

9-26-2014

## From progesterone in biopsies to estimates of pregnancy rates: Large scale reproductive patterns of two sympatric species of common dolphin, *Delphinus* spp. off California, USA and Baja, Mexico

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### Recommended Citation

Kellar, Nicholas Marc; Trego, Marisa L.; Chivers, Susan J.; Archer, Fredrick I.; and Perryman, Wayne L. (2014) "From progesterone in biopsies to estimates of pregnancy rates: Large scale reproductive patterns of two sympatric species of common dolphin, *Delphinus* spp. off California, USA and Baja, Mexico," *Bulletin of the Southern California Academy of Sciences*: Vol. 113: Iss. 2.

Available at: <https://scholar.oxy.edu/scas/vol113/iss2/2>

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# From progesterone in biopsies to estimates of pregnancy rates: Large scale reproductive patterns of two sympatric species of common dolphin, *Delphinus spp.* off California, USA and Baja, Mexico

## **Cover Page Footnote**

The authors acknowledge Amanda Bowman, James Cotton, Michael Force, Justin Garver, Siri Hakala, Candice Hall, Richard Pagen, Robert Pitman, Richard Rowlett, Juan Carlos Salinas, Heriberto Santana, Iris Segura, Corey Sheredy, Ernesto Vazquez, Sophie Webb, and Suzanne Yin, the field team, who collected biopsies and data aboard the NOAA Ship McArthur II and Jim Gilpatrick, Morgan Lynn, and Fionna Matheson who collected aerial images used in the percent-calf estimates. In addition, we thank the crew and officers of the NOAA Ship McArthur II and the NOAA Twin Otter 48. The biopsies were collected under permits 774-1714-10, MULTI-2008-003-A2, and 05599 issued by the USA's National Marine Fisheries Service and National Marine Sanctuary, and Mexico's Secretaria de Relaciones Exteriores, respectively. Finally, we want to thank James Carretta, Camryn Allen, Krista Catelani, Michelle Robbins, and Bill Perrin for their suggestions and comments refining this manuscript.

## From Progesterone in Biopsies to Estimates of Pregnancy Rates: Large Scale Reproductive Patterns of Two Sympatric Species of Common Dolphin, *Delphinus* spp. off California, USA and Baja, Mexico

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**Abstract.**—Blubber progesterone levels were measured in biopsy samples and used to predict the pregnancy status of 507 female common dolphins (204 long-beaked common dolphins, *Delphinus capensis*, and 303 short-beaked common dolphins, *D. delphis*). Samples were collected in the coastal waters of the eastern North Pacific between central California, USA and the southern end of Baja California, Mexico. The percentage of females pregnant was similar between the two species: 22.1% ( $n = 45$ ) of *D. capensis* and 28.1% ( $n = 85$ ) of *D. delphis*. For both species we found strong geographic patterns in pregnancy, suggesting that some areas were more conducive for pregnant females. A sizable drop in percent pregnant from early (38.8%,  $n = 133$ ) to late (25.3%,  $n = 91$ ) autumn was found in *D. delphis* but not in *D. capensis*. The potential for sample selectivity was examined via biopsies collected either from a large research ship or from a small, rigid-hull inflatable boat (RHIB) launched from the larger ship. An analysis of “Tandem Biopsy Sampling”, replicate biopsy effort on the same schools from each vessel/platform, yielded little evidence that disproportionately more pregnant female common dolphins were biopsied from one platform versus the other. This result plus an analysis of pregnancy status relative to the duration of biopsy operations failed to uncover strong evidence of unaccounted sampling bias with respect to pregnancy state. In total, these results demonstrate the utility of blubber progesterone concentrations to assess pregnancy status in free-ranging cetaceans and they highlight potential factors associated with population-level variation in dolphin pregnancy rates.

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### Introduction

In this study, we use hormone measurements collected from biopsy samples to evaluate pregnancy patterns in the two species of common dolphins in the eastern North Pacific. The overarching goals of this study are two-fold. The first is to investigate how pregnancy of these common dolphins varies with respect to species, season, geographic location, and schooling behavior. The second goal is to evaluate the potential sample selectivity of biopsies relative to pregnancy state. Because this is one of the first times that biopsies have been used to assess pregnancy parameters at a population level, it is important to assess whether data from these samples are suitable for such an analysis.

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### Common Dolphins and Reproduction

The short-beaked common dolphin, *Delphinus delphis*, and the long-beaked common dolphin, *D. capensis*<sup>1</sup> are remarkably similar in many aspects of their appearance, behavior, and biology (Heyning and Perrin 1994). While *D. capensis* is typically found within 185km (100nmi) of the coast from Central California south to the Gulf of California (Carretta et al. 2011, Gerrodette and Eguchi 2011), *D. delphis* has a much larger range and inhabits waters well west of the US exclusive economic zone (within 200nmi of the coast), north to Oregon and Washington and south to the southern tip of Baja California, Mexico. The distributions of the two species overlap within our study area, and on rare occasions, schools of the two species have been found immediately adjacent to one another (Heyning and Perrin 1994).

Due to its great abundance (arguably the most abundant cetacean in the world) and frequent entanglement in various fisheries worldwide, many more reproductive data have been collected from *D. delphis* than from *D. capensis*. *D. delphis*-specific reproductive parameters have been derived mainly from animals killed as bycatch in commercial fisheries in several regions of the Pacific and Atlantic oceans (Ferrero and Walker 1994, Danil and Chivers 2007, Westgate and Read 2007, Murphy et al. 2009). The percent of sexually mature females that are found pregnant within these data sets varies between the different areas, with perhaps greater rates in the tropical regions of their distribution. The percent pregnant ranges from a low of 25.0% ( $n_{\text{mature females}}=8$ ) (Ferrero and Walker 1994) in the central North Pacific, 26% ( $n_{\text{mature females}}=248$ ) (Murphy et al. 2009) and 33% ( $n_{\text{mature females}}=39$ ) (Westgate and Read 2007) in the eastern and western North Atlantic, respectively, to a high of 44.4% ( $n_{\text{mature females}}=333$ ) in the eastern tropical Pacific (Danil and Chivers 2007). Spatiotemporal variation in the occurrence of pregnancies has not been highlighted in published studies, although Murphy et al. (2009) reported that within the eastern North Atlantic region, the UK/French oceanic area had substantially higher percentage of sexually mature females pregnant: 28% ( $n_{\text{mature females}}=191$ ) than in either the Irish Sea: 20% ( $n_{\text{mature females}}=25$ ) or Iberian Sea: 19% ( $n_{\text{mature females}}=32$ ).

There is much less reproductive information regarding the other *Delphinus* species, *D. capensis*. Due to its lower worldwide abundance (Perrin 2009, Carretta et al. 2011) and only recent recognition as a separate species in the mid-1990s (Heyning and Perrin 1994, Rosel et al. 1994), no population-level pregnancy estimates have been made for this species. However, during the field effort that generated the biopsies used in the analysis for this paper, aerial images were taken and a subsequent paper estimating calving rate has been produced for *D. capensis* and *D. delphis* (Chivers et al. 2012). The percent calves out of all animals photographed (including males and females) were 4.5% and 6.7% for *D. capensis* and *D. delphis* respectively, translating into approximately 18.8% and 26.8% percent pregnant respectively, given several demographic assumptions.<sup>2</sup>

### Projectile Biopsies and Sample Selectivity

Projectile biopsies are increasingly used to address life-history questions (Mansour et al. 2002, Kellar et al. 2006, Kellar et al. 2009, Perez et al. 2011, Trego et al. 2013) including those regarding pregnancy. When interpreting results of biopsy-based

<sup>1</sup> *D. capensis* and *D. delphis* references in this paper are intended to be synonymous with the subspecies, *D. c. capensis* and *D. d. delphis*, respectively (Perrin 2011a, 2011b).

<sup>2</sup> This assumes a 1) 50:50 sex ratio, 2) 50:50 mature to immature ratio and 3) no fetal/neonatal mortality.

life-history studies in the context of population parameters, it is important to know how representative the samples are of the populations from which they are collected. Non-random sampling can lead to spurious results when conclusions from sample sets are generalized to the overall populations. Evaluating potential factors that may influence whether biopsy-obtained data represent a random sample of the population will facilitate correcting for sampling biases when estimating demographic parameters and in designing future experiments to estimate parameters.

In this study, we examine, for the first time, the likelihood of being pregnant with respect to various factors, such as vessel type, biopsy collection order, geographic location, school size, and sampling date. Some of these factors are associated with the biopsy process, which helps us assess the potential for sample selectivity, while others are aimed at examining spatiotemporal variation in pregnancy rates among species.

## Materials and Methods

### *Biopsy Collection*

We collected 507 biopsy samples from female common dolphins off Central and Southern California, USA and Baja California, Mexico in 2009 (Chivers et al. 2010) during a three-month research cruise (Leg 1: Sept. 7<sup>th</sup>–Oct. 2<sup>nd</sup> [early autumn], Leg 2: Oct. 8<sup>th</sup>–Nov. 6<sup>th</sup> [mid autumn], and Leg 3: Nov. 12<sup>th</sup>–Dec. 9<sup>th</sup> [late autumn]), using 150 lb Barnett Wildcat crossbows (Barnett Outdoors, LLC, Tarpon Springs, FL, USA) Excalibur Apex-Lite crossbows (Excalibur Crossbow, Kitchener, ON, Canada) with carbon fiber darts (Ceta-Dart, Hovedstaden, Denmark) fitted with multi-barbed stainless-steel biopsy tips. Samples included an approximately 7-mm diameter skin plug attached to an approximately 20-mm long plug of blubber. Mean mass of blubber attached to these biopsies was 0.091g (SE = 0.003g). All samples were frozen in a –80°C freezer after collection.

Most biopsies (n = 376) were collected from dolphins riding the bow of the NOAA Ship *McArthur II*, a modified 224ft T-AGOS class surveillance vessel. Targeted dolphins rode the bow between 0 and 6 meters away from the ship, most directly under the positions where biopsy collectors were stationed approximately 10 meters above the waterline. The remaining samples (n = 131) were collected from a 6-meter Zodiac rigid-hull inflatable boat (RHIB) launched from the NOAA Ship *McArthur II*. Irrespective of platform, biopsy collectors were not permitted to target suspected mother/calf pairs (identified by size characteristics and observing one individual swimming in calf or echelon position with respect to another).

There were two categories of biopsy sampling. The first was the standard method used by Southwest Fisheries Science Center research cruises over the last 20 years, in which 1–15 biopsies were gathered per school, mostly from the ship but a few also from the RHIB. These samples represented more than half of all biopsies collected for this study. The second category of sampling was dubbed “Tandem Biopsy Sampling”. During this effort, in order to minimize the effects of demographic differences inherent to schools, we collected a subset of biopsies (n = 207), from both platforms (RHIB = 111, Ship = 96) from single schools. Samples were obtained systematically, with the goal of obtaining 15 samples per platform per school. For such schools (n = 18), the sampling was done sequentially, such that approximately 15 biopsies (from both males and females; only the females were used in this study) each were obtained first from one platform and then the other, with a break in sampling on the order of 15 minutes to one hour. We alternated between starting the sampling effort on a given school from the

ship and from the smaller RHIB. Due to logistical constraints, perfect alternation was not possible, but in the aggregate, we obtained similar-sized sample sets for each platform ( $n = 10$  and 8 schools sampled from the RHIB first and ship first, respectively).

The intent in comparing the number of pregnant female dolphins sampled from the two platforms was to assess the potential sample selectivity biases of biopsy collection. Because of the limited mobility of larger research ships, the composition of samples is highly dependent on the behavior of individual animals approaching the ship and making themselves available for biopsy (i.e., bowriding). However, when sampling from the smaller and more maneuverable RHIB, biopsy collectors are less dependent on bowriding animals. Smaller boats can better maneuver through schools and biopsy collectors can target a greater range of animals, not limited to only bowriding individuals (in fact the majority of animals that bowride small boats are too close for biopsy with the crossbows we have used). From this perspective, if demographically linked bowriding behavior was contributing to a bias in percent pregnancy, we would expect that biopsies collected from a small boat to be more representative of the sampled population.

#### *Molecular Sex Determination*

DNA was extracted using the Corbett Robotics DNA Extractor (Qiagen, Valencia, CA, USA) and in some instances manually with the NaCl-‘salting out’ method. Sexes were determined using the Stratagene MX3000P qPCR (Agilent Technologies, Santa Clara, CA, USA) following the protocol established by Morin et al. (2005). Positive control tissue from one known male and one female, determined by genital inspection of reference specimens from a stranding or fishery-bycatch, was included on each assay to assess reaction quality.

#### *Blubber Progesterone Isolation*

The blubber hormone extractions followed the methods delineated in Kellar et al. (2006) with several modifications to simplify the procedure and increase consistency. Approximately 0.05g–0.15g of blubber was homogenized in 1000 $\mu$ L of 100% ethanol using a Fast Prep 24 homogenizer (MP Biomedicals, Santa Ana, CA, USA) and lysing matrix “A” tubes (MP Biomedicals, Santa Ana, CA, USA). The samples were processed seven to nine times at a speed of 5 m/s for 45-second intervals depending on sample consistency. The contents of each lysing matrix tube were poured into a glass tube and the lysing matrix tube was rinsed with 500 $\mu$ L of ethanol. The homogenate:ethanol solution was then separated from the grinding media and placed into a new glass tube. The lysing matrix tubes and grinding media were rinsed again with another 500 $\mu$ L of ethanol and combined with the homogenate. The homogenate/rinse solution combination was combined with 2 ml of 4:1 ethanol acetone. The resulting solution was vortexed and then centrifuged at 4000 rpm for 15 min. The supernatant was transferred and evaporated. Two milliliters of diethyl ether were added, vortexed and centrifuged again. The supernatant was collected and evaporated, and the residue was resuspended in 1000  $\mu$ L of acetonitrile, vortexed, and 1000  $\mu$ L of hexane added to the mixture. After the solution was vortexed and centrifuged again, the acetonitrile layer was aspirated into a new tube and the process was repeated with another 1000  $\mu$ L of hexane. The final portion of acetonitrile was collected and evaporated. The remaining residue was centrifuged at 4000 rpm for five minutes and stored at  $-20^{\circ}\text{C}$ .



### *Progesterone Enzyme Immunoassay*

To prepare the samples for the enzyme immunoassay (EIA), they were suspended in varying amounts (250 or 1,000  $\mu\text{L}$ ) of 1M phosphate buffered saline. In order to make measurements in accurate detection range on the EIA, samples were resuspended in 250  $\mu\text{L}$  of PBS then vortexed in the multitube vortex for 15 min. We used an EIA kit 900-011 (Enzo Life Sciences, Plymouth Meeting, PA, USA) that has 100% reactivity with progesterone and 5 $\alpha$ -Pregnane-3,20-dione in each sample. The assay detection limits were between 15 and 500 pg/mL. Therefore, samples that exceeded this range had to be diluted further to be accurately measured. These samples were diluted at 1:100, 1:20, 1:5, 1:3, and 1:2 depending on their original EIA measurements such that the final measurements would fall within the range of the control samples. The intra-assay coefficient of variation (CV) was between 4.9% and 7.6% and an inter-assay CV between 2.7% and 6.8%. Each sample was individually vortexed prior to quantifying progesterone concentration.

We determined the extraction efficiency using spiked samples as described in Kellar et al. (2006). The extraction control samples were spiked with 0 ng, 10 ng, and 30 ng of cold (i.e., non-radioactive) progesterone. The percentage of progesterone that was recovered after each extraction set (i.e., group of samples processed at the same time) was calculated and each assay value was adjusted to the standard prior to analysis. The mean extraction efficiency was 89.3% (SE = 4.8%). Once corrected for extraction efficiency, it was assumed that progesterone concentrations greater than 50 ng per gram of tissue were from pregnant females (Kellar et al. 2006).

“Percent pregnant” as reported here should be understood as the percent that were pregnant out of all females sampled (both mature and immature). Because maturity state was not identifiable from the field observations or biopsy sample, this is a different metric than usually reported from carcass data, which is the percent pregnant out of only the sexually mature females sampled.

### *Statistical Analysis*

We assessed a suite of factors associated with the biopsy sampling process as potential correlates with percent pregnant. They included species (*D. delphis*, *D. capensis*), latitude, longitude, platform (i.e., vessel: research ship or RHIB), school size and duration of biopsy activity/perturbation<sup>3</sup>. Platform and biopsy duration<sup>4</sup> are the two factors directly related to the biopsy process, whereas latitude, longitude, and school size were not. Julian date (more specifically the month of collection) was a factor also examined, but given its strong relationship with location during the survey, we conducted its analysis separately with a different methodology; see below.

Each of these factors’ predictive ability was assessed as part of a Bayesian model-averaging procedure using a method in which the model estimation process selects the factors to include and which to exclude for each iteration of a Markov chain Monte Carlo run (Carlin and Chib 1995). In this analysis, sets of nested logistic generalized linear

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<sup>3</sup> All independent variables were normalized to have a mean of zero and a variance of one to minimize the effects of differences in absolute values on the model results.

<sup>4</sup> Duration of biopsy activity/perturbation was measured by rank order of a biopsy in a series of samples for a particular sampling bouts (e.g., the fifth biopsy taken from a particular school would receive a rank of 5). Rank order may not be exactly comparable between biopsying bouts; the amount of time of each bout varies, so that for example, the 5<sup>th</sup> sample in one bout may have been taken much earlier relative to when the animals had begun bowriding than the 5<sup>th</sup> sample in another bout.



Table 1. Bayes factor ( $B_{10}$ ) interpretation. These are descriptive statements about standards of evidence in scientific investigation as proposed by Kass and Raftery (1995). Example: for a simple linear regression if zero lies just beyond either end of the 90% probability interval of the posterior distribution of the slope coefficient, the corresponding Bayes Factor would be nearly 20, “strong” evidence.

Bayes Factor ( $B_{10}$ )	Evidence against $H_0$
1 to 3	Not worth a mention
3 to 20	Positive
20 to 150	Strong
> 150	Very strong

models were constructed in WinBUGS (Lunn et al. 2000) with each factor multiplied by a Bernoulli selection parameter in the form<sup>5</sup>:

$$\begin{aligned} \text{logit}(p_{\text{pregnant}(i)}) = & a + \gamma_{\text{lat}} * b_{\text{lat}}(x_{i,\text{lat}}) + \gamma_{\text{long}} * b_{\text{long}}(x_{i,\text{long}}) \\ & + \gamma_{\text{platform}} * b_{\text{platform}}(x_{i,\text{platform}}) + \gamma_{\text{s\_size}} * b_{\text{s\_size}}(x_{i,\text{s\_size}}) \\ & + \gamma_{\text{b\_order}} * b_{\text{b\_order}}(x_{i,\text{b\_order}}) \end{aligned} \quad (1)$$

where  $p_{\text{pregnant}(i)}$  is the probability that individual  $i$  is female,  $b$  is the marginal slope coefficient for each factor, and  $\gamma_j$  was the selection parameter for each factor ( $j$ ) with a probability equal to the mean of  $\gamma_j$ . 300,000 iterations were used for the model runs, starting from three sets of initial points, with a burn-in period of 1,000 iterations. Every third iteration thereafter was incorporated into the parameter estimates, yielding a posterior distribution from 100,000 iterations. Vague priors were set for all marginal slope and intercept coefficients from normal distributions, each with mean = 0 and variance = 1000 (note: these prior distributions where variance > 1000 had poor convergence). The prior on each selection parameter  $\gamma_j$  was set to be  $p = 0.5$ . The weight (an indication of importance) that each factor had on the estimate of the sampled animal’s pregnancy state was equal to  $\gamma$  and directly proportional to the number of iterations in which that factor was selected in the MCMC chain. Marginal posterior probabilities of the selection parameters with median values greater than  $p = 0.5$  were those in which the weight of evidence supported their inclusion in the model.

For the “Tandem Biopsy Sampling” experiment, the difference in the proportion pregnant between platforms for each school was estimated using a Bayesian model, similar to a frequentist paired t-test (Manly 1991). By estimating the difference for each school, the analyses would not be influenced by inter-school demographic variability (e.g., nursery schools - schools disproportionately composed of mothers with calves). Difference in proportion pregnant estimations (not paired) were also used to analyze the effects of Julian date or season and to compare the proportion pregnant in three different geographic areas: north of Pt. Conception (Central California), Pt. Conception to US/Mexico border (Southern California), south of border (Baja California, Mexico).

The Bayesian results are reported as both Bayesian posterior probabilities and Bayes factors ( $B_{10}$ ). We have included a table (Table 1) with description statements for interpretation of Bayes factors adapted from Kass and Raftery (1995).

Finally, spatial analyses were conducted to examine geographical variation in pregnancy. Interpolated surfaces of proportion pregnant were mapped using inverse

<sup>5</sup>A logit link function was used, as the pregnancy data were coded as 0/1, corresponding to the probability that a sample was pregnant: 0 = not pregnant, 1 = pregnant.

distance weighting with ArcMap 9.1 (ESRI, Redlands, CA, USA)<sup>6</sup>. Statistical significance of spatial variation in proportion pregnant as determined from the biopsies (each datum equals 0 or 1, based on whether the biopsy was from a non-pregnant or pregnant female, respectively) was analyzed using permutation tests of Ripley's K (Ripley 1977, Besag and Clifford 1989), a metric that evaluates the distribution of pregnancy against a random distribution. Ripley's K is particularly useful at determining if "hot spots" (i.e., disproportional clumping) exist within an analyzed data set<sup>7</sup>.

### Results

Of the 507 biopsy samples collected, blubber progesterone concentrations of nominally pregnant females (i.e., those that were estimated to be pregnant based on blubber progesterone concentrations greater than the threshold criterion of 50 ng/g) were approximately two orders of magnitude greater than those of nominally non-pregnant ones (Figure 1 and Table 2). Using the same threshold criterion, 22.1% (45 of 204) of the biopsied female *D. capensis* were determined to be pregnant, while 28.1% (85 of 303) of biopsied female *D. delphis* were determined to be pregnant (Table 3). Varying the threshold criterion  $\pm 20$  ng had only small effects on the percent pregnant of either species (Table 4)<sup>8</sup>.

This yields a 92.9% posterior probability ( $B_{10}=13.0$ ) that the *D. delphis* in the study area had a higher proportion pregnant than the *D. capensis* in that same area. We found the percent pregnant was lower in biopsies collected from the RHIB than those collected from the ship for both species (>10 percentage point differences in both species; Table 3). Note that evidence of this difference disappears for *D. delphis* when the analysis is restrained to tandem biopsy sampling or when additional factors are incorporated along with platform into our analyses (see below).

#### *Evaluating the Effects of Biopsy-related and Biopsy-unrelated Factors on Pregnancy*

Potential factors differentiating the two species (Figure 2 and Figure 3) were highlighted in the Bayesian model averaging results. For *D. capensis*, only the posterior for the sampling platform coefficient (median = 0.527, 95% PI = 0.126 – 1.002) did not include zero within its 95% probability interval (PI), and it was selected to be included in 60% of the model iterations (Table 5). Thus, the weight of evidence, though weak, was for a final model which only included a term for platform<sup>9</sup>. Latitude and longitude had

<sup>6</sup> All default values were used in the interpolation selection process with the exception that the number of neighboring points used in the estimation was raised from the default of 5 to 50 (equivalent to approximately 10 schools) in an effort to capture broad regional patterns instead of local, likely ephemeral, heterogeneities.

<sup>7</sup> The metric was calculated for the observed samples, and then each sample set was randomized with respect to pregnancy while keeping the proportion female the same as the observed. The resulting permutation tests were constructed on 20,000 replicates, and the significance of the spatial variation in proportion female was tested against what would be expected at random given the sampling geographic

<sup>8</sup> Various blubber progesterone levels were assessed as threshold criteria to differentiate pregnant from non-pregnant individuals to illustrate the marginal effect that this has on pregnancy determination and the resulting proportion pregnant data. This is important because there is some evidence that the biopsy process itself may alter the composition of the blubber: Ryan, C., B. McHugh, I. O'Connor, and S. Berrow. 2012. Lipid content of blubber biopsies is not representative of blubber in situ for fin whales (*Balaenoptera physalus*). *Marine Mammal Science* 29: 542–547.

<sup>9</sup> Remember if a factor is included in more than 50% of the iterations then the weight of evidence is for its inclusion; however at 60% inclusion rate there are still 40% of the iterations without this factor included.

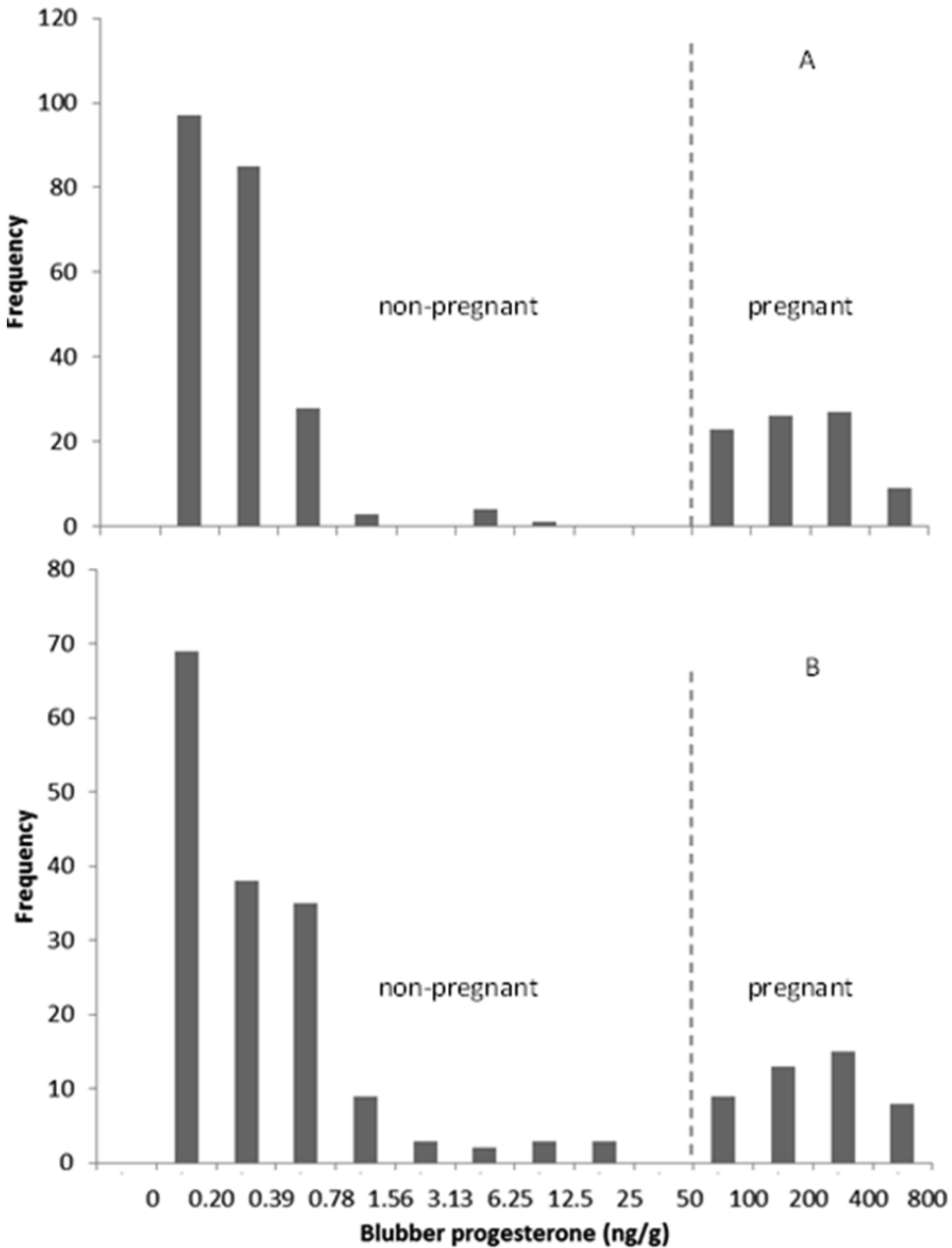


Fig. 1. *D. capensis*. Frequency distribution of blubber progesterone concentrations for *D. capensis*. (A) and *D. delphis* (B) biopsies. The abscissa is scaled via power function  $x^2$  to better visualize the two groups associated with each assessed pregnancy state.

less than majority support for inclusion, but like the evidence for platform, the statistical support was equivocal for both. There was no evidence for including biopsy order or school size for *D. capensis*. For *D. delphis*, latitude and longitude were strongly related to the proportion pregnant, as the posterior distributions for these coefficients (conditional

Table 2. Mean blubber progesterone values for nominally pregnant and non-pregnant females. Pregnancy status was determined using the 50 ng of progesterone per gram of tissue threshold value.

Nominal pregnancy status	Meanq blubber progesterone (ng/g) $\pm$ SE	
	<i>D. capensis</i>	<i>D. delphis</i>
Pregnant	241.9 $\pm$ 25.5	208.8 $\pm$ 15.4
Not pregnant	1.01 $\pm$ 0.28	0.671 $\pm$ 0.19

upon their inclusion in the model) were much greater than zero (latitude = 1.59, 95% PI = 0.904 – 2.35, increasing from south to north; longitude = 1.83, 95% PI = 1.12 – 2.60, increasing from west to east), and they were incorporated into 99.9% and 100.0% of the model iterations respectively (Table 5). For *D. delphis* there was additional strong support for inclusion of school size, which had a negative relationship with proportion pregnant. The other factors, platform and biopsy order, showed either equivocal or no evidence of a relationship with proportion pregnant (Table 5).

### Tandem Biopsy Sampling

Based on samples collected during the Tandem Biopsy Sampling, we did not find evidence of differences in the percent of dolphins pregnant between platforms (*D. capensis*: RHIB = 12.0% (6/50); research vessel = 23.9% (11/46)<sup>9</sup>; *D. delphis*: RHIB = 21.3% (13/61), research vessel = 22.0% (11/50)) (Table 6). For the 96 *D. capensis* biopsies, there is a 59.8% ( $B_{10} = 1.49$ ) probability that biopsy samples from the ship produced a higher proportion of pregnant females than biopsies from the RHIB (Figure 4). For the 111 *D. delphis* biopsies, the probability is 57.2% ( $B_{10} = 1.34$ ) that a higher proportion of pregnant females are obtained from the ship than the RHIB (Figure 4). Though the

Table 3. The number of biopsies obtained from known female (based on sexing) dolphins (*D. capensis* or *D. delphis*) that were assayed for pregnancy state and the resulting proportion identified as pregnant based on progesterone levels. The categories include all biopsies and the subsets taken from each vessel platform. A proportion of each of these samples was from the Tandem Biopsy Sampling operations in which, for a given school, biopsies were taken from one vessel platform (i.e., rigid-hull inflatable boat [RHIB] or ship) and then the other.

	Species	
	<i>D. capensis</i>	<i>D. delphis</i>
<b>ALL BIOPSIES</b>		
Total females biopsied/sexed	204	303
% pregnant	22.1%	28.1%
All biopsies from RHIB	58	73
% pregnant	10.4%	19.2%
All biopsies from ship	146	230
% pregnant	26.7%	30.9%
<b>TANDEM BIOPSY SAMPLING</b>		
Tandem trials/schools	10	8
Tandem biopsies from RHIB	50	61
% pregnant	0.120	0.213
Tandem biopsies from ship	46	50
% pregnant	0.239	0.220

Table 4. The percent pregnant as a function of the blubber progesterone criterion delineating pregnancy status. This table illustrates the limited change of the percent pregnant under different threshold criteria between 30 to 70ng/g.

Pregnancy criterion threshold	Percentage of females that are pregnant	
	<i>D. capensis</i> (n=207)	<i>D. delphis</i> (n=303)
30 ng/g	21.7% (n=45)	28.1% (n=85)
40 ng/g	21.7% (n=45)	28.1% (n=85)
50 ng/g	21.7% (n=45)	28.1% (n=85)
60 ng/g	21.3% (n=44)	27.7% (n=84)
70 ng/g	20.8% (n=43)	26.7% (n=81)

overall percentage point differences between platforms are much greater for *D. capensis* than for *D. delphis*, neither show any evidence that one platform yields a disproportionate number of pregnant female biopsies when analyzed in a paired platform manner<sup>10</sup>. Note this finding is in contrast to that found in the model averaging results which are discussed further below.

### *Spatial Patterns of Pregnancy*

Figures 5 and 6 show the geographic distributions and inverse distance-weighting interpolations of the proportion pregnant for both species. The Ripley's K analysis indicated that pregnant female *D. capensis* were more clumped than would be expected at random, at distances greater than 18.5km (10nmi) and less than 139km (75nmi) (Figure 7) and again above 185km (100nmi) and less than 741km (400nmi). We found that pregnant *D. delphis* were clumped between 93 and 278km (50 and 150nmi: Figure 8). These results suggest that there were areas in which pregnant females were disproportionately more prevalent compared with other areas. Moreover, for *D. capensis* there were two scales of clumping, indicative of both tight regional aggregations and broad inter-regional areas of elevated pregnancy rates, and for *D. delphis* there was one dominant regional aggregation where pregnancy rate was high which was in a spatial scale between the two found for *D. capensis*.

When comparing biopsies from the three geographic strata, we found slightly different results for each species using the difference-of-proportion Bayesian statistical test (again akin to a Student's t-test). The lowest percent pregnant for both species were found north of Point Conception (*D. capensis* = 0.00% (n = 8), *D. delphis* = 8.33% (n = 24)), while the highest percent pregnant was in the Southern California Bight for *D. delphis* (*D. capensis* = 20.6% (n = 155), *D. delphis* = 33.5% (n = 224)), and in Baja, Mexico for *D. capensis* (*D. capensis* = 34.1% (n = 41), *D. delphis* = 14.5% (n = 55)). The only statistical comparison that was not notably different was for *D. delphis*, between north of Point Conception and Baja California, Mexico (Table 7).

<sup>10</sup> For *D. capensis* this may seem an unlikely outcome, but the large disparity in proportion pregnant from the overall count stems from the fact that female biopsies were not evenly distributed between the platforms, and the greatest inequity in female biopsy numbers come from schools in which no pregnant animals were obtained from either platform. For example, though 0/9 from the RHIB is the same percent as 0/2 from the research vessel, those from the RHIB have a greater impact on the overall denominator than those from the research vessel.

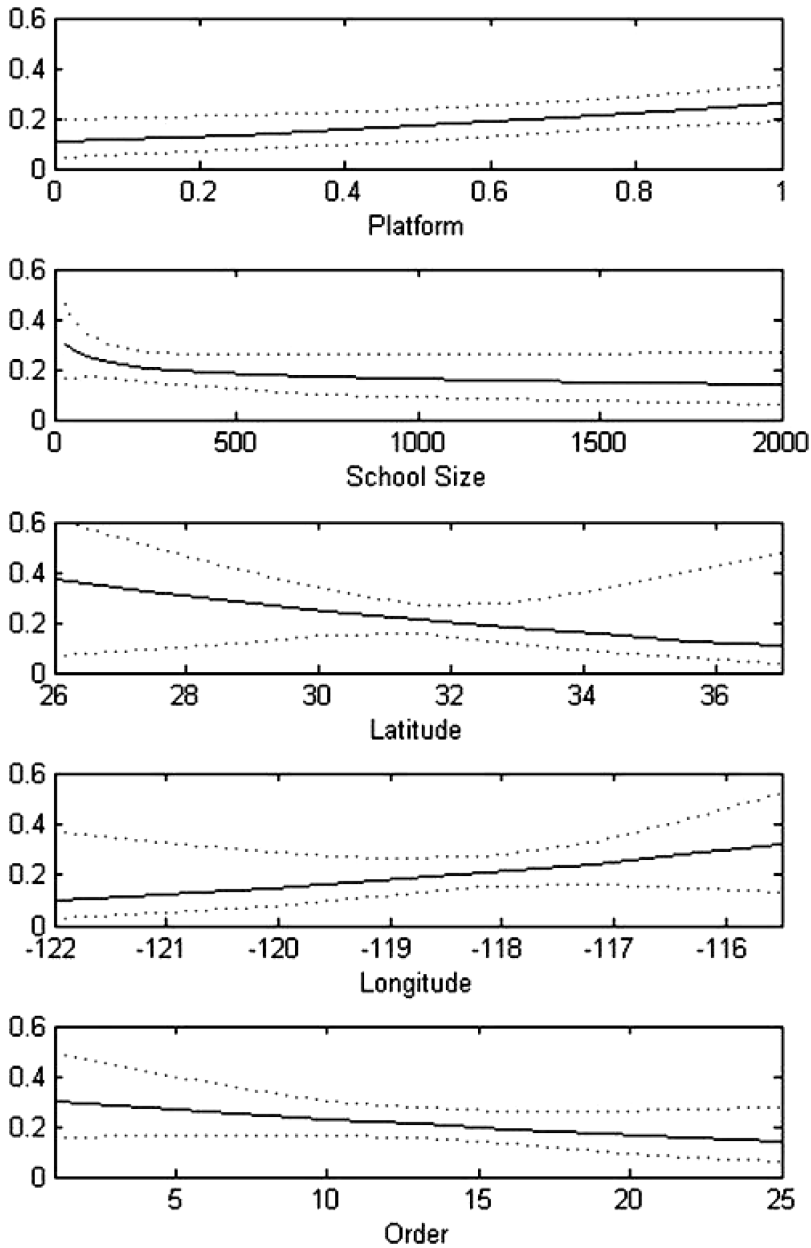


Fig. 2. *D. capensis*. Model-averaged marginal effects of platform, school size, latitude, longitude, and biopsy order, on proportion pregnant of female biopsy samples. Solid black lines: estimated marginal additive relationship, centered on mean value. Dashed black lines denote 95% probability envelope on the marginal effects. Platform value of “0” denotes the rigid-hull inflatable boat (RHIB) and “1” denotes the ship.

Seasonal differences in the proportion of pregnant females were evident between research cruise Legs 1 (early autumn) and 3 (late autumn) in *D. delphis* but not in *D. capensis* when restricting the area of both legs to their overlap in the inner Southern California Bight, the area designated as “Southern California” in Figure 6. For *D.*

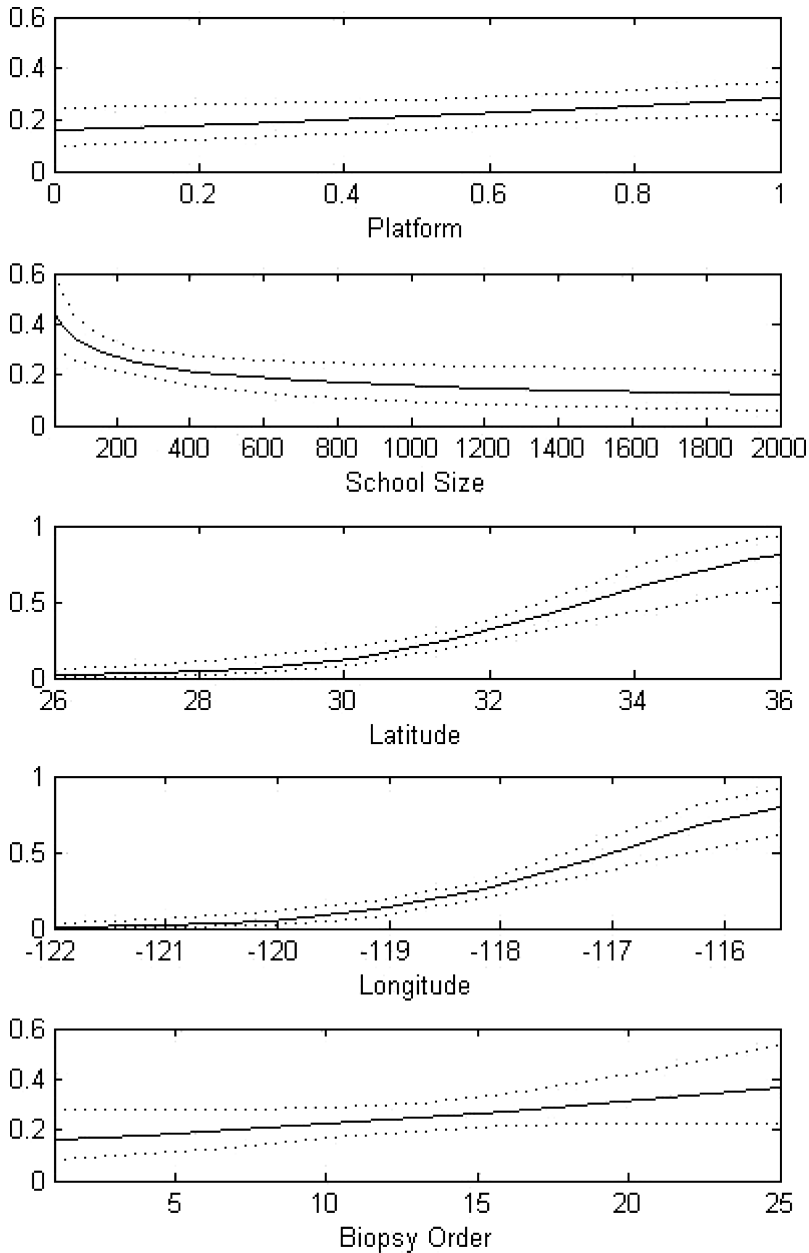


Fig. 3. *D. delphis*. Model-averaged marginal effects of platform, school size, latitude, longitude, and biopsy order on proportion pregnant of female biopsy samples. Solid black lines: estimated marginal additive relationship, centered on mean value. Dashed black lines denote 95% probability envelope on the marginal effects. Platform value of “0” denotes the rigid-hull inflatable boat (RHIB) and “1” denotes the ship.

*capensis* the percent pregnant values were 21.4% (n = 98) and 19.3% (n = 57) for Leg 1 (early autumn) and Leg 3 (late autumn) respectively, yielding little to no support for a change in pregnancy composition ( $p_{1>3} = 0.664$  ( $B_{10} = 1.97$ )) over this period. However,



Table 5. Model averaged coefficients for factors associated with the proportion pregnant, median and 95% probability interval values. % selected: percent of the iterations that each factor was selected to be included in the final model.

Species	Factor	Coefficient estimates			% selected
		2.5%	Median	97.5%	
<i>Delphinus capensis</i>					
	platform	<b>0.126</b>	<b>0.527</b>	<b>1.002</b>	<b>60.2%</b>
	latitude	-0.874	-0.399	0.621	39.4%
	longitude	-0.388	0.419	1.038	42.7%
	order	-0.639	-0.242	0.135	9.3%
	school size	-0.600	0.248	0.116	10.8%
<i>Delphinus delphis</i>					
	platform	-0.075	0.365	0.681	43.8%
	latitude	<b>0.904</b>	<b>1.594</b>	<b>2.348</b>	<b>100.0%</b>
	longitude	<b>1.117</b>	<b>1.825</b>	<b>2.600</b>	<b>99.9%</b>
	order	-0.046	0.272	0.611	13.9%
	school size	<b>-0.749</b>	<b>-0.435</b>	<b>-0.143</b>	<b>72.5%</b>

we found strong evidence for a decrease in percent pregnant in *D. delphis* ( $p_{1>3} = 0.983$  ( $B_{10} = 58.4$ )) between early and late autumn with 38.8% ( $n = 133$ ) pregnant during Leg 1 dropping to 25.3% ( $n = 91$ ) during Leg 3.

### Discussion

Overall, the small differences we observed in percent pregnant in the two species are not unexpected given the marked similarities in their behavior, ecology, and overall biology (Heyning and Perrin 1994). However, our survey encompassed the entire outer coast (i.e., outside the Gulf of California) range of *D. capensis* but only the eastern fraction of the range of *D. delphis* in the study region and is therefore less likely to be representative of the population of the latter species (Chivers et al. 2010). If the more western, un-sampled section of the *D. delphis* range contained a substantially larger or smaller percent of pregnant females, then we might have found greater differences in pregnancy rates between the two species. We note this because of 1) the strong geographic pattern in pregnancy in the sampled *D. delphis* indicating that schools sampled further west tended to have a lower proportion pregnant, and 2) the fact that in a previous study (Kellar et al. 2012) examining these same biopsy samples we found large differences in sex

Table 6. Results from the Tandem Biopsy Sampling comparison. Biopsies were collected from both the small RHIB and the large research vessel from the same schools. The difference in the fraction pregnant between these pairings is listed as mean difference in the proportion pregnant (with 95% probability interval). The number of the paired tandem trials and the aggregate proportion pregnant per platform is also reported.

	Proportion female RHIB (n)	Proportion female Ship (n)	# of tandem trials	Mean difference proportion female (95% PI)	Bayes Factor ( $B_{10}$ )
<i>D. capensis</i>	0.120 (50)	0.239 (46)	10	0.057 (-0.085 to 0.190)	1.49
<i>D. delphis</i>	0.233 (61)	0.220 (50)	8	0.024 (-0.121 to 0.161)	1.34

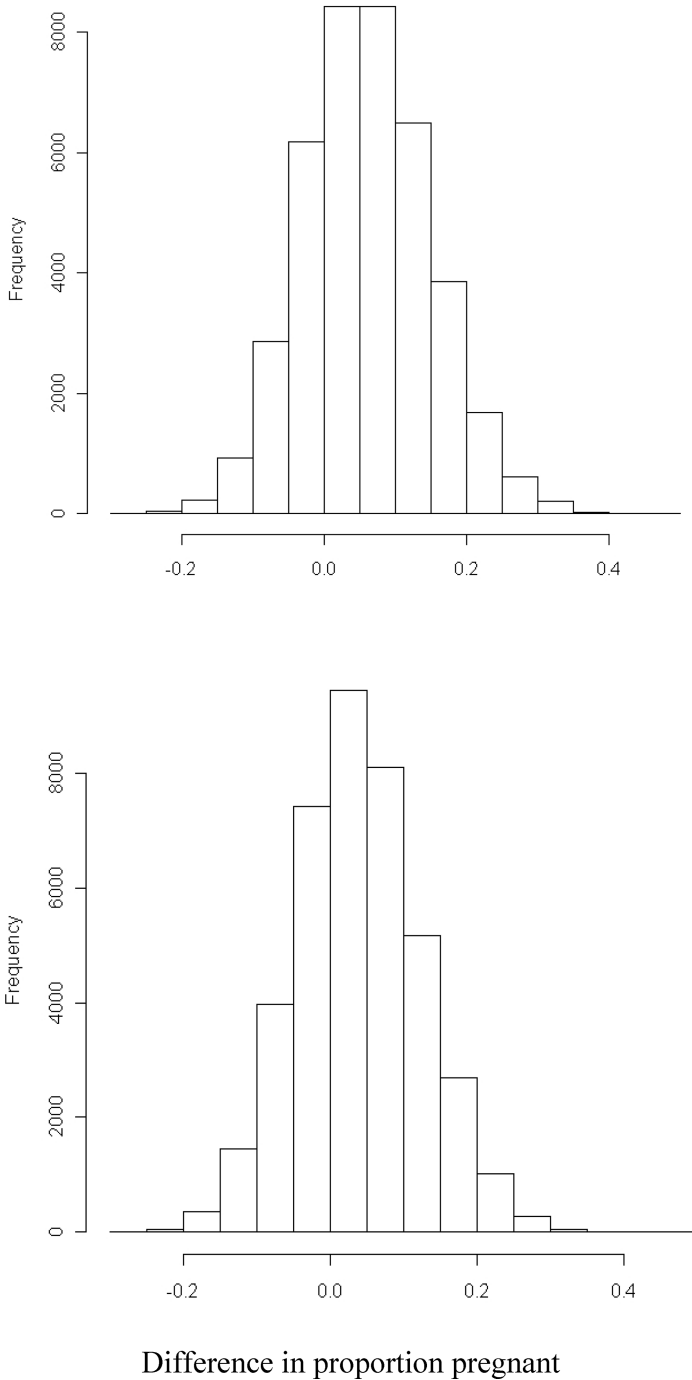


Fig. 4. Posterior probability distributions of the difference between the proportion of pregnant values for each tandem pairing (vessel: rigid-hull inflatable boat and ship) representing both species; *D. capensis* (top) and *D. delphis* (bottom).

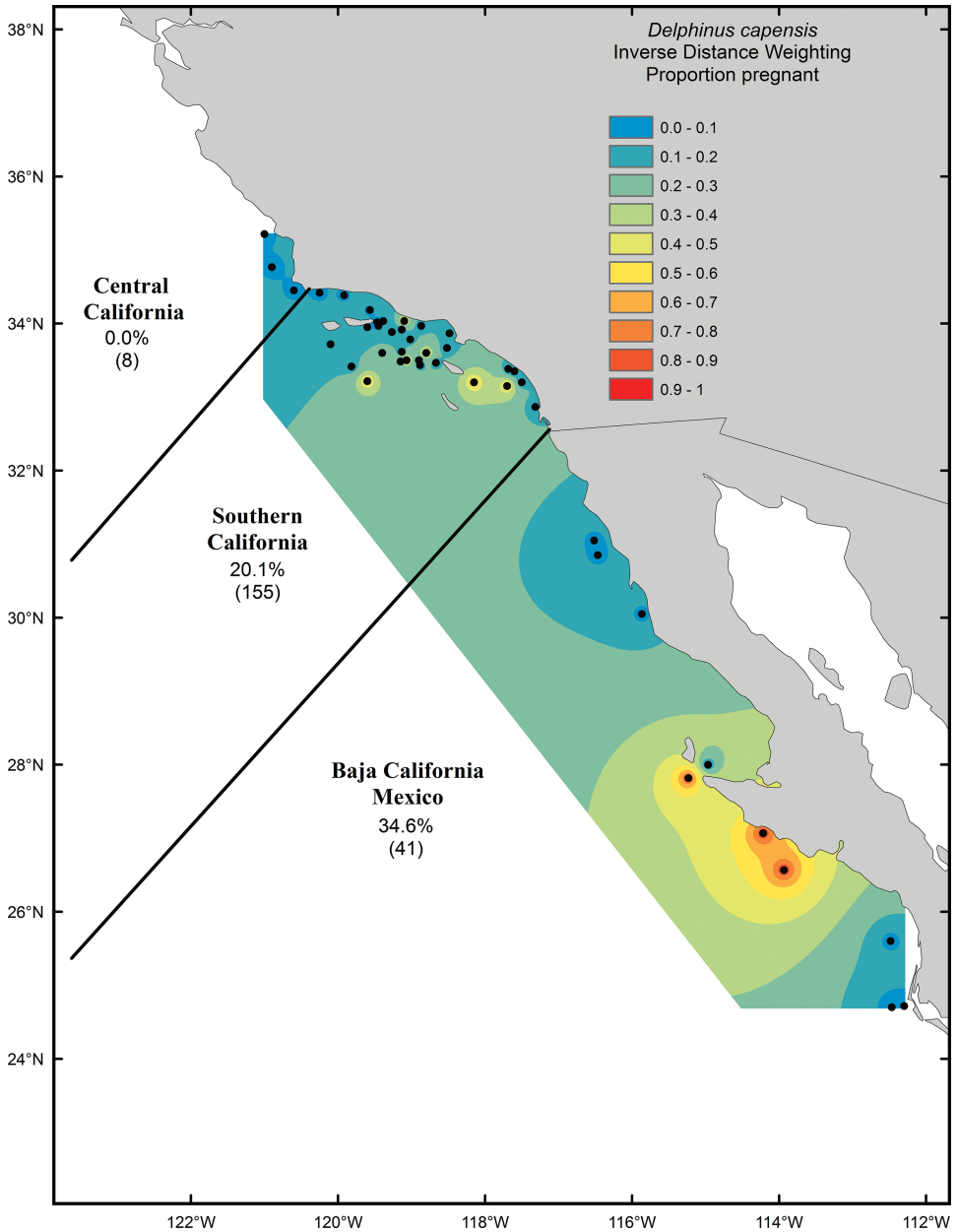


Fig. 5. *D. capensis*. Inverse distance weighted interpolation map of the proportion pregnant as measured from biopsies (locations denoted by dark circles). Diagonal lines demarcate boundaries separating three geographic strata: Central California, Southern California, and Baja, Mexico. In each stratum the proportion female and sample size (in parentheses) are indicated.

composition (*D. capensis* = 40.9% female, *D. delphis* = 66.1% female) with strong geographic relationships. Though this latter point has no direct bearing on the percent pregnant measurements made here, it does show that there are demographic parameters that are not similar between these two species.

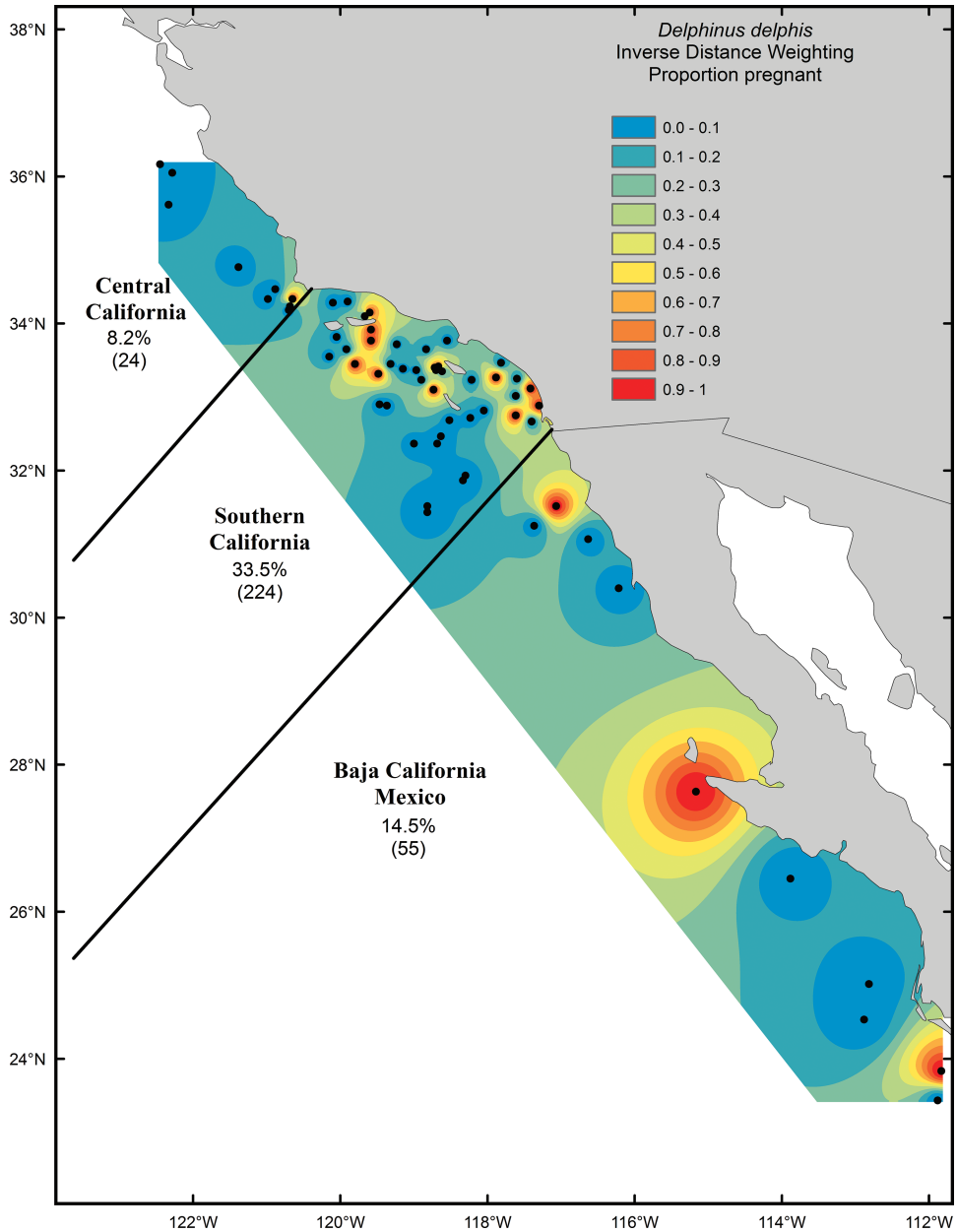


Fig. 6. *D. delphis*. Inverse distance weighted interpolation map of the proportion pregnant as measured from biopsies (locations denoted by dark circles). Diagonal lines demarcate boundaries separating three geographic strata: Central California, Southern California, and Baja, Mexico. In each stratum the proportion female and sample size (in parentheses) are indicated.

It was difficult to compare our estimates of the percent pregnant in each species (22.1% and 28.1% for *D. capensis* and *D. delphis* respectively) to other studies where estimates were derived from examinations of full carcasses for two reasons: 1) our operational restrictions prevented sampling of mothers with calves and calves, and 2) our inability to

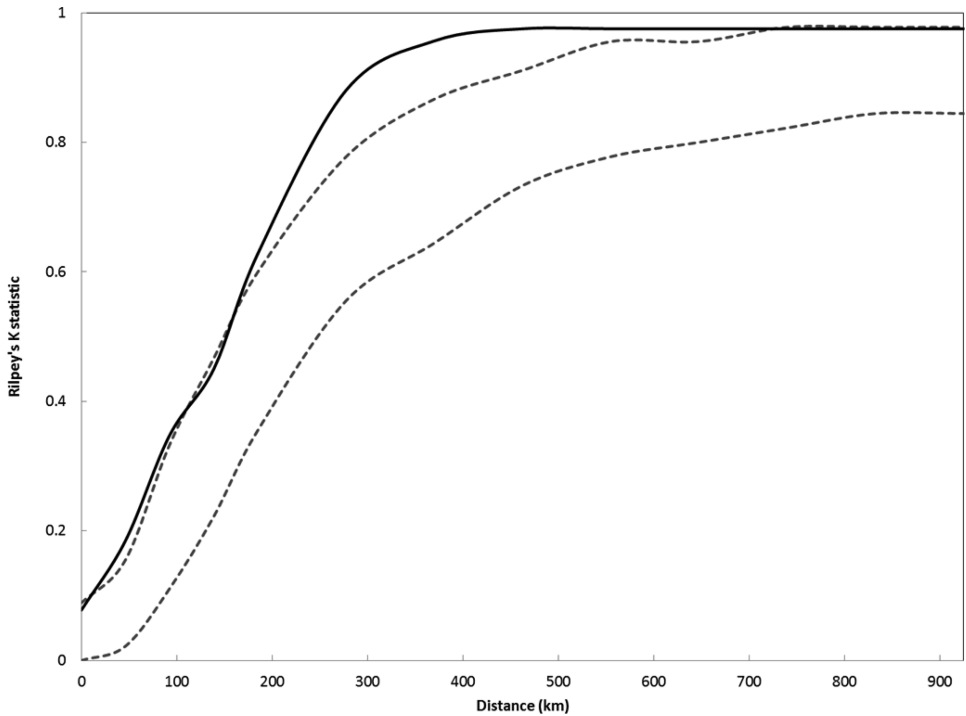


Fig. 7. Results of spatial pattern analysis of the distribution of pregnant female *D. capensis* dolphins relative to all *D. capensis* females sampled using Ripley's K statistic. The function  $K(d)$  was calculated for each 20-nmi interval (0–100nmi) and 50-nmi interval (>300nmi). The derived sample statistic  $K(d)/n_{\text{pregnant}}$  from the observed data was plotted against distance (solid line). The dotted lines represent the 95% confidence envelope for the total spatial randomness (the null distribution). The envelope was derived by permuting (10,000 simulations/distance interval) Ripley's K for the entire set of sampling sets.

reliably distinguish female maturity status from biopsy samples (most pregnancy metrics in the literature are based on the proportion of mature females that are pregnant). Thus, because we know much more about the life-history characteristics of each of the carcasses than the biopsied animals, we can more directly convert parameter estimates from these life-history studies for comparisons to those we have generated from the biopsy data. Perhaps the best comparable metric from the carcass data is:

$$p_{sp} = \frac{P_o}{P_o + R + Im - 0.5L - 0.5P_l} * 100 \quad (2)$$

where  $p_{sp}$  is the percent of solely pregnant females out of all non-lactating females,  $P_o$  is the number of animals that are pregnant and not lactating,  $R$  is the number of adult females that are resting (i.e., neither lactating nor pregnant),  $Im$  is the number of immature females,  $L$  is the number of non-pregnant lactating females, and  $P_l$  is the number of simultaneously pregnant and lactating females. By not including mothers (those lactating) and subtracting out the number of female calves (i.e., “ $-0.5L - 0.5P_l$ ”) in this metric, we can more appropriately make a comparison with the biopsy samples, which are specifically designed not to include mothers and calves. Deriving this metric from the three most recent carcass-based life-history studies of *D. delphis* yields  $p_{sp}$  values of 12.9% ( $n = 492$ ) (derived from Murphy et al. 2009) for the eastern North Atlantic,

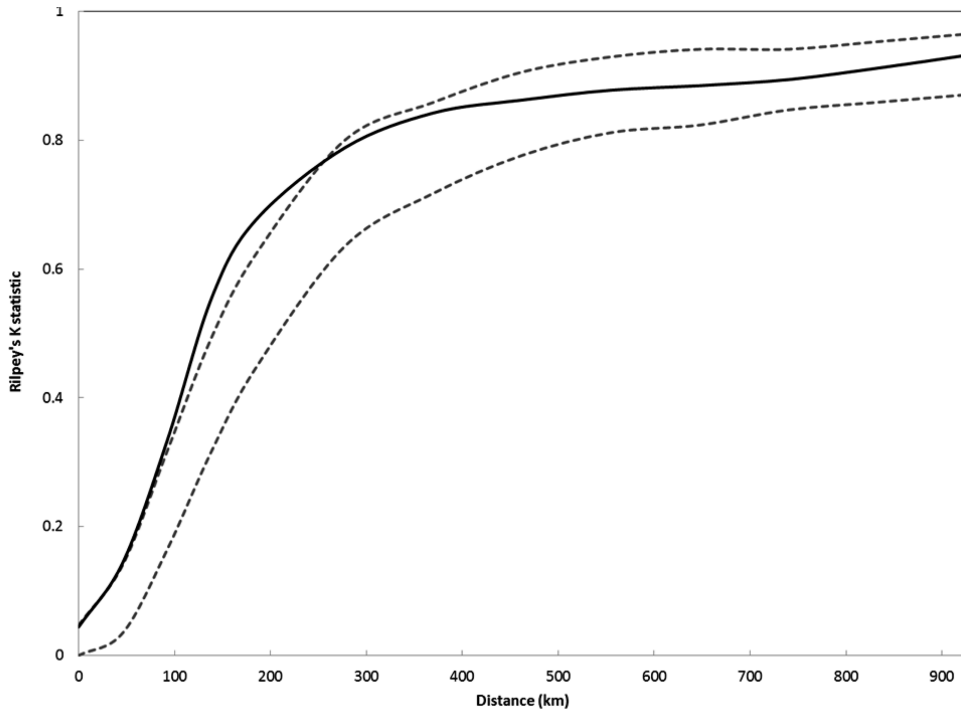


Fig. 8. Results of spatial pattern analysis of the distribution of pregnant female *D. delphis* dolphins relative to all *D. delphis* females sampled using Ripley's K statistic. The function  $K(d)$  was calculated for each 20-nmi interval (0–100nmi) and 50-nmi interval (>300nmi). The derived sample statistic  $K(d)/n_{\text{pregnant}}$  from the observed data was plotted against distance (solid line). The dotted lines represent the 95% confidence envelope for the total spatial randomness (the null distribution). The envelope was derived by permuting (10,000 simulations/distance interval) Ripley's K for the entire set of sampling sets.

22.5% (n = 22) (derived from Westgate and Reed 2009) for the western North Atlantic, and 21.9% (n = 379) (derived from Danil and Chivers 2007) for the eastern tropical Pacific. Our  $p_{sp}$  value for *D. delphis* (28.1%) in this study was higher than these, while the *D. capensis* value (22.1%) was comparable to the two higher carcass-based estimates.

We also implemented a similar conversion using estimates of the proportion of calves in a school (derived from counts on aerial photographs) to obtain suitable values for

Table 7. Comparison of the proportion pregnant between three regions in the study area. Listed are the median estimates from the posterior probability distributions of the northern area in each comparison exhibiting a smaller proportion pregnant than the southern area and the associated Bayes Factors. The lowest percent pregnant for both species were found north of Point Conception, while the highest percent pregnant was in the Southern California Bight for *D. delphis*, and in Baja, Mexico for *D. capensis*. The only statistical comparison that was not notably different was for *D. delphis*, between north of Point Conception and Baja California, Mexico.

<i>Delphinus delphis</i>	<i>Delphinus capensis</i>		
	N. of Pt. Conception	S. California Bight	Baja, Mexico
N. of Pt. Conception	n/a	0.991 (B <sub>10</sub> = 106.1)	0.998 (B <sub>10</sub> = 517.1)
S. California Bight	0.999 (B <sub>10</sub> = 665.7)	n/a	0.978 (B <sub>10</sub> = 44.85)
Baja, Mexico	0.857 (B <sub>10</sub> = 5.99)	0.997 (B <sub>10</sub> = 335.7)	n/a

comparison to our biopsy data. This conversion required estimates of proportion of females in the sampled populations. Using data collected during the same survey we have obtained estimates for percent calves from aerial photography (Chivers et al. 2012) and percent female (via sexing of biopsies) (Kellar et al. 2012) for each species (*D. capensis*: percent female = 40.9% and percent calves = 4.5%; *D. delphis*: percent female = 66.1% and percent calves = 5.7%). These values translate into percent females-with-calf values of 11.0% and 8.6% for *D. capensis* and *D. delphis* respectively. In order to compare these values to the percent pregnant from biopsies, we also need to adjust the biopsy data to compensate for the fact that we do not target mother calf pairs for biopsy. Under the assumption that for every calf present, 1.5 females are not available for biopsy (1.0 for each mother and 0.5 for each calf, assuming that every other calf is female), we estimate that 16.5% and 13.0% of all females are not available for biopsy for *D. capensis* and *D. delphis* respectively for our biopsy sample set. Therefore, when we correct for this discrepancy in the females available for each data set (aerial images versus biopsy samples), the estimates decrease to 18.4% and 24.4% for the biopsied *D. capensis* and *D. delphis* respectively. These values remain substantially greater than the females-with-calf values (11.0% for *D. capensis* and 8.6% for *D. delphis*) perhaps indicative of high perinatal mortality.

Clearly differences in the way that pregnancy rate and calving rate were measured could explain some of the difference in rates though this is difficult to assess. However, given the magnitude of these differences, we feel it is likely that additional factors play a more important role here. If perinatal mortality is responsible for the disparity between pregnancy and calf production, it possibly could be linked to anomalous oceanographic conditions observed in the California current ecosystem in the months preceding our survey (May-August 2009). Waters off California were anomalously warm, and upwelling indicators were at values lower than those observed in the previous 40 years (Melin et al. 2010). This event was thought to be a major contributing factor in the massive California sea lion (*Zalophus californianus*) mortality (e.g., ~80% pup mortality on San Miguel Island) observed within this area in 2009 (Melin et al. 2010). There is evidence that the direct cause was a radical shift in prey composition and availability associated with these anomalous conditions. Given the overlap in prey between these sea lions and common dolphins, it would be reasonable to expect some impact on the dolphins as well (Heyning and Perrin 1994, Melin et al. 2010). Higher calf mortality would not only lead to fewer calves, but it could also allow mothers who lost calves to become pregnant again sooner, thereby increasing the number of pregnancies, as lactation has an inhibitory effect on mammalian estrous cycling (Pineda 2003). We speculate that the lingering effects of this event may also be responsible for the decrease in observed pregnancy rates from Leg 1 (September) to Leg 3 (November) of the survey in *D. delphis* in the California Bight.

### *Spatial Patterns in Pregnancy*

Pregnant animals were disproportionately aggregated in specific locations for both species. For instance, the more southern areas of the Southern California Bight had higher pregnancy rates for both species; this was particularly true for *D. delphis* for which ~75% of all sampled pregnant animals were in the 220-kilometer stretch southeast of San Miguel island to the US/Mexico border, within 100 kilometers of shore. *D. capensis* had another area of high pregnancy between Cedros Island and Magdalena Bay. Both areas' oceanographic conditions are characterized by counter currents and semi-permanent



eddies creating tongues of warm sea surface temperature adjacent to strong upwelling areas (Soto-Mardones et al. 2004, Checkley and Barth 2009). These conditions promote enhanced growth and spawning of pelagic schooling fishes such as sardine, anchovy, and hake, which are all prey species of common dolphins (Hewitt 1981, Checkley and Barth 2009). It is not uncommon for mammals to segregate by demographic or reproductive condition by selecting habitats that can meet their specific nutritional requirements (Seagle and McNaughton 1992, Labisky and Fritzen 1998, Bowyer 2004, Millspaugh et al. 2004, Ciuti et al. 2006). Pregnant females, especially during mid- to late-gestation, experience large increases in energy requirements (Millar 1977, Jonsson et al. 1995, Reynolds and Kunz 2000) and changes in dietary needs that can prompt movement to more productive or more nutritionally appropriate habitats (Bonenfant et al. 2002). However, we know that the nutritional requirements of lactation are even higher (Hadjipieris and Holmes 1966, Millar 1975, 1977, Bowen et al. 2001); in some mammals the difference is as much as three times as great (Millar 1977). If nutrition is a driver for reproductive partitioning or differential location selectivity, we would not necessarily expect only pregnant animals to be disproportionately occupying the areas of highest nutritional output or highest prey concentration; lactating females would be subject to the same drive. This explanation presupposes two things. First, it assumes that there is some drawback to these areas such that when a female is not either pregnant or lactating she would be more likely to move to an area of more limited nutritional resources; otherwise one would expect that all animals, regardless of reproductive condition, would seek out areas of high productivity. Second, it also assumes that nutritional requirements are the primary drivers of spatial distribution; others include predation avoidance, thermoregulation, human activity aversion (e.g., pollution, fishery interactions, and auditory perturbations).

### *Sampling Bias*

The evidence for differences in bowriding behavior between pregnancy states is equivocal for both species. Our interpretation assumes that biopsies from the RHIB are more likely to be taken randomly with respect to pregnancy status, because the acquisition of biopsies from this platform is less dependent on bowriding behavior than acquisition from the research vessel. We found that the proportion of pregnant females sampled from the research vessel was greater than from the RHIB in both species. Though the weight of evidence supports the inclusion of platform as a predictor variable for pregnancy in *D. capensis* but not in *D. delphis*, the evidence was not definitive in either case. When the paired tandem vessel sampling was examined, there was no relationship between platform and pregnancy in either species, though the sample sizes for these comparisons were low such that we may not have had sufficient power to address this question in the tandem structure. In a previous study examining sex-linked bowriding differences we found a similar pattern (Kellar et al. 2013). In the general linear model, there was evidence that male *D. capensis* were more likely to be sampled than females from the bow of the ship than from the RHIB, but in the tandem analysis there was no evidence of differences between platforms (the sample size in Kellar et al. 2013 was larger than in the present study). In this same study, neither analysis yielded evidence of differences in sex-ratio for *D. delphis*.

The other way we examined the bowriding behavior with respect to biopsy operations was by examining the relationship between pregnancy and sample order. We found very weak or no evidence of such a relationship in either species. This finding is not consistent with the

supposition that animals of one pregnancy state or the other becomes disproportionately frightened, wary, or disinterested in bowriding the longer biopsy operations continue. Again, the (Kellar et al. 2013) study had similar results to this study when examining sex-ratio instead of pregnancy. They found no evidence that one sex disproportionately become more wary of or attracted to biopsy operations compared with the other.

### Conclusions

As cetologists continue to use biopsies to assess pregnancy patterns, they should be cognizant of the potential biases associated with biopsies of bowriding animals. However, the common dolphin pregnancy data analyzed in this study provided a series of findings that begin to illuminate population-level aspects of pregnancy patterns. Pregnancy estimates from the biopsies were comparable to those derived from full specimens, suggesting that they may be usable for future population comparisons. Differences between the two species were observed in the factors that were correlated with percent pregnant. Pregnancy was not uniform geographically, but rather there were concentrations of high pregnancy rates, perhaps in areas more beneficial for gestation. Finally, there were results consistent with a hypothesis of a period following a high natal mortality event, perhaps an indication of the effects of anomalous oceanographic conditions. Thus, this study demonstrates the utility of concentrated biopsy sampling and the techniques employed to detect pregnancy, as it would not be possible to obtain comparable data across entire populations in such a short time frame solely from bycaught and stranded specimens.

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