Using photography to determine sex in pilot whales (*Globicephala melas*) is not possible: Males and females have similar dorsal fins

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Photo-identification is used to study populations, movements and social structure (*e.g.*, Bigg *et al.* 1987, Ottensmeyer and Whitehead 2003, Oremus *et al.* 2007). All of these analyses are more informative if the sexes of the identified individuals are known. In a few ideal cases the identification photograph itself contains a strong indicator of sex. For instance the great sexual dimorphism in the size and shape of the dorsal fin in adult killer whales (*Orcinus orca*) allows sex to be determined together with individual identity from photographs (Bigg *et al.* 1987).

Long-finned pilot whales (*Globicephala melas*) are delphinids, almost entirely black or dark colored. They present three lighter areas of skin, varying from cream to white: the saddle patch, located posterior to the dorsal fin; the postorbital eye blaze, located above the eyes; and an anchor shaped patch on the throat area, extending ventrally (Sergeant 1962). Adult size length can reach up to 4.72 m for females and 6.10 m for males (Sergeant 1962). The sexual dimorphism of the species is also present in the size of the dorsal fin. Because dorsal fin size increases isometrically with body length, adult males have bigger dorsal fins than females (Bloch *et al.* 1993). It has also been suggested that dorsal fin shape differs between the sexes, with males showing a thicker edge, a more rounded contour and a more rounded tip (Sergeant 1962).

Shape can be analyzed using digital photography and shape analysis methods, such as the elliptical Fourier descriptor analysis (Kuhl and Giardina 1982). This method has been widely used to describe shape in different taxa, such as petals of Japanese primrose (*Primula sieboldii*) (Yoshioka *et al.* 2004), roots of Japanese radish (*Raphanus sativus L.*) (Iwata *et al.* 1998), wings of mosquitoes (*Ritera culicidae*) (Rohlf and Archie 1984), fish otoliths (Reig-Bolano *et al.* 2010), and dorsal fins of bottlenose dolphins (*Tursiops truncatus*) (Rowe and Dawson 2009). This method also has the advantage of analyzing shape independently of size (Kuhl and Giardina 1982).

The population of pilot whales that summers off Cape Breton, Canada, has been the subject of study since 1998 (Ottensmeyer and Whitehead 2003). Individual pilot whales have been identified using photo-identification, based on the number and location of mark points in their dorsal fins (Auger-Methe and Whitehead 2007). Saddle patch color and density were also found to be useful when identifying individual pilot whales. Although, given the high number of individual pilot whales identified in this population, the amount of photographic data collected each year,

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and that saddle patch pattern is not included in any photo-identification software, it has not been used as a photo-identification trait for this population.

Following Sergeant's (1962) suggestion, we investigated whether pilot whale dorsal fin shape, coupled with the photo identification traits saddle patch and number of mark points, were different enough between sexes for us to be able to predict sex based on photographic data.

Data were collected during July and August 2010 off Pleasant Bay, Cape Breton, Canada. Skin biopsies of 20 individuals were collected using a crossbow (Excalibur Vixen, Excalibur Crossbow, Kitchener, Canada) from a distance from 10 to 30 m to the individual. Bolts with a compressed foam stop collar were used so that penetration would not be deeper than the tip (25 mm), allowing it to rebound on impact and enabling it to float. These were fired to the mid lateral region, below and caudal to the dorsal fin. Skin samples were stored in a solution of 20% dimethylsulphoxide saturated with salt (Seutin *et al.* 1991). Photographic data were collected prior to and during biopsy using a Canon EOS 400D with a 70–300 mm lens. Only individuals that were identified in the population catalog and seen for >2 yr in the area were sampled.

DNA was extracted using the phenol:chloroform extraction method (Sambrook and Russel 2001). Sex of individuals was determined using a multiplex PCR of two primer pairs: one that amplifies a ~400 bp portion of the *ZFX/ZFY* gene (present on both sex chromosomes); and one that amplifies a ~200 bp portion of the *SRY* gene (only on the Y-chromosome) (Gilson *et al.* 1998). PCR was performed on 20 μg of purified DNA in a 20 μL reaction volume that contained 1X *Taq* polymerase PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 0.3 mM of each primer, 0.16 μg/mL BSA, and 0.05 U/μL *Taq* polymerase. PCR cycles were performed as follows: the first cycle at 94°C for 5 min, followed by 30 cycles comprised of denaturation at 94°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 1 min. A final cycle was performed at 60°C for 45 min. The PCR products were then separated and visualized using agarose gel electrophoresis in 1.5% agarose gels stained with ethidium bromide.

Photo-identification pictures collected during the biopsy protocol were classified in terms of focus, size, exposure, percentage of dorsal fin visible in the frame, and orientation of the dorsal fin according to the camera. Special attention was given to orientation of the dorsal fin, since dorsal fin shape would be distorted if the dorsal fins were not perpendicular to the axis of the camera lens. Pictures with the greatest total classification values for each individual were considered the best pictures. Dorsal fin images were extracted from the background, cropped at the base of the fin, flipped so that they were all facing the right side, and rotated so the base was horizontal (Adobe Photoshop CS5, Adobe Systems Inc., San Jose, CA). Dorsal fin base was defined as the line than runs from the anterior to the posterior insertion point of the dorsal fin (Fig. 1). The anterior insertion point is marked as the bottom of the concavity formed by the junction of dorsal fin and body. A reference line was then drawn following the main axis of the back, and the posterior insertion point was marked when it reached the dorsal fin (Rowe and Dawson 2009). All of the photographs were processed by the same person for consistency (by J.F.A.).

Dorsal fin shape was analyzed through Elliptical Fourier Description (EFD), using the software package SHAPE (Iwata and Ukai 2002). For each image, the contrasting areas between the white background and the dark dorsal fin were used to convert the image from RGB to black and white, facilitating shape detection. A closed contour of the dorsal fin was then extracted by edge detection and recorded as chain code

Figure 1. Photograph of a pilot whale dorsal fin, illustrating the line that runs from the anterior to the posterior insertion point of the dorsal fin.

(Freeman 1974). Each dorsal fin contour was saved as a set of sequential points, each a pair of x and y coordinates, measured counterclockwise from an arbitrarily set starting point (Yoshioka *et al.* 2004).

EFD coefficients were calculated from the chain-coded contours by discrete Fourier transformation (Kuhl and Giardina 1982). These were normalized to be invariant according to size, rotation and starting point of the contour (Iwata and Ukai 2002). There are two methods of normalization: the first based on the ellipse of the first harmonic (Kuhl and Giardina 1982); the second based on the longest radius—the farthest point from the centroid to the contour (Iwata and Ukai 2002). The longest radius method allows manual alignment of the contours so it was chosen for the normalization, allowing the dorsal fin bases to be horizontal during the remaining analysis.

Dorsal fin shape was determined based on the normalized EFDs using a sum of trigonometric functions, which can also be called harmonics. Each contour was approximated using the first 20 harmonics. Results were summarized in a PCA (Rohlf and Archie 1984), based on the variance-covariance matrix of the EFD coefficients. The variance explained by each component was also visualized (Furuta *et al.* 1995). The coefficients of the EFDs were recalculated, making the score for each PCA to be equal to the mean plus or minus two times the standard deviation, and the scores of the remaining components to be zero. Then, an inverse Fourier transformation was applied to create the contour corresponding to each component.

To determine whether dorsal fin shape varied according to sex of the individuals, a discriminant analysis and multivariate variance analysis (Minitab 15, Minitab Inc., State College, PA) were performed using the statistically significant ($P < 0.05$) PCA scores.

The saddle patch is a band of light pigmentation, located behind the dorsal fin (Sergeant 1962), that does not vary once individuals reach maturity. It can vary in color and pigmentation level (Auger-Methe and Whitehead 2007) between individuals. The pigmentation levels (sparse, medium, and dense) were assessed for each individual, based on the best pictures (Fig. 2). A permutation test (R 2.12.2, R Core development Team 2011) was used to determine if the distribution of pigmentation level was related to sex of the individuals. Color (gray, white, and cream) was not tested because it did not seem consistent between photographs of the same individual in different lighting conditions.

Mark points are defined as nicks and internal corners of larger notches present in the dorsal fin trailing edge (Ottensmeyer and Whitehead 2003, Auger-Methe and Whitehead 2007). They are the basis for the photo identification of different individuals in the population. The number of mark points for each individual was

Figure 2. Examples of saddle patch density. Saddle patches are within the rectangle. The left most picture represents a dense saddle patch, the center picture a medium saddle patch and the right most picture a sparse saddle patch.

Table 1. Summary of results from the Principal Component Analysis on the coefficients of the Elliptic Fourier descriptors.

Component	Eigenvalue (10^{-4})	Proportion of variance (%)	Cumulative variance (%)
1	85.9	58.2569	58.2569
2	33.2	22.4907	80.7476
3	12.5	8.4585	89.2061
4	6.52	4.4229	93.6289
5	3.64	2.4703	96.0992
6	2.45	1.6643	97.7635

determined, and a Mann-Whitney U test (Minitab 15) was applied to test whether the number of mark points was related to sex of the individuals.

From the 18 individuals sexed, 11 were males and 7 females (Fig. 3). The dorsal fin photographs of these individuals were used to calculate the standardized Elliptic Fourier coefficients. Dorsal shape variability was well summarized by the first two principal component axes that explained more than 80% of the total variance (Table 1).

How each component affects dorsal fin shape is indicated in Figure 4. The mean shape sketched for each component separately (Mean), and the mean minus (−2 SD) and plus the standard deviation (+2 SD) are presented. The left most sketches represent the overlap between the three, illustrating the variability of the component. The nonoverlapping areas represent where variability is largest.

The first component relates to the height of the dorsal fin and distance from the tip to the anterior insertion point. The second component relates to the hang of dorsal fin tip relative to the anterior insertion point and how falcate the anterior area of the dorsal fin is.

The discriminant function analysis, with cross-validation, correctly classified only 56% (with linear response) and 44% (with quadratic response) of the individuals according to the first six principal components for dorsal fin shape. Variance analysis found no significant differences between sexes for the six first principal components (MANOVA, Wilk's Lambda = 0.52, $F = 2.25$, df = 5, 12, $P = 0.119$).

There was no relation between the distribution of saddle patch density and sex (permutation test, $P = 0.17$; Fig. 5). Given that only individuals previously identified for this population were sampled, and only individuals with more than 2 mark points
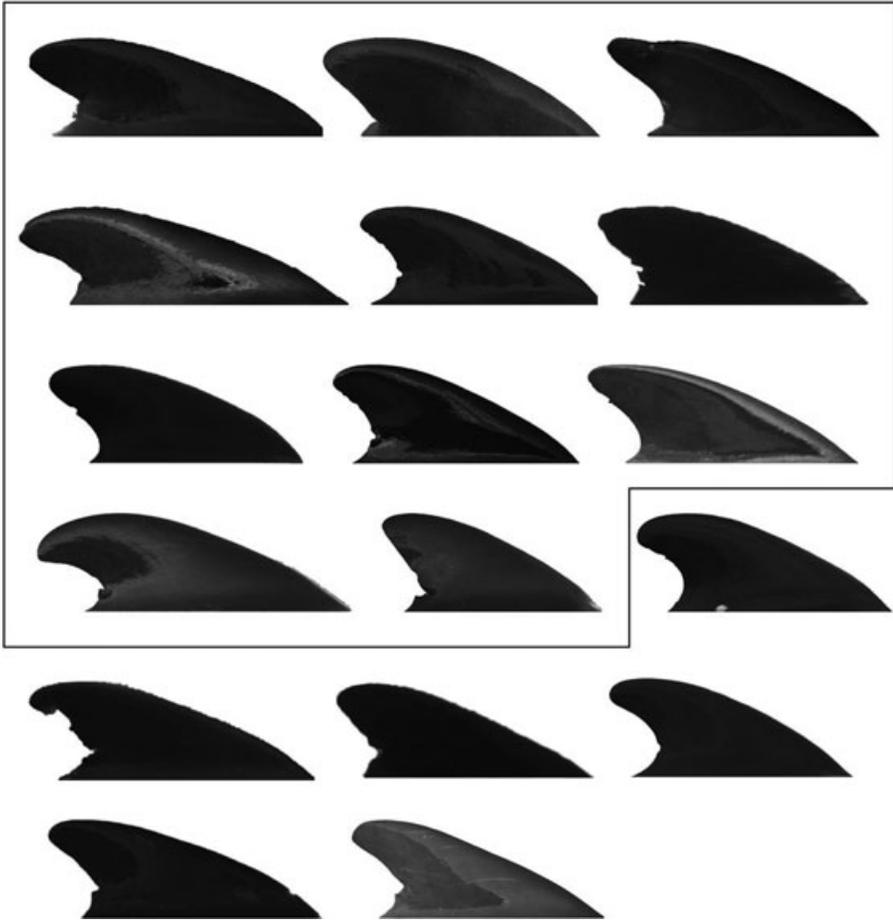
Figure 3. Dorsal fins of sampled individuals. Males are on the inside of the polygon, females on the outside.

are identifiable (Ottensmeyer and Whitehead 2003), all individuals have at least two mark points. There was no significant difference between number of mark points for males and females ($P = 0.23$; Fig. 6).

Contrary to prior suggestions (Sergeant 1962), male dorsal fins do not have a significantly more rounded contour or a more rounded tip. Male pilot whales do have larger dorsal fins than females (Sergeant 1962, Bloch *et al.* 1993) and human perception of shape can be altered by size factors (Yoshioka *et al.* 2004), so it is possible that the characteristics said to be typical of male fins appeared more prominent to the human eye because of a larger dorsal fin size. Elliptical Fourier descriptors analyze shape independently of size, so they can determine the variation in dorsal fin shape without the same biases as human perception. The number of mark points and the saddle patch density, traits used for photo-identification of individuals, also did not vary significantly between males and females.
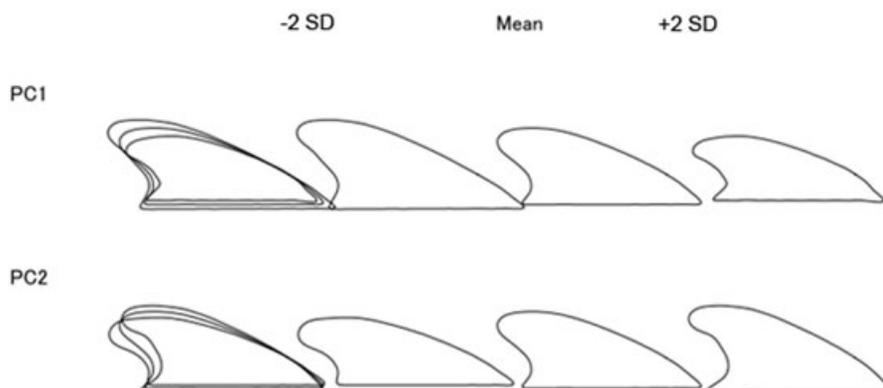
Figure 4. Variation in dorsal fin shape, explained by the first two components of the PCA. PC1 represents the first component and PC2 the second component. Mean represents the mean shape for the component, −2 SD the mean shape minus standard deviation, and +2 SD the mean plus standard deviation. The leftmost sketch is the overlap of shapes for each component.
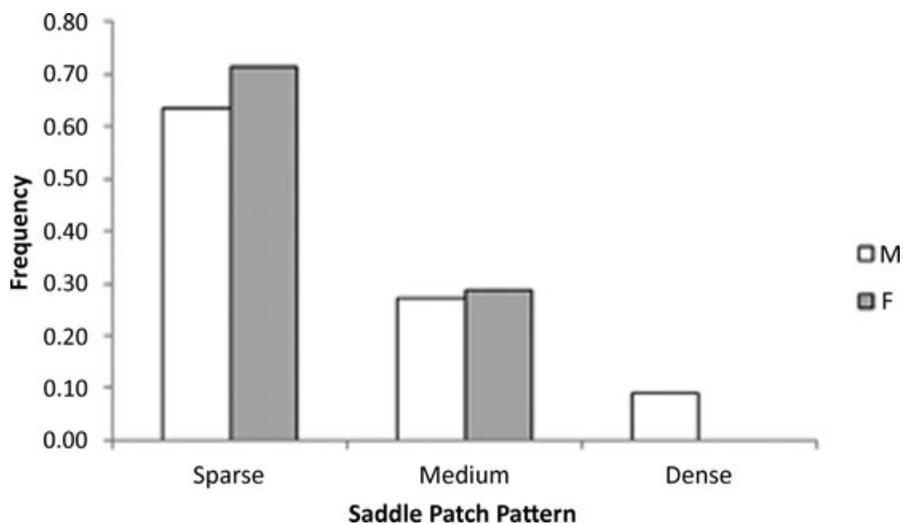
Figure 5. Saddle patch density of males (M) and females (F) in the sampled population.

In summary, we found no substantial or significant difference between males and females in any of the analyzed parameters: dorsal fin shape, saddle patch density and number of mark points. Even though our sample size was small, if dorsal fin characteristics varied with sex as markedly as referred to by Sergeant (1962), that variation would have been detected. Instead, we found that dorsal fin characteristics vary within sex. It does not seem possible, given the parameters used, to identify the sex of individuals using photo-identification photographs.
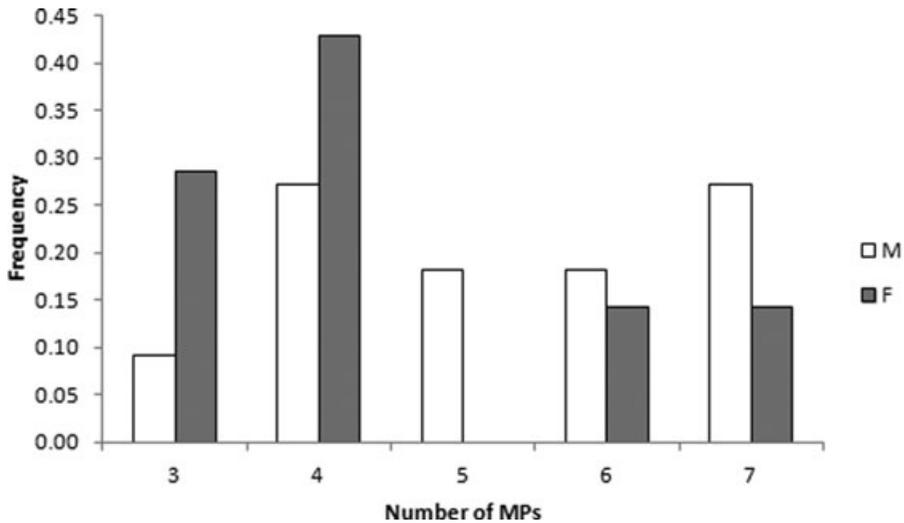
Figure 6. Frequency of the number of mark points (MPs) possessed by males (M) and females (F) in the sampled population.

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