The natural history of the humpback dolphin, *Sousa chinensis*, in KwaZulu-Natal, South Africa: Age, growth and reproduction.

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When I look into the eyes of an animal, I do not see an animal.
I see a living being.
I see a friend.
I feel a soul.

~ **A.D. Williams** ~

Abstract

Globally, inshore cetaceans are being threatened by a number of anthropogenic activities. The Indo-Pacific humpback dolphin, *Sousa chinensis*, is currently listed as ‘near threatened’ by the International Union for the Conservation of Nature (IUCN). In order to be able to advise on management and conservation strategies, knowledge on the life history of the species is required. To date very little is known about the biology of humpback dolphins. The aim of the present study was to determine basic life history parameters, including age, growth and reproduction of humpback dolphins incidentally caught in shark nets.

Age was estimated by counting the growth layer groups (GLGs) in the dentine and cementum of sectioned and stained teeth. Both a Von Bertalanffy and a Gompertz growth curve fitted well to the data, but for comparison with previous studies on *Sousa*, the Gompertz growth function was adopted to describe the relationship between length and age for KwaZulu-Natal populations. Length at birth was estimated between 104.33 and 111.57 cm for males and females, respectively. Asymptotic length was reached at 266.48 cm and 239.29 cm for males and females, respectively. This corresponds to the attainment of physical maturity at 24 GLGs in males and 16 GLGs in females. Asymptotic mass for males could not be determined, while for females it occurred around 160 kg. The maximum age estimates and recorded lengths were 24 GLGs and 279 cm for males and 17.7 GLGs and 249 cm for females. Differences in length-at-age and mass-at-age for *S. chinensis* suggest sexual dimorphism.

The attainment of sexual maturity in males occurred between 9 and 10 GLGs, corresponding to 230 cm total body length and 140 kg. The maximum combined testis mass of mature males comprised 0.42% of total body mass, and a roving male mating system was proposed. In females, sexual maturity occurred around 7.6 GLG, between 220 and 222 cm and 104 - 140 kg. The ovulation rate is estimated at 0.2 ovulations per annum, suggesting a calving interval of five years.

It is evident from the results obtained in the present study that geographical differences exist in the life history parameters of *S. chinensis*. As a result, regional conservation and management strategies are imperative. Results from this study can therefore assist in assessing the status of existing population structures in the KwaZulu-Natal coastal waters, and the implementation of regional mitigation strategies to ensure the continued survival of humpback dolphins in the region.

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Chapter 1

General Introduction

1.1 Taxonomic status

The systematics of the genus *Sousa* and the number of species belonging to the genus remains largely unresolved and somewhat controversial (Cockcroft *et al.*, 1997; Cockcroft and Smith-Goodwin, 2002; Rosenbaum *et al.*, 2002; Frère *et al*., 2008). No study has to date completely resolved the ambiguity surrounding the true number of species within the genus (Parra and Ross, 2009). This is largely due to a lack of samples from the majority of the species' range and several contradictory patterns of variation in the skeletal, external morphometric and molecular datasets (Jefferson and Waerebeek, 2004).

Current views range from the recognition of a single, but highly variable species - *Sousa chinesis* - to five nominal species: *S. chinensis* (Osbeck 1765), *S. plumbea* (G. Cuvier, 1829), *S. teuszii* (Kukenthal, 1892), *S. lentigenosa* (Owen 1866) and *S. borneensis* (Kydekker 1901) (Ross, 1984; Ross *et al.*, 1994; Parra and Ross, 2009). Based on the available information for skeletal and external morphometric data, only three of the five nominal species are commonly recognised, corresponding with their geographical range: *S. chinensis* (Eastern Indian Ocean/Western Pacific Ocean), *S. plumbea* (Western Indian Ocean) and *S. teuszii* (Atlantic Ocean) (Jefferson and Waerebeek, 2004). There is, however, some debate regarding the distinction between *S. chinensis* and *S. plumbea*, and whether they are in fact the same species. Morphologically they are very dissimilar, with the former attaining a light coloration and lack of a prominent dorsal hump, and the latter having a dark grey (adult) colouration with a prominent dorsal hump (Frère *et al*., 2008). Preliminary molecular analysis on humpback dolphin populations supports the distinction between *S. plumbea* and *S. chinensis,* suggesting high levels of genetic divergence between populations found in South African and Chinese waters (Cockcroft *et al.*, 1997; Cockcroft and Smith-Goodwin, 2002; Rosenbaum *et al.*, 2002). A more recent study, conducted by Frère *et al*. (2008) found that mitochondrial DNA lineages from South Africa, China (Hong Kong) and Australia represent three distinct, reciprocally-monophyletic clades, which all differ from one another. As a result, the current classification that lists humpback dolphins within the Indo-Pacific region as a single species is not appropriate (Frère *et al*., 2008). Furthermore, Frère *et al*. (2008) found that humpback dolphins from South Africa and China are more closely related to one another than to those from Australia. Thus the current taxonomic classification, which considers humpback dolphins from China and Australia to be the same species, may require a further revision of the genus (Frère *et al*., 2008).

If *S. plumbea* and *S. chinesis* are in fact sympatric, without the two species interbreeding, *S. chinesis* and *S. plumbea* could definitely be described as two distinct species (Jefferson and Waerebeek, 2004). However, this remains largely unconfirmed, as insufficient data are available on the status, biology, ecology and genetics of species belonging to the genus *Sousa* (Karczmarski *et al*., 2000*a,b*; Amir *et al*., 2005). Moreover, according to the list of recognised species compiled by the International Whaling Commission (IWC), only two of the five mentioned species are formally recognised: the Atlantic humpback dolphin, *S. teuszii* and the Indo-Pacific humpback dolphin *S. chinensis* (Amir *et al.*, 2005). Resultantly, due to the current taxonomic dispute and uncertainties associated with the number of species belonging to the genus *Sousa*, humpback dolphins inhabiting South African waters will, for the purpose of this study, be referred to as *Sousa chinensis* (Best, 2007).

1.2 External morphology

Humpback dolphins are easily identified by the characteristic fleshy dorsal hump that bears the dorsal fin (Best, 2007). They possess a robust, medium-sized body that is laterally compressed and becomes increasingly flat towards the flukes (Figure 1.1) (Ross, 1984; Jefferson and Karczmarski, 2001). The snout is long, slender and distinctly set off from the melon, making up roughly 6 - 10% of the total body length (Jefferson and Karczmarski, 2001). The flippers are broad near the base with a rounded apex and the flukes are moderately concave, with a distinct notch between the flukes (Leatherwood and Reeves, 1983).

Figure 1.1: Illustration of humpback dolphin, *Sousa chinensis* (Noel Ashton, 2013).

According to Ross *et al.* (1994), humpback dolphins off southern Africa and in the northern Indian Ocean, are generally larger than those of any other populations throughout the distribution range. Additionally, there is a range of phenotypic variations of *S. chinensis* which differ regionally (Parra and Ross, 2009). These include colouration and the shape and size of both the dorsal fin and the hump (Parra and Ross, 2009). In the Western Indian Ocean, the fin is elongated, thickening at the base, to form a wide dorsal ridge or characteristic hump (Figure 1.2), which covers about 40% of the total body length, with a small and slightly falcate dorsal fin (Ross *et al*., 1994; Jefferson and Karczmarski, 2001; Parra and Ross, 2009). Around the Western Pacific, animals have a slightly larger dorsal fin, which is roughly triangular, curving towards the back of the body (Parra and Ross, 2009). The fin base is comparatively smaller and smoothly blends into the dorsal surface of the body, forming a less distinct hump, and more pronounced fin (Leatherwood and Reeves, 1983; Ross *et al.*, 1994). The shape of dorsal fin and hump of Atlantic Ocean animals resembles that of the Indian Ocean populations; however, the hump tends to be more pronounced and the fin more triangular, with a rounder tip (Parra and Ross, 2009).

Coloration varies both regionally and with developmental stage (Jefferson and Karczmarski, 2001; Parra and Ross, 2009). Calves throughout the range are generally dark in colour, with a lighter ventral surface, which gradually darkens with age (Parra and Ross, 2009). Adult animals found in the Indian Ocean are a plumbeous gray on the dorsal and lateral surfaces, which gradually lightens, to off-white on the ventral surface, with occasional spotting (Ross *et al*., 1994; Jefferson and Karczmarski, 2001). Atlantic Ocean populations have a similar appearance to animals found in the Indian Ocean (Parra and Ross, 2009), while the Pacific Ocean populations differ quite markedly in colour. Sub-adults are covered in a mosaic of grey and pink, which lightens with age until the animals are eventually pure white, with a tinge of pink, resulting from intense circulation during periods of high activity (Jefferson 2000; Jefferson and Karczmarski, 2001). Some adults have dark spots on the body, forming a ring around the neck, just behind the blowhole (Jefferson and Karczmarski, 2001). In Australian populations the entire dorsal section of adult humpback dolphins is light grey, except for the rostrum, melon and dorsal fin, which whiten with age (Ross *et al*., 1994). According to Jefferson and Karczmarski (2001), the transition between dark- and light-coloured humpback dolphins is thought to occur in the eastern Indian Ocean between India and Thailand.

Figure 1.2: The dorsal hump of an Indo-Pacific humpback dolphin (*Sousa chinensis*) off Algoa Bay, Port Elizabeth, South Africa (Photo by: S. Plön).

1.3 Distribution and abundance

1.3.1 Global range

There is a general lack of global information regarding the distribution and abundance of *S. chinensis* (Amir *et al.*, 2005). However, the general distribution range of these dolphins covers the entire Indian Ocean stretching from False Bay (Western Cape, South Africa), in the Atlantic Ocean east towards Central China (South Pacific), to the northern East coast of New South Wales, Australia in the Western Pacific (Ross et al., 1994; Best, 2007). The distribution, however, is not continuous throughout this range (Leatherwood and Reeves, 1983).

S. chinensis has been recorded in South Africa, Mozambique, Tanzania, Zanzibar, Madagascar, Kenya, Comoros Islands, Somalia, Djibouti, Saudi Arabia, United Arab Emirates, Yemen, Bahrain, Oman, Qatar, Iraq, Iran, Kuwait, Pakistan, India, Sri-Lanka, Burma, Thailand, Vietnam, southern China, Taiwan, Malaysia, Indonesia, Brunei, Singapore, Papua New Guinea, eastern Australia and Korea (Ross, 1984; Jefferson and Karczmarski, 2001; Best, 2007). Countries included within this range for which no official sightings of humpback dolphins have yet been recorded include Eritrea, Sudan, Bangladesh and Cambodia (Corkeron *et al*., 1997; Jefferson and Karczmarski, 2001). The lack of sightings by nation may reflect lengths of coastline / observer effort in some cases.

In southern African waters, the distribution range stretches from the coastal waters of Mozambique (Karczmarski, 2000), south into KwaZulu-Natal and the Eastern and Southern Cape (Findlay *et al.*, 1992). The distribution seems to be more or less continuous from Mozambique into the Eastern Cape waters (Best, 2007). The most western locality where humpback dolphins occur with any regularity appears to be just south of the Danger Point peninsula (Gansbaai), Western Cape, South Africa (Best, 2007). Distribution probably extends further north than Mozambique, but sightings are patchy (Cockcroft, 1990; Jefferson and Karczmarski, 2001). The reason for this may be a reflection of observers effort. In KwaZulu-Natal waters, humpback dolphins are less common south of Richards Bay (Cockcroft, 1990).

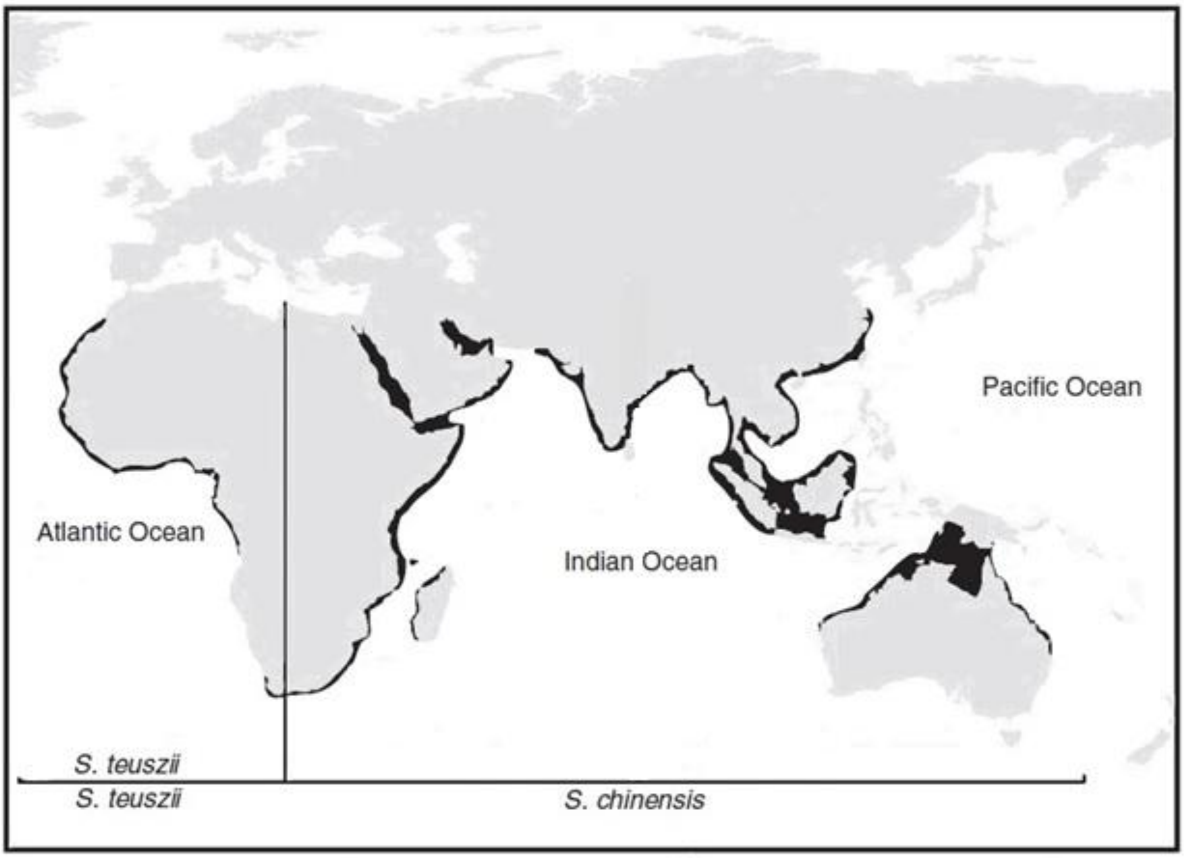


Figure 1.3: The global distribution range of the Indo-Pacific humpback dolphin (*Sousa chinensis*) (Frère *et al*., 2008).

1.3.2 Population size and abundance

There is no overall estimate of the total global population size of humpback dolphins (Reeves *et al*., 2008). In Australian waters, an estimated 100 individuals inhabit the waters of Moreton Bay and southern Queensland (Corkeron *et al.*, 1997), while in Cleveland Bay, populations numbers are thought to be fewer than 100 animals (Parra and Ross, 2009). Populations in the region are thought to be declining, primarily due to mortalities in shark nets (Corkeron *et al*., 1997). Population estimates in Western Australia are not clearly known, but it is expected that it may support a higher density than those reported for Queensland (Brown *et al*., 2012). Along the eastern Taiwan Strait there are an estimated 99 humpback dolphins, with an additional 60 individuals which were discovered only in 2002. Estimates are somewhat vague due to the limited number of sightings recorded (Wang *et al*., 2007).

In South African coastal waters, humpback dolphin populations are generally small, with no more than a few hundred individuals (Parra and Ross, 2009). Certain sub-populations are thought to be depleted, mainly as a result of habitat destruction or degradation and bycatch in fish nets (Corkeron *et al.*, 1997; Cockcroft, 1999; Reeves *et al*., 2008). Population estimates in South African waters range between 1000 and 2500 animals, based on density calculations primarily in Algoa Bay (Eastern Cape), and Richard's Bay (KwaZulu-Natal) (Karczmarski, 2000; Peddemors *et al*., 2004). Karczmarski *et al.* (1999) estimated a population size of 466 animals in Algoa Bay, of which 270 are thought to be adults. Only a small portion of this population is, however, found in the area at any given time as much of the population is widely distributed along the coast of the Eastern Cape (Jefferson and Karczmarski, 2001). In Richard's Bay, KwaZulu-Natal numbers are estimated between 150 - 244 individuals (Ross, 1982; Atkins and Atkins, 2002).

There is expected to be some sharing of stocks between South Africa and Mozambique (Peddemors *et al.*, 2004) and preliminary estimates suggest 105 humpback dolphins inhabit the waters of Maputo Bay, Mozambique (Parra and Ross, 2009). A very small population of between 58 and 65 dolphins has been recorded off the south coast of Zanzibar (Parra and Ross, 2009). Furthermore, near the south coast of Unguja, (Tanzania) around 71 individuals have been identified as resident (Stensland, 2004).

1.3.3 Habitat preference

Humpback dolphins are a tropical to sub-tropical species found in coastal waters (Parra and Ross, 2009), and show a strong preference for high water temperatures (15◦C to 36◦C), such as that of the warm eastern boundary currents of the Persian Gulf, the northern Red Sea and on the eastern coast of southern Africa and Australia (Ross *et al.*, 1994). Within their distribution range, humpback dolphins show a vast array of habitat preferences, depending on their geographical location (Best, 2007; Parra and Ross, 2009). Habitat types include sandy beaches, embayments which provide some shelter, coastal lagoons, rocky and coral reefs, mangrove swamps, and estuarine waters (Ross *et al.*, 1994; Jefferson and Karczmarski, 2001). Habitat choice among different populations is well defined and persistent in each geographical location (Jefferson and Karczmarski, 2001).

Humpback dolphins are rarely encountered more than a few kilometres offshore (Reeves *et al.*, 2008); except in Australia, where they have been recorded several kilometres offshore (Parra *et al.*, 2004). However, they are generally found in shallow water surrounding islands, reefs and reef lagoons (Parra *et al.*, 2004). In China, humpback dolphins commonly swim several kilometres up rivers to feed on estuarine-associated species (Ross *et al*., 1994) and are rarely found far from estuaries and mangrove habitats (Reeves, *et al*., 2008).

Throughout southern African waters, humpback dolphins are largely restricted to the shallow coastal waters in tropical, sub-tropical and warm temperate regions (Findlay *et al*., 1992; Ross *et al*., 1994). They occur in water depths ranging between 15 m and 50 m (Findlay *et al.*, 1992; Karczmarski *et al*., 2000*a*), corresponding to a distance of about 200 to 400 m offshore, seawards of the breaking waves (Saayman and Tayler, 1979; Karczmarski *et al*., 2000*a*). Habitat preference varies among the different populations (Durham, 1994). In KwaZulu-Natal, humpback dolphins are commonly found in the coastal waters adjacent to large, murky estuarine systems (Durham, 1994). In the Eastern Cape they are commonly found in the vicinity of rocky reefs and open sandy beaches (Saayman and Tayler, 1979; Ross, 1984; Karczmarski *et al*., 2000*a*). Unlike elsewhere along its distribution range, humpback dolphins have rarely been recorded entering estuarine systems in the south-east coast of southern Africa, as they tend to be very small, shallow and generally inaccessible (Ross, 1984; Ross *et* al., 1994; Karcmarski *et al.*, 2000*a*). However, reports do exist of lone humpback dolphins entering estuaries, such as the Swarkops River and Kowie River in the Eastern Cape Province of South Africa (S. Plön, 2012, pers. comm.).

1.4 Feeding and diet

Relatively little information is available on the diet of *Sousa*. Most of the work that has been done originates from Asia and Australia (Barros *et al*., 2004; Parra and Jedensjö, 2009). Humpback dolphins appear to show a preference for inshore, shoaling fish species (Ross *et al*. 1994). They are regarded as opportunistic generalist feeders that feed mainly on estuarine, littoral and reef-associated fish species and occasionally on cephalopods and crustaceans; both on the bottom and within the water column (Saayman and Tayler, 1979; Ross *et al*., 1994; Jefferson and Karczmarski, 2001; Parra and Ross, 2009; Venter, 2009). The most common prey species exploited by humpback dolphins in South Africa and China alike, include fishes belonging to the families Haemulidae, Clupeidae, Mugilidae, Sciaenidae and Sparidae (Parra and Ross, 2009). In South Africa, the most common prey species that form part of the diet of *S.chinensis* is *Thryssa vitrirostris* (Orangemouth anchovy), which makes up the largest percentage of their diet (Venter, 2009). This is followed by *Trichiurus lepturus* (Cutlass fish), *Pomadasys olivaceum* (Pinky grunter), *Johnius amblycephalus* (Bearded croaker) and *Otolithes ruber* (Tigertooth croaker) (Venter, 2009). Other species, consumed to a lesser extent, are *P. commersonii* (Spotted grunter), *Macrura kelee* (Kelee shad), *Liza richardsonii* (Southern mullet), *Mugil cephalus* (Flathead grey mullet), *Afroscion thorpei* (Squaretail kob), *Diplodus sargus* (White seabream), *Pachymetopon aeneum* (Blue hottentot) and *Rhabdosargus thorpei* (Bigeye stumpnose) (Ross, 1984; Barros and Cockcroft, 1991; Best, 2007; Parra and Ross, 2009; Venter, 2009). There exists some variation in the diet between *S. chinensis* inhabiting South African waters and those found in the western Pacific region, as a result of geographical differences in their distribution (Venter, 2009).

Foraging activities are generally associated with river mouths, inshore reefs and tidal channels (Parra and Ross, 2009). Humpback dolphins in Plettenberg Bay can be seen hunting at shallow depths around reefs after a drop in water temperatures, usually the result of a south-easterly wind (Saayman and Tayler, 1979). This results in the large-scale inshore movement of several pelagic fish species, which are forced into shallower waters (Saayman and Tayler, 1979). The feeding of humpback dolphins in this area is highly influenced by tidal cycles, with a marked increase in feeding activity and a decrease in group associations as the tide rises (Saayman and Tayler, 1979). Ross (1984) suggests that this type of behaviour may be a result of their inshore distribution and possibly due to the influx of various prey species during an incoming tide. Furthermore, for the duration of the high tide, fish tend to shoal in relatively compact groups, thereby increasing the chance of being captured by predators (Saayman and Tayler, 1979). Similarly, feeding behaviour in Algoa Bay demonstrates tidal, diurnal and seasonal patterns, with increased feeding during high tide and during the winter season around reefs and rocky coastal areas (Ross, 1984; Parra and Ross, 2009). In Hong Kong waters, humpback dolphins are commonly seen feeding in freshwater/saltwater mixing zones (Parra and Ross, 2009). In northern Australia and the Bazaruto Archipelago, Mozambique, humpback dolphins can be seen intentionally beaching themselves while chasing fish into shallower waters and onto sandbanks (Peddemors and Thompson, 1994). Moreover, humpback dolphins are often seen feeding behind fishing trawlers, specifically in Australia and Hong Kong (Parra and Ross, 2009).

Cooperative feeding between humpback dolphins is limited, as individuals forming part of foraging schools are generally widely dispersed (Saayman and Tayler, 1979). Individual feeding strategies may be advantageous, as fewer individuals compete for the same resources, especially if these resources are restricted to reefs or rocky outcrops in exploitable numbers (Saayman and Tayler, 1979). When feeding cooperatively, a small group of about six humpback dolphins remain in close proximity to one another and move up and down several hundred meters of coastline (Best, 2007).

1.5 Behaviour

The observed day-time behaviour of humpback dolphins includes feeding or foraging, travelling and resting (Saayman and Tayler, 1979). Foraging behaviour dominates the early daytime activity of populations in South African (Algoa Bay and Richards Bay), Chinese and Australian coastal waters (Parra and Ross, 2009). According to Karczmarski *et al*. (2000), the daylight occurrence of humpback dolphins in Algoa Bay is related to their foraging and feeding activities, which in turn is governed by the diurnal cycle of their prey species.

Saayman and Tayler (1979) categorised group behaviour of humpback dolphin in Plettenberg Bay into four major categories: group progression, feeding behaviour, social behaviour and resting behaviour. When travelling, humpback dolphins generally move together in group formations, and disperse when feeding (Saayman and Tayler, 1979). According to Karczmarski *et al*. (1997), feeding strategies, as observed in Algoa Bay populations, are limited to dispersed group feeding, with individuals moving in various directions along several hundred meters of coastline, around 200-300 m offshore. When in open sandy beaches, they tend to form larger associations, where they engage in social activities and rest (Saayman and Tayler, 1979). Social behaviour involves numerous bodily interactions between individuals, such as swimming, leaping, caressing, chasing and colliding (Saayman and Tayler, 1979). Karczmarski *et al*. (1997) reports that mating behaviour observed in Algoa Bay is more complex than that described by Saayman and Tayler (1979) and Zbinden *et al*. (1977) for humpback dolphins in Plettenberg Bay and the Indus Delta region, respectively; often involving two to six members temporarily isolated from other members of the group (Karczmarski *et al*., 1997).

1.6 Social organisation

Humpback dolphins live in a fission-fusion society, where school size and composition is variable, with only short-term affiliations between individuals (Jefferson and Karczmarski, 2001). Social bonds are not particularly strong as individuals move indiscriminately between different groups (Leatherwood and Reeves, 1983), with mother-calf bonds being the exception (Jefferson and Karczmarski, 2001). A lack of consistency in group membership is common in both South African and Hong Kong populations (Jefferson and Karczmarski, 2001). However, this cannot be considered the norm for all humpback dolphins throughout their distribution range, as strong affiliations between members of a population have been observed in Maputo Bay, Mozambique (Jefferson and Karczmarski, 2001).

Group size appears to be somewhat dependent on two major factors: the need for protection from predators, and resource availability (Karczmarski, 1999). In Algoa Bay there is a seasonal (summer and late winter) increase in group size, which coincides with the summer reproductive seasonality of humpback dolphins (Saayman and Tayler, 1979; Karczmarski, 1999). Moreover, the formation of larger nursery groups provides a protective environment for early postnatal development, learning, and alloparental care (Karczmarski, 1999).

School sizes of humpback dolphins in the Eastern Cape are up to 30 dolphins, with a mean of 6.9 individuals per pod (Ross, 1984). In KwaZulu-Natal coastal waters, group sizes range from one to 20 individuals per pod, with an average group size of 5.1 dolphins (Durham, 1994). Larger pods of between 30 - 100 individuals have been observed off the coast of Oman (Parra and Ross, 2009). Adults generally travel alone or in groups of 2 - 6 individuals (Parra and Ross, 2009), while immature humpback dolphins tend to associate with larger groups (Saayman and Tayler, 1979). Saayman and Tayler (1979) found that the majority of humpback dolphin pods consist of young adults, together with older adults, juveniles and calves, suggesting that females with calves will join a larger group rather than travel alone. This could be because larger groups provide more sensory integration, safety in numbers and are well suited for cooperative active defence (Karczmarski, 1999).

Indo-Pacific humpback dolphins do not undertake any major seasonal migrations. However, in southern Africa, distinct seasonal shifts in population sizes have been recorded (Jefferson and Karczmarski, 2001). The Eastern Cape humpback dolphins range over a wide distance along a narrow band of coastline; however, during the summer months, there is a considerable influx of dolphins into Algoa Bay (Karczmarski, 1999). In Algoa Bay, site fidelity is considered a function of prey availability (Karczmarski, 1999). Females are generally reluctant to leave a partic6ular area when in their reproductive stage or when lactating, as there are numerous physical limitations for young calves to travel extensive distances (Karczmarski, 1999). This forces lactating females to focus their activities over more limited areas (Karczmarski, 1999). In KwaZulu-Natal only females appear to show relatively strong site fidelity, whereas males tend to move between groups (Durham, 1994). In Australian and Mozambican waters, there is little seasonal variation in school size, indicating some degree of site fidelity (Saayman and Tayler, 1979; Ross *et al.*, 1994; Karczmarski, 1999; Parra and Ross, 2009). Humpback dolphins in Hong Kong are largely restricted to the vicinity of large estuaries, thus limiting seasonal movements (Jefferson and Karczmarski, 2001).

1.6.1 Mixed group interactions

Humpback dolphins share a large part of their distribution range with Indo-Pacific bottlenose dolphins, *Tursiops aduncus*, snubfin dolphins, *Orctaella heinsohni* and finless porpoises, *Neophocaena phoceanoides* (Parra and Ross, 2009). Although not common, mixed groups of humpback and bottlenose dolphins have been observed in the coastal waters of South Africa, Tanzania, Oman, Australia and China (Jefferson, 2000; Parra and Ross, 2009). Humpback dolphin groups in Algoa Bay often follow bottlenose dolphins and have been observed feeding around the periphery of these groups (Karczmarski *et al*., 1997). Single individuals may also, at times, be integrated in bottlenose dolphin schools (Best, 2007). Although these two species have been observed in close association, interactions between them are very limited (Karczmarski *et al*., 1997).

1.7 Communication

Social communication is thought to be important in mediating social interactions (van Parijs and Corkeron, 2001). Vocalisation rates are highest during foraging and socialising, and usually less evident - but not absent - during travelling (van Parijs and Corkeron, 2001). The different sounds produced have been categorized into clicks, whistles, quacks and grunts (Ross *et al*., 1994). "Clicks" and "whistles" are mainly employed during foraging activities, while "whistles", "grunts" and "quacks" serve to communicate with other humpback dolphins during social behaviour, but may also be used when foraging (van Parijs and Corkeron, 2001; Parra and Ross, 2009). "Whistle" production is particularly high in groups with a greater number of mother-calf pairs, suggesting its use as contact calls (van Parijs and Corkeron, 2001). It has also been suggested that humpback dolphins make use of their hearing ability to locate sound-producing prey by passively listening (Parra and Ross, 2009).

1.8 Threats

Humpback dolphin populations appear to be predominantly threatened by anthropogenic activities, as their near-shore distribution and preference for shallow water habitats make them susceptible to the effects of human activities (Corkeron *et al*., 1997; Parra and Ross, 2009). The greatest threats to *Sousa* throughout its range are incidental mortalities in gill and shark net gear, and habitat degradation/loss (Cockcroft and Krohn, 1994; Karczmarski, 2000; Stensland *et al*., 2006). Coastal construction influences the natural habitat of humpback dolphin populations in many ways. To date very little research has been done regarding the impact of habitat degradation on humpback dolphin populations in South Africa (Jefferson and Karczmarski, 2001). However, it is believed that coastal development is regarded as the greatest threat to their survival in the region (Jefferson and Karczmarski, 2001).

Additional threats include wildlife tourism, fishing-vessel traffic, pollution, coastal and offshore development, oil and gas exploration and human overpopulation (Jefferson, 2000; Parra and Ross, 2009). The deliberate killing of dolphins for human consumption is also known to occur in Africa and Madagascar (Amir and Jiddawi, 2001; Amir *et al.*, 2002; Stensland and Berggren, 2007; Best, 2007). Little is known, however, about the extent of human-caused mortality (Karczmarski, 2000).

Inshore dolphin species are particularly sensitive to over exploitation and recovery is difficult for already-depleted populations that are restricted to specific habitats (Cockcroft, 1990). Data available for humpback dolphin populations off the coast of KwaZulu-Natal indicate that different populations are geographically distinct from one another, suggesting that depletion of individual groups may lead to local extinction (Cockcroft and Krohn, 1994). The establishment of marine conservation areas, with strictly controlled eco-tourism and the declaration of priority sites has been suggested to be the most effective approach for conservation of humpback dolphins in South African coastal waters (Karczmarski, 2000).

1.8.1 Incidental capture in gill and shark nets

Deaths caused by the incidental capture of marine mammals in fishing gear (i.e. fishing nets and trawls) is a recurring problem, and mortalities resulting from the interaction between fisheries and marine mammal distribution is recognised as a major threat to marine mammals globally (Cockcroft, 1990). Several incidental and possibly intentional catches occur throughout most if not all of the distribution range of humpback dolphins (Jefferson and Karczmarski, 2001). The exact interaction between marine mammals and the fishing industry has not been recorded, due to difficulties in accurate monitoring (Cockcroft and Krohn, 1994). However, incidental captures by the fishing industry have been documented in Djibouti, the Arabian Gulf, the Indus Delta, the south-west coast of India and in Taiwan (Ross *et al.*, 1994; Jefferson and Karczmarski, 2001).

In South African waters, the greatest threat to local humpback dolphin populations are the non-commercial shark nets set to reduce shark-bather interactions (Cockcroft, 1990; Ross *et al.*, 1994; Dudley, 1997; Parra *et al.*, 2004). Gill nets have been deployed intermittently along 320 km of the KwaZulu-Natal coast, between Richards Bay and Port Edward, with the intention of reducing the possibility of shark-bather interactions along popular beaches (Figure 1.4) (Cockcroft, 1990; Atkins *et al*., 2013). The first nets were installed off Durban in 1952 and subsequently at other beaches along stretches of coast (Dudley and Cliff, 2010). At its maximum, the shark net program consisted of 44 km of netting. However, between 1999 and 2004 the length of netting was dramatically reduced to only 23 km, so as to minimize incidental marine animal catches (e.g. cetaceans and sea turtles) (Dudley and Cliff, 2010; Plön *et al*., 2012). The majority of nets are 213.5 m long and 6 m deep (Atkins *et al*., 2013). They run parallel to the coast, roughly 300 - 500 m offshore just beyond the surf zone (Atkins *et al*., 2013). It was not until the early 1980s that the shark netting operations became a government-controlled task. This is now controlled by the KwaZulu-Natal Sharks Board, which maintains and services the nets (Cockcroft, 1990). Between 1952 and the mid-1970s, shark nets were regulated and maintained by private tenders (Cockcroft, 1990). Resultantly, prior to the 1980s there was little assessment of the numbers of non-target species caught in the nets (Cockcroft, 1990). Currently, nets are checked 15 - 20 times per month by the KwaZulu-Natal Sharks Board (KZNSB) to remove any bycatch (Atkins *et al*., 2013).

Various non-target species, such as cetaceans, are prone to incidental capture. *Sousa chinensis* (along with *Tursiops aduncus* and *Delphinus capensis*) are particularly vulnerable due to their inshore distribution and movements (Cockcroft, 1990; Atkins *et al*., 2013). Of all small cetaceans accidentally caught in the nets, less than 1% is released alive (Cockcroft and Krohn, 1994). KZNSB have recorded 203 humpback dolphin captures over a 30 year period (1980 - 2009) (Atkins *et al*., 2013). Only 186 of these individuals were successfully sexed and measured (Atkins *et al*., 2013). It was reported that the majority of humpback dolphin captures (62%) occurred at Richard's Bay and the second highest catch rate was recorded just south of Richard's Bay at Zinkwazi, which accounts for 16% of all catches (Atkins *et al*., 2013). The remaining 33% occurred at various other netted locations along the 320 km protected coastline of KwaZulu-Natal, from Blythedale, south to Port Edward (Atkins *et al*., 2013) (Figure 1.4). A minimum of 67 humpback dolphins were captured between 1980 and 1988 (Cockcroft, 1990), and an annual average of six humpback dolphins between 2005 and 2009 (KZN Sharks Board, 2011). Atkins *et al*. (2013) reported a bycatch total of 203 humpback dolphin over a 30-year period (between 1980 and 2009), with an average of 6.8 per annum. Although this number does not appear particularly significant, when compared to the number of humpback dolphins in existing populations in KwaZulu-Natal coastal waters, this poses a concern (Cockcroft, 1990). Captures at the current rate account for a 4% mortality rate based on the population estimate made by Durham (1994) of 165 humpback dolphins, which exceeds the acceptable 2% mortality rate proposed by the International Whaling Commission (1994). However, when solely considering Richard's Bay (where the majority of catches are recorded), the mean annual catch rate results in an alarming 10% mortality rate, based on the estimate of 165 humpback dolphins in that area (Durham, 1994).

Despite the obvious impact of population declines resulting from incidental captures, mortality patters may also indirectly affect the genetics of the populations as well as the life history, demography and social systems (Atkins *et al*., 2013). This is of particular concern for species with small population sizes, such as humpback dolphins (Atkins *et al*., 2013). On-going incidental captures and mortalities may therefore lead to serious declines in humpback dolphin populations in the KwaZulu-Natal region (Ross, 1982; Cockcroft, 1990) as losses through incidental captures or entanglements may be close to or greater than the replacement rate (Karczmarski, 2000).

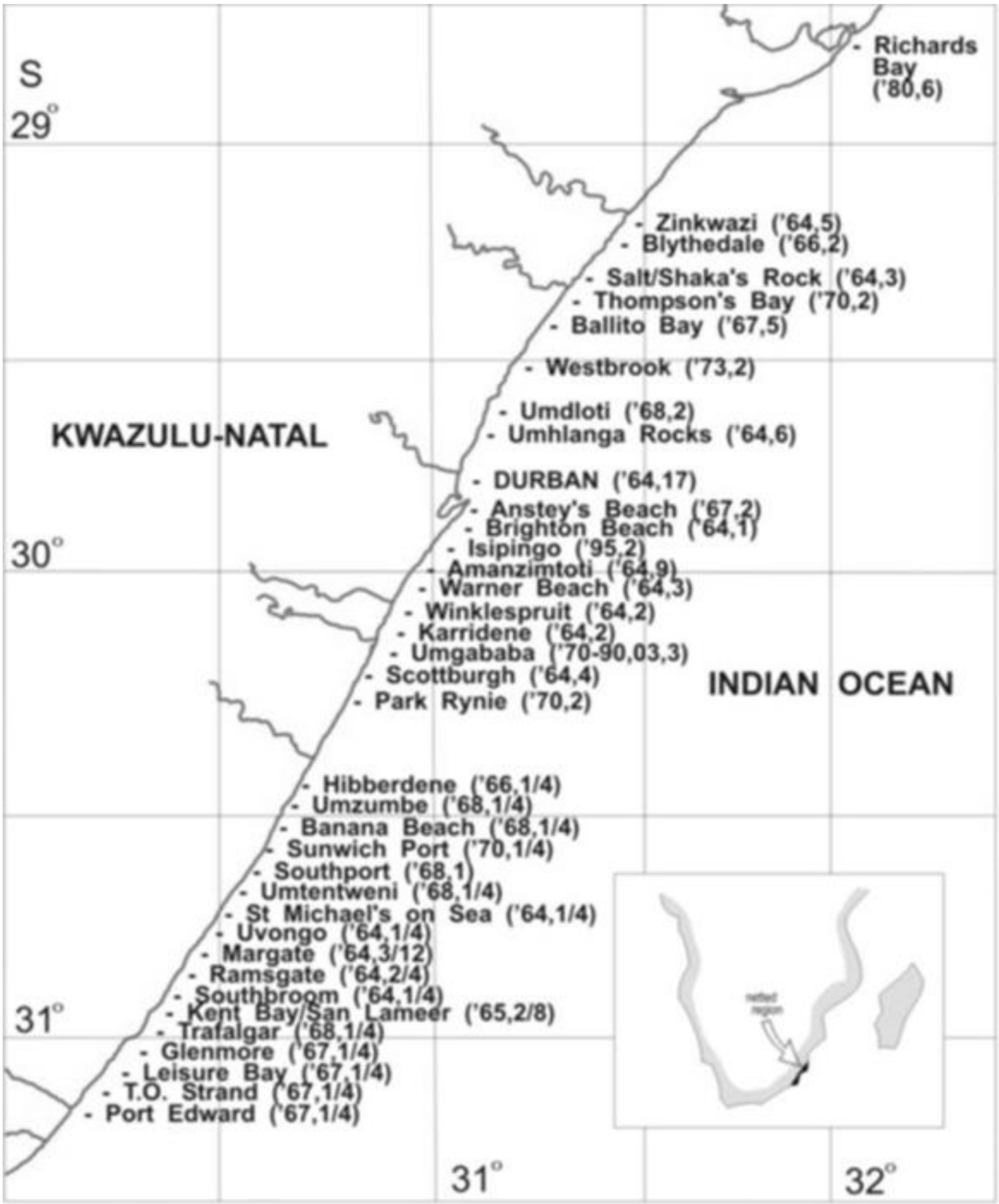


Figure 1.4: The location of shark nets deployed along the KwaZulu-Natal coastline. The first number within the parentheses indicates the year of net installation, and the second/third indicate, respectively, the number of nets/drumlines currently used. The total distance of netting along the coast is 23 km along a 320 km stretch of coast (KZN Sharks Board, 2011).

1.8.2 Pollution

Being an inshore species, humpback dolphins are exposed to run-off pollution entering the coastal environment (Karczmarski, 2000), with synthetic chlorinated hydrocarbons being reported most frequently in aquatic ecosystems (Cockcroft *et al*., 1991). These include polychlorinated biphenyls (PCBs), DDT and its metabolites DDE and DDD, Dieldrin and the chlordane group (Cockcroft *et al*., 1991). Because it is so widespread, global contamination of marine mammals has been suggested (Cockcroft *et al*., 1991), of which the highest levels have been recorded in cetaceans off Hong Kong (Reeves *et al*., 2008). Gardner *et al*. (1983) recorded high levels of PCBs and DDT in the blubber of various dolphin species, including *S. chinensis*. Moreover, Cockcroft (1999) reports that Indo-Pacific humpback dolphins inhabiting southern African waters have one of the highest concentrations of organochlorines stored in their tissue.

Prior to the discontinuation of legal DDT use in the mid-1970s, large quantities of the chemical have entered the marine system in South Africa, particularly along the KwaZulu-Natal coast, where land is used for intense agricultural purposes (Cockcroft *et al*., 1991). The continued use of DDT in northern KwaZulu-Natal for malaria control probably means that it is still entering our marine systems, albeit at a lower rate (Cockcroft *et al*., 1991). Cockcroft *et al*. (1991) found that coastal waters have greater concentrations of PCB and DDT contamination than pelagic waters, indicating that inshore species are at greater risk of contamination than pelagic marine species. Resultant pollutants concentrate in tissue, which lead to toxification over extensive periods of exposure (Cockcroft, 1999). These concentration loads can be fatal to neonates of primiparous females (Cockcroft, 1989; Jefferson, 2000). For example, females that have accumulated lipophilic pollutants up to the age of sexual maturity will transfer a large portion thereof onto their offspring through lactation (Cockcroft *et al*., 1989). Moreover, the reproductive efficiency in males may also be reduced as a result (Cockcroft, 1989; Cockcroft *et al*., 1991). The monitoring of contamination levels in marine environments should therefore be considered a priority. In Hong Kong, contaminated mud from dredging and reclamation projects has also been found to pose an indirect risk to humpback dolphins via the consumption of contaminated prey (Reeves *et al*., 2008). The ingestion of contaminated seabed sediments, prey and the transfer of toxic substances via lactation are all part of the problem (Parra and Ross, 2009). It is believed that the exceptionally high level of neonate humpback dolphin strandings in Hong Kong may be related to organochlorine contamination (Parra and Ross, 2009).

Combined with all the previously mentioned threats, increasing human population sizes and already low humpback dolphin population sizes, there is serious cause for concern over the future and the survival of this species in southern Africa (Best, 2007). Management recommendations include: habitat management, monitoring, public awareness and sustainable (non-consumptive) utilisation (i.e. boat-based dolphin watching) (Peddemors *et al*., 2004).

1.9 Conservation status

The conservation status of humpback dolphins throughout their range is largely uncertain, mainly as a result of the lack of reliable information on population size and mortalities (Corkeron *et al*., 1997; Best, 2007; Parra and Ross, 2009). The Cetacean Specialist Group re-assessed all cetacean species between 1990 and 1994. Some 38 species were classified as 'Data Deficient' (DD), including the humpback dolphins (Reeves *et al*., 2003). However *Sousa* spp. are currently classified as Near Threatened by the International

Union for Conservation of Nature (IUCN), and in the South African Red Data Book, humpback dolphins are rated as 'Vulnerable' (Peddemors *et al*., 2004). Furthermore, the Convention on International Trade in Endangered Species (CITES) has listed them as an Appendix I species, which includes species threatened with extinction.

There is a high concern regarding the survival of humpback dolphin populations inhabiting South African waters (Cockcroft, 1990). Population model growth rates for populations along the Eastern Cape coast indicate that although the population is currently stable, growth in numbers is unlikely due to negative environmental pressures and/or the destruction of natural habitats (Karczmarski, 2000). Due to the small population sizes, the detection of small and progressive population declines is difficult (Parra and Ross, 2009). Precautionary measures regarding the conservation of viable populations are necessary. Such measures should include the maintenance of high quality habitats, particularly in highly populated areas. In addition, it is important to further our understanding of the biology, ecology and taxonomy for improved conservation and management strategies (Parra and Ross, 2009).

1.10 Focus for this study

Recent conservation action plans for cetaceans recognise the urgent need for improving our understanding of *S. chinensis* (Wang *et al*, 2007)**.** According to the IUCN, humpback dolphins inhabiting coastal waters between western India and eastern South Africa especially, require serious conservation action (Reeves *et al*., 2008). However, research is needed to help formulate and design conservation programs effectively, as current conservation measures for *S. chinensis* are either meagre or non-existent (Reeves *et al*., 2008). By assessing the basic biological parameters, such as the age, growth and reproduction of humpback dolphins, we can improve our understanding and in future assist in a well-founded management plan for the conservation of *S. chinensis* off South Africa.

Some light has recently been shed on the biology of *S. chinensis* in the Pearl River Estuary off China (Jefferson *et al*., 2012), however, morphological and ecological differences exist between the geographically isolated populations (Ross *et al*., 1994). Additionally, sub-specific differences between different geographic forms have been noted, thus inter-regional comparisons should be viewed with caution.

Cetacean life-history studies enable us to better understand the basic biology of a species and its relationship to their environment, population structure, mating systems and anthropogenic effects (Danil and Chivers, 2007). Current knowledge of the life history parameters for humpback dolphins inhabiting the coastal waters of South Africa is limited to a preliminary study conducted by Cockcroft (1989). The aim of the present study is therefore to help contribute to our understanding of the biology and natural history of humpback dolphins in KwaZulu-Natal coastal waters.

Chapter 2

Age Estimation and Growth of *S. chinensis*

2.1 Introduction

2.1.1 Background information

Conducting age estimates using teeth is a recognised tool employed to assign a numerical value (i.e. age) to an animal for which the actual age is not known (Hohn, 2002). It is a fundamental component for interpreting various biological, ecological and physiological aspects of marine mammals (Luque *et al.*, 2009). By studying enough individuals, various age-related characteristics of whole dolphin populations can be identified, such as age composition, average age at sexual maturity, age at first reproduction and natural longevity (Sergeant *et al.*, 1973; Reilly *et al.*, 1983; Cockcroft and Ross, 1990; de Santos *et al.*, 2003; Mattson *et al.*, 2006; Jefferson *et al*., 2012). Moreover, age estimation provides essential information for predicting fecundity or mortality rates, through which population growth can be estimated (Hohn, 2002) and has become a standardised procedure for performing population assessments and making management decisions (Scheffer and Myrick, 1980). Age at maturation is also one of the most important and useful parameters for measuring density-dependent changes within populations (Hohn, 1990).

With the exception of a preliminary study conducted by Cockcroft (1989), this is the first in-depth study exploring the life-history-strategies of *S. chinensis* in South African waters. On a global scale, the only published data on age and growth for humpback dolphins are available for populations inhabiting southern Chinese coastal waters (Jefferson, 2000; Jefferson *et al*., 2012).

2.1.2 Age estimation using teeth

Considering the importance of obtaining age estimates, various methods and tissues for elucidating growth layers have been investigated (Perrin and Myrick, 1980; Klevezal', 1996). Investigators have been able to identify and count growth layers in both teeth and bones of several mammalian species (Klevezal' and Kleinenberg, 1967; Hohn *et al.*, 1989). The appearance of growth layers has been found to be similar among a large number of species for the same tissue (e.g. teeth) (Hohn *et al.*, 1989). As in the case of terrestrial mammals, the most commonly used, and preferred tissue for estimating the age of odontocetes, have been teeth (Klevezal' and Kleinenberg, 1967). It has been used in age estimation studies in marine mammals effectively since the 1950s (Scheffer, 1950; Scheffer and Myrick, 1980). Nishiwaki *et al*. (1958) were the first to study the periodicity of dentinal deposits in odontocetes using the teeth of sperm whales (*Physeter catodon*) of unknown age. By comparing the number of dentinal layers to total body length and the number of *corpora lutea* (a small yellow mass of tissue in the ovary that forms after an ovum has been extruded (Lawrence, 2005)) in ovaries, they concluded that two GLGs are deposited annually (Klevezal' and Kleinenberg, 1967). Sergeant (1959), however, was the first to correlate the known age of *Tursiops truncatus* with the number of GLGs deposited in dental tissue. His findings demonstrated an annual dentinal deposition rate. Similarly, Klevezal' and Kleinenberg (1967) examined teeth for nine mammalian orders, which included cetaceans, and concluded that GLGs are generally an annual event.

Determining the deposition rate is essential in obtaining accurate age estimates, and the three approaches that have been used to determine the rate at which GLGs are deposited include: 1) correlating GLGs to the known age animals, 2) using tertracycline markers on captive animals, and 3) estimating age from teeth removed at known intervals (Barlow and Hohn, 1984; Hohn, 1990). The calibration of dental layers using tetracycline labelling is performed on animals for which the minimum age is generally known; however, this method is limited to calibrations for dentinal layers only (Myrick and Cornell, 1990). To date none of these approaches has been used to confirm the deposition rate of dental growth layers (or growth layer groups) in *S. chinensis*. Regardless, the use of growth layers in age estimation for odontocetes has generally been widely accepted, despite whether or not they have been calibrated for a species (Hohn *et al.*, 1989). Species for which data were readily available and for which growth layers have been calibrated, using tetracyline labelling, include spinner dolphins, *Stenella longirostris* (Myrick *et al.*, 1984), dusky dolphins, *Lagenorhynchus obscurus* (Best, 1976), short-beaked common dolphins, *Delphinus delphis* (Gurevich *et al.*, 1980), and Atlantic Ocean bottlenose dolphins, *Tursiops truncatus* (Myrick and Cornell, 1990).

Dentinal growth layers which have been defined in delphinids have been described as similar across various species (Hohn, 1990). The similarity in growth layer patterns across species can be attributed to the mechanism responsible for regulating the layer deposition, which appears to be common among related groups of animals, as the layers look similar and represent the same amount of time (Hohn, 1990). It should therefore be theoretically possible to apply a general model of annual layering patterns using similar known-age or tetracycline-labelled species to obtain age estimates for un-calibrated species (Hohn, 1990).

Although various studies have been conducted to verify the deposition rate of growth layers in a number of odontocetes, several discrepancies do remain in the use of dentinal and cemental layers for age estimation (Hohn, 1990). Firstly, age estimates from teeth collected from animals of known age have rarely been made without prior knowledge of the age or other age-related information of the individual (Hohn, 1990). Secondly, growth layers have been calibrated in only a small number of species. Finally, growth layer calibrations have been based solely on captive animals (Hohn, 1990). It is unknown whether captivity influences the deposition rate of annual layers, as periodicity may potentially be influenced by seasonally varying factors, such as temperature, diet or endogenous factors (Hohn, 1990). Furthermore, there are various potential sources of error with the use of teeth for age estimation. Dentinal deposition may occlude the pulp cavity, whereupon dentine deposition ceases, resulting in an under-estimate of age, especially for older individuals (Klevezal' and Kleinenberg, 1967; Perrin and Myrick, 1980). Additionally, a GLG is made up of a complex unit of accessory layers of various intensities, which makes them less distinguishable. The interpretation of GLGs depends on the method of tooth preparation, as well as the method used for counting and interpreting layers (Perrin and Myrick, 1980).

2.1.3. Tooth morphology

Odontocete teeth, like other mammalian teeth, are composed of a crown which protrudes above the gum line, and a root concealed in the tooth alveolus of the jaw (Klevezal' and Kleinenberg, 1967). The majority of the tooth is composed of dentine, with a thin, prismatic layer of enamel covering the crown and an outer coat of cementum covering the root (Klevezal' and Kleinenberg, 1967) (Figure 2.1). However, odontocete teeth are different from other mammalian teeth in a number of ways. Firstly, odontocetes have homodont dentition, meaning that all teeth are the same shape, a mammalian characteristic limited to toothed whales (Myrick, 1991). Secondly, unlike the majority of other toothed mammals, which produce a set of milk/baby teeth and a subsequent set of permanent teeth (Peyer, 1968), odontocetes are monophydonts, meaning they produce a single set of teeth, which erupts shortly after birth (Myrick, 1991). Resultantly, odontocete teeth serve as natural recording devices (Myrick, 1991), making it an ideal tissue for age estimation studies, as it is a representation of a dolphin's entire ontogeny.

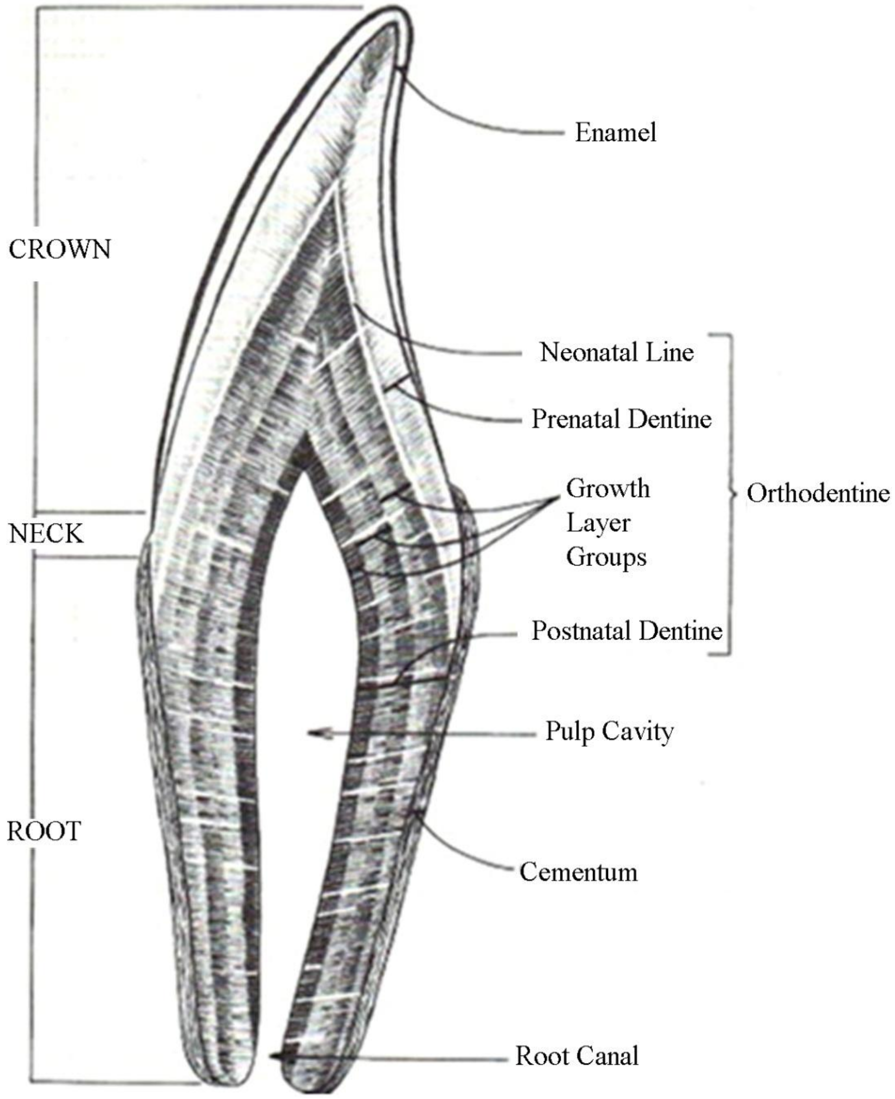


Figure 2.1: Morphology of a dolphin tooth (Perrin and Myrick, 1980).

2.1.3.1 Dental recording structures

Odontocete teeth are peg-shaped, and when newly erupted they are solely composed of a thin-walled basally-tapered cone of pre-natal dentine, covered by a thin layer of enamel encapsulating the pulp cavity or tooth canal (Figure 2.1) (Klevezal' and Kleinenberg, 1967; Boyde, 1980; Myrick *et al.*, 1983). Deposition of enamel probably starts three to four months after conception and is completed prenatally (Myrick, 1991).

Teeth retain their original shape from birth, while layers of dentine are continually being deposited postnatally throughout a dolphin's life. Prenatal dentinal and enamel deposition commences concurrently, but enamel is deposited daily, whereas dentine is deposited monthly (Myrick, 1991). Prenatally formed dentine is a hypomineralised layer in teeth that demarcates the point of parturition and represents the first of a series of prenatally deposited GLGs, i.e. the neonatal line (Figure 2.1) (Hohn, 1980; Myrick *et al*., 1983; Myrick, 1991). Experiments with tetracycline labelling have verified that the neonatal line forms at or near the time of birth (Myrick and Cornell, 1990) (Figure 2.1).

The majority of the body of a tooth is comprised of dentine, which is deposited continually throughout an animal’s life at the wall of the pulp cavity, contributing to a gradually decreasing volume (Klevezal' and Kleinenberg, 1967; Myrick *et al*., 1983; Myrick, 1991; Hohn, 2002), as well as a decrease in the widths of subsequent GLGs. Thus dentinal layers formed earlier are situated closer to the walls of the tooth, and more recently formed layers are closer to the pulp cavity (Klevezal' and Kleinenberg, 1967; Myrick, 1991; Hohn, 2002).

For older individuals, the most recently formed layers become very intricate and sometimes inconspicuous, thus making them especially difficult to differentiate, and even more so once the pulp cavity becomes occluded (Myrick *et al*., 1983). Unlike the situation in other mammals, the formation of dentine does not cease; postnatal dentinal layers continue to accumulate internally until an animal dies, or the pulp cavity becomes fully occluded, resulting in dentine-producing cells (odontoblasts) being closed off (Myrick, 1991).

Cementum is the tissue which is formed below the gumline and is composed of calcified tissue of mesodermal origin (Klevezal' and Kleinenberg, 1967; Perrin and Myrick, 1980). Functionally, it serves as an "attachment bone" which anchors the tooth to the alveolus (Perrin and Myrick, 1980; Hohn, 2002). As cementum is deposited externally in the relatively unconfined space of the tooth alveolus, it is thought to provide a continuous record of a dolphin's entire postnatal life, thereby making it adequate for estimating the maximum age of older individuals, for which dentinal estimates are not possible (Klevezal' and Kleinenberg, 1967; Myrick *et al*., 1983). However, cemental layers are very thin and often hard to differentiate (Perrin and Myrick, 1980; Myrick, 1980).

2.1.3.2 Growth layer groups

The dental tissue for all dolphins has the same basic pattern of distribution and deposition (Myrick, 1980). Depositional layers in dental tissue are defined as growth layer groups (GLGs), which is the unit of measure for performing age estimates in various mammalian species, including odontocetes. In the context of age estimation, the term "growth layer" is ambiguous (Hohn, 2002). At the International Whaling Commission workshop (1980) on age determination, it was sought to resolve the issues surrounding the definitions and terminology associated with age studies (Perrin and Myrick, 1980). Thus, a GLG was formally described as "a group of incremental layers, which may be recognised by virtue of a cyclic repetition, generally at a constant or regularly changing relative spacing" (Perrin and Myrick, 1980). In other words, a single GLG is represented by a repeating pattern of adjacent groups of varying optical densities in dentinal tissue (Klevezal' and Kleinenberg, 1967; Perrin and Myrick, 1980) and can be defined by a change from either light to dark, transparent to opaque, intensively stained to lightly stained or ridged to grooved (Perrin and Myrick, 1980).

However, a single unit may involve more than one change (Perrin and Myrick, 1980) and does not necessarily represent an annual layer (Hohn, 1990). Incremental layers, which are also referred to as "accessory layers", are discernible lines occurring within a defined GLG. They show a contrast with adjacent layers and are thought to represent lunar growth increments (Perrin and Myrick, 1980; Myrick, 1991), whose degree of clarity tends to vary in different species (Klevezal' and Kleinenberg, 1967). It was because of these incremental lines that the term ‘growth layer group’ was adopted (Hohn, 1990). Caution should be used when performing age estimates, as accessory layers may encumber estimates (Hohn, 1990). It is thus important for the investigator to define the repeating pattern and the amount of time represented by a single GLG. Nevertheless, it has been suggested that accessory layers should not be overlooked completely, as annual incremental layers formed within a GLG can serve as recording structures which reflect the physiology of the individual at the time of deposition (Klevezal, 1996). They can serve as an important tool for interpreting life history events for individuals, such as growth rates, reproductive events, and changes in the environment, which in turn may assist in distinguishing between different populations (Hohn, 2002).

2.1.4 Sexual dimorphism

Sexual dimorphism in mammals is fairly common and is generally defined as differences in shape, size or traits between males and females of the same species (Lammers *et al*., 2001). Sexual dimorphism in cetaceans is usually not displayed through secondary characteristics, but is most commonly displayed in the form of body shape and/or size (Tolley *et al*., 1995). The degree of sexual dimorphism generally increases with an increase in body size (Tolley *et al*., 1995). An extreme but good example is the sperm whale, which exhibits the greatest degree of sexual dimorphism among cetaceans, with males growing to approximately three times the weight and two times the length of females (Connor *et al.*, 1998; Ralls and Mesnick, 2002). Further examples of cetaceans in which sexual dimorphism is displayed include bottlenose dolphins, *Tursiops truncatus* (Tolley *et al*., 1995), Fraser's dolphins, *Lagenodelphis hosei* (Perrin *et al.*, 2003) and killer whales, *Orcinus orca* (Clark *et al*., 2000).

2.2 Aim of the chapter

The aim of this chapter was to obtain age estimates using teeth and to establish growth curves for *S. chinensis.* Using this information, we were able to determine various biological parameters such as length at birth, and age, length and mass at physical maturity, maximum body length, maximum body mass and longevity, which are all necessary in establishing the life history strategy employed by the species. Data on sexual dimorphism in *S. chinensis* were also examined.

2.3 Materials and methods

2.3.1 Sample

Samples employed in the present study originate exclusively from humpback dolphins incidentally caught in shark nets set in the coastal waters of KwaZulu-Natal, South Africa. Routine necropsies were performed on all humpback dolphins by various members of staff of the Port Elizabeth Museum. As a standard operating procedure, basic biological and morphological parameters were recorded as recommended by Norris (1961). Additionally, standard biological samples were taken and accessioned into the Graham Ross Marine Mammal collection at the Port Elizabeth Museum (PEM) (Port Elizabeth Museum, South Africa).

For this study samples originating from 109 humpback dolphins (71 male and 38 female), collected over a 42 year period (1972 - 2012) by the Port Elizabeth Museum (PEM), South Africa, were used for age estimation. The basic information recorded for each capture event is provided in Appendix A, and is listed in chronological order of the PEM accession number. Each capture event had the following data: date, sex, location, total body length (cm) (measured from the tip upper jaw to deepest part of notch) and mass (kg) (Norris, 1961). The earliest samples of *S. chinensis* date back to 1972 and additional samples are continually being added to the collection due to a long-standing collaboration between PEM and the KwaZulu-Natal Sharks Board.

The lengths of humpback dolphins in the sample ranged from 148 cm to 279 cm total body length (TBL) for males and 150 cm to 249 cm TBL for females. The size-frequency analysis for male *S. chinensis* showed a bimodal distribution (Figure 2.2), which indicated that the sample was dominated by males between 191 and 230 cm TBL and between 261 and

270 cm TBL. Females, on the other hand, displayed unimodal distribution, with the majority of samples ranging between 201 and 210 cm TBL. Teeth for neither male nor female humpback dolphins were available for individuals smaller than 141 cm TBL (Figure 2.2). Additionally, there were no teeth available for females larger than 250 cm TBL (Figure 2.2). The sample, therefore, showed a clear bias towards sub-adults, with males making up the majority of dolphins captured (Atkins *et al*., 2013). Most of the teeth used for analysis were stored dry, while samples collected since 2006 were stored both dry and wet (in 50% iso-propyl alcohol).

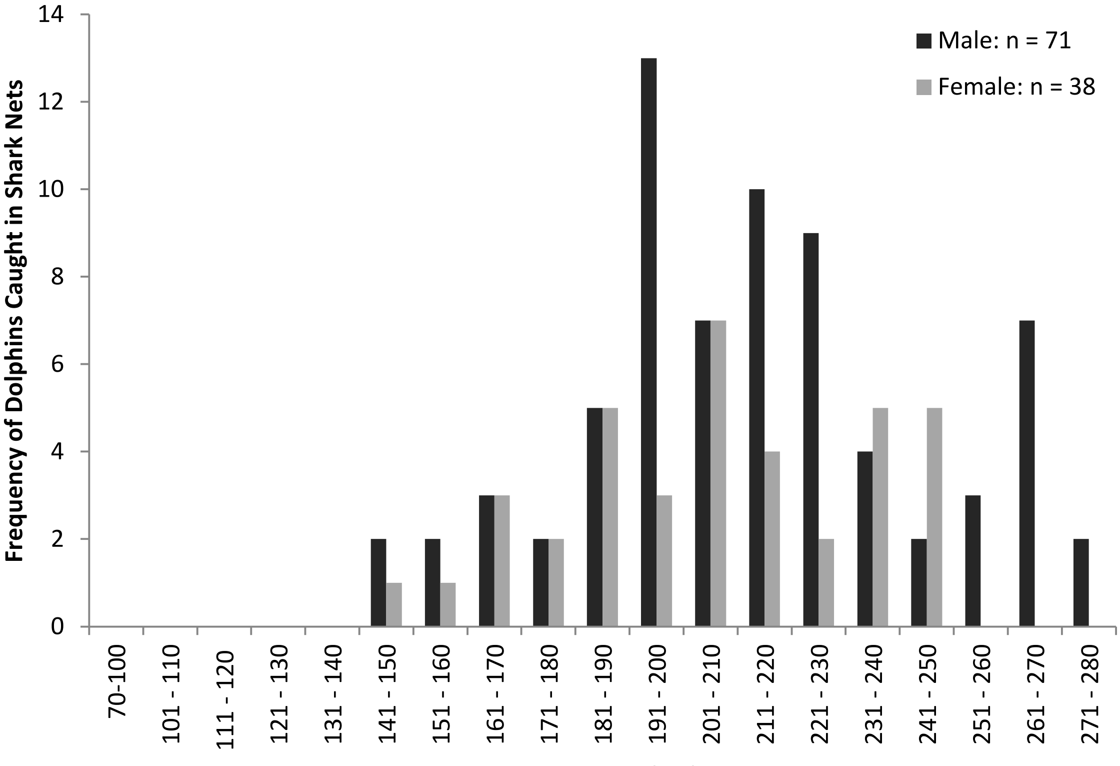


Figure 2.2: Size frequency histogram of samples from *Sousa chinensis* incidentally caught in shark nets along the KwaZulu-Natal coast used for age estimation (n = 109).

2.3.2 Preparation of tooth sections for age estimation

Age was estimated following the procedures described by Myrick *et al*. (1983) and Jefferson (2000). The staining of thin tooth sections was the preferred method for preparing teeth for age estimation as untreated, unstained teeth have been found to have a lower resolution of growth layers in both dentine and cementum (Perrin and Myrick, 1980; Hohn, 2002).

One tooth from each individual was decalcified in a commercial rapid decalcifying agent (RDO®), with hydrochloric acid as the principle active ingredient. To reduce decalcification time, teeth from larger animals were sectioned prior to decalcification using a Buehler IsoMet low-speed saw equipped with a diamond blade. Each section consisted of a 3 - 5 mm thick longitudinal section through the midline of the tooth. The method proposed by Myrick *et al*. (1983) for decalcifying teeth was slightly adjusted in this study, so that teeth were decalcified individually in 50 ml RDO per tooth. Decalcification time ranged from 1 - 52 hours, depending on the size of the tooth, and was considered complete once the tooth was adequately flexible and slightly translucent. Once that stage was reached, the teeth were rinsed under running tap-water to avoid interference with the staining procedure.

Decalcified teeth were sectioned using a Shandon mini cryostat microtome. Each tooth was mounted on a small metal stub, with a cryomatrix as embedding medium. Longitudinal sections ranged in thickness between 25 - 40 µm (Myrick *et al*., 1983; de Santos *et al*., 2003). Resulting sections were rinsed again under running tap-water to remove any residual RDO.

Only central and complete sections, cut along the midline (i.e. including the crown and maximum area of pulp cavity), were selected and stained using Mayer's haematoxylin. After a thorough rinse in tap-water, the sections were "blued" in a 0.5% solution of Ammonia to enhance the contrast of the stained layers. After the "blueing" process, the sections were thoroughly rinsed before being immersed in 50% glycerine to initiate glycerine exchange, before being transferred to 100% glycerine to complete exchange. Sections were subsequently examined under an Olympus SZ61 zoom stereo microscope at 12x magnification under transmitted light, and the best sections were selected to be mounted permanently. Sections that were cut off-centre were discarded as they were considered not suitable for age estimation purposes, as very fine layers deposited in old animals might be missed (Hohn, 2002).

2.3.3 Age estimation

Age estimates were performed following the guidelines provided in the workshop report of the International Whaling Commission (Perrin and Myrick, 1980) and Hohn *et al.* (1989). This was done by counting GLGs "blindly", without reference to additional data pertaining to the specimen to eliminate any bias in age estimation (Perrin and Myrick, 1980). Two independent methods were used for performing age estimates, which involved counting dentinal layers (Method 1) and cemental layers (Method 2).

- **Method 1 - Dentine**: a collection of incremental growth lines deposited in a repeating pattern of varying optical densities was defined by a change from either light to dark or intensively stained to lightly stained layers, with the darker layers making up the boundaries of each successive GLG. Only post-natal dentine was considered for age estimation.

- **Method 2 - Cementum**: a single opaque and translucent layer was considered to represent a single GLG in the cementum.

Although dentine was the preferred tissue for performing age estimates (Method 1), it becomes tentative once the pulp cavity becomes occluded. In this case dentinal deposition ceases or continues in the form of secondary dentine, which is ineffective for age estimation, thereby making cementum the preferred tissue for performing estimates for older individuals (Hohn *et al.*, 1996). Furthermore, counting cemental layers may be advantageous over counting dentinal layers as cemental deposition continues in some species for a considerable period even after dentinal deposition has ceased (Boyde, 1980). Growth layers in Method 1 were counted using an Olympus SZ61 zoom stereo microscope under 12x - 20x magnification. Cemental layers in Method 2 were counted using an Olympus BX50 compound microscope under 200x magnification. The most recently formed layer was compared to the previously formed layer to determine the degree of completion: if it was larger than 50% thereof, it was regarded as a complete GLG. If smaller than 50%, it was rounded to the last complete layer. Half-developed GLGs were considered as 0.5 years.

Teeth were read three times by the author and once by two independent readers (A. Bishop, Rhodes University and S. Plön, Nelson Mandela Metropolitan University) to minimise the possibility of bias in age estimates. Estimates were combined to yield a trimmed mean for each specimen (Reilly *et al.*, 1983). If the GLG counts of the different observers for the same tooth were not within 15% of each other, revised counts were performed together, until concurrence on an age estimate was reached. In addition, cemental counts were performed for 74 individuals encompassing the entire sample range, so that estimates obtained from both methods could be correlated to verify estimates and discern at which point dentinal estimates could become problematic.

Currently, no calibration for GLG patterns or the depositional rate is available for *S. chinensis*, and therefore it is assumed for this study that the rate of deposition of GLGs is equivalent to that of other odontocetes, which has been shown to be one GLG deposited per annum (Perrin and Myrick, 1980; Hohn, 1990). However, as this is only an assumption, the results obtained from this study should be treated as an estimate until calibration of the deposition rate has been performed for either or both dentine and cementum.

2.3.4 Growth models

The estimated ages of the animals were plotted against TBL and total body mass to obtain growth curves. Both a three parameter Von Bertalanffy growth function and a Gompertz growth function were used to describe the relationship between age and length/mass. The analysis was conducted using the graphical package Microsoft Excel 2007. It was decided to fit both models to the data for this study, such that results can be compared to previous studies on *S. chinensis* and for potential future comparisons to other delphinids.

A three parameter Von Bertalanffy Growth Function describes the growth curve as follow:

$$L(t) = Linf\left(1 - e^{-k(t-to)}\right)$$

Where:

L_{inf} is the asymptotic length
k is the growth constant
t is the estimated age
t_o is the age corresponding to zero length

The Gompertz Growth Function on the other hand describes the growth curve as:

$$y(t) = ae^{be^{ct}}$$

Where:

a is the asymptotic length
b is the age corresponding to zero length
c is the growth constant
t is the estimated age

An alternative method for estimating the length at birth for cetaceans is the use of the equation developed by Scott (1949): $y = 0.2411x + 44.3$. This equation can be applied to both sexes, and describes the relationship between the maximum body length of adult animals (represented by x) and the neonatal length (represented by y) (Plön, 2004).

2.4 Results

2.4.1 Tooth morphology and age estimation

Figure 2.3 shows the longitudinal sections of two *S. chinensis* teeth under transmitted light at 12x magnification. The bars represented on each illustration indicate the neonatal line as well as successive GLGs (Figure 2.3). In agreement with the literature, the neonatal line was marked by a very faintly stained layer that represents the time zero for growth layer counting purposes. This was clearly visible in all *S. chinensis* teeth and distinguishable from successive GLGs by the lack of substructures, such as incremental and accessory layers evident in postnatal dentine.

Postnatal dentine consisted of several layers that stained at varying intensities. A complete GLG was regarded as consisting of a broad lightly stained band, adjacent to a very narrow dark layer on either side, which represented the boundaries of successive GLGs. This was in accordance with Perrin and Myrick (1980). Within each lightly stained band there were several diminutive bands, which were regarded as accessory layers (Figure 2.4 (A)). The first GLG begins after the neonatal line and forms a thick, lightly stained layer comprised of numerous, clearly visible accessory layers. The boundary of the first GLG is often hard to differentiate from accessory layers, which in some instances may be more prominent than the boundary layer itself. The second GLG had similar characteristics to the first GLG, thereby making it equally difficult to differentiate. However, subsequent GLGs became increasingly identifiable, as accessory layers became less evident and the successive boundaries of GLGs more apparent. Furthermore, a prominent sub-annual layer was evident at about the midpoint of a single GLG, which became less obvious after the first three or four GLGs (Figure 2.4 (B)). Likely errors in age estimates are therefore expected to occur in the first few GLGs.

The correlation coefficient between dentinal (Method 1) and cemental (Method 2) GLG counts, using a subset of the sample (n = 74), was high (R = 0.951); thus indicating that age estimates from cemental layers may prove useful in cases where the pulp cavity has become occluded, and prevented accurate readings from dentine (Figure 2.5). Age estimates performed for 74 individuals selected at random from the sample, from both dentine and cementum, were well correlated up to about 12 GLGs. Thereafter, counts diverged with increasing age as a result of the closure of the pulp cavity (Figure 2.5). Although Method 1 was the overall preferred method for performing age estimates, generally estimates for individuals older than 12-15 GLGs were conducted using Method 2.

A greater number of older males than females, between the ages of 12 and 24 GLGs were caught in nets. As a result, individuals older than 12 GLGs were skewed towards males (Figure 2.6). The smallest male dolphin was 148 cm TBL and smallest female was 150 cm TBL. The longest male in the sample measured 279 cm TBL, and the oldest male was an estimated 24 years old and measured 267 cm TBL. The longest females measured 249 cm TBL and the oldest female in the sample was 17.7 years and measured 233 cm TBL. The majority of dolphins caught in the nets were less than 10 GLGs old (Figure 2.6) and there were no females between the ages 9 - 12 GLGs in the sample. Furthermore, there were very few animals older than 18 GLGs, and the majority of these were males between 21-24 GLGs.

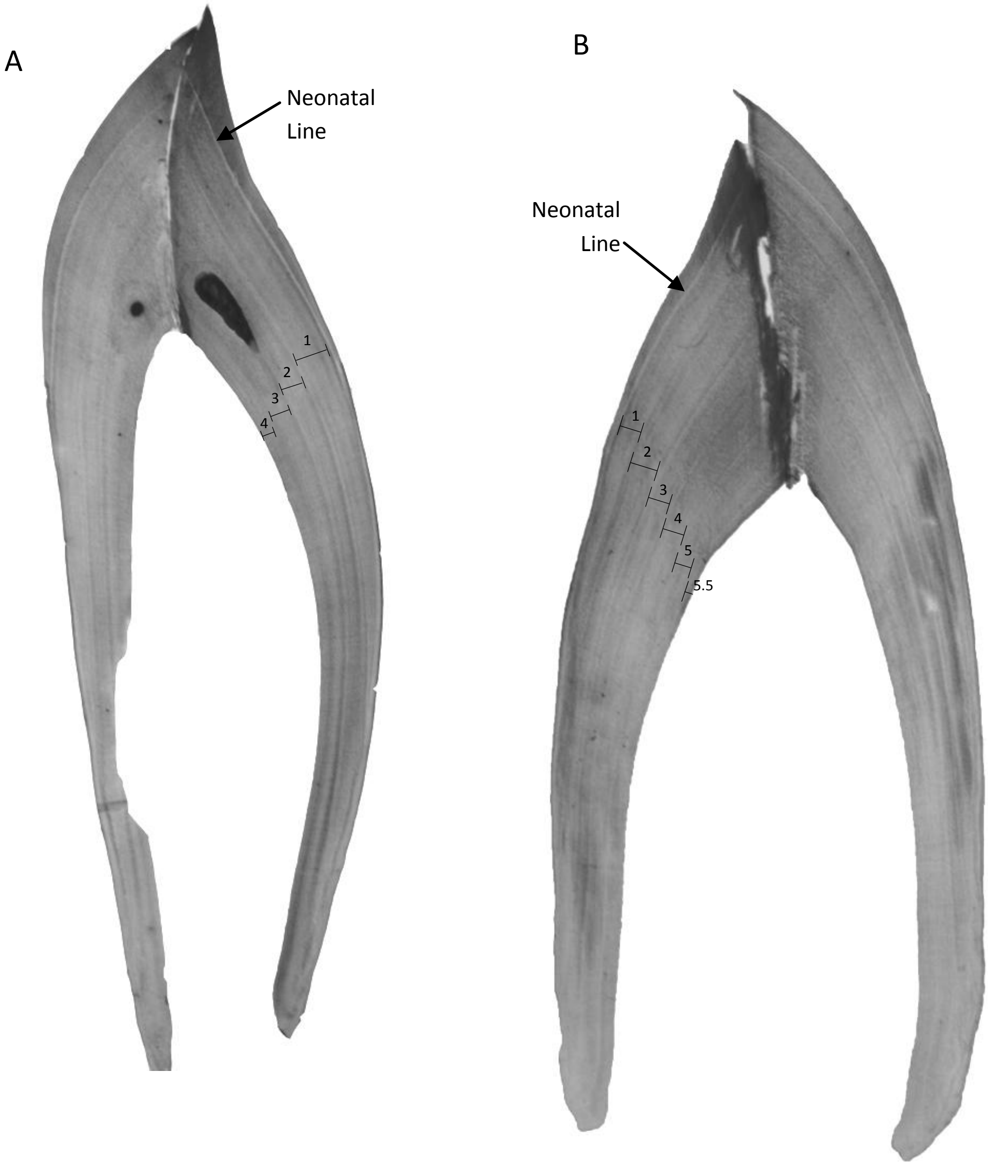


Figure 2.3: Longitudinal sections of a *Sousa chinensis* tooth viewed under transmitted light. The bars on the photograph indicate a series of successive GLGs (one GLG represents one year). Individuals A and B were an estimated four and five+ years old, respectively.

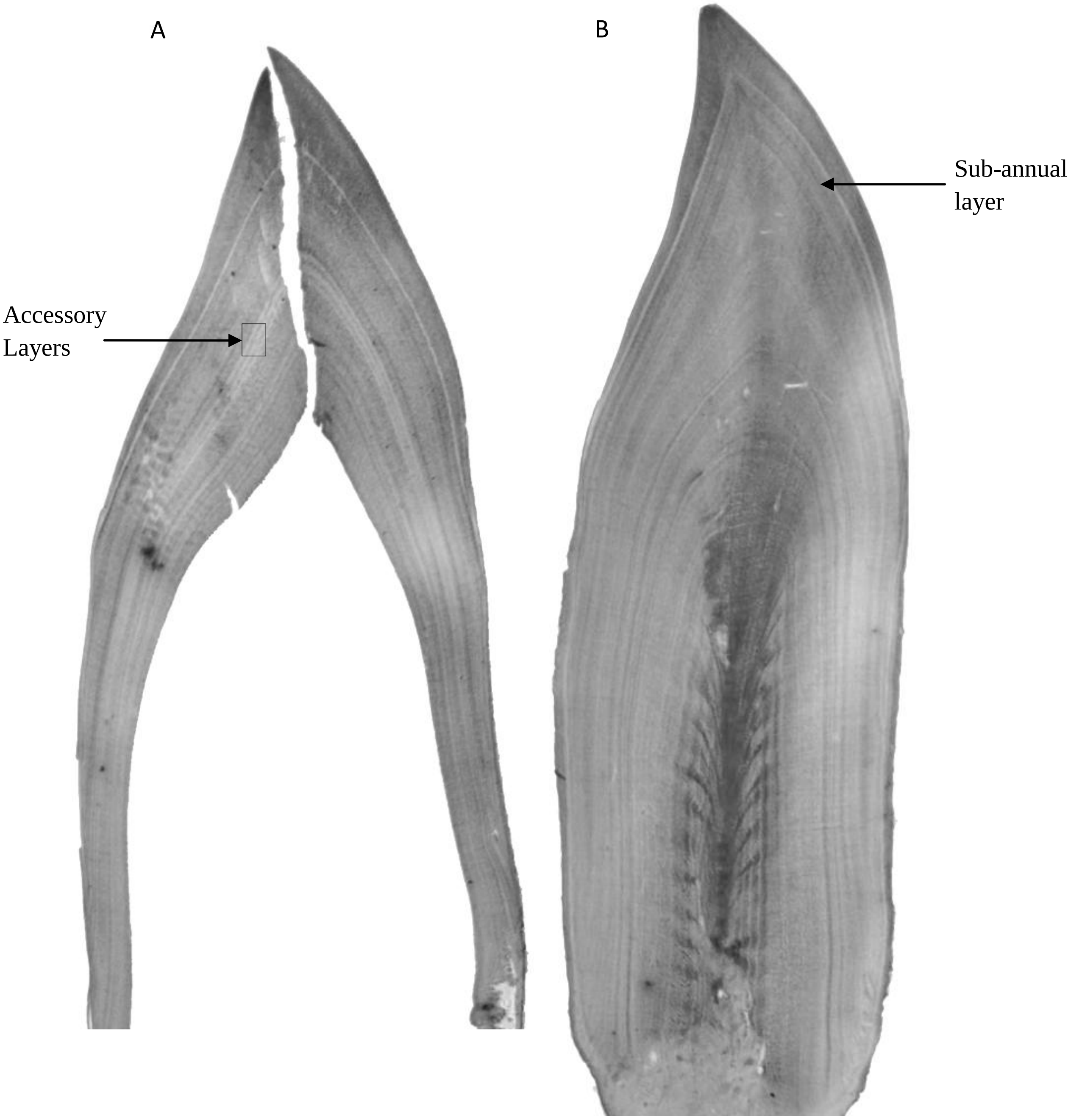


Figure 2.4: Longitudinal sections of stained teeth indicating (A) accessory layers present within each GLG, and (B) the presence of sub-annual layers present within the first GLG.

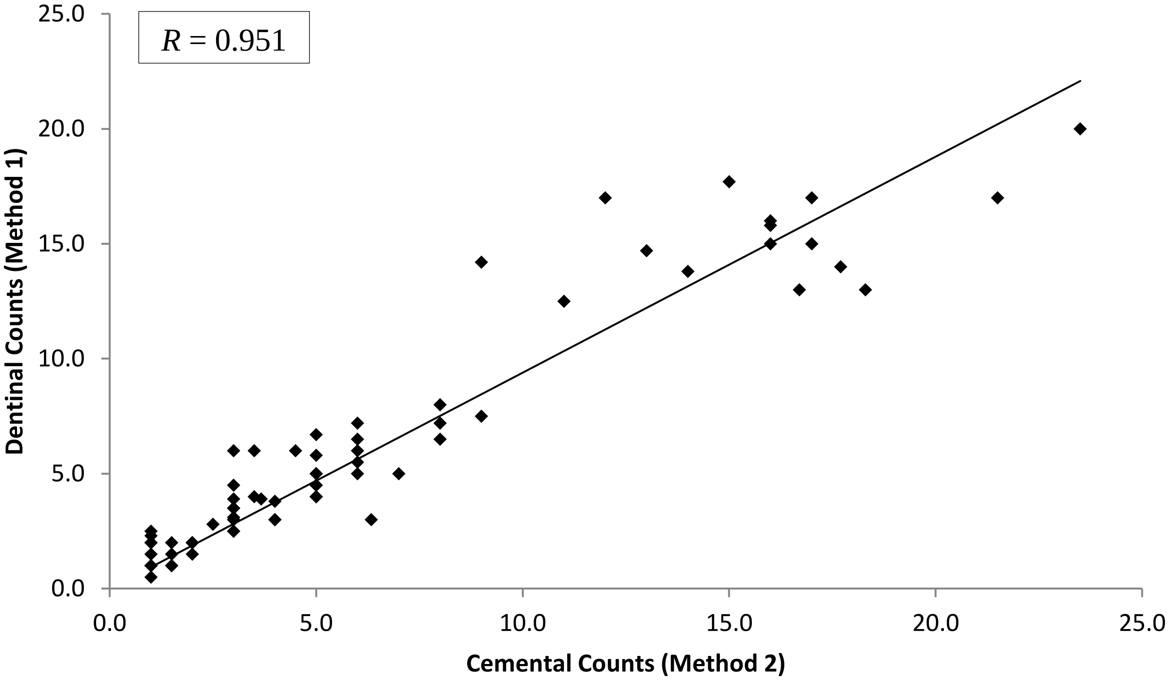


Figure 2.5: Relationship between growth layer group counts in the dentine (Method 1) and cement (Method 2) in the teeth of *S. chinensis* from KwaZulu-Natal, South Africa (n = 74).

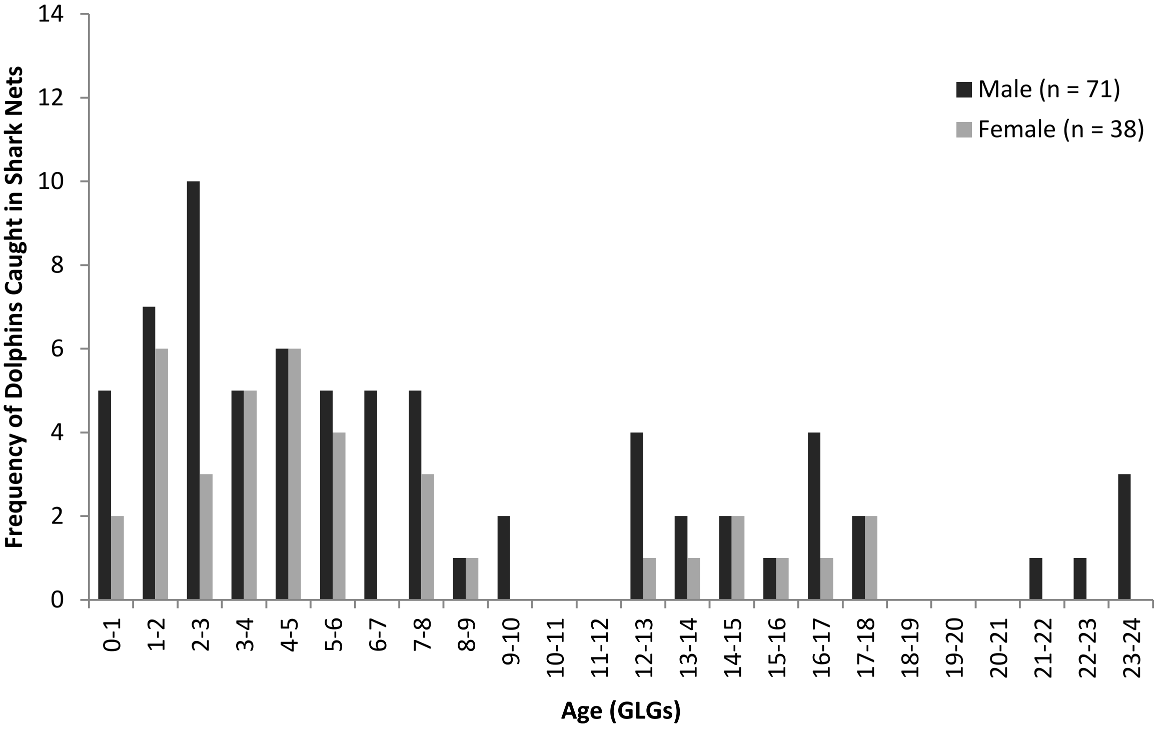


Figure 2.6: The age distribution of *S. chinensis* caught in shark nets set off the coast of KwaZulu-Natal between 1970 and 2012 (n = 109).

2.4.2 Growth curves

Von Bertalanffy and Gompertz growth curves were both fitted to the data in order to describe the relationship between age and length as well as age and mass.

2.4.2.1 The Von Bertalanffy growth function

Fitting the length-at-age data for males and females produced a good fit using the Von Bertalanffy Growth function (R^2 = 0.839 and 0.853, respectively) (Figure 2.7). Table 2.1 provides a summary of the main parameters obtained for male and female *S. chinensis*.

According to Jefferson (2000), the most reliable estimate of length at birth is obtained by the quantitative method termed "50% interpolation" by Perrin and Reilly (1984). However this method requires a moderately large sample of fetuses and neonates in overlapping length categories, which was not available for this study. Using the results obtained from the Von Bertalanffy growth function, the length at birth was estimated at 156.6 cm for males, while females were estimated to be slightly smaller at birth, measuring 116.3 cm TBL. Both are likely to be overestimates, due to the fact that samples measuring smaller than 140 cm TBL were not available for age estimation. To obtain a more likely estimate for length at birth, Scott's equation (1949) ($y = 0.2411x + 44.3$) was applied to both sexes, and predicts a more realistic neonatal length of 111.57 cm for male and 104.33 cm for female *S. chinensis*, using a maximum body length of 279 cm and 249 cm for males and females, respectively.

When fitted to the three-parameter Von Bertalanffy growth function, the asymptotic length for *S. chinensis* males was reached at 266.48 cm and 24 GLGs (Figure 2.7) and 239.29 cm at 15 GLGs for females (Figure 2.7). Most growth occurred during the first 15 years for males and the first 10 years for females, where after growth slowed until it reached an asymptote (Figure 2.7). The overall growth rate differed for males and females, such that the growth rate of females ($k = 0.292$) exceeded that of the males (k = 0.119) (Table 2.1).

Table 2.1: Parameters derived from a Von Bertalanffy growth function for male and female *S. chinensis.* A total of 71 male and 38 female dolphin teeth were analysed (n = 109).

Parameter	Male	Female
L_1	148 cm	150 cm
L_2	279 cm	249 cm
t_0	-7.432	-2.284
L_{inf}	266.48 cm	239.29 cm
k	0.119	0.292
$*L_{t0}$	111.57 cm	104.33 cm
R^2	0.839	0.853
n	**71**	**38**

L_1 = Smallest length in the sample
L_2 = Largest length in the sample
t_0 = Age corresponding to zero length/mass
L_{inf} = Asymptotic length
n = Sample size
* calculated using Scott's (1949) equation
k = Growth constant
L_{t0} = Estimated length at birth
R^2 = Data fit

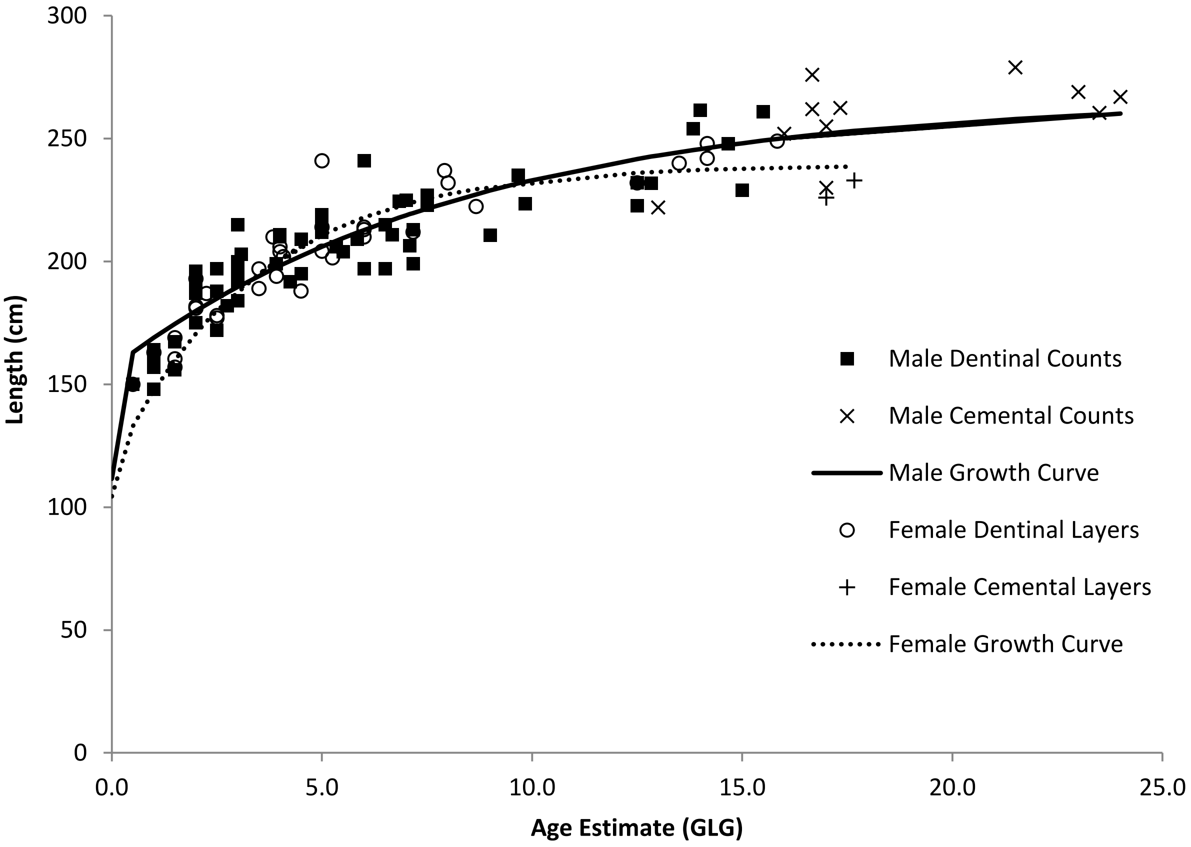


Figure 2.7: Increase in length with age (growth) for male and female *S. chinensis*, fitted to a three-parameter Von Bertalanffy growth function (n = 71♂ and 38♀).

2.4.2.2 The Gompertz growth function

Data for length at age fitted to the Gompertz growth function produced a similar fit to the Von Bertalanffy growth function for both males (R^2 = 0.835) and females (R^2 = 0.885). A summary of the parameters obtained can be found in Table 2.2.

Estimated length at birth, using the Gompertz growth function, was 159.34 cm and 122.44 cm for males and females, respectively. As in the case of the Von Bertalanffy function, both were considered likely to be overestimates, due to the fact that samples from male dolphins measuring smaller than 140 cm TBL were not available for age estimation.

Likewise, the same estimate produced by Scott's (1949) equation of 111.57 cm and 104.33 cm for males and females, respectively, was assumed.

Table 2.2: Parameters derived from a Gompertz growth function for male and female *S. chinensis* when fitted separately. A total of 71 male and 38 female dolphin teeth were analysed (n = 109).

Parameter	Male	Female
L_1	148 cm	150 cm
L_2	279 cm	249 cm
b	-0.507	-0.666
a	266.70 cm	240.96 cm
c	0.133	0.270
$*L_{t0}$	111.57 cm	104.33 cm
R^2	0.835	0.885
n	**71**	**38**

L_1 = Smallest length in the sample
L_2 = Largest length in the sample
b = Age corresponding to zero length/mass
n = Sample size
* calculated using Scott's (1949) equation
a = Asymptotic length
c = Growth constant
L_{t0} = Estimated length at birth
R^2 = Data fit

Based on the results obtained from the Gompertz growth function, the asymptotic length for males was reached at 266.70 cm and 24 GLGs (Figure 2.8) and at 240.96 cm and 16 GLGs for females (Figure 2.8). Similar to the results obtained from the Von Bertalanffy growth curve, most growth took place during the first 15 years for males, and the first 10 years for females, whereafter growth gradually slowed until it reached the asymptote (Figure

2.8). The overall growth rate of females ($k = 0.270$) exceeded that of the males ($k = 0.133$), with females evidently reaching asymptotic length well before males did.

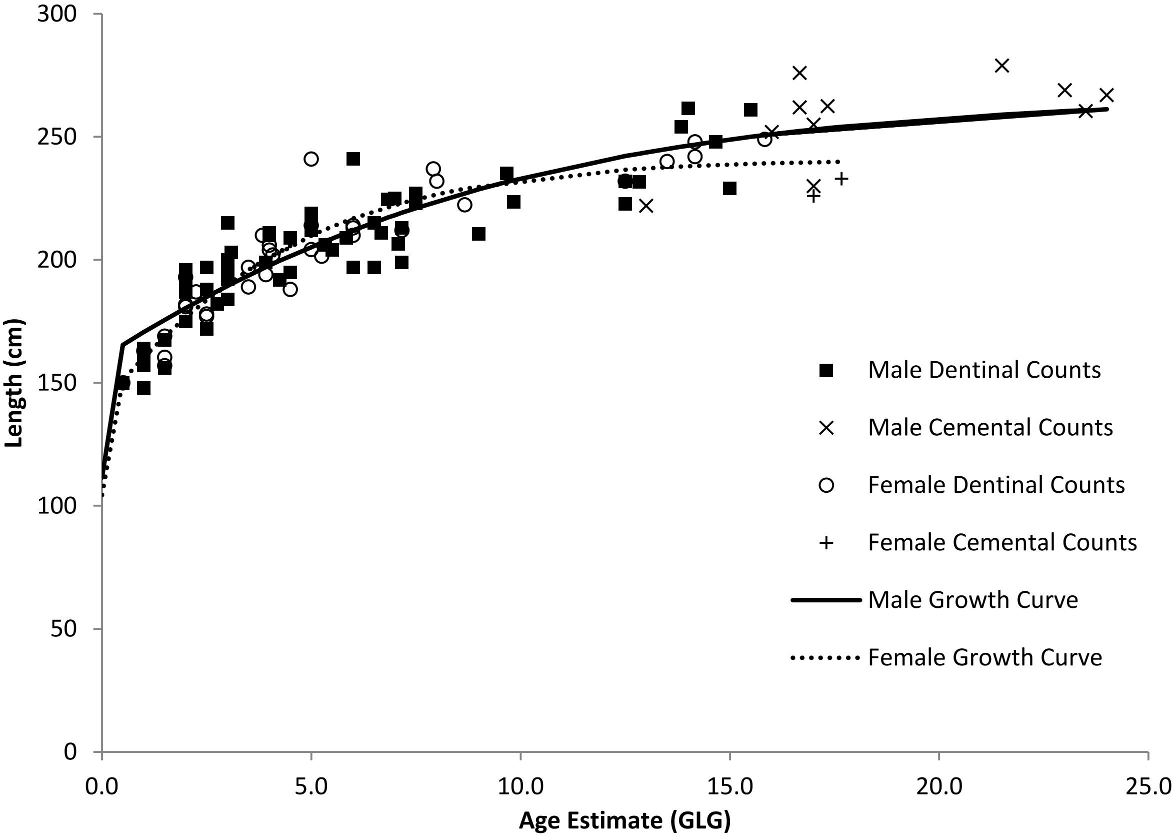


Figure 2.8: Increase in length with age (growth) for male and female *S. chinensis*, fitted to a three-parameter Gompertz growth function (n = 71♂ and 38♀).

2.4.2.3 Body mass

A regression of body length to body mass for 109 humpback dolphins (71 males and 38 females) yielded a very good correlation of $R^2 = 0.934$ (Figure 2.9). Von Bertalanffy and Gompertz growth functions were fitted to the mass-at-age data for both males and females. The Von Bertalanffy growth function provided a better fit for males ($R^2 = 0.824$) than the

Gompertz growth function ($R^2 = 0.788$), while the fit for females was identical in both models ($R^2 = 0.740$) (Figure 2.10 (A, B)).

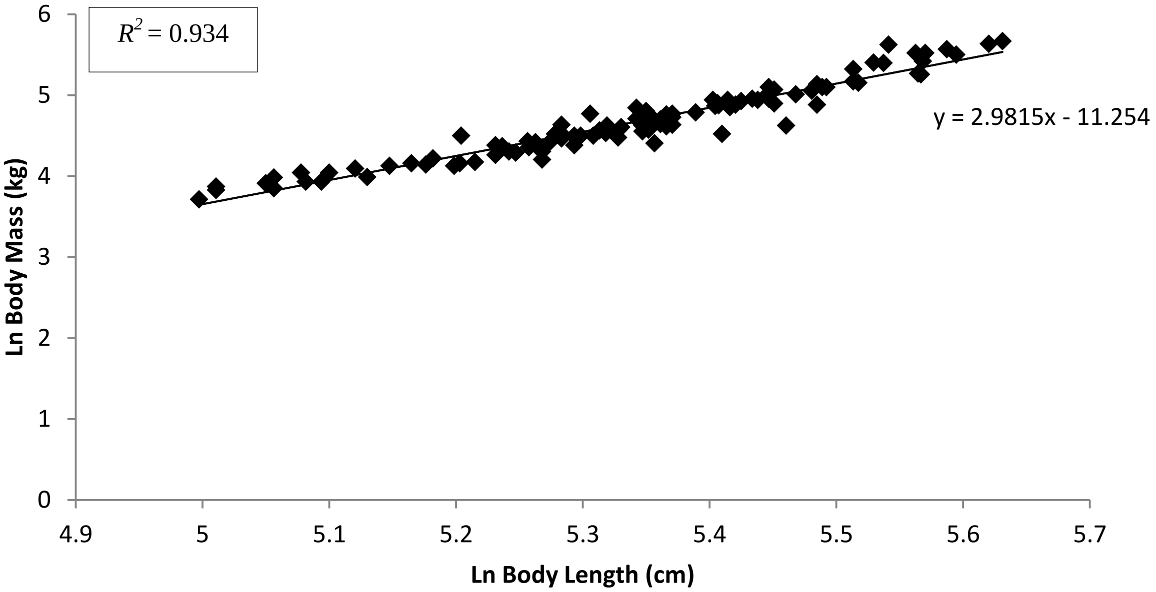
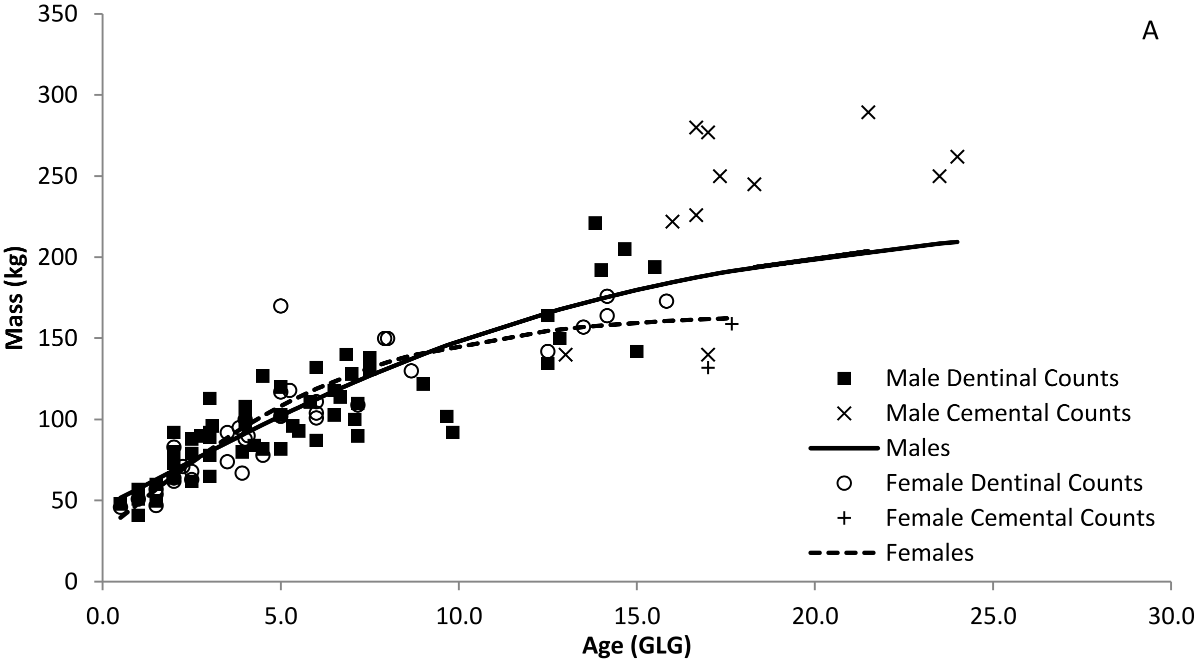


Figure 2.9: Body length in relation to body mass for *S. chinensis* ($R^2 = 0.934$).

Results for mass at age obtained from both the Von Bertalanffy growth function and the Gompertz growth function were very similar. However, unlike results obtained from the length-at-age data, growth in terms of mass continues well beyond 20 years for males. As a result, asymptotic mass cannot be predicted for males. Females, on the other hand, reach asymptotic mass well before males, around 17 GLGs and 160 kg when applying either model.

Furthermore, the Von Bertalanffy model estimated mass at birth for males at 40 kg and 30 kg for females, whereas the Gompertz growth function estimated mass at birth for males at a slightly greater mass of 50 kg and 30 kg for females. The heaviest male in the sample, estimated at 21.5 GLG, weighed 289.4 kg and measured 279 cm TBL. The heaviest



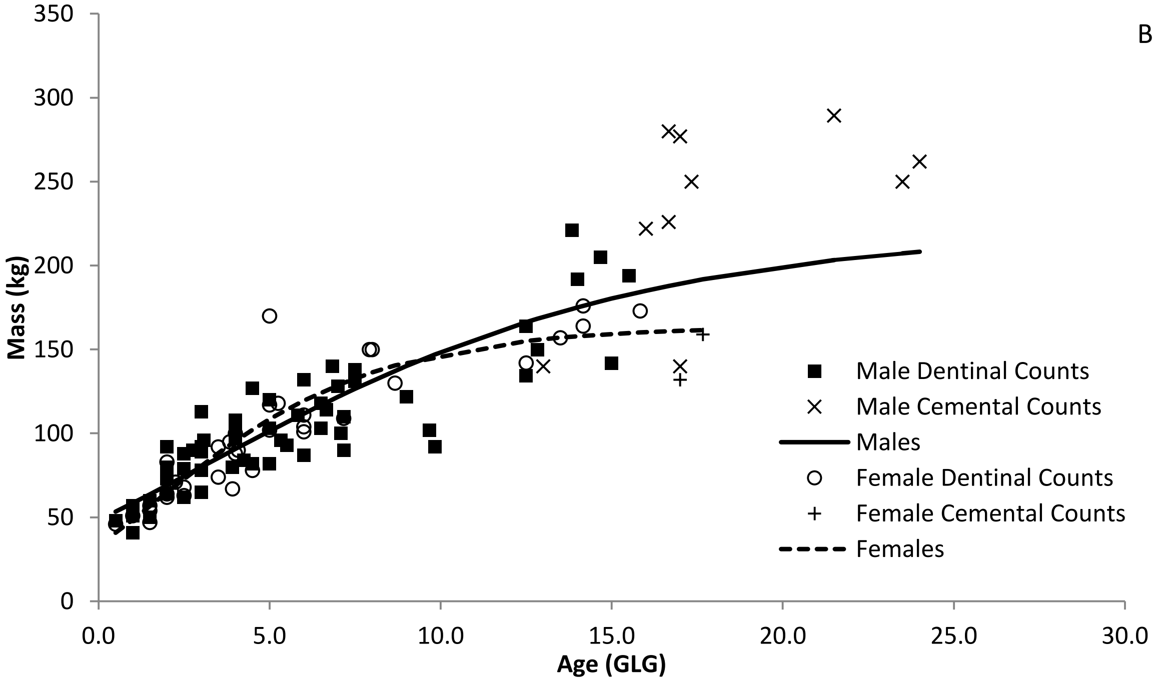


Figure 2.10: Age in relation to body mass in *S. chinensis* using the Von Bertalanffy growth model (A) and the Gompertz growth model (B) (n = 71♂ and, 38♀).

female, estimated at 14.7 GLGs, weighed 176 kg, and measured 248 cm TBL. The smallest male in the sample weighed 41 kg, measured 148 cm and had an age estimate of one GLG. The smallest female weighed 46 kg, measured 150 cm and was an estimated 0.5 GLGs old.

2.5. Discussion

2.5.1 Age estimation

According to Hohn (2002), the accuracy and precision of age estimation is influenced by the species being studied, as growth layers are more defined in some species than others. The consistency and repeatability of age estimates is increased if the sections are prepared well at each step. However, even when dealing with perfectly prepared sections, the subjective nature of the analysis could potentially result in varying age estimates (Hohn, 2002). To reduce the potential for subjectivity, two independent readers were employed (A. Bishop, Rhodes University; S. Plön, Nelson Mandela Metropolitan University) to help with age estimation and assisted in confirming estimates. However, as the rate of both dentinal and cemental deposition is not known for *S. chinensis*, it should be noted that age estimates are thus only that, an estimate.

To validate dentinal estimates in this study, age estimates were performed using cemental layers as well. For *S. chinensis* a strong correlation was found between dentinal and cemental age estimates up to the occlusion of the pulp cavity (at 15 GLGs), suggesting that layers in both tissues are deposited at the same constant rate in *S. chinensis* from KwaZulu-Natal. Dentinal counts therefore provide a reliable estimate of age up to at least 15 GLGs. Estimates beyond that, however, may produce an underestimate and should be read with caution (Gurevich *et al.*, 1980; Cockcroft and Ross, 1990). The use of cementum for performing age estimates beyond 15 GLGs is thus considered reliable (Sergeant, 1962). The time taken for the pulp cavity to become occluded varies not only among species, but also between individuals and among various teeth of a single animal (Klevezal' and Kleinenberg, 1967). On average about 10 - 12 layers are laid down in the dentine of *Delphinus delphis*, as well as the long-finned pilot whale, *Globicephala melas* before the pulp cavity becomes occluded (Sergeant, 1962; Gurevich *et al.*, 1980). Similarly, the pulp cavity for *Tursiops aduncus* closes at about 12 years (Cockcroft and Ross, 1990).

Physical maturity in females is attained at roughly the same time as the pulp cavity becomes occluded. Males on the other hand only reach physical maturity at an estimated 24 GLGs, which is well after the pulp cavity has become occluded. In the case of *S. chinensis* in the Pearl River Estuary, Jefferson *et al.* (2012) reported that the pulp cavity occluded at ages between18 and 21 GLGs, which is just after physical maturity is attained, around 14 - 17 GLGs.

2.5.2 Sexual dimorphism

Based on the results for length at age in the present study, males and females appear to exhibit clear sexual dimorphism. In other words, the length and mass at a specific age differs for males and females, based on either growth function (i.e. Von Bertalanffy or Gompertz). As suggested by the calculated growth rates for both the Gompertz and Von Bertalanffy growth function, females grow faster than males early in their ontogeny and attain greater body lengths earlier, between the ages of four and 10 GLGs. Thereafter, the growth rate decreases, reaching an asymptote around 15 - 16 GLGs. Males, on the other hand, attain greater total body lengths at ages beyond 10 GLGs. For example, at 14 GLGs the mean total body length of male humpback dolphins is 248 cm, whereas females are slightly smaller, around 237 cm TBL. The difference in size increases with age. At approximately 17 GLGs, the mean body length of males is estimated around 255 cm, whereas females are only 238 cm long. However, it should be noted that the oldest female in this sample is only 17.7 GLGs old, thus these results could be a product of the sample available. Furthermore, when considering mass-at-age for *S. chinensis*, clear sexual dimorphism is illustrated. Total body mass in males appear to increase well beyond 24 GLGs. Conversely, for females the total body mass reaches an asymptote around 17 GLGs at about 160 kg.

Cockcroft (1989) formerly also reported clear sexual dimorphism in KwaZulu-Natal humpback dolphin populations. However, a more recent study on the allometric relationship of various body measurements from humpback dolphins off KwaZulu-Natal concluded that males and females in fact do not demonstrate any degree of sexual dimorphism (Weston, 2011). This result was in agreement with *S chinensis* populations inhabiting Chinese waters (Jefferson, 2000; Jefferson *et al*., 2012). However, both Weston (2011) and the present study faced the same challenge of dealing with a generally young population, with a lack of older individuals, especially females (Trolley *et al*., 1995). The inclusion in the allometric relationship of young, physically immature individuals could possibly have influenced the results. Furthermore, Weston (2011) analysed only 11 measurements of the possible 25. Although further work is necessary to elucidate this discrepancy, based on the findings from the present study, it will be assumed that humpback dolphins off KwaZulu-Natal display clear sexual dimorphism for at least length and mass-at-age.

2.5.3 Growth

Both curves obtained from the Von Bertalanffy growth function and the Gompertz growth function described the relationships between age and length/mass equally well, and thus either can be applied. However, as previous studies on *S. chinensis* have applied the Gompertz growth function to describe the relationship between length-and-mass-at-age, this model was adopted for the discussion in the present study, to facilitate comparison with other studies on *S. chinensis*. However, it should be noted that data gathered from growth curves may not always be the most effective means of describing the biological aspects on a cetacean species' biology, especially when the sample set originates exclusively from incidental captures (de Santos *et al*., 2003).

Neither of the growth curves generated for male and female *S. chinensis* shows the expected trend in growth (of both length and mass) for cetaceans: rapid growth subsequent to birth with a gradual decrease with age, until the curve eventually reaches an asymptote, signifying mean maximum length (Hohn, 1980). The growth curves obtained from both models in the present study produced a relatively flat curve for both males and females, and failed to reach a clear asymptote. This however does not corroborate data from growth models obtained for *S. chinensis* from previously published data, nor from various other cetaceans (Barlow and Hohn, 1984; Cockcroft and Ross, 1990; Marsili *et al.*, 1997; Fernandez and Hohn, 1998; Neuenhoff *et al*., 2011; Jefferson *et al*., 2012). This may be attributed to the lack of samples for individuals smaller than 140 cm TBL, thereby probably under and over estimating the growth rates and lengths at birth, respectively. In an attempt to correct this artefact, an independent equation (Scott, 1949) was used to produce a more likely estimate for length at birth, thus forcing the curve through the y-axis.

Taking into consideration the estimate produced from Scott's (1949) equation, growth was most rapid during the first year, with animals reaching lengths of 170.59 cm and 160.31 cm and masses of 58.46 kg and 48.58 kg for males and females, respectively. Post-natal growth in southern Chinese populations appears to be rapid during the first two years, whereafter it starts levelling off (Jefferson, 2000). As derived from the growth curve obtained for *S. chinensis* in Chinese waters, a length of approximately 140 cm is attained after one year of growth by Jefferson *et al*. (2012). It should be noted that South African populations differ greatly morphologically and ecologically from those studied in southern China (Ross *et al*., 1994) and attain larger maximum TBLs than their Asian counterparts.

2.5.3.1 Length at birth

Length at birth, as predicted from Scott's equation (1949), occurs around 111 cm and 48 kg for males and 104 cm and 35 kg for females. Compared to previous findings for *S. chinensis* in South Africa (Cockcroft, 1989) and southern China (Jefferson, 2000; Jefferson *et al*., 2012), the estimated length at birth from the present study is slightly larger. Jefferson *et al*. (2012) estimated length at birth to be 101 cm for *S. chinensis* in the Pearl River Estuary (China), using a two-phased Gompertz model. The estimated length at birth for *S. chinensis* in adjacent Hong Kong (China) waters was estimated at 100 cm (Jefferson, 2000), using the same approach. Cockcroft's (1989) results for the same population of *S. chinensis* as used in the present study yielded a preliminary estimate of 100 cm. These results correlate with that of humpback dolphins found in the Pearl River Estuary and Hong Kong; however, the model from which this predication was made was not specified. Similarly, Ross (1984) assumed the length at birth is 100 cm for humpback dolphins found in South Africa, which was based on the measurements obtained from Saayman and Tayler (1979) for two stranded neonates. The difference in estimated length at birth from previous studies and the present study may well be a feature of sampling differences, as no neonates were available for age estimation in the present study. However, based on the estimates obtained from Scott's equation (1949), neonates are born roughly 39.9% of the maximum adult length (from this study) for males, and 41.0% for females. This corroborates Chivers' (2001) estimate for odontocetes, which predicts a neonatal length range between 40 - 48% maximum TBL.

This, however, does not eliminate the possibility of an overestimate for said parameter. Scott's equation (1949) has a broad application which includes both mysticetes and odontocetes; therefore, some concern lies in the possibility of potential anomalies (Plön, 2004). Consequently, the result for the estimated length at birth obtained from this study may only serve as an indicator or rough estimate, until more data for younger individuals are collected from which a more accurate estimate can be obtained.

2.5.3.2 Maximum length and age

The longest male and female *S. chinensis* in this study measured 279 cm and 249 cm respectively. These values agree with those reported by Cockcroft (1989) and Best (2007). Likewise, Ross (1984) reported the maximum lengths for South African populations to range between 254 cm and 279 cm TBL.

There was a difference in the maximum age estimates obtained in the present study and those reported by Cockcroft (1989), for the same population. The maximum age estimates obtained for *S. chinensis* in this study were 24 GLGs for males and 17.7 GLGs for females. Results based on Cockcroft's (1989) findings estimated longevity at an excess of 40 GLGs for both sexes. Similarly, Jefferson *et al*. (2012) estimated the oldest specimen of *S. chinensis* from the Pearl River Estuary to be 38 years old, and an earlier study conducted by Jefferson (2000) estimated the oldest individual in his samples from Hong Kong to be 33 GLGs. The difference in results between previous studies and the current study may be due to 1) differences in the interpretation of GLGs between the different studies, 2) the sample in the present study not being a true reflection of population age structure, thus underestimating longevity, or 3) South African populations not growing as old as southern Chinese populations. Each of the three possibilities will be discussed in more detail below.

The difference in the estimates for the maximum age for *S. chinensis* in KwaZulu-Natal coastal waters in the present study and in Cockcroft's study (1989) for the same population is difficult to addresses as the methods used to define GLGs and thus age estimates in his study were not published. Conversely, the dissimilarity to results obtained by Jefferson (2000) and Jefferson *et al*. (2012) could be attributed to the sampling artefact of a bias in shark net captures, which may have misrepresented the population structure of *S. chinensis* in KwaZulu-Natal. Differences in spatial distribution of different age classes could explain the majority of sub-adults reported in the sample (Atkins *et al*., 2013). Older dolphins reportedly do not frequent inshore netted zones due to differences in foraging behaviour and therefore do not form part of shark net bycatch (Cockcroft, 1990; Cockcroft, 1994; Atkins *et al*., 2013). Alternatively, there may be a difference in the life expectancies of different populations. However, additional samples and further analysis is required to provide more insight into these differences.

2.5.3.3 Length and age at physical maturity

Calculating the asymptotic length using the two different growth models was the only approach used in this study to assess the length/age at physical maturity. Individuals were considered physically mature if they had a standard length ≥ the asymptotic length (Cockcroft and Ross, 1990). The asymptotic length of humpback dolphins was calculated at 266.70 cm for males and 240.96 cm females; a difference of 25.74 cm. This result is very similar to the previously reported asymptotic length of 270 cm and 240 cm, respectively, by Cockcroft (1989) for the same population. In Hong Kong coastal waters, Jefferson (2000) found little differentiation in length at age and pooled data for males and females, resulting in an asymptotic length of 243 cm, which is considerably smaller than that estimated for males off the KwaZulu-Natal coast. Similarly, Jefferson *et al*. (2012) estimated the asymptotic length of male and female humpback dolphins in the Pearl River Estuary at 249 cm.

It has been reported that asymptotic length in the long-beaked common dolphin, *Delphinus capensis*, varies between different geographic populations (Mendolia, 1989). Moreover, differences in asymptotic length between various bottlenose dolphin populations have also been reported and show a fairly large range, which appears to be highly dependent on the population. It is therefore clear that differences in asymptotic lengths between different geographical populations are not unusual. Although these differences have been attributed to environmental conditions such as water temperature and diet (Cockcroft and Ross, 1990), this does not appear to explain the size differences between South African and southern Chinese humpback dolphin populations. Animals inhabiting colder waters are generally larger than animals found in more temperate regions (Cockcroft and Ross, 1990). However, coastal water temperatures off KwaZulu-Natal (23.6 ^{o}C) and southern China (24.3 ^{o}C) are very similar (Global Sea Temperature, 2013). Furthermore, the dietary preference of *S. chinensis* populations in both South Africa and southern China are very similar, with both preferentially feeding on estuarine-associated fish (Barros and Cockcroft, 1991; Jefferson, 2000).

The age at physical maturity differs for male and female *S. chinensis* in this study by eight GLGs. Males are estimated to mature physically around 24 GLGs and females around 16 GLGs, which adventitiously corresponds to the estimated maximum age attained by male (24 GLGs) and female (17.7 GLGs) *S. chinensis* in this sample. When compared to the previously estimated age at attainment of physical maturity for South African populations, males in the present study appear to become physically mature later than previously estimated by Ross (1984) (13 - 14 GLGs). In southern Chinese populations, Jefferson *et al*. (2012) estimated the age at physical maturity between 14 and 17 GLGs for humpback dolphins in Pearl River Estuary (Jefferson *et al*., 2012); and Jefferson (2000) estimated physical maturity at approximately 16 GLGs in Hong Kong waters. Both estimates from southern Chinese populations are slightly younger than the age estimated for South African *S. chinensis*.

Ecological differences between different populations may be the reason for this disparity in both age estimates and predicted asymptotic length. However, it is unsure whether the difference in estimated asymptotic length as well as age in this study, especially for males, can be attributed to environmental conditions.

2.5.3.4 Body mass

Mass increase for males is most rapid in the first 13 - 15 years, whereas for females it is during the first 10 years. Asymptotic mass for males could not be predicted, as the curve for mass-at-age did not reach an asymptote, and mass appears to continue increasing well after the age and length at attainment of physical maturity. Mass in females, on the other hand, reaches an asymptote around 17 GLGs, at approximately 160 kg and 233 cm TBL. This roughly coincides with the approximate age at attainment of physical maturity. Asymptotic mass, like length, is reached earlier in females than in males.

The maximum mass recorded for *S. chinensis* in this study was 289.4 kg for a male of an estimated 21.5 GLGs and 279 cm TBL. The maximum mass recorded for females was 176 kg, attained at 14.7 GLG and a TBL of 248 cm. Humpback dolphins found in southern Chinese waters, in both Hong Kong and the Pearl River Estuary, attain a slighter lower maximum mass of just under 250 kg, around 260 cm TBL (Jefferson, 2000), and 240 kg at 268 cm TBL (Jefferson *et al*., 2012), respectively.

Mass at birth was estimated around 50 kg for males and 30 kg for females, using the Gompertz growth curve. Currently, there appear to be no published data in the literature to which this could be compared, as this is the first published estimate for mass at birth for *Sousa.*

Chapter 3

Reproductive Biology of *S. chinensis*

3.1 Introduction

3.1.1 Background information

Studies concerning the reproductive biology of cetaceans are often limited. This is mainly due to the fact that data on the reproduction of wild populations are difficult to obtain and research results based on the reproduction of captive animals may not be a true representation of wild populations (Plön, 2004). Although a number of studies have assessed the reproduction of wild populations, this is often difficult, as reproductive behaviour mainly occurs under water (Plön and Bernard, 2007). Despite these limitations, determining the reproductive parameters of a species is an important component for both its conservation and management (Cockcroft and Ross, 1990).

The study of population dynamics allows for the understanding of human impact and its effect on all animals (Mendolia, 1989). To understand the population dynamics of odontocetes, it is imperative to know the age and size at which dolphins attain sexual maturity and start breeding (Bryden and Harrison, 1986). By determining stage and age at sexual maturity, one can correlate the information with more readily available life history data, such as length (Hohn *et al*., 1985). Data for the total body length at sexual maturity have been found to be particularly useful in field studies, mainly for animals for which no age estimates are available (Perrin and Reilly, 1984).

Age and size at attainment of sexual maturity may differ for different species and also among different forms or types of a single species (Bryden and Harrison, 1986). For example, the age at which dolphins attain sexual maturity has been found to differ geographically for the same species of both short-beaked common dolphins (Bryden and Harrison, 1986) and spinner dolphins (Perrin and Henderson, 1984). Moreover, age at sexual maturity may also vary considerably among individuals in the same population (Bryden and Harrison, 1986).

Various factors such as climate, diet, lactation, date and/or order of birth and exploitation have been demonstrated to influence changes in the basic reproductive parameters of odontocetes (Perrin and Henderson, 1984; Kasuya, 1985; Bryden and Harrison, 1986). Very little is known about the reproduction of humpback dolphins throughout their distribution range (Jefferson *et al.*, 2012). Only a single, unpublished preliminary study has to date been conducted on the growth and reproductive parameters of southern African humpback dolphins (Cockcroft, 1989).

3.1.2 Male reproductive biology

3.1.2.1 Attainment of sexual maturity

Defining the age at attainment of sexual maturity in male cetaceans is complex as there is no single criterion for describing the onset of sexual maturity (Perrin and Reilly, 1984). Approaches for determining the onset of sexual maturity range from testis histology, which involves determining the different stages of spermatogenesis and seminiferous tubule diameters, to testis mass or length, sperm abundance, the presence/absence of sperm in the epididymis, or serum testosterone levels (Plön, 2004). Of these methods, the most readily available and accurate method is testis histology, which involves determining the onset of sexual maturity by determining the state of spermatogenesis (Perrin and Donovan, 1984; Hohn *et al.*, 1985; Akin *et al.*, 1993). Sergeant (1962) defined sexual maturity in males as 'functional' at the point at which examination of the epididymis showed the presence of seminal fluids, which occur at testis masses greater than those at which spermatogenesis is detected histologically.

3.1.2.2 Spermatogenesis

Spermatogenesis is a vital indicator of the reproductive biology of male mammals (Plön and Bernard, 2007) and is a complex process of continual cell differentiation (Akin *et al*., 1993). Spermatogenic activity not only serves as an indicator of the onset of sexual maturity, but can also signify the onset of breeding in seasonally reproducing species (Plön and Bernard, 2007).

Although there is some discrepancy as to the number of stages of maturity in odontocetes, the most commonly defined states are immature, prepubescent (or pubertal), and mature (Collet and Saint Girons, 1984; Hohn *et al*., 1985; Akin *et al*., 1993). The immature stage is characterised by relatively small, circular tubules with no lumen. Only spermatogonia are present within each tubule, which are surrounded by abundant interstitial tissue (Akin *et al*., 1993). During the pubertal stage, the tubule is much larger with less interstitial tissue between adjacent tubules (Akin *et al*., 1993). Generally, only spermatogonia and spermatocytes are present (Akin *et al*., 1993). Sexual maturity, on the other hand, is characterised by fairly large tubules, surrounded by little interstitial tissue, and the presence of spermatogonia, spermatocytes, spermatids, and most importantly, spermatozoa, which are visible in virtually every tubule (Akin *et al*., 1993). Although active spermatogenesis, combined with the presence of spermatozoa in the seminiferous tubules and the epididymis, suggest fertility in males, it remains unclear whether or not adult males are capable of storing spermatozoa after sperm production has ceased; or how rapidly the onset of spermatogenesis takes place (Bryden and Harrison, 1986). In the majority of odontocetes that have been studied, both testes have been found to mature at the same rate and thus only a single testis is required for examination (Plön and Bernard, 2007).

3.1.2.3 Index of testis development

Although testis mass can be used as an indicator of sexual maturity within a population, it may vary greatly between species and populations, and therefore the calculated testis mass of one population cannot be used to determine that of another population, or species (Hohn *et al*., 1985). However, by normalising testis mass by testis length, thus determining a testis index, more accurate inter- or intra-specific comparisons can be made, allowing for direct comparisons of maturity between species (Hohn *et al*., 1985).

3.1.3 Female reproductive biology

3.1.3.1 Attainment of sexual maturity

Female delphinids generally attain sexual maturity earlier than males and at a shorter body length (Laws, 1956; Perrin and Reilly, 1984; Cockcroft and Ross, 1990). The onset of sexual maturity in females is rapid and is generally defined as the age at which a female has ovulated at least once, as evident by the presence of one *corpus luteum* (*CL*) or *corpus albicans* (*CA*) in the ovaries (Perrin and Reilly, 1984; Perrin and Donovan, 1984; Akin *et al*., 1993). *Corpora* reportedly persist throughout an animal's life, and the number of *corpora* recorded can be related to the age of an animal to determine the age at first ovulation, birth interval, as well as the female's reproductive lifespan (Perrin and Donovan, 1984; Bryden and Harrison, 1986).

The average age at attainment of sexual maturity in females can be estimated in several ways (Demaster, 1984), who lists five commonly used estimators which include 1) the mean age of first-time ovulators, 2) the mean deduced from age-specific ovulation rates, 3) graphical interpretation of a plot of percentage of mature females against their age, to determine the age when 50% of females have ovulated at least once, 4) regression of *corpora* counts versus age, and 5) the graphical interpretation of the age at which the cumulative probability of ovulating by age *x* equals the cumulative probability of not ovulating at age *x* or older (Demaster, 1984; Kasuya, 1985). Furthermore, Clazada *et al*. (1996) proposed using the sum of the fraction of immature animals, which involves the use of a pre-developed formula that estimates the sum of the fraction of immature animals in each age class, where mature and immature specimens are present (Calzada *et al*., 1996).

3.1.3.2 Ovarian cycle

The anatomy of the uterus and ovaries is similar in all cetaceans that have been studied (Brook *et al*., 2004). Immature ovaries in delphinids tend to be ellipsoid in shape and light in colour (Harrison, 1949). As age progresses and maturity is attained, the ovaries become progressively darker, less flattened, appearing as ovoid structures (Harrison, 1949; Brook *et al*., 2004). As in the case of other mammals, both ovaries in cetaceans are fully functional and females are able to ovulate from the onset of sexual maturity, onwards (Dabin *et al*., 2008). However, in delphinids it has been reported that females predominately ovulate from the left ovary (Ohsumi, 1964; Perrin *et al.*, 1977; Cockcroft and Ross, 1990; Hohn *et al.*, 1996; Murphy, 2004; Danil and Chivers, 2007).

The ovarian cycle begins with the development of a follicle within the ovary and ends with the release of an oocyte, or the degeneration of a follicle (Akin *et al*., 1993). A previous study on female *S. chinensis* revealed that developing follicles had a diameter of > 4 mm (Brook *et al*., 2004). In some instances, more than one follicle can develop simultaneously, however, only a single, dominant follicle will progress to ovulation (Brook *et al*., 2004). Ovulation is followed by the lutealisation or transformation of the follicle into a *CL* (endrocrine gland) (Akin *et al*., 1993; Dabin *et al*., 2008). In the event that a pregnancy occurs, the *CL* persists as a secretary organ, releasing various hormones necessary to maintain the pregnancy (Akin *et al*., 1993). If a foetus is found in the uterus, the *CL* is referred to as a *CL* of pregnancy or *corpus gravitatum*; whereas a *CL* that persists for a short period without a subsequent pregnancy is termed a *CL* of ovulation or *CL* of the cycle (Perrin and Donovan, 1984). The *CL* differs in appearance from the *CA* in that it forms a globular structure on the surface of the ovary (Akin *et al*., 1993). Within the globular structure, yellow granulosa cells are arranged in an intricately coiled pattern, interspersed with connective tissue and blood vessels (Akin *et al*., 1993). Following either an infertile ovulation or a parturition (in the case of a fertile ovulation), the *CL* regresses; forming a structure resembling a *CA*, which in essence is a scar that persists on the ovary (Perrin and Donovan, 1984; Bryden and Harrison, 1986; Akin *et al*., 1993). However, it is important to note that not every *CA* marks a previous pregnancy (Bryden and Harrison, 1986). *CA*s for various cetaceans have been classified into two types: one having developed from a *CL* of ovulation and the other from a *CL* of pregnancy; however, the distinction between the two has not been confirmed (Perrin and Donovan, 1984).

Unlike other mammals, the *CA*s in cetaceans remain visible on the ovaries indefinitely, thereby providing a reliable record of a female's reproductive history (i.e. ovulations) (Perrin and Reilly, 1984; Dabin *et al*., 2008). As previously stated, however, this is not reliable for assessing the number of previous pregnancies (Perrin and Reilly, 1984; Bryden and Harrison, 1986).

There are various uncertainties regarding the persistence of *CA*s, and this limits our understanding of the significance of ovarian scars (Dabin *et al*., 2008). For example, some scars may denote non-ovulatory events, such as the lutealisation of un-erupted Graafian follicles (i.e. a tertiary vesicular follicle) or infertile cyclical ovulations occurring before full breeding status is attained (Perrin and Reilly, 1984; Bryden and Harrison, 1986). Secondly, there is some ambiguity surrounding the timing of ovulation, as some species exhibit mono-ovulation, whilst others demonstrate poly-ovulation (i.e. several sequential ovulations) (Dabin *et al*., 2008). Furthermore, ovarian physiology is still poorly understood; in the case of younger females, several follicles may rupture in a single ovulation event, resulting in the development of numerous *CL*s, and the formation of several *CA*s corresponding to the same event (Dabin *et al*., 2008). Finally, there are some limitations in the methodology used to determine the attainment of sexual maturity in females. In the case of small delphinids, it has been suggested that ovarian scars resulting from either gestation or infertile ovulations cannot be discriminated either macroscopically or histologically (Dabin *et al*., 2008). Brook *et al*. (2002) found that only *CA*s resulting from gestation persisted in a bottlenose dolphin, *Tursiops aduncus,* while *CA*s resulting from unfertilised ovulations completely regressed over time. Likewise, Dabin *et al*. (2008) reported that ovarian scars in short-beaked common dolphins, *Delphinus delphis,* in the eastern North Atlantic appear rapidly soon after the attainment of sexual maturity; however, the majority regress almost completely with age.

Therefore, younger females may have more *CA*s than older females. Conversely, Danil and Chivers (2007) found that the number of *corpora* scars increase with age and persist to some degree in *D. delphis* in the Eastern Tropical Pacific, thereby offering complete insight into an individual's reproductive history. Similarly, Perrin *et al*. (1977) found that *corpora* of ovulation persisted throughout the life of spinner dolphins, *Stenella attenuata*, in the Eastern Tropical Pacific. Despite these limitations however, quantifying the number of *corpora* in the ovaries of females is currently the only method used for determining various reproductive parameters.

3.1.3.3 Ovulation rate

The number of ovulations an animal has had during the course of its reproductively active life is reflected by the number of scars present on the ovary and is therefore an indication of the ovulation rate (Plön, 2004). Ovulation rate is defined as the number of ovulations a female undergoes per annum, such that the number of *corpora* reflects an index of reproductive history in females (Perrin and Henderson, 1984). The ovulation rate can be determined by calculating the slope of the regression equation of the age of all sexually mature females versus the total number of *corpora* (Myrick *et al.*, 1986; Cockcroft and Ross, 1990). Alternatively, a Spearman's rank correlation test can also be employed to assess the relationship between number of *corpora* and age and thus the ovulation rate (Danil and Chivers, 2007).

There exists some controversy regarding the ovarian cycle in cetaceans (Perrin and Reilly, 1984). While some delphinids show spontaneous ovulations, others exhibit induced ovulations, which require either the presence of a mature male, or the event of a copulation to trigger an ovulatory event (Perrin and Reilly, 1984). It is unlikely that all females in a population will reach sexual maturity at exactly the same age and thus have the same ovulation rate (Sergeant, 1962; Perrin and Donovan, 1984; Myrick *et al*., 1986). Some variability may even exist in the number of *corpora* between females of the same age (Myrick *et al*., 1986; Cockcroft and Ross, 1990). Furthermore, ovulation rate is thought to decrease with reproductive age (Marsh and Kasuya, 1986; Myrick *et al*., 1986).

If the maximum longevity of a species is known, the ovulation rate can be used to determine an estimate for the maximum lifetime productivity per female (Plön, 2004), provided that no senescent females occur within a population. However, post-reproductive (or senescent) females have been observed in various odontocetes (Sergeant, 1962; Miyazaki, 1984; Marsh and Kasuya, 1986). According to Perrin *et al*. (1977), features commonly observed in females considered to be senescent, inactive or resting include having high *corpora* counts (greater than 10), small, withered ovaries, no developing follicles or no *corpora* indicating recent ovarian activity. Reproductive senescence has been recorded in pilot whales, *Globicephala macrorhynchus* (Marsh and Kasuya, 1986), and spotted dolphins, *Stenella attenuata* (Myrick *et al*., 1986).

Previous studies on *Sousa* in South Africa suggest that after reaching sexual maturity, females undergo rapid bursts of ovulation, which eventually settle at a rate of 0.3 ovulations per annum, suggesting a three-year calving interval (Cockcroft, 1989; Karczmarski, 1999). The gestation period of humpback dolphins in South Africa has been estimated at 10 - 12 months (Cockcroft, 1989; Karczmarski, 1999). Jefferson (2000) predicted the gestation period of *S. chinensis* in Hong Kong waters at around 11 months. Lactation lasts about two years (Cockcroft, 1989); however, mother-calf associations have been reported to persist for up to four years in *S. chinensis* in South African waters (Karczmarski, 1999) and around two years in the Pearl River Estuary (Jefferson *et al*., 2012).

3.1.4 Reproductive seasonality in both males and females

Seasonal reproduction has been widely observed in marine mammals and is largely dependent on environmental factors, the most important being food availability and its influence on energy balance (Bronson, 1989; Bronson, 2009). Generally, little is known about reproductive seasonality in marine mammals and factors influencing these patterns (Bronson, 1989, Plön and Bernard, 2007). Temperature, humidity, amount/distribution of rainfall, solar radiation/photoperiod, nutrition, productive system management, social interactions among individuals in the same population, predator-prey interactions, female body condition and parasite/pathogen-host interactions, are the main factors influencing reproduction in any animal (Plön and Bernard, 2007; Ungerfeld and Bielli, 2012). Seasonality is especially common in animals with a bigger body size and greater longevity (Ungerfeld and Bielli, 2012). However, this is not necessarily a defined rule, as diet also plays a crucial role (Ungerfeld and Bielli, 2012). Animals reliant on a reduced spectrum of food will in general breed seasonally, while more generalist feeders breed aseasonally (Ungerfeld and Bielli, 2012).

Studies involving the seasonality of reproduction in odontocetes are based on various criteria. In males, these include changes in testis mass, testis volume, seminiferous tubule diameter, sperm abundance, spermatogenic activity, Leydig cell diameter, and serum testosterone levels (Plön and Bernard, 2007). For females, seasonality is based on changes in hormone levels (Kirby and Ridgeway, 1984; Brook *et al*., 2004), conception, and birth peaks (Miyazaki, 1977; Saayman and Tayler, 1979).

Seasonal reproduction has been recorded in a number of cetaceans, including the short-beaked common dolphin, *D. delphis* (Murphy, 2004), the vaquita, *Phocoena sinus* (Hohn *et al*., 1996) and the spotted dolphin, *S. attenuata* (Perrin *et al*., 1976). Conversely, aseasonal reproduction has been observed in the Indian Ocean bottlenose dolphin, *T. aduncus* (Cockcroft and Ross, 1990), and the sperm whale, *Physeter macrocephalus (*Mitchell and Kozicki, 1984). In some humpback dolphin populations, year-round breeding has been suggested (Parra and Ross, 2009); however, births appear to predominate in summer months in some South African populations (Saayman and Tayler, 1979; Cockcroft, 1989; Karczmarski, 1999) as well as southern Chinese populations (Jefferson, 2000).

3.1.5 Mating systems

Species in which males have relatively small testes are thought to have uni-male breeding systems, which are either monogamous or polygynous (Harcourt *et al*., 1981) and involve one male maintaining a harem of females with which he alone mates (Plön and Bernard, 2007). In such instances, sexual dimorphism is great, as males fight over access to females and as a result scarring is also evident (Plön and Bernard, 2007). On the other hand, a multi-male or promiscuous mating system occurs in species where males have relatively large testes and levels of sexual dimorphism are intermediate, such that males compete with one another in the form of sperm competition (Harcourt *et al*., 1981; Mendolia, 1989; Jefferson, 1990). Larger testes are an indication of frequent copulation accompanied by sperm competition (Plön and Bernard, 2007).

Numerous factors are responsible for shaping the mating system of any species. By determining the testis mass to body mass ratio, sexual dimorphism, group size and degree of scarring from intra-sexual fighting, one can develop a hypothesis about the mating system of a species (Plön and Bernard, 2007). Different mating strategies include sperm competition, roving males, joint harem, harem, serial monogamy, a multi-male mating system and polyandry (Plön and Bernard, 2007).

3.2 Aim of the chapter

This chapter investigates reproduction in male and female *S. chinensis* such that length, age and mass at attainment of sexual maturity can be determined. This in turn will provide more detail on the reproductive biology of *S. chinensis* off KwaZulu-Natal. Additionally, more insight is given into the mating strategy and reproductive seasonality of *S. chinensis.*

3.3 Materials and methods

3.3.1 Sample

All samples used to examine the reproductive biology of *S. chinensis* originate from animals incidentally caught in the shark nets. Routine necropsies were performed on all humpback dolphins by various members of staff of the Port Elizabeth Museum. As a standard operating procedure, basic biological and morphological parameters were recorded as recommended by Norris (1961). Additionally, standard biological samples, such as gonads, were taken and accessioned into the Graham Ross Marine Mammal collection at the Port Elizabeth Museum (PEM) (Port Elizabeth, South Africa). The basic information recorded for each capture event is provided in Appendix A, and is listed in chronological order of the PEM accession number. Each capture event had the following metadata: date, sex, location, total body length (cm) (measured from the tip upper jaw to deepest part of notch) and mass (kg) (Norris, 1961).

Gonads were initially fixed in 10% formalin prior to being transferred to 50% iso-propanol for storage. The reproductive status of 53 males and 30 females was assessed in this study. The majority of male gonads were stored whole (n = 42), while for some of the larger testes only a section of the tissue was stored, in order to ensure proper penetration of the fixative into the tissue (n = 11). Weight and measurements of the reproductive organs for both males and females were only available for a limited number of specimens, as indicated by varying sample sizes in the Figures and text.

Male individuals, for which gonad samples were available, had body lengths ranging from 157 to 269 cm. There was, however, a lack of samples for males measuring between 171 and 180 cm TBL (Figure 3.1 (A)). Females for which ovaries were available ranged between 157 and 248 cm in total body length (TBL) (Figure 3.1. (A)). The age of the majority of males and females analysed ranged between one and nine GLGs (Figure 3.1 (B)). Only a few individuals older than nine GLGs were present in the sample, especially females, which presented some difficulties in determining the age at attainment of sexual maturity.

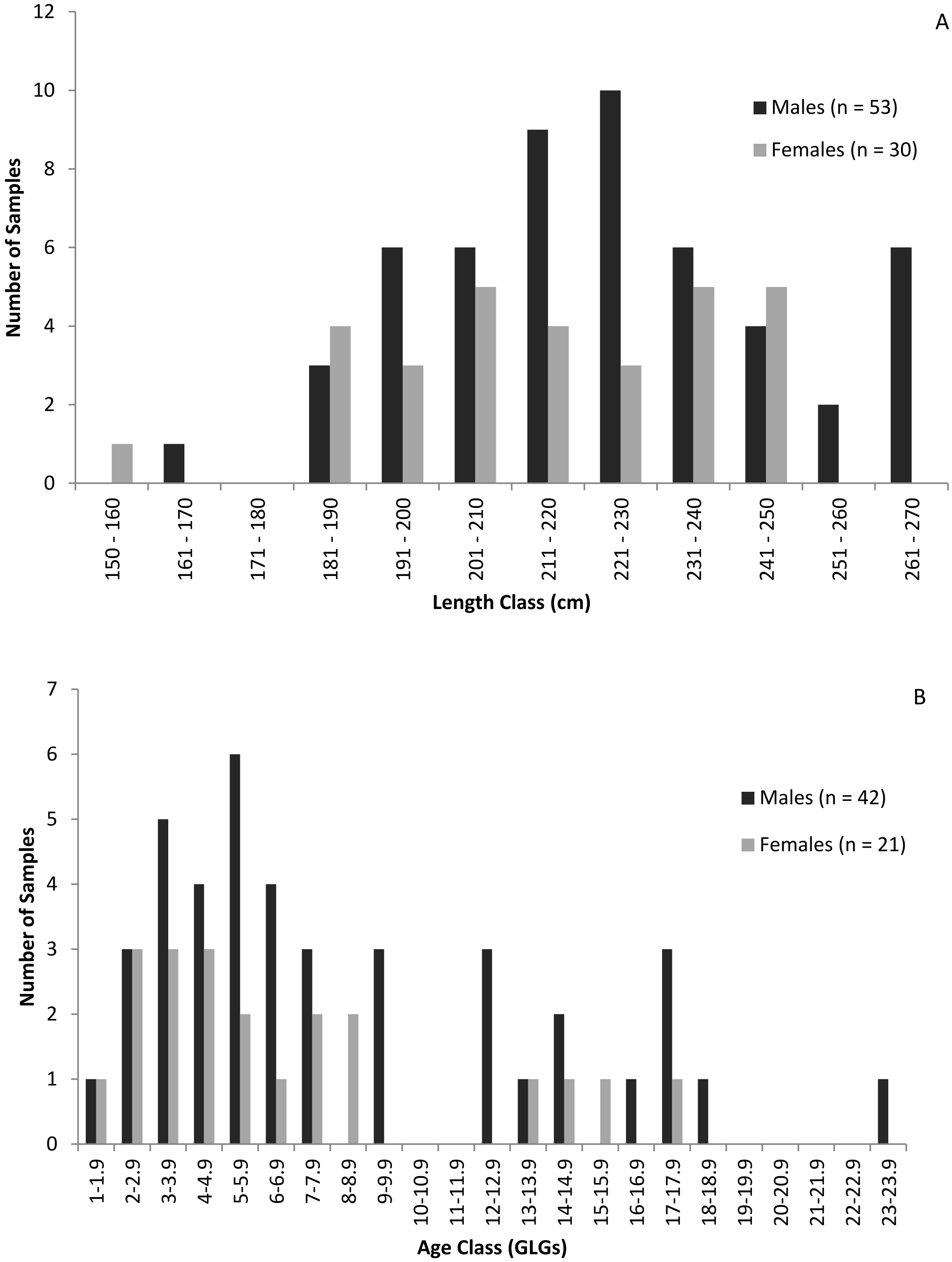


Figure 3.1: Size (A) and age (B) frequency histogram of samples from *S. chinensis* incidentally caught in shark nets set along the KwaZulu-Natal coast used for assessing the length and age at maturation.

3.3.2 Preparation of tissue and determination of maturity

3.3.2.1 Testes

Approximately 1 cm^3 of tissue was taken from the mid-section of the testis for each sample. The tissue was dehydrated in a standard ethanol series (30% to 100%), and transferred to 50% Xylene/Ethanol and 100% Xylene, before being impregnated with molten Parrafin Plus wax for at least 24 hours. Processed tissue was embedded in wax in order for sections ranging between 5 - 7 µm to be cut, using a *Leica* microtome. The sections were stained with Mayer's haematoxylin and eosin and mounted on slides using DPX mounting medium. Of the 53 samples, seven were discarded from the analysis as they could not be classified as either mature or immature due to the poor quality of the tissue, probably as a result of decay prior to sampling, or bad preservation.

A conservative approach to determining the different stages of maturity was used, as the tissue of specimens was generally poorly preserved. Unfortunately, the majority of testes were stored whole, which probably resulted in inadequate penetration of the preservative into the tissue. Poorly preserved tissue was generally very flaccid, and decay was evident by strong odour when the tissue was sectioned. Consequently, the quality of the histological sections was generally poor, which made the distinction between the different stages of sexual maturity hard to differentiate, especially between pubertal and mature individuals. Individuals were therefore solely classified as either mature or immature, as evident by the presence or absence of spermatozoa in seminiferous tubules.

Each slide was examined for the presence of spermatogonia, spermatocytes, and spermatozoa, using a BX50 compound microscope, operated at 200x to 400x magnification. Specimens were classified as immature if the following conditions were met: tubules were circular in cross section, contained no lumen, and the seminiferous epithelium comprised of spermatogonia and spermatocytes, with abundant interstitial tissue between adjacent tubules (Hohn *et al*., 1985; Akin *et al*., 1993). Maturity, on the other hand, was characterised by large seminiferous tubules, which comprised more than three layers of cells of spermatogonia, spermatocytes and, most importantly, spermatozoa (Hohn *et al*., 1985; Akin *et al*., 1993). Additionally, there was little interstitial tissue surrounding adjacent seminiferous tubules (Akin *et al*., 1993).

The seasonality of spermatogenesis was determined by measuring the seminiferous tubule diameters of 20 randomly selected tubules per specimen, using Olympus analySIS software. In order to remove any variability in testis mass, an index of testis development was determined (Hohn *et al*., 1985). This defines sexual maturity in terms of unit testis mass (g) (excluding mass of epididymis) per unit of testis length (mm) (Hohn *et al*., 1985).

3.3.2.2 Ovaries

Both left and right ovaries were routinely collected during necropsies. Both ovaries were weighed and sectioned serially at 1 - 2 mm intervals to determine the total number of Graafian follicles, *corpora lutea* (*CL*) and/or *corpora albicantia* (*CA*) macroscopically for each animal. Additionally, the length, width and height of all *CL*s and the largest *CA*s were measured using Vernier callipers in order to calculate a corpus index (mm^3) (Cockcroft and Ross, 1990). Females in which the ovaries lacked growing follicles or *corpora* were considered immature (Perrin and Donovan, 1984), while females with at least one Graafian follicle were classified as pubertal (Murphy, 2004). The presence of one or more *corpora* in one or both ovaries was used as the criterion for sexual maturity (Perrin and Donovan, 1984).

The ovulation rate of *S. chinensis* was determined based on the assumption that *CA*s persist indefinitely (Perrin and Reilly, 1984). Estimates were performed by fitting a linear regression of the *corpora* count (including both *CA*s and *CL*s) against age/length/mass, with the assumption that *corpora* form at the rate corresponding to the slope of the regression (Calzada *et al*., 1996). The regression line was determined using the least squares method from the graphical package Microsoft Excel 2007.

3.4 Results

3.4.1 Gonadal characteristics - Assessment of the stages of maturity

3.4.1.1 Males

Of the 53 samples analysed in the present study, 27 were classified as immature (Figure 3.2 (A, B)) and 19 as mature (Figure 3.2 (C)). Seven samples were discarded due to poor preservation. Left testis mass ranged from 16 g to 656 g, and right testis mass from 14 g to 595 g. There was no significant difference between the masses of the left and right testes ($x^2 = 1.67$; $P < 0.05$) (Figure 3.3). The combined testis mass (as recorded on the datasheets) ranged from 30 g (0.05% of total body mass) in an individual of 167.4 cm TBL to 1250 g (0.5% of total body mass) in an adult of 262.5 cm TBL.

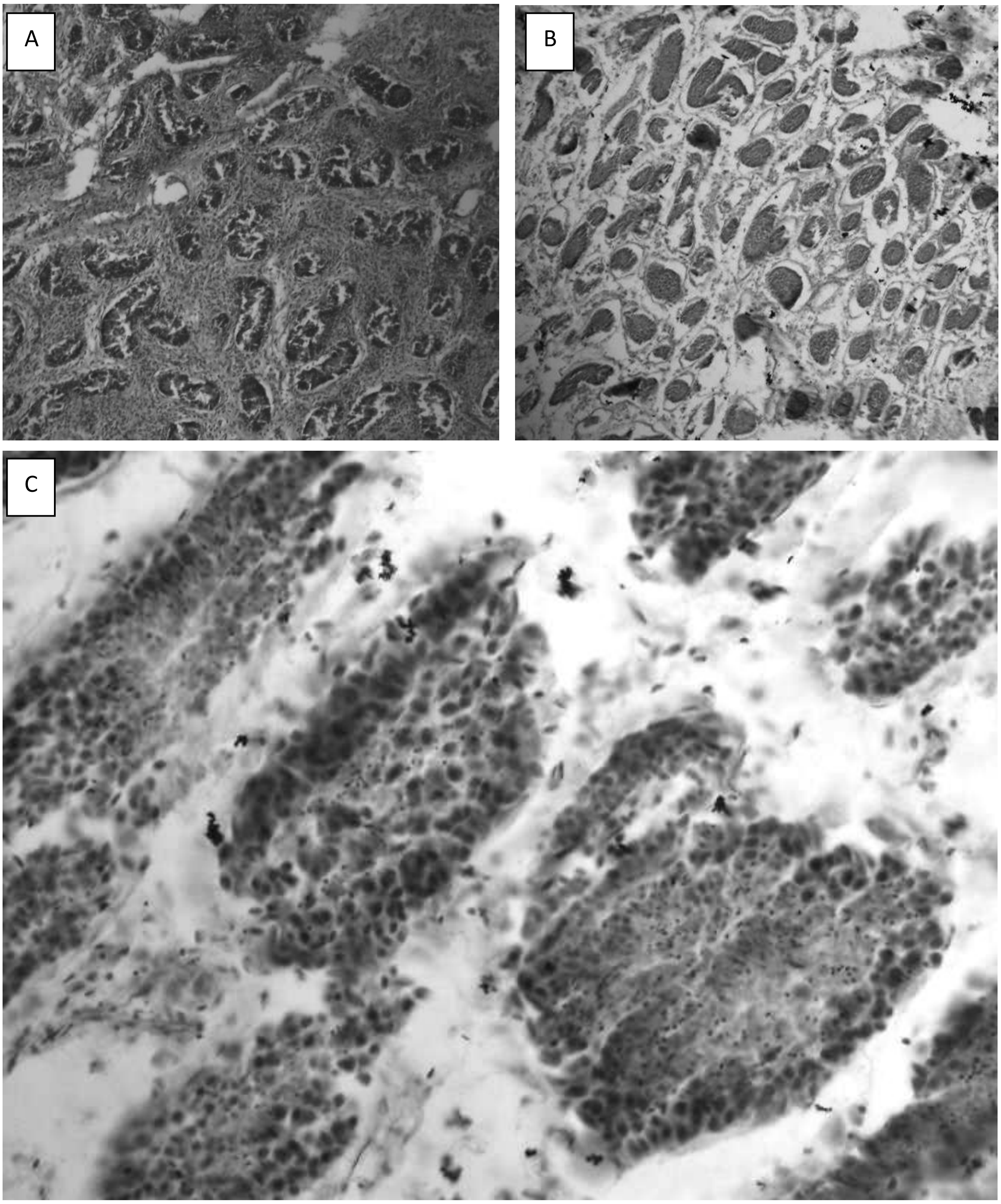


Figure 3.2: Histological preparation of different maturity stages of the testes of *S. chinensis*. (A and B). Immature testis, characterised by narrow seminiferous tubules, lacking a lumen and surrounded by abundant interstitial tissue. Photograph (C) represents late spermatogenesis/ sexual maturity, characterised by large seminiferous tubules with spermatogonia, spermatocytes and spermatids.

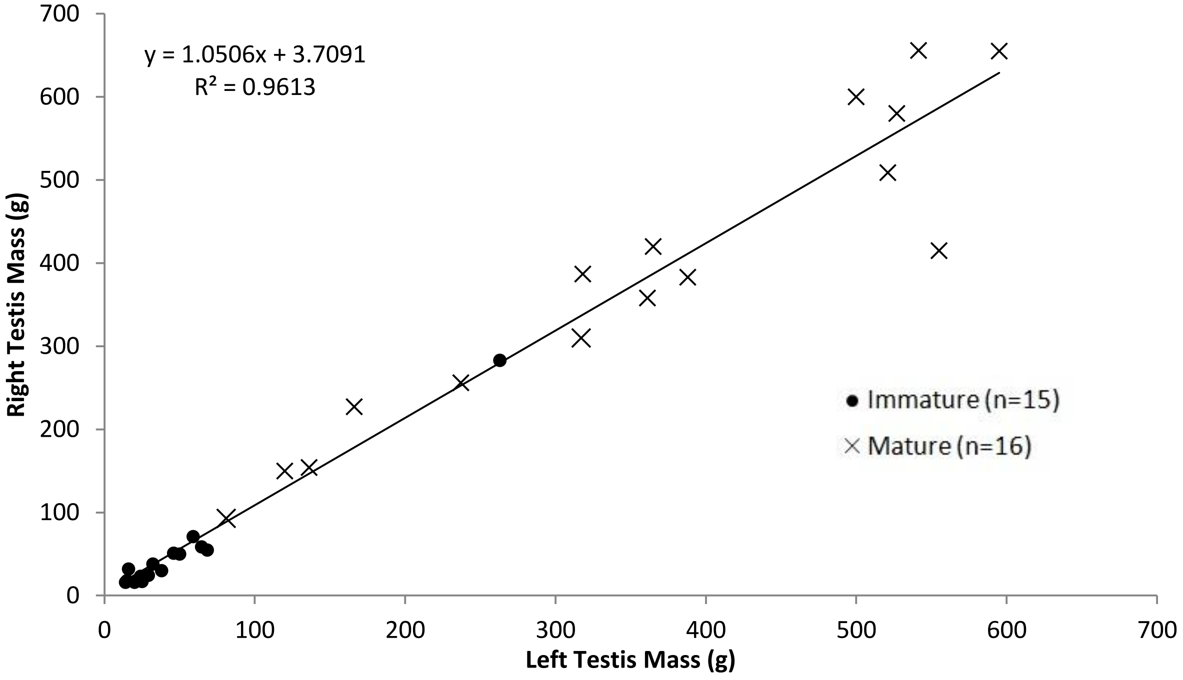


Figure 3.3: Correlation between the mass of the left and right testis in male *S. chinensis* from KwaZulu-Natal (n=31).

3.4.1.2 Females

Females were classified into one of three categories: immature, pubertal and mature. Of the 30 samples for females that were analysed, two samples were discarded due to poor preservation, 14 were immature, five pubertal and nine mature. The number of Graafian follicles in animals classified as pubertal ranged from one to 28. Females classified as mature showed evidence of at least one ovulatory event (i.e. presence of either a *CL* or *CA*) (Figure 3.5). Due to the small number of samples for mature females, and a general lack of associated information on the data sheets, especially for older individuals, mature females could not be divided into further categories, such as pregnant or resting. The number of *corpora* counted in the ovaries of mature females varied from one to five. Of the nine mature females, four were lactating at the time of death, but only one showed evidence of a *CL*. There were no pregnant females in the sample. The remaining three lactating females had only *CA*s present in their ovaries. The *CL*s recorded in the other three mature individuals were therefore probably at various stages of regression, thus no active *CL*s were recorded in any female in the present study.

Corpora scars were recorded on both ovaries in mature individuals, but more *corpora* were generally recorded on one of the ovaries. Due to the absence of metadata, no distinction could be made between left and right ovaries for the majority of samples assessed.

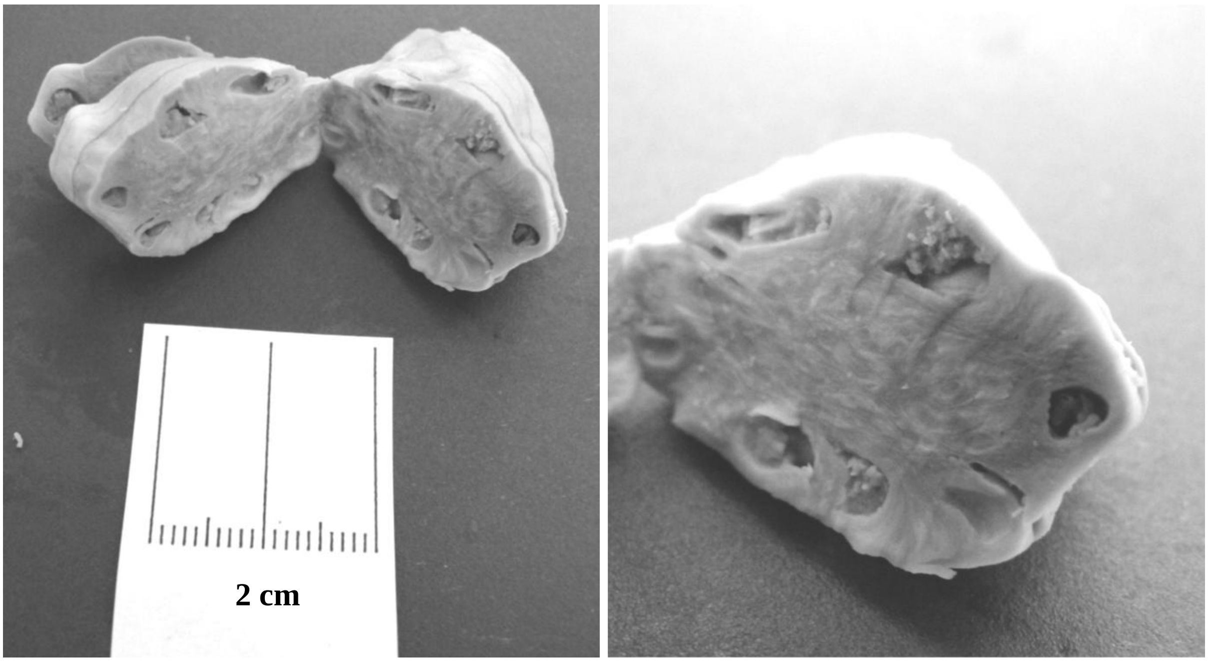


Figure 3.4: Graafian follicles as they appear in the ovarian tissue of a pubertal female.

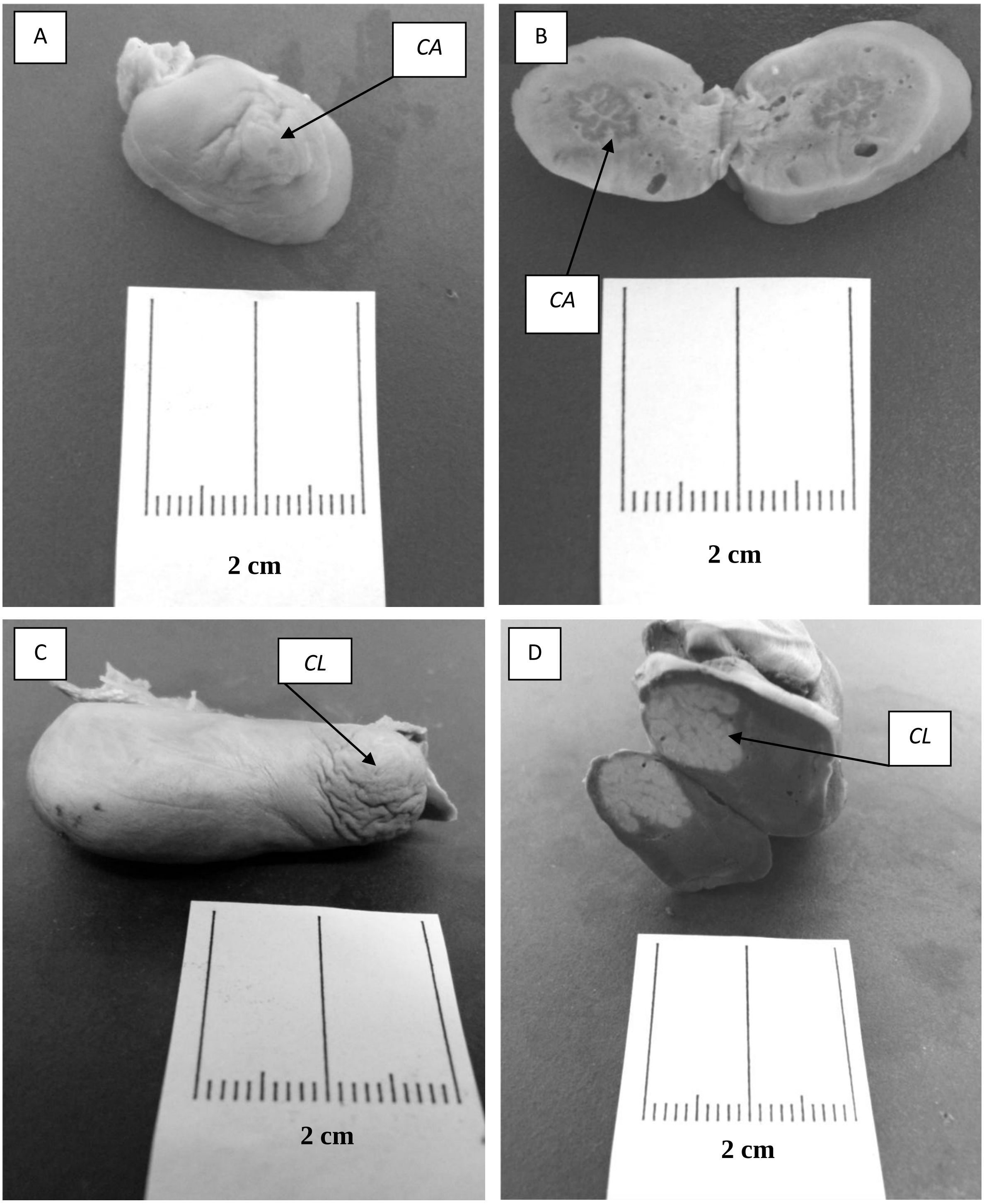


Figure 3.5: A *corpus albicans* as it appears on the surface of the ovary (A) and within the tissue (B). Figures (C) and (D) represent a *corpus luteum* as it appears on the surface of the ovary and within the tissue, respectively.

3.4.2 Attainment of sexual maturity

3.4.2.1 Males

Seminiferous tubule diameters of animals classified as immature ranged from 28.06 µm to 79.34 µm, whereas they ranged from 64.49 µm to 160.25 µm for the mature males. Mean seminiferous tubule size remained relatively constant, ranging between 28 µm and 60 µm for the immature individuals, but increased rapidly at approximately 220 cm TBL (Figure 3.6 (A)) and 9 - 10 GLGs (Figure 3.6 (B)), which probably signifies the onset of sexual maturity in male *S. chinensis*. The shortest sexually mature male measured 210.6 cm TBL and the youngest mature male was an estimated 7.5 years old.

A summary of the age, total body mass, total body length, combined testis mass (as recorded on the datasheets), mean testis length, seminiferous tubule diameter and the index of testis development for different maturity stages for male *S. chinensis* is presented in Table 3.1. The mean combined testis mass of immature individuals was 100.69 g (SD: 116.20 g); the mean testis length was 98.00 mm (SD: 13.44 mm). Those individuals classified as mature had a mean combined testis mass of 742.66 g (SD: 347.63 g) and a mean testis length of 235.88 mm (SD: 35.73 mm). The combined testis mass of *S. chinensis* was significantly positively correlated to the total body length ($R = 0.625$; $P < 0.05$), age ($R = 0.868$: $P < 0.05$) and mass ($R = 0.763$; $P < 0.05$). The combined testis mass remained relatively low between 30 - 60 g, until about 230 cm TBL (Figure 3.7 (A)), around 7.5 GLGs (Figure 3.7 (B)) and 100 kg (Figure 3.7 (C)). A noticeable increase in combined testis mass was observed at around 220 cm TBL, 140 kg and 9-10 GLGs. This corresponds to the age, length and mass at which a marked increase was recorded in the mean seminiferous tubule diameter (Figure 3.6), indicating the onset of sexual maturity.

The mean index of testis development was 0.43 for immature individuals and 2.2 for mature individuals. There was some overlap in testis mass between mature and immature individuals (Figure 3.7), which is normal in any population and evident in the present study, due to the small sample size.

Table 3.1: Summary of the age, size, and combined testis mass and seminiferous tubule diameter for different maturity stages of male *S. chinensis*. Maturity was determined based on the histological examination of testicular tissue. Only specimens for which the state of maturity could be determined are included.

	Immature		**Mature**	
Parameter	Range	*n*	Range	*n*
Estimated age (GLGs)	2 - 7.5	27	7.5 - 23.5	19
Total body mass (kg)	60 - 150	27	92 - 250	19
Total body length (cm)	167.4 - 241	27	210.6 - 269	19
Combined testis mass (g)	30 - 546	18	174 - 1250	16
Mean testis length (cm)	88.5 - 107.5	2	200 - 280	4
Mean seminiferous tubule diameter (µm)	28.06 - 79.34	20	64.49 - 160.25	10
Index of testis development (g/mm)	0.38 - 0.47	2	1.76 - 2.56	4

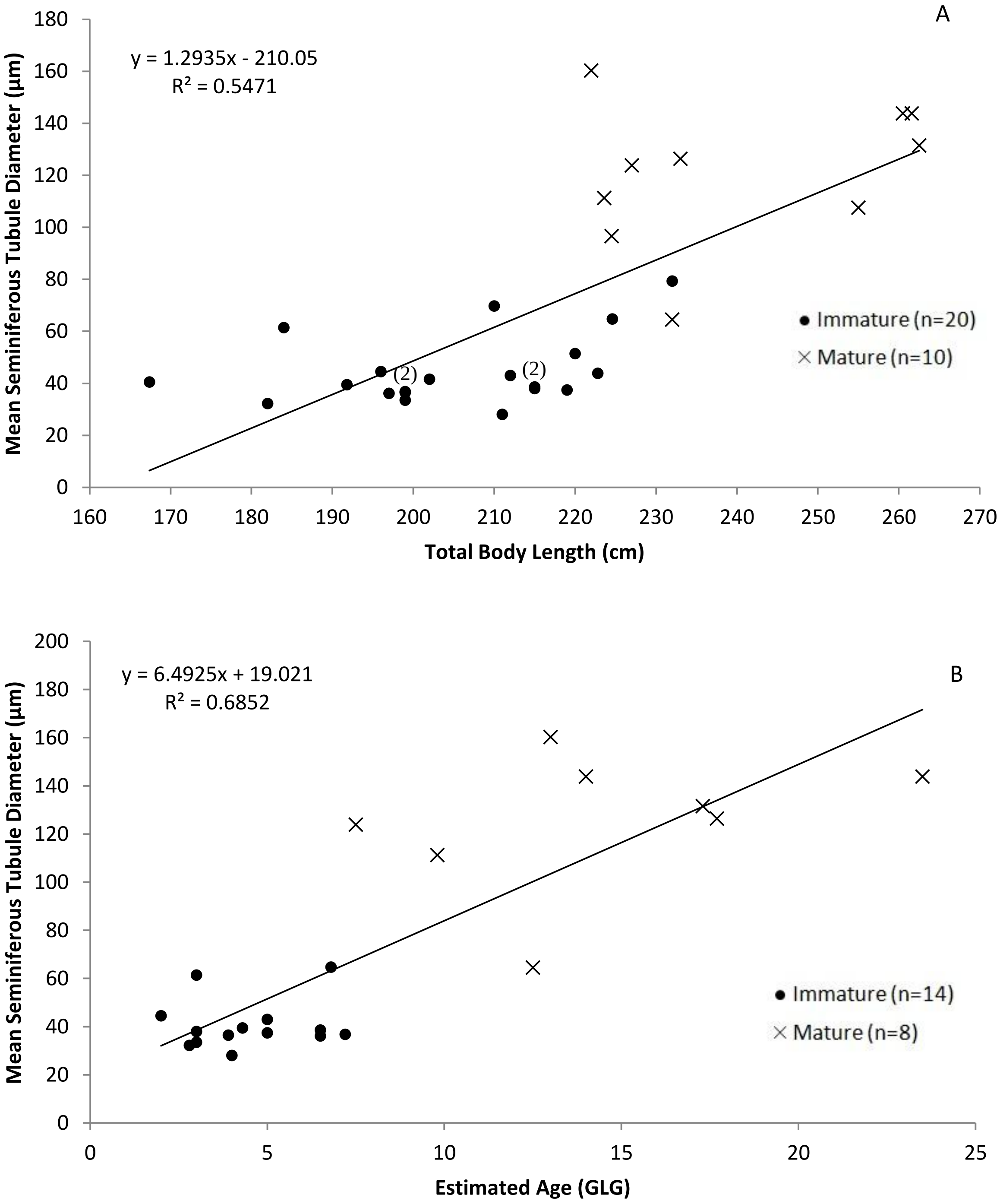


Figure 3.6: The mean seminiferous tubule diameter in relation to total body length (cm) (A) and age (GLGs) (B) of male *S. chinensis*.

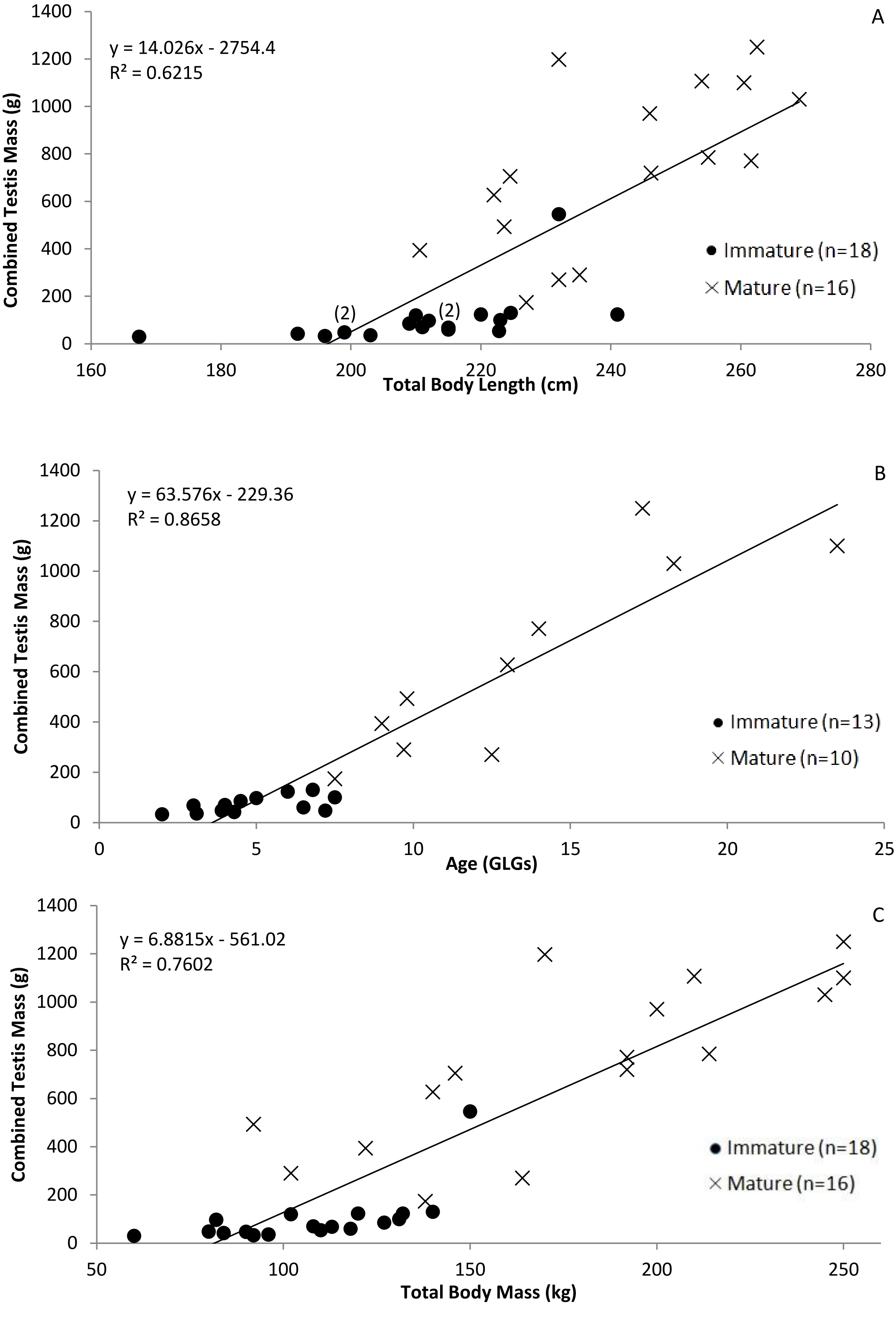


Figure 3.7: Increase in combined testis mass with body length (cm) (A), estimated age (GLG) (B) and mass (C) in male *S. chinensis*.

3.4.2.2 Females

The sample available for this study (n = 28) was too small to perform any statistical analysis for calculating the age and length at attainment of sexual maturity. Initial signs of first ovulation in pubertal individuals, characterised by the presence of enlarged Graafian follicles, were found in four females, ranging in TBL from 183.5 to 237 cm and masses between 79 and 150 kg. Age estimates were only available for three pubertal females, ranging from 5 to 8.7 GLG. The mean age at attainment of sexual maturity was calculated using the age of the youngest mature female, with one ovarian scar (8 GLGs) and the oldest immature female without any ovarian scars (7.2 GLGs) (Plön, 2004; Murphy, 2004). Using this approach, the attainment of sexual maturity in female *S. chinensis* occur around 7.6 GLGs (Figure 3.8 (A)). The heaviest and longest immature female weighed 114 kg and 222 cm TBL and the lightest and shortest mature female 140 kg and 220 cm TBL. The estimated length and mass at attainment of sexual maturity in *S. chinensis* females therefore ranges between 114 and 140 kg, and between 220 and 222 cm TBL (Figure 3.8 (B and C)). The age at first ovulation is estimated at between 5 and 8.7 GLGs.

Table 3.2: Summary of the age and size of female *S. chinensis* at different maturity stages, as determined by macroscopic examination of the ovaries.

	Immature		**Pubertal**		**Mature**	
Parameter	Range	*n*	Range	*n*	Range	*n*
Estimated age (GLGs)	1.5 - 7.2	11	5 - 8.7	3	8 - 17.3	4
Total body mass (kg)	47 - 114	14	79 - 150	5	140 - 176	8
Total body length (cm)	157 - 222	14	183.5 - 237	5	220 - 248	9
Combined ovary mass (g)	1 - 6	14	4 - 13	4	10 - 22	6

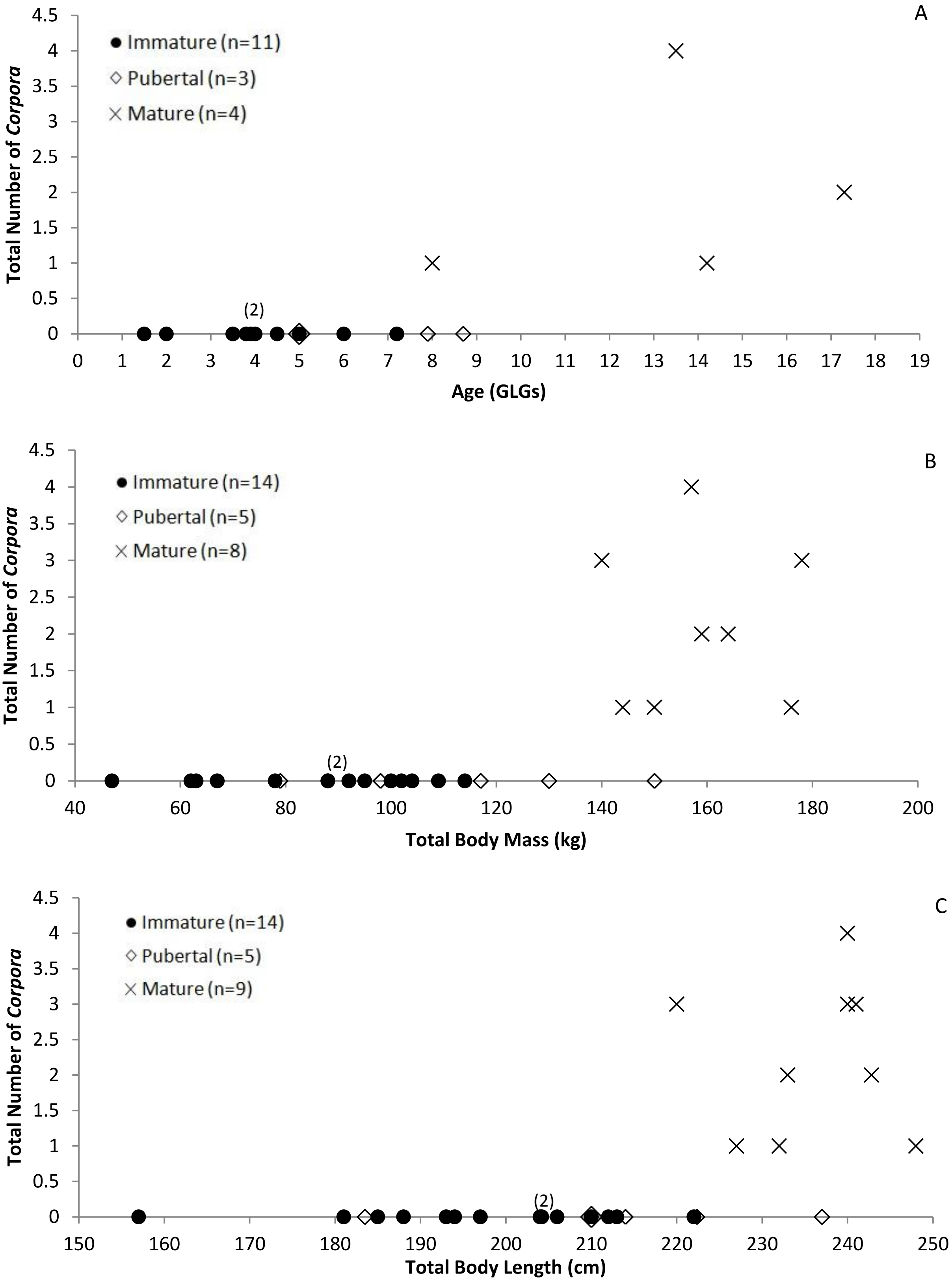


Figure 3.8: Total number of *corpora* scars present on both left and right ovaries against estimated age (GLGs) (A), body mass (kg) (B) and total body length (cm) (C) in female *S. chinensis*.

An increase in combined ovary mass was observed at a TBL of about 220 cm (Figure 3.9 (A)) and eight GLGs (Figure 3.9 (B)). However, the ovarian mass increase at the onset of sexual maturity was not as evident with age, as it was with TBL (Figure 3.9). The highest combined ovary mass in an immature female was around 6 g, at a TBL of 197 cm and 3.5 GLGs. The lowest combined ovary mass for a mature female was 10 g at 157 cm and 13.5 GLGs. This suggests that sexual maturity in female *S. chinensis* occurs between 6 g and 10 g of combined ovarian mass. Combined ovarian mass at the pubertal stage ranged between 4 and 13 g (Table 3.2).

The number of ovarian *corpora* (including both *CA*s and *CL*s) ranged from one to five in the sexually mature females. A regression of all mature females of estimated age against total number of *corpora* fitted described by the regression equation: $y = 0.15x - 0.30$, where y represents the total number of *corpora* and x the estimated age (GLGs), yielded an ovulation rate of 0.2 per year.

Insufficient data were available to make any inferences on the pregnancy rate, lactation period or resting period in *S. chinensis* in South African waters. The gestation period could also not be determined due to lack of data, as the statistical methods used to determine gestation period largely depend on foetal growth data (Perrin and Reilly, 1984), which were not available for the present study.

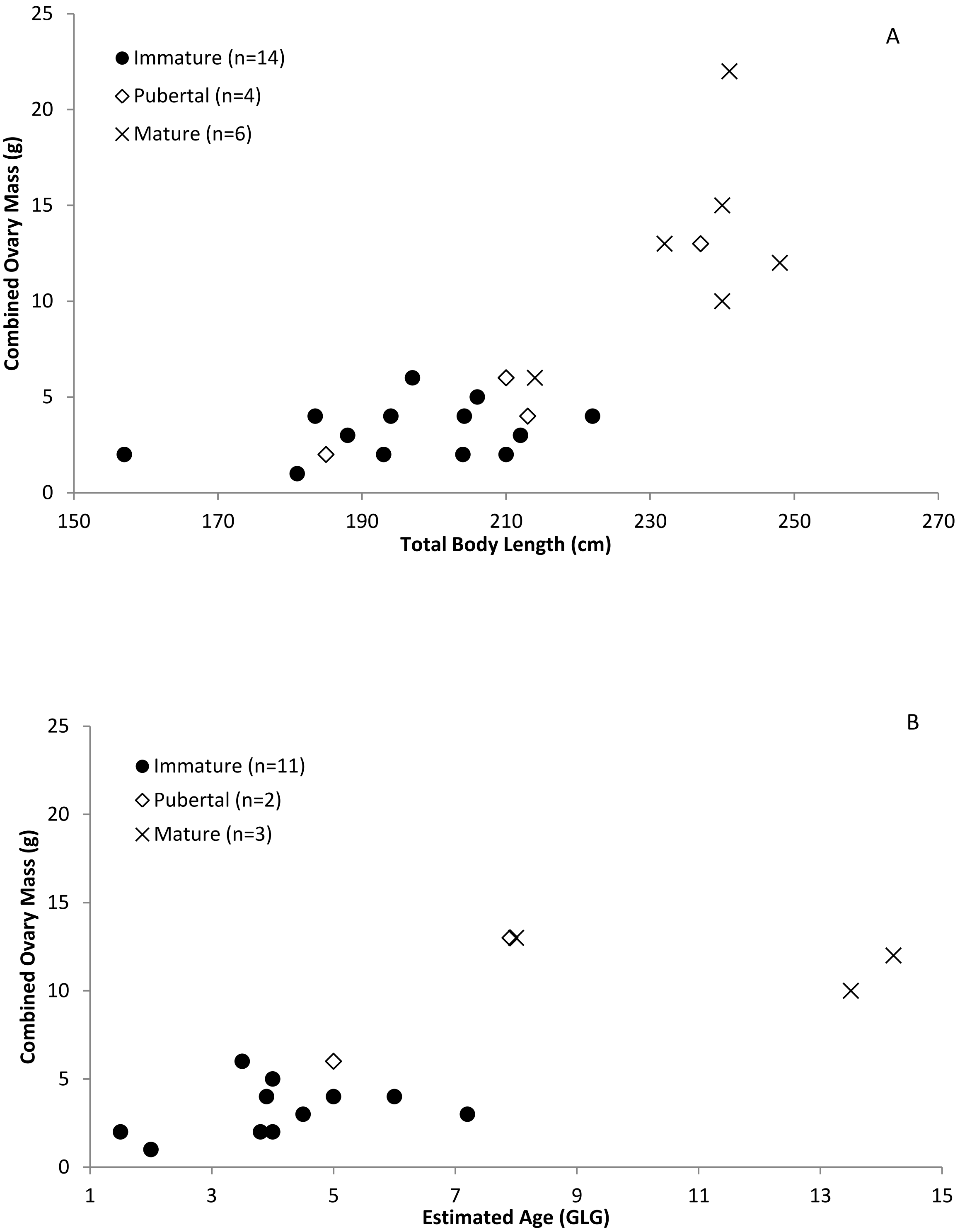


Figure 3.9: Increase of combined ovary mass with (A) body length and (B) estimated age in female *S. chinensis*.

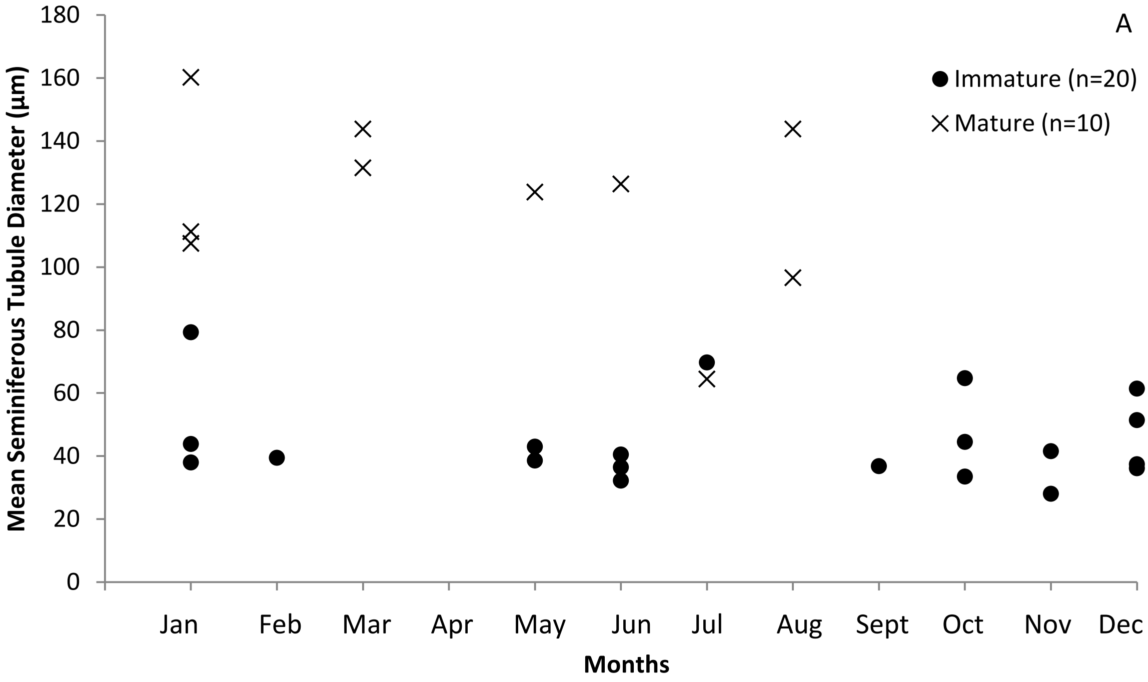
3.4.3 Reproductive seasonality

3.4.3.1 Males

Although there was an absence of seminiferous tubule diameters for mature males from September through December (Figure 3.10 (A)), due to the elimination of samples too degraded to yield accurate measurements, comparisons of testis mass (in mature males) suggest that there are no distinct seasonal changes (Figure 3.10 (B)). Furthermore, the presence of spermatozoa (i.e. individuals classified as mature) was recorded in all but three months of the year (June, November and December) (Figure 3.10 (B)). These differences are probably due to the small sample size, and are unlikely to be an indication of seasonal sperm production. Sperm production in male humpback dolphins found in KwaZulu-Natal coastal waters is therefore considered aseasonal.

3.4.3.2 Females

Results from the present study indicate no clear correlation between the corpus index and month of the year, and thus suggest that there is no seasonality in the reproduction of *S. chinensis* females (Figure 3.11). Based on data obtained from four individuals, the findings from the present study suggest that the formation of Graafian follicles is also aseasonal (Figure 3.12). Furthermore, sightings of mother-calf pairs were recorded in all but two months of the year (Figure 3.13; Atkins, 2013, pers. comm.). The absence of births in March and September could be due to the small sample size.



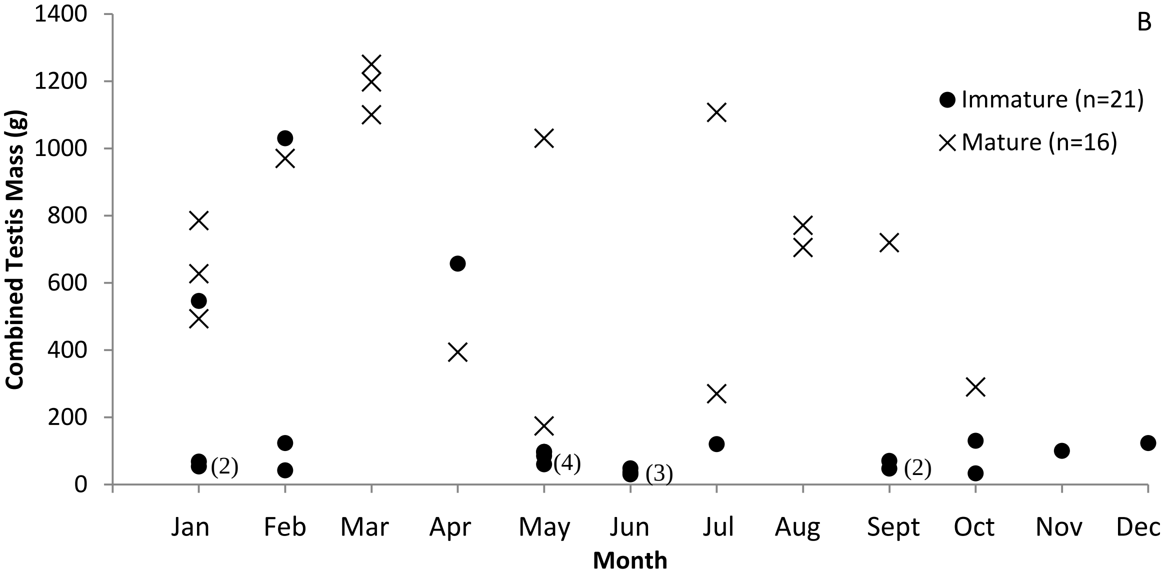


Figure 3.10: A monthly plot of seminiferous tubule diameter (A) and total testis mass (B) for the two different stages of male S. *chinensis* sexual maturity.

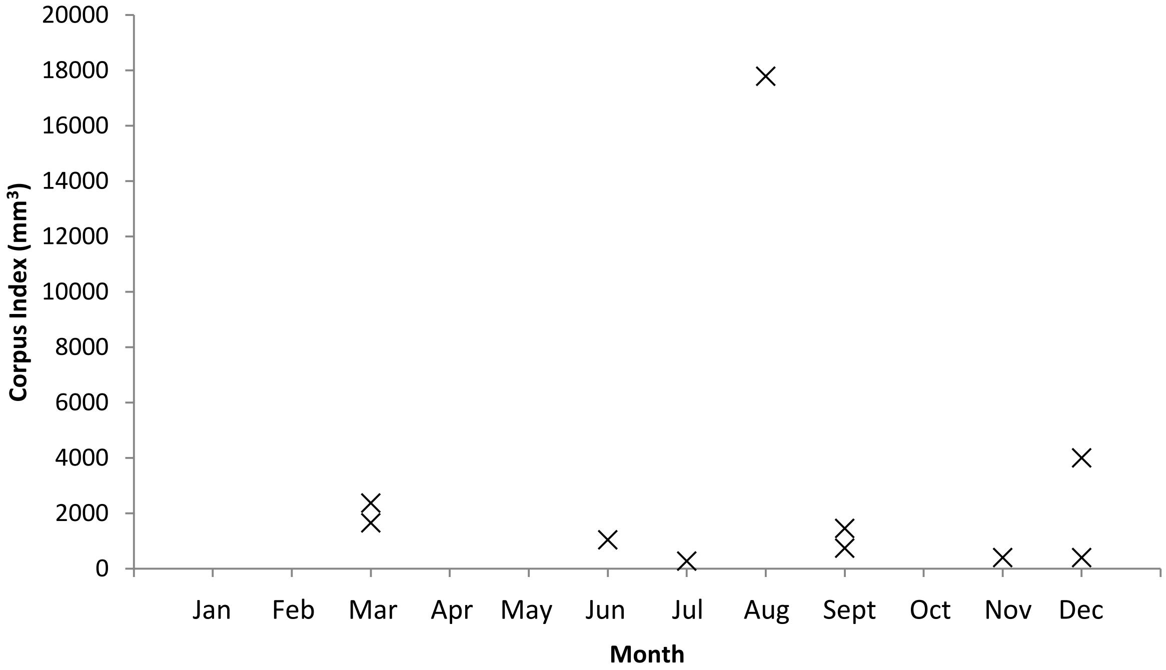


Figure 3.11: Corpus index (mm^3) of the largest *corpus* (*CL* or *CA*) in relation to month of the year in *S. chinensis* (n = 10).

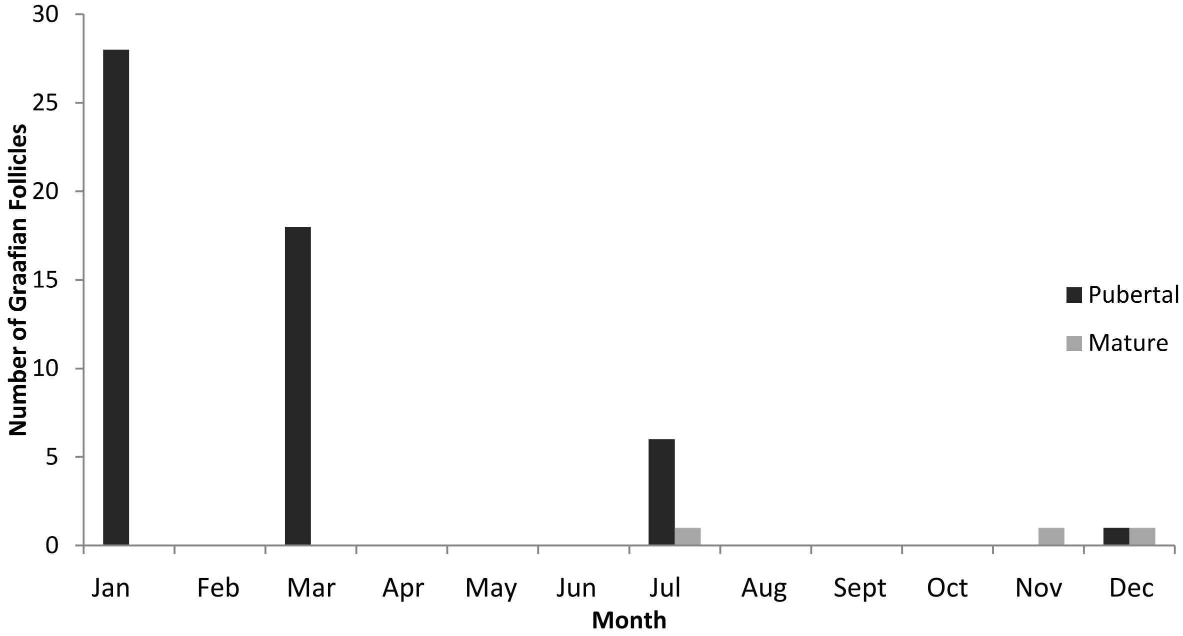


Figure 3.12: Number of Graafian follicles observed in the ovaries of seven *S. chinensis* females during different months of the year.

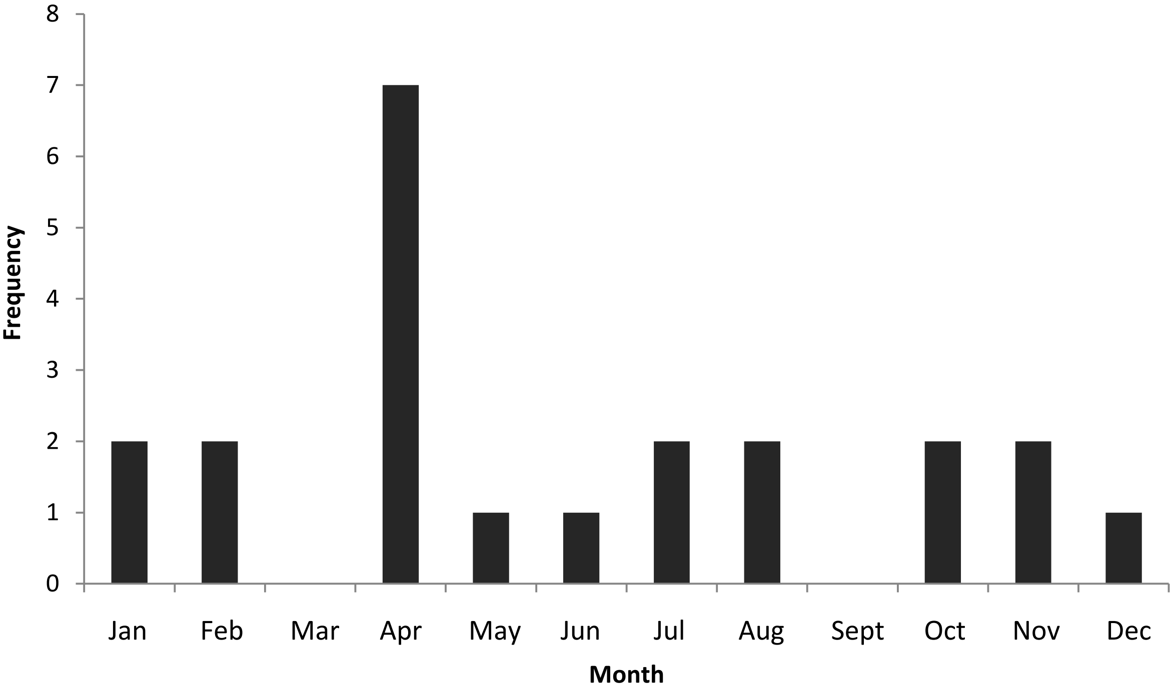


Figure 3.13: Occurrence of neonates in Richard's Bay, KwaZulu-Natal, observed between 1998 and 2005 (n=22) (Atkins, 2013, pers. comm.).

3.5 Discussion

3.5.1. Gonadal characteristics

In agreement with previous studies on other delphinids, there was no significant size difference between left and right testes in male *S. chinensis* ($P > 0.05$). This characteristic has been found in various other cetaceans, including *Tursiops aduncus* (Cockcroft and Ross, 1990) and *Delphinus capensis* (Mendolia, 1989).

Results from this study suggest that the onset of sexual maturity starts at a mean testis length of 235.9 mm and mass of 699.1 g. There are no previous records of testis mass and/or dimensions for male *S. chinensis* to allow for comparison. Mendolia (1989) found that the relationship between combined testis mass and the morphometric data of *D. capensis,* indicate no increase in testis size with body development until the attainment of sexual maturity, at which point a noticeable increase in testis size occurs. This was also the case in *S. chinensis*, together with a noticeable increase in combined testis mass at the onset of sexual maturity. However, it remains unknown at what age sexual development starts (i.e. pubertal stage) in male *S. chinensis*. Furthermore, at the onset of sexual maturity, there is an increase in size of seminiferous tubules as a result of the development of the seminiferous epithelium, accompanied by a subsequent decrease in interstitial tissue.

The mean index of testis development for *S. chinensis* was 0.43 g/mm for immature and 2.2 g/mm for mature males. These values could not be compared with those of Collet and Saint Girons (1984) or Hohn *et al*. (1985), as different methods for calculation were employed. However, fairly similar values were obtained in the present study to those of Plön (2004) for *Kogia breviceps* (immature: 0.33 g/mm; mature: 2.61) and for *K. sima* (immature: 0.41 g/mm; mature: 2.90 g/mm), using the same formula. The biological significance of these results is unknown.

3.5.2 Attainment of sexual maturity

3.5.2.1 Length

The attainment of sexual maturity often occurs at a shorter body length in females than in males of the same species (Laws, 1956; Perrin and Reilly, 1984). This was seen in the present study, with the onset of sexual maturity in male and female *S. chinensis* starting around 210 cm and between 220 and 222 cm TBL, respectively. According to Laws (1956), the length at attainment of sexual maturity is relatively constant in female cetaceans, occurring between 80.0 and 88.5% of asymptotic length, and 86% of maximum body size. Male *S. chinensis* in the present study reach sexual maturity at roughly 86% of asymptotic length (i.e. length at attainment of physical maturity) and 82 % of maximum body length, while females are a little longer at approximately 91.7% of asymptotic length and 88.8% maximum length**.** The estimate of length at attainment of sexual maturity as a percentage of asymptotic length for females was slightly higher than that proposed by Laws (1956), which was probably a result of the nature of the small sample size containing predominately immature individuals.

3.5.2.2 Age

The age at which dolphins generally attain sexual maturity differs among males and females, and it is not uncommon for females to attain sexual maturity before males (Laws, 1956; Perrin and Reilly, 1984; Cockcroft and Ross, 1990). Estimates of age at sexual maturity in the present study were made, based on the assumption that all counts of both dentinal and cemental GLGs give a precise and unbiased estimate of age for all individuals (Perrin and Reilly, 1984).

Results obtained from the current study estimate the age at onset of sexual maturity in male and female *S. chinensis*, between 9 - 10 GLGs and around 7.6 GLGs, respectively. These estimates are considerably lower than previous estimates for populations locally and elsewhere. Cockcroft (1989) estimated the age at attainment of sexual maturity for males occurring in the coastal waters of KwaZulu-Natal, between 12 and 13 GLGs and around 10 GLGs for females. Beyond this, the only other known estimate for the age at onset of sexual maturity for humpback dolphins is derived from populations inhabiting southern Chinese coastal waters (Jefferson, 2000; Jefferson *et al*., 2012). Jefferson *et al*. (2012) estimated the age at sexual maturity for male *S. chinensis* in the Pearl River Estuary at 12 - 14 years. Prior to this estimate, the only knowledge on the age at attainment of sexual maturity for *Sousa* in Chinese waters originated from two specimens, which merely suggested that males younger than 8.5 GLGs were still immature (Jefferson, 2000). Jefferson (2000) and Jefferson *et al*., (2012) found that females appear to reach sexual maturity before males, around 9 - 10 GLGs, which is in agreement with Cockcroft's (1989) preliminary estimate of 10 GLGs for females in KwaZulu-Natal coastal waters. Similarly, female bottlenose dolphins, *Tursiops aduncus* (Cockcroft and Ross, 1990), long-beaked common dolphins, *Delphinus capensis* (Mendolia, 1989) and striped dolphins, *Stenella coeruleoalba* (Kroese, 1993) in South African waters reportedly attain sexual maturity prior to males, with an age difference of two to three years between males and females. Disparity in this regard ensures more sexually mature females than males within a population (Cockcroft and Ross, 1990) and is usually prominent in animals with polygynous social systems (Evans, 1987).

Differences in the estimated age at sexual maturity between the findings of this study and that of Cockcroft (1989) could be due to a number of factors. The most probable explanation is the interpretation of growth layer groups in teeth, as there is some overlap in the samples employed in both studies, specifically samples collected between January 1980 and December 1988. However, Cockcroft (1989), unfortunately did not specify the interpretation of layers in his analysis, therefore results from his findings and that of the current study cannot be compared. Another possible explanation is that the 24- year period between the two successive studies has resulted in a reduction of the age at sexual maturity in the population. A change in reproductive parameters is a common phenomenon among mammals (Murphy *et al*., 2005). In humans, various intrinsic factors such as body weight, genetics, nutrition and contaminated foods has been found to influence the age at attainment of maturity (Murphy *et al*., 2005). Combined with the pressures of incidental exploitation, it may be that the population has experienced a decrease in the age at attainment of sexual maturity as a compensatory response. However, further analysis is required to confirm this.

Differences in age at sexual maturity between South African populations and Chinese populations are expected, as there is a marked difference in their body sizes, with South African animals reaching larger TBLs (Chapter 2). It has been noted in other cetaceans, such as the long-beaked common dolphin, *D. capensis*, that the age differences at sexual maturity between geographically isolated populations may be attributed to differences in body size, with populations attaining smaller TBLs reaching sexual maturity earlier (Mendolia, 1989). The inverse appears to be true in the case of *S. chinensis*, thus rendering this theory inept for explaining this difference. This strengthens the case for the difference in age at attainment of sexual maturity being due to exploitation. In the event of population abundance declines, compensatory responses do occur (Chivers and Myrick, 1993). For example, populations may experience an increase in pregnancy rates and a decrease in age of attainment of sexual maturity (Chivers and Myrick, 1993). According to Perrin and Reilly (1984), the age at sexual maturity is greater in less exploited *Stenella longirostris* populations in the Eastern Tropical Pacific. Moreover, the population size estimates between the different regions are substantial, with southern China accommodating more than 2500 humpback dolphins (Jefferson *et al*., 2012), while in Richard's Bay, where humpback dolphins are expected to be the most dominant (KwaZulu-Natal), the population size is estimated between 170 - 244 individuals (Atkins and Atkins, 2002); thereby theoretically making humpback dolphins in South African waters more susceptible to changes in reproductive parameters.

Estimates of age at sexual maturity in males in this study, nevertheless, are close to the estimates for age at maturation for other species of dolphins, such as *Stenella longirostris,* 8.5 to 11.5 years (Perrin *et al.*, 1977), *S. coeruleoalba*: 9 years (Kasuya, 1976), *S. attenuata*:

11.8 years (Kasuya, 1976) and *Delphinus delphis*: 8 - 13 years (Murphy *et al*., 2005). The age at onset of sexual maturity in females is similar to that of *D. delphis*, which ranges between 7.8 and 8.9 GLGs, depending on the geographical location (Danil and Chivers, 2007; Dabin *et al*., 2008).

Based on the nature of the sample, it cannot be said with certainty that the sample is representative of the population (Perrin and Reilly, 1984), as mature males and females were underrepresented. It should therefore be noted that the estimated age and length at attainment of sexual maturity in the present study may be biased and not a true reflection of these parameters. As a consequence, results from this study should serve as a guideline until more samples can be collected and further research conducted.

3.5.3 Ovulation rate

The annual ovulation rate of female *S. chinensis* in South African waters was estimated at 0.2, which suggests a calving interval of around five years. The ovulation rate of *S. chinensis* was previously estimated at 0.3 by Cockcroft (1989), and a calving interval of 3.3 years. Jefferson *et al*. (2012) estimated the mean calving interval for Chinese populations at about 5.2 years. However, they suggested that an estimated of 2-3 years was likely to be closer to the reality, and attributed the seemingly high estimate to low natural survival rates as a result of contamination, scarcity of nutritious prey resources, and an increased amount of stress from anthropogenic activities (Jefferson *et al*., 2012). The estimated ovulation rate for females in the present study is based on limited data, so it may well be true that females have a shorter calving interval, closer to that predicted by Cockcroft (1989). However, no comment can be made in this regard as further analysis is required. Furthermore, no valid deductions can be made regarding the trend in the number of ovulations in relation to age or age-specific ovulation rates (Plön, 2004).

Reproductive senescence in *S. chinensis* could not be addressed in the current study due to the unavailability of mature males and females. Based on the results, however, there was no evidence of reproductive senescence in either sex. In the case of male dolphins, combined testis mass did not decrease with age. For females, none of the individuals classified as mature showed any evidence of being senescent, which was based on the criteria described by Perrin *et al*. (1977). However, male and female dolphins are very probably capable of exceeding 24 and 17.8 years respectively (Chapter 3), as suggested by Cockcroft (1989), who estimated longevity at an excess of 40 years. Thus the possibility of senescence should not be disregarded.

3.5.4 Reproductive seasonality in *S. chinensis*

The year-round presence of spermatids recorded in seminiferous tubules of mature male *S. chinensis* suggests aseasonal reproduction, with spermatids and spermatozoa observed in seminiferous tubules in nine months of the year (no sexually mature males were present in the sample in June, November and December). These results agree with those of Reddy (1996) for *S. chinensis* in KwaZulu-Natal coastal waters. It should be noted however that her results were also based on findings obtained from a small sample, consisting of only 17 individuals.

Furthermore, results from the present study indicate no distinct seasonal cycle for an increase in testis mass or seminiferous tubule diameter in mature males. Although no such studies have been conducted on humpback dolphins in southern Chinese waters, the occurrence of year-round births would suggest aseasonal spermatogenesis (Jefferson *et al.*, 2012).

In the case of the females, Graafian follicles were recorded in only three of the 10 mature individuals and in four pubertal females. In agreement with the findings of Reddy (1996), numerous Graafian follicles, with up to 28 recorded in a single individual in the present study, were recorded in five separate months of the year: January, March, July, November and December. In Reddy's (1996) analysis, Graafian follicles were recorded in all but three months of the year (May, June and November). Given that Graafian follicles can be used as a proxy for ovulation (Reddy, 1996), this suggests that ovulation in *S. chinensis* is aseasonal. Similarly, Brook *et al*. (2004) and Jefferson *et al*. (2012) recorded no distinct or strong seasonality in the ovarian activity of captive humpback dolphins and southern Chinese populations, respectively.

Aseasonal breeding in tropical dolphins is common, and more than one calving peak per year may manifest, with parturition occurring throughout the year (Barlow, 1984). Neonate sightings recorded between 1998 and 2005 in KwaZulu-Natal, suggest that although births reportedly occur year-round, there appears to be an austral autumn peak, with the majority of neonates recorded in April (Atkins, 2013, pers. comm.). Brook *et al*. (2004) suggest that despite the year-round ovarian activity in captive *S. chinensis,* ovulations and conception predominantly occur during the winter, such that births occur during the early spring/summer months. This accords with observations previously made in southern Chinese coastal waters (Jefferson, 2000; Jefferson *et al*., 2012), as well as earlier studies on *S. chinensis* in South African waters, which reported a calving peak in late spring to early summer in Plettenberg Bay (Saayman and Tayler, 1979), KwaZulu-Natal (Cockcroft, 1989), and Algoa Bay (Karczmarski, 1999). For most delphinids, reproductive seasonality seems to be closely linked to environmental factors, such as water temperature and prey availability, and becomes increasingly pronounced as the seasonal differences in these factors increase (Karczmarski, 1999).

3.5.5 Mating system

Testis mass to body mass ratio, sexual dimorphism and group size are the three major factors used to define the mating strategy in a species (Plön and Bernard, 2007). Cockcroft (1993) previously reported that combined testis mass constitutes 0.7% of total body mass in *S. chinensis* incidentally caught in shark nets in KwaZulu-Natal coastal waters. In a more recent study by Plön *et al*. (2012), it was found that testis mass for *S. chinensis* contributes an average of 0.43% to the total body mass. Likewise, in the present study, an average of 0.42% of total body mass was calculated for mature animals. Based on the review published by Plön and Bernard (2007), small testes in relation to body mass indicate a monogamous, or extreme polygamous mating system. Therefore small testis size, in association with sexual dimorphism and small group size reported in *S. chinensis* agrees with the findings of Cockcroft (1993) and the roving male mating strategy proposed by Plön and Bernard (2007). This involves males actively searching for receptive females to maximise their reproductive opportunities, rather than monopolising and fighting over a number of females (i.e. a harem) (Plön and Bernard 2007). Small group size in *S. chinensis* thus allows for larger males to dominate smaller males and subsequently deny them access to females. Durham (1994) found that females in northern KwaZulu-Natal waters form relatively stable, resident groups, while males tend not to be resident, and move between several female groups. Similarly, Karczmarski (1999) investigated group dynamics in humpback dolphins in Algoa Bay and found groups to be somewhat more dynamic than previously suggested by Durham (1994), with only short-term affiliations between any given group of individuals. Karczmarski (1999) hypothesised that small group size (<13 animals per group, with few females available at any given time) along with a fluid social pattern, probably favours a mating strategy in which males actively search for sexually active females , as proposed in the present study.

Chapter 4

General Discussion

4.1 Introduction

The occurrence and frequency of incidental capture and subsequent death of humpback dolphins in shark nets along the KwaZulu-Natal coast has given rise to the question of the conservation status of the population(s). Special attention is needed to assess the impact of shark nets on resident populations, so that pressures can be alleviated where possible (Jefferson *et al.*, 2012). The combined characteristics of coastal distribution, small population size, and strong site fidelity render humpback dolphins particularly vulnerable to local extinction (Frere *et al.*, 2008). However, before any management strategies can be implemented, a thorough understanding of the basic biology of a species is required (Parra and Ross, 2009).

The aim of the present study was to elucidate the basic biological parameters for humpback dolphins in KwaZulu-Natal waters, with the main focus on age, growth and reproduction. Globally, humpback dolphins are not well studied, and information on the biology of the species is very limited (Cockcroft, 1989; Jefferson, 2000; Jefferson *et al.*, 2012). The results obtained from the present study will therefore contribute to our knowledge on humpback dolphin biology and a better understanding of their life history strategies. This information is necessary for the successful implementation of conservation and management plans to ensure their continued survival in South African waters.

4.2 Limitations of the present study

4.2.1 Biased sample

A concern when analysing data originating from shark-net captures is whether the sample is representative of the entire population (Cockcroft, 1990). Although the presence of predominantly immature animals in the present study may result in a skewed/biased sample, it indeed suggests that shark nets installed off the coast of South Africa are largely responsible for the removal of predominantly male juveniles and sub-adults. The underrepresentation of calves and older individuals in the by-catch may be a result of segregation within the population by age and sex, or differences in feeding habits within a population, which is generally fairly typical in large-mammal populations (Perrin and Reilly, 1984; Chivers and Myrick, 1993). Nevertheless, this could also be representative of the normal population demographic of resident humpback dolphins in the coastal waters of KwaZulu-Natal. Karczmarski (1999) reported segregation by age/sex in humpback dolphins in the Eastern Cape. Likewise, Atkins *et al*. (2013) believes this to be the reason for the higher catch rate of adolescent males in shark nets off KwaZulu-Natal. According to Cockcroft (1990), data on bycatch in shark nets along the KwaZulu-Natal coast between 1980 and 1988 suggest that young humpback dolphins and lactating females do not frequent the inshore surf zone. However, according to Atkins *et al*. (2004), all humpback dolphins in Richard's Bay predominantly feed in the Harbour Mouth region, roughly 500 m offshore (within approximately the same area in which shark nets are installed) and were rarely recorded feeding further offshore. Their diet chiefly consists of inshore estuarine, littoral and reef-associated species (Saayman and Tayler, 1979; Ross *et* al., 1994; Jefferson and Karczmarski, 2001; Parra and Ross, 2009), which suggests that feeding close to the shore, in or near netted areas, is expected to occur at least during some parts of the day, making the animals vulnerable to the possibility of incidental captures. Thus feeding behaviour in Richard's Bay gives the impression that catch rates are indeed representative of the population structure. However, based on the findings of Atkins *et al*. (2013), the higher catch rate of adolescent males in shark nets is unlikely to be representative of the age/sex structure of the resident population. Whether or not this is in fact the case, requires further attention.

4.2.2 Collection material

The advantages of conducting biological studies using collection material far outweigh the disadvantages thereof, as data and samples are representative of a longer time. Furthermore, samples needed for biological studies are often difficult to obtain due to various ethical limitations. However, there are inherent problems working with collection material. Although measurements and certain collection protocols have been standardised, various people are responsible for taking measurements and collecting samples, which may present some irregularities and inconsistencies, as was found in the present study.

4.3 Summary of results

4.3.1 Age and growth

Teeth from 109 humpback dolphins (71 males and 38 females) originating from animals incidentally caught in shark nets along the KwaZulu-Natal coast were used for age estimation in the present study. A good correlation exists between cemental and dentinal age estimates for *S. chinensis*, which suggests that either structure could be used for age estimation. Accessory layers were most evident in the first two to three GLGs, and became less evident with successive GLGs. The pulp cavity in *S. chinensis* became occluded at around 15 GLGs.

Length at birth was estimated using Scott's equation (1949), and was found to be around 111 cm for males and 104 cm for females, which corresponds to approximately 42% and 43% of the asymptotic length of males and females respectively. Furthermore, mass at birth was estimated at 50 kg and 30 kg for males and females respectively. The shortest male in the sample was 148 cm and the shortest female, 150 cm TBL. The various other growth parameters were obtained from a Gompertz Growth function. The growth rate constant was estimated at 0.133 for males and 0.270 for females, and the asymptotic length at 266.7 cm and 240.96 cm respectively. Unfortunately, data in the present study did not allow for the asymptotic mass of males to be calculated, as the mass-at-age growth curve did not reach an asymptote. For females, however, the asymptotic mass was estimated at 160 kg. The age at attainment of physical maturity was estimated at 24 GLGs for males and 16 GLGs for females. It should be noted that the underrepresentation of various age classes in the present study may have affected the estimation of the various parameters and should thus be viewed with caution.

4.3.2 Sexual dimorphism

Based on length-at-age and mass-at-age data obtained from the present study, humpback dolphins display clear sexual dimorphism. Females tend to have a faster growth rate than males early in their ontogeny, until about 10 GLGs, 230 cm TBL and 140 kg, at which point males start outgrowing females.

Cockcroft (1989) also suggests clear sexual dimorphism in *S. chinensis* in coastal KwaZulu-Natal waters; however, it is unclear how he came to this conclusion. Weston (2011), on the other hand, working on the same population, recorded no sexual dimorphism based on the allometric relationship of various body measurements. However, she examined only 11 external morphometric measurements from a possible 25 in her study (Weston, 2011). Further work is therefore required to elucidate this aspect. However, based on the results obtained from the present study, sexual dimorphism was assumed for *S. chinensis* in KwaZulu-Natal waters.

4.3.3 Reproduction

The reproductive status of 46 males was successfully determined in the present study. The poor preservation of testicular tissue did not allow for a distinction to be made between the different reproductive stages of sexual development in males. The distinction between immature and mature was based on the absence or presence of sperm in seminiferous tubules, the mean seminiferous tubule diameter and combined testis mass.

The histology of testes for immature and mature males was described, and histological and morphological parameters were determined. Combined testis mass in males increased rapidly at the onset of sexual maturity. The attainment of sexual maturity in males occurred between 9 and 10 GLGs, 210 - 220 cm and 140 kg. No seasonal cycle was recorded in the combined testis mass, presence of sperm, or mean seminiferous tubule diameter in mature males. These results suggest aseasonal testicular activity. The maximum combined testis mass made up 0.42% of body mass, and a roving male mating strategy has been proposed for *S. chinensis* (Plön and Bernard, 2007). This is characteristic of species with small testes, displaying clear sexual dimorphism, and forming small social groups, and is in accordance with previous findings for *S. chinensis* (Plön and Bernard, 2007).

A total of 28 females were successfully classed as immature, pubertal or mature. Puberty was determined by the presence of at least one Graafian follicle in either left or right ovary, while females classified as mature showed evidence of at least one ovulatory event (either in the form of a c*orpus luteum* or *corpus albicans*). A lack of either *corpora* or Graafian follicles was characteristic of immaturity in females.

Females commonly ovulated from both ovaries. Unfortunately due to a lack of metadata, ovulatory prevalence in either the left or right ovary could not be determined. The onset of sexual maturity occurred around 7.6 GLGs, between 220 and 222 cm and 104 - 140 kg. The ovulation rate was estimated at 0.2 ovulations per annum, indicating a calving interval of five years. Females appeared to display no reproductive seasonality. Furthermore, Atkins (2013, pers. comm.), recorded sightings of young calves in almost every month of the year (except March, November and December) over a seven-year period, the majority of which were recorded in April.

Insufficient data were available in the present study to make any comment on the pregnancy rate, lactation period, resting period or the possibility of reproductive senescence in *S. chinensis*.

4.4 Variation to the life history strategies of *S. chinensis*

4.4.1 Intra-specific variation

4.4.1.1 Within the same population

The discrepancy between the life history parameters estimated in the present study and those reported by Cockcroft (1989) is evident throughout the thesis. The reasons for these differences are difficult to address, however, as Cockcroft's (1989) findings are only discussed preliminarily in the form of an abstract.

The estimated length at birth in the present study was slightly larger than Cockcroft's (1989) estimate of 100 cm (Table 4.2). The reason for this difference is probably due to the use of Scott's equation (1949) for estimating the length at birth in the present study. The estimated age at attainment of sexual maturity found in the present study was three years younger than that proposed by Cockcroft (1989) (♂: 12-13 GLGs; ♀: 10 GLGs) for both males and females (Table 4.2). In an attempt to elucidate this disparity, I compared the data for samples employed in the present study, collected between January 1980 and December 1988, to Cockcroft's (1989) results (Table 4.1). Given that both studies employed samples from the same collection (Graham Ross Marine Mammal Collection, Port Elizabeth Museum), results for the same period (1980-1988) are expected to be similar. However, this appeared not to be the case. There was also a marked difference in the sample size, with Cockcroft (1989) having almost twice as many samples (n = 67). In the present study, only samples for 45 individuals were accounted for in the collection. Of the 45 animals making up the sub-sample, teeth were available for only 43 individuals (29 males and 14 females) in the collection, of which 36 (26 males and 10 females) were processed for the purpose of this study. A total of 22 reproductive organs (15 testes and 7 ovaries) were available for that period, however five (4 male and 1 female) had to be discarded from the sample due to poor quality/preservation (Chapter 3). Subsequently, both teeth and reproductive organs were available for only 15 (11 male and 4 female) individuals in this subset, and were thus the only samples that could effectively be used to determine the age at attainment of sexual maturity for dolphins caught between January 1980 and December 1988 (Table 4.1).

The onset of sexual maturity in males, as estimated from this sub-sample, is between 17.3 and 23.5 GLGs, compared to 12 - 13 GLGs estimated by Cockcroft (1989). Females, on the other hand, are estimated to reach sexual maturity at around 17.7 GLGs, which is 7.7 years older than Cockcroft (1989) previously estimated. This variance is unlikely to be due to a difference in the interpretation of GLGs between the two successive studies. When considering the difference in the estimated age at attainment of sexual maturity, as calculated from the sub-sample and the whole sample in the present study, it seems more likely that no recently matured animals were caught during that period. This would appear to indicate that the majority of animals caught between 1980 - 1988 were larger/older than those caught in subsequent years, resulting in demographic change in the population structure over time. However, this is not the case (Figure 4.1).

Thus the most probable explanation for this disparity is the difference in the size of the sub-sample in the present study (n = 36) from that used by Cockcroft (1989) (n = 67), which suggests he had additional samples available. It could be that Cockcroft (1989) included samples collected from stranded animals, or ones that are not accessioned into the collection held at the Port Elizabeth Museum, which possibly included mature animals. This would also explain the difference in the estimates of longevity between the present study and that of Cockcroft (1989), who reported humpback dolphins to live up to 40 GLGs, compared to only 24 GLGs in the present study (Table 4.2).

Alternatively, there is also the prospect of a change in the age at attainment of sexual maturity due to the effects of exploitation. It has been shown that compensatory response, such as variation in the age at attainment of sexual maturity, can occur in response to exploitation (Perrin and Reilly, 1984; Chivers and Myrick, 1993). This phenomenon has been reported in spinner dolphins, *Stenella longirostris* in the Eastern Tropical Pacific (Perrin and Reilly, 1984). However, it may also be possible that there was a difference in the interpretation of GLGs, which would have resulted in differences in the estimated ages of the same specimens.

Table 4.1: A comparison of the sample used (January 1980 - December 1988) for determining the age at attainment of sexual maturity in the present study and that of Cockcroft (1989).

			Present study	**Cockcroft (1989)**
Total sample size between January 1980 - December 1988			36	67
Number of individuals for which reproductive organs and teeth were available	♂	Immature	8	unknown
		Mature	3	
	♀	Immature	3	
		Mature	1	
Age range (GLGs)	♂	Immature	2.8 - 6.5	Unknown
		Mature	17.3 - 23.5	12 - 13
	♀	Immature	2 - 4.5	Unknown
		Mature	17.7	10

Lastly there was also a discrepancy in the estimated ovulation rate between the two studies. Based on the results from the present study, females ovulate less frequently and as a result have a higher calving interval. As with the various aforementioned parameter estimates, this is probably due to the sampling bias and the availability of data, or the result of a difference in the interpretation of GLGs.

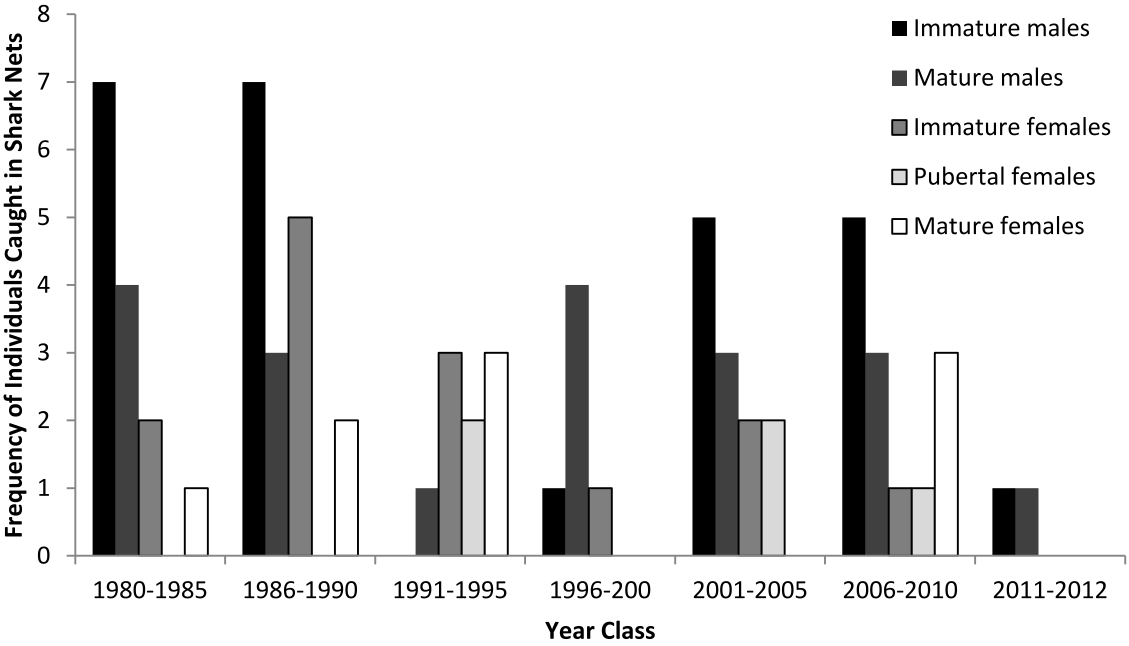


Figure 4.1: The frequency of humpback dolphins caught in shark nets between 1980 and 2012.

4.4.1.2 Geographical variation

Table 4.2 presents a summary of the data from studies conducted on *S. chinensis* in southern China (Hong Kong (Jefferson, 2000)), the Pearl River Estuary (Jefferson *et al*., 2012), and South Africa (KwaZulu-Natal (Cockcroft, 1989; and present study)).

Various life history parameters in cetaceans can vary within species, and appear to depend largely on environmental conditions (Laws, 1956). As indicated by results obtained in the present study, humpback dolphins in South Africa differ from humpback dolphins in southern Chinese waters in a number of ways. Firstly, there is a difference in the overall body size between the different populations, with South African males reaching maximum body lengths of 279 cm and females 249 cm. Southern Chinese populations are slightly smaller, attaining maximum lengths of between 260 and 265 cm (Jefferson, 2000; Jefferson *et al*., 2012). Furthermore, South African populations display clear sexual dimorphism (present study; Cockcroft, 1989), with males attaining greater lengths and mass than females. In contrast, humpback dolphins in southern China do not appear to be sexually dimorphic (Jefferson, 2000; Jefferson *et al*., 2012). The age and length at attainment of physical maturity also differs geographically, with males in southern Chinese populations becoming physically mature earlier than males found in KwaZulu-Natal coastal waters. In the Pearl River Estuary, physical maturity occurs between 14 and 17 GLGs and 249 cm TBL (Jefferson *et al*., 2012), while in KwaZulu-Natal waters, males only reach physical maturity at around 24 GLGs and 266.7 cm TBL. Female humpback dolphins in KwaZulu-Natal waters, however, appear to become physically mature at roughly the same age and length (16 GLGs and 241.96 cm TBL) as their Asian counterparts.

Furthermore, there is also a difference in age at attainment of sexual maturity, with KwaZulu-Natal populations maturing around three years earlier than southern Chinese humpback dolphins. In KwaZulu-Natal waters, sexual maturity in males occurs between 9 and 10 GLGs, and around 7.6 GLGs in females. Cockcroft (1989) previously estimated the age at attainment of sexual maturity between 12 and 13 GLGs in males and 10 GLGs for females, which is very similar to the estimates for humpback dolphins in the Pearl River

Estuary, of between 12 and 14 GLGs and 9 - 10 GLGs for males and females, respectively (Jefferson, 2000; Jefferson *et al*., 2012) (Table 4.2).

The calving interval for South African humpback dolphins is in agreement with a 5.2-year interval estimated for humpback dolphins in the Pearl River Estuary (Jefferson *et al*. 2012). However, the ovulation rate obtained in the present study may perhaps be underestimated, as mature females were largely under-represented in the sample. Although year-round breeding has been reported in both KwaZulu-Natal and southern China (Jefferson, 2000; Jefferson *et al*., 2012), there is evidence of apparent calving peaks in Hong Kong as well as the Pearl River Estuary and KwaZulu-Natal. Jefferson (2000) initially recorded a late boreal winter to summer calving peak (between January and August) for the Hong Kong population (Jefferson, 2000). However, in a later study conducted in the Pearl River Estuary, Jefferson *et al*. (2012) recorded a boreal spring to early summer calving peak (between March and June). Based on the observations made by Atkins (2013, pers. comm.), the KwaZulu-Natal population shows an early austral autumn (April) peak in births, while Cockcroft (1989) recorded an austral summer peak.

Table 4.2: Geographical variation in growth and reproductive parameters of three different populations of *S. chinensis.*

		*** South Africa (KwaZulu-Natal)**	**South Africa (KwaZulu-Natal)**	*** China (Pearl River Estuary)**	*** China (Hong Kong)**
Parameter		Present study	Cockcroft, 1989	Jefferson *et al.*, 2012	Jefferson, 2000
Length at birth (cm)	♂	111	100	101	100
	♀	104			
Growth Rate	♂	0.119	-	-	-
	♀	0.292	-	-	-
Age at sexual maturity (GLGs)	♂	9-10	12-13	12-14	-
	♀	7.6	10	9-10	9-10
Length at attainment of sexual maturity (cm)	♂	230	-	-	-
	♀	220-222	-	-	235
Asymptotic length (cm)	♂	266.7	270	249	243
	♀	240.96	240		
Asymptotic mass (kg)	♂	-	260	-	-
	♀	160	170	-	-
Age at physical maturity	♂	24	-	14-17	16
	♀	16	-		
Maximum age	♂	> 24	> 40	38+	-
	♀	> 17.7			
Maximum length	♂	279	> 270	265	260
	♀	249	> 240		
Maximum mass	♂	289.4	> 260	240	< 250
	♀	176	> 170		
Sexual Dimorphism		Present	Present	Absent	Absent
Reproductive Seasonality		Aseasonal	Seasonal	Aseasonal	Aseasonal
Peak calving season		Austral Autumn	Austral Summer	Boreal Spring to Summer	Boreal Winter to Summer
Calving interval		5 years	3 years	5 years	-
Ovulation Rate		0.2	-	-	-

*** Study in which results pertaining to growth are based on a Gompertz Growth Function**

4.4.2 Inter-specific variation

Little comparative work has been done on the life history strategies of cetaceans (Kasuya, 1995) and there is particularly a dearth of published information on the natural history of inshore delphinids (Plön, 2004), with the exception of *T. truncatus*. In South African waters, *S. chinensis,* along with *Tursiops aduncus,* share much of the same distribution and may occasionally be found together in a single group, with one or more humpback dolphins generally joining a larger pod of bottlenose dolphins (Karczmarski *et al.*, 1997; Parra and Ross, 2009, Koper and Plön, *in prep*.). The maximum recorded body length of humpback dolphins is similar to that of bottlenose dolphins (Table 4.3; Plön *et al*., 2012). Taking into consideration Cockcroft's (1989) estimate of the longevity (> 40 GLGs) for this species, humpback dolphins appear to grow roughly as old as Indian Ocean bottlenose dolphins *T. aduncus* (Cockcroft and Ross, 1990) (Table 4.3). However, based on results from the present study, *S. chinensis* has a lifespan (17 - 24 GLGs) more similar to that of the short-beaked common dolphin, *Delphinus delphis* (20 - 25 GLGs) (Perrin and Reilly, 1984; Murphy, 2004) (Table 4.3). Additionally, sexual maturity is attained at an age comparable to that of the striped dolphin, *Stenella coeruleoalba* (Perrin and Reilly, 1984; Kroese, 1993), with male humpback and striped dolphins becoming sexually mature between 9 - 10 GLGs and 10 - 12 GLGs, respectively, while females mature at a younger age - around 7.6 GLGs and 8.9 GLGs, respectively (Table 4.3). Likewise, the age at which physical maturity is attained in males also corresponds to that of the striped dolphin, *S. coeruleoalba* (Kroese, 1993), and is reached around 24 GLGs (Table 4.3). Females, on the other hand, reach sexual maturity around 16 GLGs, which is roughly the same as that of *T. aduncus* (12 - 15 GLGs) (Cockcroft and Ross, 1990) (Table 4.3). Likewise, the ovulation rate of *S. chinensis* is most comparable to that of *T. aduncus* (0.29) (Cockcroft ad Ross, 1990) with 0.2 ovulations per annum.

Humpback dolphins share some life history characteristics with other inshore delphinids, such as the bottlenose dolphins, *T. aduncus* (Cockcroft and Ross, 1990; Siciliano *et al.*, 2007). This can probably be ascribed to their having similar body sizes, and to their inshore distribution, as they are exposed to similar environmental factors, such as water temperature. Some parameter estimates, such as age at attainment of sexual and physical maturity, are more analogous to pelagic delphinids. Previous estimates of age at attainment of physical/sexual maturity for *S. chinensis*, locally (Cockcroft, 1989) and elsewhere (Jefferson, 2000; Jefferson *et al.*, 2012) (Table 4.2) are similar to those found in Atlantic Ocean bottlenose dolphins, *T. truncatus* (Siciliano *et al.*, 2007) and Indian Ocean bottlenose dolphins *T. aduncus* (Cockcroft and Ross, 1990) as well as striped dolphins, *S. coeruleoalba* (Kroese, 1993), and short-beaked common dolphins, *D. delphis* (Murphy, 2004) (Table 4.2).

Table 4.3: Various life history parameters for a number of delphinids.

Species	Age at Sexual Maturity (GLGs)		Age at Physical Maturity (GLGs)		Maximum Age (GLGs)		Maximum Length (cm)		Ovulation Rate	Source
	♂	♀	♂	♀	♂	♀	♂	♀		
Sousa chinensis **(Indo-Pacific humpback dolphin)**	9-10	7.6	24	16	24	17.7	279	249	0.2	Present study
Tursiops aduncus **(Indian Ocean bottlenose dolphin)**	14.5	10.5	12-15		42	43	257	249	0.29	Cockcroft and Ross, 1990
T. truncatus **(Atlantic Ocean bottlenose dolphin)**	-	-	20		26		280	260	-	Siciliano *et al*., 2007
	12	-	-	-	25	27	381	367	-	Perrin and Reilly, 1984
	-	-	-	-	33	41	-	-	-	Fernandez and Hohn, 1998
	-	-	-	-	27	30	-	-	-	Mattson *et al*.,2006
	-	-	-	-	44	38	-	-	-	Neuenhoff *et al*., 2011
	-	-	-	-	35		-	-	-	Stolen *et al*., 2002
Grampus griseus **(Risso's dolphin)**	-	>9	-	-	>13	>17	383	366	-	Perrin and Reilly, 1984

Table 4.3: *Continued*

Species	Age at Sexual Maturity (GLGs)		Age at Physical Maturity (GLGs)		Maximum Age (GLGs)		Maximum Length (cm)		Ovulation Rate	Source
	♂	♀	♂	♀	♂	♀	♂	♀		
Delphinus delphis **(Short beaked Common dolphin)**	3	4	-	-	22	20	260	230	-	Perrin and Reilly, 1984
	11.86	9-11	11.86	9-11	25		231		0.7	Murphy, 2004
Stenella coeruleoalba **(Striped dolphin)**	>12	7	-	-	45.5	37.5	256	245	-	Perrin and Reilly, 1984
	10-12	8.9	25	20	47	42	256	240	0.43	Kroese, 1993
S. attenuata **(Spotted dolphin)**	16	11.4	-	-	40	46	257	242	0.61	Perrin and Reilly, 1984; Hohn *et al*., 1985; Myrick *et al*., 1986
S. longirostris **(Spinner dolphin)**	>9	>8	-	-	12-19	15-23	235	204	-	Perrin and Reilly, 1984
Cephalorhynchus commersonii **(Commerson's dolphin)**	-	-	-	-	15	10	166.5	174	-	Perrin and Reilly, 1984
Lagenorhynchus obscurus **(Dusky dolphin)**	-	-	-	-	7	21	211	193	-	Perrin and Reilly, 1984

4.5 Shark nets

Bycatch rates in shark nets along the KwaZulu-Natal coast indicate that the majority of incidental catches of humpback dolphins is concentrated in the northern region of KwaZulu-Natal, namely Richard's Bay, and to a lesser extend Zinkwazi (Atkins *et al*., 2013). *S. chinensis* appears to be resident throughout the year in this region; therefore mortalities may be localised and thus intense, with an expected marginal effect on adjacent groups (Ross, 1982). Atkins *et al*. (2013) state that the lack of decline in the catch rate of *S. chinensis* in KwaZulu-Natal waters reflects the population's persistence in the sub-region. A reduction in population numbers in one location may, however, lead to the immigration of other animals into that area, which in turn would result in increased losses through incidental capture and the depletion of the local *S. chinensis* population (Ross, 1982).

By-catch data presented in the present study, which indicate a strong bias in the entanglement of immature and, to a lesser extent, young sexually mature animals (particularly males), poses some concern for the continued survival of *S. chinensis*. The removal of males from a population exaggerates a female bias, which can in turn influence male-male competition and affect the genetic diversity within a population (Atkins *et al*., 2013). It is therefore advisable to revise existing management strategies to attempt to further decrease catches in shark nets, especially since the largest population of *S. chinensis* appears to be resident in the northern parts of KwaZulu-Natal waters.

A number of management strategies have already been developed and employed in KwaZulu-Natal coastal waters to reduce the incidental capture of non-target marine species (KwaZulu-Natal Sharks Board, 2011). Actions currently taken by the KwaZulu-Natal Sharks Board (KZNSB) include the permanent removal of all nets at selected beaches that are not heavily used by bathers, reducing the number of nets at individual beaches, replacing nets with drumlines where possible, and installing acoustic deterrents such as 'pingers' and sonar reflecting floats (KwaZulu-Natal Sharks Board, 2011). Although further efforts are currently made to manage and preserve existing populations, such as the seasonal removal of nets during the 'sardine run', it appears to have little impact on humpback dolphin catches (Peddemors, 2006), as Richard's Bay and Zinkwazi are outside the usual range of the 'sardine run' (Dudley and Cliff, 2010). The obvious solution, therefore, is to remove some if not all of the shark nets within the region (Cockcroft, 1990). Realistically, however, this is not a feasible option as it will jeopardise the multi-million Rand tourism industry in KwaZulu-Natal, which relies on the safety provided by these shark nets (Cockcroft, 1994). Atkins *et al*. (2004) suggested removing the nets that coincide with the feeding area of resident humpback dolphins in Richard's Bay. Entanglement is probably the result of dolphins not being aware of the location of nets, as the turbidity of inshore waters in and around Richard's Bay is generally poor (Atkins *et al*., 2013). However, this again is impractical due to the beach infrastructure (Atkins *et al*., 2004). According to Peddemors (2006), 'pingers' installed in nets along the coast reduced humpback dolphin catches by an estimated 60%. However, after having been tested at Richard's Bay alone, they appeared not to have the desired effect, and it is reported that the sound may even have attracted dolphins to the nets (Atkins *et al*., 2013). Another proposed alternative to reduce by-catch is to increase the mesh size of nets to possibly reduce the capture of at least smaller individuals (Cockcroft, 1990). Cockcroft (1994) suggested, however, that this might not prove to be as effective, since even the smallest humpback dolphins caught were still substantially larger than the net mesh.

4.6 Conclusion and future considerations

Despite the findings of the present study, the life history of *S. chinensis* in South African waters remains poorly understood. This is primarily due to the fact that samples available for the study probably underrepresented the different age, length and mass classes within the population. The data were skewed towards immature and early mature males, and largely underrepresented females. Results were further complicated by the various discrepancies in life history parameters estimated in the present study and by Cockcroft (1989), for the same population.

Data on age and growth in relation to reproductive parameters provides information vital to the potential survival of a species (Scheffer and Myrick, 1980). Deviations from baseline growth parameters may serve as a useful indication of possible perturbations within populations (Neuenhoff *et al.*, 2011). For example, increased adult mortality has been associated with sexual maturity attained at too early an age (Neuenhoff *et al.*, 2011). The results obtained from the present study should therefore be used only as a baseline for assessing *S. chinensis* population structure.

Results from the present study did indicate that different populations of *S. chinensis* are geographically distinct from one another, and this should be taken into consideration when assessing future conservation and management strategies. However, the management and thus protection of humpback dolphins, particularly along the KwaZulu-Natal coast, requires accurate population census, accompanied by accurate and unbiased estimates of both age and the sex structure (Cockcroft and Ross, 1990). Census data for humpback dolphins in southern African coastal waters are out-dated (Durham, 1994; Karczmarski, 1996). Thus it remains unclear whether local populations are facing declines, or are stable. The Endangered

Wildlife Trust (EWT) is currently reassessing the Red Data Book for South Africa, and the IUCN (International Union for the Conservation of Nature) Cetacean Specialist Groups is also reassessing the status of, among others, humpback dolphins (Plön, 2013, pers. com.). Should bycatch data be a true representation of the population structure, there is serious cause for concern, as the survival of adolescent or young mature individuals is the most important element for the continued existence of a dolphin population (Jefferson *et al*., 2012). It is therefore recommended to continue the assessment of humpback dolphins in the region and to use the data obtained from this study to assess population structure, such that the necessary actions can be taken to best conserve the population.

Future considerations for assessing the life history strategies of *S. chinensis* in South African waters should include employing a larger sample size, with a greater spectrum of individuals of different age and length classes (especially neonates and older mature individuals). This would help to achieve more reliable estimates of the various life history parameters of *S. chinensis*.

References

Akin, P.A., Peltier, K.M. and Miller, R.B. 1993. Techniques for the Preparation and Examination of Reproductive Samples Collected from Dolphins in the Eastern Tropical Pacific. NOAA Technical Memorandum NMFS. NOAA-TM-NMFS-SWFSC-192. La Jolla, California.

Amir, O.A. and Jiddawi, N.S. 2001. Dolphin Tourism and Community Participation in Kizimkazi Village, Zanzibar. In: Richmond, M.D. and Francis, J. (eds.), *Marine Science Development in Tanzania and Eastern Africa. Proceedings of the 20th Anniversary Conference on Advances in Marine Science in Tanzania.* Institute of Marine Sciences, University of Dar Es Salaam/Western Indian Ocean Marine Science Association, Zanzibar, Tanzania.

Amir, O.A., Berggren, P. and Jiddawi, N.S. 2002. The Incidental Catch of Dolphins in Gillnet Fisheries in Zanzibar, Tanzania. *Western Indian Ocean Journal of Marine Science* 1: 155–162.

Amir, O.A., Jiddawi, N.S. and Berggren, P. 2005. The Occurrence and Distribution of Dolphins in Zanzibar, Tanzania, with Comments on the Differences Between Two Species of Tursiops. *Western Indian Ocean Journal of Marine Science* 4(1): 85-93.

Ashton, N. 2013. Noel Ashton Inspired By Nature. Available [online]: http://www.noelashton.com/printsbooks/prints-illustrations-archive/whale-dolphin-paintings-and-scientific-llustrations/scientific-illustrations/dolphin-scientific-illustrations/ [Accessed: 06/04/2013].

Atkins, S. and Atkins, B.L. 2002. Abundance and Site Fidelity of Indo-Pacific Hump-Backed Dolphins (*Sousa chinensis*) at Richard's Bay, South Africa. *Report of the International Whaling Commission (Special Issue 15)*: 317-328.

Atkins, S., Pillay, N. and Peddemors, V.M. 2004. Spatial Distribution of Indo-Pacific Humpback Dolphins (*Sousa chinensis*) at Richards Bay, South Africa: Environmental Influences and Behavioural Patterns. *Aquatic Mammals* 30(1): 84-93.

Atkins, S., Cliff, G. and Pillay, N. 2013. Humpback Dolphin Bycatch in the Shark Nets in KwaZulu-Natal, South Africa. *Biological Conservation* 159: 442-449.

Barlow, J. 1984. Reproductive Seasonality in Pelagic Dolphins (*Stenella* spp.): Implications for Measuring Rates. *Report of the International Whaling Commission (Special Issue 6)*: 191-198.

Barlow, J. and Hohn, A.A. 1984. Interpreting Spotted Dolphin Age Distributions. NOAA Technical Memorandum NMFS. NOAA-TM-NMFS-SWFSC-48. La Jolla, California.

Barros, N.M. and Cockcroft, V.G. 1991. Prey of Humpback Dolphins (*Sousa plumbea*) Stranded in the Eastern Cape Province, South Africa. *Aquatic Mammals* 17(3): 134-36.

Barros, N.B., Jefferson, T.A. and Parsons, E.C.M. 2004. Feeding Habits of Indo-Pacific Humpback dolphins (*Sousa chinensis*) Stranded in Hong Kong. *Aquatic Mammals* 30(1): 179-188.

Best, P.B. 1976. Tetracycline Marking and the Rate of Growth Layer Formation in the Teeth of a Dolphin (*Lagenorhynchus obscurus*). *South African Journal of Science* 72: 216-218.

Best, P.B. 2007. *Whales and Dolphins of the Southern African Subregion*. Cambridge University Press, Cape Town.

Boyde, A. 1980. Histological Studies of the Dental Tissue of Odontocetes. *Report of the International Whaling Commission (Special Issue 3)*: 65-69.

Bronson, F.H. 1989. *Mammalian Reproductive Biology*. The University of Chicago Press, Chicago.

Bronson F.H. 2009. Climate Change and Seasonal Reproduction in Mammals. *Philosophical Transactions of the Royal Society of London Biological Sciences* 364: 3331–3340.

Brook, F.M., Kinoshita, R. and Benirschke, K. 2002. Histology of the Ovaries of a Bottlenose Dolphin, *Tursiops truncatus*, of Known Reproductive History. *Marine Mammal Science* 18: 540-543.

Brook, F.M., Lim, E.H.T., Chua, F.H.C. and Mackay, B. 2004. Assessment of the Reproductive Cycle of the Indo-Pacific Humpback Dolphin, *Sousa chinensis*, Using Ultrasonography. *Aquatic Mammals* 30(1): 137-148.

Brown, A., Bejder, L., Gagnazzi, D., Parra, G.J. and Allen, S.J. 2012. The North West Cape, Western Australia: A Potential Hotspot for Indo-Pacific Humpback Dolphins *Sousa chinensis*? *Pacific Conservation Biology* 18: 240-246.

Bryden, M.M. and Harrison, R.J. 1986. Gonads and Reproduction. In: Bryden, M.M. and Harrison, R.J. (eds.), *Research on Dolphins*. Claredon Press, Oxford.

Calzada, N., Aguilar, A., Sørensen, T.B. and Lockyer, C. 1996. Reproductive Biology of Female Striped Dolphin (*Stenella coeruleoalba*) from the Western Mediterranean. *Journal of Zoology, London* 240: 581-591.

Chivers, S.J. 2001. Cetacean Life Histories. In: Perrin, W.F., Würsig, B. and Thewissen, J.G.M. (eds.), *The Encyclopedia of Marine Mammals*. Academic Press, San Diego.

Chivers, S.J. and Myrick Jr., A.C. 1993. Comparison of Age at Sexual Maturity and Other Reproductive Parameters for Two Stocks of Spotted Dolphin, *Stenella attenuata*. *Fishery Bulletin* 91: 611-618.

Clark, S.T., Odell, D.K. and Lacinak, C.T. 2000. Aspects of Growth in Captive Killer Whales (*Orcinus orca). Marine Mammal Science* 16: 110-123.

Cockcroft, V.G. 1989. Biology of Indo-Pacific Humpback Dolphins (*Sousa plumbea*) off Natal, South Africa. In: *Abstract, Eight Biennial Conference on the Biology of Marine Mammals*. *Pacific Grove, California, U.S.A*: 13.

Cockcroft, V.G. 1990. Dolphin Catches in the Natal Shark Nets, 1980 to 1988. *South African Journal of Wildlife Research* 20(2): 44-51.

Cockcroft, V.G. 1991. Incidence of Shark Bites on Indian Ocean Humpback Dolphins (*Sousa chinensis*) off Natal, South Africa. In: Leatherwood, S. and Donovan, G.P. (eds.), *Cetaceans and Cetacean Research in the Indian Ocean Sanctuary, Marine Mammal Technical Report Number* 3. Nairobi, United Nations Environment Program.

Cockcroft, V.G. 1993. Size: The Only Male Reproductive Strategy. In *Abstract*, *Tenth Biennial Conference on the Biology of Marine Mammals*. *Galveston, Texas, U.S.A.*

Cockcroft, V.G. 1994. Is There a Common Cause for Dolphin Captures in Gillnets? A Review of Dolphin Captures in Shark Nets off Natal, South Africa. *Report of the International Whaling Commission (Special Issue 15)*: 541-547.

Cockcroft, V.G. 1999. Organochlorine Levels in Cetaceans from South Africa: A Review. In: Reijnders, P.J.H, Aguilar, A. and Donovan, G.P. (eds.), Chemical Pollutants and Cetaceans. *Journal of Cetacean Research and Management.*

Cockcroft, V.G., De Kock, A.C., Lord, D.A. and Ross, G.J.B. 1989. Organochlorines in Bottlenose Dolphins *Tursiops truncatus* from the East Coast of South Africa. *South African Journal of Marine Science* 8*:* 207–217.

Cockcroft, V.G. and Ross, G.J.B. 1990. Age, Growth and Reproduction of Bottlenose Dolphins *Tursiops truncatus* from the East Coast of Southern Africa. *Fishery Bulletin* 88(2): 289-302.

Cockcroft, V.G., Ross, G.J.B., Cornell, A.D. and Butler, A.C. 1991. Occurrence of Organochlorines in Stranded Cetaceans and Seals from the East Coast of Southern Africa. In: Leatherwood, S. and Donovan, G.P. (eds.), *Cetaceans and Cetacean Research in the Indian Ocean Sanctuary, Marine Mammal Technical Report Number 3*. Nairobi, United Nations Environment Program.

Cockcroft, V.G. and Krohn, R. 1994. Passive Gear Fisheries of the Southwestern Indian and Southeastern Atlantic Oceans: An Assessment of the Possible Impact on Cetaceans. *Report of the International Whaling Commission (Special Issue 15)*: 317–328.

Cockcroft, V.G., Leatherwood, S., Goodwin, J. and Porter, L.J. 1997. The Phylogeny of Humpback Dolphins Genus *Sousa*: Insights Through mtDNA Analyses. *Report to the Scientific Committee of the International Whaling Commission* (SC/49/SM25). The Red House, Cambridge.

Cockcroft, V.G. and Smith-Goodwin, J. 2002. The Phylogeny of the Humpback Dolphins Genus *Sousa*. *Report to the Scientific Committee of the International Whaling Commission* (IWC SC/54/SM23). The Red House, Cambridge.

Collet, A. and Saint Girons, H. 1984. Preliminary Study of the Male Reproductive Cycle in Common Dolphins, *Delphinus delphis*, in the Eastern North Atlantic. *Report of the International Whaling Commission (Special Issue 6)*: 355-360.

Connor, R.C., Mann, J., Tyack, P.L. and Whitehead, H. 1998. Social Evolution in Toothed Whales. *Trends in Ecology and Evolution* 13: 228-232.

Corkeron, P.J., Morissette, N.M., Porter, L. and Helene, M. 1997. Distribution and Status of Hump-Backed Dolphins *Sousa chinensis* in Australian Waters. *Asian Marine Biology* 14: 49-59.

Dabin, W., Cossais, F., Pierce, G.J. and Ridoux, V. 2008. Do Ovarian Scars Persist With Age in all Cetaceans: New Insight from the Short-Beaked Common Dolphin (*Delphinus delphis* Linnaeus, 1758). *Marine Biology* 156: 127-139.

Danil, K. and Chivers, S.J. 2007. Growth and Reproduction of Female Short-Beaked Common Dolphins, *Delphinus delphis*, in the Eastern Tropical Pacific. *Canadian Journal of Zoology* 85: 108-121.

Demaster, D.P. 1984. Review of Techniques Used to Estimate the Average Age at Attainment of sexual Maturity in Marine Mammals. *Report of the International Whaling Commission (Special Issue 6)*: 175-179.

de Santos, M.C., Rosso, S. and Ramos, R.M.A. 2003. Age Estimation of Marine Tucuxi Dolphins (*Sotalia fluviatilis*) in South-Eastern Brazil. *Journal of Marine Biological Association of the United Kingdom* 83: 233-236.

Dudley, S.F.J. 1997. A Comparison of the Shark Control Programs of New South Wales and Queensland (Australia) and KwaZulu-Natal (South Africa). *Ocean and Coastal Management* 34: 1-27.

Dudley, S.F.J. and Cliff, G. 2010. Influence of the Annual Sardine Run on Catches of Large Sharks in the Protective Gillnets off KwaZulu-Natal, South Africa, and the Occurrence of Sardines in Shark Diet. *African Journal of Marine Science* 32(2): 383-397.

Durham, B. 1994. The Distribution and Abundance of Humpback Dolphins (*Sousa chinensis*) Along the Natal Coast, South Africa. MSc Thesis, University of Natal. Durban, South Africa.

Evans, P.G.H. 1987. *The Natural History of Whales and Dolphins*. Christopher Helm, London.

Fernandez, S. and Hohn, A.A. 1998. Age, Growth and Calving Season of Bottlenose Dolphins, *Tursiops truncatus*, off Coastal Texas. *Fishery Bulletin* 96(2): 357-365.

Findlay, K.P., Best, P.B., Ross, G.J.B. and Cockcroft, V.G. 1992. The Distribution of Small Odontocete Cetaceans off the Coasts of South Africa and Namibia. *South African Journal Of Marine Science* 12(1): 237-270.

Frère, C.H., Hale, P.T., Porter, L., Cockcroft, V.G. and Dalebout, M.L. 2008. Phylogenetic Analysis of mtDNA Sequences Suggests Revision of Humpback Dolphin (*Sousa* spp.) Taxonomy is Needed. *Marine and Freshwater Research* 59: 259-268.

Gardner, B.D., Cornell, A.D., Eagle, G.A., Molden, A.G.S., Oliff, W.D., Orren, M.J. and Watling, R.J. 1983. South African Marine Pollution Survey Report 1976-1979. *South African National Scientific Programmes Report* 73.

Global Sea Temperature. 2013. World Sea Temperatures. Available [online]: http://www.seatemperature.org/ [Accessed: 18/11/2013].

Gurevich, V.S., Stewart, B.S. and Cornell, L.H. 1980. The Use of Tetracycline in Age Determination of Common Dolphins, *Delphinus delphis*. *Report of the International Whaling Commission (Special Issue 3)*: 165-169.

Harcourt, A.R., Harvey, S.G., Larson, S.G. and Short, R.V. 1981. Testes Weight, Body Weight and Breeding System in Primates. *Nature, London* 293: 55-57.

Harrison, R.J. 1949. Observations on the Female Reproductive Organs of the Ca'aing Whale *Globicephala melaena* Traill. *Journal of Anatomy* 83(3): 238-253.

Hohn, A.A. 1980. Age Determination and Age Related Factors in the Teeth of Western North Atlantic Bottlenose Dolphins. *Scientific Report of the Whales Research Institute, Tokyo* 32: 39-66.

Hohn, A.A. 1990. Reading Between the Lines: Analysis of Age Estimation in Dolphins. In: Leatherwood, S. and Reeves, R.R. (eds.), *The Bottlenose Dolphin*. Academic Press, New York.

Hohn, A.A. 2002. Age Estimation. In: Perrin, W.F., Wűrsig, B. and Thewissen, J.G.M. (eds.), *Encyclopedia of Marine Mammals*. Academic Press, San Diego.

Hohn, A.A., Chivers, S.J. and Barlow, J. 1985. Reproductive Maturity and Seasonality of Male Spotted Dolphins, *Stenella attenuata*, in the Eastern Tropical Pacific. *Marine Mammal Science* 1(4): 273-293.

Hohn, A.A., Scott, M.D., Wells, R.S., Sweeney, J.C. and Irvine, A.B. 1989. Growth Layers in the Teeth from Known-Age, Free-Ranging Bottlenose Dolphins. *Marine Mammal Science* 5: 315-342.

Hohn, A.A., Read, A.J., Fernández, S., Vidal, O. and Findley, T. 1996. Life History of the Vaquita, *Phocoena sinus* (Phocoenidae, Cetacea). *Journal of Zoology*, *London* 239: 235-251.

International Whaling Commission. 1994. Report on the Workshop on the Mortality of Cetaceans in Passive Fishing Nets and Traps. *Report to the International Whaling Commission*: 1-71.

Jefferson, T.A. 1990. Sexual Dimorphism and Development of External Features in Dall's Porpoise *Phocoenoides dalli*. *Fishery Bulleting* 88(1): 119-132.

Jefferson, T.A. 2000. Population Biology of the Indo-Pacific Hump-Backed Dolphin in Hong Kong Waters. *Wildlife Monographs* 144: 1-65.

Jefferson, T.A. and Karczmarski, L. 2001. *Sousa chinensis*. *American Society of Mammalogists* 655: 1-9.

Jefferson, T.A. and van Waerebeek, K. 2004. Geographic Variation in Skull Morphology of Humpback Dolphins (*Sousa* spp.). *Aquatic Mammals* 30(1): 3-17.

Jefferson, T.A., Hung, S.K., Robertson, K.M. and Archer, F.I. 2012. Life History of the Indo-Pacific Humpback Dolphin in the Pearl River Estuary, Southern China. *Marine Mammal Science* 28(1): 84-104.

Karczmarski, L. 1996. Ecological Studies of Humpback Dolphins *Sousa chinensis* in the Algoa Bay region, Eastern Cape, South Africa. PhD Thesis, University of Port Elizabeth. Port Elizabeth, South Africa.

Karczmarski, L. 1999. Group Dynamics of Humpback Dolphins (Sousa *chinensis*) in the Algoa Bay region, South Africa. *Journal of Zoology* 249(3): 283-293.

Karczmarski, L. 2000. Conservation and Management of Humpback Dolphins: The South African Perspective. *Oryx* 34(3): 207-216.

Karczmarski, L., Thornton, M. and Cockcroft, V.G. 1997. Description of Selected Behaviours of Humpback Dolphins *Sousa chinensis*. *Aquatic Mammals* 23(3): 127-133.

Karczmarski, L., Winter, P.E.D., Cockcroft, V.G. and McLachlan, A. 1999. Population Analyses of Indo-Pacific Humpback Dolphins *Sousa chinensis* in Algoa Bay, Eastern Cape, South Africa. *Marine Mammal Science* 15(4): 1115-1123.

Karczmarski, L., Cockcroft, V.G. and Mclachlan, A. 2000*a*. Habitat Use and Preference of Indo-Pacific Humpback Dolphins *Sousa chinensis* in Algoa Bay, South Africa. *Marine Mammal Science* 16(1): 65-79.

Karczmarski, L., Thornton, M. and Cockcroft, V.G. 2000*b*. Daylight Occurrence of Humpback Dolphins *Sousa chinensis* in Algoa Bay, South Africa. *East African Wildlife Society* 38: 86-90.

Kasuya, T. 1976. Reconsideration of Life History Parameters of the Spotted and Striped Dolphins Based on Cemental Layers. *Scientific Reports of the Whales Research Institute*, *Tokyo* 28: 73-106.

Kasuya, T. 1985. Effect of Exploitation on Reproductive Parameters of the Spotted and Striped Dolphins off the Pacific Coast of Japan. *Scientific Report of the Whales Researcher Institute*, *Tokyo* 36: 107-138.

Kasuya, T. 1995. Overview of cetacean life histories: an essay on their evolution. In: Blix, A. S., Walløc, L., Ulltang, Ø. (eds), *Developments in Marine Biology*, *Vol. 4*. Elsèvier, Amsterdam.

Kirby, V.L. and Ridgway, S.H. 1984. Hormonal Evidence of Spontaneous Ovulation in Captive Dolphins *Tursiops truncatus* and *Delphinus delphis*. *Reports of the International Whaling Commission (Special Issue 6)*: 459-464.

Klevezal', G.A. 1996. *Recording Structures of Mammals: Determination of Age and Reconstruction of Life History*. *Balkema* Publishing House, Rotterdam.

Klevezal’, G.A. and Kleinenberg, S.E. 1967. Age Determination of Mammals by Layered Structures in Teeth and Bone. Akad. Nauk, SSSR, Inst. Morf. Zhivot. im. A.N. Severtsova. Izat Nauka., Moskow.

Koper, R.P. and Plön, S. (*in prep*.) Interspecific Behavioural Interactions in Cetaceans in Algoa Bay, South Africa. Intended journal: *Aquatic Mammals*.

Kroese, M. 1993. Age, Growth and Reproductive Biology of Striped Dolphins, *Stenella coeruleoalba* (Meyen, 1833) off the Coast of Southern Africa. MSc Thesis, University of Port Elizabeth. Port Elizabeth, South Africa.

KwaZulu-Natal Sharks Board, 2011. Shark Nets, Drumlines, and Safe Swimming. Available [online]: www.shark.co.za/SharksNetsMore/ [Accessed: 16/10/2012].

Lammers, A.R., Dziech, H.A. and German, R.Z. 2001. Ontogeny of Sexual Dimorphism in *Chinchilla lanigera* (Rodentia: Chinchillidae). *Journal of Mammalogy* 82: 179-189.

Lawrence, E. 2005. *Henderson's Dictionary of Biology Thirteenth Edition*. Pearson's Education Limited, Malaysia.

Laws, R. M. 1956. Growth and Sexual Maturity in Aquatic Mammals. *Nature* 178: 193-194.

Leatherwood, S. and Reeves, R. 1983. Indo-Pacific Humpback Dolphin. *The Sierra Club Handbook of Whales and Dolphins*. Sierra Club Books, San Francisco.

Luque, P.L., Learmonth, J.A., Santos, M.B., Ieno, E. and Pierce, G.J. 2009. Comparison of Two Histological Techniques for Age Determination in Small Cetaceans. *Marine Mammal Science* 25(4): 902 - 919.

Marsh, H. and Kasuya, T. 1986. Evidence for Reproductive Senescence in Female Cetaceans. *Report International Whaling Commission (Special Issue 8)*: 57-74.

Marsili, L., Casini, C., Marini, L., Regoli, A. and Focardi, S. 1997. Age, Growth and Organochlorines (HCB, DDTs and PCBs) in Mediterranean Striped Dolphins *Stenella coeruleoalba* Stranded in 1988-1994 on the Coast of Italy. *Marine Ecology Progress Series* 151: 273-282.

Mattson, M.C., Mullin, K.D., Ingram, G.W. and Hoggard, W. 2006. Age Structure and Growth of the Bottlenose Dolphin (*Tursiops Truncatus*) from Strandings in the Mississippi Sound Region of the North-Central Gulf of Mexico From 1986 to 2003. *Marine Mammal Science 22*(3): 654–666.

Mendolia, C. 1989. Reproductive Biology of Common Dolphins (*Delphinus delphis*, *Lineneaeus*) off the South East Coast of Southern Africa. MSc Thesis, University of Port Elizabeth. Port Elizabeth, South Africa.

Mitchell, E. and Kozicki, V.M. 1984. Reproductive Condition of Male Sperm Whales, *Physeter macrocephalus*, Taken off Nova Scotia. *Report of the International Whaling Commission (Special Issue 6)*: 243-251.

Miyazaki, N. 1977. Growth and Reproduction of *Stenella coeruleoalba* off the Pacific Coast of Japan. *Scientific Report of the Whales Research Institute* 29: 21-48.

Miyazaki, N. 1984. Further Analyses of Reproduction in the Striped Dolphin, *Stenella coeruleoalba*, off the Pacific Coast of Japan. *Report of the International Whaling Commission (Special Issue 6)*: 343-353.

Murphy, S. 2004. The Biology of the Short-Beaked Common Dolphin *Delphinus delphis* in the North-East Atlantic. PhD Thesis, University College Cork, National University of Ireland. Cork, Ireland.

Murphy, S., Collet, A and Rogan, E. 2005. Mating Strategy in the Male Common Dolphin (*Delphinus delphis*): What Gonadal Analysis Tells Us. *Journal of Mammmalogy* 86(6): 1247-1258.

Myrick, A.C. 1980. Examination of Layered Tissue of Odontocetes for Age Determination Using Polarized Light Microscopy. *Report of the International Whaling Commission (Special Issue 3)*: 95-97.

Myrick, A.C. 1991. Some New and Potential Uses of Dental Layers in Studying Delphinid Populations. In: Pryor, K. and Norris, K.S. (eds.), *Dolphin Societies - Discoveries and Puzzles*. University of California Press, Berkeley.

Myrick, A.C., Hohn, A.A., Sloan, P.A., Kimjura, M. and Stanley, D.D. 1983. Estimating Age of Spotted and Spinner Dolphins (*Stenella attenuata* and *Stenella longirostris*) from teeth. NOAA Technical Memorandum NMFS-SWFC-30. La Jolla, California.

Myrick, A.C., Shallenberger, E.W., Kang, I. and MacKay, D.B. 1984. Calibrations of Dental Layers in Seven Captive Spinner Dolphins, *Stenella longirostris*, Based on Tetracycline Labelling. *Fishery Bulletin* 82(1): 207-255.

Myrick, A.C., Hohn, A.A., Barlow, J. and Sloan, P.A. 1986. Reproductive Biology of Female Spotted Dolphins, *Stenella attenuata*, from the Eastern Tropical Pacific. *Fishery Bulletin* 84(2): 247-259.

Myrick, A.C. and Cornell, L.H. 1990. Calibrating Dental Layers in Captive Bottlenose Dolphins from Serial Tetracycline Labels and Tooth Extractions. In: Leatherwood, S. and Reeves, R.R. (eds.), *The Bottlenose Dolphin*. Academic Press, New York.

Neuenhoff, R.D., Cowan, D.F., Whitehead, H. and Marshall, C.D. 2011. Prenatal Data Impacts Common Bottlenose Dolphin (*Tursiops truncatus*) Growth Parameters Estimated by Length-at-Age Curves. *Marine Mammal Science* 27(1): 195-216.

Nishiwaki, M., Hibiya, T. and Ohsumi, S. 1958. Age Study of Sperm Whale Based on Reading of Tooth Laminations. *Scientific Reports of the Whales Research Institute, Tokyo* 13: 135-153.

Norris, K.S. 1961. Standardized Methods for Measuring and Recording Data on the Smaller Cetaceans. *Journal of Mammalogy* 42: 417-476.

Ohsumi, S. 1964. Comparison of Maturity and Accumulation Rate of *Corpora albicantia* Between the Left and Right Ovaries in Cetacea. *Scientific Report of the Whales Research Institute, Tokyo* 18: 123-148.

Parra, G.J., Corkeron, P.J. and Marsh, H. 2004. The Indo-Pacific Humpback Dolphin, *Sousa plumbea* (Osbeck, 1765), in Australian Waters: A Summary of Current Knowledge. *Aquatic Mammals* 30(1): 197-206.

Parra, G.J. and Jedensjö, M. 2009. Feeding Habits of Australian Snubfin (*Orcaella heinsohni*) and Indo-Pacific Humpback dolphins (*Sousa chinensis*). *Project Report to the Great Barrier Reef and Rainforest Research Centre Limited*. Cairns, Australia.

Parra, G.J. and Ross, G.J.B. 2009 . Humpback Dolphins *S. chinesis* and *S. teuszii*. In: Perrin, W.F., Würsig, B and Thewissen, J.G.M. (2nd eds.), *Encyclopedia of Marine Mammals.* Academic Press, San Diego.

Peddemors, V. 2006. Sharks and Netting - Some South African Perspectives. In *Scientific Shark Protection Summit*, 10 April 2006. NSW Department of Primary Industries and Sydney Aquarium, Sydney.

Peddemors, V. M. and Thompson, G., 1994. Beaching Behaviour During Shallow Water Feeding by Humpback Dolphins *Sousa plumbea*. *Aquatic Mammals* 20 (2): 65-67.

Peddemors, V.M., Atkins, S. and Oosthuizen, W.H. 2004. South African Mammal CAMP 2002/3: *Sousa plumbea*. *Red Data Book of the Mammals of South Africa: A Conservation Assessment*. Endangered Wildlife Trust and CBSG (IUCN/SCC).

Perrin, W.F., Coe, J.M. and Zweifel, J.R. 1976. Growth and Reproduction of the Spotted Porpoise, *Stenella attenuata,* in the Offshore Eastern Tropical Pacific. *Fishery Bulletin* 72(2): 229-269.

Perrin, W.F., Holts, D.B. and Miller, R.B. 1977. Growth and Reproduction of the Eastern Spinner Dolphin, a Geographical Form of *Stenella longirostris* in the Eastern Tropical Pacific. *Fishery Bulletin* 75(4): 725-750.

Perrin, W.F. and Myrick, A.C. 1980. Age Determination of Toothed Whales and Sirenians. *Report of the International Whaling Commission (Special Issue 3)*: 1-50.

Perrin, W.F. and Donovan, G.P. 1984. Report of the workshop. *Report of the International Whaling Commission (Special Issue 6)*: 1-24.

Perrin, W.F. and Henderson, J.R. 1984. Growth and Reproduction in Two Populations of Spinner Dolphins, *Stenella longirostris,* With Different Histories of Exploitation. *Report of the International Whaling Commission (Special Issue 6)*: 417-430.

Perrin, W.F. and Reilly, S.B. 1984. Reproductive Parameters of Dolphins and Small Whales of the Family Delphinidae. *Report of the International Whaling Commission (Special Issue 6)*: 97-133.

Perrin, W.F., Dolar, M.L.L., Amano, M. and Hayano, A. 2003. Cranial Sexual Dimorphism and Geographic Variation in Fraser's Dolphin, *lagenodelphis hosei. Marine Mammal Science* 19: 484-501.

Peyer, B. 1968. *Comparative Odontology*. University of Chicago Press, Chicago.

Plön, S. 2004. The Status and Natural History of Pygmy (*Kogia breviceps*) and Dwarf (*K. sima*) Sperm Whales off Southern Africa. PhD Thesis, Rhodes University. Grahamstown, South Africa.

Plön, S. and Bernard, R.T.F. 2007. Testis, Spermatogenesis, and Testicular Cycles. In: Miller, D.L. (eds.), R*eproductive Biology and Phylogeny of Cetacea - Whales, Dolphins and Porpoises*. Science Publishers, Enfield, New Hampshire.

Plön, S., Albrecht, K.H., Cliff, G. and Froneman, P.W. 2012 Organ Weights of Three Dolphin Species (*Sousa chinensis*, *Tursiops aduncus* and *Delphinus capensis*) from South Africa: Implications for Ecological Adaptation? *Journal of Cetacean Research and Management* 12: 265-276.

Ralls, K. and Mesnick, S.L. 2002. Sexual Dimorphism. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (eds.), *The Encyclopedia of Marine Mammals*. Academic Press, San Diego.

Reddy, K. 1996. The Reproductive Biology of Four Species of South African Dolphins. MSc Thesis, Rhodes University. Grahamstown, South Africa.

Reeves, R.R., Smith, B.D., Crespo, E.A., and Notarbartolo di Sciara, G. 2003. *2002-2010 Conservation Action Plan for the World's Cetaceans: Dolphins, Whales and Porpoises*. IUCN/SCC Cetacean Specialist Group. Cambridge, United Kingdom.

Reeves, R.R., Dalebout, M.L., Jefferson, T.A., Karczmarski, L., Laidre, K., O'Corry-Crowe, G., Rojas-Bracho, L., Secchi, E.R., Slooten, E., Smith, B.D., Wang, J.Y. and Zhou, K. 2008. *Sousa chinensis*. In: IUCN 2013. IUCN Red List of Threatened Species. Available [online]: www.iucnredlist.org [Accessed: 17 October 2013].

Reilly, S.B., Hohn, A.A. and Myrick, A.C. 1983. Precision of Age Determination of Northern Offshore Spotted Dolphins. NOAA Technical Memorandum NMFS. NOAA-TM NMFS-SWFC-35. La Jolla, California.

Ross, G.J.B. 1982. A Preliminary Survey of Bottlenosed Dolphin *Tursiops aduncus* and Humpback Dolphin *Sousa plumbea* Populations on the South East Coast of Southern Africa. *Interim Report to S.A Nature Foundation.*

Ross, G.J.B. 1984. The Smaller Cetaceans of the South East Coast of Southern Africa. Annals of the Cape Provincial Museums. *Natural History* 15: 173-410.

Ross, G.J.B., Heinsohn, G.E. and Cockcroft, V.G. 1994. Humpback Dolphins *Sousa chinensis* (Osbeck, 1765), *Sousa plumbea* (G. Cuvier, 1829) and *Sousa teuszii* (Kukenthal, 1892). In: Ridgeway, S.H. and Harrison, R. (eds.), *Handbook of Marine Mammals, Volume 5: The First Book of Dolphins.* Academic Press, San Diego.

Rosenbaum, H., Glaberman, S., Jefferson, T.A, Collins, T., Minton, G., Peddemors, V. and Baldwin, R. 2002. Phylogenetic Relationships and Population Structure among Humpback Dolphins Based on mtDNA Variation. *International Whaling Commission Scientific Committee Report* (SC/54/SM34). The Red House, Cambridge.

Saayman, G.S. and Tayler, C.K. 1979. The Sociobiology of Humpback Dolphins (*Sousa* sp.). In: Winn, H.E. and Olla, B.L. (eds.), *Behaviour of Marine Animals 3: Cetacea.* Plenum Press, New York.

Scheffer, V.B. 1950. Growth Layers in the Pinnipedia as an Indicator of Age. *Science* 112 (2907): 309-311.

Scheffer, V.B. and Myrick, A.C. 1980. A Review of Studies to 1970 of Growth Layers in the Teeth of Marine Mammals. *Report of the International Whaling Commission (Special Issue 3*): 51-63.

Scott, E.O.G. 1949. Neonatal Length as a Function of Adult Length in Cetacea. *Papers and Proceedings. Royal Society of Tasmania*: 75-93.

Sergeant, D.E. 1959. Age Determination of Odontocete Whales from Dentinal Growth Layers. *Norsk Hvalfangsttid* 6: 273-288.

Sergeant, D.E. 1962. The Biology of the Pilot or Pothead Whale *Globicephala melaena* (Traill) in Newfoundland waters. *Bulletin of the Fisheries Research Board of Canada* 132: 1-84.

Sergeant, D.E., Caldwell, D.K. and Caldwell, M.C. 1973. Age, Growth and Maturity of Bottlnosed Dolphins (*Tursiops truncatus*) from Northeast Florida. *Journal of the Fisheries Research Board of Canada* 30: 1009-1011.

Siciliano, S., Ramos, R.M., Di Beneditto, A.P.M., de Santos, M.C.O., Fragoso, A.B., Brito, J.L. Jr., Azevedo, A.F., Civente, A.F.C., Zampirolli, E., Alvarenga, F.S., Barbosa, L. and Lima, N.R.W. 2007. Age and Growth of Some Delphinids in South-Eastern Brazil. *Journal of the Marine Biological Association, U.K.* 87: 293-303.

Stensland, E. 2004. Behavioural Ecology of the Indo-Pacific Bottlenose and Humpback Dolphins. PhD Thesis, Stockholm University. Stockholm, Sweden.

Stensland, E., Carlen, I., Sarnblad, A., Bignert, A. and Berggren, P. 2006. Population Size, Distribution, and Behavior of Indo-Pacific Bottlenose (*Tursiops aduncus*) and Humpback (*Sousa chinensis*) dolphins off the south coast of Zanzibar. *Marine Mammal Science* 22(3): 667-682.

Stensland, E. and Berggren, P. 2007. Behavioural Changes in Female Indo-Pacific Bottlenose Dolphins in Response to Boat-Based Tourism. *Marine Ecology Progress Series* 332: 225-234.

Stolen, M.K., Odell, D.K. and Barros, N.B. 2002. Growth of Bottlenose Dolphins (*Tursiops truncatus*) from the Indian River Lagoon System, Florida, U.S.A. *Marine Mammal Science* 18(2): 348-357.

Tolley, K.A., Read, A.J., Wells, R.S., Urian, K.W., Scott, M.D., Irvine, A.B. and Hohn, A.A. 1995. Sexual Dimorphism in Wild and Captive Bottlenose Dolphins (*Tursiops trunctatus)* from Sarasota, Florida. *Journal of Mammalogy* 76: 1190-1198.

Ungerfeld, R. and Bielli A. 2012. Seasonal and Social Factors Affecting Reproduction. In: González-Bulnes, A. and Astiz, S. (eds.), *Livestock Reproduction: Bovine, Swine, and Ruminants. Encyclopedia of Life Support Systems (EOLSS).* Available [online]: www.eolss.net [Accessed: 20 May 2013].

Van Parijs, S.M. and Corkeron, P.J. 2001. Vocalizations and Behaviour of Pacific Humpback Dolphins *Sousa chinensis*. *Ethology* 107: 701-716.

Venter, K. 2009. Diet of Humpback Dolphins (*Sousa plumbea*) Along the South-Eastern Coast of South Africa. Unpublished Manuscript, Rhodes University. Grahamstown, South Africa.

Wang, J.Y., Chu Yang, S., Hung, S.K. and Jefferson, T.A. 2007. Distribution, Abundance and Conservation Status of the Eastern Taiwan Strait Population Of Indo-Pacific Humpback Dolphins, *Sousa chinensis. Mammalia* 71(4): 157-165.

Weston, L.F. 2011. Allometric Relationships and Sexual Dimorphism in the Indo-Pacific Humpback Dolphin (*Sousa chinensis*) in South African Waters. Unpublished Manuscript, Rhodes University. Grahamstown, South Africa.

Zbinden, K., Pilleri, G., Kraus, C. and Bernath, O. 1977. Observations on the Behaviour and the Underwater Sounds of the Plumbeous Dolphin (*Sousa plumbea* G. Cuvier, 1829) in the Indus Delta Region. *Investigations on Cetacea* 8, 259–286.

Appendices

APPENDIX A

Specimens, selected metadata and associated samples available for the present study, along with estimates of age and reproductive status obtained from the present study.

PEM: Port Elizabeth Museum

PEM Number	NSB Number	Sex	Date of Death	Location	Length (cm)	Mass (kg)	Teeth	Gonads	Age Estimate	Reproductive State
N0181	-	M	20/07/1972	Umhlanga Rocks	279	289.4	x	0	21.5	-
N0486	MS 22	M	09/10/1980	Richards Bay	211	114	x	0	6.7	-
N0487	MS 223	F	09/10/1980	Richards Bay	210	111	x	0	6.0	-
N0536	-	M	28/11/1980	Richards Bay	157	53.7	x	0	1.0	-
N0739	MS 540	M	23/06/1981	Richards Bay	225	128	x	0	7.0	-
N0742	MS 522	M	08/07/1981	Richards Bay	254	221	x	0	13.8	-
N0801	VC 3	M	13/08/1981	Richards Bay	209	111	x	0	5.8	-
N0802	VC 1	F	25/09/1981	Warner Beach	163	51	x	0	1.0	-
N0803	VC 2	M	19/10/1982	Richards Bay	197	87	x	0	6.0	-
N0804	MA 36	M	13/01/1982	Richards Bay	195	82	x	0	4.5	-
N0805	DB 31	F	22/07/1981	Richards Bay	242	164	x	0	14.2	-
N0825	CS 305	M	25/01/1982	Amanzimtoti	215	113	x	x	3.0	Im

N0826	RB 82/316	M	05/02/1982	Amanzimtoti	248	205	x	x*	14.7	-
N0850	RB 16	M	20/04/1982	Richards Bay	188	79	x	x*	2.5	-
N0868	MZA 35	M	11/08/1982	Mzamba	164	57	x	0	1.0	-
N0971	RAM 83/12	F	08/08/1983	Ramsgate	197	92	x	x	3.5	Im
N1002	RB 83/75	M	28/10/1983	Richards Bay	199	89	x	x	3.0	Im
N1033	TIN 84/2	F	10/02/1984	Tinley Manor	249	173	x	x*	15.8	-
N1036	TRA 84/2	M	10/01/1984	Trafalgar	210	102	x	0	4.0	-
N1038	BLY 84/14	M	29/03/1984	Blythedale Beach	260.5	250	x	x	23.5	Mat
N1041	VC 84 05 2	M	22/12/1983	NASMB	184	65	x	x	3.0	Im
N1045	RAM 83/20	F	22/12/1983	Ramsgate	188	78	x	x	4.5	Im
N1086	BAL84/17	F	02/10/1984	Ballito Bay	232	142	x	0	12.5	-
N1121	MUZ 138	M	06/12/1984	Mtazami	267	262	x	x*	24.0	-
N1122	RB 84/001	M	11/12/1984	Richards Bay	204	93	x	x*	5.5	-
N1123	WIN 84/25	M	04/01/1985	Winkelspruit	232	150	0	x	-	Im
N1147	MZA 85/25	M	11/03/1985	Mazuba	262.5	250	x	x	17.3	Mat
N1179	RB 85/07	M	22/0519/85	Richards Bay	269	245	x	x	18.3	Mat
N1219	ZIN 85/54	M	02/08/1985	Zinkwazi	224.5	146	0	x	-	Mat
N1242	RB 85/54	M	13/06/1985	Richards Bay	182	90	x	x	2.8	Im
N1266	RB 85/176	F	19/12/1985	Richards Bay	233	159	x	x	17.7	Mat
N1267	SAN 85/37	M	10/12/1985	San Lameer	219	120	x	x	5.0	Im
N1271	RB 86/02	M	02/12/1985	Richards Bay	197	103	x	x	6.5	Im
N1315	ZIN 86/57	F	05/09/1986	Zinkwazi	169	54	x	0	1.5	-
N1316	RB 86/73	F	11/08/1986	Richards Bay	185	63	0	x	-	Im
N1364	RB 87/07	M	16/06/1987	Richards Bay	233	134	x	x	17.7	Mat
N1408	RB 87/02	F	14/04/1987	Richards Bay	222	114	0	x	-	Im
N1473	RB 87/05	M	17/11/1987	Richards Bay	211	97	x	x	4.0	Im
N1474	BLY 88/03	F	04/01/1988	Blythedale Beach	181	62	x	x	2.0	Im
N1498	RB 88/05	M	04/05/1888	Richards Bay	215	118	x	x	6.5	Im

N1499	HQ 88/01	F	08/03/1988	Umhlanga Rocks	214	101	x	0	6.0	-
N1521	PE 88/13	F	07/07/1988	Port Edward	193	83	x	0	2.0	-
N1576	TIN 89/09	F	17/01/1989	Tinley Manor	240	157	x	x	13.5	Mat
N1577	UMD 88/05	F	18/10/1988	Umdloti Beach	213	104	x	x	6.0	Im
N1578	RB 88/08	M	01/12/1988	Richards Bay	206	96	x	x	5.3	Im
N1579	SAL 89/01	M	03/01/1989	Salt Rock	215	103	x	x	5.0	Im
N1580	TIN 89/10	M	17/01/1989	Tinley Manor	156	50	x	0	1.5	-
N1582	RB 89/13	M	21/02/1989	Richards Bay	246	200	0	x	-	Mat
N1583	RB 89/12	M	16/01/1989	Richards Bay	194	74	x	0	2.0	-
N1593	SAL 89/14	M	11/05/1989	Salt Rock	262	226	x	x	16.7	Mat
N1600	RB 89/14	M	29/05/1989	Richards Bay	209	127	x	x	4.5	Im
N1610	ZIN 89/19	M	24/07/1989	Zinkwazi	210	102	0	x	-	Im
N1631	RB 89/16	M	18/08/1989	Richards Bay	161	51	x	0	1.0	-
N1671	RB 89/17	M	13/02/1990	Richards Bay	187	80	x	0	2.0	-
N1684	ST 90/10	F	22/03/1990	St. Michael's	212	109	x	x	7.2	Im
N1777	ZIN 90/21	M	18/07/1990	Zinkwazi	148	41	x	0	1.0	-
N1778	ZIN 90/20	F	18/07/1990	Zinkwazi	227	144	0	x	-	Mat
N1791	RB 90/19	M	04/06/1990	Richards Bay	190	73	x	0	2.0	-
N1792	SAL 90/10	F	22/09/1990	Salt Rock	187	71	x	0	2.3	-
N1825	RB 90/20	M	10/11/1990	Richards Bay	223	131	x	x	7.5	Im
N1913	RB 92/29	F	13/07/1992	Richards Bay	189	74	x	0	3.5	-
N1934	RB 92/30	F	15/07/1992	Richards Bay	210	95	x	x	3.8	Im
N1935	RB 92/31	F	27/07/1992	Richards Bay	150	46	x	0	0.5	-
N1945	RB 92/25	M	13/08/1992	Richards Bay	200	90	x	0	3.0	-
N1950	ZIN 92/31	F	06/07/1992	Zinkwazi	248	176	x	x	14.2	Mat
N1951	RB 92/28	M	01/07/1992	Richards Bay	252	222	x	0	16.0	-
N1954	UVO 92/19	F	19/06/1992	Uvongo	177	63	x	0	2.5	-
N1962	RB 91/23	M	16/09/1991	Richards Bay	229	142	x	0	15.0	-

N1963	RB 91/24	M	21/10/1991	Richards Bay	197	92	x	0	3.0	-
N1978	ZIN 92/27	F	27/01/1992	Zinkwazi	240	178	0	x	-	Mat
N2059	RB 93/34	F	17/06/1993	Richards Bay	226	132	x	0	17.0	-
N2088	TIN 93/08	F	27/07/1993	Tinley Manor	178	68	x	x*	2.5	-
N2089	RB 93/37	M	19/07/1993	Richards Bay	213	110	x	0	7.2	-
N2090	RB 93/38	M	19/07/1993	Richards Bay	225	128	0	x	-	Mat
N2091	RB 93/39	F	26/08/1993	Richards Bay	194	67	x	x	3.9	Im
N2130	ZIN 93/36	M	22/10/1993	Zinkwazi	175	64	x	0	2.0	-
N2166	RB 92/35/3	F	27/07/1992	Richards Bay	157	47	x	x	1.5	Im
N2470	RB 95/47	F	30/06/1995	Richards Bay	220	140	0	x	-	Mat
N2490	MAR 95/04	F	28/12/1995	Marina Beach	183.5	79	0	x	-	Pb
N2607	ZIN 96/13	M	05/08/1996	Zinkwazi	197	88	x	0	2.5	-
N2608	ZIN 95/11	F	13/09/1995	Zinkwazi	237	150	x	x	7.9	Pb
N2609	SCO 396/37	F	22/02/1996	Scottburgh	201.5	118	x	0	5.3	-
N2611	RB 96/56	M	07/02/1996	Richards Bay	206.5	100	x	0	7.1	-
N2715	RB 71	M	-	Richards Bay	230	140	x	x*	17.0	-
N2742	ZIN 97/15	M	-	Zinkwazi	220	120	0	x	-	Im
N2743	ZIN 97/16	M	11/07/1997	Zinkwazi	254	210	0	x	-	Mat
N2766	RB 99/84	M	26/01/1999	Richards Bay	222	140	x	x	13.0	Mat
N2768	DUR 99/293	M	26/01/1999	Durban	276	280	x	0	16.7	-
N2832	ZIN 99/26	M	14/07/1999	Zinkwazi	255	277	x	0	17.0	-
N2844	RB 99/90	M	18/06/1999	Richards Bay	203	96	x	x	3.1	Im
N2845	RB 99/92	F	03/09/1999	Richards Bay	204	100	x	x	4.0	Im
N2847	RB 99/88	M	20/04/1999	Richards Bay	261	194	x	0	15.5	-
N2849	RB 99/86	M	31/05/1999	Richards Bay	192	78	x	0	3.0	-
N2862	BLY 00/19	M	06/01/2000	Blythedale Beach	255	214	0	x	-	Mat
N2870	RB 99/91	M	21/07/1999	Richards Bay	232	164	x	x	12.5	Mat
N2974	RB 02/96	M	12/08/2002	Richards Bay	172	62	x	0	2.5	-

N2991	RB 02/95	M	26/06/2002	Richards Bay	199	80	x	x	3.9	Im
N2992	ZIN02/ 36	F	15/07/2002	Zinkwazi	193	88	0	x	-	Im
N3050	RB 03/120	F	24/03/2003	Richards Bay	206	88	x	x	4.0	Im
N3052	ZIN 03/41	F	13/12/2002	Zinkwazi	210	98	0	x	-	Pb
N3092	RB 03/124	F	11/11/2003	Richards Bay	214	117	x	x	5.0	Pb
N3112	ZIN 04/54	M	24/05/2004	Zinkwazi	227	138	x	x	7.5	Mat
N3169	RB 04/133	M	07/09/2004	Richards Bay	199	90	x	x	7.2	Im
N3176	RB 04/136	F	11/11/2004	Richards Bay	202	90	x	0	4.1	-
N3177	BLY 04/29	M	22/10/2004	Blythedale Beach	235.2	102	x	x	9.7	Mat
N3178	DUR 04/472	M	15/10/2004	Durban	196	92	x	x	2.0	Im
N3259	RB 05/151	M	07/09/2005	Richards Bay	246.2	192	0	x	-	Mat
N3274	RB 06/161	M	06/06/2002	Richards Bay	167.4	60	x	x	1.5	Im
N3275	ZIN 06/75	F	23/08/2006	Zinkwazi	242.8	164	x	x	17.3	Mat
N3306	RB 07/168	M	13/02/2007	Richards Bay	191.8	84	x	x	4.3	Im
N3310	RB 07/170	M	26/03/2007	Richards Bay	232	170	0	x	-	Mat
N3311	RB 07/164	M	09/01/2007	Richards Bay	222.8	110	0	x	-	Im
N3312	RB 07/165	M	10/01/2007	Richards Bay	223.6	92	x	x	9.8	Mat
N3314	RB 07/172	M	10/05/2007	Richards Bay	212	82	x	x	5.0	Im
N3322	ZIN 07/89	M	25/06/2007	Zinkwazi	222.8	134.5	x	x*	12.5	-
N3323	RB 07/179	M	04/10/2007	Richards Bay	224.6	140	x	x	6.8	Im
N3324	RB 07/176	M	23/08/2007	Richards Bay	261.6	192	x	x	14.0	Mat
N3343	RB 08/184	F	10/03/2008	Richards Bay	241	-	0	x	-	Mat
N3348	RB 08/186	F	10/03/2008	Richards Bay	204.2	102	x	x	5.0	Im
N3360	RB 08/185	F	10/03/2008	Richards Bay	181.8	64	x	0	2.0	-
N3408	MG 08/108	F	28/07/2008	Margate	232	150	x	x	8.0	Mat
N3426	MG 08/09	M	28/07/2008	Margate	150	48	x	0	0.5	-
N3584	RB 10/03	M	29/04/2010	Richards Bay	231.8	150	x	x*	12.8	-
N4341	RB 10/04	M	17/09/2010	Richards Bay	211	108	x	x	4.0	Im

N4351	RB 10/06	F	02/08/2010	Richards Bay	222.4	130	x	x	8.7	Pb
N4542	-	M	14/04/2011	Unknown	210.6	122	x	x	9.0	Mat
N4644	RB 12/03	M	02/02/2012	Richards Bay	241	132	x	x	6.0	Im

*** indicates samples that were excluded/discarded**