



Sexing free-ranging adult Cuvier's beaked whales (*Ziphius cavirostris*) using natural marking thresholds and pigmentation patterns

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Sex identification of adult cetaceans is an important ecological parameter that should be incorporated into studies such as population dynamics and animal behavior. In Cuvier's beaked whale (*Ziphius cavirostris*), sex determination may be achieved through genetics, observation of genitals, the presence/absence of erupted teeth, and calf association. However, these features are difficult to ascertain due to the shy behavior of this species. Therefore, this study aimed to create a robust sex identification method using only external characteristics. Particularly, this work analyzed pigmentation patterns and levels of natural marks from adult individuals of known sex in order to identify gender differences, using frequency analysis and generalized linear models. Photographic captures of 73 free-ranging animals were utilized. The frequencies of the individual pigmentation patterns were found to be sex dependent. The 63% of the animals could be classified into either a "soft" or "sharp" pigmentation cluster. The "soft" cluster was only displayed by females, while the "sharp" cluster was present in both the sexes. However, the model selection process indicated that natural marking is the best determinative factor for sex classification. The density of the visible intraspecific natural marks was found to differ between the sexes (P value < 0.001) and was incorporated as a predictor variable into several candidate models. All candidate models had a high predictive power (mean area under the curve 0.973) and correctly predicted the sex, by means of a density threshold value, in 85–90% of the analyzed animals. The density threshold ranged from 4.1% to 6.4% according to the different body area analyzed. These density threshold values represent a robust post hoc sexing method to classify individuals to sex from opportunistic photos in the absence of other sexing methods.

Key words: beaked whales, cluster analysis, GLM, natural marking, pigmentation patterns, sexual coercion, sexual dimorphism, *Ziphius cavirostris*

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Identification of an animal's sex is an important aspect of a species population ecology. Thus, the improvement of sex identification methods would be a valuable tool, especially for nonsexually dimorphic species. The adult sex ratio is an important ecological parameter that is required in several areas of biological research (Székely et al. 2014). This factor can deeply influence multiple aspects of a species' biology including behavior and mating systems, evolutionary biology, physiology, extinction risk, and conservation (e.g., Bessa-Gomes et al. 2004; Sæther et al. 2004; Garel et al. 2006; Ewen et al. 2011; Székely et al. 2014). Therefore, the sex of an animal is an important variable which can greatly improve population dynamics and social analysis studies (Whitehead 2009). For

example, Rankin and Kokko (2007) found that incorporating the numbers of both sexes has a substantial effect on the outcome of population dynamic models. However, sex recognition of free-ranging cetaceans is difficult to investigate since many species have no obvious sexual dimorphism (Miller 2007).

Cuvier's beaked whale (*Ziphius cavirostris*, Cuvier 1823) is a sensitive species that is susceptible to anthropogenic pressures (e.g., Cox et al. 2006) and additional ecological data are urgently needed for conservation purposes (e.g., ACCOBAMS 2004). However, it exhibits no obvious sexual size dimorphism (MacLeod 2006) and like many other species of beaked whale can only be positively identified to sex through direct genital observation, genetics, the presence/absence of erupted teeth,

and/or close association with a calf (Heyning 1989; Mead 2002). This species performs long dives, is inconspicuous at the surface, and is considered to avoid vessels (Heyning 1989). Due to this behavior, the previously stated ecological parameters may be biased by the fact that it is only possible to sex a proportion of the animals using the main sex determination methods.

Head butting during aggressive interactions is a widespread behavior among cetaceans (Carrier et al. 2002) and *Z. cavirostris* like other beaked whales is believed to commonly use this behavior in aggressive interactions (Gowans and Rendell 1999; MacLeod 2002). As in most beaked whales, the erupted teeth of *Z. cavirostris* are only present in the adult males and having been freed from the biological constraint of prey capture, through the use of suction-feeding techniques (Heyning and Mead 1996), they have evolved into weapons. The erupted teeth of adult males during intraspecific agonistic interactions cause natural marks in the form of linear, often parallel scarring (McCann 1974; Heyning 1984). It has been proposed that the level of scarring in some odontocete species may act as an indicator of male quality (MacLeod 1998). Moreover, in several beaked whales, the accumulation of scars has been shown to have an overall gain rate with time (Hobson and Martin 1996; Gowans and Whitehead 2001; Rosso et al. 2011).

Intraspecific scarring has previously been described as being dimorphic with some male odontocetes having a higher prevalence of scarring compared with females (Gerson and Hickie 1985; Heyning 1989; Gowans and Whitehead 2001; MacLeod 2002; Scott et al. 2005; Martin and Da Silva 2006; Rowe and Dawson 2009; Rosso et al. 2011). It has been proposed that this level of intraspecific scarring could be used as a possible sex determination cue (Marley et al. 2013). This assumption has been used in previous studies to classify the sex of adult *Z. cavirostris* that could not be sexed by the presence or absence of erupted teeth or close association with a calf (McSweeney et al. 2007; Falcone et al. 2009).

The pigmentation patterns found in Cuvier's beaked whale have been described to differ slightly between the sexes (Heyning 1989). It is generally stated that coloration in adult males is dark gray with the females varying in color from a dark gray to reddish brown (Heyning 1989). Both sexes have a distinctive white pigmentation that is present on the head of adult animals and may extend posterior along the dorsum (e.g., Heyning 1989; Shirihai and Jarret 2006). However, the white pigmentation pattern present on the females has been described as being not as dramatic in contrast and extending posterior to a lesser degree than that of the males (e.g., Heyning 1989). These findings were derived from historic reports conducted on a few stranded specimens; therefore, a more substantial analysis of the pigmentation patterns of living animals would better complement the work of Heyning (1989).

The aim of this work was to present new insights into the external characteristics of Cuvier's beaked whales. A large photo-id catalogue of free-ranging whales from the Mediterranean Sea was analyzed. A robust and feasible methodology was created to measure scarring density within standardized whale body

regions. Quantitative and qualitative analyses of the scarring and pigmentation of *Z. cavirostris* were conducted between animals of known sex. The results were then used to create a reliable noninvasive sexing model for adults when the main sex determination methods were not possible (i.e., presence or absence of teeth, calf association, genital observation, and genetics).

MATERIALS AND METHODS

Photographic collection and selection.—Photographic sequences of Cuvier's beaked whale flanks were collected in the North-West Mediterranean Sea from 2004 to 2013, using auto-focus digital cameras equipped with a 80–300 mm stabilized zoom lens. Whenever possible, photographic sequences were taken in order to capture the whole exposed flank, from the snout to the tail stock of both sides of the animal. A typical sequence was composed of up to 8 consecutive photographs, with the same zoom factor from individuals less than 30 ms from the photographer. Each sequence and photograph was assigned a quality rating (Q-value) from 1 to 6, based on the focus, angle of the animal relative to the sensor plane, the proportion of the frames filled by the animal, and the exposure (e.g., Gowans and Whitehead 2001). The Q-value was independent of the amount of markings on the individual and $Q \geq 5$ represented the highest photographic qualities. The photographed individuals were separated into 3 age categories: juveniles, sub-adults, and adults following the method of Rosso et al. (2011). The teeth erupt only in mature males (Leatherwood et al. 1982; Heyning 1989; Mead 2002); therefore, animals were categorized as adult males when a high quality photograph of the snout displayed erupted teeth. Toothless adults in close association with a calf (dark black individual less than two-thirds the adult length swimming in infant position) were categorized as adult females. Only sequences of adult individuals were considered for the purpose of this work.

In order to prevent pseudoreplication, only individuals identified on both sides were selected where at least one side was described by a $Q \geq 3$ sequence. Particularly, only one $Q \geq 3$ sequence per individual was analyzed. In the case that the individuals had both sides described by 1 or more $Q \geq 3$ sequences, the side with the highest Q-value was selected. In the case of identical Q-values, the sequence was chosen randomly.

Pigmentation patterns description.—All the $Q \geq 3$ sequences selected were used for pigmentation pattern analysis to investigate possible differences between genders in adults. Only the pigmentation patterns visible during a normal surfacing behavior were recorded (i.e., the dark eye patch was not included in the following analyses). The investigated pigmentations were 1) the presence and distinctiveness of a highly variable pair of dark stripes found slightly posterior to the blowhole, defined here as “dark crescents” (Leatherwood et al. 1982); 2) the presence of a distinctive dark pigmentation among the pale coloration of the melon, defined here as “dark oval patch” using the definition found in Heyning (1989); 3) the extension and distinctiveness of the white depigmentation starting from

the snout and going posterior along the animal's back, here defined as "white cape"; 4) the flank coloration not covered by the white cape, classified into 3 categories: "dark" ranging from dark gray to black, "brownish" comprising all the shades of brown ranging from chestnut brown to light brown, and "light" which comprised the light-gray shades (Fig. 1).

To define patterns in pigmentation, a cluster analysis using "simple ratio" association index (Ginsberg and Young 1992) was applied to quantify the frequency of association among pigmentation variables. We defined *null* simple ratio index (SRI_{null}) as the threshold value obtained if a variable associates randomly (equation 1).

$$SRI_{null} = n_{associate} / (N - 1) \quad (1)$$

$n_{associate}$ is the mean number of pigmentation variables shown by individuals and N the total number of variables used for the analysis. The associations between variables occurring more often than expected by chance were defined when the SRI was greater than the SRI_{null} . All associations which have a SRI value greater than SRI_{null} form a cluster.

Scarring assessment.—Among the $Q \geq 3$ sequences selected for the pigmentation analysis, only $Q \geq 5$ sequences were considered for the scarring assessment (Fig. 2A) since only the latter were confirmed to have enough quality for studies on natural marking in this species (Rosso et al. 2011). The preparation and analysis of every photograph of the selected sequences was conducted using the open source java imaging processing platform, Image J (Schneider et al. 2012). To ensure consistency and eliminate observer bias, all photographic quality categorizations, selections, manipulations, and measurements were conducted by the same researcher (FC).

The 1st step was to extract the visible body area of the whale (i.e., the part of the animal above the water) from the background pixels of each photograph. Background pixels were removed by creating an outline mask of the whale using color pixel threshold techniques. Inaccuracies that could have occurred during the automated threshold procedure have then been checked and corrected with a subsequent freehand selection manipulation (Fig. 2B). The 2nd step required the division

of the visible body area into comparable subdivisions, called regions of interest (ROIs). The subdivision was achieved using specific reference points along the body of the animal. The 1st reference points were the anterior insertion point of the dorsal fin (Rowe and Dawson 2009) and the blowhole (Fig. 2C). This nonlinear distance (x) was measured using a smooth curve. This distance is equivalent to roughly 7 times the average length of the base of the dorsal fin for this species (Heyning 1989). Thus, by dividing this length by 7, a division unit length ($x/7$; Fig. 2C) was calculated. This unit was then used to create further reference points along the dorsal edge of the animal. At each reference point, lines were created using the angle tool to divide the animal into a set number of ROIs. The lines were drawn at one-half the angle between the previous and subsequent reference points, except for the head where the angle between the most prominent point on the melon and the subsequent reference point was used. Therefore, each ROI can be considered comparable between individuals as the reference lengths and points are equivalent for each animal.

To provide a measure of the level of scarring, the density of scarred to unscarred pixels was measured for each ROI. If a particular ROI was visible in more than 1 photograph within a sequence, the ROI with the highest portion of visible animal body was selected (Fig. 2D). Natural markings due to intraspecific interactions, here after referred to as "scarring" (see Rosso et al. 2011), were then traced using the same techniques described in Rowe and Dawson (2009). During tracing, additional photographs were manipulated including 1) inverted polarized view, which aided scar tracing within pale areas of animals; 2) red–green–blue stack separation, to remove obstruction by the diatom film; and 3) photos of enhanced contrast and brightness or combination of them to aid in mark identification. An independent experienced adjudicator (MR) double checked the scar selections to verify that no scars were missed, or other scarring caused by parasites or anthropogenic processes was classified as intraspecific. The traced scarring was then measured as the number of scarred pixels for each ROI using threshold techniques (Fig. 2D). The scarring density was expressed as the proportion of scarred to unscarred pixels, and

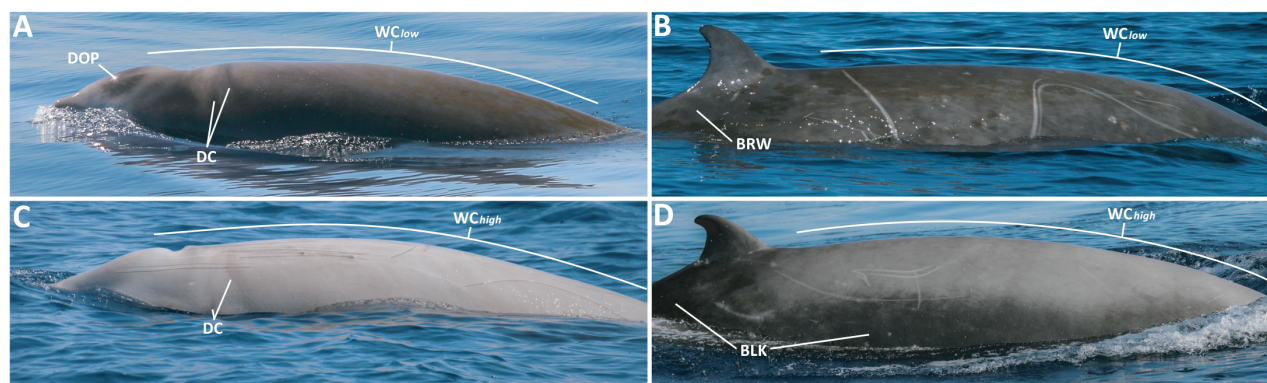


Fig. 1.—An example of the pigmentation patterns considered in this study: A) individual showing dark oval patch (DOP), distinctive dark crescents (DC), and nonuniform white cape (WC_{low}); B) brown-colored (BRW) individual with low-contrasted heterogeneous white cape (WC_{low}); C) individual showing indistinct dark crescents (DC) and uniform white cape (WC_{high}); D) dark-colored (DRK) individual with high-contrasted white cape (WC_{high}).

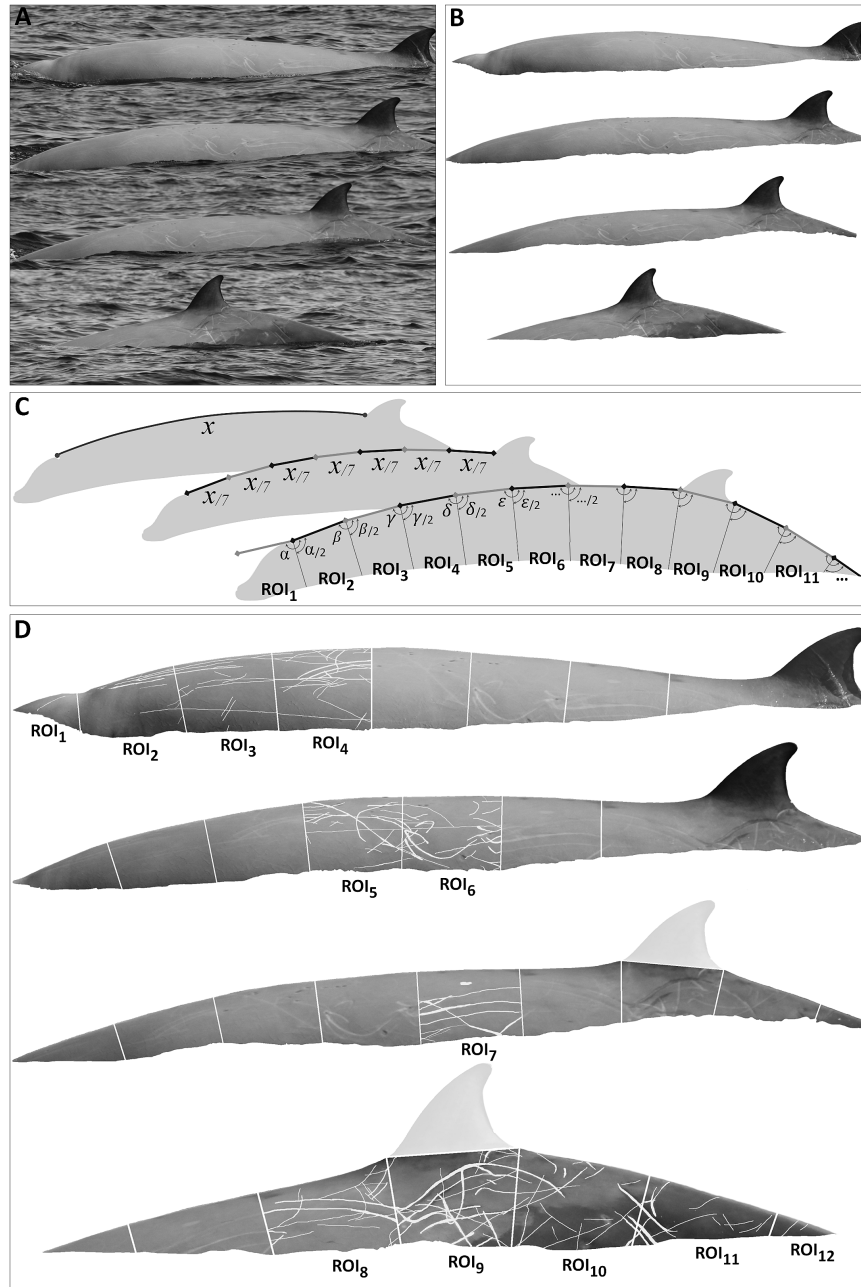


Fig. 2.—The sequential procedures used to subdivide an animal and to trace the visible scarring from a photographic sequence. A) The original $Q \geq 5$ photographic sequence; B) extraction of the visible body area from the background pixels; C) the subdivision into different regions of interest (ROIs): firstly, the measured smooth curve (x) delimited by the blowhole and the anterior insertion joint of the dorsal fin; secondly, the subdivision of the smooth curve to create 7 division units ($x/7$) and 8 reference points; thirdly, drawing the division lines to demark the ROIs; D) the scar tracing of the selected ROIs using the methodology of [Rowe and Dawson \(2009\)](#).

it was calculated for each ROI or any amalgamation of ROIs. The amalgamation of all the individual ROIs in one sequence has been classified as the total visible body area (TVB).

Descriptive statistics and generalized linear model.—Qualitative and quantitative analyses were conducted on the level of scarring in individual ROIs and their relation to the mean TVB scarring, in order to assess if there was any apparent scarring pattern (i.e., localized areas of high or low relative scarring densities). To test the significance of differences in scarring levels and the pigmentation pattern frequencies, a Kruskal–Wallis 1-way

analysis of variance (ANOVA) and Fisher's test were used, respectively.

The external characteristics that were considered to sex an animal were assessed using a generalized linear model (GLM) framework ([Nelder and Wedderburn 1972](#)). A logistic GLM with a logit link function was fitted in R version 3.1.0 ([R Development Core Team 2014](#)) given its ability to predict a binary outcome from a set of continuous predictor variables. In the GLMs, the sex was the response variable, while the ROI (and amalgamations of ROIs) scarring density and the pigmentation patterns

were predictor variables. A set of optimal candidate models were selected using a stepwise procedure based on the Akaike information criterion (Sakamoto et al. 1986). The significance of each predictor variables inclusion or exclusion was assessed using an ANOVA (library car in R—Fox and Weisberg 2011) for every possible combination of uncorrelated predictor variables. The best models were then selected from the candidate models using the Bayesian information criterion (BIC—Schwarz 1978), as candidate models were nonnested but had the same response variable. To test the significance in fit between the best models, Vuong's test (Vuong 1989) was applied. To determine the predictive power of the model and the optimal threshold value, area under receiver operating curve (AUROC) methods were used (library ROC in R—Sing et al. 2005). AUROC statistics are useful in evaluating binary class decisions and cutoff parameterization based on a models' sensitivity (the true positive rate) and specificity (the true negative rate) at various threshold values.

RESULTS

Pigmentation patterns.—A total of 73 different adult individuals (represented by 73 $Q \geq 3$ individual sequences) were selected for the pigmentation pattern analysis, 34 of the right side and 39 of the left side. The number of photos per sequence ranged from 2 to 8 with a modal number of 4, for a total of 328 $Q \geq 3$ photographs. The sequences selected described 38 and 35 adult males and adult females, respectively. The frequencies of pigmentation patterns within the 2 sexes are listed in Table 1.

The Fisher's test highlighted some significant differences in pigmentation pattern between the sexes (Table 1). The biggest differences were in the frequency of the dark oval patch and the brownish coloration of the body: these patterns were common in adult females, while these were rare in adult males. Moreover, all the males analyzed showed a contrasted and uniform white cape which was evident in only the 54% of the females. One or 2 dark crescents were present in nearly all the animals (except for 2 almost white males) and displayed no significant differences between the sexes.

The cluster analysis result identified 2 pigmentation clusters ($n_{\text{associate}} = 4.662$; $N = 9$; $\text{SRI}_{\text{null}} = 0.583$), the "soft" cluster and the "sharp" cluster (Fig. 3). The "soft" cluster is characterized by a brownish coloration of the body covered by both a low contrast white cape and dark crescents (e.g., Figs. 1A and 1B). In this pattern, the white cape is more evident on the head in respect to the rest of the body and the dark oval patch pigmentation is always present on the melon. The "soft" cluster was evident in the 40% of the adult females analyzed (14 individuals), while no adult males showed this cluster. The "sharp" cluster

is characterized by a dark coloration of the body covered by a strong, contrasted, and homogeneous white cape (e.g., Figs. 1C and 1D). No dark oval patch is present on the melon and the dark crescents are visible but not particularly distinctive. The "sharp" cluster was displayed by the 34% of the adult females (12 individuals) and by the 53% of the adult males (20 individuals); however, the frequency of this cluster was not significantly different between the sexes (Fisher's test, P value = 0.157). The dark oval patch was present in 83% of "sharp" females ($n = 10$) and only in 10% of the "sharp" males ($n = 2$). In females, the frequency of the "sharp" cluster and the "soft" cluster was similar (Fisher's test, P value = 1).

The white cape was found to be significantly shorter in "soft" females, which had an average white cape length of 4 ROIs, while the average length in the rest of females was 8.5 ROIs ($F_1 = 6.8382$, P value = 0.0089) and of 9.2 ROIs in males ($F_1 = 11.329$, P value = 0.0008). No difference in cape length was observed between "sharp" females and "sharp" males ($F_1 = 0.3157$, P value = 0.5742).

The remaining adults not classified to a pigmentation cluster (18 males and 9 females) had varying degrees of the different pigmentation patterns combinations. Males generally displayed the same pigmentation pattern grouping characteristics of the "sharp" cluster except they had "light" body color. While unclassified females shared pigmentation patterns from both clusters but did not conform strongly to one cluster or the other. Two males and 1 female showed an almost complete white coloration over the entire (visible) body but were not classified as "sharp individuals" only due to the absence of dark body coloration.

The length of the white cape was found not to be related to the level of TVB scarring for both females ($F_{1,21} = 1.364$, P value = 0.2559) and males ($F_{1,23} = 1.01$, P value = 0.3253). Moreover, there were no differences in level of TVB scarring between "soft" and "sharp" females ($F_1 = 1.3393$, P value = 0.2472).

Scarring assessment and differences between sexes.—Among the $Q \geq 3$ sequences selected, 48 individual $Q \geq 5$ sequences were used for the scarring analysis (25 adult males and 23 adult females). Among the 48 selected individuals, there were 8 females and 8 males showing the "sharp" cluster and 7 females showing the "soft" cluster. The modal Q -value of the sequences selected was Q_6 and the Q -value was found to have no significant effect on the measured scar density ($F_3 = 5.04$, P value = 0.1688) with no significant difference in quality between the sexes ($F_1 = 1.47$, P value = 0.2253). Within the sequences, it was possible to identify up to 13 separate identifiable ROIs, going from the head (ROI_1), the trunk (ROI_2 to ROI_7), the dorsal fin zone (ROI_8 to ROI_{10}) to the rest of the tail

Table 1.—Frequencies of the pigmentation variables in adult females and males Cuvier's beaked whales. Fisher's test P values from inter-gender comparisons are also shown.

	Dark oval patch	Low contrasted white cape	High contrasted white cape	Uniform white cape	Dark crescents presence	Distinctive dark crescents	Dark body coloration	Brownish body coloration	Light body coloration
Female	0.829	0.429	0.571	0.543	1.000	0.600	0.343	0.514	0.143
Male	0.105	0.000	1.000	1.000	0.921	0.395	0.526	0.026	0.447
Fisher's P value	<0.0001	<0.0001	<0.0001	<0.0001	0.2627	0.1623	0.1067	<0.0001	0.0097

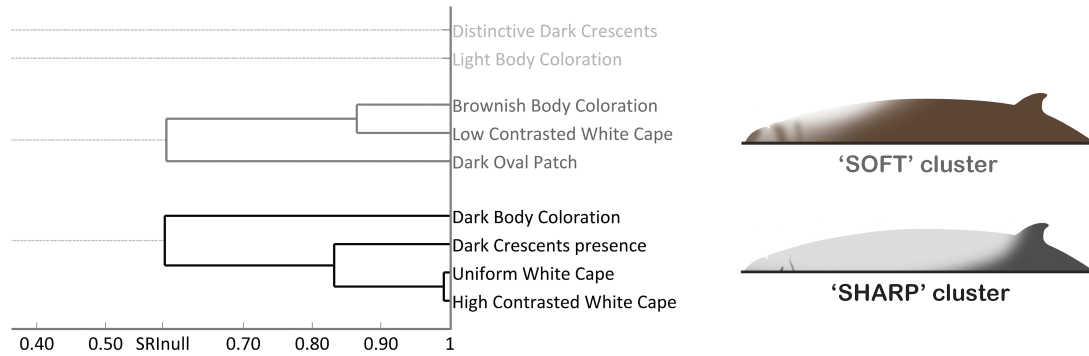


Fig. 3.—Cluster analysis of the pigmentation patterns in adult Cuvier’s beaked whales. Simple ratio index (SRI) values are indicated on x axis, while pigmentation pattern variables are indicated on the y axis. Clusters are represented by the bold lines. Two main clusters arise from the analysis ($SRI_{null} = 0.583$; cluster correlation coefficient = 0.811), the “SOFT” cluster and the “SHARP” cluster which are comprised of 3 and 4 pigmentation variables, respectively. Drawing examples of the 2 clusters are shown.

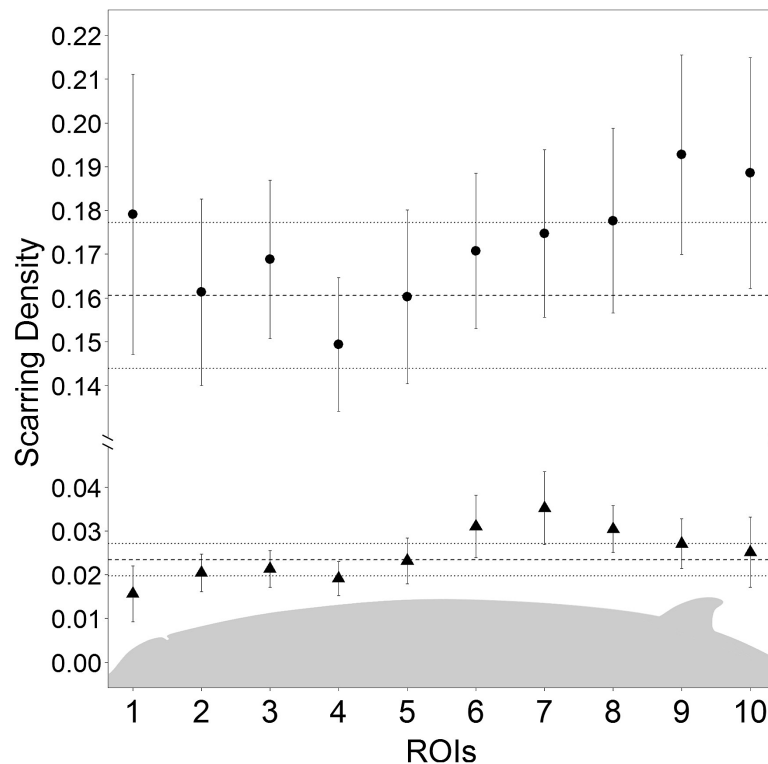


Fig. 4.—The relative scarring density of each region of interest (ROI), compared with the TVB global mean across the 2 sexes. Circles and triangles represent the mean scarring density for each ROI in the males and females, respectively, with attached SE bars of the sample. The horizontal dashed lines are the mean TVB scarring level and SE s of the different sexes. TVB = total visible body area.

stock (ROI₁₁ to ROI₁₃). However, not all ROIs were represented in all the animal sequences and many of the tail stock ROIs had small percentages of visible body. Therefore, all subsequent analyses and amalgamations only considered ROI₁ to ROI₁₀ as these were available for every analyzed individual.

The level of TVB scarring between the left and right side sequences was found to be nonsignificant for both adult males ($F_1 = 2.08$, P value = 0.1488) and adult females ($F_1 = 0.0026$, P value = 0.959). However, the males were found to have a significantly higher level of TVB scarring in relation to the females ($F_1 = 30.4713$, P value < 0.0001), with a mean scarring level of 0.160 ± 0.083 and 0.023 ± 0.018 for males and females, respectively.

The amount of scarring among the different ROIs is generally correlated ($\bar{X} = 0.673$; range 0.318–0.914 for males and $\bar{X} = 0.433$; range 0.055–0.910 for females), with adjacent ROIs displaying the highest correlation. However, there was an indication to areas of localized high and low relative scarring levels that appeared to be sex dependent (Fig. 4). Adult males had the highest relative scarring density in the head and beside the dorsal fin (ROI_{8–10}). Females on the other hand showed a low relative scarring density on the head that gradually increases progressively posterior, reaching the maximum around the midpoint between the snout and the fluke (ROI₇). The individual ROI scarring levels deviated from the mean TVB by a higher degree in females than in males (i.e., ROI₇ was almost double the TVB in females, while

in males, the highest deviance—ROI₉—was less than 1.2 the TVB; see Fig. 4). However, the Kruskal–Wallis tests conducted between the scarring levels of the separate ROIs showed no significant differences for males ($F_9 = 2.827$, P value = 0.9710) and the significant difference found in the females ($F_9 = 17.277$, P value = 0.0441) was due to the low scarring on the 1st ROIs (Tukey honest significant difference).

A total of 9 optimal candidate models were fitted (Table 2) and based on the lowest BIC and the Vuong’s test statistics, 6 models were considered the best for predicting an animal’s sex from the scarring density of the different ROI amalgamations (Fig. 5). These 6 model area under

the curve statistics ranged from 0.967 to 0.981 with a scarring density threshold that ranged from 0.041 to 0.064 with generally a lower threshold on the anterior amalgamations and a high threshold on the posterior ROI amalgamations (Table 2). An animal was classified to an adult male when the scarring density was greater than the model scarring density threshold. Thirty-eight out of 48 individuals (79%) were correctly classified by all the best 6 candidate models, while the modal success in sex classification per each of the 6 models was 43 animals (90%; Fig. 6). However, no model performed better than any other model. Only 2 animals (4%) were never identified to the correct sex.

Table 2.—The summary statistics of the optimal candidate models after stepwise selection. All candidate models are presented here with information relating to the model selection (BIC) and predictive power (AUC). The top 6 best models based on the BIC value are all within 2 points and according to Vuong’s test are indistinguishable and the scarring density threshold values are presented for these models. AUC = area under the curve; BIC = Bayesian information criterion; ROI = region of interest; TVB = total visible body area.

Model	Logit (y)	Scarring density threshold	AUC	BIC	ΔBIC
Sex ~ (ROI ₄₋₆)	$-4.432 + 70.786 \cdot (\text{ROI}_{4-6})$	0.047	0.976	26.274	
Sex ~ (ROI ₃₋₅)	$-4.521 + 79.930 \cdot (\text{ROI}_{3-5})$	0.041	0.981	26.387	0.113
Sex ~ (TVB)	$-4.532 + 79.897 \cdot (\text{TVB})$	0.046	0.972	26.714	0.440
Sex ~ (ROI ₇₋₉)	$-4.716 + 63.371 \cdot (\text{ROI}_{7-9})$	0.064	0.974	27.357	1.083
Sex ~ (ROI ₁₋₆)	$-4.100 + 74.759 \cdot (\text{ROI}_{1-6})$	0.044	0.967	28.086	1.812
Sex ~ (ROI ₇₋₁₀)	$-4.333 + 59.043 \cdot (\text{ROI}_{7-10})$	0.058	0.969	28.174	1.900
Sex ~ (ROI ₉₋₁₀) + (ROI ₇)	$-3.974 + 33.148 \cdot (\text{ROI}_{9-10}) + 19.075 \cdot (\text{ROI}_7)$		0.963	33.430	7.156
Sex ~ (ROI ₈) + (ROI ₁₀)	$-4.693 + 31.714 \cdot (\text{ROI}_8) + 39.775 \cdot (\text{ROI}_{10})$		0.977	33.725	7.451
Sex ~ (ROI ₇₋₈) + (ROI ₁₀)	$-3.908 + 36.557 \cdot (\text{ROI}_{7-8}) + 16.458 \cdot (\text{ROI}_{10})$		0.962	34.540	8.266

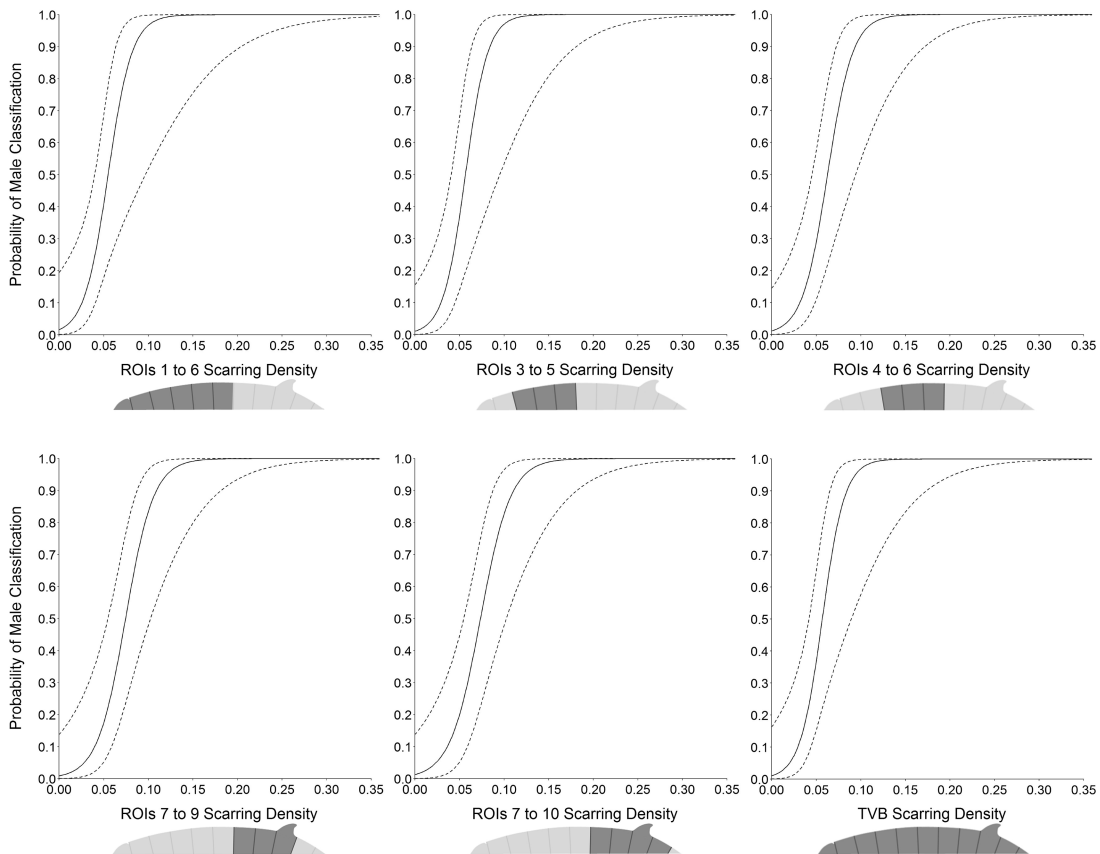


Fig. 5.—Predictions from the GLM for the top 6 candidate models. Dashed lines represent the 95% CIs. GLM = generalized linear model; ROI = region of interest; TVB = total visible body area.

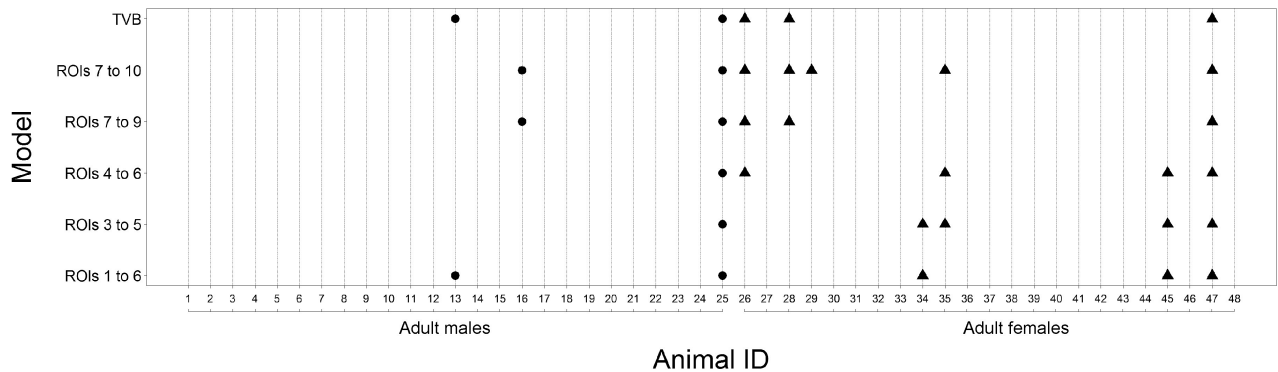


Fig. 6.—Errors in sex classification from each of the top 6 candidate models. Individuals analyzed are indicated along the horizontal axis, the candidates models are indicated on the vertical axis. Circles represent false negatives (mode = 2 out of 25), while triangle represent false positives (mode = 4 out of 23). ROI = region of interest; TVB = total visible body area.

DISCUSSION

Sex determination in Cuvier's beaked whales may be identified through genetics, direct observation of the genital area, and in adult animals by the presence or absence of erupted teeth or a close association with a calf. Given this species offshore habitat (Moulins et al. 2007; Correia et al. 2015), its long dive times with short surface interval (e.g., Tyack et al. 2006), and its shy reactions to boats (Heyning 1989), researchers can usually only classify a proportion of all the animals through the methods cited before. Therefore, gender assessment of many animals has been done through assumptions related to the scarring densities and pigmentation patterns of adult animals (e.g., McSweeney et al. 2007). However, many of these assumptions have been made from historic reports on stranded animals and conducted on a limited number of specimens that were fresh enough for a useful analysis (e.g., Houck 1958). Baseline species description mainly refers back to Heyning's (1989) document which made a review of the reports available at that time. For the 1st time, in order to complement the work of Heyning (1989), our paper analyzed a large sample size of sexed living individuals inhabiting the Mediterranean Sea with the aim to increase the reliability of assumptions on animal sex through species external characteristics. Since the analysis has been carried out on living animals, no bias due to postmortem pigmentation obscuration affected the study.

Overall, our results of pigmentation analysis confirmed the general past findings on Cuvier's beaked whale pigmentation patterns described by Heyning (1989) but allowed a finer description of individual pigmentation frequency and heterogeneity between the sexes. The adult males always showed contrasted and uniform white cape which usually extended until the dorsal fin zone. The adult male body coloration varied individually from dark to almost white; however, there were similar frequencies of the dark and light colorations (light gray to almost white). A new finding of this work was that more than 1 adult female out of 3 showed very similar pigmentation patterns to those of adult males ("sharp" pattern, e.g., Fig. 8). These females were similar to male also in the white cape contrast and length (P value > 0.05) but differed with a much higher frequency of dark oval patch presence (P value < 0.001). However, the dark oval patch pigmentation itself may not be

useful for sex assessment in this case because it may be under-represented in adult males due to scarring superimposition. Moreover, the dark oval patch has been observed in subadult males (M. Rosso, CIMA Research Foundation, pers. comm.). Overall, these females cannot be distinguished from the males (and vice versa) by using the pigmentation pattern alone. However, the majority of adult females showed pigmentation related to a "soft" cluster characterized by a brownish coloration of the body, ranging from chestnut brown to light brown. The anterior part of these females was usually covered by a soft low contrasting white cape (similar to the description of Heyning 1989). In this pattern, the white cape extended typically until the 1st third of the animal body being shorter than those recorded in the other adults (P value < 0.01). In these females, the white cape was more evident on the head in respect to the rest of the body and the dark oval patch pigmentation was always present on the melon.

In past studies, the Cuvier's beaked whale scarring has been demonstrated to be a proxy of animal age since it has a net gain rate ranging from 1 to 6 marks per adult individual per year (Rosso et al. 2011). Three quarters of the adults analyzed in this work had an extended white cape; however, in both sexes, the extension of the white cape cannot be used as a proxy of the animal age since it was noncorrelated with the scarring density ($r = 0.379$). Particularly, in our sample, there were males with extended white cape that had very limited scarring, thus "young" adult animals. In marine mammals, coloration typically changes between birth and adulthood and in some cases appearance changes radically (Perrin et al. 2002). Our results indicated that for non-"soft" Cuvier's beaked whales (which represent ~80% of individuals analyzed in this paper), the change in dorsal coloration from the subadult to the adult pattering may be rapid. To support this finding, we show the photos of consecutive ($Q \leq 4$) photographic captures of an adult individual (Fig. 7). The animal changed from a primitive brown coloration with a paling head (subadult form) to a dark body coloration with an extended white cape in less than 2 years which was the time gap between the 2 captures. On the other hand, the "soft" cluster had similar pigmentations of subadult animals (i.e., brown body coloration and paling melon) but it is not necessarily a previous stage of the "sharp"



Fig. 7.—Two consecutive photographic captures of the same individual within a 2-year timeframe. A) and B) are, respectively, the right and left side identifications at the 2 captures. A) and B) top sequences were taken in July 2004, while A) and B) bottom sequences were taken in May 2006.

cluster since there were no strong differences in level of (age-related) scarring density between “soft” and “sharp” females (P value = 0.2472). Moreover, at least 2 “soft” females were actually older than 3 “sharp” females (M. Rosso, CIMA Research Foundation, pers. comm.). These findings may indicate that a part of females (i.e., “soft” females) do not rapidly change from the primitive coloration of immature whales when they get adults, while all (toothed) adult males do. Therefore, it is not possible to assess the sex of these females (which represent ~20% of the adult individuals analyzed in this paper) by looking at the pigmentation pattern only. Pigmentation heterogeneity has been observed in other beaked whales, for example, O’Brien and Whitehead (2013) found that not all adult male *Hyperoodon ampullatus* developed the typical white melon coloration. In some documents, it has been reported that only adult males Cuvier’s beaked whales can appear almost all white (e.g., Shirihai and Jarret 2006). One of the 3 almost all white individuals analyzed here was a female; therefore, a very pale coloration is a pattern present in both sexes.

No pigmentation patterns were retained in the candidate model selection procedure and their omission always resulted in a lower BIC value. Therefore, the scarring density is likely a more predictive model parameter in respect to the pigmentation pattern for sexing Cuvier’s beaked whale adults. The top 6 predictive models obtained were able to correctly assess the sex from 85% to 90% of the individuals analyzed, supporting the use of scarring level as a reliable sexing methodology for this species. However, the models predicted better for adult males since the number of false positives was higher than the number of false negatives (Fisher’s test; P value = 0.0123). This may be explained by the age-dependent level of scarring; therefore,



Fig. 8.—Photos of the entire visible flank of a “sharp-clustered” female (#35; see Fig. 6). The scarring density level along the body make this individual a typical “threshold” individual ($ROI_{1-6} = 0.034$; $ROI_{3-5} = 0.042$; $ROI_{4-6} = 0.049$; $ROI_{7-9} = 0.060$; $ROI_{7-10} = 0.062$; $TVB = 0.044$). Note that the scarring density is higher in the posterior part of the animal; this scarring pattern has been observed to be a common pattern in adult females. According to the model results, individuals showing a more severe scarring of this “threshold” example are more likely adult males. ROI = region of interest; TVB = total visible body area.

“old” females are more likely to be classified as males. The method used in this study represents a novel approach to the division of the TVB of a cetacean species and the adaptation of an existing scar tracing methodology (Rowe and Dawson 2009). This method allowed for comparisons between the different regions of an animal’s body, where the relative scarring was found to display sexual differences (Fig. 4). Females have localized area of high scarring just anterior of the dorsal fin and overall they tended to have higher posterior scarring levels relative to the anterior, especially to the 1st 4 ROIs. Where males as a group tend toward having higher relative levels of scarring around the head and dorsal fin zone. Since the scarring is age-dependent, these patterns may become more distinctive with age. Therefore, for a “threshold” animal (i.e., an animal having a TVB scarring density close to 0.046; Fig. 8), the relative scarring level among the ROIs might be used as a secondary cue for sex determination in order to reduce false positive/negative classifications.

The results presented in this study clearly confirm that adult males are distinctly more scarred by intraspecific marks than adult females, being 7 times more scarred than the counterparts (averaged TVB scarring density = 0.160 in males, 0.023 in females). Similar inter-gender differences in scarring were recorded in other species of odontocetes including narwhals (Gerson and Hickie 1985), bottlenose whales (Gowans and Whitehead 2001), dense beaked whales (MacLeod 2002), bottlenose dolphins (Scott et al. 2005; Rowe and Dawson 2009; Marley et al. 2013), and the Amazon river dolphins (Martin and

Da Silva 2006). Conspecific aggression is likely to have a large impact on the socioecology, social structure, and relationship in Cuvier’s beaked whales as well as in other odontocetes. It has been hypothesized that intraspecific scarring may act as a display of dominance to other males during aggressive social interactions (MacLeod 1998). However, this study has found that females accumulate scarring caused by toothed males, mainly around the lumbar flanks. This intraspecific scarring pattern has been already recorded in indo-pacific bottlenose dolphins (*Tursiops aduncus*) and it is plausible that it is caused by sexual coercion. Males indo-pacific bottlenose dolphins in Shark Bay have been observed to form male association that aggressively coerce females through harassment and intimidation, behaviors that have included biting and resulted in tooth rakes on the female (Scott et al. 2005). Male sexual aggression appears to be most common in gregarious and polygynous species, such as pinnipeds (e.g., Le Boeuf and Mesnick 1990) and bottlenose dolphins (e.g., Connor et al. 1996), in which females do not form long-term bonds with a single male but encounter several that compete for access to her (Smuts and Smuts 1993). Therefore, the scarring patterns of Cuvier’s beaked whale might indicate a social ecology resembling a fission fusion organization, already observed in other beaked whale species (Gowans et al. 2001). This may be further supported by the fact that recorded group composition of Cuvier’s beaked whales includes more than 1 male within a group (Falcone et al. 2009).

In conclusion, the scarring density was revealed to be both a useful and main predictive parameter for sexing adult Cuvier’s

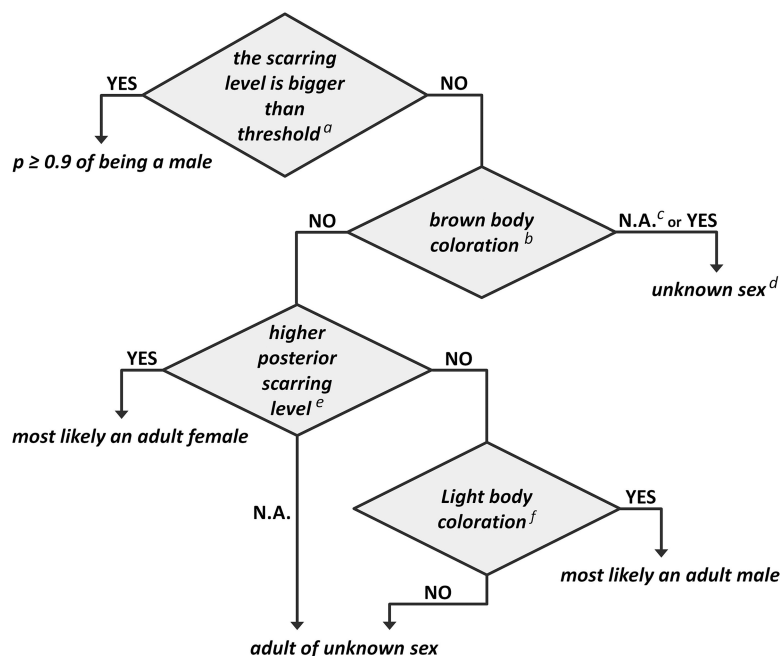


Fig. 9.—Flow chart describing the best logical flow to sex whales from photographic capture(s) of animal flank-only according to the findings of this paper. a—the 1st step is to assess the scarring density present on the flank portion visible in the photo(s); to do, so refer to the scarring thresholds indicated in Table 2 (according to the flank portion visible in the photo) or Figure 8 for a crude visual comparison. b—is intended as the primitive coloration of this species, ranging from chestnut to dark brown. c—as the case of diatom film covering the entire flank portion visible in the photo(s) and making color assessment imprecise. d—note that some “soft” clustered adult females may fall into this category since their coloration is similar to the subadult coloration pattern. e—the average scarring density of ROI₆₋₉ may be 200% the density of ROI₂₋₄ in adult females, while it may be up to 115% only in adult males, see Fig. 4. f—the flank coloration not covered by the white cape. ROI = region of interest.

beaked whales. Particularly, the predictive models presented in this study allowed identifying reliable scarring density thresholds across different whale body areas and added support to the proposal that scarring level can be used as a reliable sexing methodology in odontocetes. This methodology can be utilized even if the only information available is one opportunistic photo of an animal. On the other hand, the results obtained clearly indicate that pigmentation patterns alone cannot be considered as a reliable cue in sex determination since adult females showed very similar pigmentation patterns to both those of adult males (“sharp” pattern) and those of subadult males (“soft” pattern). In Fig. 9, we present a flow chart that can be used as an aid in sexing adult Cuvier’s beaked whales from photographic capture(s), where erupted teeth are not visible. The chart uses a stepwise process of the absolute and relative scarring density in combination with the color patterns to provide an additive sex determination procedure. According to the individuals sample analyzed in this paper, this chart may allow for reliable sex classification of more than 80% of adult individuals photographed.

ACKNOWLEDGMENTS

This work was partially funded by the Italian “Ministero dell’Ambiente e della Tutela del Territorio e del Mare” (MATTM) and the Pelagos Sanctuary Permanent Secretariat. This work would not have been possible without the enthusiasm of all the students and researchers who participated in the surveys on board the research vessel “Leon Pancaldo.” Particular thanks to X. Wang, S. Cominelli, A. M. Correia, D. Horta, and C. Morgado.

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Submitted 18 May 2015. Accepted 22 January 2016.

Associate Editor was Jeanette A. Thomas.