Life history of the Indo-Pacific humpback dolphin in the Pearl River Estuary, southern China

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ABSTRACT

We studied life history characteristics of the Hong Kong/Pearl River Estuary population of Indo-Pacific humpback dolphins (*Sousa chinensis*), based on data from 120 specimens stranded between 1995 and 2009, 40 individuals biopsied at sea, and a long-term (14+ yr) photo-identification study. Ages were determined for 112 specimens by thin-sectioning teeth and counting growth layer groups. Estimated length at birth was 101 cm. Longevity was at least 38 yr, and there was little difference in growth patterns of males and females. Growth was described by a Bayesian two-phase Gompertz model; asymptotic length was reached at 249 cm. The tooth pulp cavity filled at an average of 18.5 yr of age. Physical maturity was reached at between 14 and 17 yr of age, apparently a few years after attainment of sexual maturity. Maximum lengths and weights of about 268 cm and 240 kg were attained. Females appear to lose all their spots by 30 yr, although males may retain some spotting throughout life. Calving occurred throughout the year, with a broad peak from March to June. Of 60 females monitored at sea for >14 yr of the study, none were documented to have more than three calves, suggestive of low reproductive output or low calf survival.

Key words: age, growth, reproduction, sexual maturity, age/sex classes, calving seasonality, Hong Kong, Pearl River Estuary, Southeast Asia.

Knowledge of the growth, reproduction, and demography of wildlife populations is critically important to their management and conservation. Efforts to protect

the animals from exploitation and habitat loss/deterioration are generally hampered when such information is lacking. Humpback dolphins (*Sousa* spp.) have not been well studied in most parts of their range, and thus very little is known about the life history of dolphins of this genus of coastal small cetaceans. Only a single study has analyzed growth and reproductive parameters based on a large sample of specimens, in this case a population of Indo-Pacific humpback dolphins in South Africa (Cockcroft 1989); however, the results have not been published in the peer-reviewed literature. Elsewhere, only scattered details of the life history of other humpback dolphin populations have been available, usually based on opportunistic records and very small samples (*e.g.*, Lal Mohan 1982, Wang and Sun 1982, Ross 1984).

In Hong Kong waters, where Indo-Pacific humpback dolphins have been studied perhaps more intensively than in any other single location of the species' range, apparently a single population exists (Chen *et al*. 2010). These animals range throughout all of the adjacent Pearl River Estuary (PRE), southern China (Hung and Jefferson 2004), although they have not been as well studied in mainland Chinese waters. It has recently been found that the population numbers >2,500 individuals (Chen *et al*. 2010), making it the largest known population of the species or the genus.

Despite the intensive ecological studies in Hong Kong, life history of this population is still not well understood. Jefferson (2000) conducted a preliminary analysis of growth and reproduction, based largely on specimens stranded over a 4 yr period. A preliminary growth curve was presented, but the sample of known-sex specimens was not adequate to evaluate possible sexual differences, nor to construct a detailed growth curve. Calving seasonality and recruitment rates were evaluated from small samples. Data on length and age at attainment of sexual maturity were almost nonexistent, owing to the fact that most specimens available for the study were badly decomposed carcasses.

The present study reevaluates most of the issues and parameters that Jefferson (2000) examined, based on much larger sample sizes ($n = 120$ strandings *vs.* 34 in Jefferson 2000). In addition, the present study incorporates data from a long-term (>14 yr) photo-identification study and recent biopsy sampling of free-living dolphins, thus allowing a better assessment of most life history parameters. Unfortunately, due to the prevalence of young specimens among the strandings and the decomposed nature of most specimens, we were not able to improve our estimates of age and length at sexual maturity.

MATERIALS AND METHODS

Collection of Data and Samples from Strandings

Sporadic data on cetacean "strandings" (including both specimens collected as floating and beach-cast carcasses) in Hong Kong date back to 1954 (see Romer 1958), although it was not until 1973 that regular attempts were made by the Hong Kong Government to document cetacean strandings. In 1995, a systematic stranding recovery program was set up by the senior author, and since then there has been a concerted effort to examine and sample virtually every marine mammal stranding discovered in the Hong Kong Special Administrative Region (see also Parsons and Jefferson 2000). A total of 120 specimens of *Sousa chinensis* were documented as

stranded in Hong Kong between 1973 and 2004. Most data on humpback dolphins for this study come from carcasses stranded in Hong Kong between 1995 and 2009; for some analyses, data, and photos from previous Hong Kong strandings were also used, but only when the data were deemed reliable (see Parsons *et al*. 1995).

Necropsies were performed either in the laboratory (for fresh specimens) or in the field (for those that were badly decomposed or in relatively inaccessible locations). Basic biological data and samples were collected (see Parsons and Jefferson 2000 for a detailed discussion of the stranding program and sampling procedures). Specimens were classified as to their level of decomposition, using the codes outlined in Geraci and Lounsbury (2005). Total length for each specimen was measured in a straight line, using a taut tape measure, from the tip of the upper jaw to the notch in the tail flukes (Norris 1961). Two to three teeth were collected from the middle (or nearby in some cases) of the lower jaw and stored in water or alcohol. Reproductive tracts of fresh, noncalf specimens (generally codes 2 and 3) were examined, and gonads were collected and stored in 10% formalin.

Many stranded specimens (81%) were very badly decomposed (codes 4 or 5 of Geraci and Lounsbury 2005). For most of these, decomposition was too advanced to properly analyze reproductive status, but specimens for which an accurate length could still be obtained were used in the growth analyses. For some of these decomposed specimens, sex was impossible to identify in the field and was determined using DNA analyses (see below).

Collection of Data and Samples from Living Dolphins

Data from photo-identified living dolphins were used to determine color pattern development, reproduction, and calving seasonality and intervals (these analyses also incorporated data from strandings and biopsy sampling). When dolphins were sighted during at-sea surveys, observers typically went off-effort and the vessel approached the dolphin group for accurate estimation of group size and for photo-identification (see Jefferson 2000). Over 100,000 frames were taken of dolphins for the purposes of photo-identification and age/class assessment in this study. Photographs were taken with a variety of Nikon and Canon 35 mm SLR autofocus cameras (most recently a Canon 7D model), equipped with databacks or digital data recorders and 100–400 mm telephoto or zoom lenses. If possible, we photographed both sides of the dolphin, because the coloration markings are not completely symmetrical. Dolphins were identified by coloration and scars on their backs and dorsal fins (Würsig and Jefferson 1990, Jefferson 2000).

Identified dolphins were cataloged and given a unique catalog number with an alphanumeric code for the area where they were first identified (*e.g.*, NL02, EL07, SL30). As of early 2010, the photo-ID catalog contained data on 679 different individuals sighted between 1994 and 2009, with between 1 and 130 sighting records for each dolphin. Each individual was placed into an age class, based on its size, external morphology, color pattern, and behavior. There were six classes (Unspotted Calf, Unspotted Juvenile, Mottled, Speckled, Spotted Adult, and Unspotted Adult), as described in Jefferson (2000). Earlier reference to the Spotted Juvenile and Spotted Subadult categories are referable to the Mottled and Speckled classes, respectively (see Jefferson and Leatherwood 1997).

Between October 2004 and December 2008, we collected biopsy samples remotely using a Barnett Ranger RX-150 crossbow from 40 living specimens, focusing on photo-identified individuals (two individuals were inadvertently sampled twice) (see Jefferson and Hung 2008 for a description of biopsy methods).

Sex Determination

For freshly stranded specimens, we determined sex by direct examination of re-productive organs. For some photo-identified individuals, we could determine their sex as female by repeated, very close association over several months or years with young calves (these were associations much closer than those typical for other members of the group). For badly decomposed strandings and for biopsied individuals, we determined sex using DNA analyses at the Southwest Fisheries Science Center (SWFSC, NOAA, NMFS, La Jolla, CA). The sample of specimens in which DNA sex determination was attempted consisted of a total of 58 specimens. For most of these, decomposition was too advanced to determine sex even by laboratory methods.

To determine sex in the lab, standard protocols were used for DNA extraction and polymerase chain reaction (PCR) sequencing (Qiagen DNeasy #69506; Palumbi *et al*. 1991, Sambrook *et al*. 1989). Whether the sample came from a male or female was determined by a real-time qPCR assay following the methods in Morin *et al*. (2005). Sex-specificity of this technique has been demonstrated for 33 cetacean species in nine different families; 86 samples from specimens of known sex were tested, and the effectiveness of the technique was confirmed in each case (Morin *et al*. 2005).

Age Determination

We estimated age of specimens at the SWFSC, generally following the procedures described by Myrick *et al*. (1983) and Jefferson (2000). For each specimen, one or two teeth were decalcified in rapid decalcifying solution (RDO), a commercial rapid-decalcifying agent. Immersion times in the RDO were approximately 1.5–19.0 h, depending on the length of the animal. Teeth from the larger specimens were often first "wafered" with a diamond-edge saw to reduce decalcification times. The wafer consisted of a 2–3 mm thick longitudinal section through the middle of the tooth, encompassing the tip and pulp cavity. The decalcified teeth were sectioned longitudinally into 26 μm thick sections using a sledge-type sliding microtome, with a freezing stage. Sections through the center of the tooth and encompassing the entire pulp cavity were stained with Mayer's hematoxylin stain. After an ammonia rinse of 30–60 s, 4–5 sections from each specimen were mounted on glass microscope slides.

Tooth sections were then examined under a compound microscope (4–40× power), without reference to specimen length or other biological data. We assumed that one growth layer group (GLG) represented 1 yr. This assumption is supported by calibration studies on captive spinner dolphins (*Stenella longirostris*, Myrick *et al*. 1984) and is widely used in small-cetacean life history studies. We counted GLGs in the postnatal dentine. For some older specimens, the pulp cavity was completely occluded, making the dentinal GLGs indistinct. In these specimens, cementum layers were counted and were used to assess the age of the individual. The teeth were read independently by two of the authors (Jefferson and Robertson) 3–4 times each on days separated by at least 48 h. After the readings were complete, the readers compared their data and agreed on an age for each specimen. If the readers' GLG

counts did not agree, tooth sections were reexamined simultaneously by both readers and a final age was decided upon after discussion.

For some specimens, there was only a portion of the first GLG present. Clearly, these animals were less than 1 yr old, but in such cases it is difficult to accurately determine a precise age in months. Therefore, the age of these animals was listed as "<1" in the database.

Fitting of Growth Curves

We conducted a Bayesian estimation of the parameters of a two-phase Gompertz growth model (Laird 1966, Kaufmann 1981) using the samples for which we had both age and length estimates. Because this was a two-phase model, the change point (c) between the phases, and the parameters of the first and second curves were estimated using the following normal likelihood functions:

$$x_i < c : l_i \sim \text{Normal}\left(L_0 \cdot e^{k_1\left(1-e^{-g_1 \cdot x_i}\right)}, \tau_1\right)$$
$$x_i \geq c : l_i \sim \text{Normal}\left(L_c \cdot e^{k_2\left(1-e^{-g_2(x_i-c)}\right)}, \tau_2\right),$$

where x_i and l_i are the age (yr) and length (cm) of the ith sample. The remaining parameters and their priors are defined as:

$$c = \text{age at change point between first and second curves: } \textit{Uniform}(7, 13)$$
$$L_0 = \text{length at birth: } \textit{Uniform}(80, 120)$$
$$L_c = \text{length at change point given } L_0, k_1, \text{ and } g_1,$$
$$k_1, k_2 = \text{scale parameter of curve 1 or 2: } \textit{Uniform}(0, 100)$$
$$g_1, g_2 = \text{slope parameter of curve 1 or 2: } \textit{Uniform}(0, 100)$$
$$\tau_1, \tau_2 = \text{precision of curve 1 or 2: } \textit{Gamma}(10^{-4}, 10^{-4})$$

The prior on c was set based on available literature reporting estimates of age at sexual maturity from 7 to 13 yr for most closely related species of delphinids (see Perrin and Reilly 1984).

Length at birth was estimated in the same Bayesian analysis using a logistic likelihood function and including fetuses for which we had length measurements. The likelihood function was defined as:

$$\text{Pr}(birth)_j \sim \textit{Bernoulli}\left(\frac{e^{L_0+x_j}}{1+e^{L_0+x_j}}\right),$$

where $\text{Pr}(birth)_j$ was 0 for fetuses, and 1 for all other postnatal specimens. The Markov chain Monte Carlo (MCMC) run consisted of 10 independent chains, each starting with 50,000 burn-in iterations followed by 10,000,000 iterations with samples taken every 100th iteration, thus generating a total of 100,000 samples from the posterior distribution.

The age at which the pulp cavity filled (A_f) was estimated using a Bayesian logistic model similar to the length-at-birth model. The likelihood function was defined as:

$$\text{Pr}(filled)_j \sim \textit{Bernoulli}\left(\frac{e^{A_f+x_j}}{1+e^{A_f+x_j}}\right),$$

where $Pr(filled)_j$ was 0 for specimens with open pulp cavities, and 1 for those with filled cavities. The MCMC run consisted of three independent chains, each starting with 10,000 burn-in iterations followed by 10,000 iterations with samples taken every 10th iteration, thus generating a total of 1,000 samples from the posterior distribution. All Bayesian analyses were conducted with R v2.10.1 (R Development Core Team 2009) and the *BRugs* v0.5–3 package.

Determination of Physical Maturity

Physical maturity was determined for 10 large stranded specimens by extracting a section of 3–5 adjacent vertebrae from the center of the thoracic section of the vertebral column. The vertebral sections were cleaned by water maceration, and the epiphyses were checked for fusion to the centra (*i.e.*, all examined vertebrae had epiphyses completely fused to the adjacent centra). If all epiphyses were fully fused, then the specimen was classified as physically mature. If any of the epiphyses were unfused, the specimen was scored as physically immature.

Determination of Calving Seasonality

Calving seasonality was determined by computing an estimated birth date for each stranded calf of the year (<137 cm in length, see below) and fetus in the sample, based on the average length at birth, along with fetal and early neonatal growth rates from the literature. Birth dates for neonates were estimated using 101 cm as the average length at birth, and the length at 1 yr as 137 cm (after Jefferson 2000). Assuming linear growth in the first year, this translates to a first-year growth rate of 3.08 cm/mo. For fetuses, linear growth was assumed (based on the presence of only a very short, nonlinear phase, see Perrin and Reilly 1984). Assuming an 11 mo gestation period (Jefferson 2000), this corresponds to a fetal growth rate of 9.09 cm/mo. The estimated month of birth was then calculated from the average length at birth and the appropriate growth rate.

RESULTS

Age and Growth

We estimated age for a total of 112 specimens (102 from Hong Kong, five from Xiamen,[1,2] and five from nearby locations in the PRE), measuring from 100 to 268 cm. For the length-at-birth model, there were eight fetuses measuring from 46 to 100 cm. Of the total postnatal specimens, 44 (39%) of them were <1yr old. The oldest specimen was estimated to be 38 yr old (a female) and 78% of specimens were sexed (Fig. 1).

[1] While it is unknown if dolphins from the PRE and Xiamen are part of the same population (see Wang *et al.* 2008), recently analyses of mitochondrial DNA have suggested that there may be some exchange of genetic material between the two areas (Chen *et al.* 2008).

[2] Hung, S. K. 2009. Monitoring of marine mammals in Hong Kong waters—data collection (2008–09): Final report. Unpublished contract report to the Hong Kong Agriculture, Fisheries and Conservation Department. Available from the authors.
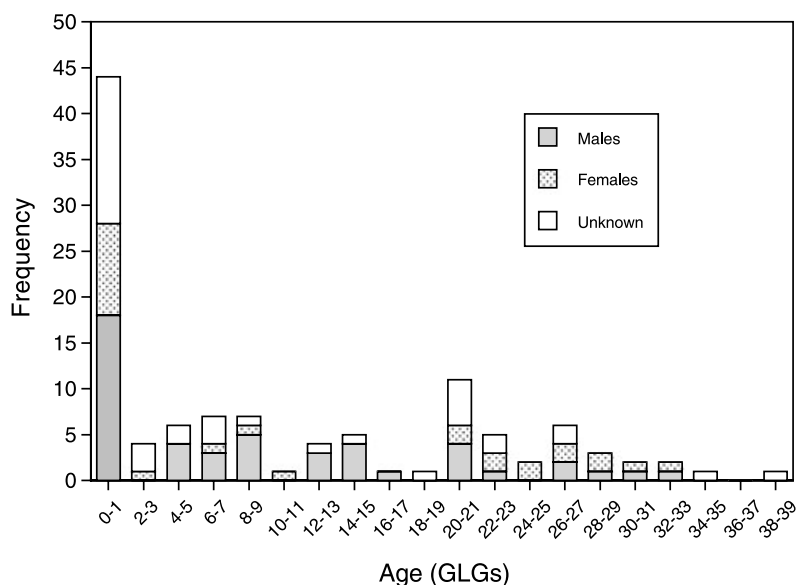
Figure 1. Age distribution of the sample of stranded specimens used for examining growth and reproduction.

The Bayesian MCMC two-phase Gompertz growth model showed good mixing within chains with no significant autocorrelation in the already thinned samples. Traces of the posterior sample and posterior distributions of each parameter are available in online Figures S1 and S2. There was little evidence of sexual dimorphism, with males and females clustering and showing little differentiation in length-at-age. For this reason, a single curve was constructed, with males, females, and unsexed specimens pooled, and an asymptote was reached at 249 cm.

The median estimated length at birth was 101 cm (Fig. 2) with 95% CI of 99–102 cm. Figure 3 shows the fit of the two-phase Gompertz model to the age-length data. The median estimated change point was 10 yr, with a 95% CI equal to its prior (7–13 yr). However, the posterior distribution of the change point is not uniform like its prior, but rather has some modal peaks, indicating that the data are only somewhat informative about its location. As can be seen in Figure 3, there are few samples between the ages of 9 and 13, which is where the uncertainty in length is greatest, immediately following the median change point estimate. This is also reflected by the range of the posterior distribution of g_2, the slope parameter for the second curve, which is equivalent to its prior distribution. Thus, this lack of data near the likely location of the change point hampers the model's ability to estimate the change point with any greater precision. After this point, growth continued into adulthood at an estimated median asymptotic length of 249 cm (95% CI = 244–267 cm).

Information on vertebral fusion was available for only 10 specimens. Four had vertebrae that were not fully fused, and they ranged from 230 to 249 cm in length and from 7 to 14 yr in age. Six specimens had fully fused vertebrae, and they ranged from 238 to 252 cm in length and from 17 to 32 yr in age. Therefore, based on
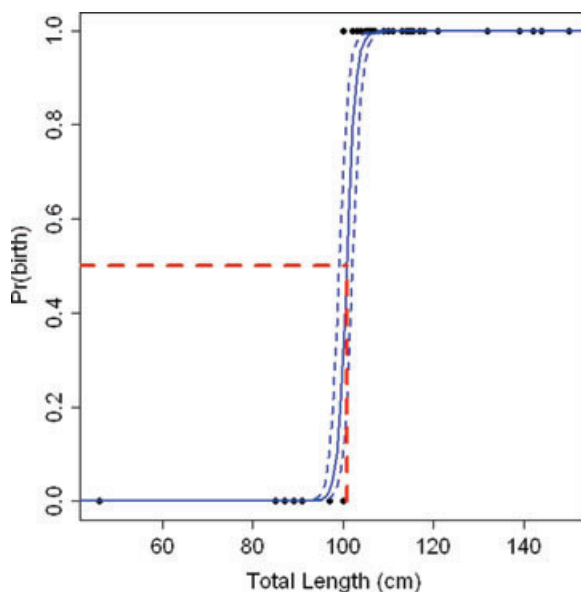
Figure 2. Posterior of logistic estimate of probability of birth *vs.* total length. Solid blue line is median of estimated probability from posterior distribution, and dashed blue lines are upper and lower 95 percentiles. Dashed red line indicates estimated length at birth (Pr[*birth*] = 0.5).
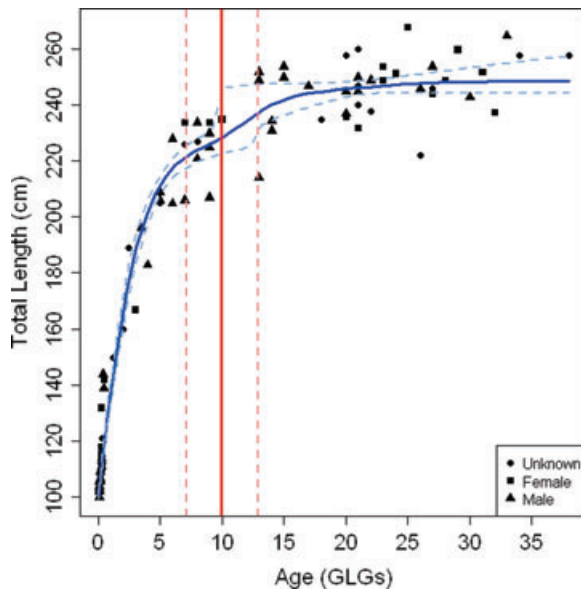

Figure 3. Bayesian two-phase Gompertz growth curve with estimated change point. Solid blue line is median of estimated length for each age from posterior distribution; dashed blue lines are upper and lower 95 percentiles. Solid red line is median of change point; dashed red lines are upper and lower 95 percentiles.
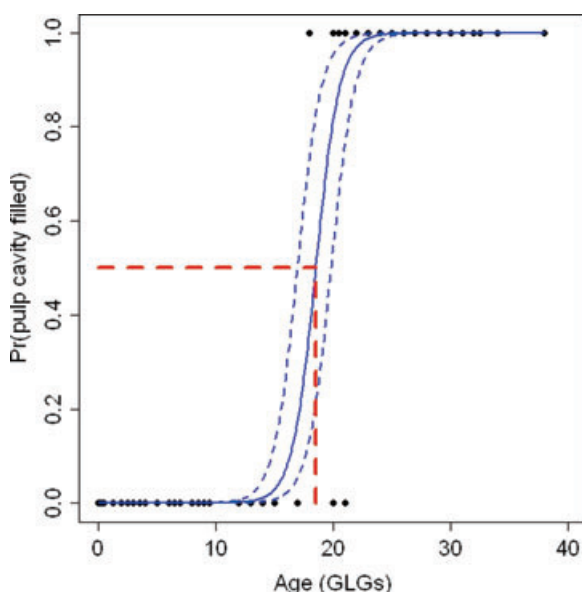
Figure 4. Posterior of logistic estimate of average length at which the pulp cavity fills (dashed red line). Solid blue line is median of estimated probability from posterior distribution; dashed blue lines are upper and lower 95 percentiles.

vertebral fusion, physical maturity is estimated to occur at between 14 and 17 yr of age, and at lengths between approximately 238 and 249 cm.

The pulp cavity in the center of the tooth filled in and became occluded at ages ranging between 18 and 21 yr (Fig. 4), which is just after physical maturity is reached (see above). The average age at which the pulp cavity filled was estimated to be 18.5 yr with a 95% CI of 17.0–19.8. Of five specimens with an open pulp cavity in which physical maturity was evaluated, four (80%) were classified as immature; and all four (100%) of those with a filled pulp cavity were mature, based on vertebral fusion.

The weight/length relationship is shown in Figure 5, for both fetal and postnatal specimens. There appears to be a relatively continuous curve, with the maximum weight reached at around 240 kg.

Color Pattern Development

In the PRE population, there are six age classes (summary in Table 1), and it should be noted that there appears to be some age overlap among the different classes (*i.e.*, animals of a particular age may fall into more than one age class). The first is Unspotted Calf, and these animals of both sexes are clearly newborns <1 yr old. A near-term female fetus (98 cm long) collected from a 254 cm female (age = 23 GLGs) looked very similar to an Unspotted Calf. However, its coloration was somewhat lighter gray than that of most UCs, suggesting that newborns may darken dramatically immediately after birth (Fig. 6). We have only examined four Unspotted Juveniles. These animals are clearly young (although older than newborns), and are much smaller than adults. There is no indication of a difference between the sexes at
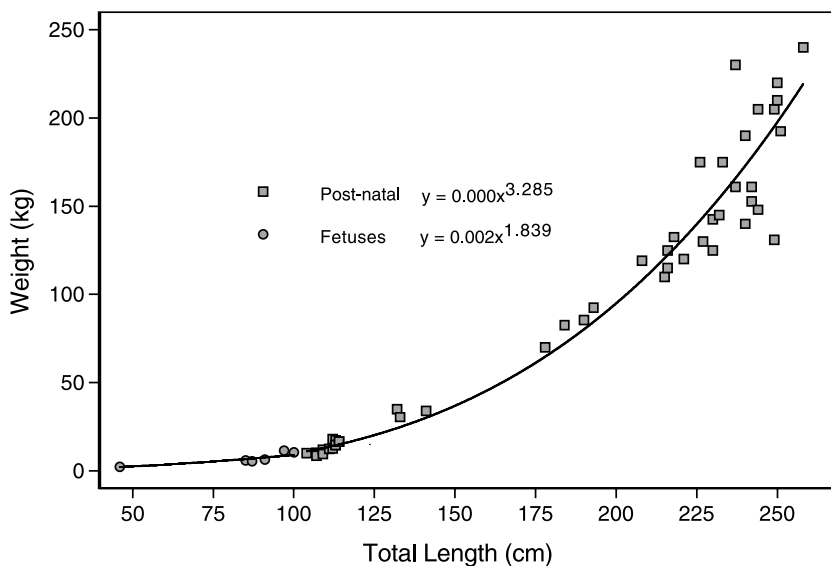
Figure 5. Weight/length relationship of Indo-Pacific humpback dolphins from Hong Kong and Pearl River Estuary.

this life stage. Although the two that had age estimates were both <1 yr in age, a larger sample may show that Unspotted Juveniles may span several years in age.

Mottled animals (which used to be called Spotted Juveniles) are slightly less than adult size and appear to contain more males than females. They appear to be mostly older subadults, although some young adults may also be in this category (ages range from at least 7 to >13). Speckled animals (which used to be called Spotted Subadults) are nearly adult size, although the small amount of data suggests that most are probably at ages around puberty. Some are likely young adults (the oldest was 20 yr old), and both males and females are represented.

Spotted Adults and Unspotted Adults are both of adult size, and we are quite certain that virtually all of these animals are sexually mature. Most in our sample were females, although there are clearly some males in the Spotted Adult category. Almost all Unpotted Adults appear to be females. In fact, we have only documented one (out of 13) Unspotted Adult that was a male (EL01, sex determined from biopsy sampling). This individual may have been anomalous (perhaps an albino) or, alternately, it may have been a very old male. Unspotted Adults are older animals; most are over 25 yr of age.

Reproduction

Due to a paucity of fresh noncalf specimens, no new data (see Jefferson 2000) on the age and/or length at attainment of sexual maturity have been obtained in this study (see Discussion below). Length at birth is estimated to be 101 cm, based on Bayesian analysis (Fig. 2). Projected birth dates of fetuses and neonates strongly suggest that some births occur in every month of the year (the absence of records for October is probably related to small sample sizes), but with a large, although

Table 1. Summary of age/sex classes and characteristics of specimens that have been iden-
tified as in a particular class. The scarring column gives the approximate proportion of
individuals with moderate to major nicks and/or scarring on the dorsal fin and tailstock.
These classes apply to the Pearl River Estuary population of humpback dolphins, and dol-
phins from other areas in Chinese waters may have very different patterns (see Wang *et al.*
2008).

Age class	Abbreviation	Sex			Total length (cm)	Age (yr)	Scarring
		Male	Female	Unknown			
Unspotted Calf	UC	6	8	2	102–130	<1[a]	None
Unspotted Juvenile	UJ	3	1	0	139–209	<1	None or light
Mottled	SJ	8	5	1	204–234	7–>13	Often with significant scarring
Speckled	SS	14	10	0	207–250	4–20	Often with significant scarring
Spotted Adult	SA	5	14	0	235–265	9.5–32.5[b]	Scarring tends to be light
Unspotted Adult	UA	1	12	0	238–268	25–32	Scarring tends to be light

[a]Most < 6 mo.
[b]Most > 20 yr.

somewhat protracted, peak in the spring and early summer months (from March to
June, Fig. 7). During this third of the year, when air and water temperatures are
rising rapidly, 61% of the calves were born. The pattern of estimated birth dates
closely follows the overall distribution of strandings throughout the year, which
is not surprising, since a large percentage (53%) of all strandings are neonates (see
Jefferson *et al.* 2006). At-sea sightings of Unspotted Calves followed a broadly similar
distribution, with a peak between April and July (or lagged 1 mo behind the peak of
birth dates, which is not surprising since mothers with very young neonates appear
to actively avoid vessels) (Fig. 8).

Several dolphins known to be females from our photo-ID and biopsy studies
have shown an interesting pattern of calf production. In the photo-ID catalog, a
total of 60 females have records of calves since 1996, and the period of female-
calf association and calving interval were examined. Among these 60 females,
50 of them were only seen with one calf during the study period. Ten other
females had records of two calves, and most of these are residents of Hong Kong with
relatively long-sighting histories. Notably, many calves (40 of the 70 calves) were
seen only once with their mothers. Using the subsample of females that had been
observed over a period of at least 5 yr, the general pattern is the same: 26 females
were documented to have only a single calf, nine of them were documented to have
two calves, and only a single one was documented to have three calves.

It is possible that for some of these calves, their mothers are not residents of Hong
Kong and do not occur there frequently enough to be resighted again during the
period of female-calf association. Another plausible explanation is that the calves did
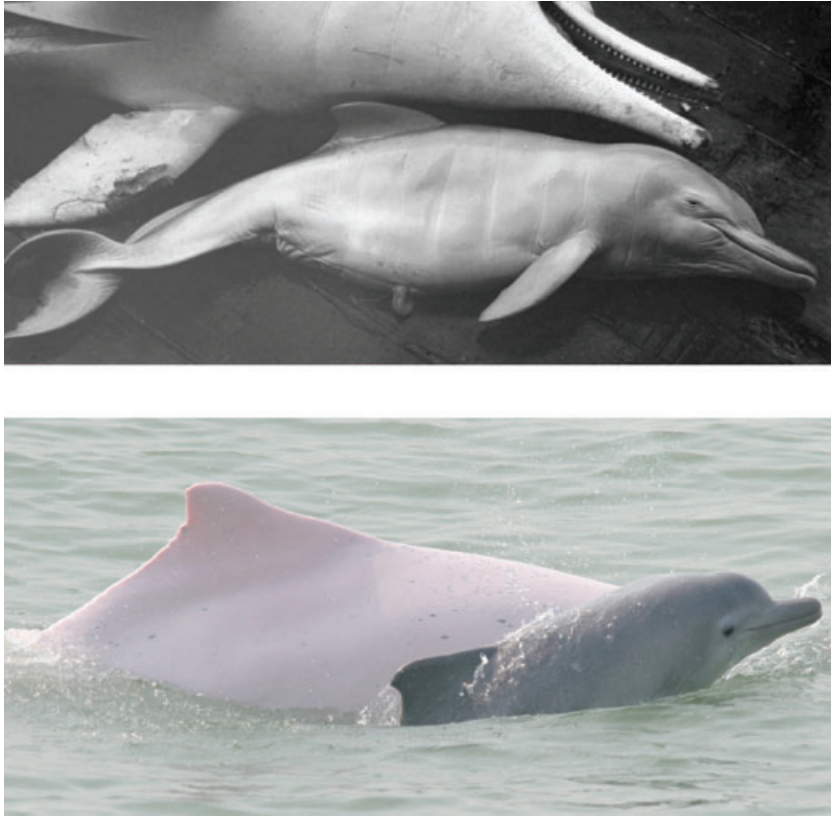not survive long enough to be seen at the next sighting of their mothers. In fact,

Figure 6. Near-term fetus (98 cm female) collected from a specimen found floating west of Lantau Island (SC98–03/06, upper), and neonate (Unspotted Calf) observed at sea in Hong Kong waters (lower).

epimeletic behaviors were observed on a number of occasions in the past, with healthy individuals supporting dead, newborn calves by carrying them in their mouth or on their back (TAJ and SKH, unpublished observations). The potentially low survival rate of calves is also exemplified from the stranding data. Among the 137 stranding records of Indo-Pacific humpback dolphins from 1996 to 2006, 44 and 12 had body lengths of <120 cm (presumably newborn calves) and 120–180 cm (presumably older calves), respectively, providing preliminary evidence that many calves did not survive long after birth.

A total of 10 females had records of two calves since 1996, and the maximum calving intervals were estimated. Again, it should be cautioned that the estimated calving intervals are probably overestimates, as the first calves may still associate with their mothers after the last sightings, while the second calves may have already associated with the same females well before their first sightings were made. Moreover, there were also possibilities that some females might have given birth again during the interval, but have gone unnoticed. Nevertheless, the maximum interval between the two births for these 10 mothers ranged from 18 to 165 mo, with an average of 62.6 ± 47.76 mo (median = 54.5 mo).
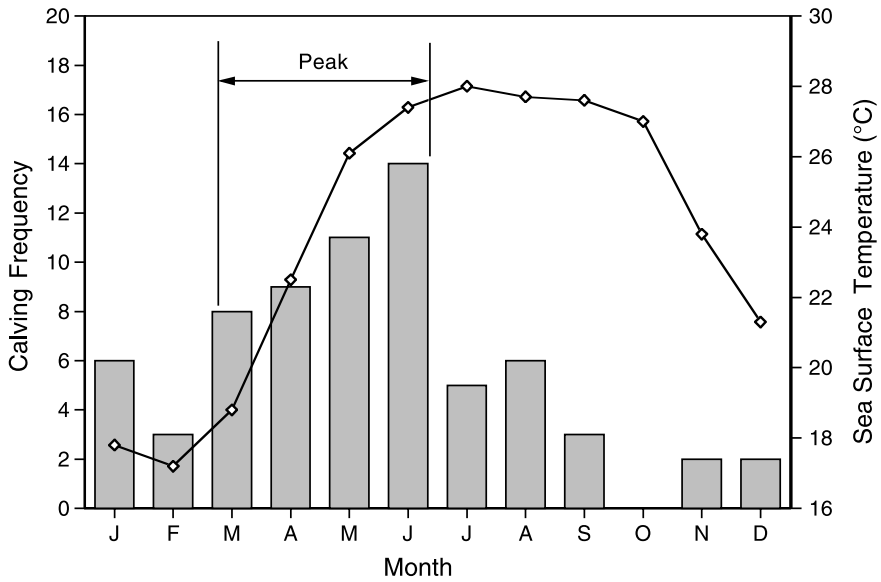
Figure 7. Monthly distribution of projected birth months for fetal and neonatal specimens, showing the estimated peak in calving. Also, shown are average sea surface temperatures for Hong Kong waters.
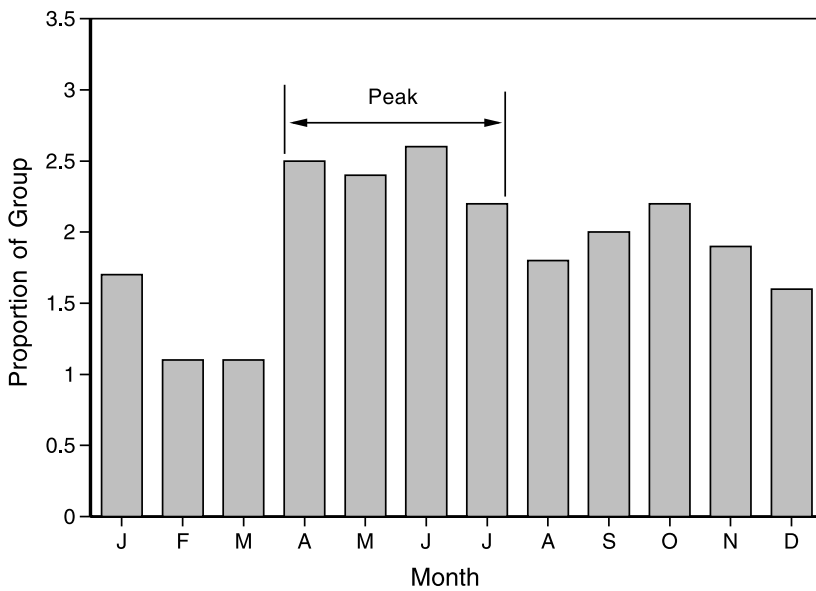

Figure 8. Monthly distribution of Unspotted Calves from at-sea surveys (as a proportion of individuals in each group).

<center>DISCUSSION</center>

Age and Growth

The analyses of growth indicate that a two-phase model describes growth well in this population and suggest that a growth spurt occurs at a point near the age at which dolphins become sexually mature. Unfortunately the change point falls in a data-poor region of the age/length plot. This is reflected in the posterior distribution of g_2, which is equal to the prior.

In a previous study, it was found that 42 of 79 (53%) humpback dolphin strandings in Hong Kong were young-of-the-year (<1 yr old, Jefferson *et al*. 2006). The present study also supports a high neonatal mortality rate. These statistics indicate a high proportion of young (and especially neonate) specimens among the stranded sample, and this fact is possibly related to high concentrations of organochlorines in dolphin tissues (Parsons and Chan 1998, Parsons 2004, Jefferson *et al*. 2006).

Color Pattern Development

The updated analysis of this paper reinforces our belief that once animals develop spotting and pass out of the Unspotted Juvenile class, they progressively lose spotting throughout their lives, with females doing so more quickly and reaching a relatively unspotted stage much sooner than males. It is still unclear whether males become completely unspotted in old age, and further research is needed to clarify this. The oldest females (>25 yr) are clearly unspotted.

The observations above suggest that males and females do not begin to differ much in appearance until they are in the Unspotted Juvenile stage (probably between 2 and 6 yr of age). At that point, it appears that females may lose the majority of their spots very quickly and most probably move rapidly to the Spotted Adult stage, then proceed to lose their remaining spots more slowly until they become virtually unspotted in old age (over 25–30 yr). Males, on the other hand, appear to retain heavy spotting much longer, although at least some lose their spots completely (*e.g.*, EL01). So far, with this one exception, all old (25+ yr old) males appeared to retain a moderate amount of spotting.

There are two major issues that still need to be resolved to clarify this picture and to determine if we can reliably distinguish males from females at sea. First, do very old males typically get to a point where they lose all or most of their spotting? Second, what happens during the period from about 2–6 yr of age? We have very little data on external appearance during this period, when animals are growing and probably changing rapidly. At what specific point do males and females begin to differ, and do they follow the same pattern of spot loss (at very different rates), or is there some qualitative difference in how they appear in the years leading up to sexual maturity (at about 8–13 yr, see below)? There is some suggestion from live sightings at sea of two different patterns of coloration development among older Unspotted Juveniles, but this remains unconfirmed (as these animals rarely strand). Continued long-term monitoring of individuals in Hong Kong may provide information on how the color pattern changes as individuals move from the UJ to the older age classes.

Age classes have also been recognized for humpback dolphins of South Africa, but they do not correspond to those for dolphins from southern China, which have very different patterns of growth, sexual dimorphism, coloration, and external morphology

(see Saayman and Tayler 1979). South African animals appear to grow quite a bit larger (Jefferson and Van Waerebeek 2004), have significant sexual dimorphism (Cockcroft 1989), are dark gray in color as adults and do not lighten significantly with age (Jefferson and Karczmarski 2001), and possess a large hump of connective tissue on the back (Saayman and Tayler 1979, Jefferson and Karczmarski 2001). Clearly, they are very different animals from those in the PRE, although there are also some very interesting similarities (see below).

It is interesting to compare the color pattern development of Chinese humpback dolphins with that of the only other delphinids that have a similarly extensive pattern of spotting, the pantropical and Atlantic spotted dolphins (*Stenella attenuata* and *S. frontalis*). In both species of spotted dolphins, calves are born unspotted with a two-tone pattern consisting of a white belly and dark cape. They first develop dark spots on the white ventral surface in the speckled stage, and are not yet sexually mature. In the mottled stage, the ventral spots become more extensive and light dorsal spots appear on the cape (dolphins reach puberty in this stage). In *S. attenuata* sexually mature specimens enter the fused stage, in which the dorsal spotting continues to develop, and the ventral spots become so large and extensive that they fuse and then lighten to yield a uniform gray ventral surface (Perrin 1969, Perrin *et al.* 1976). In *S. frontalis*, older animals remain mottled (Herzing 1997). The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) also has spotting, but generally only on the ventral surface (Wang and Yang 2009).

Color pattern development appears to be very different in humpback dolphins. Although newborns are also unspotted, their coloration is more nearly uniform dark gray at birth (but with a lighter belly). The dark ground color lightens (UJs are lighter in color than UCs) before spotting begins to develop at the Mottled stage, and this does not appear to occur in the spotted dolphins (*Stenella* spp.). The main difference between the two groups is that in spotted dolphins, spotting develops through the appearance of contrasting spots on a dark or light surface. In humpback dolphins, the spots are only dark and appear to develop through the loss of pigmentation in the surrounding area. While in spotted dolphins, the oldest adults are also the most heavily spotted, in humpback dolphins, the most heavily spotted animals are apparently subadults and young adults. Once they appear, there is a progressive loss of spots (albeit quite slowly) in older humpback dolphins, which appears to result in the complete loss of all spotting in old females (and at least some males).

Thus, the spotting of the two groups of dolphins may look quite similar superficially. However, the patterns are in many ways the exact opposite. Spotted dolphins gain spots throughout their life, while humpback dolphins progressively lose them (with the exception of the calves and juveniles).

Reproduction

A previous study on humpback dolphins of Hong Kong evaluated reproduction based on a very small sample of specimens (Jefferson 2000). In that study birth length was estimated at about 100 cm, and age at sexual maturity for females approximately 9–10 yr. Although there were not enough data to estimate them empirically, gestation was assumed to be about 11 mo, and male sexual maturity was thought to occur at about 12–14 yr (Jefferson 2000). Although the present study was able to reevaluate growth and physical maturity parameters with much larger sample sizes, the paucity of fresh adult-sized specimens prevented us from determining sexual maturity more

precisely. We may be able to do so in the future using reproductive steroid analysis of biopsy samples. Due to lack of funding, most biopsy samples collected from Hong Kong have not been analyzed to determine reproductive steroid concentrations. Little is known about the reproductive physiology of this species, but in a study of captive specimens at Underwater World in Singapore, Brook *et al.* (2004) found an irregular pattern of ovarian cycling. There was no distinct or strong seasonality, which is generally consistent with our studies.

Most calves were associated with their mothers for less than 24 mo, but there were a few exceptions. Both NL202 and SL40 were seen with their calves for more than 3 yr, and WL 25 was seen with her calf for more than 4 yr. NL18 presented the most extreme case, as she was accompanied by her calf (NL259) from March 2000 to January 2009, a total of almost 9 yr. This individual was first identified at the start of the study in 1995 and was already of adult size at that time, suggesting that she may be an older individual. Therefore, it is possible that this calf was the last offspring of NL18, and therefore this long-term maternal relationship may be longer than usual.

If a high proportion of calves die shortly after birth, this may be a serious issue for the continued survival of the PRE dolphin population. It has long been speculated that mortality of young calves can be linked to the negative impacts of water pollution, as heavy loads of pollutants (*e.g.*, dichlorodiphenyltrichloroethane [DDTs], polychlorinated biphenyl [PCBs]) have been found among some stranded dolphin calves in Hong Kong (Parsons and Jefferson 2000, Jefferson *et al.* 2006). The increasing acoustic disturbance from vessel traffic and dolphin-watching activities at Tai O (in western Hong Kong), an area with a high density of mother/calf pairs, are additional stressors (Hung 2008, 2009). Special attention should be paid to alleviate these negative impacts, as the survival of calves is the most important element for the long-term survival of the dolphin population. Important habitats that recorded high density of calves, such as the entire west coast of Lantau Island, should receive urgent protection in order to safeguard mother–calf pairs from further disturbance, and to provide them with sufficient prey resources to cope with various threats.

The photo-identification records of known-female dolphins that have been frequently seen over the course of the study give cause for concern about calf production and survival. It appears that relatively few calves are being successfully weaned. Wells (2000) found that the likelihood of calf survival in bottlenose dolphins (*Tursiops truncatus*) was dependent on the mother's age, with younger females much less likely to successfully rear a calf to weaning. We assume that the same is true for humpback dolphins in Hong Kong, but we have no specific data to test this hypothesis. However, it is also very possible, even likely, that the extremely high concentrations of PCBs and DDTs in the tissues of these animals are compromising their reproductive potential. Evidence in support of this hypothesis was discussed recently by Jefferson *et al.* (2006), and the present study is consistent with this hypothesis.

From the data presented, the apparent calving interval for this dolphin population should be about 2–3 yr, and could potentially be up to 4–6 yr. The average calving interval of 5.2 yr is near the upper range of calving intervals reported for bottlenose dolphins of 2–6 yr (Connor *et al.* 2000). Although these dolphins likely enjoy long life spans, they appear to have long calving intervals, and their calf survival rate and fecundity appear to be fairly low. This is further supported by the evidence that most female year-round residents that have been consistently sighted in the past 15 yr have only successfully produced 1–2 offspring during that time. The low fecundity suggested by preliminary data may be caused by a number of factors, such as low natural survival rates of calves in this population, the negative effects

of environmental contamination, scarcity of nutritious prey resources, and increased amount of stress from anthropogenic disturbance.

Assuming that the maximum age seen in this study (38 yr) is typical for females in this population, and that there is no period of reproductive senescence, females would appear to have about a 28 yr reproductive life span. A 2.5 yr average calving interval would result in a reproductive output of 11 calves, and a 5-yr interval would result in only about 5 calves being produced in a lifetime. Reproductive senescence (which appears typical of most long-lived odontocetes, see Marsh and Kasuya 1986) and a shorter average life span would reduce the reproductive output of females even further. If the above analyses of calf production are accurate, then this means a potentially low reproductive output for females in this population. The calving history and calving interval should be closely monitored in future years of photo-identification work, to verify these findings with larger sample sizes.

Geographical Variation in Growth and Reproductive Parameters

There have been very few studies of the life history of humpback dolphins from anywhere else in the range of the genus *Sousa*. For the Indo-Pacific species, other work has only been done in South Africa (Cockcroft 1989) and to a lesser extent in central China (Wang 1965, 1995). No detailed life history studies have been conducted on the Atlantic humpback dolphin (*Sousa teuszii*) (see Van Waerebeek *et al.* 2004).

Table 2 summarizes the current best estimate of various life history parameters for the Hong Kong/PRE humpback dolphin population, and provides some comparisons with South African results. The oldest specimen in our study had 38 GLGs, which is similar to the situation in South Africa, where longevity reaches at least 40 yr (Cockcroft 1989). A peak in calving during the warmer months of the year may be typical for the species. Other studies of humpback dolphins have also found a peak of calving in summer (South Africa, Cockcroft 1989) or spring/summer (Xiamen; Wang 1965, 1995).

We found little evidence of differences in size between males and females in this study. This is quite different from the situation in South Africa, in which

Table 2. A summary of estimated life history parameters for the Pearl River Estuary population of humpback dolphins, compared with values for South Africa (from Cockcroft 1989).

Parameter	Pearl River Estuary	South Africa
Length at birth	101 cm	100 cm
Age at sexual maturity (females)	9–10 yr[a]	10 yr
Age at sexual maturity (males)	12–14 yr[a]	12–13 yr
Asymptotic length	249 cm	240 cm (F), 270 cm (M)
Age at physical maturity	14–17 yr	n/d
Maximum longevity	38+ yr	40+ yr
Maximum length	265 cm	>270 cm
Maximum weight	240 kg	260 kg
Peak calving season	March–June	Summer
Calving interval	5 yr	3 yr

[a]Not estimated empirically from large samples.

there is extensive sexual dimorphism, with females reaching an asymptotic length of 240 cm and males 270 cm (Cockcroft 1989). South African males also develop a larger dorsal hump and distinct keels on the tailstock, and such dimorphism is not apparent in humpback dolphins from Chinese waters, nor in other parts of the range in southeast Asia. Wang (1965, 1995) stated that female humpback dolphins in Xiamen, southern China, were somewhat larger than males, but this may simply have been an artifact of small sample sizes. All indications from our data are that there is little, if any, sexual dimorphism in the growth of humpback dolphins from Chinese waters.

Conclusions

The role of long-term cetacean study was recently and quite elegantly demonstrated by Wells (2003), who outlined the subtleties and complexities of bottlenose dolphin ecology, life history, behavior, social organization, and population biology that have been elucidated through 35 yr of such research in Florida waters. Our research program has only been in place for about 15 yr, but we are beginning to see some of the same (albeit preliminary) benefits of such an approach.

Clearly, there is still a need to resolve the major uncertainties in development of the color pattern, potential differences between males and females, and to calculate age and length at sexual maturity. It may be possible to assign a sex to many of the adult-size individuals in our photo-ID catalog if we can confirm what appears to be sexual dimorphism in the color pattern. Biopsy sampling will undoubtedly be an invaluable tool in this research. By targeting specific individuals (*e.g.*, those showing particular age/sex patterns of interest) from our photo-ID catalog for biopsy and using the skin sample to determine sex, we can test hypotheses on the development of the color pattern. Through the continued combination of data obtained from many different research techniques, we hope to clarify the life history of dolphins in this population, and clear up many of the remaining uncertainties that have plagued management authorities for years.

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SUPPORTING INFORMATION

The following supporting information is available for this article online:

Figure S1: MCMC traces of parameters of Bayesian two-phase Gompertz growth model. Each trace is composed of 10 chains of 10,000 samples each.

Figure S2: Posterior distributions of parameters of Bayesian two-phase Gompertz growth model. Each distribution is composed of 100,000 samples.