]<sub>Y</sub> and [N]<sub>Z</sub>. The isotopic signatures ( $\delta^{15}$ N and  $\delta^{13}$ C) of total diet ( $\delta^{15}$ N Diet<sup>T</sup> and  $\delta^{13}$ C Diet<sup>T</sup>), corrected for C and N concentration, were then calculated as:

$$\delta^{15}$$
N Diet<sup>T</sup> =  $f_{X,N} \delta^{15} N_X + f_{Y,N} \delta^{15} N_Y + f_{Z,N} \delta^{15} N_Z$ ; and (8)

$$\delta^{13} C \text{ Diet}^{T} = f_{X,C} \,\delta^{13} C_X + f_{Y,C} \,\delta^{13} C_Y + f_{Z,C} \,\delta^{13} C_Z \tag{9}$$

where  $\delta^{15}N$  Diet <sup>T</sup> or  $\delta^{13}C$  Diet <sup>T</sup> equal the total diet isotopic signature expressed as stable isotopes of nitrogen and carbon and  $\delta^{15}N_X$ ,  $\delta^{15}N_Y$ ,  $\delta^{15}N_Z$ ,  $\delta^{13}C_X$ ,  $\delta^{13}C_Y$  and  $\delta^{13}C_Z$  are the  $\delta^{15}N$  and  $\delta^{13}C$  values of prey items X, Y and Z. Both BULK diet (DIET<sup>T</sup><sub>BULK</sub>) and LE diet (DIET<sup>T</sup><sub>LE</sub>) were calculated.

Diet discrimination factors ( $\Delta^{15}$ N and  $\Delta^{13}$ C) for each shark BULK and LE white muscle tissue, BULK and LE liver tissue, vertebral cartilage and LE organ were calculated as:

$$\Delta^{15} N = (\delta^{15} N_{\text{tissue}} - \delta^{15} N \text{ Diet}^{\mathrm{T}})$$
(10)

$$\Delta^{13}C = (\delta^{13}C_{\text{tissue}} - \delta^{13}C \text{ Diet }^{\text{T}})$$
(11)

where  $\delta^{15}N_{tissue}$  and  $\delta^{13}C_{tissue}$  is the nitrogen and carbon isotope value of the BULK or LE shark muscle tissue, BULK or LE liver tissue, vertebral cartilage and LE organ of an individual shark. Data are presented as mean  $\pm$  SD.



**Fig. 1.** Percent gravimetric contribution of prey items to total diet of the Deep Sea World (DSW) sand tiger (*Carcharias taurus*), The Deep (TD) sand tiger, The Blue Planet (BP) sand tiger and the Blue Planet (BP) lemon shark (*Negaprion brevirostris*) for the 12 month period prior to euthanasia. Note hake (*Merluccius merluccius*) constituted 0.40% to total diet of the BP lemon shark; whiting (*Merlangius merlangus*) and mullet (*Liza ramada*) constituted 1.20% and 0.67% to total diet of the BP sand tiger, respectively.

### RESULTS

Mean annual tank temperatures were  $16.27^{\circ}C \pm 1.97$ ,  $24.37^{\circ}C \pm 0.96$ , and  $24.29^{\circ}C \pm 1.39$  for DSW, TD and BP, respectively. The diet of the TD sand tiger, BP sand tiger and BP lemon shark consisted predominantly of single prey items, haddock (*Melanogrammus aeglefinus*), trevally (*Pseudocaranx dentex*) and octopus (*Eledone cirrhosa*), respectively, while the DSW sand tiger diet was composed of trevally, saithe (*Pollachius virens*) and mackerel (*Scomber scombrus*) in descending order of importance (Fig. 1).

Lipid extracted and bulk muscle tissue  $\delta^{15}$ N, N,  $\delta^{13}$ C, C and C:N ratios of all prey items fed to sharks are presented in Table 2. For  $\delta^{15}$ N and  $\delta^{13}$ C there was minimal variance among prey samples, with the exception of TD haddock, BP mullet and BP octopus (SD >1; Table 2). The proximate composition of fish prey items was similar based on C and N values; cephalopod prey was depleted in both C and N relative to fish (Table 2). As a result of lipid extraction,  $\delta^{13}$ C values of prey items were enriched and C depleted relative to BULK values. For  $\delta^{15}$ N, LE prey samples were enriched by 0.47 ± 0.10 ‰ (mean ± SD) when compared to BULK. Total N of most prey items significantly increased following lipid extraction (Table 3; Fig 2a).

There was minimal variance in  $\delta^{15}N$  and  $\delta^{13}C$  of BULK and LE muscle tissue with sampling location along the length of each shark ( $\delta^{15}N$  and  $\delta^{13}C$  SD range: 0.14 – 0.30 and 0.24 – 0.78, respectively; Table 4). Lipid extraction resulted in an increase in  $\delta^{15}N$  and a corresponding decrease in N of shark muscle tissue, while  $\delta^{13}C$ , C and C:N ratio increased (Table 4; Fig. 2b).

Equally, there was minimal variance in  $\delta^{15}N$  and  $\delta^{13}C$  of bulk and lipid extracted liver tissue sampled from upper, mid and lower sections of each lobe of the three sharks where data were available ( $\delta^{15}N$  and  $\delta^{13}C$  SD range: 0.06 – 0.22 and 0.17 – 0.91, respectively; Table 4). Neither  $\delta^{15}N$  nor  $\delta^{13}C$  differed between right or left liver lobe of the TD sand tiger ( $\delta^{15}N$ : T<sub>3</sub> = 1.0, p = 0.42;  $\delta^{13}C$ : T<sub>3</sub> = 0.98, p = 0.43, respectively), BP sand tiger ( $\delta^{15}N$ : T<sub>3</sub> = -0.18, p = 0.87;  $\delta^{13}C$ : T<sub>3</sub> = -1.77, p =0.22, respectively) or BP lemon shark ( $\delta^{15}N$ : T<sub>3</sub> = 0.24, p = 0.83;  $\delta^{13}C$ : T<sub>3</sub> = 0.41, p = 0.72, respectively). Lipid

**Table 2:** Mean ( $\pm$  SD) lipid extracted (LE) and non-lipid extracted (BULK) stable nitrogen and carbon ( $\delta^{15}$ N and  $\delta^{13}$ C), total nitrogen and carbon (%N and %C), and C:N ratio in tissues of shark prey items sampled from Deep Sea World (DSW), The Deep (TD) and the Blue Planet (BP) aquaria and calculated  $\delta^{15}$ N and  $\delta^{13}$ C total diet isotopic signatures for the four sharks (see text). Mean mass (g) and fork length (cm) of prey samples are provided.

Prey item	N	Mass (g)	Fork length (cm)	$\delta^{15}N$	%N	$\delta^{13}C$	%C	C:N
DSW - sand tiger (C. taurus)								
LE saithe (P. virens)	15	$2310.0 \pm 1703.0$	$52.20 \pm 7.04$	$11.97\pm0.74$	$14.15\pm0.11$	$-19.11 \pm 0.79$	$44.81\pm0.30$	$3.17\pm0.03$
BULK saithe (P. virens)				$11.52 \pm 0.71$	$13.93\pm0.11$	$-19.34 \pm 0.84$	$44.87\pm0.43$	$3.22 \pm 0.02$
LE mackerel (S. scombrus)	6	$432.0 \pm 287.0$	$30.16 \pm 2.14$	$13.13 \pm 0.16$	$14.12 \pm 0.12$	$-18.04 \pm 0.11$	$45.01 \pm 0.26$	$3.19 \pm 0.04$
BULK mackerel (S. scombrus)				$12.56 \pm 0.10$	$12.88 \pm 0.78$	$-18.68 \pm 0.52$	$46.85 \pm 1.18$	$3.65 \pm 0.33$
LE trevally (P. dentex)	9	$772.2 \pm 258.7$	$33.78 \pm 4.79$	$14.41 \pm 0.48$	$14.33 \pm 0.11$	$-16.82 \pm 0.24$	$45.34 \pm 0.26$	$3.16 \pm 0.03$
BULK trevally (P. dentex)				$13.77 \pm 0.40$	$13.85 \pm 0.44$	$-17.11 \pm 0.43$	$45.91 \pm 1.28$	$3.32 \pm 0.20$
Calculated total diet (LE   BULK)				13.27   12.71		-17.89   -18.25		
<b>TD</b> - sand tiger ( <i>C. taurus</i> )								
LE haddock (M. aeglefinus)	26	$978.3 \pm 119.5$	$45.62 \pm 2.47$	$13.37 \pm 1.06$	$14.17\pm0.20$	$-16.97 \pm 0.76$	$44.76\pm0.40$	$3.16\pm0.06$
BULK haddock (M. aeglefinus)				$12.95 \pm 1.09$	$14.11\pm0.23$	$-17.30 \pm 0.72$	$45.08\pm0.42$	$3.20\pm0.05$
LE trevally (P. dentex)	7	$1246.9 \pm 130.7$	$39.71 \pm 1.76$	$13.16 \pm 0.22$	$14.27\pm0.18$	$-16.50 \pm 0.15$	$44.99 \pm 0.30$	$3.15\pm0.03$
BULK trevally (P. dentex)				$12.67\pm0.23$	$14.12\pm0.13$	$-16.69 \pm 0.13$	$45.61\pm0.56$	$3.23\pm0.06$
Calculated total diet (LE   BULK)				13.32   12.89		-16.87   -17.17		
<b>BP</b> – sand tiger ( <i>C. taurus</i> )								
LE trevally ( <i>P. dentex</i> )	18	$768.8 \pm 169.3$	$32.32 \pm 2.27$	$14.34\pm0.64$	$14.17\pm0.19$	$-16.94 \pm 0.62$	$44.68 \pm 0.48$	$3.15\pm0.05$
BULK trevally (P. dentex)				$13.93 \pm 0.63$	$14.45\pm0.10$	$-16.93 \pm 0.55$	$45.94\pm0.38$	$3.18\pm0.04$
LE grey mullet (L. ramada)	7	$907.0 \pm 447.0$	$37.62 \pm 2.21$	$16.62 \pm 3.17$	$13.81\pm0.13$	$-14.48 \pm 1.25$	$45.09\pm0.80$	$3.27\pm0.08$
BULK grey mullet (L. ramada)				$16.28 \pm 3.20$	$13.54\pm0.30$	$-14.65 \pm 1.21$	$46.85 \pm 1.07$	$3.46\pm0.14$

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LE whiting ( <i>M. merlangus</i> ) BULK whiting ( <i>M. merlangus</i> ) Calculated total diet (LE   BULK)	5	560 ± 84.0	32.03 ± 3.18	$16.28 \pm 0.75 \\ 15.79 \pm 0.78 \\ 14.40 \mid 13.99$	$13.63 \pm 0.15$ $14.16 \pm 0.07$	-16.61 ± 0.29 -16.57 ± 0.26 -16.90   -16.89	$\begin{array}{l} 43.54 \pm 0.36 \\ 44.92 \pm 0.20 \end{array}$	$3.19 \pm 0.01$ $3.17 \pm 0.01$
BP - lemon shark (N. brevirostris)								
LE giant squid (Teuthoida)	3	$662.5 \pm 53.0$	$30 \pm 1.4$	$14.46\pm0.42$	$12.14\pm0.06$	$-15.36 \pm 0.13$	$37.82 \pm 0.14$	$3.11 \pm 0.00$
BULK giant squid (Teuthoida)				$13.91 \pm 0.53$	$11.17\pm0.39$	$-17.05 \pm 0.24$	$41.35 \pm 0.19$	$3.70 \pm 0.15$
LE octopus (E. cirrhosa)	14	$433.9\pm100.8$	$12.09\pm2.08$	$10.11 \pm 1.02$	$12.67\pm0.41$	$-17.15 \pm 0.48$	$40.72 \pm 1.52$	$3.22 \pm 0.16$
BULK octopus (E. cirrhosa)				$9.51 \pm 1.14$	$12.03 \pm 0.59$	$-17.85 \pm 0.48$	$42.07 \pm 1.65$	$3.51 \pm 0.28$
LE squid (L. opalescens)	5	$40.42 \pm 11.22$	$10.76\pm1.54$	$13.57 \pm 0.74$	$12.86\pm0.42$	$-17.00 \pm 0.59$	$41.12 \pm 0.67$	$3.20 \pm 0.14$
BULK squid (L. opalescens)				$13.08\pm0.49$	$12.15\pm0.16$	$-17.83 \pm 0.51$	$42.74 \pm 0.35$	$3.52 \pm 0.03$
hake (M. merluccius)			9-15 <sup>a</sup>	11.84 <sup>a</sup>		-18.42 <sup>a</sup>		
horse mackerel (T.trachurus)				12.33 <sup>a</sup>		-18.14 <sup>a</sup>		
Calculated total diet (LE   BULK)				11.05   10.50		-17.05   -17.81		

<sup>a</sup>Values obtained from Le Loc'h et al. (2005).

	n	δ <sup>13</sup> C		%C		$\delta^{15}N$		%N	
		<i>t</i> -value	р	t-value	р	t-value	р	t-value	р
DSW prey									
Saithe	15	-7.82	<0.0001	0.75	0.466	-19.18	<0.0001	-5.96	<0.0001
Mackerel	6	-3.08	0.027	3.84	0.012	-7.95	0.001	-3.93	0.011
Trevally	9	-2.38	0.045	1.49	0.174	-12.64	<0.0001	-3.80	0.005
TD prey									
Haddock	26	-13.93	<0.0001	5.01	<0.0001	-14.71	<0.0001	-2.33	0.029
Trevally	7	-2.93	0.026	2.88	0.028	-8,98	<0.0001	-2.38	0.055
BP ST prey									
Trevally	18	0.14	0.891	8.09	<0.0001	-11.63	<0.0001	5.60	<0.0001
Mullet	7	-1.36	0.223	4.55	0.004	-17.99	<0.0001	-2.26	0.065
Whiting	5	0.58	0.591	6.15	0.004	-4.48	0.011	7.52	0.002
BP L prey									
Giant Squid	3	-9.35	0.011	20.98	0.002	-38.94	0.001	-7.09	0.019
Octopus	14	-7.95	<0.0001	6.48	<0.0001	-9.77	<0.0001	-8.69	<0.0001
Squid	5	-4.88	0.008	5.98	0.004	-3.26	0.031	-3.46	0.026

**Table 3:** The effects of lipid extraction on  $\delta^{13}$ C, %C,  $\delta^{15}$ N and %N values of prey items fed to sharks. Paired t-tests were used to compare non-lipid extracted (BULK) and lipid extracted (LE) data. Bold values indicate significant *t*-test results.

extraction of shark liver tissue resulted in an increase in total N and  $\delta^{13}$ C, a decrease in total C and C:N ratio and no change in  $\delta^{15}$ N (Table 4; Fig 2c).



Fig. 2. The effect of the lipid extraction process on  $\delta^{13}$ C, %C,  $\delta^{15}$ N, %N and C:N ratio of (a) muscle tissue of prey items fed to the shark, (b) shark muscle tissue and, (c) shark liver tissue sampled from aquaria under controlled conditions. Note: LE are values for lipid extracted tissues and BULK are for non-lipid extracted tissue.
**Table 4:** Stable nitrogen and carbon ( $\delta^{15}$ N and  $\delta^{13}$ C), total nitrogen and carbon (%N and %C) and C:N ratio in tissues, vertebral material and organs of sand tiger sharks (*Carcharias taurus*) and a lemon shark (*Negaprion brevirostris*) sampled from Deep Sea World (DSW), and The Deep (TD) and the Blue Planet (BP) aquaria. Corresponding diet-tissue discrimination factors are detailed ( $\Delta^{15}$ N and  $\Delta^{13}$ C in bold) for both lipid extracted diet (DIET<sub>LE</sub>) and non-lipid extracted diet (DIET<sub>BULK</sub>). Note, for muscle tissue, n is the number of samples analysed along the length of each shark and for liver is upper/mid and lower samples of each lobe. Data are ± 1 SD. Note: BULK are values for non-lipid extracted tissue and LE for lipid extracted tissue.

Tissue	n	$\delta^{15}N$	%N	$\Delta^{15}N$	$\delta^{13}C$	%C	$\Delta^{13}C$	C:N
				$DIET_{LE} \mid DIET_{BULK}$			$DIET_{LE} \mid DIET_{BULK}$	
DSW sand tiger								
BULK muscle	4 <sup>a</sup>	$14.99 \pm 0.19$	$16.31\pm0.16$	1.72   2.28	$-17.12 \pm 0.08$	$43.26 \pm 0.28$	0.77   1.13	$2.65\pm0.03$
LE muscle	4 <sup>a</sup>	$15.54 \pm 0.16$	$14.85 \pm 0.05$	2.27   2.83	$-16.63 \pm 0.11$	$44.70 \pm 0.24$	1.26   1.62	$3.01\pm0.01$
Vertebrae EDGE		14.18	6.67	0.91  1.47	-14.20	18.32	3.69   4.05	2.75
TD sand tiger								
BULK muscle	7	$15.64 \pm 0.19$	$16.01\pm0.11$	2.32   2.75	$-16.31 \pm 0.08$	$42.51 \pm 0.78$	0.56   0.86	$2.65\pm0.05$
LE muscle	7	$15.47\pm0.30$	$14.87\pm0.12$	2.15   2.58	$-15.80 \pm 0.19$	$43.24 \pm 0.40$	1.07   1.37	$2.91\pm0.05$
BULK liver	6	$15.27 \pm 0.07$	$3.44\pm0.70$	1.95   2.38	$-22.30 \pm 0.63$	$64.21 \pm 11.94$	-5.42   -5.13	$19.88 \pm 7.06$
LE liver	6	$15.36 \pm 0.21$	$10.48\pm2.80$	2.04   2.47	$-15.52 \pm 0.27$	$40.74 \pm 5.26$	1.35   1.65	$3.33\pm0.09$
LE heart	1	18.14	13.89	4.82   5.25	-15.46	43.08	1.41   1.71	3.10
LE kidney	1	15.67	13.69	2.35   2.78	-15.36	38.95	1.51   1.81	2.84
LE spleen	1	15.02	13.85	2.22   2.65	-15.21	41.32	1.86   2.16	2.98
LE spiral valve	1	15.54	14.35	1.70   2.13	-15.01	41.15	1.66   1.96	2.87
LE stomach	1	15.24	13.69	1.92   2.35	-14.04	40.38	2.84   3.13	2.95
BP sand tiger								
BULK muscle	4 <sup>b</sup>	$16.26 \pm 0.18$	$15.94 \pm 0.09$	1.86   2.27	$-16.31 \pm 0.09$	$42.74 \pm 0.25$	0.59   0.58	$2.68 \pm 0.01$
LE muscle	4 <sup>b</sup>	$16.54 \pm 0.22$	$13.83\pm0.27$	2.14   2.55	$-16.20 \pm 0.08$	$43.09\pm0.51$	0.70   0.70	$3.12\pm0.02$

BULK liver	6	$15.56 \pm 0.22$	$2.87\pm0.50$	1.16   1.57	$-22.21 \pm 0.17$	$73.97 \pm 5.92$	-5.31   -5.32	$26.39 \pm 4.41$
LE liver	6	$15.36 \pm 0.09$	$11.26 \pm 0.99$	0.96   1.37	$-16.58 \pm 0.91$	$45.14 \pm 2.45$	0.32   0.31	$4.06 \pm 0.61$
LE heart	1	17.26	12.89	2.86   3.27	-16.00	42.27	0.90   0.90	3.28
Vertebrae EDGE		15.72	9.24	1.32   1.73	-13.56	24.29	3.34   3.33	2.63
LE kidney	1	15.51	13.90	1.11   1.52	-14.74	39.84	2.16   2.16	2.87
LE spleen	1	15.16	14.06	0.76   1.17	-15.46	42.32	1.44   1.43	3.01
LE spiral valve	1	15.92	14.10	1.52   1.93	-14.86	42.19	2.04   2.04	2.99
LE stomach	1	16.53	14.32	2.12   2.53	-14.44	40.66	2.46   2.46	2.84
LE rectal gland	1	15.75	13.73	1.35   1.76	-15.59	41.03	1.31   1.31	2.99
LE pancreas	1	15.46	14.06	1.06   1.47	-15.23	42.22	1.67   1.66	3.00
LE testes	1	15.40	14.05	1.00   1.41	-14.51	40.35	2.39   2.39	2.87
BP lemon								
BULK muscle	5	$12.94 \pm 0.23$	$15.37\pm0.15$	1.88   2.44	$-17.02 \pm 0.15$	$41.90 \pm 0.30$	0.03   0.79	$2.73\pm0.04$
LE muscle	5	$13.65\pm0.14$	$14.54\pm0.12$	2.60   3.15	$-16.50 \pm 0.24$	$44.40 \pm 0.30$	0.55   1.31	$3.05 \pm 0.04$
BULK liver	6	$12.05 \pm 0.19$	$2.21 \pm 0.30$	0.99   1.55	$-24.41 \pm 0.18$	$69.90 \pm 1.67$	-7.36   -6.60	$32.22 \pm 5.0$
LE liver	6	$12.55\pm0.06$	$9.32\pm0.22$	1.50   2.05	$-18.05 \pm 0.19$	$44.17\pm0.63$	-1.00   -0.24	$4.74 \pm 0.12$
Vertebrae EDGE		13.16	6.35	2.11   2.66	-12.83	17.02	4.22   4.98	2.68
LE heart	1	15.09	14.03	4.04   4.59	-16.73	44.93	0.32   1.08	3.20
LE kidney	1	12.60	12.34	1.54   2.10	-17.30	42.57	-0.26   0.50	3.45
LE spleen	1	11.85	14.13	0.79   1.35	-16.96	42.54	0.09   0.85	3.01
LE spiral valve	1	13.41	14.14	2.36   2.91	-15.90	43.35	1.15   1.91	3.07
LE stomach	1	13.62	14.15	2.57   3.12	-15.98	43.17	1.07   1.83	3.05
LE rectal gland	1	14.59	13.08	3.54   4.10	-17.41	44.76	-0.36   0.40	3.42
LE pancreas	1	11.89	13.87	0.83   1.39	-17.03	42.42	0.02   0.78	3.06
LE testes	1	10.87	13.80	-0.18   0.38	-17.37	42.11	-0.32   0.44	3.05

<sup>a</sup> Note muscle section 2 (at ~ 20cm posterior to gill slits) and muscle section 4 (at ~ 60 cm posterior to gill slits) were lost due to freezer failure

<sup>b</sup> Four muscle samples were taken: posterior to gills / anterior to dorsal fin / posterior to second caudal fin and anterior to caudal fin.

Mean BULK and LE muscle tissue  $\delta^{15}N$  of the DSW, TD and BP sand tigers were higher than the BP lemon shark;  $\delta^{13}C$  values were similar between all sharks (Table 4). For the TD sand tiger, the LE liver  $\delta^{15}N$  value was similar to LE muscle, while the LE liver  $\delta^{15}N$  values of the BP sand tiger and BP lemon were depleted. For  $\delta^{13}C$ , LE liver values of the TD and BP sand tigers were similar to LE muscle, but the BP lemon  $\delta^{13}C$ was depleted (Table 4). The  $\delta^{15}N$  values of the outer edge of vertebral centra, i.e. vertebral cartilage, were lower than LE muscle tissue for all three shark sampled;  $\delta^{13}C$ values were enriched (Table 4). Values of  $\delta^{15}N$  and  $\delta^{13}C$  in most LE organs were similar or slightly depleted in  $\delta^{15}N$  and enriched in  $\delta^{13}C$  relative to LE muscle tissue (accepting a higher degree of variability in the BP lemon), but  $\delta^{15}N$  LE heart tissue of both species was consistently enriched by ~ 1 - 2.5% (Table 4).

C:N ratios, total C and the difference between BULK and LE  $\delta^{13}$ C values indicated that lipid extraction of internal organs was required and produced adequate results (C:N ~3.0; Table 4; Figs. 3b). For most LE organs,  $\delta^{15}$ N either remained the same (within analytical error) or there was a marginal increase, with the exception of spleen tissue, which was consistently depleted in  $\delta^{15}$ N in all three animals sampled (Fig. 3a)

Individual shark and mean  $\Delta^{15}$ N and  $\Delta^{13}$ C values for BULK and LE muscle and liver tissue, LE organs and vertebral cartilage calculated from both DIET<sub>BULK</sub> and DIET<sub>LE</sub> are presented in Tables 4 and 5, respectively. There was an observed difference in  $\Delta^{15}$ N values of LE white muscle tissue among the two species of sharks fed on different diets; the  $\Delta^{15}$ N of the BP lemon shark fed on cephalopods of 2.60‰ was higher than that of the DSW, TD and BP sand tigers fed on teleost prey (2.27‰, 2.15‰ and 2.14‰, respectively; Table 4). For  $\Delta^{13}$ C, the LE muscle tissue of the BP lemon was depleted relative to the three sand tiger sharks (Table 4). Accepting that  $\Delta^{13}$ C values of the BP lemon shark LE organs were highly variable and negative discrimination factors were calculated from DIET<sub>LE</sub>, this animal was excluded from the mean organ  $\Delta^{13}$ C summary in Table 5.



Fig. 3. The effect of the lipid extraction process on (a)  $\delta^{15}$ N and (b)  $\delta^{13}$ C of the organs of sand tiger (*Carcharias taurus*) and lemon (*Negaprion brevirostris*) sharks sampled from aquaria under controlled conditions. Note: LE are values for lipid extracted tissues and BULK are for non-lipid extracted tissue.

### DISCUSSION

The mean  $\Delta^{15}$ N values for LE shark muscle tissue, using LE prey data, of 2.29‰ ± 0.22 (all sharks) and 2.19‰ ± 0.07 (sand tiger sharks only), were lower than the widely applied values (3.4‰) of Minagawa & Wada (1984) and Post (2002) and lower, but more similar, to the mean values of 2.96‰ and ~2.5‰ reported for fish muscle by Vanderklift *et al.* (2003) and Caut *et al.* (2009), respectively. Sharks are unusual in that they retain levels of urea and trimethylamine oxide (TMAO) in their tissues for osmoregulatory purposes (Olson 1999). Fisk *et al.* (2002) suggested that as urea is a metabolic waste product, it would be isotopically light therefore rendering shark muscle

**Table 5:** Summary diet-tissue discrimination factors for non-lipid extracted (BULK) and lipid extracted (LE) muscle and liver tissue and vertebral cartilage for; (i) all sharks (fish and cephalopod diet) and (ii) sand tiger sharks only (fish diet), calculated from both LE and BULK total diet (DIET<sub>LE</sub> and DIET<sub>BULK</sub>). Summary diet-tissue discrimination factors for lipid extracted (LE) organs are also presented. Data  $\pm$  1SD.

SUMMARY					
Tissue	n	$\Delta^{15}N$ DIET <sub>LE</sub>	DIET <sub>BULK</sub>	$\Delta^{13}C$ DIET <sub>LE</sub>	DIETBULK
All sharks					
BULK muscle	4	$1.95 \pm 0.26$	$2.44 \pm 0.22$	$0.49 \pm 0.32$	$0.84 \pm 0.23$
LE muscle	4	$2.29 \pm 0.22$	$2.78\pm0.28$	$0.90 \pm 0.33$	$1.25 \pm 0.39$
BULK liver	3	$1.37 \pm 0.51$	$1.83 \pm 0.47$	$-6.03 \pm 1.15$	$-5.68 \pm 0.80$
LE liver	3	$1.50\pm0.54$	$1.96\pm0.56$	$0.22\pm1.18$	$0.57\pm0.97$
Vertebrae EDGE	3	$1.45\pm0.61$	$1.95\pm0.63$	$3.75 \pm 0.44$	$4.12\pm0.83$
Sand tiger sharks only					
BULK muscle		$1.97 \pm 0.31$	$2.43 \pm 0.27$	$0.85 \pm 0.20$	$0.86 \pm 0.28$
LE muscle	3	$2.19 \pm 0.07$	$2.65 \pm 0.15$	$1.01 \pm 0.29$	$1.23\pm0.48$
BULK liver	2	1.56	1.98	-5.37	-5.23
LE liver	2	1.50	1.92	0.84	0.98
Vertebrae EDGE	2	1.12	1.60	3.52	3.69
LE heart	3	$3.91 \pm 0.99$	$4.37 \pm 1.01$	1.16 <sup>a</sup>	1.31 <sup>a</sup>
LE kidney	3	$1.67 \pm 0.63$	$2.13 \pm 0.63$	$1.84^{\mathrm{a}}$	1.99 <sup>a</sup>
LE spleen	3	$1.26 \pm 0.83$	$1.72 \pm 0.81$	1.65 <sup>a</sup>	$1.80^{\mathrm{a}}$
LE spiral valve	3	$1.86 \pm 0.44$	$2.32\pm0.52$	$1.85^{\mathrm{a}}$	$2.00^{a}$
LE stomach	3	$2.20 \pm 0.33$	$2.67 \pm 0.40$	2.65 <sup>a</sup>	$2.80^{a}$

tissue artificially low in  $\delta^{15}$ N if not removed. For sharks an increase in  $\delta^{15}$ N of ~0.5‰ in muscle tissue was observed following lipid extraction. This effect on  $\delta^{15}$ N was similar to both that of teleost prey items in this study (Table 3) and the difference between BULK and LE  $\delta^{15}$ N muscle tissue previously reported by Sotiropoulos *et al.* (2004), Ingram *et al.* (2007) and Logan *et al.* (2008). Murray *et al.* (2006) and Ingram *et al.* (2007) suggested this increase in  $\delta^{15}$ N may be due to the leaching of nitrogenous metabolites or waste, principally ammonia and ammonium, via the lipid extraction process. Christie (1993) stated that chloroform-methanol may act to remove urea, and thus the observed increase in  $\delta^{15}$ N shark muscle tissue may be due to this effect. For shark muscle, there was also a large decrease in total N following lipid extraction, further providing evidence for the removal of nitrogenous waste products.

For the teleost and cephalopod prey items, there was a significant increase in  $\delta^{13}$ C and associated decrease in total C following lipid extraction as would be expected for tissue containing lipids. For shark muscle tissue, there was a marginal increase in  $\delta^{13}$ C, indicating low lipid content (Bone & Roberts 1969), but in contrast to the prey tissue, C increased. The diametric directional trends of total C and N of shark and prey muscle indicate that the prey had proportionally higher lipid content than nitrogenous waste, in contrast to shark muscle. The issue of increasing  $\delta^{15}$ N following lipid extraction and whether this correlates with urea/ammonia/ammonium removal, however, requires further investigation. Work undertaken by Moeri *et al.* (2003), may suggest that the urea signature is labelled in muscle tissue at the amino acid level.

White muscle  $\Delta^{15}$ N values of the three sand tiger sharks were similar but lower than that of the lemon shark. These  $\Delta^{15}$ N differences could be species-specific (Kurle 2002), but might also be explained by diet. The BP lemon shark was fed on a predominantly cephalopod diet with lower  $\delta^{15}$ N and total N values that contrast the teleost diet of the three sand tigers (Table 2). The significant negative relationship between  $\Delta^{15}$ N values and  $\delta^{15}$ N diet values reported by Felicetti *et al.* (2003), Caut *et al.* (2008b, 2009) and Overmyer *et al.* (2008) may therefore explain the enriched  $\Delta^{15}$ N value recorded for the BP lemon shark.

For  $\Delta^{13}$ C, the mean value for shark muscle tissue of 0.90‰ ± 0.33 (all sharks) and 1.01‰ ± 0.29 (sand tigers only) were similar to the widely accepted value of ~1.0‰ but lower than recent estimates for muscle tissue of 2‰ based on a controlled study in European sea bass, *Dicentrarchus labrax* (Barnes *et al.* 2007) and 1.8‰ based on a review of 41 estimates (Caut *et al.* 2009).

Mean  $\delta^{15}$ N and  $\delta^{13}$ C and calculated  $\Delta^{15}$ N and  $\Delta^{13}$ C values of LE liver were depleted relative to white muscle tissue in agreement with data for marine and freshwater fish (Pinnegar & Polunin 1999; MacNeil *et al.* 2005; Sweeting *et al.* 2007a). Previous work has suggested that lower  $\Delta^{15}$ N values in liver tissue reflect the larger proportion of essential amino acids in liver protein (Pinnegar & Polunin 1999; Kurle & Worthy 2002). There was no observed change in  $\delta^{15}N$  of liver tissue following lipid extraction in agreement with the findings of Logan *et al.* (2008). Whether this reflects low nitrogenous waste and/or urea concentrations (Ballantyne 1997) requires further investigation. Ingram *et al.* (2007) reported that the effect of lipid extraction on  $\delta^{15}N$ was reduced in muscle tissue with higher lipid content.

Total C values of LE liver tissue were of a similar magnitude to muscle, but there was greater variance in C concentrations of LE liver tissue among sharks. This may indicate variable success of the soxhlet extraction process. Additionally, total N values increased following lipid extraction, in contrast to muscle tissue. Similar to prey muscle tissue, this may be a result of the imbalance of removing large volumes of lipids and hence total C. The variable N and C concentrations in LE shark liver tissue, however, resulted in C:N ratios ranging from 3.33 to 4.74. This may suggest that previously reported C:N ratios for lipid extracted fish liver of 6.36-6.47 (Sweeting et al. 2006) and 3.2-6.4 (Logan et al. 2008), are either influenced by nitrogenous waste content (i.e. N concentration) or that standard lipid extraction methods are not effective for high lipid content liver tissue resulting in both variable C and N values. In previous experimental trials it was found that standard chloroform-methanol extraction of shark liver tissue produced highly variable results when compared to soxhlet extraction (Hussey & Fisk unpubl. data). Variable LE liver  $\Delta^{13}$ C values among the three sharks sampled, including a negative  $\Delta^{13}$ C value for the BP lemon shark, further indicate the complications of; (i) effectively standardising high lipid content tissues and; (ii) the variable metabolic nature of liver tissue in sharks (Hoffmayer et al. 2004; Hussey et al. 2009b).

The estimated  $\Delta^{15}$ N values of 1.47‰ ± 0.66 (all sharks) and 1.12 (sand tigers only), for vertebral cartilage, were lower than those for LE muscle tissue in this study and the mean value for collagen (~ 2‰) reported by Caut *et al.* (2009). In agreement with this data, MacNeil *et al.* (2005) found that  $\delta^{15}$ N cartilage values drilled from vertebral centra of blue (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*) and thresher sharks (*Alopias vulpinus*) were depleted relative to muscle tissue. These authors suggested that sampling across the vertebral surface may have resulted in a bias of the  $\delta^{15}$ N values by incorporating the larger growth bands of younger animals. The data derived in this study would suggest this may not be the case and that the use of a  $\Delta^{15}$ N of 3.4‰ for cartilage (Estrada *et al.* 2006; Kerr *et al.* 2006) may be inappropriate. The  $\Delta^{13}$ C

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estimates for vertebral cartilage were highly enriched compared to other tissue types, emphasising the need for future work to isolate the organic collagen portion of the matrix prior to  $\delta^{13}$ C analysis (Kerr *et al.* 2006).

With the exception of  $\delta^{13}$ C in the BP lemon shark LE kidney, LE rectal gland and LE testes, all other LE organs were enriched in <sup>15</sup>N and <sup>13</sup>C relative to diet; however the enrichment for other organs of the BP lemon was less pronounced. Generally the  $\delta^{15}$ N and  $\delta^{13}$ C LE organ data were similar between individuals but there was a degree of variability in  $\Delta^{13}$ C and  $\Delta^{15}$ N values of LE organs between individual sharks. It is well understood that isotopes contained in different dietary components are routed differentially to specific tissues (i.e. isotopic routing; Tieszen & Fagre, 1993). It is therefore possible that the varied and mixed diets of the individual experimental sharks may have resulted in variable isotopic routing to organs, producing variable diet-tissue discrimination factors. Of all organs sampled, LE heart tissue was considerably enriched in  $\delta^{15}$ N. This result was in agreement with Hobson *et al.* (1996) for captive harp seals (*Pagophilus groenlandicus*) and may reflect the abundance of non-essential amino acids in heart tissue (Wilson & Poe 1974).

Accepting the small sample size, these data provide the first estimates of  $\Delta^{15}$ N and  $\Delta^{13}$ C values determined under semi-controlled conditions for white muscle tissue, liver tissue, vertebral cartilage and internal organs of large sharks. The approach used is simplistic, similar to Hobson *et al.* (1996) and Kurle (2002), in that the sample size of individual animals was low and they received a varied diet. However, the mass contribution to diet,  $\delta^{15}$ N and  $\delta^{13}$ C signatures of prey items and N and C concentrations in individual prey were quantified in the diet calculation of all four sharks prior to euthanasia and considered in the estimates for diet-tissue discrimination factors. The inclusion of multiple dietary components, of mixed proximate composition, also provides the first realistic comparison to the diet of wild sharks. Given the threatened status of global stocks of sand tiger sharks (Musick *et al.* 2000b; Otway *et al.* 2004), these data are of particular importance.

Considering the known effects of growth rate on  $\Delta^{15}N$  and  $\Delta^{13}C$  values (Gaye-Siessegger *et al.* 2003; Trueman *et al.* 2005), future work should aim to investigate stable isotopes in both slow and fast growing juvenile sharks; dusky, *Carcharhinus* 

obscurus and bonnethead sharks, Sphyrna tiburo, may be suitable candidates. The relatively small size at birth of these sharks will enable a detailed examination of diettissue discrimination factors under controlled laboratory conditions. Accepting that sharks typically consume a diverse prey base [teleost, cephalopod, crustacean, marine mammal, elasmobranch and bird (Wetherbee & Cortés 2004)], further aquaria/laboratory investigation in to the effects of diets with variable  $\delta^{15}$ N, N and  $\delta^{13}$ C, C values on derived  $\Delta^{15}$ N and  $\Delta^{13}$ C estimates is of critical importance to advancing our knowledge of stable isotopes in this unique group of marine vertebrates.

# **CHAPTER SIX**

 $\delta^{15}$ N and  $\delta^{13}$ C ontogenetic profiles of large marine predators: viable indicators of trophic and movement ecology in sharks?

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### ABSTRACT

Stable isotopes are viewed as an important tool to examine human impacts on community structure but ontogenetic variation of individual species is generally not accounted for.  $\delta^{15}N$  and  $\delta^{13}C$  profiles of two large predatory sharks, the scalloped hammerhead (*Sphyrna lewini*) and the dusky (*Carcharhinus obscurus*) were used in conjunction with a comprehensive archival stomach content data set to examine species, size, gender, movement and method specific effects on calculated trophic position (TP) through ontogeny. Diet,  $\delta^{15}N$  and  $\delta^{13}C$  data revealed ontogenetic- and gender-specific shifts in diet composition indicating resource partitioning for and between both species.

Calculated TP for the two sharks varied by method, either  $\delta^{15}N$  (TP<sub>SIA</sub>) or stomach content (TPr), but in general increased with size for S. lewini and for C. obscurus, large animals fed at a similar TP to small animals. These results, together with large discrepancies between TP<sub>SIA</sub> and TP<sub>r</sub> for reproductively active C. obscurus females. reflect (i) the integrative periods of the two approaches, (ii) the inclusion of TP values for broad diet functional prey groups in the calculation of TPr, and (iii) movement of sharks between isotopically distinct food webs and the use of a single source isotope model in the calculation of TP<sub>SIA</sub>. With knowledge of systematic geographical variation in  $\delta^{13}$ C baseline values, ontogenetic  $\delta^{13}$ C profiles of S. lewini identified sexual segregation and habitat partitioning of individuals measuring  $\sim 130 - 160$  cm precaudal length. Large C. obscurus, in particular pregnant and postpartum sharks, had depleted  $\delta^{13}$ C signatures identifying periods of time foraging in offshore food webs. Therefore  $\delta^{13}$ C profiles in sharks can provide valuable insights into their movements. When considering community structure, large predatory sharks can influence multiple ecosystems, and the proportional effects on a single system vary by species, size and gender. Ontogenetic variation in TP and the associated interplay between  $\delta^{15}N$  and  $\delta^{13}C$ therefore need to be accounted for to refine community structure modelling. Nitrogen and carbon isotopes are a valuable tool, but caution in their interpretation is required and multifaceted research programmes are recommended.

# INTRODUCTION

Body size has long been recognised to have an important influence on the structural and functional complexity of aquatic food webs (Elton 1927). As predators are typically larger than their prey, trophic position is expected to increase with body size (Cohen *et al.* 1993). Size as a surrogate measure of trophic position has therefore been used as a tool to examine human induced impacts on community structure (Pinnegar *et al.* 2002; Dulvy *et al.* 2004a and b), alterations in trophic linkages (Pauly *et al.* 1998; Pauly & Watson 2005) and/or cascading effects of predator removal (Myers *et al.* 2007; Mumby *et al.* 2006). Assessments of community based stable isotope trophic structuring, however, are based on the simplified relationship between size and  $\delta^{15}$ N trophic position of the community as a whole (Jennings *et al.* 2001; Jennings & Mackinson 2003; Layman *et al.* 2005; Al-Habsi *et al.* 2008), and within-species differences, including ontogenetic variation, associated ontogenetic effects on different ecosystems and interplay between  $\delta^{15}$ N and  $\delta^{13}$ C are not clearly defined and/or generally accounted for.

High order consumers or large predators are typically species which exert top-down control and have a fundamental influence on the structure and function of marine food webs (Heithaus *et al.* 2008). These predators often exhibit a large alteration in size with maturation and consequently variation in their ontogenetic trophic profiles may directly influence inferences on food web structure and impacts on community structure over time (Myers & Worm 2003; Myers *et al.* 2007; Heithaus *et al.* 2008). This is of particular concern for large sharks, where gender and/or size classes are targeted by area-specific fishing practices (Mucientes *et al.* 2009). Furthermore, the removal of large individuals (Ward & Myers 2005) and the resulting reduced predation effect may ultimately lead to an abundance of smaller individuals of a species (Van der Elst 1979) and/or a population explosion of mesopredator prey populations (Myers *et al.* 2007). A more thorough understanding of species-specific trophic dynamics of large predators with relation to changes in body size is therefore required (Layman *et al.* 2005).

For organisms which undergo considerable change in body size between birth and maturation, ontogenetic switches in diet and/or habitat use are common (Post 2003).

The factors determining these ontogenetic diet shifts and corresponding changes in trophic position estimates are not clearly defined but a combination of factors may exert control, including: (1) reduced competition between sympatric species through inter-specific resource partitioning (Werner & Gilliam 1984; Papastiamatiou *et al.* 2006); (2) minimisation of predation risk by habitat selection and/or partitioning (Werner *et al.* 1983) and; (3) realisation of maximum growth potential (including reproductive growth) through exploiting different prey resources, both by type and size (Olson 1996).

Diet and trophic ecology of fish has been traditionally studied using stomach content analysis (Hyslop 1980; Cortés 1999). However, for sharks, as with many large highly mobile predatory fish, stomach content analysis is hindered on several scales by high vacuity rates, insufficient sampling of size classes, incomplete sampling across the geographic range of the animal both in temporal and spatial domains and by differences in detectability and digestibility among prey species. Coupled with this is the concern over the global decline of shark populations (Baum *et al.* 2003) and therefore the difficulty of ethically obtaining the required sample sizes for stomach content analysis. More recently, the stable isotopes of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) have proven to be a valuable tool in elucidating trophic relations in aquatic food webs (Vander Zanden *et al.* 1997; Post *et al.* 2000, 2002). The acceptance and development of this biochemical approach has led to a series of studies in which the trophic ecology of animals is examined by undertaking a holistic approach that combines the complementary aspects of traditional stomach content analysis and stable isotope analysis of long term integrative tissue (Vander Zanden *et al.* 1997; Post 2002).

The conservative fractionation of  $\delta^{13}$ C between primary producers and top level predators also provides a tool to track animal movements between regions with differing baseline sources of  $\delta^{13}$ C (Hobson 1999; Rubenstein *et al.* 2004). Considering the logistical difficulties and the expenses involved in the examination of fine scale movement patterns through the application of archival/satellite tracking telemetry (Block *et al.* 2001; Bonfil *et al.* 2005) there is a requirement to target specific species, sexes and life-stages to provide the required insights for effective management of exploited species. Ontogenetic  $\delta^{13}$ C data may provide coarse resolution movement profiles to guide the application of telemetry methods. The aim of the current study was therefore to examine size- and gender-specific variations in diet and estimated trophic position of the scalloped hammerhead (*Sphyrna lewini*) and dusky shark (*Carcharhinus obscurus*) by combining both stomach content analysis (from a long-term archived data base) and stable isotope analysis of muscle tissue. The utility of  $\delta^{13}$ C as an indicator of coarse resolution ontogenetic movement profiles in these sharks, and a third species, the spinner shark (*Carcharhinus brevipinna*) with nursery habitat in KZN, was also assessed because  $\delta^{13}$ C values have been shown to vary systematically across the geographic range of these species. Both  $\delta^{15}$ N and  $\delta^{13}$ C were used to investigate possible effects of resource and habitat partitioning over ontogeny.

#### MATERIALS AND METHODS

#### Location and sampling

All sharks included in the following analyses were sampled from animals incidentally caught in beach protection nets in KwaZulu-Natal (KZN), South Africa, with the exception of five juvenile scalloped hammerhead sharks and five juvenile spinner sharks, sampled from commercial trawl fisheries on the Tugela banks (Fig. 1). Beach net installations are set parallel to the shoreline, approximately 300-500m from the beach in 10-14m depth of water (Dudley *et al.* 2005). Net servicing is undertaken by the KwaZulu-Natal Sharks Board (KZNSB) and all dead sharks retrieved in good condition are transported to the KZNSB laboratory where they are stored frozen (-20°C) until public dissection. On arrival at the laboratory, basic data on species, sex and morphological measurements, including total body mass (TBM - kg), and precaudal length (PCL - cm) are recorded.

#### Stomach content analysis

The diets of the scalloped hammerhead and dusky shark were quantified using stomach content data accessed from the KZNSB archived database (1983 - 2006). A total of



**Fig. 1**. The study region, including the area protected by beach protection nets and the Tugela Banks in KwaZulu-Natal. The netted region is currently comprised of 38 individual net installations (see Dudley et al. 2005 for specific details on locations).

1018 scalloped hammerhead and 900 dusky sharks contained stomach contents and were included in the dietary analysis. During dissection, the complete stomach was removed from the shark, each prey item was identified to the lowest possible taxon and the number and mass (to 0.1g) of each prey item recorded. To summarise the stomach content data, all prey items were grouped to family level and then further divided into eight functional prey groups according to Cortés (1999) defined as; elasmobranch, teleost, cephalopod, crustacean, mollusc, mammal, bird and miscellaneous items. The percent gravimetric mass contribution (% M) of each functional prey group to the diet of the two shark species was then calculated according to Hyslop (1980). To determine gender-ontogenetic shifts in diet for the two species, the data were divided by sex and into 3 size classes: for scalloped hammerheads sharks; small (<110 cm), medium (110-140 cm) and large (> 140 cm): for dusky sharks; small (<100 cm), medium (100-209 cm) and large ( $\geq$  210 cm). Large female dusky sharks were further divided into

reproductively inactive ( $L_{inact}$ ), pregnant ( $L_{preg}$ ) and postpartum ( $L_{post}$ ) sharks. No reproductively active, pregnant or postpartum scalloped hammerhead sharks were included in the analysis. The size classes selected approximate to maturity stage and relate to species-specific life-strategies (Compagno 1984).

The percent gravimetric mass contribution of the functional prey groups for the two species of sharks divided by sex and the predefined size classes were (i) plotted in the form of histograms to enable visual examination of the important prey contributions to diet and (ii) subjected to non-metric multi dimensional scaling (MDS) ordination, to infer the overall influence of species, sex and body size on diet composition. A common problem when examining the diet of sharks is the limited number of prey items/types per individual stomach. The approach of White *et al.* (2004a) and Huveneers *et al.* (2007) was therefore adopted and dietary data for groups of individual animals (approx. 10 per group) were pooled, herein referred to as dietary samples. Prior to MDS ordination, the dietary samples were ARCSIN transformed and a similarity matrix was constructed using the Bray-Curtis similarity coefficient in PRIMER. One way analyses of similarities (ANOSIM) tests were then employed to determine if statistical differences in diet composition occurred between the two species, by sex and predefined size classes.

#### Stable isotope analysis

All net caught scalloped hammerhead (n = 38) and dusky (n = 63) sharks were sampled between 2005 and 2007. Trawl fishery derived scalloped hammerhead (n = 5) and spinner sharks (n = 5) were sampled in 2006. A section of white muscle tissue was excised from the muscle block anterior to the first dorsal fin adjacent to the vertebral column and stored frozen (-20°C). The muscle tissue was then freeze-dried and homogenised. Lipid extraction was undertaken by twice agitating the dried powdered muscle tissue in a 2:1 chloroform-methanol solution for 24 hrs. The tissue and solvent were then filtered through 25mm GF/F filter papers and the resulting residue/filter paper dried at 60°C for 48 hours to evaporate the remaining solvent. Between 400-600  $\mu$ g of tissue was weighed into tin capsules and stable carbon and nitrogen isotope ratios were provided from a continuous flow isotope ratio mass spectrometer (IRMS, Finnigan MAT Delta<sup>plus</sup>, Thermo Finnigan, San Jose, CA, USA). Stable isotope abundances are expressed in delta ( $\delta$ ) values as the deviation from standards in parts per thousand ( $\infty$ ) using the following equation:

$$\delta \mathbf{X} = \left[ \left( \mathbf{R}_{\text{sample}} / \mathbf{R}_{\text{standard}} \right) \cdot \mathbf{1} \right] \times 1000 \tag{1}$$

where X is <sup>15</sup>N or <sup>13</sup>C and R is the ratio <sup>15</sup>N/<sup>14</sup>N or <sup>13</sup>C/<sup>12</sup>C. The standard reference material was Pee Dee Belemnite carbonate for CO<sub>2</sub> and atmospheric nitrogen for N<sub>2</sub>. The analytical precision for  $\delta^{15}$ N was <0.22‰ and for  $\delta^{13}$ C was <0.24‰ based on more than 100 analyses of a NIST standard (bovine muscle) across multiple runs and was 0.14‰ for  $\delta^{15}$ N and 0.05‰ for  $\delta^{13}$ C based on a single run of NIST standard sucrose (n = 13) and ammonium sulphate (n = 13).

To visually display ontogenetic  $\delta^{15}$ N profiles,  $\delta^{15}$ N values vs. PCL were plotted for each individual shark per species. Regression models were then fitted to the data by species and sex.

### Trophic level estimation: diet data and $\delta^{15}N$

To facilitate a comparison between methods of calculating trophic position (TP) for each species by sex and predefined size class, diet trophic position (TP<sub>r</sub>) and  $\delta^{15}$ N trophic position (TP<sub>SIA</sub>) were calculated. Dietary samples for each shark species in conjunction with the estimated TP of functional prey groups were used to calculate TP<sub>r</sub> using the following equation (Cortés 1999):

$$TP_r = 1 + (\sum_{i=1}^{7} p_i \ge TP_i)$$
(2)

where TP<sub>r</sub> is the trophic position of the species in question,  $p_i$  is the proportion of each prey category in the total diet (expressed as %M) and TP<sub>i</sub> is the trophic position for each functional prey category. TP of functional prey groups were defined as: elasmobranch (3.65), teleost (3.24), cephalopod (3.2), crustacean (2.52), mollusc (2.1), bird (3.87), and mammal (4.02) based on Cortés (1999). The miscellaneous functional prey group was

excluded from all trophic level calculations. A measure of associated error was provided through calculating TP<sub>r</sub> for dietary samples.

To determine an absolute measure of shark TP using  $\delta^{15}N$  (TP<sub>SIA</sub>), a baseline value for  $\delta^{15}N$  (7‰; Hill *et al.* 2006) of a primary consumer (TP = 2) was used, the inshore unionid mussel, *Perna perna*, in KZN coastal waters, thus correcting for  $\delta^{15}N$  seasonal variation (Cabana & Rasmussen 1996; Vander Zanden & Rasmussen 1999). To enable accurate inferences on TP within food webs requires knowledge of species-specific diet discrimination factor ( $\Delta^{15}N = \delta^{15}N_{shark} - \delta^{15}N_{food}$ ). A  $\Delta^{15}N$  value of 2.29 ± 0.22‰ for large sharks was used according to Hussey *et al.* (2009a). For secondary consumers (i.e. teleosts, TP = 3), the mean enrichment of  $\delta^{15}N$  for muscle tissue of 2.96‰ was assumed (Vanderklift 2003). TP<sub>SIA</sub> for each shark  $\delta^{15}N$  value was then calculated following the derivation detailed in Hobson *et al.* (1995):

$$TP_{SIA shark} = 3 + (\delta^{15}N_{shark} - \delta^{15}N_{estimated primary consumer})/2.3$$
(3)

where  $\delta^{15}N_{\text{shark}}$  is the direct measurement of  $\delta^{15}N$  for individual sharks and  $\delta^{15}N_{\text{estimated}}$ primary consumer was that of the unionid mussel modified by the addition of the mean tertiary consumer enrichment equating to a value of 9.96‰. Sharks are highly mobile and therefore likely feed within several food webs (i.e. coastal vs. offshore pelagic), thus a simple single source food web was used to estimate TP based on: (i) all sampled sharks were caught in KZN coastal waters, (ii) reported minimal variation in  $\delta^{15}N$  of suspended particulate matter (SPM) between inshore and offshore environments of South Africa (Hill et al. 2006), and (iii) our lack of knowledge regarding the movement patterns of the species in question would provide ambiguous estimates of the percent time spent in different food webs to apply a multi source model. Through the use of a model that considered a single primary productive source, the TP estimates were standardised for species/size class comparisons.  $\delta^{13}C$  ontogenetic profiles were also used to determine the influence of foraging environment on calculated TP. A general linear model (GLM) with adjusted Bonferroni pairwise comparisons was used to examine effects and interactions of sex, size and method of calculating TP ( $TP_r$  and TP<sub>SIA</sub>) on derived TP values for both shark species. Data were unequal in variance (Levine's test: p < 0.05), but observation of the residual data indicated adequate conformity to homoscedasticity. Accepting the introduction of type 1 errors, statistical

results were only deemed reliable if p < 0.01. All effects were further tested using nonparametric statistics and results were in agreement with our GLM.

# Movement: ontogenetic $\delta^{13}$ C profiles

To interpret regional coarse resolution movement/residency patterns of the two shark species over ontogeny using  $\delta^{13}$ C, two assumptions were made:

- The core nursery habitat of juvenile scalloped hammerheads (< 58cm PCL) and spinner sharks (< 60cm PCL) is located in KZN coastal waters (Bass *et al.* 1973) and home-ranges are restricted (White *et al.* 2004b; Duncan & Holland 2006). The nursery habitat of dusky sharks (<100 cm PCL) is more extensive, stretching along the entire coastline of KZN and newborn/juveniles may undertake long distance migrations (Bass *et al.* 1973; Hussey *et al.* 2009c).
- Distinct systematic latitudinal and inshore-offshore gradients in δ<sup>13</sup>C occur off South Africa. With increasing distance from KZN to the Western Cape (WC) there is a marked increase in δ<sup>13</sup>C in coastal waters (Fig. 1; Hill *et al.* 2006, 2008). Measurements of SPM in offshore waters were significantly depleted in δ<sup>13</sup>C compared to coastal waters (Hill *et al.* 2006).

To visually display ontogenetic  $\delta^{13}$ C profiles,  $\delta^{13}$ C values *vs.* PCL were plotted for each individual shark by species and sex. Large sharks may be highly mobile, undertaking large unidirectional migrations, but also rapid return migrations (Bonfil *et al.* 2005). Muscle tissue  $\delta^{13}$ C values integrate the  $\delta^{13}$ C signatures of diet over long periods (MacNeil *et al.* 2005) and therefore the identification of distinct  $\delta^{13}$ C profiles as previously described for large migration marine mammals, for example bowhead whales, *Eubalaena australis* (Best & Schell 1996), would not be expected unless animals remain resident in core environments, which are isotopically distinct, for extended periods of time.



Fig. 2. Percent gravimetric contribution of functional prey categories defined by Cortés (1999) to the diet of scalloped hammerhead and dusky sharks by size class and sex. Large female dusky sharks are further subdivided into inactive, pregnant and postpartum animals. Numbers indicate sample sizes per size class.

### RESULTS

#### Multivariate analysis of stomach content data

Ontogenetic shifts in diet were observed for both male and female scalloped hammerhead and dusky sharks (Fig. 2; see Appendices S1 and S2 for detail of stomach contents). ANOSIM found that overall dietary composition differed significantly between species (R statistic = 0.611; p = 0.001). Teleosts were most important to the diet of small male scalloped hammerhead sharks but this decreased with size (Fig. 2); members of the families Sparidae, Sciaenidae, Haemulidae, Scombridae and Teraponidae were the most important teleost contributions to the diet of all male size classes (Appendix S1). The elasmobranch component increased but cephalopod prey decreased with increasing size class of the male scalloped hammerheads, with members of the families Scyliorhinidae and Rhinobatidae the principal elasmobranch prey (Fig. 2: Appendix S1). The cephalopod diet of small and medium male scalloped hammerheads consisted of cuttlefish (Sepiidae) and octopus (Octopodidae) but large males preved on members of the family Octopoteuthidae (Appendix S1). MDS ordination of male scalloped hammerhead diet data found a high degree of dietary overlap for all three size classes of shark, although several large male dietary samples were located to the right of the plot frame identifying the importance of elasmobranchs (Fig 3; Table 1). Similar to males, teleosts were the dominant prey item for female scalloped hammerhead sharks but these decreased with size (Fig. 2); the principal teleost families were Scombridae, Sparidae, Trichiuridae and for small sharks, Synodontidae (Appendix S1). In contrast to male sharks, cephalopods dominated the diet of large female scalloped hammerhead sharks, specifically the families Enoploteuthidae and Octopoteuthidae (Fig. 2; Appendix S1) and elasmobranchs constituted a small proportion of the diet and were absent in the diet of large sharks (Fig. 2; Appendix S1). MDS ordination of the female diet data demonstrated a high degree of overlap although data for medium and large sharks were more highly clustered, indicating a transition to a cephalopod dominated diet (Fig. 3). ANOSIM detected no significant differences in diet between any of the female size classes (Table 1), but was likely a result of the small sample size of large females.



Fig. 3. Non-metric multi dimensional scaling (MDS) ordination of the percent gravimetric dietary samples for the scalloped hammerhead and dusky sharks by size class and sex. For scalloped hammerhead and dusky sharks,  $\bullet = \text{small}$ ,  $\blacktriangle = \text{medium}$  and  $\Box = \text{large inactive sharks}$ . For female dusky sharks,  $\bullet = \text{pregnant}$  and  $\diamond = \text{postpartum}$ . For the definition of size classes see methods. Ellipses mark the outer bounds of dietary samples for small  $\bigcirc$ , medium  $\bigcirc$ , large/inactive  $\bigcirc$ , pregnant  $\bigcirc$  and postpartum  $\bigcirc$  size classes. Arrows indicate the ontogenetic trends in diet. For male scalloped hammerhead sharks, the shaded grey circle highlights large animals focused on an elasmobranch diet.

For male dusky sharks the elasmobranch component of the diet increased between small and medium sharks but decreased in the largest sharks (Fig. 2; Appendix S2). Teleosts were of similar importance to small and medium sharks, particularly members of the families Scombridae, Sciaenidae and Clupeidae (Appendix S2). The diet of large dusky sharks was dominated by teleosts; Clupeidae was the principal component (Fig. 2; Appendix S2). MDS ordination of the male dusky shark dietary samples found a clear transition in diet between the 3 size classes (Fig 3; Table 1). For female *C. obscurus*, the proportion of elasmobranch prey in the diet increased markedly with increasing size class and was dominated by members of the families Sphyrnidae and Carcharhinidae (Fig. 2; Appendix S2). Non-active/immature sharks preyed on a higher percentage of teleosts, principally Clupeidae, but elasmobranchs were the dominant prey item (Fig. 2; Appendix S2). In agreement, MDS ordination found that with increasing female size class, data points were located further to the left of the plot frame (Fig. 3). Pregnant/postpartum female data were highly clustered to the left while non-active/immature females exhibited a high degree of overlap with all size classes (Fig. 3). ANOSIM pairwise comparisons were highly significant for female dusky sharks (Table 1).

**Table 1.** ANOSIM pairwise comparisons of the percent gravimetric dietary samples for the scalloped hammerhead and dusky shark by sex and size class.  $F_{Ina}$  = large inactive female,  $F_{preg}$  = pregnant,  $F_{post}$  = postpartum. Levels of significance are; \*\*\* = p<0.001, \*\* = p<0.01 and \* = p<0.05.

					Sex	and size	class				
		Dusky shark									
			$M_{\mathrm{small}}$	$M_{\mathrm{medium}}$	$M_{\text{large}}$	$\mathbf{F}_{\mathbf{small}}$	$\mathbf{F}_{medium}$	$F_{Ina}$	$F_{\text{Preg}}$	F <sub>Post</sub>	
	*	M <sub>small</sub>		0.103	0.207	-0.045	0.308 *	0.594 **	0.893 ***	0.902 ***	
Sex and size class Scalloped hammerhead sharl	M <sub>medium</sub>	0.05		0.434 **	0.182 **	0.049	0.507 ***	0.707 ***	0.748 ***		
	chead	M <sub>large</sub>	0.334 ***	0.223 **		0.16	0.551 *	0.22	0.936 **	0.926 ***	
	F <sub>1</sub>	0.027	0.113	0.198 **		0.381 ***	0.639 ***	0.798 ***	0.866 ***		
	F <sub>2</sub>	-0.029	-0.058	0.226 **	-0.086		0.325 *	0.256 *	0.329 *		
	allop	F <sub>Ina</sub>	0.044	0.238 **	0.29 **	-0.117	-0.014		0.013	0.038	
	F <sub>Preg</sub>		<del></del>	-	Ħ		-		-0.065		
		F <sub>Post</sub>	37	<del></del>	÷	-		<del></del>	<b>.</b>		

# Ontogenetic δ<sup>15</sup>N profiles

For scalloped hammerhead sharks, both sexes showed a significant increase in  $\delta^{15}$ N with increasing PCL, however accepting the smaller sample size, the female increase was not as prominent ( $F_{1,23} = 53.3$ , p < 0.0001 and  $F_{1,5} = 9.08$ , p = 0.030; male and female respectively) (Fig 4). Values of  $\delta^{15}$ N in male and female dusky sharks increased between the smallest animals and medium sharks measuring up to approximately 130cm PCL, although a high degree of  $\delta^{15}$ N variability was observed (Fig. 4). The  $\delta^{15}$ N values of medium and large dusky sharks of both sexes >130 cm PCL were similar to small animals <100cm PCL. Excluding pregnant and postpartum animals from the analysis, no significant linear relationship between  $\delta^{15}$ N and PCL for either sex of dusky was found ( $F_{1,30} = 0.11$ , p = 0.75 and  $F_{1,20} = 0.66$ , p = 0.43; male and female respectively). A third order regression model provided a better fit to the data for both sexes (Fig. 4). Pregnant and postpartum dusky sharks had marginally lower  $\delta^{15}$ N values than inactive large sharks but exhibited a degree of variability with  $\delta^{15}$ N values ranging from 10 to 12.9‰ ( $T_{17} = 2.16$ , p = 0.046; Fig. 4).

# Trophic level estimation: dietary analysis and δ<sup>15</sup>N

Mean TP<sub>r</sub> ( $\pm 1$  S.E.) of scalloped hammerhead and dusky sharks for both sexes and all size class combined were 4.26  $\pm$  0.01 and 4.47  $\pm$  0.02, respectively. Mean TP<sub>SIA</sub> of scalloped hammerhead and dusky sharks were 4.53  $\pm$  0.06 and 4.31  $\pm$  0.05 respectively.

For male scalloped hammerhead sharks  $TP_r$  and  $TP_{SIA}$  increased with increasing animal size, whereas for female sharks the  $TP_r$  value was similar across size classes but  $TP_{SIA}$  increased with size (Fig. 5). Intra size class variability between calculated  $TP_r$  and  $TP_{SIA}$  of both sexes of scalloped hammerhead sharks was evident (Fig. 5). A GLM on the TP data found that male scalloped hammerhead sharks had higher estimated TP values than females (Table 2; Fig. 5). For both sexes, larger animals fed at a higher trophic level but the significance level was marginal for females (significant size x sex interaction; Table 2). Method  $TP_{SIA}$  provided significantly higher TP estimates for large scalloped hammerhead sharks than method  $TP_r$  (significant size x method interaction; Table 2). Note, no  $TP_{SIA}$  male scalloped hammerhead data and a single female data point precluded size class 2 animals from the GLM.



Fig. 4.  $\delta^{15}$ N ontogenetic profiles of scalloped hammerhead and dusky sharks by sex ,• = male, = female and • = unknown; for dusky shark;  $\Delta$  = pregnant and = postpartum sharks. Linear regression models were fitted to scalloped hammerhead sharks by sex. Third order regression models provided a better fit for the dusky shark data by sex.



Fig. 5. Mean calculated trophic position ( $\pm 1$  SE) by diet (TP<sub>r</sub> – striped bars) and  $\delta^{15}$ N (TP<sub>SIA</sub> – solid bars) for each sex and size class of the scalloped hammerhead (**■**) and dusky (**■**) shark.

The trend of calculated TP for male dusky sharks was similar across size classes for both methods (Table 2). For female dusky sharks, clear differences in estimated TP by TP<sub>r</sub> and TP<sub>SIA</sub> were observed between the  $L_{inact}$ ,  $L_{preg}$  and  $L_{post}$  sharks (Fig. 5). A GLM testing only small, medium and  $L_{inact}$  sharks found that medium sharks (both sexes and methods combined) had significantly higher TP than small sharks (significant size; Table 2).

	Sc	alloped h	ammerhe	ad	Dusky			
Source	d.f.	MS	F	р	d.f.	MS	F	р
Size	1	1.401	59,56	< 0.0001	2	0.261	5.25	0.007
Sex	1	0.440	18.70	<0.0001	1	0.006	0.11	0.738
Method	1	0.893	37.95	<0.0001	1	0.148	2.97	0.088
Size x Sex	1	0.175	7.44	0.008	2	0.005	0.11	0.898
Size x method	1	0.648	27.55	<0.0001	2	0.084	1.68	0.191
Sex x method	1	0.030	1.31	0.256	1	0.226	4.54	0.035
Size x sex x method	1	0.006	0.26	0.614	2	0.059	1.18	0.312
Residual	95	23.3			111	49.01		

**Table 2.** General linear model testing the effects of size, sex, method of calculating trophic position (TP) and associated interactions on estimated TP for the scalloped hammerhead and dusky shark.

# Ontogenetic $\delta^{13}$ C profiles

The mean  $\delta^{13}$ C values of small scalloped hammerhead, dusky and spinner sharks were similar (Fig 6;  $F_{2,26} = 0.95$ , p = 0.40). This provides confidence that the range of  $\delta^{13}C$ values exhibited by small scalloped hammerhead and spinner sharks (min: -16.22 and max: -15.48) was indicative of  $\delta^{13}$ C signature in their nursery habitat in KZN. Dusky sharks <130 cm PCL had variable  $\delta^{13}$ C values but the majority of individuals were within the range of the small scalloped hammerhead and spinner sharks. For scalloped hammerhead sharks, the  $\delta^{13}$ C values of males and females between 120 and 160 cm PCL showed a marked sexual segregation of ~2.5‰ (accepting the small sample size,  $T_4 = -19.44$ , p < 0.0001). The  $\delta^{13}C$  signatures of male scalloped hammerheads >160.0 cm PCL then showed a gradual depletion to sharks of >200cm PCL where the  $\delta^{13}$ C signatures of both sexes were similar to that of the small shark (Fig 6). For the dusky shark, several individuals <160cm PCL were enriched in  $\delta^{13}$ C relative to the juvenile hammerhead and spinner sharks. Dusky sharks >200cm PCL of both sexes had  $\delta^{13}$ C values similar to small sharks, although most individuals were at the more depleted end of the  $\delta^{13}$ C range of the nursery habitat (Fig 6). Pregnant and postpartum C. obscurus  $\delta^{13}$ C values were variable and more depleted than small sharks (min: -17.21 and max: -15.55).



Fig. 6.  $\delta^{13}$ C ontogenetic profiles of scalloped hammerhead and dusky sharks by sex, • = male, • = female and • = unknown; for dusky shark;  $\Delta$  = pregnant and  $\Box$  = postpartum sharks. Grey bar depicts the predicted  $\delta^{13}$ C range of the KZN nursery habitat of scalloped hammerhead and spinner sharks ( $\diamond$ ).

### DISCUSSION

Knowledge of ontogenetic trophic profiles of large marine predators is vital to our understanding of ecosystem structure and function and to predict the effects of the removal of species, specifically the targeting of size classes and sexes by commercial fishing operations (Ward & Myers 2005). The stable isotopes of nitrogen have proven to be a reliable indicator of the TP of fish when, (i) suitable diet discrimination factor/s appropriate to the study organism are applied (Vanderklift & Ponsard 2003; Caut et al. 2009), (ii) there is accurate knowledge of  $\delta^{15}$ N baseline values of the system in question (Vander Zanden et al. 1997) and (iii) multiple diet discrimination factors are incorporated into the TP<sub>SIA</sub> calculation (Hobson et al. 1995). Stable isotopes and stomach content data archive a record of diet and TP and, when combined, provide a method to examine variations in ontogenetic trophic profiles over different integration time periods and an assessment of resource partitioning between species. With knowledge of systematic  $\delta^{13}$ C variation of baseline organisms across the geographic range of the study species,  $\delta^{13}C$  ontogenetic profiles provide insight into coarse resolution movement patterns and the effect of habitat partitioning on observed trophic profiles. To date, our principal understanding of the TP of sharks has stemmed from stomach content analysis as a stand alone proxy (Cortés 1999; Vögler et al. 2009) and initial work investigating the application of stable isotopes (Fisk et al. 2002; Estrada et al. 2003, 2006; MacNeil et al. 2005). This study presents the first comparative analysis of ontogenetic trophic profiling derived from stomach content data and both  $\delta^{15}N$  and  $\delta^{13}$ C data and provides empirical evidence for ontogenetic inter and intra species variation in TP of two large predators, but suggests a cautionary approach when interpreting stable isotope data in ecological research.

# Ontogenetic trophic profiles: diet data and $\delta^{15}N/\delta^{13}C$

The mean trophic position (TP > 4) of the scalloped hammerhead and dusky shark, calculated by TP<sub>r</sub> and TP<sub>SIA</sub> in this study were similar, but higher than those previously reported by Cortés (1999). In contrast to these results, Estrada *et al.* (2003) reported agreement between TP<sub>SIA</sub> for five shark species in the North Atlantic and standardised diet trophic level calculations by Cortés (1999), although TP evaluation of stomach contents was not undertaken. TP values calculated by both Cortés (1999) and Estrada *et* 

*al.* (2003) may be underestimated by the bias of larger numbers of smaller individuals in the TP<sub>r</sub> value and the use of a diet discrimination factor that was too large in the calculation of TP<sub>SIA</sub>. The latter point is based on a recent controlled feeding study using large sharks (Hussey *et al.* 2009a).

Inter-specific differences in calculated TP by size class were evident and would be expected considering size, diet and movement characteristics of the two species. For example, the higher trophic position, by both  $TP_{SIA}$  and  $TP_{r_c}$  of small dusky relative to small scalloped hammerhead sharks likely reflected the higher percentage contribution of elasmobranchs to the diet of the dusky. On the premise that juvenile sharks are apex predators within their nursery habitat, size of sympatric elasmobranch species at birth governs TP through associated differences in gape size and manoeuvrability. Neonates born at a larger size can rapidly expand their home range over larger spatial scales. This, in conjunction with larger gape size, can influence foraging success through both the diversity of encountered prey and the range of prey sizes a predator is able to manipulate. Dusky sharks are born at a larger size than scalloped hammerhead sharks (Dudley *et al.* 2005; Duncan and Holland 2006) and rapidly expand their home range (Hussey *et al.* 2009c). This may enable young dusky sharks to exploit seasonally abundant prey resources (Hussey *et al.* 2009c) that young scalloped hammerheads cannot, resulting in resource partitioning via habitat expansion.

In contrast to the smaller size classes, large scalloped hammerheads fed at a significantly higher TP, calculated by TP<sub>SIA</sub>, than corresponding dusky sharks, while TP<sub>r</sub> estimated similar TPs. Klimley (1987) identified qualitative differences in the diet of small and large male and female scalloped hammerhead sharks and attributed this to a switch in resource type determined by an ontogenetic driven movement from coastal waters to a pelagic environment. In agreement with the diet data in this study, neritic and pelagic cephalopods were an important component of the diet of large female sharks (Klimley 1987, 1993). Concordantly, Clarke (1971) reported an ontogenetic shift in the diet of scalloped hammerhead sharks from small reef fish and crustaceans to cephalopods, but data were not examined by sex. Reported  $\delta^{15}$ N values for pelagic ommastrephid squids are of the order of 10-15‰ (Parry 2008; Cherel *et al.* 2009a). Considering a diet discrimination factor of 2.29‰ for large sharks (Hussey *et al.* 2009a), the  $\delta^{15}$ N values of large female hammerhead sharks and associated TP<sub>SIA</sub> in

conjunction with both diet data and the shift in  $\delta^{13}$ C (see discussion on movement below) indicate a switch in both habitat and resource use. TP<sub>r</sub> of the large female hammerheads was likely underestimated due to the broad functional prey group of cephalopods, encompassing larger voracious pelagic species and smaller coastal species. For large male scalloped hammerhead sharks, the high percentage mass of elasmobranchs in the diet was unusual (Klimley 1987; Steven & Lyle 1989) but supported the observed increase in  $\delta^{15}$ N and TP<sub>SIA</sub>. The marginal discrepancy between TP<sub>SIA</sub> of large male and female scalloped hammerhead sharks may be a result of the elasmobranch diet component of male sharks and/or variable  $\delta^{13}$ C profiles between sexes.

Intra size class variability of TP<sub>SIA</sub> and TP<sub>r</sub> values were evident for both species of shark. The difference in magnitude between TPr and TPSIA for inactive, pregnant and postpartum dusky sharks was likely influenced by the integrative period of the two TP methods. Dudley et al. (2005) reported a scarcity of pregnant dusky sharks other than those at or near term in NSB net catches, suggesting individuals are only present in the coastal region at the point of parturition. Furthermore, the residency period of postpartum animals is likely limited (Hussey et al. 2009b). KZN coastal waters constitute known nursery grounds for several elasmobranch species (Bass et al. 1973, 1975) and a high incidence of elasmobranchs in the diet of large sharks is reported (Cliff et al. 1989; Cliff & Dudley 1991a, 1991b). The higher TPr of pregnant/postpartum dusky sharks may, therefore, have reflected the snapshot sampling of stomach content analysis (possibly 2-3 weeks) compared with the long-term integrated signature of TP<sub>SIA</sub>. It is important to note that the observed low  $\delta^{15}N$  values and calculated TP<sub>SIA</sub> of pregnant and postpartum females may also be slightly biased by two possible factors. Corresponding  $\delta^{13}$ C values of these sharks were depleted relative to inactive sharks. Based on the assumption that pelagic food webs are more depleted in  $\delta^{13}$ C than coastal food webs (Hill *et al.* 2006), and associated shifts in  $\delta^{15}$ N are reported (Sherwood & Rose 2005), these sharks may either be feeding offshore or migrating to KZN for parturition from other geographic localities, i.e. movement from isotopically distinct food chains. Alternatively, the low  $\delta^{15}N$  and calculated TP<sub>SIA</sub> may be a result of preferential <sup>15</sup>N excretion from the mother to near term young through the placental connection. Enriched  $\delta^{15}N$  values in near term pups relative to mothers have been observed for both carcharhinid and lamnid sharks (McMeans et al. 2009, Hussey & Fisk, unpubl. data).



Fig. 7. Trend of jaw width with increasing length (PCL) of the scalloped hammerhead ( $\cdot$  n = 624) and dusky shark ( $\cdot$  n = 1009). Inset graphics include upper and lower jaw tooth form for each species.

With the exception of large female dusky sharks, the overall ontogenetic  $TP_r$  and  $TP_{SIA}$  trends for each species were comparable. Ontogenetic shifts in diet have been reported for several elasmobranchs (see review by Wetherbee & Cortes 2004) and a corresponding increase in  $\delta^{15}N$  with size of animal has been shown for several marine taxa; teleosts (Deudero *et al.* 2004; Graham *et al.* 2007), sharks (Estrada *et al.* 2006), turtles (Godley *et al.* 1998), marine mammals (Mendes *et al.* 2007; Newsome *et al.* 2009) and cephalopods (Cherel *et al.* 2009; Parry 2008). This is in general agreement with our scalloped hammerhead shark data where  $\delta^{15}N$  increased with size but contrasts the results in the dusky shark, where  $\delta^{15}N$  and  $TP_r$  decreased with size, with the exception of pregnant and postpartum females. Lower  $TP_r$  estimates of the large inactive male and female dusky sharks may be explained by a bias to sampling this size class during the annual sardine run; the principal period when these sharks are present in coastal waters (Dudley *et al.* 2005). The annual sardine migration provides an important prey resource for multiple top predators off southeast Africa (Armstrong *et al.* 1991). For  $TP_{SIA}$ , the ontogenetic profile in the dusky shark is more puzzling. Compared

to scalloped hammerheads, large dusky sharks are equipped with a substantially larger gape size and serrated teeth (Fig. 7), similar to white sharks (Carcharodon carcharias), typically associated with predators feeding and/or scavenging on large prey. Predatorprey dynamics suggest that prey sizes consumed by a predator generally increase with predator size (Bethea et al. 2004). Most predator-prey relationships are however, asymmetric, in that maximum prey size increases, while minimum prey size often exhibits minimal variation (Scharf et al. 2000). Spear fishermen in northern KZN report frequent sightings of large dusky sharks throughout the year on or near the edge of the continental shelf (Heydorn pers. comm.). There are therefore two possibilities for the observed TP<sub>SIA</sub> ontogenetic trend in dusky sharks: (i) small schooling fish are abundant in the region, the resulting predator-prey encounter rates are high and energetic costs of foraging (prey chase down and manipulation) are minimal, providing large dusky sharks with an adequate prey base which may be supplemented by occasional predation on larger prev items, particularly opportunistically scavenged prev. Small dusky sharks incorporate an elasmobranch component in their diet, due to the abundance of elasmobranch prey in the coastal environment and therefore overall TP values are similar between life-stages; (ii) large dusky sharks are incorporating larger prey in their diets, but these prev are from the pelagic environment on the edge of the continental shelf. Therefore the  $\delta^{15}N$  signature and TP<sub>SIA</sub> are biased by combining dietary signatures from isotopically distinct environments. Considering a degree of variation between the small and large inactive dusky shark  $\delta^{13}$ C signatures (more pronounced for pregnant and postpartum females) it is likely that a combination of the above two possibilities results in dilution of the observed  $\delta^{13}$ C and  $\delta^{15}$ N values.

# Coarse resolution ontogenetic profiles: $\delta^{13}C$

Small scalloped hammerhead shark  $\delta^{13}$ C values were clustered with those of both small dusky and spinner sharks indicating residency in a core area of nursery habitat in KZN. Fennessy (1994) reported high catches of small scalloped hammerheads in the prawn trawl fishery on the Tugela banks in KZN. These observations support the assumption of a core nursery area previously described for the species in Hawaii (Clarke 1971; Duncan & Holland 2006). In offshore waters of the Gulf of Mexico, Klimley (1987) reported that schools of scalloped hammerhead sharks consisted of predominantly female animals of 140-180 cm TL. The  $\delta^{13}$ C values of female sharks of a similar size in

this study were depleted relative to the nursery habitat, suggesting an offshore origin, and supporting the findings of Klimley (1987). In agreement, field observations by both spear fishermen and regional dive operators report the occurrence of schooling hammerheads on submerged pillars/banks located on the edge of the continental shelf in KZN. It cannot be ruled out, however, that these female scalloped hammerhead sharks may be moving further north into  $\delta^{13}$ C depleted waters in conjunction with offshore movement.

For male scalloped hammerhead sharks of ~80cm PCL.  $\delta^{13}$ C values followed a trend of enrichment with increasing size. Male sharks of ~140-160 cm PCL were enriched ~1‰ to the nursery habitat and 2.5% to corresponding female sharks. When considering the  $\delta^{13}$ C gradient in secondary consumers in South African waters (Hill *et al.* 2006), these enriched  $\delta^{13}$ C values may suggest that male sharks remain on the continental shelf but undertake southerly movement with a period of residency in the northern Eastern Cape region (Fig. 1). The high catch rate of male scalloped hammerhead sharks relative to females in KZNSB net installations further supports sexual segregation in this species with the more frequent occurrence of males in coastal waters (de Bruyn et al. 2005). In addition, Bass et al. (1975) reported the occurrence of juvenile and adolescent scalloped hammerhead sharks, principally males, in Natal throughout the year, but in greater numbers during the warmer months. Alternatively, the associated increase in  $\delta^{13}$ C may represent an increase in the trophic level of prey consumed by these male sharks, for example large benthic prey, within the continental shelf food web. Surprisingly, the  $\delta^{13}$ C signatures of large individuals of both sexes were similar to small animals resident in KZN nursery habitat. Our current knowledge of the ecology of large scalloped hammerheads is limited. Bass et al. (1975) reported that adult females were rare in KZN waters, while large males were common during summer. Clarke (1971), Branstetter (1987) and Stevens & Lyle (1989) reported that large female scalloped hammerheads were rare in coastal waters of Hawaii (except at time of parturition), north-western Gulf of Mexico and off Northern Australia, respectively. In schools of hammerhead sharks in the Gulf of California, Klimley (1987) found that few animals of both sexes larger than 200cm TL were present. Clarke (1971) suggested that large hammerheads may remain offshore, but the data in this study do not confirm this point. The observed  $\delta^{13}C$ signatures may suggest these animals are highly migratory both by latitude and between

inshore and offshore environments, incorporating  $\delta^{13}C$  signatures from various isotopically distinct regions which result in a value similar to those of the small animals.

For dusky sharks,  $\delta^{13}C$  values of small animals were more variable, with some individuals up to ~130cm PCL enriched in  $\delta^{13}$ C relative to scalloped hammerhead and spinner sharks. This enrichment may reflect the southern migration of small dusky sharks to the EC region and a period of residency in EC waters (Bass et al. 1973; Smale 1991, Hussey et al. 2009c).  $\delta^{13}$ C values for smooth hound sharks (Mustelus mustelus) resident in the EC region of  $-14.4 \pm 0.10$  (Singh, unpubl. data), provide further evidence to support the observed trend. Variability in the  $\delta^{13}$ C signature of this size range of dusky sharks may also support observations derived from tag-recapture data, that small dusky sharks adopt different life strategies, with some 'nomadic' individuals undertaking migrations, while others remain resident in KZN throughout the year (Hussev et al. 2009c). The fact that the trends in  $\delta^{13}$ C values of these size sharks were similar between sexes would suggest that the occurrence of sexual segregation, proposed by Bass et al. (1973), is not true. Similar to the scalloped hammerhead shark however, it is not possible to rule out that the observed increase in  $\delta^{13}$ C is an associated increase in trophic level of prev consumed on the continental shelf. The overall trend of decreasing  $\delta^{13}$ C with increasing size would suggest larger animals of both sexes are spending periods of time foraging in offshore depleted waters. This is in agreement with Compagno (1984) who reported that large dusky sharks occupy an intermediate offshore coastal habitat. Large dusky sharks are also recorded further north off Mozambique and Madagascar (Fourmanoir 1961; McVean et al. 2006). When considering the  $\delta^{13}$ C gradient along the southeast coast of Africa (Hill *et al.* 2006); it is possible that the waters of Mozambique/Madagascar are depleted in  $\delta^{13}$ C providing evidence for a northerly movement, but a strong latitudinal gradient in  $\delta^{13}$ C in tropical waters would not be expected. The more pronounced depletion of  $\delta^{13}C$  values and the range of variability of pregnant and postpartum dusky sharks may be a result of: (i) a reproductive separation within the large female size class, with pregnant females spending proportionally more time in offshore waters (or moving further north). Gestating females may adopt this strategy to enhance the development of young through profitable foraging and reduced competitive interactions with conspecifics, (ii) it may be indicative of the immigration of pregnant females to KZN for parturition from multiple geographic localities and/or, (iii) as previously discussed it may be a result of a physiological process of isotope transfer between mother and pups.

#### CONSIDERATIONS AND CONCLUSION

Understanding both TPr and TPSIA ontogenetic profiles of large predators in the marine environment is complex. Parameters affecting calculated TPr are well documented, but the complexities associated with stable isotope trophic profiling are less well understood. Multiple factors may influence the calculations and interpretations of data including: (i) movement between isotopically distinct food webs: coastal - pelagic, by latitude (our  $\delta^{13}$ C profiles, Queiroz et al. 2005; Weng et al. 2005), (ii) knowledge that sharks undertake variable movement strategies: diel movement (Klimley & Nelson 1984; Klimley et al. 1988; Cartamil et al. 2003), seasonal migrations (Weng et al. 2005, Domeier & Nasby-Lucas 2008; Hussey et al. 2009c) large scale movement and transoceanic migrations (Bonfil et al. 2005; Gore et al. 2008) (iii) ontogenetic-gender physiological changes, for example growth rate (Natanson et al. 2002; Piercy et al. 2006), (iv) mother-developing young interactions (McMeans et al. 2009) and (v) feeding on a diverse range of functional prey groups within a single food web: coastal [demersal to mid water], offshore [shallow pelagic to mesopelagic] (Wetherbee & Cortés 2004). As a result, to accurately interpret stable isotope data of large mobile predators requires the disentangling of the connectivity and interplay between the  $\delta^{15}N$ and  $\delta^{13}$ C ontogenetic profiles (Layman et al. 2007). Previous work has determined the importance of applying multiple source models accounting for variability in baseline producers from isotopically distinct environments when estimating TP (Post 2002). For large predators such as sharks, however, both our lack of understanding of their movement between and residency within these isotopic environments and the availability of accurate isoscapes, restrict the approach that can be undertaken without introducing further bias and error in the calculations. In many instances in the literature, no attention is given to the importance of variable  $\delta^{13}C$  values on observed  $\delta^{15}N$  trends and hence trophic profiles (France et al. 1998; Parry 2008). Furthermore, in the analysis of white shark (Carcharodon carcharias) vertebrae from the North West Atlantic, Estrada et al. (2006) generally found an increase in  $\delta^{15}N$  with increasing size of individual animal with no statistical change in  $\delta^{13}$ C. In contrast, for white sharks in the
eastern north Pacific, Kerr *et al.* (2006) found no increase in  $\delta^{15}$ N, but noted a decrease in  $\delta^{13}$ C with size. Thus it would appear that understanding ontogenetic  $\delta^{15}$ N profiles of highly mobile shark species may well be complicated by both movements between isotopically distinct regions, variation in regional isotopic gradients and intra-species variability in life-history strategies of geographically separated populations.

Although this study utilised a simplistic single source model to estimate TP<sub>SIA</sub>, by adopting a multifaceted approach encompassing stomach content data and  $\delta^{15}N$  and  $\delta^{13}C$  ontogenetic profiles, the level of confidence in the conclusions drawn from the data were increased. It is important to reiterate the point that although stable isotopes are a valuable tool to examine population level ecology, they must be applied in conjunction with multiple methodological approaches (Hobson 1999; Rubenstein & Hobson 2004) including tracking data, stomach content data and other biogeochemical/contaminant tags (Fisk *et al.* 2002) to provide a reliable metric for management and conservation (Layman *et al.* 2007).

Where discrete geographical gradients in baseline  $\delta^{13}$ C values occur,  $\delta^{13}$ C profiles of long term integrative muscle tissue of large predatory sharks provide accurate insights into archived coarse resolution ontogenetic movement patterns as previously described for marine mammals (for example, Best & Schell 1996). Understanding movement and defining migration corridors of large marine vertebrates is critical for species protection and management (Weng *et al.* 2005). Satellite/acoustic tracking technologies provide a comprehensive tool to define high resolution movement patterns of difficult to study marine species but they are restricted by cost in their application to studies at the population level. The  $\delta^{13}$ C ontogenetic profiles in this study identified species-, genderand size-specific movements which require further investigation. These data may provide an important platform for the development of *a priori* hypotheses for the efficient design and implementation of species-specific satellite tagging programmes.

This study indicates that sharks as top predators influence multiple food webs or 'ecosystems' and raises important questions over the proportional influence exerted on a single food web by a specific species, gender and size class. There is therefore a requirement to better understand and incorporate accurate ontogenetic profiles of large predators to refine community based isotope modelling approaches. Furthermore, with regard to community based approaches, if large individuals only are sampled (Jennings *et al.* 2001; Al-Habsi *et al.* 2008), trophic position results may be biased by associated  $\delta^{13}$ C values. With the recent advancement of isoscapes (Bearhop *et al.* 2005), and through directed satellite tagging programmes, future work will address the development of multisource productivity/baseline consumer models in conjunction with movement/residency time periods of large sharks to improve isotopic predictions of TP.

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## **CHAPTER SEVEN**

General Discussion

## **GENERAL DISCUSSION**

It is widely accepted that large marine predator populations have undergone rapid worldwide declines and that current levels of exploitation are unsustainable (Baum et al. 2003; Ward & Myers 2005). Scientists are now raising concerns over the ecological consequences of this large scale exploitation and possible eventual removal of large marine predator on ecosystem structure, function and stability (Baum & Worm 2009; Heithaus et al. 2008). The low economic importance of sharks to global fisheries, in terms of flesh value, has however, traditionally resulted in their low priority designation when considering research, conservation and structured management (Walker 1998; Fowler et al. 2005). Shark research has consequently lagged behind that of teleosts, specifically the commercially important species. Acknowledging the continued growth of elasmobranch exploitation, there has been an increased awareness of the need to generate species and life-stage specific biological and ecological knowledge for large marine predators to assist regional management plans and to enable accurate stock assessments to be undertaken (Dulvy et al. 2008; Kinney & Simpfendorfer 2009). Considering their unique life-history strategies (Hoenig & Gruber 1990), it is also recognised that commonly applied teleost-based management approaches must be advanced and modified to provide a suitable tool for the management of sharks (Kinney & Simpfendorfer 2009). The study of large highly mobile marine predators is inherently difficult and poses a unique challenge to fisheries managers. This study demonstrates that long-term archived data sets and the application of modern chemical tracer techniques provide important tools to advance our ecological knowledge base of large sharks.

The recapture rate of dusky sharks (*Carcharhinus obscurus*) in this study of 6.7% [a value comparable with most tag recapture studies (Kohler & Turner 2001)], highlights the level of effort required to generate sufficient data for meaningful biological interpretation. With the advancement of modern technological tracking techniques, for example the development of acoustic receivers (Heupel & Simpfendorfer 2005) and application of both acoustic and satellite tags (PSATs – Pop up archival satellite tags) (Block *et al.* 2001; Bonfil *et al.* 2005), field scientists are beginning to understand fine scale movement patterns (Gore *et al.* 2008; Skomal *et al.* 2009) and

behavioural decisions of sharks (Sims et al. 2008). These approaches provide important insights, but much of these inferences are based on few individuals (Gore et al. 2008; Pade et al. 2009). The analysis of a comprehensive archival tag-recapture data set on dusky sharks off South Africa (Chapter Two), comprising the tagging of 9746 individuals, revealed important data on core nursery habitat regions, both localised and regional movements and growth rates at the population level. Although dusky sharks select open water nursery regions, which are typically more complex to manage, core activity zones were identified in both KwaZulu-Natal and the Eastern/Western Cape region. This in conjunction with a defined seasonal migration between these two core habitats has important implications for the monitoring and regulation of both the commercial and the well developed recreational fishing industries (Dudley & Simpfendorfer 2006; Pradervand et al. 2007). Furthermore, defining nursery habitat selection, coastal migrations and growth rates provide the necessary data to advance regional demographic analyses which are required for effective management (Cortés 1998; Romine et al. 2009). This is pertinent considering the global status of dusky sharks in the northwest Atlantic (Musick et al. 1993), questions raised over the sustainability of the commercial fishery for juvenile animals off south-western Australia (McAuley et al. 2007) and the recent development of commercial industries exploiting juvenile dusky sharks off Southern Africa (Dudley & Simpfendorfer 2006; Dudley pers. comm.). With the establishment of the Ocean Tracking Network<sup>1</sup> and the regional expansion of an acoustic receiver array in South Africa, future work will examine fine-scale movements of juvenile dusky sharks in coastal waters using passive telemetry techniques. Considering the expense of applying these technologies, the data in this thesis will enable the development of a priori hypotheses and provide an important guide for the effective positioning of acoustic receivers to maximise the efficiency of the study techniques.

Defining and protecting the nursery habitat of juvenile sharks, i.e. the new recruits, is now recognised as an essential component of shark management to ensure viable stock recruitment (Bonfil 1997; Heupel *et al.* 2007; McCandless *et al.* 2008). With an increase in research effort focused on juvenile animals, measures of the health or condition of sharks have become an increasingly important tool to quantify

<sup>&</sup>lt;sup>1</sup> The Ocean Tracking Network is a global research initiative to study the migration patterns of fish and marine mammals (http://oceantrackingnetwork.org/)

anthropogenic impacts and to investigate life-history strategies (Duncan & Holland 2006: Hoffmayer et al. 2006: Dibattista et al. 2007). Measuring fish condition is a standard practice in the management of teleost fishes both at the individual and population level (Stevenson & Woods 2006), but the comparative analysis of several condition indices to sharks requires a rigorous evaluation. This thesis presented a unique data set, consisting of 2120 dusky sharks, to enable the comparison of multiple condition indices for a large marine predator (Chapter Three). The various morphometric condition measures [condition factor (CF), relative condition (Kn) and residual condition (Rr<sub>PCL</sub>)] provided comparable results for sharks, but, hepatosomatic index, i.e. liver mass vs. body mass, provided the most sensitive and rapid indicator of their seasonal energetic state. In the case of small sharks, HSI of newborn and >1 year old sharks demonstrated distinct seasonal patterns, indicating that newborn sharks may be provisioned with enlarged livers as a form of maternal investment. When considering the larger liver size of newborn animals and the relatively small liver size of pregnant and postpartum sharks, this study highlighted the importance of carefully defining life-stages and reproductive states to enable accurate interpretation of condition data. This is of particular importance for HSI which is also affected by increasing size of animal. The morphometric measures of condition were unable to differentiate between the condition of the two small size classes of shark and were also less sensitive to short term variations in condition over a seasonal cycle. With current concern over the exploitation of shark stocks (e.g. Baum et al. 2003), future research should investigate minor invasive methods of measuring HSI. Portable ultrasound may provide an effective tool to measure the HSI of juvenile sharks, without the requirement for large numbers of mortalities within a given nursery bound population. Net caught sharks from South Africa would provide a robust data set by which the application of ultrasound approaches could be validated. Additionally, minor invasive methods for measuring HSI could be combined with contaminant studies and biochemical condition measures to further our understanding of the early life-stages of sharks in coastal regions. Applying these methods to the study of impacted and so called pristine nursery populations would also provide valuable insights in to the factors that determine the viability and survival of juvenile sharks.

The identification of an enlarged liver in newborn dusky sharks, through the above comparative condition analyses, raised an important question over maternal

investment strategies in carcharhinid sharks. At the point of parturition, neonatal sharks have fully functional jaws and teeth and have long been considered independent from maternal resource allocation (Wourms 1977; Carrier et al. 2004). Through the examination of a large archived data set, it was possible in this thesis to provide the first empirical evidence for a decrease in liver size with umbilical scar stage, in other words the utilisation of liver reserves over the first few weeks/months of life (Chapter Four). HSI and CF data were also in agreement, identifying the role of the enlarged liver as a form of maternal head-start in newborn sharks. The identification of this maternal head-start has important implications for the study of juvenile shark populations. Previously, concern has been raised over the weight loss of newborn sharks and how this may relate to density-dependent mechanisms in the nursery population and/or prey limitation (Duncan & Holland 2006; Heupel et al. 2007). This has led field workers to conclude that nursery-bound shark populations may be exposed to high natural mortality rates (Lowe 2002; Duncan & Holland 2006). As neonatal sharks use the provisioned liver reserves in the first few weeks/months of life, it is natural that weight loss will occur. Further investigation into density-dependent effects on nursery populations, taking into account natural weight loss due to utilisation of liver reserves, is therefore required. Future work should also investigate; i) how maternal investment, in the form of an enlarged liver, differs between reproductive modes, ii) variation in maternal investment within single litters and between individual mothers and; iii) the relationship between the condition of the mother and condition of near-term pups. For teleost fish and marine reptiles, the variable allocation of maternal reserves has raised interesting lines of inquiry over the growth and survival of young with implications for general stock recruitment (Berkeley et al. 2004; Gagliano & McCormick et al. 2007; Donelson et al. 2008). It is likely that the documenting of maternal investment in carcharhinid sharks in this study will raise similar questions which will further our understanding of maternal investment strategies within this large group of vertebrates.

To address the knowledge gap over maternal resource allocation in sharks, this thesis undertook a quantitative modelling approach to investigate variation in reproductive output with maternal size of two carcharhinid species (Chapter Four). This was possible through access to one of the world's largest data sets on near-term pregnant sharks and their associated near-term pups. To date, most studies examining the reproductive output of sharks have been based on single factor analysis, for example litter size vs. maternal size and pup mass vs. maternal size (Parsons 1993; Allen & Cliff 2000; Dudley et al. 2005). Furthermore, given the difficulty of obtaining sufficient samples of large sharks, most analyses are typically limited by available data. Understanding the reproductive potential of shark populations, however, is critical for accurate demographic modelling and to further our understanding of juvenile to adult stock recruitment curves (Cortés 1998; Simpfendorfer 2005). Through the application of mixed effects models it was possible to examine the effect of multiple interacting factors on near-term pup condition in order to gain an overall understanding of the mechanisms controlling reproductive output. The model results found that total reproductive output of carcharhinid sharks increases with maternal size as would be expected, but with evidence for a moderate decline at the larger sizes. This is the first reported evidence for a decline in reproduction with size (age) in sharks. For the dusky shark, there was also evidence for a trade off between litter size and pup mass, although it is likely that both species select litter size within a confined range and observed variation in litter size may not necessarily be linked to maternal size as previously thought. Interestingly, pup mass increased with month for both species which may indicate variable parturition strategies, as reported for teleost fish (Karr & Kaufman 2009), but may also provide the first evidence for sharks exhibiting flexible gestation periods. With the knowledge that a small increase in fishing mortality can have a disproportionally large effect on population viability (Walters & Martell 2004), the implications of a peak reproductive size in commercially harvested shark species is of fundamental importance. Accounting for size-specific reproductive output in large carcharhinid sharks within future demographic analyses will therefore be critical. This work presents initial model development to address key questions over reproductive output. Future work will build on the modelling approaches presented to examine patterns in reproductive output across a range of species and to determine if the findings reported for these two carcharhinid sharks are ubiquitous.

Pursuing the application of new methods to investigate large marine predators is fundamental to the advancement of our ecological knowledge base and to improve management considerations. Stable isotopes are a well established tool for investigating the trophic, feeding and movement ecology of both terrestrial and marine organisms (Koch et al. 1995; Burton & Koch 1999; Bearhop et al. 2005; Caut et al. 2008a) and initial work on sharks has showed promise (Fisk et al. 2002; Estrada et al. 2003, 2006; MacNeil et al. 2005, 2006). In recent years, our understanding of stable isotopes has improved but this has resulted in a need to address certain key assumptions, for example species-specific diet-tissue discrimination factors (Caut et al. 2009; Martinez del Rio et al. 2009; Wolf et al. 2009). For many organisms. controlled feeding experiments are practical but for many large sharks this approach is complicated by their large size and the difficulties of maintaining such animals under an experimental design. Through a long-term study involving the main commercial aquaria in the U.K., this thesis was able to opportunistically sample multiple tissues from large sharks [sand tiger (Carcharias taurus) and lemon shark (Negaprion brevirostris)] to derive the first diet-tissue discrimination factors for a large ureosmotic organism (Chapter Five). Although we observed variability between the organs of the four monitored sharks, values for muscle tissue, the most commonly analysed tissue for stable isotope analysis, were comparable between individuals and were lower than those reported for teleost fish. The derived values of  $2.29\% \pm 0.22$ and 0.90‰ ± 0.33 for  $\delta^{15}N$  and  $\delta^{13}C$  of muscle tissue respectively should provide a reliable estimate for the study of wild populations. Our analysis of liver tissue, demonstrated that high lipid content tissues are problematic and further work is required to improve lipid extraction procedures and to standardise high-lipid tissues prior to stable isotope analysis. This is of particular concern for the analysis of  $\delta^{13}C$ , which is heavily influenced by lipid content (Logan et al. 2008). Additionally, our understanding of the effects of lipid extraction on  $\delta^{15}N$  of shark muscle tissue [similar to that reported for teleost fish (Sotiropoulos et al. 2005; Ingram et al. 2007; Logan et al. 2008)] and the possible effect of urea in shark muscle tissue, requires ongoing investigation. As our understanding of the factors regulating diet-discrimination factors increases, future work will also need to investigate the effect of growth rate (for juvenile sharks), variable diets and environmental controls (Trueman et al. 2005; McCutchan et al. 2003; Barnes et al. 2007). The issue of variable diets and environmental conditions, specifically temperature, are of particular interest considering that sharks inhabit polar, temperate, sub-tropical and tropical environments (Compagno 1994) and consume a diverse prey base (Wetherbee & Cortés 2004).

The final chapter of this thesis (Chapter Six) applied the derived aquaria  $\delta^{15}N$  diettissue discrimination factor (Chapter Five) in conjunction with  $\delta^{15}N$ ,  $\delta^{13}C$  values of wild caught sharks, a comprehensive archived stomach content data set and knowledge of movement patterns (Chapter Two) to examine the utility of ontogenetic isotopic profiling of the dusky and scalloped hammerhead (Sphyrna lewini) sharks. Adopting a multifaceted approach to investigate the ecology of marine animals is widely recognised and considered necessary to validate ecobiogeochemical techniques (Hobson 1999; Rubenstein & Hobson 2004). Ontogenetic trophic profiles (TP) were generally similar between those derived from  $\delta^{15}N$  values (TP<sub>SIA</sub>) and those calculated from stomach contents (TPr) providing confidence that correctly applied, stable isotopes provide a reasonable estimate of trophic position in sharks. Α discrepancy between estimated TP<sub>SIA</sub> and TP<sub>r</sub> for pregnant dusky sharks, however, suggests that multiple methodological approaches are required. For the pregnant dusky sharks, it is likely that stomach content data reflects a short term integration period of feeding habits when the animals move inshore to pup. Similarly, the higher TP<sub>SIA</sub> values for the scalloped hammerhead shark, when compared to TP<sub>r</sub> estimates, suggest that the broad trophic categories used in TPr calculations (Cortés 1999) may be too coarse and reduce TP accuracy. Additionally, the examination of stable isotopes in sharks is complicated by their movement between isotopically distinct food webs and further work is required to quantify the trophic structure of coastal and pelagic environments off Southern Africa to improve TP<sub>SIA</sub> estimates. With prior knowledge of the systematic variations in  $\delta^{13}$ C along the coast of South Africa and between inshore and offshore environments (Hill et al. 2006), the ontogenetic  $\delta^{13}C$ profiles of the dusky and scalloped hammerhead sharks were in agreement with known information on the movement patterns of these two species (Chapter Two; Klimley 1987; Duncan & Holland 2006). Considering the relative inexpense of stable isotope analysis and the possibility of minor invasive sampling via biopsy,  $\delta^{13}C$ ontogenetic profiles may therefore provide an important tool to generate coarse resolution movement patterns of little known/studied species. Additionally, these ontogenetic profiles could be used to generate a priori hypotheses to direct the application of more expensive tracking technologies, for example satellite tags. With respect to community-based stable isotope modelling, it is necessary to understand ontogenetic variation in TP of large marine predators (Layman et al. 2005) in conjunction with more detailed knowledge of their interaction times in coastal and pelagic food webs. With the understanding that nitrogen and carbon stable isotopes are intrinsically inter-connected, it is important that ontogenetic profiles of both  $\delta^{15}N$ and  $\delta^{13}C$  in large marine predators are presented. Although the interplay between  $\delta^{15}N$ and  $\delta^{13}C$  values complicates interpretation of the data (Layman *et al.* 2007), accounting for both profiles presents a more accurate picture of the trophic, feeding and movement ecology of sharks.

The study of sharks presents a complex but rewarding challenge. Relative to teleost fish, their low levels of abundance, migratory nature and longevity coupled with the logistical difficulties of studying large animals in marine systems, entails long-term investment in study. This thesis took advantage of large archival data sets and the sampling of tissues from sharks caught in beach protection nets to further our ecological knowledge of the dusky shark off Southern Africa. Although, there is much that remains unknown regarding the ecology and life-history of the dusky shark, this thesis provides important data and raises specific questions relevant to improving monitoring and analysis techniques and will assist in regional management planning.

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## APPENDICES

## **APPENDICES**

**Appendix A:** The diet of the scalloped hammerhead shark (*Sphyrna lewini*) off KwaZulu-Natal, South Africa from archived stomach content data (1983 - 2006). Data are broken into size classes and sex and percent gravimetric mass (%Mass) and percent index of relative importance (%IRI) are presented. For sample sizes see Fig. 2.

	Small	111100			Medium				Large			
	%Mass		%IRI		%Mass		%IRI		%Mass		%IRI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
TELEOST	62.98	67.37	55.63	83.87	52.35	56.28	54.80	57.56	49.24	49.76	64.21	68.37
Acantheridae	0.56		0.03									
Anguillidae	4.37	2.17	0.33	0.21	1.82	0.24	0.29	0.01		0.90		0.21
Ariidae	0.72	0.77	0.07	0.04	0.49	2.25	0.15	0.33		1.91		0.53
Balistidae												
Belonidae						0.07		0.00				
Carangidae	0.73	1.88	0.07	0.34		0.08		0.04		0.05		0.01
Cheilodactyleidae					3.57	0.58	0.22	0.01				
Chirocentridae												
Chloropthalmidae												
Cichlidae												
Clupeidae	1.58	0.24	0.24	0.01		1.22		0.16		1.37		0.37
Congridae	0.26		0.01									
Cynoglossidae												
Dactylopteridae									0.75		0.15	
Elopidae												
Engraulidae		0.09		0.00						0.04		0.03
Ephippidae												
Exocetidae		1.57		0.06		0.32		0.01				
Exocoetidae												
Gempylidae												
Gerreidae												
Haemulidae	0.39	3.75	0.04	2.95	2.54	1.31	1.40	1.30		15.81		13.66
Istiophoridae												

Leiognathidae	0.06	1.30	0.02	0.15		0.26		0.01		0.06		0.01
Mugilidae						0.22		0.01		0.15		0.02
Mullidae	2.89	1.39	0.12	0.02		1.05		0.02				
Muraenidae												
Myctophidae					0.06		0.03					
Oplegnathidae						0.14		0.00		1.74		0.63
Ostraciidae	0.52		0.02			0.21		0.01				
Ostraciidae		0.09		0.00								
Peristediidae									1.94		0.26	
Platycephalidae		0.14		0.01		2.38		0.08				
Pleuronectidae												
Plotosidae	1.36	1.05	0.13	0.17		3.70		0.52		4.46		0.65
Pomacentriidae	0.19		0.03									
Pomatomidae												
Priancathidae		1.28		0.02								
Sciaenidae	2.31	5.19	0.43	0.77	1.85	8.52	0.30	6.23		1.81		1.89
Scombridae	6.22	4.13	0.87	0.45	10.66	1.70	3.16	0.37		8.36		1.13
Scorpididae												
Serranidae		0.91		0.07		0.18		0.00		0.35		0.03
Sparidae	3.21	12.16	1.59	8.89	4.16	9.56	1.84	10.49	7.50	0.81	2.38	1.63
Sphyraenidae		0.84		0.09	0.42	0.10	0.14	0.01	0.50		0.21	
Sygnathidae		0.04		0.00		0.01		0.00				
Synodontidae	13.37	2.71	3.31	0.16	0.49	0.20	0.15	0.02	0.60		0.14	
Teraponidae	3.37	4.57	0.42	0.73	3.86	2.11	1.31	0.53	6.38	0.13	0.68	0.02
Trichiuridae	4.40	4.10	0.31	0.65	4.69	8.11	2.58	5.91		0.79		0.16
Triglidae	0.04		0.01									
Unidentified teleost	16.43	17.03	47.58	68.08	17.74	11.77	43.23	31.47	31.56	11.01	60.38	47.39
ELASMOBRANCH	6.05	6.52	1.28	0.58	7.56	16.01	1.34	5.43	0.00	37.96	0.00	9.74
Carcharhinidae										1.25		0.14
Dasyatidae						1.27		0.02		2.56		0.24
Gymnuridae						2.76		0.09		2.25		0.22
Lamnidae												
Mobulidae												
Myliobatidae												
Odontaspididae												

Rajidae	1.88		0.14		3.02		0.65						
Rhinobatidae	0.44	2.76	0.02	0.11	1.63	5.34	0.18	2.12		11.54		1.42	
Rhinopeteridae													
Scyliorhinidae	3.51	2.39	1.09	0.28	1.11	6.64	0.12	3.19		8.01		3.59	
Sphyrnidae													
Squalidae					1.80		0.39						
Squatinidae										4.80		0.19	
Unidentified batoid		0.29		0.01									
Unidentified shark Unidentified										6.96		2.98	
elasmobranch	0.21	1.07	0.03	0.17						0.58		0.95	
CEPHALOPOD	18.87	24.34	29.00	15.23	39.07	23.71	43.53	33.83	50.69	11.72	35.62	21.82	
Coleoidae	1.79	1.65	0.18	0.38		0.37		0.08					
Enoploteuthidae					1.41	1.24	0.15	0.20	31.56	0.09	3.35	0.03	
Loligo spp.													
Lycoteuthidae		0.18		0.01									
Octopodidae	10.40	7.59	25.03	7.81	2.93	4.69	1.58	3.72	4.89	0.43	6.10	0.08	
Octopoteuthidae	0.41	4.63	0.10	1.13	16.44	0.20	3.55	0.03	13.22	9.71	25.91	12.83	
Ommastrephidae													
Sepiidae (cuttlefish)	6.28	10.20	3.70	5.90	2.11	3.09	2.64	3.58	1.02	1.50	0.26	8.88	
Teuthoidea (squids)						13.90		26.22					
Unidentified cephalopod		0.07		0.00	16.17	0.22	35.60	0.01					
CRUSTACEAN	12.10	1.75	14.09	0.32	0.99	4.01	0.25	3.17	0.06	0.00	0.09	0.00	
Anomura (hermit crab)													
Brachyura (crabs)	0.11		0.03		0.09	2.05	0.02	2.55					
Macrura (rock lobsters)		1.17		0.22	0.48	0.04	0.10	0.01	0.06		0.09		
Mantis shrimp	11.90		14.06			1.56		0.51					
Unidentified prawn		0.37		0.09	0.36	0.27	0.12	0.08					
Unidentified crustacean	0.09	0.21	0.01	0.01	0.06	0.09	0.01	0.02					

MAMMAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tursiops aduncus												
unidentified dolphin												
unidentified cetacean												
unidentified mammal												
unidentified mysticeti												
unidentified seal												
BIRD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00
Unidentified bird						0.00		0.00	0.01		0.08	
MISCELLANEOUS	0.00	0.03	0.00	0.00	0.04	0.00	0.07	0.00	0.00	0.00	0.00	0.00
Bivalve					0.04		0.07					
Gastropod		0.03		0.00								
Fisherman's bait												

**Appendix B:** The diet of the dusky shark (*Carcharhinus obscurus*) off KwaZulu-Natal South Africa from archived stomach content data (1983 - 2006). Data are broken into size classes and sex and percent gravimetric mass (%Mass) and percent index of relative importance (%IRI) are presented. For sample sizes see Fig. 2.

	Small %Mass		%IRI		Medium %Mass		%IRI		Large %Mass		%IRI	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
TELEOST	65.67	54.27	91.80	82.46	31.98	43.28	64.64	67.93	12.06	78.95	78.13	99.06
Acantheridae												
Anguillidae	1.44		0.12		0.21		0.01					
Ariidae					0.19		0.02					
Balistidae	0.16		0.02		0.35	0.09	0.01	0.00				

Belonidae												
Carangidae	0.31	0.97	0.16	0.11	2.16	0.92	0.17	0.05	0.01		0.00	
Cheilodactyleidae					0.34	0.24	0.02	0.01				
Chirocentridae												
Chloropthalmidae	0.39		0.02									
Cichlidae					0.22	0.15	0.01	0.00				
Clupeidae	2.23	1.99	0.17	1.01	11.88	32.84	36.10	60.67	10.39	78.74	77.08	99.03
Congridae												
Cynoglossidae	0.04		0.01									
Dactylopteridae												
Elopidae												
Engraulidae	0.03		0.01		1.57	0.06	5.25	0.01				
Ephippidae					1.25		0.03					
Exocetidae												
Exocoetidae						0.59		0.02				
Gempylidae		1.67		0.06								
Gerreidae	0.97		0.05									
Haemulidae	0.41	0.44	0.35	0.63	0.24	0.24	0.13	0.03	0.57		0.02	
Istiophoridae									0.23		0.01	
Leiognathidae	3.97		0.11			0.14		0.01				
Mugilidae	1.58	1.11	0.12	0.46		0.38		0.01				
Mullidae						0.04		0.00				
Muraenidae		0.91		0.04	2.16							
Myctophidae												
Oplegnathidae					0.55	0.04	0.01	0.00				
Ostraciidae					0.02		0.00					
Ostraciidae												
Peristediidae												
Platycephalidae												
Pleuronectidae		0.27		0.02		0.05		0.01				
Plotosidae		1.54		0.14					0.21		0.01	
Pomacentriidae												

Pomatomidae	2.28	0.73	0.31	0.10	0.11		0.00		0.11		0.00	
Priancathidae												
Sciaenidae	1.15	7.53	0.19	1.01	0.49	0.13	0.10	0.00	0.17		0.02	
Scombridae	18.40	11.08	5.58	1.79	4.22	3.91	1.75	0.86	0.02	0.09	0.00	0.00
Scorpididae	0.11		0.01									
Serranidae												
Sparidae	5.74	1.17	3.21	0.15	0.32	0.06	0.13	0.00	0.14		0.02	
Sphyraenidae					0.08		0.00					
Sygnathidae												
Synodontidae		0.08		0.02								
Teraponidae												
Trichiuridae					0.01		0.00					
Triglidae												
Unidentified teleost	26.46	24.79	81.37	76.93	5.63	3.40	20.90	6.22	0.21	0.11	0.97	0.03
ELASMOBRANCH	21.11	36.14	2.19	14.79	65.26	48.97	32.65	24.35	78.25	14.26	20.92	0.85
Carcharhinidae	1.20	1.66	0.04	0.06	17.74	4.84	8.34	0.51	29.91	8.12	9.50	0.55
Dasyatidae	4.70	11.04	0.86	3.77	10.03	3.38	4.79	0.55	0.85	0.23	0.05	0.00
Gymnuridae						0.86		0.02				
Lamnidae									5.58		0.29	
Mobulidae	0.68		0.08		0.85		0.05					
Myliobatidae					1.85		0.15		1.75		0.05	
Odontaspididae									6.76		0.54	
Rajidae									0.00		0.00	
Rhinobatidae		0.80		0.04	1.77		0.05		0.23		0.01	
Rhinopeteridae	2.89		0.08						0.39		0.01	
Scyliorhinidae					0.14		0.01		6.62		0.17	
Sphyrnidae	5.27	2.86	0.30	0.21	16.96	6.51	4.35	0.50	7.18	0.52	0.96	0.01
Squalidae												
Squatinidae					0.76	0.73	0.02	0.02	0.00		0.00	
Unidentified batoid	0.62	0.24	0.08	0.02	0.08	1.71	0.01	0.04				
Unidentified	0.01	0.90	0.01	0.14	1.22	0.30	0.21	0.03	0.01		0.00	

elasmobranch												
Unidentified shark	5.74	18.65	0.74	10.56	13.86	30.64	14.69	22.66	18.98	5.39	9.34	0.29
CEPHALOPODS	5.78	4.68	1.99	1.97	2.29	2.37	1.76	0.84	0.47	0.01	0.17	0.00
Coleoidae												
Enoploteuthidae												
Loligo spp.	0.05	0.01	0.01	0.02								
Lycoteuthidae												
Octopodidae	0.41	1.82	0.09	0.28	1.52	1.49	0.72	0.27	0.42		0.03	
Octopoteuthidae												
Ommastrephidae	0.02		0.01									
Sepiidae (cuttlefish)	1.71	1.32	0.68	1.26	0.65	0.62	1.01	0.55	0.05	0.01	0.14	0.00
Teuthoidea (squids)	0.41	0.01	0.23	0.02	0.09	0.09	0.03	0.00				
Unidentified cephalopod	3.17	1.52	0.96	0.40	0.02	0.17	0.00	0.02				
CRUSTACEANS	0.43	0.68	0.13	0.06	1.47	3.57	0.87	6.68	0.01	0.00	0.01	0.00
Anomura (hermit crab)												
Brachyura (crabs)	0.07		0.05		0.63	1.10	0.35	0.48	0.01		0.01	
Macrura (rock lobsters)	0.12	0.60	0.05	0.03	0.08	0.18	0.03	0.04				
Mantis shrimp					0.75	2.29	0.49	6.16				
Unidentified prawn	0.17	0.06	0.02	0.02	0.02		0.00					
Unidentified crustacean	0.07	0.02	0.01	0.02	0.00	0.00	0.00	0.00				
MAMMALS	1.33	3.73	0.11	0.42	1.12	1.82	0.06	0.20	9.20	2.98	0.77	0.04
Tursiops aduncus									3.27	2.98	0.17	0.04
unidentified dolphin	1.33	3.73	0.11	0.42	0.46	1.82	0.03	0.20	0.27		0.01	
unidentified mysticeti									5.36		0.58	
unidentified seal									0.30		0.01	
unidentified cetacean												
unidentified mammal					0.65		0.04					
BIRDS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Unidentified birds												
MISCELLANEOUS	5.69	0.50	3.78	0.30	0.04	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Bivalve												
Gastropods					0.01		0.01					
Fisherman's bait	5.69	0.50	3.78	0.30	0.03		0.00					