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RRH: EMMONS *ET AL.*: VARIATION IN KILLER WHALE MORPHOLOGY

Quantifying variation in killer whale (*Orcinus orca)* morphology using elliptical Fourier analysis

CANDICE K. EMMONS[1] and **JEFFREY J. HARD**, Conservation Biology Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, Washington 98112, U.S.A.; **MARILYN E. DAHLHEIM** and **JANICE M. WAITE**, Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 7600 Sand Point Way Northeast, Seattle, Washington 98115, U.S.A.

ABSTRACT

In the northeastern Pacific Ocean, there are three "ecotypes" of killer whales that differ in diet, ecology, behavior, acoustics, genetics, and morphology. Previous attempts to describe the morphological differences among populations of killer whales (*Orcinus orca*) have been limited to descriptive accounts or categorical studies. We used elliptical Fourier analysis (EFA) to quantify shape differences of dorsal fins and pigmentation patterns among the ecotypes from photo-identification data of more than 500 individuals. Variation in shapes of the dorsal fin, saddle patch, and eye patch were successfully quantified using EFA, and there were highly significant ($P < 0.01$) differences among the ecotypes in all three morphological traits. The ability of EFA to discriminate ecotypes based on dorsal fin and eye patch shapes was substantial, while it did not perform as well for saddle patches. Visualization of the shape variation along principal component axes mirrored previous descriptions of the differences among ecotypes. Although the degree of inheritance of morphology in killer whales has not been determined, these results are consistent with previous inference of reduced gene flow between the ecotypes, and introduces elliptical Fourier analysis to the study of cetacean morphometrics.

Key words: killer whale, *Orcinus orca*, morphological variation, elliptical Fourier analysis.

Killer whales (*Orcinus orca*) are currently recognized as one species globally despite extensive descriptions of unique “forms” or “ecotypes” existing in sympatry (de Bruyn *et al*. 2013). In the northeastern Pacific Ocean, three ecotypes have been described: resident, offshore, and Bigg’s killer whale (Ford *et al*. 2000, Krahn *et al*. 2004). Over 40 yr of study have shown that while these ecotypes overlap in range, they are socially and genetically isolated from each other (Hoelzel *et al*. 2007, Morin *et al*. 2010, Foote *et al*. 2011). This reproductive isolation is associated with fundamental differences in their ecology (Bigg *et al*. 1987), acoustic behavior (Barrett-Lennard 1996, Foote and Nystuen 2008), and diet (Ford *et al*. 1998, Herman *et al*. 2005). Resident killer whales live in large, stable family groups of 5–40 whales and eat fish, primarily salmonids (Ford and Ellis 2006, Hanson *et al*. 2010, Ford *et al*. 2016). Bigg’s (transient) killer whales live in smaller groups of 1–10 whales, and other marine mammals are their preferred prey (Ford *et al*. 1998, Dahlheim and White 2010). Less is known about the offshore ecotype, since they are rarely encountered in the nearshore waters. They are often found in large social groups of up to 100 individuals, and bony fish and elasmobranchs are thought to be their primary prey (Dahlheim *et al*. 2008, Ford *et al*. 2011). The genetic, ecological, and behavioral differences between resident and transient killer whales are well established and constant across multiple populations throughout the North Pacific (Dahlheim *et al*. 2009, Ford *et al.* 1998, Herman *et al.* 2005, Hoelzel *et al.* 2007, Matkin *et al*. 2012, Parsons *et al*. 2013), but data supporting morphological differentiation are limited in the published literature (Perrin *et al*. 2009).

There is some uncertainty as to whether killer whale ecotypes meet the criteria for subspecies or species designation (Reeves *et al*. 2004). Taxonomic uncertainty presents a practical problem for implementing conservation laws like the U.S. Endangered Species Act of 1973 (ESA), which require the designation of a "species" for protection. The ESA protects intraspecific taxa by expanding the definition of species to include "any subspecies of fish or wildlife of plants" and "any distinct population segment (*i.e*., DPS) of any species of vertebrate fish or wildlife, which interbreeds when mature" (U.S. Federal Register 1996). ESA listings of intraspecific taxa, like southern resident killer whales, have become increasingly contentious (Haig *et al*. 2006). After biological and legal reviews, the southern residents were listed as a distinct population segment (DPS) of the North Pacific residents, an unnamed subspecies of *Orcinus orca* (Krahn *et al*.

2004). Legal challenges to this listing have focused on whether southern residents constitute a DPS and on taxonomic issues, and petitioners have cited a lack of quantitative analysis of morphological differences between the ecotypes (Ford 2013).

Globally, quantitative studies of the morphological differences among killer whale ecotypes have been limited (Baird and Stacey 1988, Visser and Mäkeläinen 2000, Mäkeläinen *et al.* 2014). These studies have utilized the frequency of occurrence of broad pigmentation patterns to examine differences between killer whale populations. Other authors have qualitatively described the differences in dorsal fin shape and variation in the shape of the saddle patch among resident, Bigg’s, and offshore killer whales in the northeastern Pacific Ocean (Evans *et al.* 1982, Baird 2000, Ford *et al.* 2000, Dahlheim *et al.* 2008). Zerbini *et al.* (2007) demonstrated that these differences were sufficient for experienced observers to correctly assign unknown whales in photographs to ecotype. However, the qualitative nature and subjectivity of these accounts make it difficult to rely on them as definitive ecotype descriptions (de Bruyn *et al.* 2013).

One of the challenges to studying cetacean morphology is access to specimens. Photo-identification studies provide extensive documentation of the morphological variation within and among killer whale ecotypes, but methods for quantifying the extent of these differences or the variation within an ecotype have not been readily available. Morphometric studies of two-dimensional shapes have typically used the geometric configuration of landmarks, or points along the form that are biologically and structurally homologous between individuals, to quantify variation (Bookstein 1986). Aside from the tip of the dorsal fin, killer whale dorsal fins and pigmentation patterns do not have identifiable homologous features. An alternate approach is Fourier-based analysis, which provides a description of shape without reference to structural landmarks (Bonhomme *et al*. 2014). One such approach is elliptical Fourier analysis (EFA), in which complex shapes can be decomposed stepwise using a harmonic series of ellipses generated along the shape's outline without requiring structural landmarks.

Elliptical Fourier analysis (Kuhl and Giardina 1982) has previously been used to comprehensively depict and quantify shapes of highly complex objects: violins (Chitwood 2014), watershed boundaries (Bonhomme *et al*. 2013), otoliths (Tracey *et al*. 2006, Keating *et al.* 2014), octocoral sclerites (Carlo *et al*. 2011), caudal skeletons in birds (Felice and O'Connor 2014), human teeth (Ferrario *et al*. 1999), human body shape (Courtiol *et al*. 2010), and leaves (Neto *et al*. 2006). These studies have shown that these shape differences can provide tools for stock identification (Tracey *et al*. 2006), species identification (Neto *et al*. 2006, Carlo *et al*. 2011) and understanding evolutionary processes (Felice and O'Connor 2014).

Shape refers to the boundary outline independent from its orientation, relation to reference planes, or size (Lestrel 1989), and it can be quantified by mathematical methods such as the Fourier series (Ferrario *et al*. 1999). The EFA method consists of decomposing the outline of an object into a sum of harmonically related ellipses (harmonics) of increasing order (Lestrel 1989). When pooled, many harmonics can be utilized to describe complex shapes (Felice and O'Connor 2014). EFA was chosen because it does not require structurally defined landmarks, and virtually any closed outline can be fitted. The coefficients can be estimated independently of outline position and normalized for size and orientation. Lastly, EFA results in quantification of the geometry of the outlines that can be analyzed with classical multivariate tools, including principal component analysis, linear discriminant analysis, and multivariate analysis of variance (Bonhomme *et al*. 2014).

Here we describe and examine a method based on elliptical Fourier analysis to quantify shape differences of the dorsal fins and pigmentation patterns among resident, Bigg's, and offshore killer whales in the northeastern Pacific Ocean using standard and easily obtainable photo-identification data. Our primary aim in this study was to assess shape variation in dorsal fins and pigmentation patterns within and among these three killer whale ecotypes to determine to what extent analysis of shape variation among cataloged individuals can correctly identify an individual's ecotype. This effort represents important information for evaluating morphological differences between killer whale ecotypes, and provides quantitative support for the morphological differences between the ecotypes described in previous studies.

METHODS

Photograph Selection

Identification photographs of individuals from all three ecotypes were collected from multiple locations in three regions of the northeastern Pacific Ocean: western Alaska, southeastern Alaska, and Washington State. For the resident ecotype, the photographs were also obtained from multiple resident communities (northern residents, southern residents, southeast Alaska residents, and western Alaska residents), and for the Bigg's ecotype both west coast and Gulf of Alaska communities were included. These photographs were taken both opportunistically and as part of dedicated photo-identification studies over a 20 yr period. Prior to our study, all photographs had been matched to catalogs of previously photo-identified individuals of known ecotype (Dahlheim *et al*. 1997; Ford *et al*. 2000; Ellis *et al*. 2011; Towers *et al*. 2012, 2015). In the case of western Alaska, where whales are encountered less frequently, individuals were included only if their ecotype had been confirmed by genetic analysis or association with individuals of known ecotype (Zerbini *et al*. 2007). Since the goal of our study was to quantify the observed morphological variation of the ecotypes as they are currently defined, we did not reclassify any individuals prior to our analysis.

Dorsal fin shape and the pigmentation patterns of the saddle and eye patches (Fig. 1) show individual variation and are used for identification of individual killer whales (Bigg *et al*. 1987, Visser and Mäkeläinen 2000). In the Southern Ocean and the North Atlantic Ocean, the orientation and relative size of the eye patch differs among killer whale populations (Pitman and Ensor 2003, Pitman *et al*. 2010, Mäkeläinen *et al*. 2014). Typical identification photographs are of the dorsal fin and saddle patch. Eye patches are especially useful in identifying young whales, since saddle patch pigmentation develops during the first year of life. But as whales age it becomes more difficult to photograph eye patches consistently. Photographs were quality graded based on the clarity, angle, distance, and proportion of the dorsal fin, saddle patch, or eye patch visible. Images were required to be in sharp focus, well lit, and show the feature of interest in full to be included in the analyses. Only photographs where the whale was parallel to the photographic plane were used. Additionally, photographs were not included if there were any obstructions, like glare or water spots, that would alter the shape of the feature of interest. Age and sex classes were determined from long term photo-identification studies of known individuals and the rate of development of the dorsal fin (Olesiuk *et al.* 1990). Only adults were included in the analyses of dorsal fins, to avoid the confounding effect of growth on overall shape. All age classes, except young of the year, were included in the analyses of pigmentation patterns, since once developed they remain unchanged for the duration of a whale’s life (Bigg 1982).

Image Processing

Prior to this study, all images were archived digitally. Eye patches, dorsal fins, and saddle patches were analyzed separately and therefore processed separately. The entire eye patch and saddle patch were used to define the shape. The shape of the dorsal fin required defining the base as the breakpoint of the bodyline and dorsal fin. The breakpoint was determined by tracing a line along the body fore and aft of the dorsal fin. The point at which the dorsal fin deviated vertically from this line was used to define the base. Using the program ImageJ (National Institutes of Health 2015), images were cropped so the shape of interest was centered and filled the frame. If needed, brightness and contrast levels were adjusted and then all images were converted to grayscale 8-bit images, resulting in a black shape on a white background. The paintbrush tool was used to remove any artifacts like glare spots or water spray from the background or center of the shape, while taking care not to alter the shape (Fig. 2).

Analysis

For each analysis, images were passed into Momocs (Bonhomme *et al*. 2014), a package developed to analyze the closed outlines of shapes in R v. 3.3.1 (R Core Team 2017) by linking the shape descriptors of each outline to a Fourier series. Outlines of each shape were extracted as a series of *x*-, *y*-coordinates in Momocs using the algorithm presented in Rohlf (1990) and implemented in R by Claude (2008) (Fig. 2). These coordinates are used to estimate four coefficients for each harmonic ellipse that describe its size, shape, and orientation. All normalization, Fourier transformation, visualization of the resulting harmonic coefficients, and statistical analyses were performed in Momocs v. 0.2 (Bonhomme 2015). Each set of images was normalized *a priori* by scaling, centering, and aligning outlines to the first fitted ellipse of the shape. In advance of elliptical Fourier analysis, homologous pseudolandmarks generated along the outline were defined during a Procrustes alignment of the outlines using a multiple-point superimposition (Friess and Baylac 2003).

Equations to Estimate the Coefficients of Harmonic Ellipses

To estimate the harmonic coefficients for each shape, the *x*- and *y*-coordinates of each closed outline were projected onto a plane. This outline was then approximated by the partial sum of a periodic function with period *T* (equivalent to the outline's perimeter) sampled *N* times, which is given by, for *x*:

$$x(t) = \frac{a_0}{2} + \sum_{n=1}^{N} a_n \cos(\omega n t) + b_n \sin(\omega n t)$$

where $\omega = 2\pi/T$ (the frequency of the function), and is the *x*-coordinate of the centroid of the outline, with

$$a_n = \frac{2}{T} + \int_o^T x(t)\cos(\omega n t)\,dt \text{ and } b_n = \frac{2}{T} + \int_0^T x(t)\sin(\omega n t)\,dt.$$

For *y*:

$$y(t)=\frac{c_0}{2}+\sum_{n=1}^{N}c_n\cos(\omega nt)+d_n\sin(\omega nt)$$

where is the *y*-coordinate of the centroid of the outline, with

$$c_n=\frac{2}{T}+\int_0^T y(t)\cos(\omega nt)\,dt \quad \text{and} \quad d_n=\frac{2}{T}+\int_0^T y(t)\sin(\omega nt)\,dt.$$

The harmonic coefficients were estimated from points (pseudolandmarks) sampled along the outline. Four coefficients were estimated for each harmonic ellipse, two for *x* and two for *y*, which describe its size, shape, and orientation. The coefficients were normalized to remove the influences of size, location, and rotation, which allowed them to be aligned to the first fitted ellipse:

$$\begin{pmatrix} A_n & B_n \\ C_n & D_n \end{pmatrix}=\frac{1}{\lambda}\begin{pmatrix} \cos\psi & \sin\psi \\ -\sin\psi & \cos\psi \end{pmatrix}\begin{pmatrix} a_n & b_n \\ c_n & d_n \end{pmatrix}\begin{pmatrix} \cos n\theta & -\sin n\theta \\ \sin n\theta & \cos n\theta \end{pmatrix}$$

where *λ* is the scale (size of semimajor axis) of the first harmonic ellipse, *ψ* its rotation angle, and *θ* the rotation of the starting point to the end of the ellipse. These parameters were estimated from (Ferson *et al.* 1985):

$$\lambda=\sqrt{a_1\cos\psi+b_1\sin\psi+c_1\cos\psi+d_1\sin\psi}$$

$$\psi = 0.5 \arctan\left(\frac{2(a_1 b_1 + c_1 d_1}{a_1^2 + c_1^2 - b_1^2 - d_1^2}\right)$$

$$\theta = \arctan\left(c^* / a^*\right)$$

Outline Processing

In addition, outlines were smoothed and the number of smoothing iterations needed was assessed qualitatively. A total of 1,000 smoothing iterations were used for dorsal fins to minimize the effects of digitization and nicks on the overall shape. For both saddle patches and eye patches, ten smoothing iterations were used to minimize the effects of digitization. Outlines were Fourier transformed resulting in four coefficients (Elliptical Fourier Descriptors) for each harmonic, two for *x*-coordinates and two for *y*-coordinates (Felice and O'Connor 2014). For each shape, the spectrum of harmonic Fourier power was used to estimate the number of harmonics statistically sufficient for analysis. Harmonic power is proportional to the harmonic amplitude and is a measure of the shape information in an outline (Bonhomme *et al.* 2014). The number required to retain 99.9% of the cumulative harmonic power in reconstructing the outline (Fig. 3) determined the number of harmonics retained: 14 for female dorsal fins, 16 for male dorsal fins, 16 for saddle patches, and 19 for eye patches.

Multivariate Analyses

Principal component analysis (PCA) of Euclidean distances between shape coordinates was performed, and shape variation along the principal component axes was visualized using the *PCcontrib* function in Momocs. Principal component (PC) scores were evaluated using both multivariate analysis of variance (MANOVA) and pairwise MANOVA with Bonferroni correction, using ecotype (and sex, in the case of dorsal fins) as the grouping factor. The PC scores for each shape were used as the dependent variables in the MANOVAs. The PCA indicated that the first ten axes combined explained 99% of the total variance, and these ten axes were retained for the MANOVAs. For both female and male dorsal fins, the first two axes explained more than 75% of the variation in morphology (Fig. 4), and 65% for saddle and eye patches (Fig. 5). The shape of the scree plots demonstrated that the geometric structures of these shapes, as reflected by the correlation structure of the coordinate data, were relatively simple for dorsal fins and eye patches but often more complex for saddle patches.

Linear discriminant analysis (LDA) was also applied to investigate how well EFA could discriminate ecotypes with each shape. Leave-one-out cross-validation was used as a means of evaluating the fit of the modeled estimates to the data. This method uses one part of the data set (all but one point) to fit the model and then compares the prediction at the point left out to the actual value, providing a measure of how well the model will generalize to independent data. Pairwise comparisons were also done to further investigate the differences between the ecotypes.

Killer whales exhibit pronounced sexual dimorphism, with males having a greater ratio of dorsal fin height to base length than females (Clark and Odell 1999), indicating that female and male dorsal fins may require separate analyses. Previous studies of killer whale pigmentation patterns have combined both sexes when looking for ecotype differences (Baird and Stacey 1988, Mäkeläinen *et al*. 2013), and Mäkeläinen *et al*. (2014) found no effect of sex on eye patch size. The same analyses were performed to test for sex differences in dorsal fins, saddle patches, and eye patches, and determine whether it was appropriate to analyze them separately.

RESULTS

High quality photographs of dorsal fins were available for 335 females and 176 males. Saddle patch photographs were available for 207 individuals, and 82 photographs were available for eye patches (Table 1).

As predicted, there was a significant difference in dorsal fin shape between males and females, but this was not the case for saddle and eye patches (Table 2). Male and female dorsal fins were differentiated with high accuracy, with leave-one-out cross-validation indicating 99.2% and 90.6% correct classification for females and males, respectively. For these reasons, all analyses of dorsal fin shape were completed separately for males and females, while sexes were combined for saddle and eye patch analyses.

There were highly significant ($P < 0.01$) differences in the shapes of the dorsal fin, saddle patch, and eye patch among the ecotypes (Table 3). Pairwise comparisons between the resident and offshore ecotypes were also significantly different for each shape ($P < 0.0033$) except for saddle patches, while the differences between the Bigg's ecotype and both residents and offshores were significant for all shapes ($P < 0.0033$).

Visualization of the shape variation along the primary PC axes was informative for the relatively simple shapes of the dorsal fin (Fig. 6) and eye patch (Fig. 7), but less so for the more complex saddle patch shape. Female fin variation appears to be driven first by how falcate or “hooked” the fin is and secondly the ratio of height to width. This ratio is also the primary driver of male fin variation, followed by the angle of the fin's trailing edge. Height to width was also the primary driver of eye patch variation, followed by the direction that the eye patch narrows: vertically or horizontally. The degree to which a saddle patch was smooth, notched, or hooked (Baird and Stacey 1988) was a component of both the first and second PC axes. However, these two PC axes also incorporated thickness of the saddle patch and whether it extended forward or ventrally.

There was substantial variation along the principal components within each ecotype and among the ecotypes for all three shapes (Fig. 8, 9). Linear discriminant analysis and leave-one-out cross-validation of dorsal fin morphology indicated 70.1% correct classification for females and 73.9% for males among ecotypes (Table 4). Eye patches showed a similar level of variation among ecotypes, with 78% correct classification. The ability to discriminate ecotypes with saddle patches was more limited, with only 58.5% correct classification (Table 4). This result reflected the substantial variation observed in saddle patch shape within the ecotypes. Residents had the highest correct classification rate for each shape except saddle patches, while offshores were incorrectly classified most often.

DISCUSSION

Killer whales exhibit individual variation in the shape of their dorsal fins, saddle patches, and eye patches that have been used to identify individuals and qualitatively describe morphological differences among ecotypes. This study demonstrates that this variation can be quantified using EFA and provides evidence that the morphological variation observed in killer whales in the northeastern Pacific Ocean is related to ecotype, indicating phenotypic differentiation is consistent with differences in habitat, behavior, diet, and inferred levels of gene flow between the ecotypes.

The ability of EFA to discriminate ecotypes based on dorsal fin and eye patch shapes was substantial, but this was not the case for saddle patches. This may in part be due to the inability to reliably discriminate offshores from residents because of the diversity of saddle patch patterns observed in these ecotypes. Baird and Stacey (1988) described five different saddle patch patterns among residents and Bigg’s in British Columbia, Alaska and Washington State: vertical notch, horizontal notch, smooth, hook, and bump. All five patterns were seen in residents, but only two were seen in Bigg’s killer whales (bump and smooth). Similarly, residents, and to a lesser extent offshores, exhibited substantial variation across the first two PC axes, while Bigg’s killer whales fell into two distinct clusters (Fig. 9).

The inability to correctly differentiate residents and offshores may have been influenced by a smaller sample size for offshores, since they incorrectly classified most often (Table 4). Pairwise comparisons of the ecotypes indicate otherwise. Pairwise comparisons and leave-one-out cross-validation of the offshore, resident, or combination of the offshore and resident ecotypes with the Bigg's ecotype resulted in higher rates of correct classification across all shapes. For example, when the two fish-eating ecotypes (resident and offshore) combined were compared with Bigg's killer whales, correct classification rates increased from 58.5% to 68.6% for saddle patches, 70.1% to 84.8% for female dorsal fins, 73.9% to 86.4% for male dorsal fins, and 78% to 86.6% for eye patches. Given that residents and offshores are more closely related to each other than either are to Bigg's killer whales (Morin *et al.* 2010), these results are not surprising.

The components of dorsal fin shape that describe most of the variation in this analysis mirror the qualitative descriptions of the differences among ecotypes (Ford *et al.* 2000), especially the degree to which a dorsal fin is rounded or falcate. Eye patches in these ecotypes have not been described previously, but shape components were similar to categories used to describe the eye patches of killer whales in New Zealand (Visser and Mäkeläinen 2000). This indicates that an assessment of the prevalence of these categories within each ecotype is warranted, similar to that done with saddle patch patterns by Baird and Stacey (1988). The agreement of these analyses with prior descriptions and qualitative studies of killer whale ecotypes further demonstrate that EFA is an effective tool for quantifying variation in cetacean morphology.

The biological importance and degree of inheritance in the variation of these shapes is not clear. Morphological variation can result from environmental factors and from evolutionary processes such as natural selection and genetic drift. Caro *et al*. (2012) suggests that striking coloration, like the eye patch, is associated with diet but the mechanism is unclear. These markings may also play a role in group cohesion and individual recognition (Caro *et al*. 2011). Mäkeläinen *et al*. (2013) demonstrated that the saddle patches of resident killer whales are the most asymmetric, followed by offshores then Bigg’s killer whales, and suggested that genetic factors like small population size and reproductive isolation were most likely responsible.

Whether these ecotypes constitute a single species or multiple species or subspecies has not been completely resolved, but our results are consistent with the previous inference of reduced gene flow between the ecotypes (Morin *et* al. 2010, Parsons *et al.* 2013, Pilot *et al.* 2010). This work also fills a need for a rigorous quantitative analysis of the morphological differences among these ecotypes.

Access to cetacean specimens for morphological studies is limited, but photo-identification is a commonly used method in field studies. Our study demonstrates that these identification photographs are adequate for investigating and quantifying morphological variation within and among cetacean populations using EFA. The method is relatively quick and inexpensive, especially with advances in digital photography. By introducing EFA, this study adds another tool to the suite of methods used for evaluating population variation and understanding ecological and evolutionary processes in living cetaceans.

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Figure 1. Examples of female dorsal fin, saddle patch, and eye patch in killer whales.

Figure 2. Process of converting each identification photo first to an 8-bit grayscale image and then to a closed outline in the R package Momocs.

Figure 3. Process of reconstructing dorsal fin shape using harmonic ellipses (left) and the cumulative power for each harmonic in approximating the closed outline (right). In this case, three harmonics are required to achieve 95% power; between 10 and 20 harmonics are required to achieve 99.9% power.

Figure 4. Distributions of scores for each ecotype on the first five principal components for shapes of female dorsal fins (left) and male dorsal fins (right). For female dorsal fins, the variance explained by each principal component was the following: PC1-59.4%, PC2-23.5%, PC3-6.4%, PC4-3.3%, PC5-2.0%, and for male dorsal fins: PC1-45.0%, PC2-30.6%, PC3-7.6%, PC4-5.2%, PC5-3.5%.

Figure 5. Distributions of scores for each ecotype on the first five principal components for shapes of saddle patches

(left) and eye patches (right). For saddle patches, the variance explained by each principal component was the following: PC1-43.8%, PC2-19.5%, PC3-10.6%, PC4-8.6%, PC5-4.1%, and for eye patches: PC1-49.8%, PC2-18.0%, PC3-10.5%, PC4-5.2%, PC5-3.5%.

Figure 6. Standardized shape variation of female dorsal fins (left) and male dorsal fins (right) along the first four principal component axes, explaining 95.7% and 94.4% of the variance in shape. See text for more discussion.

Figure 7. Standardized shape variation of eye patches along the first four principal component axes, explaining 89.6% of the variance in shape. See text for more discussion.

Figure 8. Principal component analysis (PCA) plots of variation within and among ecotypes for female (left) and male (right) dorsal fin shape. Ellipses represent 95% of the data. Red = offshore, blue = resident, and green = Bigg’s killer whale.

Figure 9. Principal component analysis (PCA) plots of variation within and among ecotypes for saddle patch shape (left) and eye patch shape (right). Ellipses represent 95% of the data. Red = offshore, blue = resident, and green = Bigg’s killer whale.

[1] Corresponding author (e-mail: candice.emmons@noaa.gov).

Table 1. Images included in the Elliptical Fourier Analysis.

Shape	Ecotype	No. images[a]
Female dorsal fin	Offshore	84 (37 SEAK, 36 WAK, 11 WA)
Female dorsal fin	Resident	157 (112 WAK, 45 WA)
Female dorsal fin	Bigg’s	94 (41 WAK, 53 WA)
Male dorsal fin	Offshore	32 (12 SEAK, 17 WAK, 3 WA)
Male dorsal fin	Resident	112 (90 WAK, 22 WA)
Male dorsal fin	Bigg’s	32 (17 WAK, 15 WA)
Saddle patch (male and female)	Offshore	43 (11 SEAK, 22 WAK, 10 WA)
Saddle patch (male and female)	Resident	93 (1 SEAK, 43 WAK, 49 WA)
Saddle patch (male and female)	Bigg’s	71 (3 SEAK, 8 WAK, 60 WA)
Eye patch (male and female)	Offshore	10 (1 SEAK, 9 WA)
Eye patch (male and female)	Resident	42 (12 WAK, 30 WA)
Eye patch (male and female)	Bigg’s	30 (1 WAK, 29 WA)

[a] SEAK = Southeast Alaska, WAK = Western Alaska, WA = Washington State.

Table 2. MANOVA results for sexual dimorphism in shapes of dorsal fins, saddle patches, and eye patches.

Shape	Grouping	Degrees of freedom	Hotelling-Lawley T^2	Approximate *F* statistic	*P*	Significance
All dorsal fins	Gender	1, 343	3.9392	100.3	2.2^{-16}	$P < 0.001$
All saddle patches	Gender	1, 205	0.12902	1.079	0.373	$P > 0.05$[a]
All eye patches	Gender	1, 54	0.98239	1.7192	0.078	$P > 0.05$[a]

[a] Not significant.

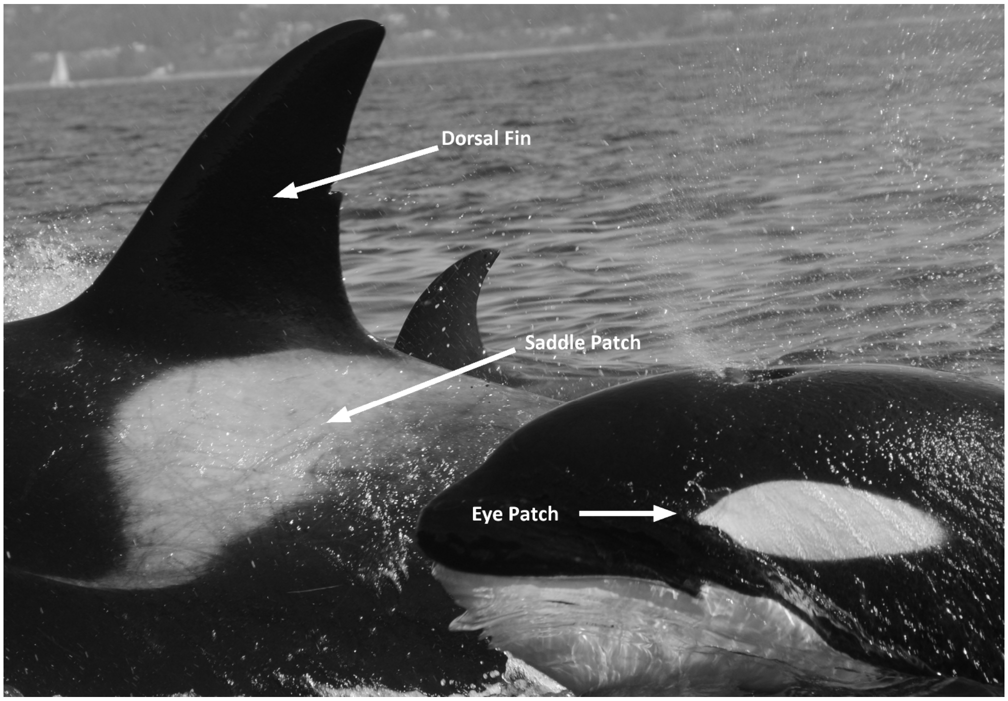
Table 3. MANOVA results for variation among ecotypes in shapes of dorsal fins, saddle patches, and eye patches.

Data set	Grouping	Degrees of freedom	Hotelling-Lawley T^2	Approximate F statistic	P	Significance
Female dorsal fins	Ecotype	2, 332	2.1658	26.572	2.2^{-16}	$P < 0.001$
Male dorsal fins	Ecotype	2, 93	1.4069	6.9024	2.2^{-16}	$P < 0.001$
All saddle patches	Ecotype	2, 126	0.5839	2.4154	4.8^{-6}	$P < 0.001$
All eye patches	Ecotype	2, 60	3.1636	4.8892	5.34^{-13}	$P < 0.001$

Table 4. Leave-one-out cross-validation results evaluating ecotypic variation for all shapes.

Actual ecotype	Predicted			% correct
	Offshore	Resident	Transient	
Offshore female fins	44	31	9	52
Resident female fins	20	129	8	82
Bigg’s female fins	13	19	62	66
Offshore male fins	20	10	2	62
Resident male fins	11	94	7	84
Bigg’s male fins	0	16	16	50
Offshore saddle patches	2	24	17	5
Resident saddle patches	2	65	25	71
Bigg’s saddle patches	0	18	54	75
Offshore eye patches	4	4	2	40
Resident eye patches	2	37	3	88

Bigg’s eye patches	2	5	23	77



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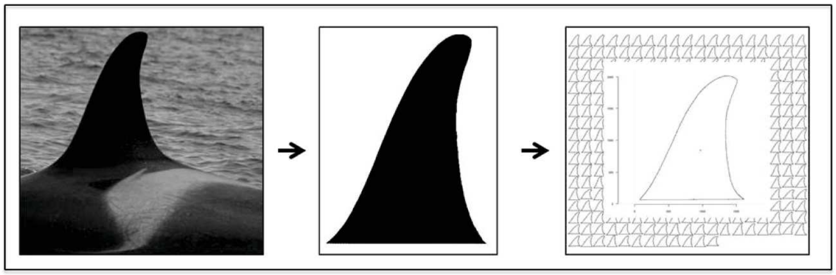

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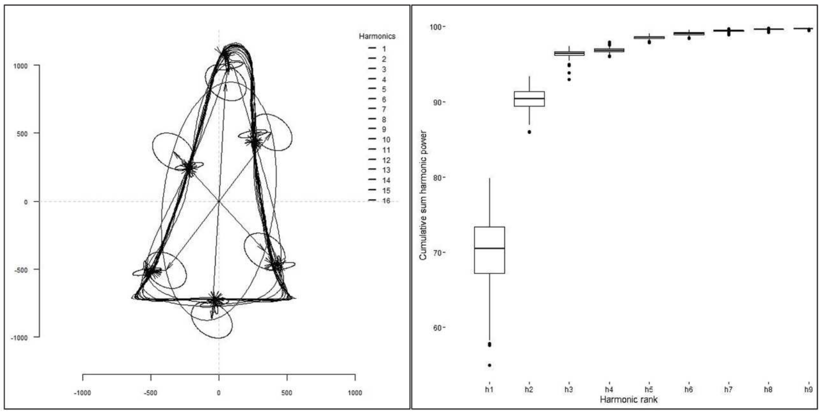

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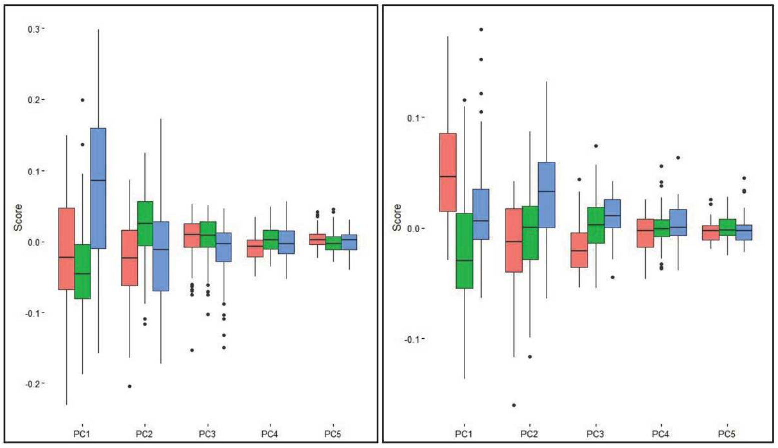

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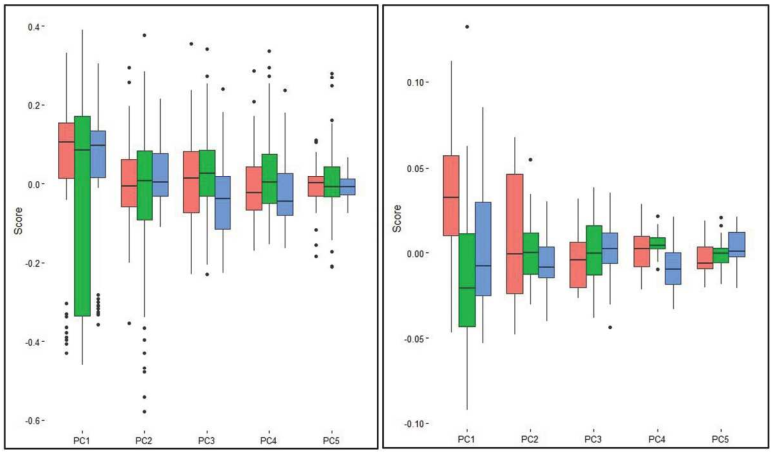

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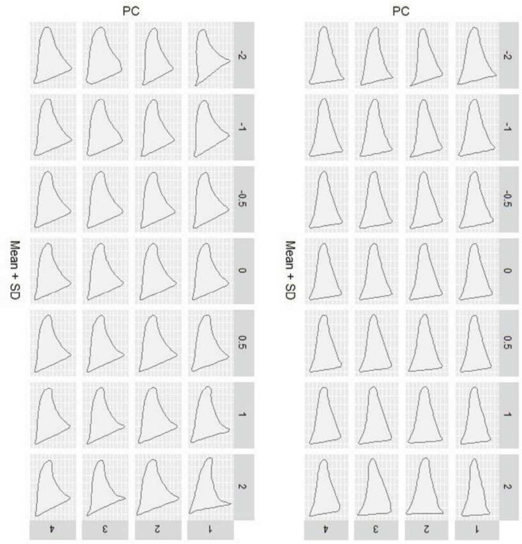

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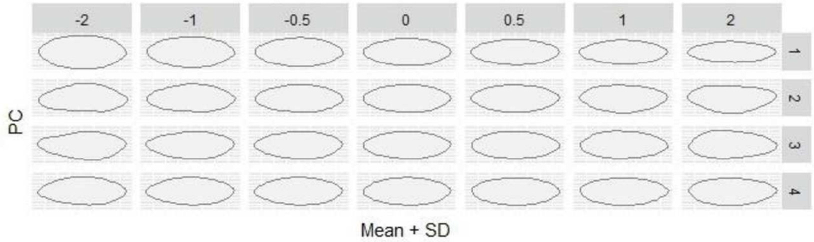

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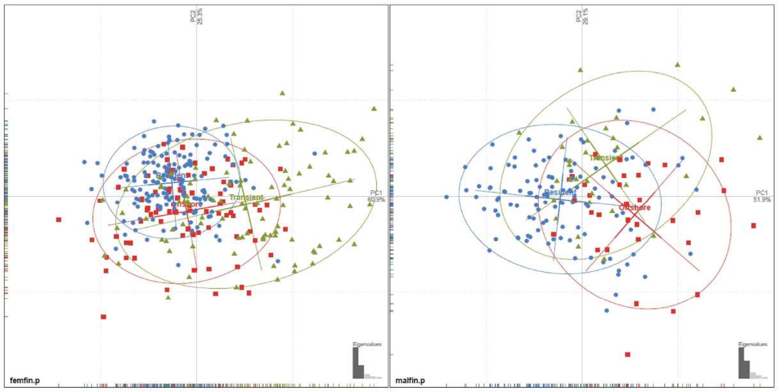

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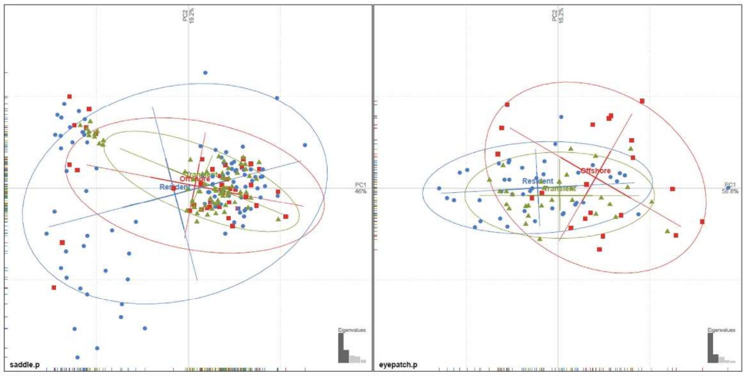

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