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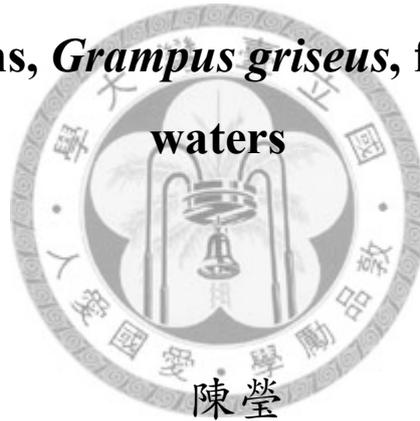
College of Life Science

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Master Thesis

台灣海域瑞氏海豚生長及骨骼成熟模式

**Patterns of growth and maturation of the skeleton in
Risso's dolphins, *Grampus griseus*, from Taiwanese**



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中文摘要

瑞氏海豚為一種中小型齒鯨，廣泛分布於全球溫帶及熱帶大洋水域，同時也是台灣東部海域最常見的鯨豚種類之一。由於他們的生活史資料闕如，本研究之目的為由判定牙齒年齡及頭骨、胸鰭骨骼成熟度，來探究台灣瑞氏海豚族群之年齡、體長及骨骼成熟度之間的關係。研究材料來自1994年至2008年間測量並解剖之死亡擱淺或誤捕致死海豚99隻（體長範圍124 – 290公分）。骨骼成熟度由67隻胸鰭（來自34隻海豚）的X光片顯影上橈骨（radius）及尺骨（ulna）末端軟骨骨化癒合（epiphyseo-metaphyseal fusion）程度，以及33顆處理過之乾燥頭骨上20個骨骼相接處（sutures/synchondroses/symphysis）癒合程度評估之。28隻海豚年齡透過牙齒組織切片的生長輪紋（growth-layer-groups, GLG）判定。初步結果發現台灣的瑞氏海豚在體長的成長模式上沒有明顯性別差異，雌性性成熟估計始於體長240 – 255公分，雄性則為253 – 265公分，雌雄性成熟年齡始於約10歲左右；性成熟體型略小於日本及南非的族群，而遠小於北大西洋族群。當海豚胸鰭長度停止生長時，尺骨及橈骨末端軟骨完成骨化癒合，此時其體長介於248 – 255公分，推測當時約為性成熟年齡。在胸鰭骨骼成熟過程中橈骨與尺骨末端的發育不完全同步，橈骨先熟於尺骨，但左右胸鰭及性別上並無先後差異。在頭骨方面，發育過程中背後側頭蓋骨（basioccipital-exoccipital synchondrosis and supraoccipital-exoccipital suture）先行癒合，而位於項脊（nuchal crest）之骨骼交接處（fronto-parietal, fronto-interparietal sutures）則較晚癒合；癒合過程也無左右差異。本研究建議頭骨項脊上骨骼交界、鼻骨與額骨前緣交界（rostral nasal-frontal suture）和上頷骨與前頷骨交界先端（distal maxilla-incisive suture）處癒合狀態可作為判定性成熟個體之指標，因這些癒合線在體長大於250公分的成熟海豚的頭骨中普遍都已進入高度癒合狀態。

關鍵字：瑞氏海豚，台灣，頭骨，胸鰭，牙齒，生活史，年齡鑑定

English Abstract

Risso's dolphins (*Grampus griseus*) are moderately small odontocetes, widely distributed throughout temperate to tropical pelagic regions of the world's oceans and are one of the most frequently encountered cetaceans in eastern Taiwanese coastal waters. Since their life history is poorly known, the goal of this study was to investigate the maturation of the skull and flipper bones, and to estimate age based on growth layers in tooth sections, and to determine the relationship between the age, body length and skeletal maturity for this Taiwanese population. Ninety-nine carcasses of dead-stranded or incidentally bycaught dolphins were measured and dissected during 1994 – 2008 (total body length, TBL: 124 – 290 cm). Skeletal maturity was assessed by a radiographic examination of the fusion of the distal epiphyseo-metaphyseal junction in the distal radius and ulna of 67 flippers (from 34 dolphins), and by an examination of the fusion stages of 20 sutures/synchondroses/symphysis on 33, cleaned dry skulls. Age was estimated by counting growth-layer-groups in routine histologically prepared tooth sections from 28 dolphins. Preliminary analysis revealed no sexual dimorphism in body length, and the onset of sexual maturity occurred at 240 – 255 cm in females and 253 – 265 cm in males, which was at about 10 years of age for both sexes. Body length at sexual maturity was slightly shorter than those from Japan and South African waters, but much shorter than those from northeast Atlantic Ocean. Cessation of longitudinal flipper growth in epiphyseo-metaphyseal ossification in the distal radius and ulna occurred at 248 – 255 cm TBL, about the age at sexual maturity. The distal radius closed earlier than distal ulna; bilateral asymmetry and sexual dimorphism was not detected. On the skull, the bones of the

caudoventral brain case fused early in development (basioccipital-exoccipital synchondrosis and supraoccipital-exoccipital suture), whereas fusion along the nuchal crest (fronto-parietal, fronto-interparietal sutures) occurred later; bilateral asymmetry of the fusion process was not detected. At TBL > 250 cm, advanced fusion occurred along the nuchal crest, rostral nasal-frontal, and distal maxilla-incisive sutures which may be useful indicators of sexual maturity.

Keywords: *Grampus griseus*, Risso's dolphin, Taiwan, skull, flipper, teeth, GLG, life history, age estimation



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Chapter 1: Introduction to Patterns of growth and maturation of the skeleton in Risso's dolphins, *Grampus griseus*, from Taiwanese waters

Risso's dolphins (*Grampus griseus*) are moderately small toothed whales, widely distributed throughout the temperate to tropical pelagic regions of all oceans (Kruse *et al.* 1999, Baird 2002). The type specimen of Risso's dolphin, from the western English Channel, was described by M. Risso to Cuvier in 1812, and later the osteology of the skull, vertebral column, and the thoracic limb was established through a detailed morphological description based on 2 female dolphins (3.2 m adult, 1.85 m immature calf) from the English Channel off southern England (Flower 1874). Afterwards, several studies also investigated skull and vertebral morphology, although the focus was mainly on the numbers of bones to aid in clarification of the taxonomic status of the species (True 1889, Mizue & Yoshida 1962, Ross 1984). To my knowledge, neither the development nor the maturation of the skeleton in Risso's dolphins has been published.

Knowing the age of individuals is the first step in constructing fundamental information to study developmental biology, life history, and population biology of any species under study. Age in odontocetes is generally determined by counting the growth layer groups in dentine or cementum in longitudinal sections of teeth (Kasuya 1976, Ross 1984, Hohn 1990). The annual deposition of growth layer groups in teeth has been confirmed in numerous species (Myrick *et al.* 1983, Hohn *et al.* 1989, Luque *et al.* 2007), and now has become the commonly acceptable assumption for odontocetes in general (Slooten 1991, Amano *et al.* 1996, Lockyer *et al.* 2001). Subsequent studies on

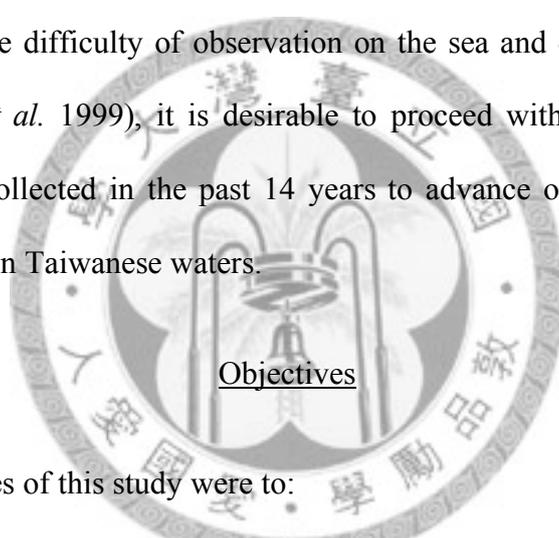
the age estimation of Risso's dolphins were also taken under this assumption (Ross 1984, Kruse 1989, Kruse *et al.* 1999, Amano & Miyazaki 2004).

Currently the life history of Risso's dolphins is poorly known, because Risso's dolphins seldom mass strand, and live in the offshore pelagic habitat where it is demanding for observations (Kruse *et al.* 1999). Limited studies report the age, body length and reproductive status of individual stranded Risso's dolphins (Paul 1968, Stroud 1968, Kruse 1989, Lawson & Eddington 1998), although a few contained enough specimens to estimate an age-growth relationship (Japan: Kasuya & Izumizawa 1981, Amano & Miyazaki 2004; South Africa: Ross 1984). The age of sexual maturity was inferred to be 8-10 years old and occurred at 270 cm in body length in Japanese specimens (Amano & Miyazaki 2004), however the Risso's dolphin in Taiwan has not been studied.

Risso's dolphins are considered to be plentiful in Spanish Mediterranean (de Segura *et al.* 2006), north Atlantic (Mullin & Fulling 2003), and Californian waters (Forney *et al.* 1995), and are one of the most common cetacean species encountered off the east coast of Taiwan (Chen 2001, Yeh 2001, Wang 2003). The overall abundance of Risso's dolphins in Taiwan waters is unclear, but it is estimated to have 218 dolphins (CV=29.39%) in Ilan waters (northeast Taiwan), and could be more in Taitung waters (southeast Taiwan) based on the relative occurrence of whale watching sightings (Chen 2001). Warm but deep-water environment such as the east coast of Taiwan is considered to be ideal habitat for Risso's dolphins feeding and nursing offspring (Yeh 2001), where the suspicion was supported by a previous study in Californian waters (Kruse 1989). Since they commonly consume cephalopods (Tsutsumi *et al.* 1961,

Wang 2003), Risso's dolphins are often encountered in squid fisheries, and where they are directly or incidentally killed (Kasuya & Izumizawa 1981, Chou 2007).

Risso's dolphins may also suffer from the impact of whale watching tourism (Bejder *et al.* 2006, Stensland & Berggren 2007), especially when the whale watching industry in Taiwan is rapidly expanding (Hoyt 2001), and these dolphins are one of the most frequently targeted species at the eastern and southeastern coastal waters (Chou 2007). Therefore it will be a basic requirement for the information on their basic biology in order to build a baseline data for further conservation and management programs. Due to the difficulty of observation on the sea and dead carcass specimen availability (Kruse *et al.* 1999), it is desirable to proceed with this study on limited samples have been collected in the past 14 years to advance our knowledge of these mysterious creatures in Taiwanese waters.



Objectives

The specific objectives of this study were to:

1. review the literature on the life history of Risso's dolphins and in particular their tooth age, body growth, reproductive status, and distribution; make histological examination of growth layer groups in tooth sections to estimate the age of each dolphin, and to analyze the relationship between body length and estimated age; (Chapter 2)
2. review the literature on the maturation of flipper skeleton in odontocetes; examine the stages of epiphyseo-metaphyseal maturation of the distal radius and ulna in radiographs of flippers of Risso's dolphins, and correlate the maturity to the body length and estimated age information; (Chapter 3)
3. review the literature on the maturation of skull skeleton in odontocetes; examine

the degree of fusion between the bony elements as seen on dry, cleaned skulls of Risso's dolphins preserved in museum, and analyze the relationship between the skull maturity, body length and estimated age; (Chapter 4).



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Chapter 2: Estimation of age and growth in Risso's dolphins, *Grampus griseus*, from Taiwanese waters

ABSTRACT

Risso's dolphins (*Grampus griseus*) are widely distributed throughout temperate to tropical pelagic waters of the world and are one of the most frequently encountered cetaceans in eastern Taiwanese coastal waters. Since their life history is poorly known, the goal of this study was to investigate the relationship between age, body length, and sexual maturity of Risso's dolphins stranded or bycaught in Taiwanese waters. Ninety-two carcasses of dead-stranded or incidentally killed dolphins (1994 – 2008) were measured and dissected (total body length 124 – 290 cm); sexual maturity was assessed in 32 dolphins; and age was estimated by counting dentinal growth layer groups in routine histologically prepared tooth sections of 28 dolphins. Sexual dimorphism in TBL growth was not detected. The onset of sexually maturity occurred at 240 – 255 cm in females and 253 – 265 cm in males, which was at about 10 years of age for both sexes. Based on stranding and bycatch records, Risso's dolphins likely occur year-round in Taiwanese waters, and have a summer calving season. The similar range of life history parameters and calving season in those from Japanese waters likely indicates a common west Pacific population of Risso's dolphins.

Key words: body length, TBL, age-estimation, teeth, GLG, sexual maturation, seasonality, Risso's dolphin, *Grampus griseus*, Taiwan

INTRODUCTION

Risso's dolphins (*Grampus griseus*) are moderately small toothed whales, widely distributed throughout the temperate to tropical pelagic regions of all oceans, although their life history is poorly known (Kruse *et al.* 1999, Baird 2002, Taylor *et al.* 2008). Since Risso's dolphins seldom mass strand, and live in the offshore pelagic habitat where it is demanding for observations (Kruse *et al.* 1999), there are few life history studies documenting age, body length, and reproductive status for this species (reviewed in Perrin & Reilly 1984, Kruse *et al.* 1999).

Knowing the age of individual animals is the first step in constructing fundamental information to study developmental biology, life history, and population ecology of any species under study. Age estimation in marine mammals has been usually conducted by examining the numbers of growth layer groups (GLG) in tooth sections (Hohn 2002). Tooth sections from approximately 100 Risso's dolphins from four major oceanographic regions of the world have been examined with some success to estimate the age (Stroud 1968, Ross 1984, Kruse 1989, Amano & Miyazaki 2004, Mazzatenta *et al.* 2005) (Table 2.1). The validation of annual deposition of GLG in Risso's dolphins through tetracycline labeling of teeth, examination of teeth from captive Risso's dolphins, or through the investigation of the seasonal accumulation pattern of GLG has not been scrutinized. However, based on previous investigations in related delphinids (Myrick *et al.* 1983, Myrick *et al.* 1988, Hohn *et al.* 1989, Myrick & Cornell 1990, Lockyer 1993), the current consensus has established one GLG per calendar year in odontocetes and thus the expectation that GLG deposition in Risso's dolphins follows

this typical manner (Kim *et al.* 1996, Kruse *et al.* 1999, Frodello *et al.* 2000, Amano & Miyazaki 2004, Mazzatenta *et al.* 2005).

Various microscopic techniques had been employed on the teeth of Risso's dolphins to gain satisfying resolution of GLGs for age estimation. Undecalcified thick sections (200-300 μm) examined under reflected or transmitted light were used in an early study (Ross 1984), although decalcification techniques are now more commonly used. Decalcification by commercial acid, sectioning at 25 μm with a cryostat microtome, and staining with Mayer's hematoxylin was used for age-estimation of Risso's dolphins from Californian waters (Kruse 1989 – based on technique of Myrick *et al.* 1983). In more recent studies on Risso's dolphins from Japanese waters the teeth were decalcified with formic acid, after they were prepared to 40-50 μm by diamond saw and polishing by whetstones, and then stained with Mayer's hematoxylin (Kim *et al.* 1996, Amano & Miyazaki 2004). Although the effects of these different histological techniques on the results of age estimation remain unclear in Risso's dolphins, in other delphinids, the differences between various technical modifications including decalcification or not (Kasuya 1976), frozen-sectioning or paraffin-embedded sectioning (Luque *et al.* 2005), and different dyes (Molina & Oporto 1993), resulted in GLG counts that were largely similar under most circumstances.

The age of sexual maturity in Risso's dolphins from Japanese waters is 8-12 years of age and occurs at 270 cm in body length (Amano & Miyazaki 2004). However, Risso's dolphins from Taiwanese waters have not been investigated. The aim of this study was to examine life history traits of Risso's dolphins in Taiwanese waters including their reproductive status and growth, and in particular to make histological

examinations of the growth layer groups in sectioned teeth to estimate their age, and to analyze the relationship between body length and estimated age.

MATERIALS AND METHODS

Specimens

Carcasses from 92 dead Risso's dolphins that either stranded on the coast or were incidentally caught in the local fishery of Taiwan (1994 – 2008) were examined. There were 43 female, 47 male, and two of undetermined sex, with total body lengths (TBL) of 124 – 290 cm; 75 dolphins were from the east coast (Appendix 2.1). Standard external morphological data including TBL, length from tip of upper jaw to center of anus (Rostrum – Anus Length, RAL), sex, and date of salvage, were recorded during the necropsy (Norris 1961).

Thirteen dolphins (7 female, 6 male) were salvaged with amputated caudal regions – thus a complete TBL could not be measured. A power regression equation was used to estimate the full body length: $y = ax^b$, where y = estimated TBL, x = RAL, and the constants a , b , were derived from dot pairs of known TBL and RAL. To minimize any potential sexual dimorphism in length measurements, separate estimations with different equations, were made for male and female.

Sexual Maturity Assessment

Reproductive organs were examined for sexual maturity in 14 female and 18 male dolphins: this assessment was based on the veterinarian's inspection during necropsy. Females were classified as sexually mature if either milk was found in a mammary

gland or a corpus luteum or corpus albicans was present in an ovary on gross visual examination. Males were classified as sexually mature if either testicular fluid was detected in a testis, or if a testis weighed more than 300 g (Kasuya & Izumizawa 1981, Amano & Miyazaki 2004), or if it measured more than 40 cm in length (Ross 1984).

Age Estimation – Tooth Sectioning

Age was estimated in 28 dolphins (12 female, 14 male, 2 unknown sex) by determining the number of growth layer groups (GLG) in routine histological preparations of longitudinally sectioned teeth. At necropsy, a tooth was extracted from the lower jaw, either left or right side, usually the second or third tooth¹, selecting one with the least worn-off crown, and then stored in 70% ethanol. The smaller teeth with wide-open pulp cavities were not slab-sectioned. Larger teeth, in which the pulp cavity was closed, or almost so, were slab-sectioned with a low-speed diamond saw (Buehler Isomet). These teeth were mounted in a commercial plastic resin (Epofix²) before a 2 – 3 mm longitudinal slab was sawn from the central axis of the tooth. The small teeth and the slabs were decalcified by a commercial histological decalcifying agent (RDO³) following an established protocol (Myrick *et al.* 1983) but modified by first pre-washing specimens under running water for at least six hours to remove the ethanol. Decalcification time varied: the smaller teeth took 4 – 6 hours; the slabs took up to 14 hours depending on thickness and size. The end point of decalcification was determined by testing the solution with an equal volume of 5% ammonium hydroxide and 5%

¹ Risso's dolphins in Taiwanese waters typically had three to four teeth in each side of tooth row.

² Electron Microscopy Sciences, P.O. Box 550, 1560 Industry Road, Hatfield, PA 19440, USA

³ Apex Engineering Products Corporation, 1241 Shoreline Drive Aurora, IL 60504, USA

ammonium oxalate (Luna 1992). Decalcified specimens were rinsed under running tap water for at least three hours to remove residual RDO, and then stored in 70% ethanol.

Decalcified tooth specimens were processed for microscopy by a routine paraffin-embedding histological method, sectioned at 8 μm (Luque *et al.* 2005), stained by Mayer's hematoxylin, and mounted in 100% glycerin on standard glass slides. Growth layer groups in the dentine were examined with a low-power binocular dissection microscope and assessment was based on an established protocol in bottlenose dolphins (Hohn *et al.* 1989). To avoid empirical bias, each slide was examined at least twice, with a 10-day interval between the first and second examinations. If the GLG counts were different, the slide was reexamined and or reviewed by a second reader.

Each dolphin was classified into a "year-age class" based on the readable dentinal GLG in its sectioned tooth. Only complete GLGs were counted; developing and incomplete layers lining the pulp cavity were not counted as a single GLG. One GLG was taken to represent one calendar year of age.

Data Analysis

Statistical software Microsoft Office Excel 2003 (Microsoft corporation) and R (version 2.5.0, <http://www.r-project.org/>) were used to establish a database, and to perform comparison, correlation coefficient detection and regression model construction. Pearson's moment correlation coefficient (r) was determined for TBL-RAL correlation; tests were considered to be statistically significant when $P > 0.05$.

RESULTS

Calculation of Missing TBL

Seventeen dolphins had incomplete tails and thus incomplete TBL. To estimate TBL, separate male and female regression models were constructed from known TBL and RAL of 35 female and 40 male intact dolphins (Appendix 2.1). The correlation coefficient (r) for TBL-RAL correlation in these males and females was 0.99, with significant association ($P < 0.01$, Pearson's moment correlation coefficient). The power regression equations for RAL estimating TBL in female was $y = 1.4914x^{1.004}$, and for male was $y = 1.3809x^{1.0199}$ (Fig. 2.1). The coefficient of determination (R^2) for both regression lines was 0.99, indicating a high level of probability in correctly estimating TBL from RAL. Hence, the 13 dolphins (seven female, six male) with missing tails had their TBL estimated by these equations and these TBLs were used in subsequent analyses (Appendix 2.1).

TBL Distribution

The range of TBL in the 43 females was 143 – 289 cm, with most of them (83 %) measuring within the length of 140 – 200 cm (43%) or 250 – 290 cm (40%) (Fig. 2.2A). Likewise, the range of TBL in the 47 males was similar to that in the females, being 124 – 290 cm, with most of them (76%) between 140 – 190 cm (43%) and 250 – 280 cm (33%) (Fig. 2.2B). Note the shortest male was estimated at 124 cm from his RAL, and the second shortest male had a known TBL of 132 cm.

Sexual Maturity Assessment

Fourteen females were assessed for sexual maturity (150 – 284 cm): six were immature (150 – 255 cm), and eight were mature (240 – 289 cm) (Table 2.2). In these dolphins the range of TBL for onset of sexual mature was 240 – 255 cm; the shortest sexually mature female minimally had four ovarian corpora albicantia (one in left, three in right ovary), as well as milk in her mammary glands. The majority of corpora occurred in the right ovary: in five of the seven females more were found in the right than in the left, in one there was equal number in both (seven), and in one other female she only had one corpus luteum in her left ovary. Moreover, corpora were present in all ovaries examined in females that had milk in their mammary gland. Six of eight mature females were lactating; three were incidentally caught in early summer (June / July), two in fall (October), and one in early spring (February).

Eighteen males were assessed for sexual maturity (TBL 148 – 290 cm). Testis length (8.1 – 65 cm) increased with TBL (Fig. 2.3; Table 2.3). There were 11 dolphins classified as sexually immature: 10 of these had short testis lengths (8.1 – 17.5 cm) and also short body lengths (148 – 265 cm); in seven of these 10 dolphins (with testis length measurement) and one other (without testis length measurement) their single testis weight was below 40 g. In the longest sexually immature dolphin (TBL 265 cm), the testis length was 13.7 cm, and in the next longest dolphin (229 cm) its left testis was 13.5 cm and weighed 39.7 g.

Seven others males (TBL 253 – 290 cm) were classified as sexual mature based on their longer testis lengths (43 – 65 cm), with three of these had single testis weights of 980 g, 2650 g, and 3100g. One dolphin (TBL 278 cm) had a low single testis weight of 280 g, but it was 51.4 cm long and testicular fluid was present on the cut surface of the

testis at necropsy, therefore he was considered sexually mature. The shortest sexually mature male was 253 cm with a 55 cm testis length; the next shortest mature male was 254 cm with a testis 49 cm long and weighing 2650 g.

Seasonality

Distribution of all stranded and bycatch dolphins by month of year demonstrates that Risso's dolphins occur in Taiwanese waters year-round, although fewest dolphins were salvaged in May ($n = 2$), August ($n = 2$) and September ($n = 1$) (Fig. 2.4). Sampling was neither systematic nor consistent throughout the year and thus this seasonal distribution was interpreted with caution. The three shortest dolphins were recovered during the summer months of June and July (TBL 124 – 136 cm): the shortest dolphin (124 cm) had a flaccid dorsal fin and fetal fold marks were present on another small dolphin (136 cm). Five slightly larger dolphins (TBL 143 – 150 cm) were salvaged three months later in October, although the conditions of their dorsal fins or trunk skin were not recorded.

Age Estimation

The stained teeth sections revealed the key morphological landmarks including dentinal layers (GLGs) (Fig. 2.5 – 2.7). These reference characteristics included the unstained, or poorly stained, neonatal line present in the crown of the tooth (Fig. 2.5), which separated the outer-most prenatal dentine from the rest of the tooth, and internal to this, the postnatal dentine of alternating stained sequential bands deposited in GLGs around the central gap of the pulp cavity (Fig. 2.6).

Twelve female dolphins (TBL 154 – 275 cm) had estimated ages of 0 – 14 years (Table 2.4, Fig. 2.10). The youngest, and also the shortest female (154 cm), salvaged in August, had a clear neonatal line and a well defined postnatal dentine layer approximately two-thirds the width of the prenatal dentine layer (Fig. 2.5). Six other females (TBL < 230 cm) were all younger than 5 years old (Fig. 2.6); three longer females (TBL at 240 – 265 cm) were estimated to be 11 – 13 years old. The longest female (275 cm) had an estimated age of 14 years: this was also one of the oldest estimated age in the females (the other one was 272 cm, 14 year-old), and even though her tooth slab was not fully decalcified and the section was damaged during histological preparation, a full crown with neonatal line was present and the pulp cavity was identified and not obliterated by dentine. Sexual maturity was not examined directly in any of these 12 females.

The TBL range in 14 age-estimated males was 150 – 290 cm, and the age range was 0 – 34 years old (Table 2.4). The three short males (TBL 150 – 173 cm) had estimated ages of less than 1 year: the shortest of which was a bycatch in late October and its neonatal line was difficult to identify since there was almost no postnatal dentine deposited; the other two infant males were caught in December and January, their neonatal line was recognizable and the postnatal dentine was about half the width of the prenatal dentine. Four other short males (TBL 162 – 191 cm) had estimated ages of 0 – 1 year old. One longer male (TBL 234 cm) was estimated to be six years of age. Seven males with longer TBL (TBL 248 – 290 cm) were nine or more years of age. The oldest, also the longest male (TBL 290 cm), had an estimated age of at least 34 years (Fig. 2.7). The pulp cavity in this male was recognized as a small fissure enclosed by dentine and there were 34 readable GLGs in this tooth section. The crown of this tooth

was worn off however, and consequently the neonatal line could not be identified, thus the age of this dolphin was likely under-estimated. In two other long males (TBL 269, 267 cm) the crown of their tooth was also worn off and neither the neonatal line nor the pulp cavity could be identified. Their estimated ages were minimally 14 and 16 years respectively. Out of 18 males with sexual maturity identified, the age was examined in only four of them: two immature males were age 0 (162 cm) and age 6 year (234 cm), and two others were sexually mature at age 14+ (269 cm) and age 34 years (290 cm).

DISCUSSION

For the first time, vital growth and maturation life history parameters based on body length, estimated-age, and sexual maturity data of the Risso's dolphins off the coast of Taiwan have been established through this study of 92 dolphins collected over 14 years.

TBL Distribution and Population Identity

Risso's dolphins sampled from Taiwanese waters ranged in body length from 124 – 290 cm (females, 143 – 289 cm; males, 124 – 290 cm), included neonates to mature adults, and as a group was closely similar to those from Japanese waters (Table 2.1: Mizue & Yoshida 1962, Kasuya & Izumizawa 1981, Kim *et al.* 1996, Amano & Miyazaki 2004) with females at 180 – 289 cm and males at 146 – 309 cm, which all came from a directed harvest. Lack of shorter females in these Japanese studies probably resulted from a sampling bias (see below). With the exception of five large males (TBL 290 – 309 cm) caught off Iki Island in the eastern Korean Strait (Kasuya & Izumizawa 1981) and one live-stranded male (310 cm) in northern Taiwan (Yang &

Chou 2001), the Risso's dolphins from Japanese waters and those in this current study from Taiwanese waters are all equal to or shorter than 290 cm TBL, with a mode about 250 – 270 cm (Fig. 2.2) (Amano & Miyazaki 2004). It has been suggested that Risso's dolphins in Japanese waters are shorter than those in other areas, although this whaling data may be biased by sampling method (direct catch vs. stranding) (Amano & Miyazaki 2004).

Nevertheless, Risso's dolphins from the Mediterranean Sea (Pilleri & Gahr 1969), and the east and west Atlantic Ocean are the longest on record (median TBL 325 cm) (Ross 1984), and those stranded from the west Indian Ocean being significantly shorter (275 cm) (Ross 1984). Based on these current data Risso's dolphins in the west Pacific Ocean are on average shorter than those from other oceanographic regions (Table 2.1).

Classical morphological studies suggests that Risso's dolphins from the various oceanographic regions (Atlantic and western Indian Oceans, Mediterranean Sea) have significant similarity of skeletal characters and therefore should be grouped as a single species (Rice 1998), although at this time a world-wide review is unavailable, impeded by limited information on specimens from the west Pacific (True 1889, Ross 1984). Even so, based on morphological analysis of few skeletons, Risso's dolphins can be separated into different populations within or between different ocean basins (Mizue & Yoshida 1962, Kruse *et al.* 1999, Baird 2002, Amano & Miyazaki 2004). Furthermore, this regional differentiation is supported by a recent genetic study that examined microsatellite DNA markers and mitochondrial DNA sequences from dolphins from UK waters (Western Isles, Scotland) and the Mediterranean Sea, revealing clear population separation and a lower genetic diversity of those in UK

waters (Gaspari *et al.* 2007). Even so, sequence variation in the cytochrome *b* gene between a single east coast and a west coast United States Risso's dolphin was comparatively low (0.2%) (LeDuc *et al.* 1999). Morphological and genetic/molecular aspects of Risso's dolphins in Taiwanese waters await study and should help clarify their population status, and the possible uniqueness of the west Pacific population.

Further suggestive support for the Risso's dolphins off the east coast of Taiwan being part of a larger west Pacific Ocean group, and thus sharing affinity with those from Japanese waters, comes from these two groups having similar life history parameters – their TBLs and ages of attaining sexual maturity are similar in females and males (see below), but both are similarly dissimilar to those from other oceanographic regions. Low re-sighting rates of photo-identified Risso's dolphins off east coast of Taiwan suggest their distribution range is wider than the studied area⁴ (Kuo 2002, Lin 2003), and the potential for north-south migration of Risso's dolphins between Japan and Taiwan has been demonstrated by one rehabilitated adult male (310 cm) Risso's dolphin released from northern Taiwan that was sighted two weeks later off the Ryukyu Islands 770 km from its release site (Yang & Chou 2001). Risso's dolphins around the south-eastern United States have been tagged as traveling more than 3300 km from the Gulf of Mexico northward into the western North Atlantic Ocean (Wells *et al.* 2009). The wide-ranging movements of Risso's dolphins in the west Pacific Ocean remain unknown. Further information from individual photo identification and molecular phylogeny would assist in understanding the residency of Risso's dolphin in Taiwanese waters and their relationship with others in the west Pacific Ocean.

⁴ Kuo 2002: southeastern coast (22°85' - 23°30' N, 121°2' - 121°4' E – as from map); Lin 2003: central-east coast (23°16' - 23°44' N, 121°24' - 121°44' E). These two studies did not compare their photographs to check identifications for any dolphins between study sites.

Sexual Maturity

The onset of sexual maturation in female Risso's dolphins from Taiwanese waters was at TBL of 240 – 255 cm. Because none of the females examined for sexual maturity had teeth available for age estimation, the available plots of age and TBL from other individuals suggested that females of 240 – 250 cm were about 10 years of age (Fig. 2.10). Female Risso's dolphins from Japanese waters also reach sexual maturity at estimated ages of 8 – 10 years (Amano & Miyazaki 2004), one from the east Pacific Ocean was sexually mature at an estimated age of 8 – 9 years (Kruse 1989), and two sexually mature females from the western Indian Ocean were 9+ and 17+ years of age (Ross 1984). These data, which are consistent with those found in Taiwanese dolphins, indicate that the onset of sexual maturity in Risso's dolphins is likely to occur at 8 – 10 years of age. The length at sexual maturity in females, conversely, is not consistent amongst the oceanographic regions. Those from the Atlantic Ocean reach sexual maturity when greater than 300 cm (Perrin & Reilly 1984), those from the western Indian Ocean at 277 cm (Ross 1984), and those from Japanese waters at 265 – 270 cm (Amano & Miyazaki 2004), which is a little longer than those from Taiwanese waters in this study (TBL 240 – 250 cm). Among the various studies in which age and maturity were examined, those from Japan and Taiwan were the only two with reasonable sample size ($n > 40$ for each sex), whereas in other studies the number of specimens of each sex is less than ten. Currently, these conclusions are restricted by sampling biases for oceanographic or regional comparisons.

From the male aspect, the onset of sexual maturity of Risso's dolphins from Taiwanese waters was at TBL of 250 – 265 cm, although estimated age of onset of

sexually maturity was not determined due to limited sample size ($n = 4$) and records on reproductive organs. The oldest sexually immature male had an estimated age of 6 years (TBL 234 cm), and youngest sexually mature had an estimated age of 14+ years (TBL 269 cm). Moreover, males older than 9 years revealed a wide individual variation in TBL (248 – 290 cm) that was not consistent with increasing age (Table 2.4). Partial information from other regions is available on the age of onset of sexual maturity for male Risso's dolphin. Those from Japanese waters attain sexual maturity at 10 – 12 years of age (TBL *ca.* 270 cm) (Amano & Miyazaki 2004). Limited data suggests an estimated age at sexual maturity of Risso's dolphins in Taiwanese waters of about 10 years of age, although the TBL at sexual maturity reported in these dolphins from the present study (250 – 265 cm) is shorter than those from Japanese waters (*ca.* 270 cm) (Amano & Miyazaki 2004), and shorter than those from the western Indian Ocean (260 – 300 cm), and a supposedly sexually mature “adult length” of 260 – 360 cm suggested for Risso's dolphins in the Mediterranean Sea (Pilleri & Gahr 1969).

Longevity

Longevity of Risso's dolphins is seldom reported. In the present study the oldest was a male at 34 years (290 cm) – the oldest female was 14 years (275 cm). Similar ages are known for Risso's from Japanese waters – the oldest was a female at 34.5 years and a male at 16.5 years of age (from a single school harvest) (Amano & Miyazaki 2004), while a single stranded male Risso's dolphin (361 cm) from the east Pacific Ocean had an estimated age of 30+ years (Kruse 1989) (Table 2.1). In addition, one, well-documented famous, wild Risso's dolphin, Pelorus Jack, from New Zealand, who regularly accompanied steam ships over a particular stretch of coastal water for 24 years (1888 – 1912) and was reliably estimated to be 335 – 365 cm TBL (Alpers 1961).

Another long-lived Risso's dolphin was "Yon," a female held in the Enoshima Aquarium, Japan, until her death (1961 – 2003), and who was estimated to be at least 43 years old (Hori 2008). In combination, these reports offer strong support that both male and female Risso's dolphins do live to at least 30 years of age (Baird 2002).

Seasonality

Risso's dolphins occur year-round in Taiwanese waters, although the speculation is based on intermittent salvage of beach strandings and incidental bycatch survey, they were least abundant during May, August, and September. Likewise, Risso's dolphins were present off-shore along the central-eastern coast of Taiwan from April to September (survey period), and group sizes were largest during summer months (Lin 2003). Together, these findings indicate a year-round occurrence of Risso's dolphins in Taiwanese waters. Risso's dolphins are also sighted year-round in the tropical-temperate waters of the northeastern Pacific and western Atlantic Oceans, UK waters and around the Azores (Leatherwood *et al.* 1980, Kruse *et al.* 1999, Hartman *et al.* 2008, Pereira 2008). Those in Azorean waters show high site fidelity (63% resighted, one-third resighted each year over a 3-year photo-ID survey period) suggesting they may be part of a resident local population (Hartman *et al.* 2008). On the other hand, only 9% of Risso's dolphins identified in eastern Taiwanese waters were resighted during at least two consecutive months (May – September) (Lin 2003), and a similar low resighting rate (5%) was reported in adjacent southeastern Taiwanese waters (July – September) (Kuo 2002). Long-term observations over more inclusive study areas are needed to evaluate the residency of Risso's dolphins in Taiwanese waters.

Risso's dolphins are considered migratory in Japanese waters (Kruse *et al.* 1999). Catches from whaling studies along the west coast of Kyushu suggest that these Risso's dolphins have a summer "parturient migration" and a winter "feeding migration" (Tsutsumi *et al.* 1961, Mizue & Yoshida 1962), although the migration direction and locale were not indicated. Water temperature might be a critical factor influencing the distribution and migration of Risso's dolphins (Davies 1963, Kruse *et al.* 1999), since they were "frail" when in water temperatures under 10°C (Nishiwaki 1965). If Risso's dolphins in Taiwanese and Japanese waters are from one single western Pacific population (see above), its distribution could vary during different seasons with a core distribution area in the west tropical Pacific near Taiwan and away from Japanese waters in winter months — further field studies including long-term photo identification and population genetics are required to test this hypothesis.

Calf Size and Calving Season(s)

In dolphins, a flaccid dorsal fin and fetal folds on the body are indicators for newborns, although fetal-fold marks may persist longer after birth than the flaccidity of the dorsal fin (Mead & Potter 1990). Two Risso's dolphins from Taiwanese waters had evidence of their newborn status: the shortest (124 cm) in July had a flaccid dorsal fin, and the third shortest (136 cm) in June had fetal folds, which corresponds with field observations of most calves and mother-calf pairs being sighted between June and September (Kuo 2002, Lin 2003). This very limited data tentatively suggests a calving season in summer for Risso's dolphins in Taiwanese waters.

Based on the longest fetuses recovered from Japanese female Risso's dolphins caught in July, a birth length of 110 – 120 cm was suggested (Mizue & Yoshida 1962),

although another calculation based on a survey of fetal lengths suggested a birth length of 130 cm (Kasuya 1985, cited in Amano and Miyazaki 2004). The shortest Risso's dolphin hunted in Taiji, Japan, in November, was 146 cm TBL, and by back-calculating based on a proposed fetal growth rate (Huggett & Widdas 1951) a natal length of 130 – 140 cm is suggested, with a calving season off Japan during July to October (Amano & Miyazaki 2004).

The shortest, tooth-age estimated dolphin from Taiwanese waters (150 cm) was salvaged in late October, and had very little postnatal dentine deposited in its sectioned tooth. In the same way, the shortest dolphin (146 cm) caught off Taiji, Japan, in November, likewise had very little postnatal dentine, confirming its neonatal age (Amano & Miyazaki 2004).

Taken together, these reports with the data from Risso's dolphins from Taiwanese waters, support a summer – fall calving period in the west Pacific Ocean (Mizue & Yoshida 1962, Amano & Miyazaki 2004). A similar July – August calving season is reported in Azorean Islands (Pereira 2008), conversely, a fall – winter calving season is reported for Risso's dolphins along the California coast (Kruse 1989).

Sexual Dimorphism and Reproductive Strategies

The limited data in this study projected an asymptotic length for Risso's dolphins in Taiwanese water at 260 – 300 cm, with no clear difference between females and males. Additionally, a difference in TBL or age of onset sexual maturation was not detected between males and females, although there was a trend that males were slightly longer than females at sexual maturity. Furthermore, Risso's dolphins from Japanese waters

have a similar, although better defined, estimated asymptotic length at about 270 cm for both males and females and a similar length at sexual maturity in both sexes, although females reach sexual maturity at an earlier age (8 – 10 years) than males (10 – 12 years) (Amano & Miyazaki 2004). In general, these two studies on western Pacific Risso's dolphins support the notion that there is no significant sexual dimorphism in body length (Kruse *et al.* 1999), and that there may be some wide range in age and length at sexual maturity in both sexes.

The apparent lack of sexual dimorphism in body length and differences in age and length at sexual maturity, suggests that Risso's dolphins might have an atypical reproductive strategy. In other medium- to large-sized odontocetes, typically the males are larger than the females in body size (Kasuya & Marsh 1984, Read *et al.* 1993, Murphy & Rogan 2006) — larger body size offers benefit in intraspecific male-male aggression for access to reproductive females and female monopolization. Alternatively, some species that are monomorphic in body length usually have large testes and engage in multiple-male mating behaviors, and it thus is suggested that such species employ a sperm competition mating strategy (Ralls & Mesnick 2002). Male short-beaked common dolphins (*Delphinus delphis*) in the western North Atlantic have a moderate degree of sexual dimorphism in body length: they are slightly longer and slightly older age at sexual maturity than females, and during the breeding season they have remarkably large testes (combined testes weight at 2.3 – 4.4% of body weight) (Westgate & Read 2007), which is of comparable high relative weight with other cetaceans proposed to have sperm competition strategy (Murphy *et al.* 2005, Neuenhagen *et al.* 2007). Similar to common dolphins, Risso's dolphins showed mild degree of sexual dimorphism (no significant TBL difference between both sexes,

slightly older age of males at sexual maturity), relatively large testis size (43 – 86 cm, 15.9 – 26.5 % of TBL) (Present study, Orr 1966, Ross 1984) and high testis weight in mature males (one male in current study had an estimated combined testes weight of about 3% of body weight), and likely seasonality of breeding (see above), could suggest these two species share a similarity of reproductive strategy, *i.e.*, both feature sperm competition as part of the male mating strategy.

Nevertheless, the extensive tooth-mark scarring that characterizes older male Risso's dolphins seems conspecific in origin (Kruse *et al.* 1999) and likely results from male-male aggression (fighting) (MacLeod 1998). Male Risso's dolphins form long-term highly stable "clusters" ($n = 3 - 12$) with cooperative behaviors (Hartman *et al.* 2008), which could function in female monopolization behaviors as seen in reproductively active male bottlenose dolphins in Shark Bay, Australia (Scott *et al.* 2005). This may be contradictory to the concept of sperm competition strategy. Nevertheless, cluster formation in male Risso's dolphins is thought to have evolved due to three, separate, but collaborative, adaptive benefits: first is for foraging cooperation, second is for access to reproductive females, and a third and new benefit is "habitat defense" with inter-cluster and inter-species competition (Hartman *et al.* 2008). The extent of dimorphic coloration between the sexes however, and its potential advantage is still unclear (Kruse *et al.* 1999, Hartman *et al.* 2008). Although this current study and other reports on the biology of Risso's dolphins add support to the behavioral observations in Azores that Risso's dolphins feature a sperm competition based reproductive strategy, further behavioral studies, especially of intra- and inter-sexual interactions and mating seasonality, physiological studies in reproductive biology, and a mechanistic understanding of the development of skin coloration in Risso's dolphins are

needed to shed more light on the apparently odontocete unique reproductive strategy in Risso's dolphins.

Data Gap

The distribution of dolphin TBL was discontinuous with a separation into a short class (120 – 200 cm) and a long class (250 – 290 cm). There was a paucity of both females and males in the gap of 200 – 250 cm. This data gap was across TBL of the most possible TBL for the onset sexual maturation in these Risso's dolphins from Taiwanese waters. A similar TBL gap (215 – 245 cm) existed in a group of 43 Risso's dolphins harvested in a whale fishery off Japan (Kasuya & Izumizawa 1981). In addition, a similar pre-pubertal gap in TBL distribution was reported in Risso's dolphins from the Mediterranean (Razzauti 1910, summarized in Pilleri & Gahr 1969) and in bycatch common dolphins off California (Hui 1979). This data gap in TBL corresponds to a gap in age: lack of females at from 5 – 10 years of age and males from 2 – 8 years of age in the present study with limited sample size. Another group harvested off Japan had few dolphins of 5 – 10 years old (but did not have TBL gap in specimens). This gap was considered due to the pubertal male and female dolphins leaving their natal school after about 5 years of age, and therefore not being caught in this harvest of a single school (Amano & Miyazaki 2004). More recent observations however, reveal that male Risso's dolphins around the Azores Islands do not disperse from their natal grounds (Hartman *et al.* 2008), although the mechanism to avoid interbreeding is unknown.

Limitations of Study

The major limitation in this study was the discontinuous distribution of dolphins over the full range of known body lengths. This was particularly limiting because of the small numbers of dolphins salvaged over the probable TBL range of onset of sexual maturity, while considering the importance of this parameter in attempting to establish a specific life history model for Risso's dolphins in Taiwanese waters. In addition, the lack of consistent examination of reproductive organs and tooth collection from earlier years constrained satisfactory analyses on age-dependent body growth and reproductive status. These age and TBL conclusions in this study are thus to be viewed with caution until further studies can be based on a larger, fully sampled, database of Risso's dolphins from all Taiwanese waters.

Although estimation of age in odontocetes based on dentinal GLGs is well established (Klevezal' & Kleineberg 1967, Klezeval' 1980, Myrick *et al.* 1983, Hohn 1990, Hohn 2002), some individual Risso's dolphins in this study may have underestimated ages due to particular processing difficulties. Parallel study of cemental GLGs may provide additional confirmatory data, especially in "older" dolphins with worn crowns (Kasuya 1976, Myrick *et al.* 1983, Kasuya & Matsui 1984, Myrick & Cornell 1990).

FIGURES WITH LEGENDS

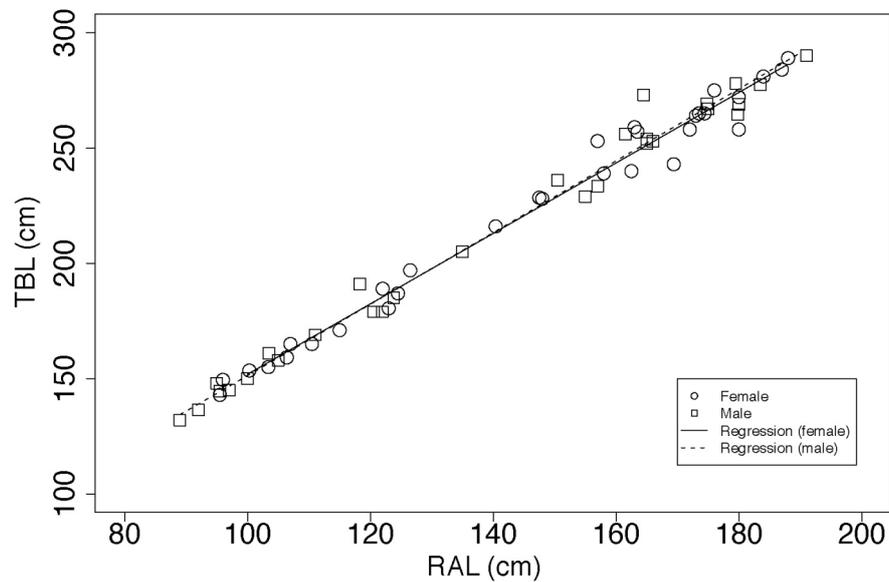


Figure 2.1. Relationship between length from tip of upper jaw to center of anus (Rostrum – Anus Length, RAL) and Total Body Length (TBL) in 31 female and 29 male intact Risso's dolphins from Taiwanese waters. Solid line indicates power regression equation for females ($TBL = 1.4914 \times RAL^{1.004}$) and dashed line for males ($TBL = 1.3809 \times RAL^{1.0199}$; $R^2 = 0.99$ for both sexes).

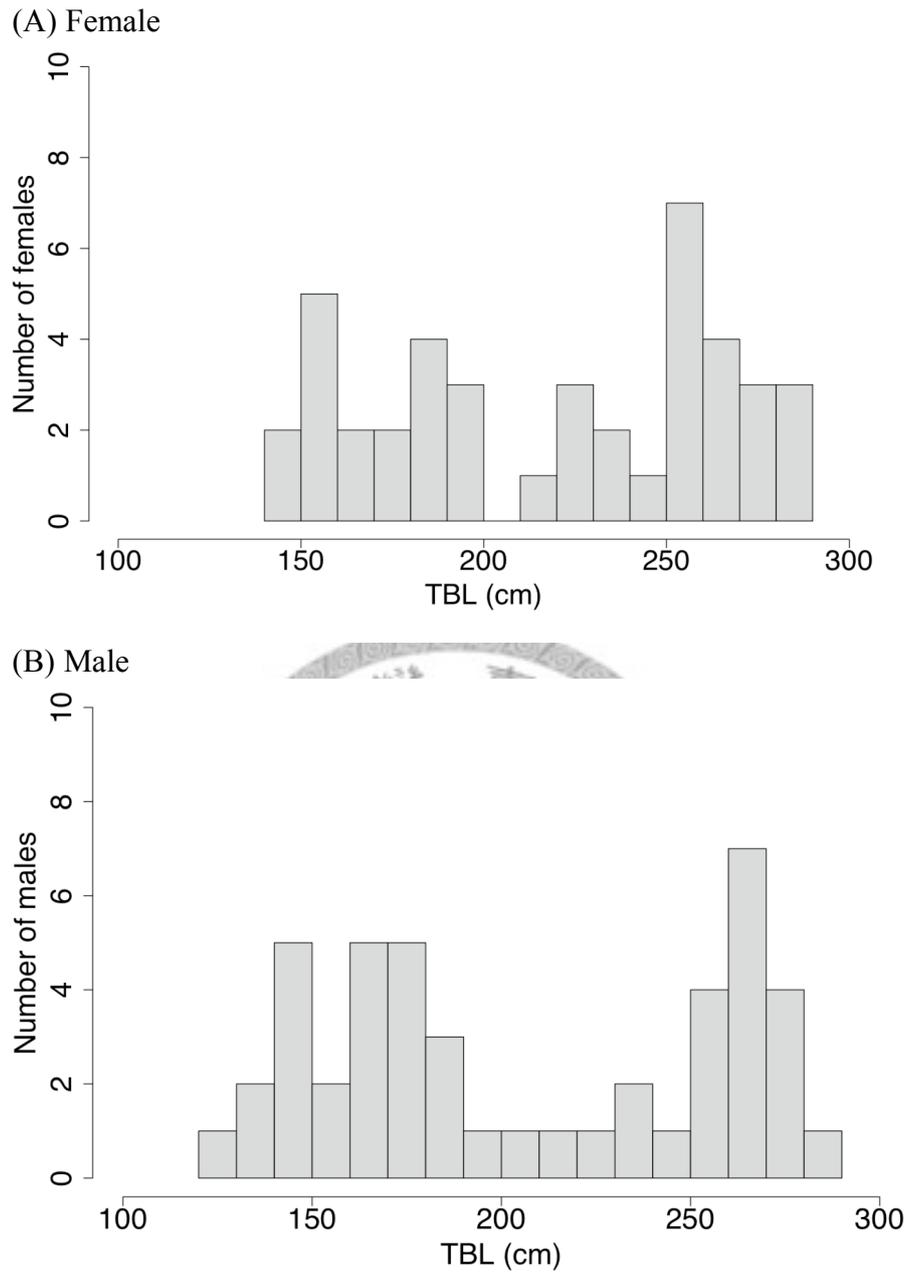


Figure 2.2. Distribution of 43 female (A) and 47 male (B) Risso's dolphins by TBL in increments of 10 cm.

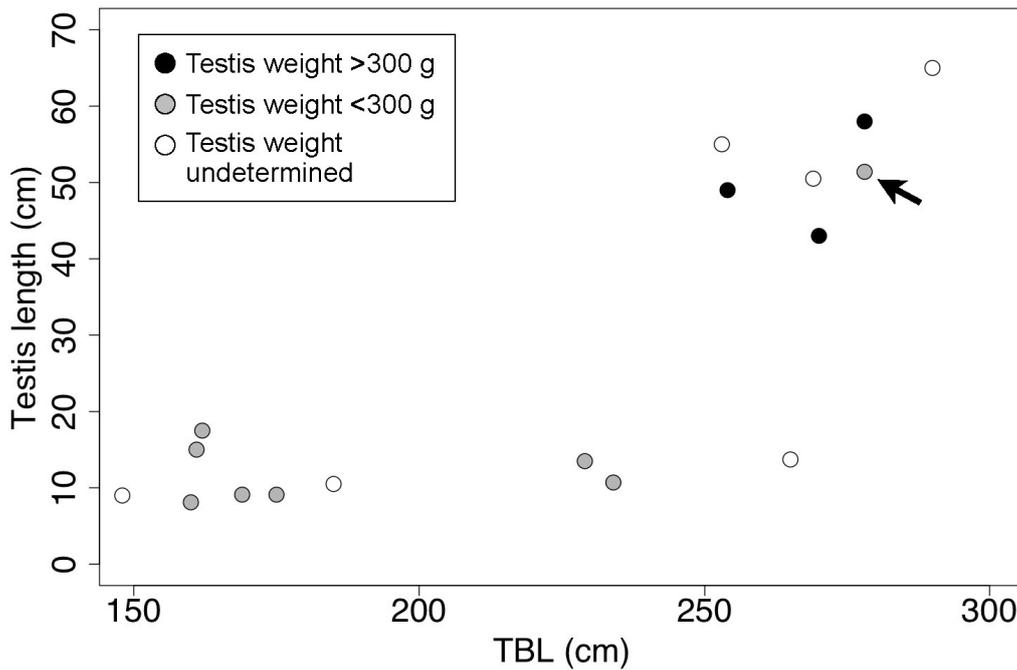


Figure 2.3. Testis length compared to TBL for 17 male Risso's dolphins with testis weight. Sexually mature males were those with single testis weight >300 g (black dot), immature males with single testis <300 g (most 5 – 20 g) (gray dot), testis weight unavailable (white dot). One male (HU98007, 278 cm) whose testis length was 51.4 cm, weight 280 g, but had testicular fluid present at necropsy was classified as mature (gray dot at arrow). Additional quantitative data for testes in Table 2.3.

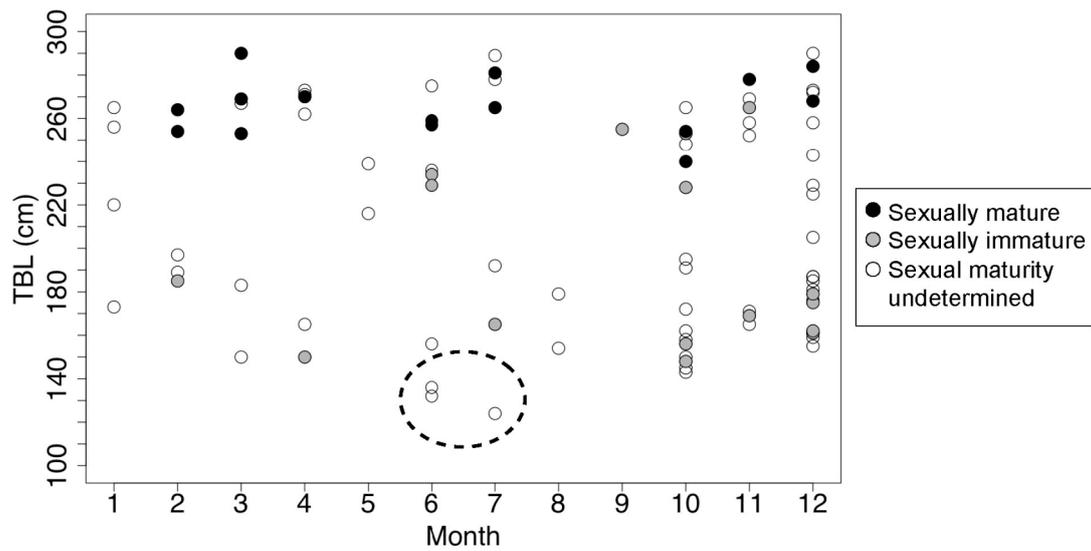


Figure 2.4. Distribution of 92 Risso's dolphins recovered from Taiwanese waters each month of the year plotted by TBL and sexual maturity: sexually mature (solid dot), immature (gray dot), and sexual maturity undetermined (white dot). The three shortest dolphins were neonates (TBL 124, 132, 136 cm; see details in text) and were recovered during summer (June, July) (encircled by dashed line).

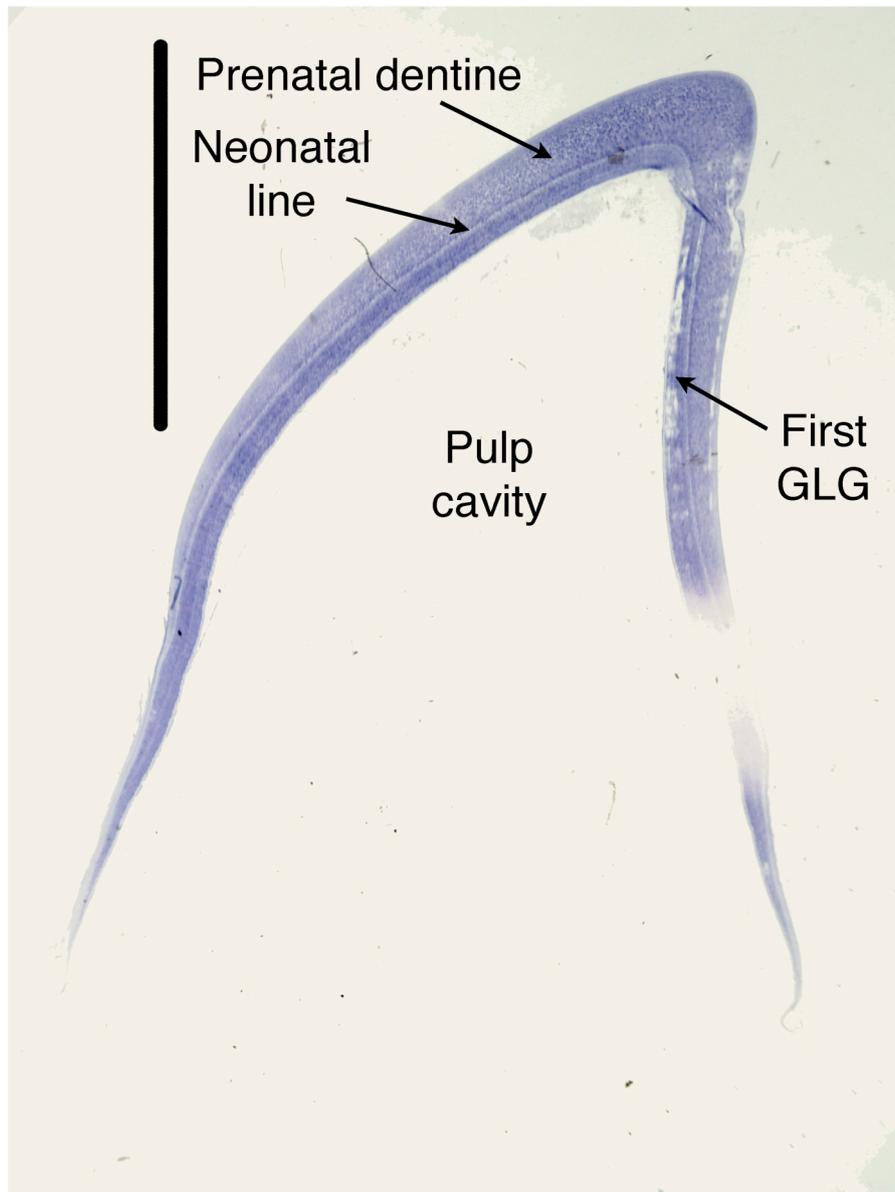


Figure 2.5. Tooth section from a 154 cm female, estimated age 0 (TD2006001).

Neonatal line is well defined as lightly stained pale line in the crown, which separates the outer prenatal dentine and a partial GLG of the postnatal dentine.

Scale bar = 5 mm.

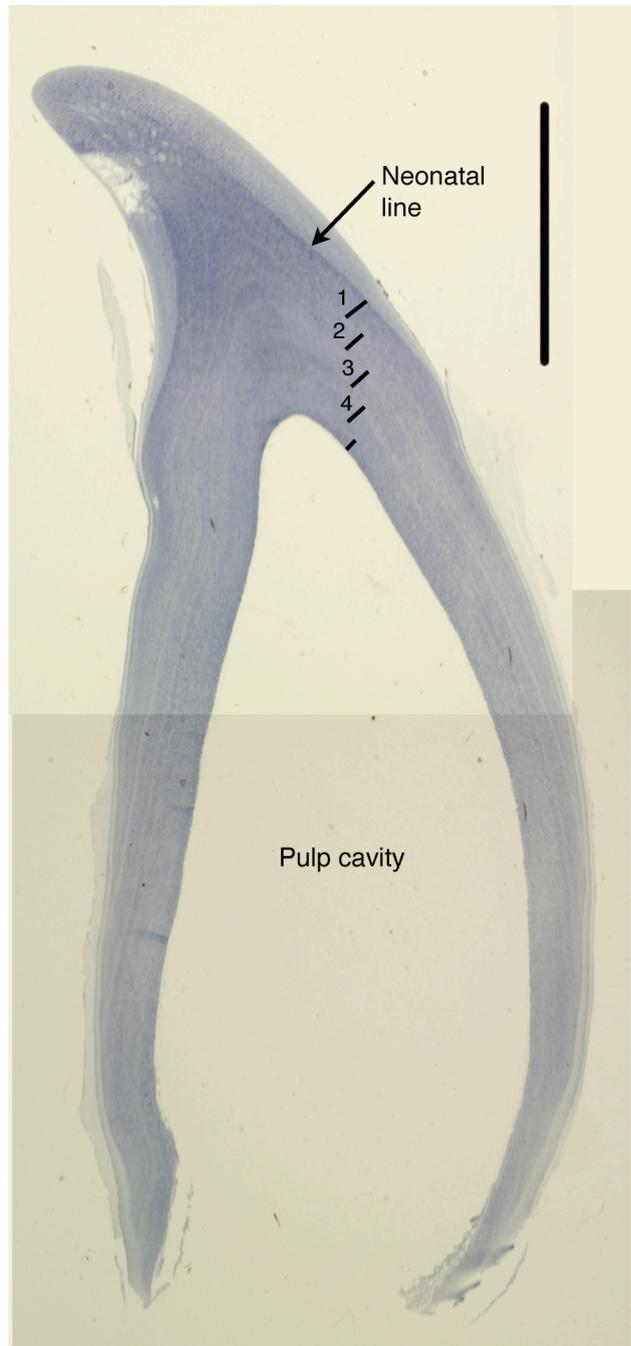


Figure 2.6. Tooth section from a 192 cm (estimated) female, estimated age 4 years (IL20080707). Prenatal dentine over the crown superficial to the neonatal line is lightly stained. The small bars with numbers in the postnatal dentine mark likely GLGs. Scale bar = 5 mm.

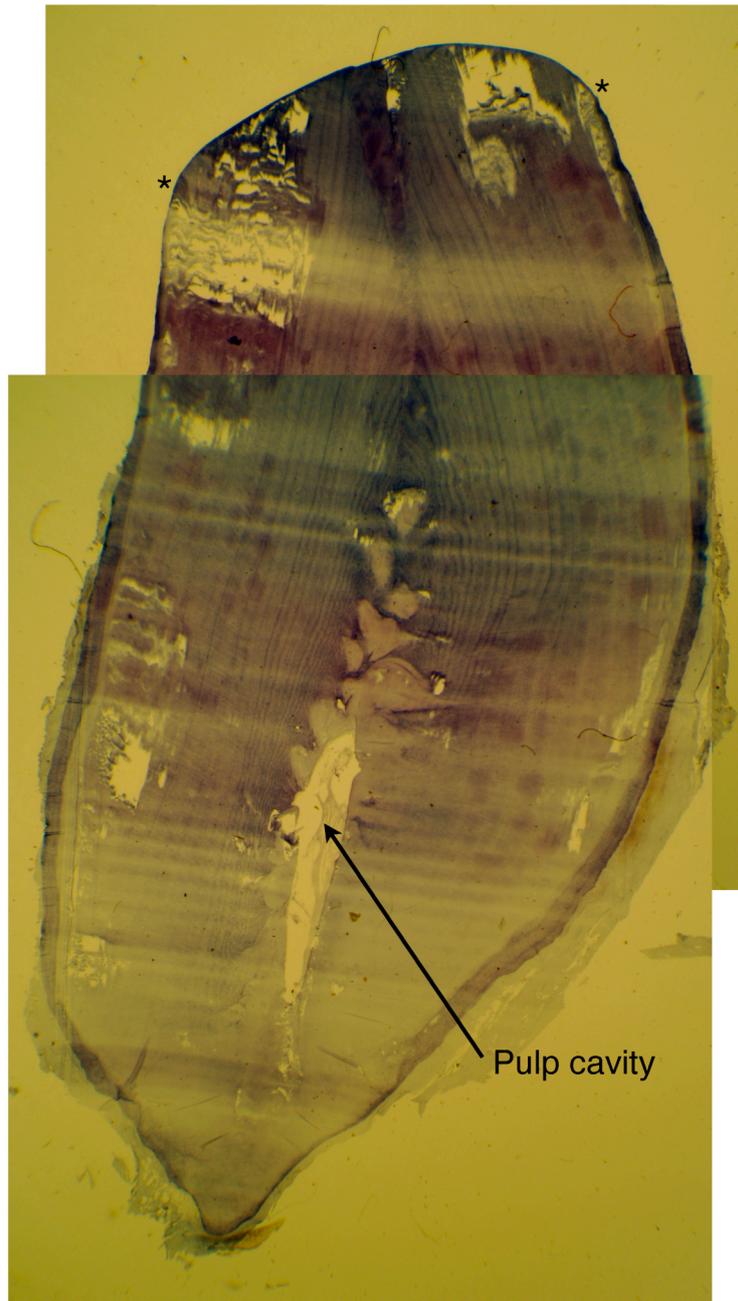


Figure 2.7. Tooth section from a 290 cm male, estimated age at least 34 years (neonatal line absent since most of crown worn down, and pulp cavity almost obliterated by dentine layers) (TY20070314-1). Section shows artifacts due to poor decalcification. * * gingival edge of cementum layer covering tooth root.

Table 2.1. Body length (TBL), sex, and age (GLG) of Risso's dolphins from different oceanographic regions.

Region	Sex	n	TBL (cm)	Age	Source
East Atlantic Ocean					
United Kingdom	F	3	185 – 320	--	Murie 1871, Flower 1874
Shetland	M?	4	262 – 318		Turner 1892
Portugal	M	1	147	Infant (fetal fold)	Nachtigall <i>et al.</i> 2005
Eastern North Atlantic	U	57	240 – 409	--	summarized in Ross 1984
West Atlantic Ocean					
Eastern Canada coast	M	1	277	13	Lawson & Eddington 1998
Massachusetts	F	1	330	--	True 1889
	U	2	173, 185	--	
Gulf of Mexico	M	1	283	--	Paul 1968
Brazil	M	1	162	--	Geise & Borobia 1987
East Pacific Ocean					
Eastern North Pacific (British Columbia – Baja California)	M	10	143 – 361	<1 – 30+ (n = 4, 143 – 361 cm)	Orr 1966, Stroud 1968, Leatherwood <i>et al.</i> 1980, Kruse 1989
	F	9	270 – c.400	5 – 13-14 (n = 3, 274 – 312 cm)	
	U	2	334, c.400	--	
West Pacific Ocean					
Japan	M	54	146 – 309	0 – 16.5 (n = 30, 146 – 287 cm)	Mizue & Yoshida 1962, Kasuya & Izumizawa 1981, Kim <i>et al.</i> 1996, Amano & Miyazaki 2004
	F	79	180 – 289	0.5 – 34.5 (n = 49, 183 – 284 cm)	
Taiwan	M	47	124e – 290	0 – 34 (n = 14, 150 – 290 cm)	Present study
	F	43	143 – 289	0 – 14 (n = 12, 154 – 275 cm)	
	U	2	165e	1 (165e cm) 8 (TBL undetermined)	
Indian Ocean					
S. Africa	M	7	163 – 325	<1 – 13+ (n = 6, 163 – 306s cm)	Ross 1984
	F	7	212 – 300s	<1 – 17+ (n = 6, 212 – 291s cm)	
	U	3	84 (Fetus), 295, 297	12+ (297 cm)	
Mediterranean Sea					
Western & eastern Mediterranean Sea	U	37	135 – 181 (young) 260 – 360 (adult)	--	Razzauti 1910, summarized in Pilleri & Gahr, 1969.
Italy	M	3	290 – 298	--	Marsili & Focardi 1997, Jimenez <i>et al.</i> 2000, Zucca <i>et al.</i> 2004
	F	2	299, 311	--	
Corsica	F	1	310	2.5	Frodello <i>et al.</i> 2000

Spain	M	6	172 – 308	--	Blanco <i>et al.</i> 2006
	F	9	165 – 320	<1 (165 cm)	
Israel	M	1	320	--	Shoham-Frider <i>et al.</i> 2002
	F	1	335	19	

e: estimated by RAL, see Material & Methods

s: estimated by CBL , equation provided in Ross 1984.



Table 2.2. Month of salvage, number of corpora found in ovaries, and presence of milk in mammary glands of 14 female Risso's dolphins from Taiwanese waters assessed for sexual maturity.

Catalog number	Month of bycatch/ stranded	TBL (cm)	Corpora albicantia counts (L/R)	Corpora lutea counts (L/R)	Milk in mammary glands	Sexual Maturity ^a
IL2000003	April	150	-- ^b / --	-- / --	Absent	I
HU98006	October	156e	-- / --	-- / --	Absent	I
HU2000009	July	165	0 / 0	0 / 0	--	I
PE2001003	October	228	0 / 0	-- / --	--	I
HU2000033	October	240	>1 / >3	-- / --	Present	M
IL98003	October	253	-- / --	0 / 0	Absent	I
HU2001001	February	254	>1 / >2	0 / 0	Present	M
HU97002	September	255	0 / 0	0 / 0	Absent	I
CK97005	June	257	>1 / >1	-- / --	Present	M
CK97016	June	259	-- / 0	1 / 0	Present	M
HU2001007	February	264	-- / --	-- / --	Present	M
HC2002003	July	265	>7 / 7	-- / --	--	M
HU99002	July	281	0 / 3	0 / 1	Present	M
HU2000048	December	284	0 / 0	0 / 1	Absent	M

a: I, immature; M, mature

b: data not available

Table 2.3. Testicular dimensions^a, weight^a, and presence of testicular fluid of 18 male Risso's dolphins from Taiwanese waters assessed for sexual maturity.

Catalog number	TBL (cm)	Testis dimensions ^b (L / W / D, cm)	Testis weight (g)	Testicular fluid	Age (GLGs)	Sexual maturity ^c
HU2001015	148	9/1.2/- ^d	--	--	--	I
CK94003	161e	8.1/1.3/0.7	5.9	--	--	I
CK20051208-2	161	15.0/--/--	9.5	--	--	I
CK20051208-3	162e	17.5/--/--	15.6	--	0	I
SU94114	169	17.5/--/--	4.9	Absent	--	I
TP95001	175	9.1/1.2/0.6	8	--	--	I
CK94002	179	--	4.14	--	--	I
HU2001002	185	10.5/1.1/0.8	--	--	--	I
CK97014	229	13.5/2.5/1.7	39.7	Absent	--	I
SU95093	234	10.7/1.6/1.2	19.8	--	6	I
TY20070314-2	253	55/14/14	--	--	--	M
TC2000001	254	49/11.5/6	2650	--	--	M
HU98008	265	13.7/2.4/1.8	--	Absent	--	I
ST20051214	268e	58/15/6.5	3100	--	--	M
TY20070314-3	269	50.5/13.5/7	--	--	14+	M
TP20080430	270	43/10/10 ^e	980 ^e	Absent	--	M
HU98007	278	51.4/13.5/4.9	280	Present	--	M
TY20070314-1	290	65/15/15	--	--	34	M

a: data from examination on left testis.

b: testis length (L), maximum width (W), and maximum depth (D)

c: I: immature; M: mature

d: Data not available.

e: right testis (L45.2/W12/D10, cm)

Table 2.4. Total length, sex, stranding/by catch month, and sexual maturity for 28 age-estimated Risso's dolphins from Taiwanese waters.

Catalog number	TBL (cm)	Catch/stranded month	Sex	Estimated age	Sexual maturity
TD-2006-001	154	August	F	0	--
CK20061209-1	155	December	F	0	--
ST20041101-01	--	November	F	0	--
ST20041030-01	172	October	F	1	--
IL20080707	192e	July	F	4	--
ST20041028-01	195	October	F	2	--
CK-94-005	229	December	F	5	--
ST-2005-12-11	243	December	F	13	--
CK061117-1	258	November	F	11	--
PE-97-012	262	April	F	11	--
CK-94-004	272	December	F	14	--
HU-98-004	275	June	F	14	--
HU-2000-035	150	October	M	0	--
CK-2005-12-03	162	December	M	0	I
ST20041012-02	162e	October	M	1	--
ST-2006-001	173e	January	M	0	--
ST-2005-006	176	December	M	1	--
ST-2005-001	191	October	M	1	--
SU-95-093	234	June	M	6	I
ST20041023-02	248	October	M	15+	--
ST-2005-005	252	November	M	9	--
ST20041023-01	265	October	M	26+	--
TY20070314-4	267	March	M	16	--
TY20070314-3	269	March	M	14+	M
ST-2005-004	269	November	M	15	--
TY20070314-1	290	March	M	34	M
CK061117-2	165e	November	U	1	--
IL-2006-0312	--	March	U	8	--

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Appendix 2.1. Catalog number, salvage year, month, source, sex, total body length (TBL), length from tip of upper jaw to center of anus (RAL), and estimated TBL (see text for equations) of 92 Risso's dolphins from Taiwanese waters.

Catalog number	Year	Month	Source	Sex	TBL (cm)	RAL (cm)	TBL _e (cm)
HU-99-010	1999	10	bycatch	F	143	95.5	--
IL2000-003	2000	4	stranding	F	150	96	--
TD-2006-001	2006	8	bycatch?	F	154	100.3	--
CK20061209-1	2006	12	bycatch	F	155	103.4	--
CK20061209-3	2006	12	bycatch	F	159	106.4	--
PD-99-001	1999	4	stranding	F	165	107	--
HU-2000-009	2000	7	bycatch	F	165	110.5	--
HU-2000-044	2000	11	bycatch	F	171	115	--
ST20041030-01	2004	10	bycatch	F	172	NA	--
CK-94-001	1994	12	bycatch	F	181	123	--
HU-2000-050	2000	12	bycatch	F	187	124.5	--
HU2001-005	2001	2	bycatch	F	189	122	--
ST20041028-01	2004	10	bycatch	F	195	NA	--
HU2001-004	2001	2	bycatch	F	197	126.5	--
HU-98-003	1998	5	bycatch	F	216	140.4	--
PE-2001-003	2001	10	stranding	F	228	148	--
CK-94-005	1994	12	bycatch	F	229	147.5	--
ML-99-001	1999	5	stranding	F	239	158	--
HU-2000-033	2000	10	bycatch	F	240	162.5	--
ST-2005-12-11	2005	12	bycatch	F	243	169.4	--
IL-98-003	1998	10	stranding	F	253	157	--
HC-97-002	1997	9	bycatch	F	255	NA	--
CK-97-005	1997	6	bycatch	F	257	163.5	--
CK061117-1	2006	11	bycatch	F	258	180	--
HU-2000-051	2000	12	bycatch	F	258	172	--
CK-97-016	1997	6	bycatch	F	259	163	--
PE-97-012	1997	4	stranding	F	262	161?	--
HU2001-007	2001	2	bycatch	F	264	173	--
HU-98-001	1998	1	bycatch?	F	265	173.5	--
HC2002003	2002	7	stranding	F	265	174.4	--
CK-94-004	1994	12	bycatch	F	272	180	--
HU-98-004	1998	6	bycatch	F	275	176	--
HU-99-002	1999	7	bycatch	F	281	184	--
HU-2000-048	2000	12	bycatch	F	284	187	--
HC2002002	2002	7	stranding	F	289	188	--
CK-2005-12-08-1	2005	12	bycatch	F	NA	147.8	225
CK20061209-2	2006	12	bycatch	F	NA	122	185
IL20080707	2008	7	stranding	F	NA	126	192
TD20040405	2004	4	stranding	F	NA	178	271
HU2001-001	2001	2	bycatch	F	NA	166.6	254
HU-2000-005	2000	6	bycatch	F	NA	102.5	156

HU-98-006	1998	10	bycatch	F	NA	102.5	156
ST20041101-01	2004	11	bycatch	F	NA	NA	NA
HU-2000-002	2000	6	bycatch	M	132	89	--
HU-2000-006	2000	6	bycatch	M	136	92	--
HU-2000-031	2000	10	bycatch	M	145	95.5	--
HU-2000-040	2000	10	bycatch	M	145	97	--
HU2001-015	2001	10	bycatch	M	148	95	--
HU2001010	2001	3	bycatch	M	150	NA	--
HU-2000-035	2000	10	bycatch	M	150	100	--
TD-97-002	1997	NA	Stranding	M	154	NA	--
HU-2000-014	2000	10	bycatch	M	158	105	--
CK-2005-12-08-2	2005	12	bycatch	M	161	103.5	--
ST20041012-02	2004	10	bycatch	M	162	NA	--
SU-94-114	1994	11	by catch	M	169	111	--
TP-95-001	1995	12	stranding	M	175	117	--
ST-2005-006	2005	12	bycatch	M	176	162	--
CK-94-002	1994	12	bycatch	M	179	120.5	--
HU-99-008	1999	8	bycatch	M	179	122	--
HU2001-009	2001	3	bycatch	M	183	NA	--
HU2001-002	2001	2	bycatch	M	185	123.8	--
ST-2005-001	2005	10	bycatch	M	191	118.3	--
HU-2000-049	2000	12	bycatch	M	205	135	--
PD2002-001	2002	1	stranding	M	220	NA	--
CK-97-014	1997	6	bycatch	M	229	155	--
SU-95-093	1995	6	bycatch	M	234	157	--
CK-97-004	1997	6	bycatch	M	236	150.5	--
ST20041023-02	2004	10	bycatch	M	248	NA	--
ST-2005-005	2005	11	bycatch	M	252	165	--
TY20070314-2	2007	3	stranding	M	253	166	--
TC2000-001	2000	10	stranding	M	254	165	--
TD-98-001	1998	1	stranding	M	256	161.5	--
HU-98-008	1998	11	bycatch	M	265	179.8	--
ST20041023-01	2004	10	bycatch	M	265	NA	--
TY20070314-4	2007	3	stranding	M	267	175	--
TY20070314-3	2007	3	stranding	M	269	180	--
ST-2005-004	2005	11	bycatch	M	269	174.8	--
TP20080430	2008	4	stranding	M	270	NA	--
ML-98-003	1998	4	stranding	M	273	164.5	--
KD-94-001	1994	12	stranding	M	273	NA	--
HU-98-007	1998	11	bycatch	M	278	183.5	--
HU-2000-007	2000	7	bycatch	M	278	179.5	--
TY20070314-1	2007	3	stranding	M	290	191	--
ST-2006-001	2006	1	bycatch	M	NA	114.2	173
CK-2005-12-03	2005	12	bycatch	M	NA	106.6	162
CK20061231	2006	12	bycatch	M	NA	123	187
ST-2005-12-14	2005	12	bycatch	M	NA	175	268
CK-94-003	1994	12	bycatch	M	NA	106	161
HU-99-004	1999	7	bycatch	M	NA	82.5	124
HU-20011231	2001	12	stranding	M	NA	NA	NA

CK061117-2	2006	11	bycatch	U	NA	109	165
IL-2006-0312	2006	3	stranding	U	NA	NA	NA



Chapter 3: Maturation of flipper skeleton in Risso's dolphins, *Grampus griseus*, from Taiwanese waters

ABSTRACT

Risso's dolphins (*Grampus griseus*) are one of the most frequently encountered cetaceans in eastern Taiwanese coastal waters. Since their life history is poorly known, and knowledge on their skeletal maturation is not available, we examined the relationship of skeletal maturation, estimated tooth age, and total body length (TBL) on dead-stranded or incidentally killed Risso's dolphins collected in 1994 – 2008 from Taiwanese waters. Skeletal maturity in the flippers was assessed radiographically by evaluating fusion of the distal epiphyseo-metaphyseal junction in the distal radius and ulna (67 flippers; 34 dolphins; TBL 148 – 290 cm). Cessation of longitudinal flipper growth in the distal radius and ulna and the onset of flipper maturity occurred at 248 – 255 cm TBL, when dolphins were about 10 years old. This timing was coincident with their sexual maturity. The development of the epiphyseal ossification on the distal radius in individual dolphins was usually more advanced than on the distal ulna. Bilateral asymmetry and sexual dimorphism was not detected. This radiography-based data on skeletal maturity of flippers in Risso's dolphins can provide a useful foundation for a simple and non-invasive method to estimate age of live animals and thus an indicator of sexual maturity.

Key words: skeletal maturation, epiphysis, flipper, radiography, Risso's dolphin, *Grampus griseus*, Taiwan

INTRODUCTION

Many features of the skeleton change through growth and maturation and therefore can provide useful age-related information (Rommel & Reynolds 2002). Furthermore, investigation of the chondro-osseous growth pattern provides the potential to evaluate the health status and approximate age of individual animals, especially when chronological age is uncertain, as is usually the case in museum specimens or with beached whales and dolphins (Ogden *et al.* 1981, Galatius *et al.* 2009).

The bones of the flipper are often studied for skeletal maturation in small cetaceans and several methods have been applied to evaluate flipper skeleton maturity. One of the earliest studies, based on radiographs from 87 common dolphins (*Delphinus delphis*) from southern California, evaluated the fusion of bones in the distal flipper and proposed a combined flipper epiphyseal fusion index⁵ (Hui 1979). This index revealed a strong correlation with the combined testes weight, but is poorly correlated with the number of ovarian scars, suggesting that this index could be a reliable technique to determine sexual maturity only in males. However, the scoring technique was complex and failed to attract further application.

Another simpler radiograph-based method was developed (Ogden *et al.* 1981). In this study, radiographs of flippers from Dall's porpoise (*Phocoenoides dalli dalli*) ($n = 114$) and short-finned pilot whales (*Globicephala macrorhynchus*) ($n = 154$) were

⁵ The degree of epiphyseal fusions in the distal radius and ulna, metacarpals, and phalanges were scored from 0 (epiphysis absent) to 3 (epiphysis fused with adjacent metaphysis), then summed the scores in both flippers into a single flipper index value for each dolphin, to quantify the flipper maturity of 35 males and 52 females.

examined and the authors concluded that the developmental process of the distal epiphyseal ossification of the radius and ulna could be a good indicator for skeletal maturity. They established 7 stages (0 – 6) to describe this cartilage-to-bone process (detailed below in Materials and Methods). This evaluation method has become more widely applied and adapted to assess skeletal maturation in a number of studies in small odontocetes including bottlenose dolphins (*Tursiops truncatus*) from west Atlantic Ocean (Mead & Potter 1990), striped dolphins (*Stenella coeruleoalba*) from Mediterranean Sea (Calzada & Aguilar 1996, DiGiancamillo *et al.* 1998), harbor porpoises (*Phocoena phocoena*) in Danish waters (Galatius *et al.* 2006), and recently also applied to a baleen whale, Bryde's whale (*Balaenoptera brydei*) in New Zealand waters (Stockin *et al.* 2008).

Skeletal maturity of the flipper in harbor porpoises has also been assessed by examination of dry, cleaned skeletons in museum collections (Galatius & Kinze 2003). Herein the authors, using a simplified 4-stage classification with tooth-aged specimens, concluded that timing of epiphyseal fusion in the flipper was more consistently correlated with age than that in the vertebral column. This conclusion was confirmed by a later study, which evaluated skeletal maturation in radiographs of flippers from the same species (Galatius *et al.* 2006).

More recently the mineral density of cleaned arm and forearm bones has been used to investigate chronological age in striped dolphins (Guglielmini *et al.* 2002) and in bottlenose dolphins (Butti *et al.* 2007). Using sophisticated scanning and software protocols they found that bone mineral density was a reliable predictor of age in these

dolphins, although this method may be constrained by the limited availability of equipment and its untested applicability for intact cetacean flippers.

Risso's dolphins (*Grampus griseus*), the subject of this present skeletal maturation study, are moderately small toothed whales, widely distributed and abundant throughout the temperate to tropical pelagic regions of all oceans (Kruse *et al.* 1999, Baird 2002, Taylor *et al.* 2008), and commonly encountered in eastern coastal waters of Taiwan (Yeh 2001, Kuo 2002, Lin 2003). Early studies on the skeletons of Risso's dolphins mostly focused on the morphological variation and the numbers of bones to aid in clarification of the taxonomic status of the species (Flower 1874, True 1889, Mizue & Yoshida 1962, Ross 1984). In none of these studies on Risso's dolphins was the development of the skeleton or its maturation investigated.

The objectives of this study were: (1) to examine the stages of epiphyseo-metaphyseal maturation of the distal radius and ulna in radiographs of flippers from Risso's dolphins from Taiwanese waters; (2) to correlate this flipper skeletal maturity to body length, estimated age, and sexual maturity of these dolphins; and (3) to explore the variation as well as bilateral asymmetry between flippers and between sexes.

MATERIALS AND METHODS

Specimens

Thirty-four Risso's dolphin carcasses with total body lengths (TBL) of 148 – 290 cm were salvaged from either stranded on the coast or caught incidentally in a local fishery of Taiwan (1994 – 2008) (Table 3.1). Date, sex, and standard external

morphological data were recorded (Norris 1961). Reproductive maturity was assessed in 14 dolphins (three female, 11 male): females were classified sexually mature if either milk was found in a mammary gland or a corpus luteum or corpus albicans was present in an ovary (Perrin & Reilly 1984); males were classified as sexually mature if either a testis weighed more than 300 g (Kasuya & Izumizawa 1981, Amano & Miyazaki 2004) or if it measured more than 40 cm in length (Ross 1984, Chapter 2). Age was estimated in 17 dolphins (six female, nine male, two unknown sex) by determining the number of growth layer groups (GLG) in a routinely histological preparation of a longitudinally sectioned tooth, with a consensus assumption that one GLG represented one calendar year (Kruse *et al.* 1999, Amano & Miyazaki 2004, Chapter 2). Flippers were disarticulated from the trunk at the scapulo-humeral joint, labeled, and frozen: 67 flippers (34 left, 33 right) from 13 female, 19 male, and from two unknown sex dolphins were salvaged and radiographed.

Radiography

Each flipper was defrosted, cleaned of extraneous blood, dirt, and sand, soft tissue was removed from around the proximal end of the humerus to attach an identity tag, and then placed in a thin plastic bag (Fig. 3.1A). Each flipper, in its bag was placed directly on a 36 x 43 cm non-grid cassette⁶ and ventrodorsal plain-film radiographs taken with a film-to-source distance of 100 – 130 cm and exposures of 40 – 47 kVp and 100 – 220 mA for 0.06 – 0.11 second.

⁶ Twenty-eight radiographs were taken at the Taipei City Zoo, Taipei, which incorporated an Okamoto LUS intensifying screen and films were hand processed; 32 were taken at the Chang-Hua Hospital, Chang-Hua, which used a Konica Minolta KR-II intensifying screen and were processed in an automatic processor; seven were provided by Dr. Tzong-Fu Kuo (School of Veterinary Medicine, National Taiwan University).

Maturity Evaluation

Skeletal maturation of the flipper skeleton was evaluated on radiographs by examining the degree of ossification of the distal epiphysis on the radius and ulna. The developmental progression of epiphyseo-metaphyseal fusion has been categorized into 7 stages (stage 0 – 6, according to Ogden *et al.* 1981) and a stage 7 was added in this study:

Stage 0: epiphyseal ossification center absent;

Stage 1: epiphyseal ossification center present, but less than 50% of the craniocaudal latitudinal width of the adjacent metaphysis;

Stage 2: epiphyseal ossification center well established, from 50% to 100% width of the metaphysis. There was a distinct cartilage growth plate, seen as a radiolucent band between the epiphyseal center and the metaphysis;

Stage 3: radiolucent cartilage growth plate was thinner, and more radiodense juxtaphyseal osseous plates in the metaphysis and epiphyseal ossification center were formed;

Stage 4: closing of the cartilage growth plate apparent, with visible formation of trabecular osseous bridges between the epiphyseal ossification center and the metaphysis;

Stage 5: closure of the cartilage growth plate complete, but the closure scar of dense bone tissue traversing 50 – 100% of the latitudinal width of the bone could be seen;

Stage 6: less than 50% evidence of the transverse scar remnant;

Stage 7: transverse scar remnant absent, and metaphyseal area fully remodeled.

The onset of flipper skeletal maturation was defined as the transition in the ossification process between stages 4 and 5.

All films were examined with the unaided eye on a standard radiographic illuminator. A common plastic ruler was used to measure the width of the epiphyseal ossification center and metaphysis to assess whether development was greater or less than 50% width. The width ratio and maturity stage of distal radius and distal ulna were evaluated independently in each radiograph. To avoid empirical bias, each radiograph was placed in an envelope without TBL or sex information on it, and was examined at least twice, with a 10-day interval between the first and second examinations. If the maturity stage was different between the two readings, the radiograph was reviewed by a second person.

Data Analysis

Statistical software Microsoft Office Excel 2003 (Microsoft corporation) and R (version 2.5.0, <http://www.r-project.org/>) were used to establish a database, and to perform comparison and correlation coefficient detection.

Wilcoxon signed-rank test was used to detect any asymmetry in the maturity stage between distal radius and distal ulna, and between left and right flippers. Student's *t*-test was applied to detect any difference in TBL between male and female in different maturity stages. Pearson's product-moment correlation was used to determine the correlation coefficient (*r*) of the maturity-TBL, and maturity-age relationship. All tests were considered statistically significant at $P \leq 0.05$.

RESULTS

Skeletal Topography

The topographical arrangement and general shape of the bones in the flipper of Risso's dolphin revealed a common pattern with other odontocetes: a shortened humerus, dorsoventrally flattened radius and ulna and both in same plane, a series of six carpal bones in two rows, five metacarpals, and five digits with varying numbers of phalanges (Fig. 3.1B). The distal epiphyses of the radius and ulna underwent a characteristic ossification progression as their respective distal cartilages were each transformed into a distinct and separate bony epiphysis, which in turn underwent a bony fusion process to unite with its adjacent metaphysis (Fig. 3.2A-E). In the three shortest dolphins, 148 – 150 cm, the epiphyseal ossification center was either absent or small in both the radius and ulna – stage 0 or 1 (Fig. 3.2A), and in 12 dolphins at 155 – 195 cm TBL these ossifications were much larger and wider, covering 50 – 100% the craniocaudal width of their respective metaphysis and a distinct radiolucent band of the cartilage growth plate was present in the longest individuals – stage 2 (Fig. 3.2B). The growth plate showed evidence of thinning and early closure with osseous bridges established between epiphyseal and metaphyseal ossifications in five dolphins at 225 – 255 cm — stage 3 and 4 (Fig. 3.2C and D). There were few dolphins in the sample between 200 and 250 cm and thus the stages of growth plate closing and closure were poorly represented and not well defined. Dolphins longer than 250 cm, in particular 248 – 290 cm, showed varying degrees of advanced epiphyseo-metaphyseal fusion – stages 5 – 7 (Fig. 3.2E and F), and advanced stages were not necessarily so-graded in longest dolphins: the dolphins with distal radius graded as stage 7 were 257 and 290 cm TBL (Fig. 3.3A).

Estimated Age, TBL and Flipper Maturity

Flipper maturity in both the distal radius and distal ulna was significantly correlated with TBL (Fig. 3.3) (radius: $r = 0.94$; $P < 0.01$; ulna: $r = 0.95$, $P < 0.01$; Pearson's product-moment correlation). Estimated tooth age (data from Chapter 2) was also significantly correlated with flipper maturity (Fig. 3.4) ($r = 0.91$; $P < 0.01$ for both radius and ulna, Pearson's product-moment correlation). In dolphins shorter than 195 cm TBL, their epiphyseal ossifications were either under developed (stage 0 - 1) or present as a distinctive bony center adjacent to its metaphysis (stage 2), and less than two years of age. In dolphins at 225 – 255 cm TBL, the epiphyseal ossifications reached maximum widths and started to fuse with their metaphysis (stage 3 - 4); one stage 3 dolphin was estimated to be eight years of age, although its TBL was undetermined. The cessation of longitudinal flipper growth through the closure of the epiphyseo-metaphyseal growth plate in the distal radius and ulna, the onset of flipper skeletal maturation, occurred at 248 – 255 cm (between the longest stage 4 dolphin and shortest stage 5 dolphin) (Fig. 3.3) and at about 10 years of age (Fig. 3.4), and complete closure with remodeling occurred at 248 – 271 cm TBL and at ages greater than 10 years. Full remodeling and loss of the fusion scar in the distal radius and ulna (stage 6 - 7) occurred in two specimens, and one of those was the oldest dolphin with an estimated age of 34 years (290 cm).

Sexual Maturity and Flipper Maturity

Eleven male dolphins were available for joint assessment of sexual and flipper maturation (Table 3.1). Four sexually immature dolphins had flippers at stage 1 – 2 (TBL <200 cm) and at stage 3 (TBL 229 cm). Six sexually mature dolphins (TBL

253 – 270 cm) had flippers at stages 5 – 6, while a seventh sexually mature male and the longest dolphin (TY20070314-1, 290 cm) had a flipper maturity of stage 7 in the distal radius of the right flipper, and stage 6 for the right ulna and both bones in the left flipper. Three females were jointly assessed: one was sexually immature (HC97002, 255 cm) and had a flipper maturity of stage 4, whereas the two sexually mature dolphins had either a flipper maturity of stage 5 (CK97016, 259 cm, one corpus luteum) or stage 6 – 7 (CK97005, 257 cm, two corpora albicantia) (Table 3.1).

Sexual Dimorphism

There was no sexual dimorphism in TBL within the stages of epiphyseo-metaphyseal fusion in either the distal radius and or in the distal ulna. In particular, the TBLs were not significantly different between males and females in both the immature⁷ and in the mature dolphins⁸, for both the distal radius and ulna (Table 3.2). There was no significant difference in TBL between males and females for the closure period of epiphyseo-metaphyseal fusion⁹, although the sample size might be arguably small.

In the zero-year age class there were two females and one male and their epiphyseo-metaphyseal development on both distal radius and ulna was similar (distal radius, stage 1 – 2; distal ulna, 0 - 1). In the one-year olds, there were two females and two males all with similar flipper maturity stage (distal radius, stage 2; distal ulna, stage 1 - 2). There was only one female dolphin with mature flippers and an estimated age

⁷ Stage 1 – 2, male: mean TBL 168.9 cm, *SD* = 16.3; female: 170.0 cm, *SD* = 16.3; *t* = -0.1704, *df* = 24, *P* = 0.8662, Student's *t*-test

⁸ Stage 5 – 7, male: mean TBL 265.2 cm, *SD* = 11.8; female: 265.2 cm, *SD* = 7.9; *t* = -0.0156, *df* = 25, *P* = 0.9877, Student's *t*-test

⁹ Stage 3 – 4, male: mean TBL 232.5 cm, *SD* = 4.0; female: 240.0 cm, *SD* = 17.3; *t* = -0.8434, *df* = 3.326, *P* = 0.4554, Student's *t*-test)

(flipper maturity: radius 6, ulna 5; age 11 years) therefore we were unable to compare the age of dolphins with mature flippers between sexes.

Advanced Epiphyseal Ossification in Radius

Within flippers, the epiphyseo-metaphyseal ossification was significantly more advanced in the distal radius than in the distal ulna ($P < 0.01$, Wilcoxon Signed-Rank Test) (Fig. 3.5). Of 30 flippers graded stage 0 – 2, there were 13 flippers in which the epiphyseal ossification on the distal radius was well established and was greater than 50% of the width of the adjacent metaphysis (stage 2), but the epiphyseal ossification on the distal ulna was smaller and less than 50% of the width of its adjacent metaphysis (stage 1) (Fig. 3.2B). In four other flippers, the epiphyseal ossification was established in distal radius (stage 1) but not in distal ulna (stage 0) (Fig. 3.2A). Similar “advanced radius” ossification was also found in the mature stages: metaphyseal remodeling and loss of the closure scar was advanced in the distal radius (stage 6) when the fusion scar was still largely present across the distal ulna (stage 5) in five flippers; and in two flippers without any remnants of the transverse closure scar (stage 7) in the distal radius, some transverse scar remained in distal ulna (stage 6) (Fig. 3.2F).

Bilateral Symmetry

Between left and right flippers of a pair, there was no significant difference in the fusion stage in either the distal radius ($P > 0.05$, Wilcoxon Signed-Rank Test) or in the distal ulna ($P > 0.05$, Wilcoxon Signed-Rank Test) (Fig. 3.6).

DISCUSSION

Flipper and Sexual Maturity

This radiographic study established that the onset of skeletal maturation of the distal radius and ulna of Risso's dolphin coincided with the TBL of sexual maturity (Fig. 3.7). The timing of this transition in the ossification process occurred between stages 4 and 5. The longest stage 4 had a TBL of 255 cm while the shortest stage 5 had a TBL of 248 cm: a range which was similar to the TBL for the onset of sexual maturity in Risso's dolphins from Taiwanese waters (females at 240 – 255 cm, males at 250 – 265 cm; see Chapter 2). The estimated age of sexual maturity for these dolphins was at 10 years of age (Chapter 2), which is similar to that for Risso's dolphins from Japanese waters (Amano & Miyazaki 2004). This concordance of sexual maturity occurring at about the same TBL as onset of flipper maturity is also seen in Dall's porpoises and short-finned pilot whales (Ogden *et al.* 1981), bottlenose dolphins from the western Atlantic Ocean (Mead & Potter 1990), and in harbor porpoises from the Baltic and North Seas (Galatius & Kinze 2003). On the other hand, in striped dolphins (*Stenella coeruleoalba*), flipper maturity (stage 5) occurs somewhat earlier (5 – 6 years in females, 8 – 9 years in males) than the age of sexual maturity (10 – 15 years) (Calzada *et al.* 1996, Calzada *et al.* 1997), and a similar maturation sequence likely exists in spotted (*Stenella attenuata*) and spinner dolphins (*Stenella longirostris*) (Perrin 1975). There might be other unknown mechanisms influencing flipper skeletal maturation: perhaps this is a special inherent biological character in *Stenella*, or related to their highly energetic and acrobatic locomotory life style in the pelagic habitat (Norris *et al.* 1994), which may have driven their developmental adaptation for their early skeletal

maturation before sexual maturity in comparison to that known in other odontocete species (as above).

Sexual Dimorphism

Sexual dimorphism in flipper maturation was not detected in these Risso's dolphins. However, the limitation in sampling size in the stages immediately prior to full growth plate closure (*i.e.*, stages 3 and 4) and at around 250 cm TBL may have precluded the detection of any possible affect due to sex, since sexual maturity occurred seemly coincident with flipper maturation. Obvious sexual dimorphism in body length has not been detected in Risso's dolphins (Chapter 2) (Kruse *et al.* 1999, Baird 2002, Amano & Miyazaki 2004), although a small difference in age of sexual maturity between the sexes occurs in Risso's dolphins from Japanese waters (Amano & Miyazaki 2004). On the other hand, striped dolphins are sexually dimorphic in body length: in that males are always longer in asymptotic length and reach sexual maturation at ages older than females (Ito & Miyazaki 1990, Calzada *et al.* 1997), and this phenomenon appears as a common pattern in a number of delphinids (Perrin *et al.* 1977, Kasuya & Marsh 1984, Read *et al.* 1993, Amano *et al.* 1996, Murphy & Rogan 2006). Coincidentally, maturation of the flipper skeleton in striped dolphins from the Mediterranean Sea is also sexually dimorphic (Calzada & Aguilar 1996, DiGiancamillo *et al.* 1998), which adds support to the concept that sexual dimorphism reveals divergent patterns in energy allocation to growth and reproduction between the sexes of different species (Costa 2002).

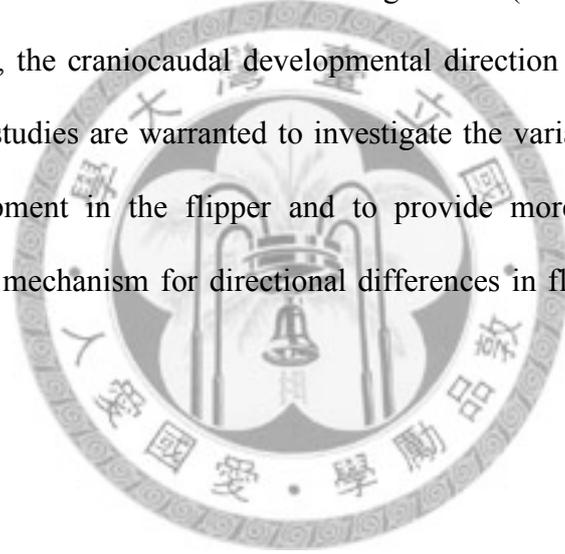
Bilateral Symmetry of Flipper Skeleton and Maturation

Bilateral asymmetry of the maturation process in the flipper skeleton was not detected in this study of Risso's dolphins, which parallels the symmetry in the common dolphin (Hui 1979), Dall's porpoise and short-finned pilot whale (Ogden *et al.* 1981), and in the striped dolphin (DiGiancamillo *et al.* 1998). However, there is bilateral asymmetry of the size of flipper bones in harbor porpoises, in which the diameter/length ratios are significantly larger in the right humerus and ulna than in the left (Galatius 2005). Similar findings are seen in white-beaked dolphins (*Lagenorhynchus albirostris*) (Galatius 2006). These two studies suggest that preferential use of the right flipper in these two small odontocetes might induce relatively more mechanical stress, as the physiological osteogenic inducer, thereby leading to morphological asymmetry. Indeed, lateralized behaviors are known in some cetaceans: *e.g.*, captive and wild bottlenose dolphin (Marino & Stowe 1997b, Sakai *et al.* 2006) and captive beluga (*Delphinapterus leucas*) (Marino & Stowe 1997a), but lateralized flipper use is only known in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Sakai *et al.* 2006) and humpback whales (*Megaptera novaeangliae*) (Clapham *et al.* 1995); and none has been reported for harbor porpoises or Risso's dolphins. Further studies investigating flipper bone morphometrics and field observation for lateralized behaviors are necessary to clarify this behavior-physiological interaction hypothesis in cetaceans.

Craniocaudal Direction of Flipper Maturation

In this study, the epiphyseo-metaphyseal ossification process in the distal radius was more advanced than in distal ulna at almost each stage of development. This demonstrates a cranial-to-caudal direction of maturation at this level in the flipper, which is seen in the same way in the eight radiographic images from a developmental series of Dall's porpoise flippers (Ogden *et al.* 1981) and also remarked upon in

Commerson's dolphins (*Cephalorhynchus commersonii*) (de Buffrénil and Robineau 1984). In the harbor porpoise, however, the direction of maturation is reversed since the distal ulna reaches full maturity at younger age than distal radius (Dawson 2003, Galatius *et al.* 2006). The overall sequence of epiphyseal ossification and fusion in the development in the flipper skeleton of small odontocetes follows the usual terrestrial mammalian directional pattern of proximal to distal (Felts 1966, Calzada & Aguilar 1996, Sedmera *et al.* 1997), but flipper maturation in the craniocaudal dimension has not been investigated. Since the proximal-distal direction of flipper maturation is under genetic developmental control and hormone regulation (Johnson & Tabin 1997, Sedmera *et al.* 1997), the craniocaudal developmental direction is likely under similar control. Additional studies are warranted to investigate the variance among species of craniocaudal development in the flipper and to provide more information for our understanding of the mechanism for directional differences in flipper chondro-osseous development.



Practical Application

This radiography-based data on skeletal maturity of flippers in Risso's dolphins will provide a useful foundation for a simple and non-invasive method for evaluating age of live animals and thus also an estimate of sexual maturity status. Its use in captive or stranded dolphins would be of value in assisting decisions for suitable husbandry or veterinary care, since Risso's dolphins are commonly displayed in ocean parks (Tsutsumi *et al.* 1961, Kruse *et al.* 1999). This would be preferred to the more accurate tooth-based estimation of age, since Risso's have only 2 – 7 teeth in each side of their lower jaw (Kruse *et al.* 1999), and tooth extraction is not benign. Furthermore, since this study has revealed a similar sequence of maturation in the distal radius and in the

ulna, it would be convenient to examine only one bone, preferably the radius, as it is the larger of the two and lies cranial in the flipper making it most favorable for study.

Limitations of the Study

The major limitation in this study of Risso's dolphins from Taiwanese waters was the small total number of dolphins available for study and the discontinuous distribution of dolphins across all body-length/age classes. This primarily reflects the collection time, the logistical difficulties in acquiring bycatch carcasses from local fishermen, and the timely salvage of beach-stranded dolphins. There were no dolphins in the 200 – 220 cm TBL range, and only four in the 220 – 250 cm TBL range. These are significant gaps in the sample since dolphins across these lengths are undergoing sexual maturation and the early part of growth plate closure in the distal radius and ulna. Similarly, pre-pubertal individuals were missing in a study on common dolphins (Hui 1979), which suggested that young male dolphins tend to herd separately or have a better capacity to avoid being caught. Likewise, in Risso's dolphins from Japanese waters a similar data gap was considered due to the pre-pubertal male and female dolphins leaving their natal school, after about five years of age (Amano & Miyazaki 2004).

FIGURES WITH LEGENDS



Figure 3.1. A. Dorsal view of disarticulated left flipper of a 154 cm female Risso's dolphin, cleaned and ready for radiography; proximal end of humerus is to the top and cranial (leading) edge of flipper is to the left. Scale in cm.

B. Dorsoventral radiograph of flipper in "A" showing topographical arrangement of bones: humerus (H), distal metaphyseal zone of radius (R), distal metaphyseal zone of ulna (U), distal epiphyseal ossification centers of radius and ulna

(*), carpal bones (c), metacarpal bones (mc), and first phalanges (ph) of the digits I - IV.

Scale bar = 5 cm.



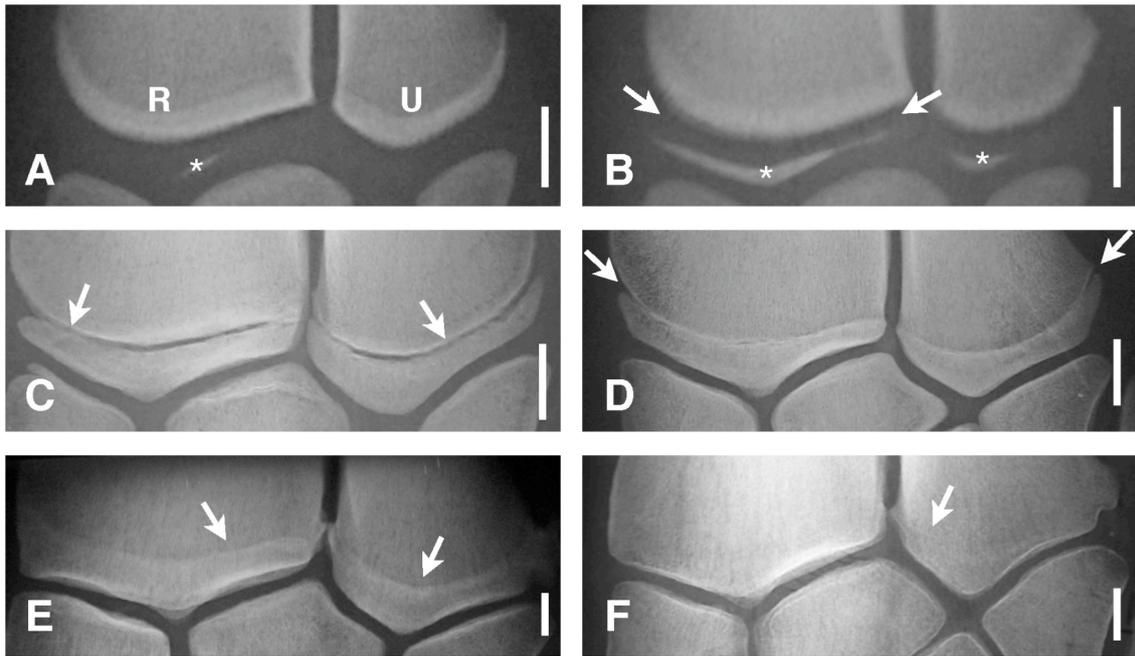


Figure 3.2. Radiographic views of the distal radius and ulna illustrating the normal sequence of epiphyseo-metaphyseal ossification (radiographs rotated as necessary so that all views show the radius on the left for standardized viewing). Note that in many dolphins, the stage of development in the radius (R) is more advanced than that in the ulna (U). Scale bar = 1 cm.

A. Stage 1 in distal radius; stage 0 in distal ulna (148 cm, male, right flipper), early ossification in distal epiphysis of radius (*).

B. Stage 2 in distal radius; stage 1 in distal ulna (154 cm, female, right); broad cartilage growth plate recognized as well defined radiolucent transverse band (between arrows) between the distal metaphysis of radius and ulna and their respective epiphyseal ossification centers (*).

C. Stage 3 (late) in both distal radius ulna (undetermined TBL and sex, left); more radiodense juxtaphyseal osseous plates in the metaphyses (arrows) and epiphyseal ossification centers were formed.

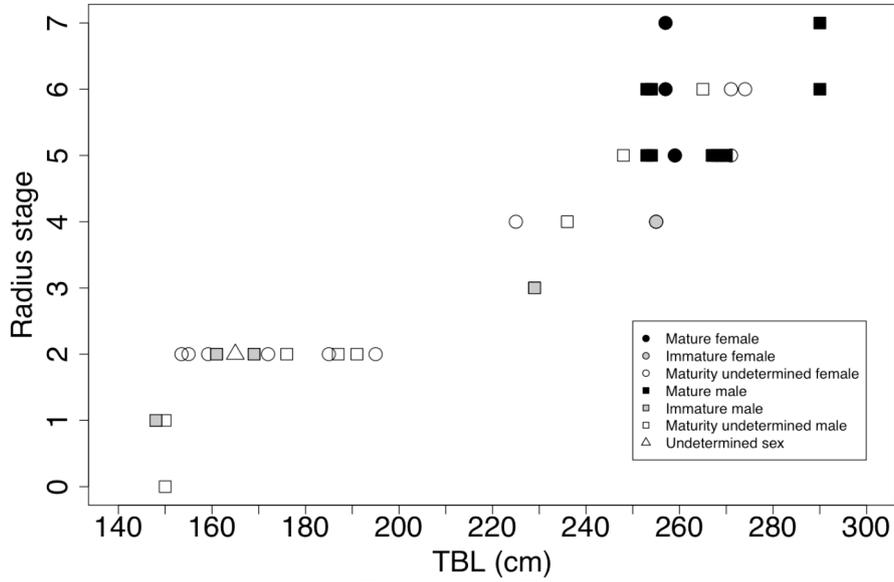
D. Stage 4 (late) in both distal radius and ulna (255 cm, female, left); both cartilage growth plates closing but not completed (arrows).

E. Stage 5 in distal radius; stage 5 in distal ulna (269 cm, male, right); transverse metaphyseal closure scar (arrows); fully present in distal ulna.

F. Stage 7 in distal radius; stage 6 (late) in distal ulna (257 cm, female, right); transverse metaphyseal closure scar in distal ulna less than 50% present (arrow).



(A) Radius



(B) Ulna

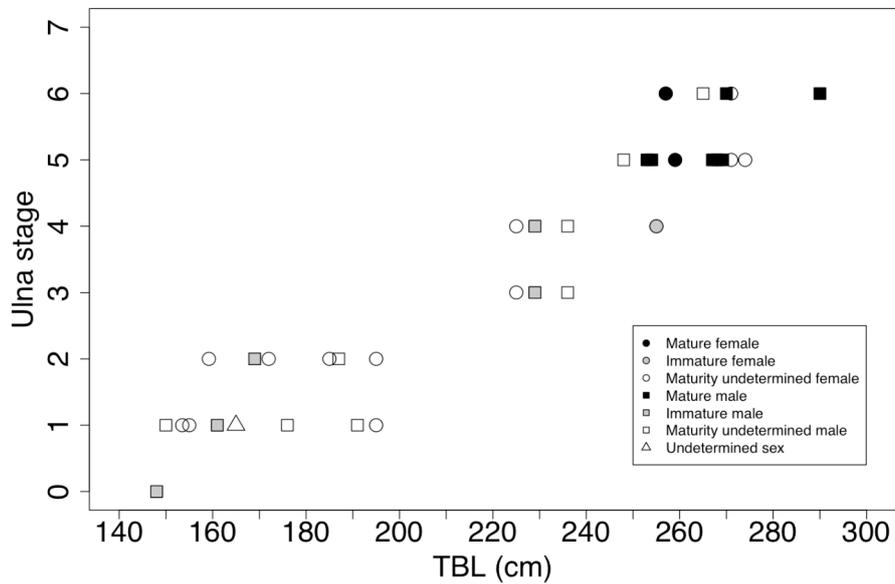
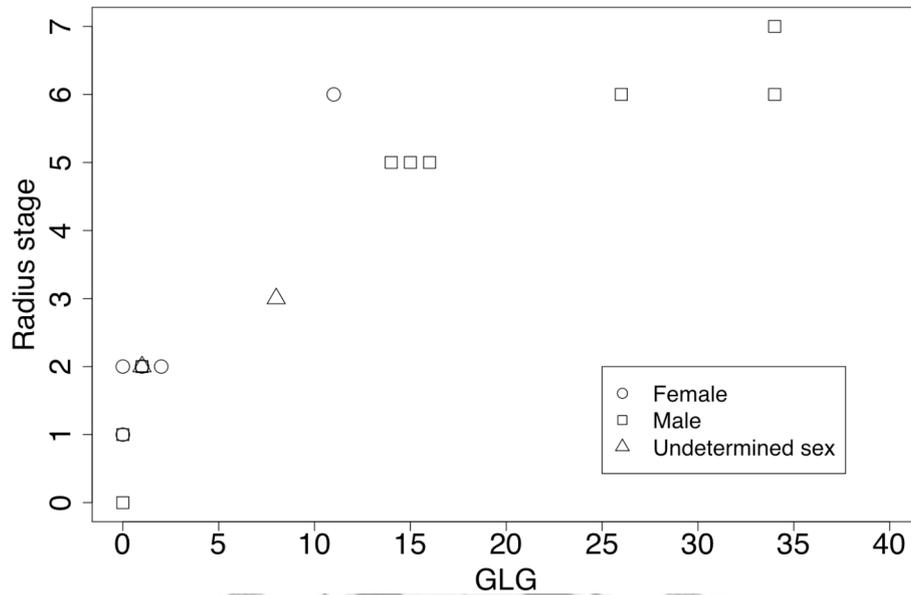


Figure 3.3. Flipper skeletal maturation in Risso's dolphins as evaluated by the stage of epiphyseo-metaphyseal ossification (0 – 7) in the distal radius (A) and ulna (B) in relation to TBL (cm). Each dot represents one flipper. Note paucity of dolphins in 200 – 250 cm TBL.

(A) Radius



(B) Ulna

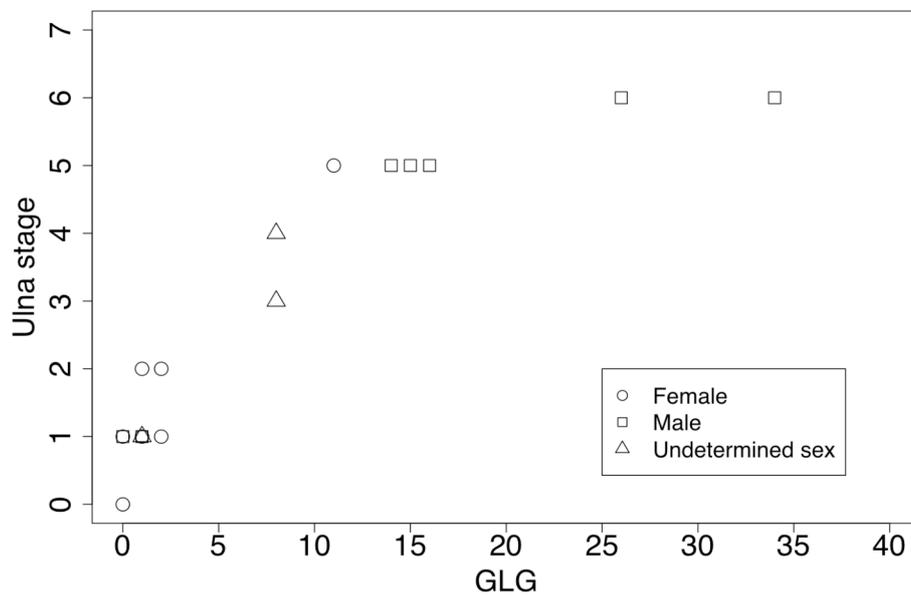


Figure 3.4. Flipper skeletal maturation in Risso's dolphins as evaluated by the stage of epiphyseo-metaphyseal ossification (0 – 7) in the distal radius (A) and ulna (B) in relation to estimated tooth age (GLG).

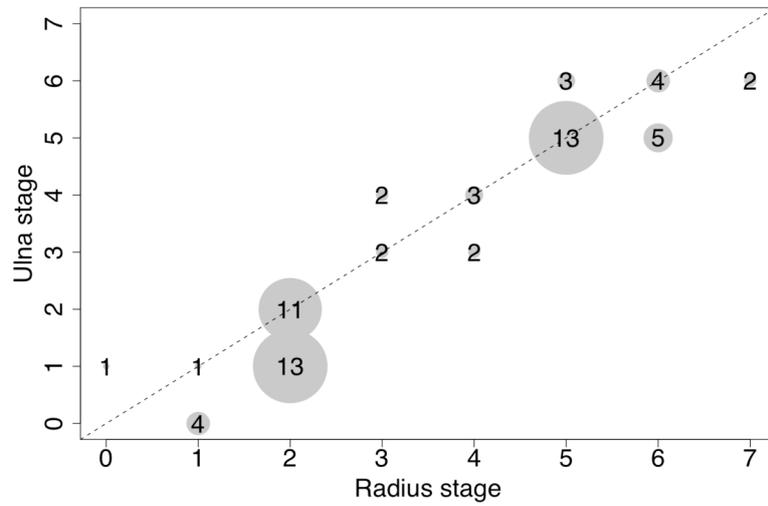
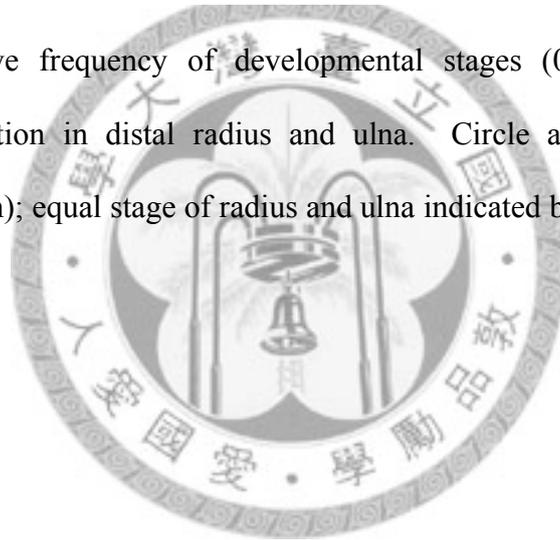
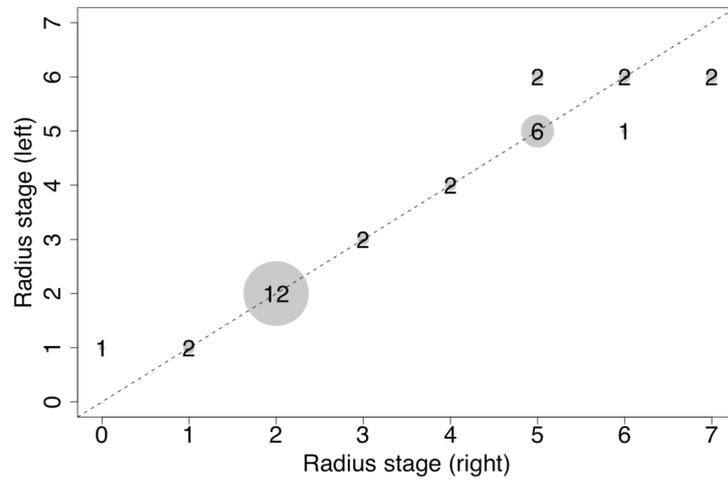


Figure 3.5. Relative frequency of developmental stages (0 – 7) of epiphyseo-metaphyseal ossification in distal radius and ulna. Circle area is proportional to frequency (n is shown); equal stage of radius and ulna indicated by dashed line.



(A) Radius



(B) Ulna

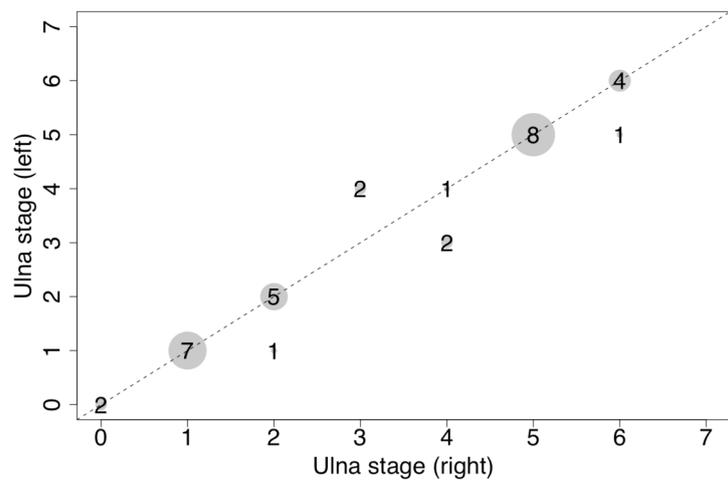


Figure 3.6. Correlation of epiphyseo-metaphyseal ossification stages (0 – 7) in right flipper by left flipper, revealing no stage difference – *i.e.*, bilateral symmetry of flipper maturation – in both distal radius (A) and distal ulna (B). Circle area is proportional to frequency (n is shown); equal stage of radius and ulna indicated by dashed line.

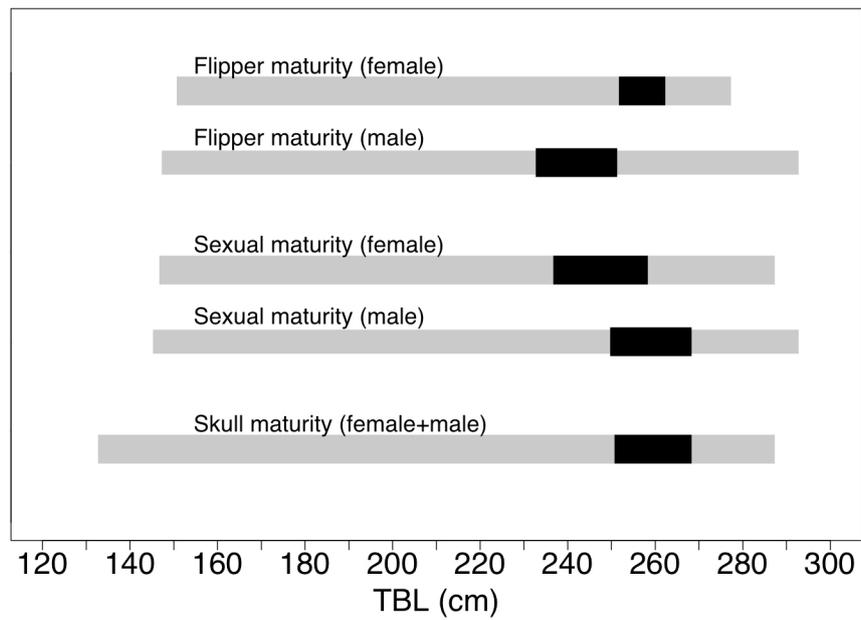


Figure 3.7. Graphic relationship of body lengths (TBL) at flipper skeletal maturity, sexual maturity (Chapter 2), and skull maturity (referred to fronto-occipital suture; Chapter 4) in male and female Risso's dolphins from Taiwanese waters. Gray bar – total range of sample, black bar – range of lengths between longest immature and shortest mature dolphin.



Table 3.1. Field collection data and estimated tooth age for 34 Risso's dolphins from Taiwanese waters with paired flippers radiographed for determination of flipper maturity. Arranged in ascending total body length (TBL).

Catalogue number	Sex	TBL (cm)	Sexual maturity	Estimated age ^a
ST20041101-1	F	-- ^b	--	0
HU20011027-2	M	148	Immature	--
HU2000035	M	150	--	0
TD20060812	F	154	--	0
CK20061209-1	F	155	--	0
CK20061209-3	F	159	--	--
CK20051208-2	M	161	Immature	--
TD20061117-2	U	165 ^c	--	1
SU94113	M	169	Immature	--
ST20041030-1	F	172	--	1
ST20051202	M	176	--	1
CK20061209-2	F	185 ^e	--	--
CK20070106	M	187 ^e	--	--
ST20051012	M	191	--	1
HU20041028-1	F	195	--	2
CK20051208-1	F	225 ^e	--	--
CK97014	M	229	Immature	--
IL20060312	U	--	--	8
CK97004	M	236	--	--
ST20041023-2	M	248	--	15+
TY20070314-2	M	253	Mature	--
TC2000001	M	254	Mature	--
HC97002	F	255	Immature	--
CK97005	F	257	Mature	--
CK97016	F	259	Mature	--
ST20041023-1	M	265	--	26+
TY20070314-4	M	267	Mature	16
ST20051214 ^d	M	268 ^e	Mature	--
TY20070314-3	M	269	Mature	14+
ST20051115-4	M	269	--	15
TP20080430	M	270	Mature	--
TT20040405	F	271 ^e	--	--
TD20061117-1	F	274 ^e	--	11
TY20070314-1	M	290	Mature	34

F – female, M - male

^a as determined by GLG evaluation (Chapter 2)

^b data not available

^c TBL estimated from TBL-RAL correlation equations (Chapter 2)

^d only left flipper examined.

Table 3.2. Mean TBL (cm) for male and female Risso's dolphins from Taiwanese waters at different stages in flipper maturity (0 – 7) of the distal radius and ulna (n = number of flippers). Student's t -test applied to test for significant differences in TBL between groups.

	Radius			Ulna		
	Male	Female	P value	Male	Female	P value
Stage 0	150 $n=2$	-- ^a	--	148 $n=2$	--	--
Stage 1	149 $n=4$	--	--	170 $n=10$	162 $n=5$	0.48
Stage 2	177 $n=10$	170 $n=12$	0.28	178 $n=4$	175 $n=7$	0.75
Stage 3	229 $n=2$	--	--	233 $n=2$	225 $n=1$	--
Stage 4	236 $n=1$	240 $n=4$	--	233 $n=2$	245 $n=3$	0.41
Stage 5	263 $n=17$	263 $n=4$	0.97	261 $n=16$	267 $n=7$	0.16
Stage 6	265 $n=7$	269 $n=5$	0.42	275 $n=9$	262 $n=3$	0.13
Stage 7	290 $n=1$	257 $n=1$	--	--	--	--

^a data not available

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Chapter 4: Maturation of skull in Risso's dolphins, *Grampus griseus*, from Taiwanese waters

ABSTRACT

The degree of fusion of sutures on the skull is often a useful indicator of skeletal and sexual maturity for data deficient cetacean specimens in museums. The aim of this study was to examine the degree of fusion between bony elements in skulls of Risso's dolphins (*Grampus griseus*) from Taiwanese waters, and to analyze the relationship between skull maturity, body length, sexual maturity, and estimated age, with the aim of determining an appropriate skull indicator for maturity in Risso's dolphins. The stage of fusion of 20 superficial sutures/synchondroses/symphysis between selected skull bones was examined on 33 clean, dry skulls, which were salvaged from stranded or bycatch dead Risso's dolphins in Taiwanese waters during the years of 1994 – 2001. The bones of the caudoventral brain case fused early in development (basioccipital-exoccipital synchondrosis and supraoccipital-exoccipital suture), whereas fusion along the nuchal crest (fronto-parietal and fronto-interparietal sutures) occurred later, and some sutures may remain open in adulthood in these specimens (lacrima/maxilla-frontal, squamosal-parietal, squamosal-exoccipital sutures, intermandibular symphysis); bilateral asymmetry of the fusion process was not detected. At total body length > 250 cm (at sexual maturity) advanced fusion occurred along the medial aspect of nuchal crest, in the rostral nasal-frontal, and distal maxilla-incisive sutures which may be useful indicators of sexual maturity.

Key words: Risso's dolphin, *Grampus griseus*, Taiwan, skull, development, maturity, sutures



INTRODUCITON

Skulls are routinely studied as part of many investigations of mammalian biology. The characters examined include both qualitative and quantitative features, which are analyzed to discern the presence of and the degree of sexual dimorphism, ontogenetic and individual variation as well as geographic/oceanographic variation within and amongst populations (Perrin 1975). Such data contributes substantially towards resolution of questions of taxonomic, ecologic, and evolutionary importance. Since these characters typically change during growth, an adult skull with relatively fixed characteristics is preferred for non-developmental aspects of these examinations (Perrin & Heyning 1993). For many museum skull specimens however, the size, reproductive status or other age-related information is often not available, and thus the degree of fusion of sutures on the skull becomes a useful indicator of maturity for such specimens (Dailey & Perrin 1973).

A pioneering and comprehensive study in spotted and spinner dolphins (genus *Stenella*) from the eastern tropical Pacific and Hawaiian waters related chronological age to skull maturity (Perrin 1975). One part of this was the innovative analysis of the postnatal development of the skull (spotted dolphins: $n = 69$; spinner dolphins: $n = 28$) by partitioning it into assemblages of bones, an apparatus, according to some common function: the most precocious in development was the hearing apparatus, followed in order by the vision apparatus, the braincase, the breathing and sound-producing apparatus, and finally the feeding apparatus. Skulls of spotted dolphins reached “adult configuration” by five years of age (five or more postnatal dentinal layers or GLGs) and at four years of age in spinner dolphins.

Although a specific bony suture can serve as an indicator for maturity, the developmental process of each suture has its own chronological variation, and thus empiric baseline data needs to be established for each species, and perhaps for each regional population. A simpler technique that rather than examining complex bony apparatuses, just examined the degree of fusion of the distal rostrum proved a fairly reliable indicator of skeletally immature specimens in bottlenose dolphins (*Tursiops truncatus*) ($n = 128$) from the western North Atlantic Ocean (Mead & Potter 1990). This study examined the maxilla-premaxillary suture (referred to hereafter as maxilla-incisive suture in current study) and cautioned that it needed careful scrutiny to clarify the degree of fusion. A study in striped dolphins (*Stenella coeruleoalba*) ($n = 15$) (Calzada *et al.* 1997) from the western Mediterranean Sea also focused on fusion of the distal portion of the maxilla-incisive suture, as well as the nasal bones, based on an earlier protocol for spotted (*Stenella attenuata*) and spinner dolphins (*Stenella longirostris*) (Dailey & Perrin 1973). This assessment of skull maturity in striped dolphins determined that although skull fusion occurred between 13 – 20 years of age, sexual maturity occurred at about 12 years of age, and that females reached full skull maturity earlier than males. In short-beaked common dolphins (*Delphinus delphis*) however, fusion of the distal maxilla-incisive suture is an inaccurate marker of cranial maturity – the rostrum tends to elongate after this fusion begins, and both sexually mature and sexually immature dolphins have fusion (Perrin & Heyning 1993). In dusky dolphins (*Lagenorhynchus obscurus*) ($n = 116$) advanced fusion of the frontal-supraoccipital suture is the most reliable skull maturity reference to predict sexual maturity (95% efficiency) (Van Waerebeek 1993). In addition, fusion of the zygomatic-parietal/exoccipital and the lacrimal-maxilla/frontal sutures are accurate maturity

indicators (89% and 85% respectively), although advanced fusion of the distal maxilla-incisive suture only occurred in 25% of adult skulls. The unreliability of fusion in distal maxilla-incisive suture as an indicator of maturity was likewise noted in Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Walker *et al.* 1986).

In Risso's dolphins, *Grampus griseus*, however, there are few studies on the skeleton, and especially on its skull. An early morphological account described the skull of two females (3.2 m adult, 1.85 m immature calf) (Flower 1874), and subsequent accounts detailed other skull features in order to clarify the taxonomic status of the species (True 1889, Mizue & Yoshida 1962, Ross 1984). Other than a brief note that the palatine-maxillary and maxilla-incisive sutures are fully fused in old specimens (True 1889), we are not aware of studies on the development nor on the maturation of the skeleton, including its skull, in Risso's dolphins.

The aim of present study therefore, was to investigate the maturation of the skull in Risso's dolphins from Taiwanese waters, and in particular to examine the degree of fusion between the bony elements as seen on their dried, cleaned skulls preserved as museum specimens, and to analyze the relationship between skull maturity, body length, sexual maturity, and estimated age, with the aim of determining an appropriate skull indicator of maturity.

MATERIALS AND METHODS

Specimens

Thirty-three clean, dry skulls (13 females, 11 males, nine of undermined sex), which were salvaged from stranded or bycatch dead Risso's dolphins (1994 – 2001), were prepared and preserved in the National Museum of Natural Science (Tai-Chung, Taiwan). Date, sex, and standard external morphological data were recorded for 13 females and 11 males (Norris 1961). Reproductive maturity was assessed in nine dolphins (four female, five male): females were classified sexually mature if either milk was found in a mammary gland or a corpus luteum or corpus albicans was present in an ovary (Perrin & Reilly 1984); males were classified as sexually mature if either a testis weighed more than 300 g (Kasuya & Izumizawa 1981, Amano & Miyazaki 2004) or if it measured more than 40 cm in length (Ross 1984)(Chapter 2). Age was estimated in three dolphins (one female, two males) by determining the number of growth layer groups (GLG) in routinely histological preparations of longitudinally sectioned teeth, with the assumption that one GLG represented one calendar year (Kruse *et al.* 1999, Amano & Miyazaki 2004) (Chapter 2). The condylobasal length (CBL) was measured on the intact skulls from 30 dolphins as a straight line from the tip of rostrum (incisive bone) to the occipital condyle (Perrin 1975). In the case of nine dolphins without TBL and known sex records, we considered that they were sexually mature when $CBL > 465$ mm ($n = 3$) and immature when $CBL < 465$ mm ($n = 6$) (Mizue & Yoshida 1962).

Maturity Examination

Maturation of the skull of Risso's dolphins was evaluated by examination of the fusion of 20 superficial sutures/synchondroses/symphysis of the bones forming the skull

(Fig. 4.1). These particular fusion sites were selected based on our preliminary study of Risso's skulls and a review of studies on cranial maturity in other odontocetes (Mead & Potter 1990, Van Waerebeek 1993, Calzada *et al.* 1997). We adapted chosen sites and definitions as follows:

SYM: the full circumferential line of symphysis between the left and right mandibles;

MX-INdi: the bony rostrum between the antorbital notch and the rostral tips of incisive bone; the distal-most, or most rostral one-third (**MX-INdi-di**) and the middle-third (**MX-INdi-mi**) of the maxilla-incisive suture were evaluated along the dorsal surface of the rostrum (left and right);

MX-MX: the intermaxillary suture on the caudal half of the ventral surface cranium;

PT-PL: the pterygoid-palatine suture evaluated around its full periphery on the external surface (left and right);

PT-BO: the pterygoid-basioccipital suture on the ventral surface of the basicranium (left and right);

LC: the lacrimal/maxilla-frontal suture (left and right);

NS: the nasal-frontal sutures, recorded as **NSro** (from rostrally end of **NS-NS** to the rostral-lateral corner), **NSlt** (the rostral-lateral corner to the caudal-lateral corner) and **NScd** (from the caudal-lateral corner to the caudal end of **NS-NS**), both left and right were examined;

NS-NS: the internasal suture on the dorsal cranium;

FR-FR: the interfrontal suture, examined on the most rostral extent visible superficially (**FR-FRro**) and the caudal extent (**FR-FRcd**), but not the middle section which covered by the nasal bones;

FR-PR: the fronto-parietal suture at the nuchal crest (left and right side);

FR-IP: the fronto-interparietal suture on the dorsal surface of the cranium, medially between left and right **FR-PR**;

SQ-PR: the squamosal-parietal suture (left and right);

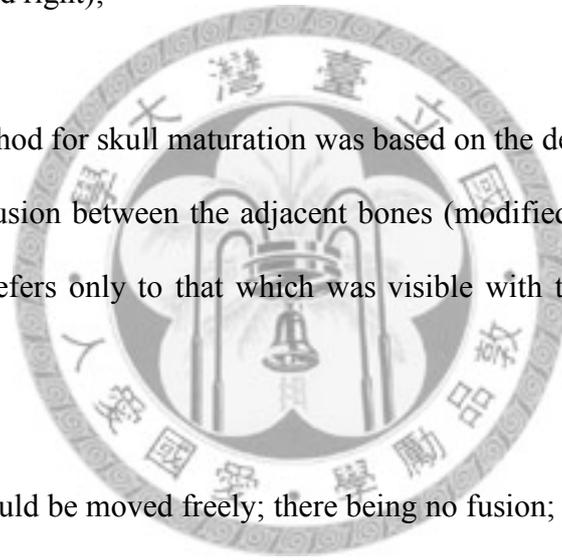
SQ-EO: the squamosal-exoccipital suture (left and right);

PR-EO: the parietal-exoccipital suture (left and right);

SO-EO: the supraoccipital-exoccipital suture on the external surface of the caudal cranium (left and right);

BO-EO: the basioccipital-exoccipital synchondrosis on the ventral surface of the cranium (left and right);

The scoring method for skull maturation was based on the degree of movement and the degree of bony fusion between the adjacent bones (modified after Van Waerebeek 1993). Here fusion refers only to that which was visible with the unaided eye on the external surfaces.



Stage 0: the bones could be moved freely; there being no fusion;

Stage 1: the bones could not be moved, and the junction between the bony elements or suture was clearly visible at all points;

Stage 2: partial obliteration of the suture line, due to advancing fusion;

Stage 3: complete obliteration of the suture line.

Data Analysis

Statistical software Microsoft Office Excel 2003 (Microsoft corporation) and R (version 2.5.0, <http://www.r-project.org/>) were used to establish a database, and to perform comparisons. Efficiency of fusion stage in skull sutures as indicators for sex

maturity was calculated (after Mead & Potter 1990). Wilcoxon signed-rank test was used to detect the bilateral asymmetry in the maturity stage between the left and right side of the sutures/synchondroses. Tests were considered statistically significant at $P < 0.05$.

RESULTS

Skull Maturation Process

Generally, the fusion of sutures was more advanced in dolphins with longer TBL. The least suture fusion was seen in one small male dolphin (136 cm TBL) – his skull was completely disarticulated, all skull bony elements were separated (stage 0) (Fig. 4.2A); the most developmentally mature skulls were from one male (254 cm) and one female (272 cm), both were sexually mature and in which all sutures were fused (at least reached stage 1) and many of them at advanced fusion (stage 2-3) (Fig. 4.2D).

In the shortest dolphins (136 – 234 cm) eight sutures (LC, MX-MX, NS, NS-NS, PT-BO, PT-PL, SQ-EO, SQ-PR) and the intermandibular symphysis (SYM) remained unfused (stage 0), whereas in longer dolphins (254 – 284 cm) many of these (MX-MX, NS, NS-NS, PT-BO, PT-PL, and SYM) were fused in different degrees (stage 1 – 3) (Fig. 4.3). The degree of fusion in three other sutures however (LC, SQ-EO, SQ-PR), showed no consistent relationship with increasing TBL in dolphins greater than 234 cm. In contrast, three other sutures had a consistent pattern of fusion: for PR-EO, MX-INdi and FR-FR, the onset of stage 0 occurred at TBL 145 – 181 cm, were consistently stage 1 at TBL of 183 – 234 cm, and were generally at advanced stages of fusion (stage 2 or 3) in dolphins with TBL > 254 cm. The sutures on the caudoventral braincase (SO-EO,

BO-EO) remained open only in short dolphins (TBL 145 – 165 cm), and both reached stage 2 or 3 in dolphins longer than 171 cm.

Ages were estimated in three dolphins: the suture fusion stages in a five-year-old female (TBL 229 cm) and a six-year-old male (TBL 236 cm) were almost the same, although more advanced fusion was seen in the six year-old than in the five year-old dolphin on the LC, MX-INdi-mi, FR-FRro, FR-PR, SO-EO, MX-MX, and SYM. In the skull of a 14-year-old female (TBL 272 cm) all examined sutures/synchondroses/symphysis were fused and most had reached advanced fusion stages, except NS-NS and PT-PL, which were at stage 1.

Sexual Maturity Indicators

Sexual maturity was only directly examined in nine dolphins: six were sexually immature (150 – 234 cm) and three were sexually mature (254 – 284 cm). Advanced fusion (Stage 2 or 3) in PR-EO, MX-INdi-di, NSro, FR-IP, and SYM was found in all sexually mature dolphins, and none in immature dolphins. On the other hand, if the onset of sexual maturity was taken to occur at TBL 250 cm (Chapter 2), then the efficiency of fusion stage in skull sutures as indicators for sexual maturity was greatest, i.e., lowest misclassification rate for FR-IP (0%), NSro (3.9% for <250 cm; 0% for >250 cm), MX-INdi-mi (7.4% for <250 cm; 0% for >250 cm) and PR-EO sutures (0% for <250 cm; 11.1% for >250 cm), but the highest misclassification rate in SYM (7.7% for <250 cm; 14.3% for >250 cm) (Table 4.2).

Bilateral Symmetry in Maturation

No significant difference in the fusion pattern of all the sutures between the left and right sides of the skull was detected by Wilcoxon signed-rank test ($n = 32-33$, $P < 0.01$).

DISCUSSION

Skull Maturation Process

In Risso's dolphins we found that various groups of bones had different fusion patterns. The earliest onset of fusions occurred between the bones of the caudoventral braincase and their sutures reached an advanced fusion stage early in development before the onset of sexual maturity (BO-EO, basioccipital-exoccipital synchondrosis and SO-EO, supraoccipital-exoccipital sutures). The interfrontal (FR-FR) and maxilla-incisive (MX-INdi) sutures however, commenced fusion at about a similar TBL but took a longer time for fuller fusion, and reached an advanced fusion stage at the TBL of sexual maturity. And the fusion along the nuchal crest (FR-PR, fronto-parietal and FR-IP, fronto-interparietal sutures) occurred relatively later, but reached an advanced fusion stage when the dolphins attained a TBL similar to that for the onset of sexual maturity. On the other hand, some sutures remained open, at least in part, in adulthood (LC, lacrimal/maxilla-frontal; SQ-PR, squamosal-parietal; SQ-EO, squamosal-exoccipital sutures). Overall, the order of postnatal development of skull in these Risso's dolphins was similar to a sequence of skull maturation, based on the bony functional apparatuses, for spinner and spotted dolphins (Perrin 1975) and harbor porpoises (Gol'din 2007). In particular in Risso's dolphins, the caudoventral braincase bones fused before the interfrontal and maxilla-incisive sutures, which are topographically closely associated to some breathing/sound producing structures of the skull (Perrin 1975, Rommel *et al.*

2002), which in turn fused before the intermandibular symphysis as functionally part of the feeding apparatus, and late in maturation.

Nevertheless, full skull maturation in Risso's dolphins apparently takes longer than in *Stenella* spp., since the fusion of at least six sutures is not complete until after sexual maturity (Fig. 4.3), which is somewhat similar to that in harbor porpoises (Gol'din 2007). Whereas, in spotted and spinner dolphins, most skull elements matured to adult state and size (4 – 5 years of age) before onset of sexual maturity (9 – 11 years) (Perrin 1975, Chivers & Myrick 1993). Even so, some caution is suggested in this interspecies comparison since the examination methods were different among these studies (see above).

Suture Fusion as Indicator of Sexual Maturity

Our limited data in Risso's dolphin, reveals that the suture at the medial aspect of the nuchal crest (fronto-interparietal suture) was a highly accurate predictor for sexual maturity (0% misclassified), which substantiates that previously validated for dusky dolphin (their so-called frontal-supraoccipital suture)(Van Waerebeek 1993). In addition, the bony maturity of the suture along distal rostrum (distal maxilla-incisive suture) might also be a good predictor of sexual maturity. This study is now added to the list of five small odontocete species in which advanced fusion of the distal portion of the maxilla-incisive suture is acceptable indicator for sexual maturity: bottlenose dolphins (Mead & Potter 1990, Van Waerebeek *et al.* 1990, Turner & Worthy 2003), spotted dolphins (Dailey & Perrin 1973, Douglas *et al.* 1984, Schnell *et al.* 1985, Yao *et al.* 2008), striped dolphins (Calzada *et al.* 1997), spinner dolphins (Dailey & Perrin 1973, Douglas *et al.* 1986, Perrin *et al.* 1999), and Fraser's dolphins (*Lagenodelphis hosei*) (Perrin *et al.* 2003).

Overall, the sutures at the medial aspect of the nuchal crest (fronto-interparietal), along distal rostrum (distal maxilla-incisive suture) and at the rostral side of the nasal bones (rostral nasal-frontal sutures, NSro) might be good indicators for sexual maturity. They were all relatively consistent to identify in fusion stage and had a low miscalculation rate. The parietal-exoccipital suture and the intermandibular symphysis also had advanced stage of fusion at about sexual maturity, but their usefulness was limited because of the visual difficulty in clearly identifying the stage of fusion and the high miscalculation rate.

Bilateral Symmetry in Maturation

Bilateral asymmetry of the skull is a well-recognized and canonical character of odontocetes (Ness 1967, Rommel *et al.* 2002) as it is fundamentally associated the development of echolocation capability (Cranford *et al.* 1996). Nevertheless, bilateral asymmetry had not been recognized in the development or in the maturation of the bones of the odontocete skull – this current study in Risso's dolphins likewise was unable to detect any asymmetry in the skull maturation process, as was also not detected in the maturation of the flipper bones (Chapter 3).

Limitations of this Study / Further Study

This study was intended as a preliminary examination into the maturation pattern of skulls of Risso's dolphins from Taiwanese waters. The limited number of available skulls with applicable data including sex, TBL, and chronological age, has restricted the strength of conclusions in this study. This was particularly a concern in the lack of bountiful adult specimens with reliable sexual maturity information as this reduced the

accuracy of skeletal maturity predictors (flipper and skull) for sexual maturity. Further investigations are required, especially with larger numbers of longer and sexually mature dolphins that have teeth available for estimation of age. Such specimens and information would assist confirmation of our suggestions about the life history and biology of Risso's dolphins in Taiwanese waters.



FIGURES AND LEGENDS

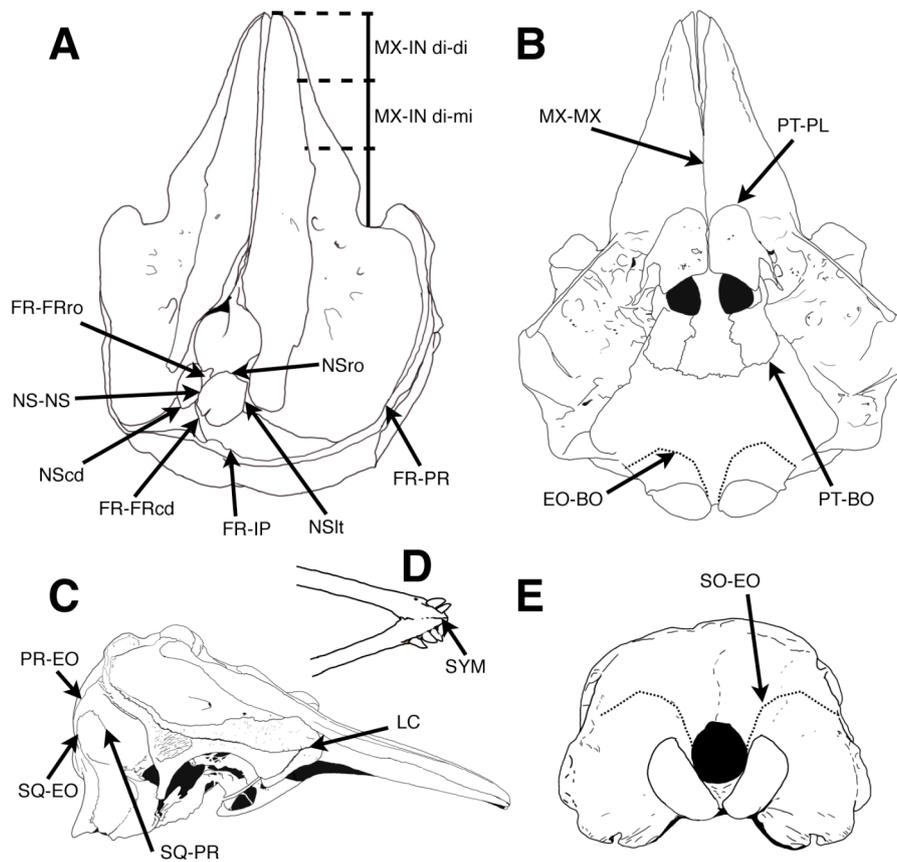


Figure 4.1. Superficial sutures/synchondroses/symphysis examined for fusion states on the skulls of Risso's dolphin from Taiwanese waters, in dorsal view (A), ventral view (B), lateral view (C), ventral view of rostral ends of mandibles (D), and caudal view of skull (E). Suture/synchondrosis/symphysis name abbreviations as per Materials and Methods.

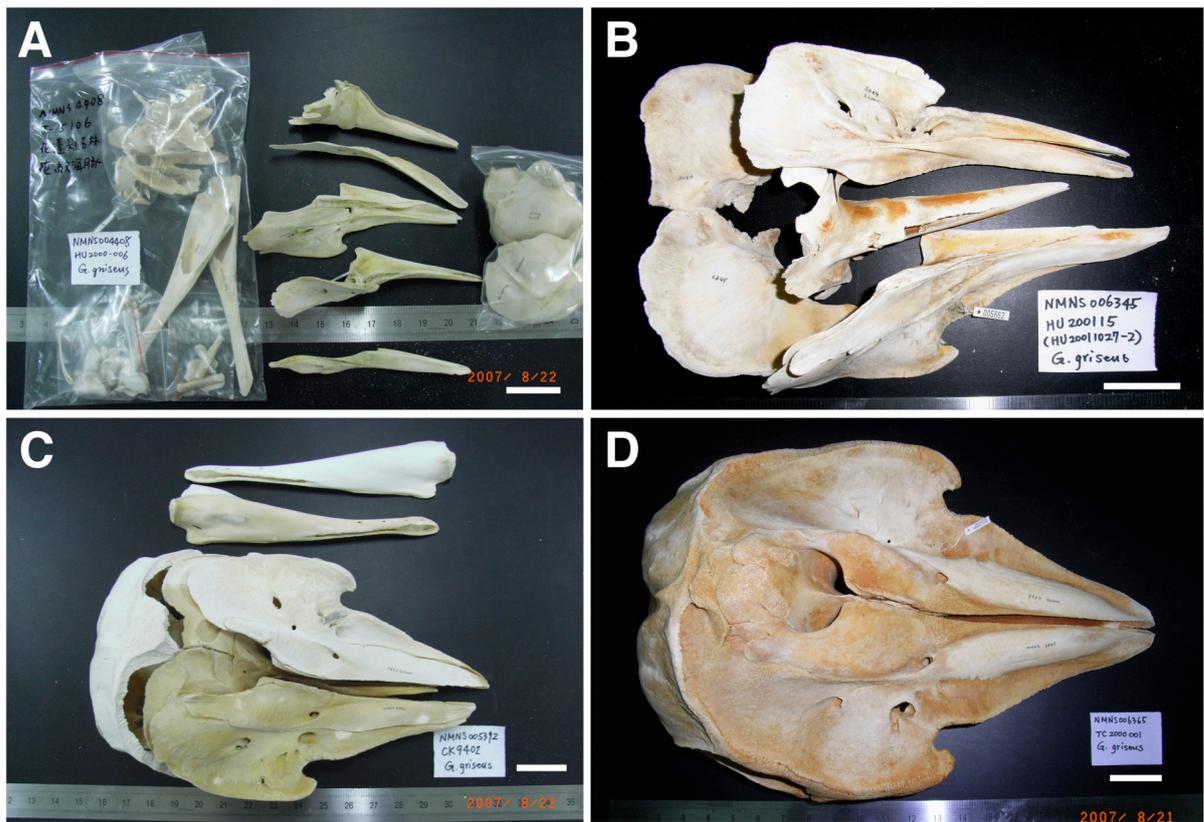


Figure 4.2. Photographs of skulls of Risso's dolphins showing varying degrees of fusion, in dorsal view. Scale bar = 5 cm.

- A: All bones completely disarticulated, 136 cm, male, sexual maturity undetermined;
- B: Some fusion between incisive and maxilla of rostrum, 148 cm, sexually immature male;
- C: Intermediate fusion showing caudal skull separation from facial and cranial braincase bones (sutures along the nuchal crest remained opened), left and right mandibles unfused, 179 cm, sexually immature male;
- D: Advanced fusion, many sutures at stage 2-3, 254 cm, sexually mature male.

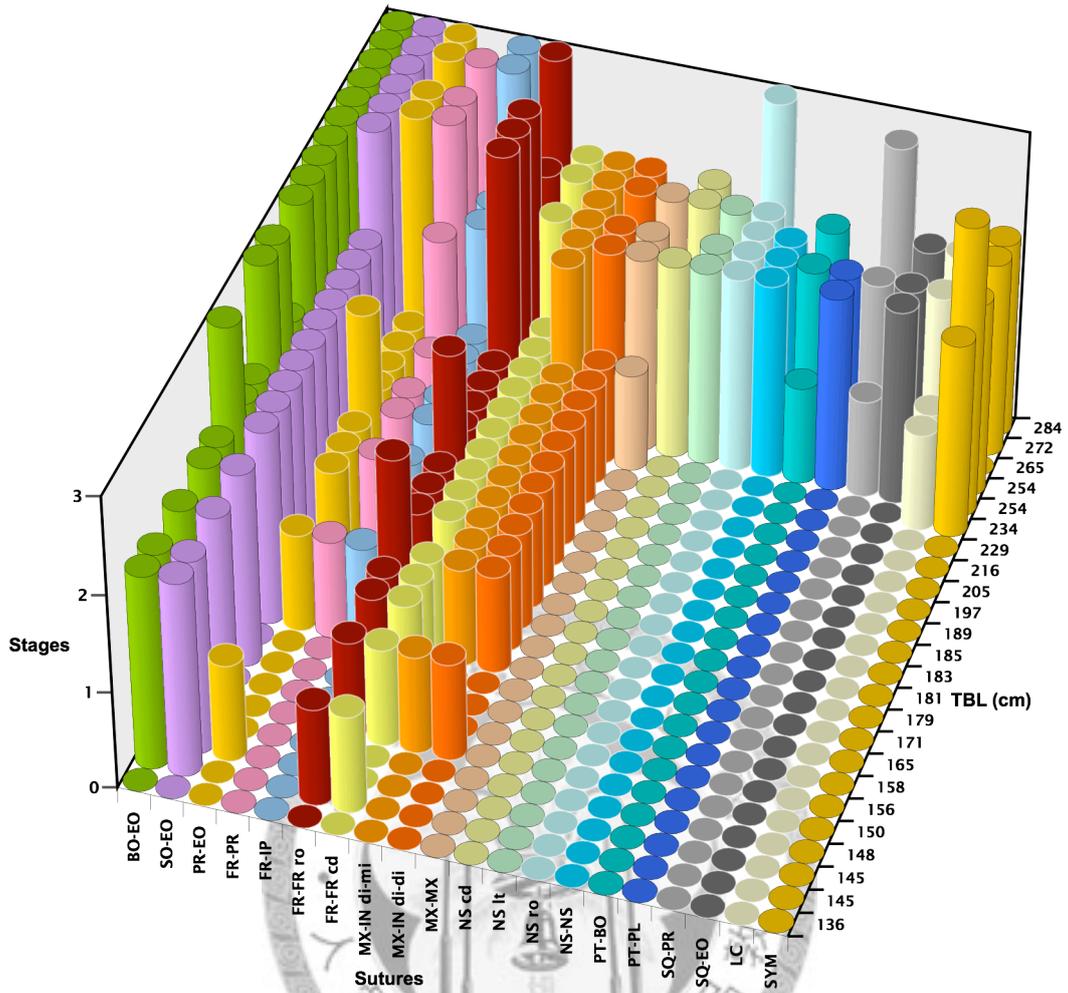
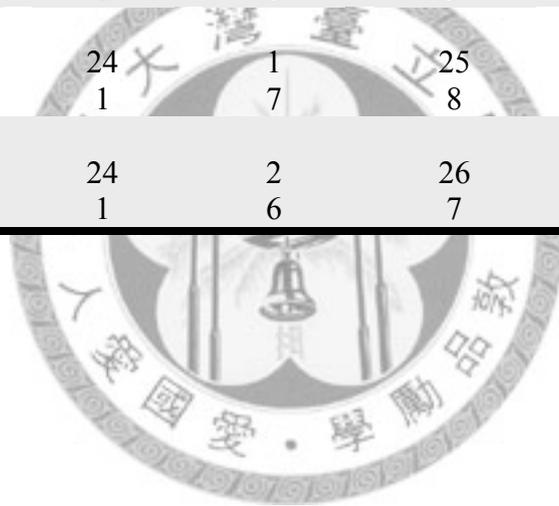


Figure 4.3. Graphic relationship of 24 Risso's dolphins (TBL) and their skull suture fusion stages for 20 superficial sutures/synchondroses/symphysis.

Suture/synchondrosis/symphysis name abbreviations as per Materials and Methods.

Table 4.2. Efficiency of fusion stage of skull sutures as indicator for sexual maturity, set at TBL of 250 cm (Chapter 2) (after Mead & Potter, 1990).

Suture & Fusion Stage	Sample <250 cm	Sample >250 cm	Total Sample (n)	Percentage misclassified
<u>FR-IP</u>				
Stage 0, 1	25	0	25	0
Stage 2, 3	0	8	8	0
<u>NSro (L)</u>				
Stage 0, 1	25	1	26	3.9
Stage 2, 3	0	7	7	0
<u>MX-INdi-di (L)</u>				
Stage 0, 1	25	2	27	7.4
Stage 2, 3	0	6	6	0
<u>PR-EO (L)</u>				
Stage 0, 1	24	0	24	0
Stage 2, 3	1	8	9	11.1
<u>FR-PR (L)</u>				
Stage 0, 1	24	1	25	4
Stage 2, 3	1	7	8	12.5
<u>SYM</u>				
Stage 0, 1	24	2	26	7.7
Stage 2, 3	1	6	7	14.3



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