Status review update of Southern Resident killer whales

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We are much more interested in conserving actual morphological, ecological and genetic diversity than in structuring conservation around a nebulous taxonomic level about which, in the past, there has been so much disagreement – Mallet 1995

Acknowledgements

Introduction

On August 2, 2012, the National Marine Fisheries Service received a petition submitted by the Pacific Legal Foundation on behalf of the Center for Environmental Science Accuracy and Reliability, Empresas DelBosque, and Coburn Ranch to delist the endangered Southern Resident killer whale (SRKW) distinct population segment (DPS) under the Endangered Species Act (ESA). On November 28, 2012, NMFS published a 90-day finding (77 FR 70773) that the petition presented substantial scientific information indicating that the petitioned action may be warranted and that NMFS would initiate a status review. The petition focused specifically on issues of taxonomy and whether the SRKW constituted a DPS, and NMFS therefore determined that the status review would also focus on these issues rather than on the extinction risk status of the SRKW more broadly.

On March 21, 2013, after a public comment period on the 90-day finding, the NMFS Northwest Region requested that the Northwest Fisheries Science Center conduct a scientific review and evaluation of the petition, the key scientific papers cited in the petition, the biological information received from the public, and any other best available relevant information. Specifically, the Northwest Region requested the Center to consider if there is new best available information that would lead to different conclusions from those of the 2004 BRT (Krahn *et al.* 2004) regarding the existence of a North Pacific resident killer whale taxon (species or subspecies) or the discreteness or significance of the SRKW with reference to this taxon. This report is intended to address the Northwest Region's request.

Summary of taxonomic issues addressed by the 2004 BRT

In evaluating the status of the southern resident killer whales (SRKW), the previous NMFS biological review teams (BRTs) had to explicitly address the issue of the uncertain taxonomy of the killer whale. These issues are discussed extensively in the BRT reports (Krahn *et al.* 2004; Krahn *et al.* 2002) and in the report of the NMFS Workshop on Cetacean Taxonomy (Reeves *et al.* 2004). Briefly, at the time of the first SRKW status review (Krahn *et al.* 2002), the most recently published taxonomy of killer whales placed them in a single polytypic species, *Orcinus orca*, as described by Linnaeus in 1758 (Heyning *et al.* 1988; Rice 1998). However, the 2002 BRT report stated that killer whale taxonomy was uncertain and that several authors had recently proposed new *Orcinus* species on the basis of morphological variation and potential reproductive isolation among ecologically distinct populations of killer whales in Antarctica (Berzin *et al.* 1983; Mikhalev *et al.* 1981) and the North Pacific (Baird 2000). Even general reviews of *O. orca* taxonomy, while ultimately concluding that *O. orca* should probably be considered a single species, also emphasized the uncertain taxonomy. For example, Heyning and Dahlheim (1988, p.

5, emphasis added) noted that "The genus *Orcinus currently* is considered monotypic by most authorities with geographic variation noted in size and color pattern, but *a worldwide systematic review is needed*" and "Until more substantial data are presented, a *conservative* view of recognizing only one highly variable species *probably* is warranted."

Faced with this taxonomic uncertainty, the 2002 BRT evaluated a wide variety of potential taxonomic scenarios and considered the DPS status of SRKW within hypothesized taxa (see Table 8 in Krahn *et al.* 2002). Ultimately, the BRT remained uncertain about both the global taxonomy of killer whales and whether or not the SRKW met the criteria to be considered a DPS, and faced with this uncertainty NMFS concluded that listing the SRKW under the ESA was not warranted. The agency noted the taxonomic uncertainty described by the BRT, and as a result indicated it would reassess its decision after a reconsideration of killer whale taxonomy (NMFS 2002).

Subsequent to the 2002 "not warranted" finding, in 2004 NMFS initiated another status review in response to a finding by a U.S. District Court that in using a possibly outdated taxonomy, NMFS failed to make use of the best data available. In addition to initiating a new status review, NMFS also held a cetacean taxonomy workshop that, in part, reviewed and summarized information relating to the uncertainties surrounding killer whale taxonomy (Reeves *et al.* 2004). Based on the findings of the workshop and new genetic data analyzed after the 2002 status review, the 2004 BRT concluded that the North Pacific resident killer whales satisfied Reeve's *et al.* (2004) criteria for being a subspecies (Krahn *et al.* 2004, p. 41). Specifically, the BRT cited studies noting differences between the resident and transient ecotypes in external morphology, reproductive isolation in sympatry, foraging behavior and diet; acoustic dialects and vocal behavior, and mtDNA and nuclear genetic characteristics (see Krahn *et al.* 2002; 2004). The 2004 BRT further concluded that the SRKW population met the USFWS & NMFS (1996) criteria for being a DPS of the North Pacific resident subspecies, citing differences between the SRKW and other resident populations in ecological setting, range, genetic variation, and behavioral and cultural traits (Krahn *et al.* 2004). The BRT emphasized, however, that there was some scientific uncertainty related to both the taxonomic and DPS conclusions.

Summary of the substantive points made in the petition

After a brief summary of killer whale natural history, the petition notes that there are varying scientific opinions regarding the definition of species, and that the definitions of sub-species and other intraspecific terms such as Distinct Population Segments (DPS) are subject to even greater uncertainty and scientific debate. The petition notes that splitting taxa ever more finely does not necessarily result in conservation benefits and may result in a false perception of risk.

The petition then briefly summarizes the current *Orcinus* taxonomy, followed by a more extensive summary of the Workshop on Cetacean Taxonomy convened by

NMFS in 2004 (Reeves *et al.* 2004). After summarizing some of the conclusions in the workshop report, the petition concludes that the workshop participants were unable to identify additional species within the currently recognized species *O. orca*. The petition further indicates that in the petitioners' opinion NMFS contradicted the workshop's recommendations when it concluded that the North Pacific fish-eating ('resident') killer whales are a subspecies of *O. orca*, and that the southern resident population is a DPS of this subspecies.

The petition follows with considerable discussion questioning whether the ESA allows for identification of DPS within subspecies, a legal question beyond the scope of this biological review.

Finally, the petition reviews some published studies related to the question of whether the North Pacific resident killer whales meet the criteria for subspecies designation, focusing on the lack of a Latin trinomial name for the proposed subspecies, and the genetic, morphological or ecological evidence as it relates to the question of subspecies status. The review focuses considerable attention (nearly six pages) on a recent genetic study by Pilot *et al.* (2010), arguing that the study provides clear evidence that the putative North Pacific resident killer whale subspecies is not genetically isolated from other killer whale populations. The petition concludes by reviewing some of the morphological, behavioral, and ecological differences among the North Pacific killer whale ecotypes, arguing that these are likely to be largely learned behaviors and therefore not important to consider when identifying subspecies or conservation units. See the Appendix for a detailed review of the biological arguments made in the petition.

Summary of public comments

The public comment period on the 90-day finding closed on January 28, 2013. The Northwest Region received over 2,750 comments. Despite the request for specific scientific and commercial information, the vast majority of commenters simply noted their opposition to the petition to delist SRKWs, while a handful of comments supported the petition. The Northwest Region did, however, receive several substantive comments regarding both the biological and legal aspects of the DPS determination as raised in the petition. The substantive points raised in the comments are briefly summarized in Table 1.

Table 1 -- Summary of Substantive Public Comments Received on Pacific Legal Foundation (PLF) 2012 Petition to Delist Southern Resident Killer Whale (SRKW) Distinct Population Segment (DPS).

Organization/ Commenter	Summary of comments
Marine Mammal Commission (MMC)	• Disagrees that the petition may be warranted; recommends reversing 90-day finding and devoting resources to higher priorities • Listing SRKW as a DPS of a subspecies is appropriate, using 2nd prong of Chevron analysis (*Chevron USA v. Natural Resources Defense Council*) as applied to the definition of a DPS • Recommends that consistent with NMFS precedent and applicable case law, NMFS interpret ESA definition of "species" to include DPSs of both species and subspecies • Research has identified multiple, geographically distinct populations of killer whales that have unique behavioral and ecological traits • MMC believes PLF's arguments related to Pilot *et al.* 2010 are incorrect and inconsistent; references several new papers on genetics and speciation • Pilot *et al.* 2010 findings are not sufficient to refute treatment of North Pacific residents as a putative subspecies or the designation of SRKWs as a DPS • Pilot *et al.* 2010 does not provide conclusive evidence of recent mating between SRKWs and other resident populations or between resident killer whales and any other regional ecotype; used unusually liberal criteria to assign parentage based on genetic data • Parsons *et al.* in review found that "estimates of genetic distance between two predominant North Pacific ecotypes [resident and transient] indicate negligible levels of gene flow."
Humane Society of the United States	• Opposes further consideration of the petition as it does not present substantial scientific information that the listing is no longer warranted; population is appropriately listed as endangered • Disagrees with the petitioners that SRKWs are an unlistable entity under the ESA • Basing conclusion that the population is not a subspecies on limited male-mediated gene flow between populations from Pilot *et al.* 2010 ignores more recent work by Ford *et al.* 2011 that detected no gene flow among populations
Center for Biological Diversity	• Petition fails to present substantial information that SRKWs are not a DPS; does not comport with ESA's plain language, ignores NMFS policy, and disregards scientific record that indicates significant speciation of the global taxon • ESA allows NMFS to designate a DPS of a subspecies; if ESA were ambiguous, NMFS' DPS policy allows designation of a subspecies and deserves deference; case law cited by petitioners does not support their claim • Data and information support speciation for North Pacific and SRKW populations such as genetic data; morphological data, including body size; behavioral variation including vocalization, food preference, and social organization
Animal Legal Defense Fund (ALDF)	• Opposes delisting petition on legal as well as scientific bases • Petition mischaracterizes Pilot *et al.* 2010 and Morin *et al.* 2010 and took conclusions out of context • Petitioners legal argument is inconsistent with case law and statutory interpretation

	• ALDF counters the three primary assumptions in the petition – (1) ESA does not require formal taxonomic recognition, (2) Pilot *et al.* 2010 does not contradict a subspecies designation, (3) Morin *et al.* 2010 unequivocally urges a subspecies designation • ALDF also organized a comment campaign, we received hundreds of individual comments opposing the delisting
Rus Hoelzel	• Clarifies Pilot *et al.* 2010 conclusions • Does not believe a subspecies must be defined before designating a DPS; see examples in Fallon *et al.* 2007 using genetic markers to designate DPSs where a subspecies has not been designated • Notes that gene flow is allowed when determining discreteness • Notes the petition does not address significance • Supports current DPS listing
The Whale Museum	• Opposes delisting petition; supports 2004 status review and listing • Pilot *et al.* 2010 do not reference cross ecotype mating involving SRKWs; Barrett-Lennard *et al.* 2000 supports reproductive isolation too • SRKW DPS is both discrete and significant
Orca Conservancy	• Opposes delisting petition • MMPA does not provide adequate protection for SRKWs; ESA allows protection from indirect threats, requires section 7 consultations and permits, allows more citizen oversight and recourse • Morin *et al.* 2010 is more reliable than Pilot *et al.* 2010 because it relies on more base pairs and more microsatellites, which contradict conclusion of interbreeding in modern times
Northwest Environmental Defense Center	• Petition is inconsistent with science, court decisions on the prior listing, and the ESA. • Economic concerns listed in the petition cannot be considered and would not be resolved even with delisting • NMFS is within its statutory authority to list SRKW DPS • Current science supports and requires the continued protection of SRKW DPS – pinnipeds can tell residents apart from transients based on acoustics; SRKWs are a demographically closed population; best available science has not changed much since 2005 • MMPA protections alone are insufficient to protect and recover – procedural issues (jeopardy and adverse mod), takings, and legal tools in ESA
Miami Seaquarium	• Agrees with petitioner that SRKW DPS is not a listable entity; ESA does not authorize listing a DPS of a subspecies; North Pacific subspecies itself is a "nonexistent and scientifically unjustifiable" listing unit • "Taxonomic inflation" is occurring – unjustified elevation of subspecies to species and populations to subspecies or DPSs • 2005 listing of SRKW DPS as endangered resulted in collateral issues including impacts on CA farmers and whether to include Lolita in the listing. Notes that PLF filed its petition to delist SRKW DPS "long before" PETA/ALDF filed their petition to add Lolita to the SRKW DPS. NMFS should carefully and promptly consider the PLF petition, which if granted would negate the need to consider these collateral issues.
Animal Welfare Institute, CBD, Center for Whale Research, EarthJustice,	• Petition is based on a narrow and incorrect construction of ESA and the best scientific and commercial data available; incorrect legal arguments and one-sided interpretation of science; do not, and cannot, address or demonstrate that status has improved or threats have been reduced • ESA defines "species" broadly; authorizes listing a DPS of a subspecies -

Friends of the Earth, Friends of the San Juans, International Marine Mammal Project of Earth Island Institute, Marine Mammal Connection Society, NRDC, Oceana, Orca Network, Dr. David Bain, Will Anderson, Dr. Samuel Wasser	Congress did not intend DPSs to be constrained by taxonomy; designating DPSs of subspecies is consistent with longstanding agency interpretations • PLF arguments lack merit; the justification included does not support those arguments • Focus on genetics and interbreeding is misplaced as genetic data is not the sole evidence for determining "markedly separate" populations • Significant scientific evidence supports designation of SRKW population as a DPS – physical separation from other KW populations; morphological data, including body size, supports speciation of NP and SRKW populations; and behavioral variation, including vocalization, food preference, and social organization meet DPS criteria • SRKWs meet the ESA listing criteria – EarthJustice provides a five factor analysis
Whale and Dolphin Conservation	• Opposes petition; threats continue and delisting is not appropriate • Notes the ESA definition of "species" and NMFS' interpretation unambiguously refute PLF's legal argument as has been the case with their recent attempts to challenge other ESA listings • Notes that the DPS policy does not prohibit listing if occasional gene flow occurs beyond the listed population; Pilot *et al.*'s main conclusion from their data emphasized social cohesion of killer whales to produce genetic differences between populations despite capacity for dispersal outside their groups.
Change.org – Bruce Gorcyzcki	• J, K, and L pods don't associate or interbreed with other ecotypes in the North Pacific • SRKWs have been determined as a discrete population with their own social groupings, dialect and behaviors • SRKWs' absence from the ecosystem would upset the balance
Individual – Ruth Muzzin	• Petition should be denied as it does not present new information, such as population numbers, and does not demonstrate that the DPS has recovered or become extinct; none of the delisting criteria are met • NMFS has listed a DPS of a subspecies previously – e.g., ringed seals, bearded seals, and Atlantic sturgeon
Individual – David Bain	• Describes characteristics of "newer" and "older" species in an evolutionary sense with respect to reproductive isolation, morphology (dorsal fin and jaw sizes), and geographic isolation • Transients are older species and distinct in all ways species are expected to differ • Residents and offshore have reached a plateau, but additional differentiation would be expected over evolutionary time, though reproductive isolation is occurring; overlap in color patters and range; SRKWs appear the only group of residents to use the CA current system thereby giving them a slightly different ecological niche. • Morin *et al.* 2010 found the evidence of interbreeding in Pilot *et al.* 2010 was an artifact attributable to incomplete DNA sequencing • SRKWs should be considered a subspecies and are eligible for ESA listing regardless of whether a DPS of a subspecies is eligible. Endangered status should be retained.
Individual – Sharon Grace	• Petition is without merit • Commenter references many threats and effects on population abundance and social structure • Notes Pilot *et al.* 2010 examples are not SRKWs; some inbreeding is okay for DPS designation

| Individual – Jodi Smith | • Morin *et al.* 2010 confirms that genes are slow to change over time, making differentiation difficult even though it happens
• In addition to genetic isolation, SRKWs are distinct based on social organization, dietary preference, and behavior. Recent evidence from a review of Southern Hemisphere killer whale populations is likely to conclude distinction as well (de Bruyn *et al.* 2013)
• Delisting SRKWs will not alleviate water restrictions for CA farmers as many other threats exist for CA spawning salmon |

Taxonomic issues, general principles

The petition states that it is motivated in part by a general concern about "taxonomic inflation", or the tendency to increasingly split taxa into smaller subunits based on minor differences between putative taxa (petition p. 11). The petition notes that this can be a problem even at the species level, but seems particularly concerned with the incorrect identification of subspecies, due in part to a lack of consistent and rigorous subspecies definitions in the scientific literature (petition, p. 11).

The petition is correct in its conclusion that taxonomic uncertainty is a practical and conceptual problem for implementing conservation policy, particularly under laws such as the Endangered Species Act that rely on designation of particular species or intraspecific groups of organisms for special protections. Even the definition of a species is subject to ongoing scientific debate, with dozens of species concepts circulating in the scientific literature and debate about whether species are 'real' entities or simply categories invented for human convenience (Hey *et al.* 2003; Mallet 1995). As the petition notes, subspecies concepts have been subject to less intensive theoretical treatment than have species, but even so there are numerous definitions of subspecies in the scientific literature (reviewed by Haig *et al.* 2006). Other definitions of intraspecific groupings, such as Evolutionarily Significant Units (e.g., Crandall *et al.* 2000; Moritz 1994; Waples 1991), Distinct Population Segments (DPS; USFWS *et al.* 1996), and stocks (Dizon *et al.* 1992; McElhany *et al.* 2000) have also been the subject of considerable scientific debate and controversy (reviewed by Ford 2003; Fraser *et al.* 2001).

The petition focuses considerable attention on the societal costs associated with designating insufficiently discrete taxa, but does not discuss the converse conservation problem of failing to identify discrete taxa when they exist. Failure to identify species, subspecies or other intraspecific varieties when they do in fact exist has clear conservation costs, mostly notably the potential loss of such unique groups through failure to protect them. This problem has been extensively discussed in the scientific literature, and has provided the motivation for several explicit definitions of both subspecies and ESUs (Avise *et al.* 1990; Crandall *et al.* 2000). The potential for outdated or incorrect taxonomy, particularly at the subspecies level, has been a

motivation for more explicit subspecies definitions and suggestions to review outdated taxonomic designations (Haig *et al.* 2006). For example, with regard to designation of cetacean species and subspecies, Reeves *et al.* (2004) noted that

> *There has been a tendency to err in the direction of avoiding designating too many taxa rather than making sure that all potentially recognized taxa have been designated. In other words, the direction of precaution toward stability in traditional taxonomy has not been appropriate for conservation.*

 and

> *Cetacean taxonomy in the latter half of the 20th century was conservative in part as an over-reaction to the excessive splitting that occurred during the 19th century.* (p. 30)

In other words, at least in Reeves *et al.*'s view, the currently accepted cetacean taxonomy tends to err on the side of lumping discrete taxa together rather than splitting them apart. To facilitate accurate designation of new cetacean taxa, particularly at the subspecies level, Reeve's *et al.* recommended the following definition of subspecies:

> *In addition to the use of morphology to define subspecies, the subspecies concept should be understood to embrace groups of organisms that appear to have been on independent evolutionary trajectories (with minor continuing gene flow), as demonstrated by morphological evidence or at least one line of appropriate genetic evidence. Geographical or behavioral differences can complement morphological and genetic evidence for establishing subspecies. As such, subspecies could be geographical forms or incipient species.* (p. 7).

Based on the discussion above, the problem of how to deal with taxonomic uncertainty in applying laws such as the ESA is not a new issue. Neither are concerns about wasting resources or causing economic harm through listing of inappropriately designated taxa. For example, the issue of balancing the competing tensions of conserving genetic resources but doing so when only biologically warranted was a motivating factor in the development of both the NMFS ESU concept (Waples 1991) and the joint USFWS & NMFS DPS policy (USFWS *et al.* 1996). It is beyond the scope of this review to attempt to resolve all of the bigger picture issues surrounding the intersection of taxonomy and conservation status. In developing and applying its policy on DPS, however, NMFS did explicitly consider the need to identify conservation units under the ESA at an appropriate scale.

New information since 2004

In this section we briefly summarize information relevant to both the taxonomic and DPS questions that has been published in the scientific literature since the 2004 status review.

Morphology and color variation

The only published quantitative analysis of variation in pigmentation patterns in North Pacific killer whales remains that of Baird and Stacey (1988), which found significant differences between residents and transients and among resident populations in the frequencies of alternative saddle patch patterns. Several authors (Baird 2000; Dahlheim *et al.* 2008; Ford *et al.* 2000) have also described qualitative differences in morphology among the three Pacific ecotypes. All of these studies except for Dahlheim *et al.* (2008) were considered by the 2004 BRT in their status review report.

While not describing morphological variation *per se*, a study by Zerbini *et al.* (2007) demonstrated that the ecotypes can be unambiguously distinguished based on visual appearance of dorsal fin shape and saddle patch pigmentation. In that study, ecotype determination of unknown groups of whales was made independently by both visual examination of photographs and genetic analysis of the mtDNA control region. In all 32 cases where both photographs and genetic data were available, the ecotype designation based on the photographs matched that based on the mtDNA control region.

Since 2004, there have been multiple studies published on morphological and ecological variation among Antarctic killer whales, confirming and extending the more preliminary information that was available to the 2004 BRT. Pitman and Ensor (2003) describe field observations and descriptions of three distinct types of Antarctic killer whale (designated A, B, and C) differentiated by size, pigmentation, habitat and apparent prey preferences. The C type appeared to correspond to *O. glacialis*, a dwarf form of killer whale previous described by Berzin and Vladimirov (1983) but not generally accepted as a distinct species due to small sample size and lack of a type specimen (Heyning *et al.* 1988). Pitman *et al.* (2007) used aerial photographs to quantify the length distribution of a sample of 221 Type C whales, and confirmed this type as smaller than the Type A whales. Based on historical and contemporary photographs, Pitman *et al.* (2011) described a new "Type D" killer whale characterized by a very small eye patch and somewhat bulbous head and inhabiting the Southern Ocean between 40 and 60 degree south. More recently, Olsen *et al.* (2012) observed groups of east Antarctic killer whales that were intermediate in some morphological characters between types B and C.

Feeding ecology and diet

Since the 2004 BRT report, several additional studies have been published on the diet and feeding ecology of North Pacific killer whales. Herman *et al.* (2005) and Krahn *et al.* (2007) examined variation in organic contaminants and fatty acid composition of blubber biopsy samples and carbon and nitrogen stable isotope ratios in dermal samples from 169 samples (between the two studies), obtained primarily from the Gulf of Alaska and the Aleutian Islands but including some samples from Puget Sound and the U.S. west coast. All three ecotypes were represented, although the number of offshore samples was small (4 in the 2005 study and 9 in the 2007 study). The studies found significant variation among the three ecotypes in fatty acid profiles and contaminant burdens and ratios, likely reflective of different diets and foraging locations (Figure 1). Nitrogen stable isotope ratios also differed significantly between transients and residents, with transients having more enriched ^{15}N levels consistent with a marine mammal diet. Offshores had nitrogen ratios that were between residents and transients, and not significantly different from either. Alaskan residents sampled from different areas also varied considerably in both nitrogen and carbon stable isotope profiles, presumably reflecting differences in foraging location and/or prey types.

Ford and Ellis (2006) and Hanson *et al.* (2010) conducted field observations of resident killer whale predation combined with genetic analysis of prey remains and field collected fecal samples to evaluate resident killer whale diets in the Salish Sea. Both studies observed predation of only fish, and analysis of prey remains and fecal DNA indicated a summer diet dominated by Chinook salmon (*Oncorhynchus tshawytscha*). Dahlheim and White (2010) describe foraging behavior and prey preferences for Alaskan transient killer whales. Killer whale diet information, including considerable unpublished data, was further reviewed by an independent science panel in 2012 (Hilborn *et al.* 2012; NMFS 2013).

In the Antarctic, Pitman and Durban (2012) described a field study of foraging behavior of Type B killer whales, documenting predation of primarily Weddell seals (*Leptonychotes weddellii*) using a cooperative hunting behavior that involved washing the seals off of ice flows. Olsen *et al.* (2012) described Type A and B killer whales in a common feeding aggregation. Foote *et al.* (2009) describe variation in stable isotope ratios and tooth wear potentially indicative of two killer whale foraging types in the North Atlantic.
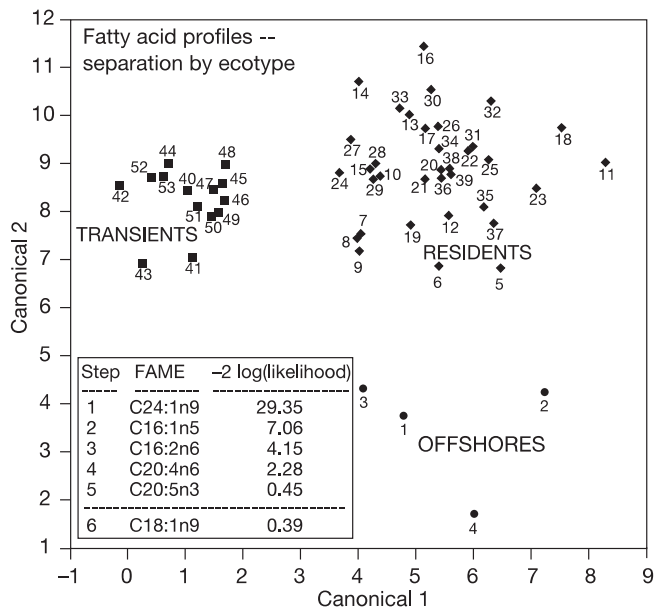
Figure 1 -- Orcinus orca. First 2 discriminant functions showing separation of killer whale ecotypes based on fatty acid profiles of the blubber biopsies. Reproduced from Herman *et al.* (2005).

Deecke *et al.* (2005) found significant differences in the acoustic behavior of transients and residents during foraging events, with transients calling significantly less frequently than residents. The difference appears to be related to hearing abilities of their preferred prey; marine mammals have excellent hearing in the frequency range of the killer whale calls while fish do not. These results are consistent with earlier work (Deecke *et al.* 2002) demonstrating that harbor seals displayed predator avoidance behavior during playback experiments using transient calls but not during experiments using resident calls. Deecke *et al.* (2011) and Beck *et al.* (2011) found differences in group size and acoustic behavior between seal-eating and fish-eating killer whales in the North Atlantic. Based on the phylogenetic relationships between the Atlantic and Pacific populations (Foote *et al.* 2011b; Morin *et al.* 2010), Beck *et al.* (2011) concluded that such foraging specialization and associated behaviors must have arisen independently in both oceans and be fairly plastic traits. Dahlheim *et al.* (2008) describe foraging behavior of offshore killer whales including highly worn teeth suggesting feeding on abrasive prey such as sharks. Ford *et al.* (2011a) collected prey samples from offshore killer whales and identified the prey as Pacific sleeper sharks (*Somniosus pacificus*). On a research cruise off the Oregon and Washington coasts in spring of 2013, an offshore whale was observed foraging on Chinook salmon (NWFWC unpublished data).

Genetics

The genetic information available at the time of the 2004 status review consisted of several studies focusing on variation in the mtDNA control region and at multiple

nuclear microsatellite loci (see tables 1 and 2 of Krahn *et al.* 2004). Two studies, both in the form of preliminary reports, were cited as being particularly influential due to their large sample sizes: a global study of 211 killer whales analyzed at 17 microsatellite loci (Hoelzel 2004), and a similar study of 219 whales sequenced at the mtDNA control region (LeDuc *et al.* 2004). Both studies produced a somewhat inconclusive picture of population structure, as summarized by Krahn *et al.* (2004, p. 15-16):

> *The understanding of killer whale population genetic structure has expanded considerably since the 2002 status review. In particular, the mtDNA differentiation among eastern North Pacific resident, transient, and offshore populations can now be seen in the context of variation worldwide. The most notable result from the new mtDNA data is the lack of strong mtDNA structure worldwide, suggesting that the current distribution of killer whales populations may be relatively young on an evolutionary scale (e.g., several hundred thousand years compared to the ≈5 million year old age of the Orcinus genus [Waples and Clapham 2004]) and possibly associated with a population bottleneck followed by a worldwide expansion. With respect to identifying conservation units, one of the implications of the new data is that the relative degree of mtDNA divergence among populations is not necessarily a good predictor of the length of time that the populations have evolved independently. For example, killer whales with the same haplotype as in Southern Residents have also been found in Alaska, Russia, Newfoundland, and the United Kingdom (Figure 2). Evolutionarily, these whales with the southern resident haplotype are almost certainly more closely related to other geographically proximate populations than to each other (a hypothesis supported by the microsatellite data, Table 3) and therefore, share a mtDNA haplotype purely by chance. Because of this finding, it would be inappropriate to rely heavily on simple mtDNA divergence as a criterion for identifying conservation units, especially on a global scale. On a local scale, however, mtDNA clearly remains useful for helping to identify populations, especially when combined with other types of information.*

> *In addition to more mtDNA data, the amount of nuclear microsatellite data has expanded greatly in the last 2 years, both in terms of whales and loci analyzed. Within the eastern North Pacific, both the mtDNA and microsatellite data remain consistent with a hypothesis of four to five resident populations, at least two to three transient populations and at least one offshore population (Figure 1). The issue of whether any contemporary gene flow occurs among eastern North Pacific populations remains unresolved, but the microsatellite data are consistent with either low levels of gene flow (at most a few mating events among populations per generation) or divergence times of at least several hundred to several thousand years (M. Ford 2004, Hoelzel 2004). Despite some uncertainty about the evolutionary history that produced the current patterns of variation, both the mtDNA and the microsatellite data indicate a high degree*

> *of contemporary reproductive isolation among eastern North Pacific killer whale populations.*

As we discuss below, our understanding of global killer whale population structure has improved considerably since 2004, although some uncertainties remain.

We identified 10 studies of the genetic population structure of killer whales that have been published since the 2004 status review (Table 2). Three of these – Hoelzel *et al.* (2007), LeDuc *et al.* (2008), and Pilot *et al.* (2010) – are expanded and published versions of the preliminary reports considered by the 2004 BRT (Hoelzel 2004, LeDuc and Taylor 2004).

Hoelzel *et al.* (2007) analyzed 203 killer whales sampled from the North Pacific (including samples of the resident, transient and offshore ecotypes) and Iceland at 16 microsatellite loci and the mtDNA control region (~1000 bp). Similar to preliminary results reported to the 2004 BRT (Hoelzel 2004), they found significant differentiation among all groups of samples but estimated that rates of gene flow among most groups, including between ecotypes, was significantly greater than zero. Among North Pacific resident groups, they found that genetic differentiation at microsatellite loci was proportional to geographic distance between the groups. The most geographically distant resident groups had similar levels of genetic divergence to that between the residents and the transients. Using genetic assignment tests, they identified 5 putative migrant individuals, but none between residents and transients. In fitting a model of divergence with migration, they estimated low but non-zero (< 1 migrant/generation) rates of gene flow between residents and transients, and between the Alaskan resident and Icelandic groups. From the same type of analysis, they estimated that the divergence time between residents and transients was 4000 – 36,000 years ago, depending on mutation rate assumptions, and hypothesized that most if not all of the population structure observed evolved after the most recent glacial maximum.

Using the same data, Pilot *et al.* (2010) expanded upon Hoelzel *et al.*'s (2007) results by conducting a parentage analysis within and among populations in order to directly estimate contemporary gene flow. The study also extended the assignment test analyses of Hoelzel *et al.* (2010) using two additional methods. Out of 213 samples, they found a total of 3 putative first generation migrants (individuals sampled from a population but with a genetic profile more similar to a different population), and 8 putative second generation migrants (individuals inferred to be the offspring of a first generation migrant). Of these 11 putative migrants, 8 were within the same ecotype (exchanges between California and Alaska transients, or between Alaskan and Russian residents), 2 were between transients and the Icelandic group (both second generation), and 1 was between transients and offshores (second generation). Using a model fitting approach, rates of gene flow between residents and transients and from the offshores into residents and transients were estimated to be <1% per generation. Rates of gene flow from both residents and transients into the offshore group were estimated to 2.2 – 3.6%. Gene

flow rates between resident populations were estimated to be 0.5% - 2.4%, except for the rates between Russian and Bering Sea groups and between Bering Sea and Alaskan groups which were much higher (14% - 28%).

Pilot et al's parentage analysis identified at least one parent for 95 individuals, but more than half these (57) were rejected by the authors as spurious. The remaining parentage assignments suggested low dispersal (42/43 maternal assignments were to a mother within the offspring's population) and very high male-mediated gene flow (10/22 paternal assignments were to a male not in the offspring's population). No parentage assignments were made between members of different ecotypes. The authors suggested that the discrepancy between the low rates of intra-ecotype gene flow estimated by using assignment tests and model-fitting and the high rates estimated from parentage analysis could be explained by a recent range expansion leading to increasing contact among formally isolated populations. Another possible explanation, suggested by the large number of assignments rejected as spurious, is that the parentage analysis may not have had sufficient power to exclude all false paternity assignments.

Ford *et al.* (2011b) conducted a similar parentage and assignment test analysis, but focused the parentage analysis exclusively on the southern resident population and did not attempt to identify potential parents outside of this population. The authors did test for the presence of first generation immigrants into the SRKW population, however, and found no evidence of recent gene flow into the SRKW population.

Another significant development in our understanding of global killer whale population structure has resulted from sequencing full ~16,390 bp mitochondrial genomes from a large number of individuals (Morin *et al.* 2010). Sequencing the full mitogenome has increased the number mtDNA base pairs examined by over 16 fold compared to the earlier studies that focused exclusively on the ~1000 bp control region. This increase in sequence evaluated has greatly improved the resolution of the estimated mtDNA gene trees, and significantly altered our understanding of killer whale population structure, particularly as it relates to the degree of divergence among some of the known ecotypes.

Morin *et al.* (2010) sequenced and analyzed full mitochondrial genomes from 139 killer whales sampled primarily from the North Pacific, North Atlantic, and Antarctic areas, with a smaller number of additional samples from the tropical Pacific. In contrast to earlier results based on only the control region, the phylogenetic tree constructed from the full length mitogenome sequences showed strong genetic structure associated with many of the previously identified ecotypes (Figure 2). In particular, the North Pacific residents, North Pacific transients, North Pacific offshores, and Antarctic type B and type C groups each formed distinct monophyletic clades. The North Pacific transients were particularly divergent from most other killer whale groups, including the sympatric residents and offshores. For example, there were 57 fixed sequence differences between the transients and the residents and offshores. The estimated time to the most recent common ancestor of

all of the mtDNA haplotypes was ~700,000 years, and the divergence time between the haplotypes characterizing the residents and those characterizing the offshores was 177,000 years ago. Haplotypes characterizing the Antarctic B and C types were estimated to share a common ancestor 155,000 years ago. The Antarctic B and C types were also each found to have a sequence substitution inferred to be due to natural selection (Foote *et al.* 2011a). Based on the clear genetic divergence among ecotypes, combined with divergence at microsatellite loci and previously reported morphological and ecological differences, Morin *et al.* (2010) concluded that the North Pacific transients and Antarctic B and C types each met criteria for being considered full species, and the other known ecotypes (North Pacific residents, offshores, North Atlantic populations, and the Antarctic A type) each met criteria for being considered distinct subspecies, but could be elevated to species with if additional data supported evolutionary distinctiveness.

Utilizing the same dataset of mitogenome sequences, Foote *et al.* (2011b) conducted additional analyses on the relationship between North Pacific and North Atlantic populations. Based on the structure of the mitogenome tree, they suggested that over the past ~300,000 years there have been several episodes of migration of whales between the Pacific and Atlantic oceans. The timing and pattern of these inferred episodes further suggested that the Pacific resident and transient ecotypes may have initially diverged in allopatry (transients in Pacific, residents in Atlantic), and then subsequently came into contact following a migration event of residents back into the Pacific. Using the same isolation-divergence model used by Hoelzel *et al.* (2007), Foote *et al.* (2011b) also found non-zero but extremely low rates of bi-directional female gene flow between the Atlantic and Pacific (< 1 migrant / 150,000 years).

Foote *et al.* (Foote *et al.* 2009; Foote *et al.* 2011c)conducted analyses focused on understanding killer whale population structure within the North Atlantic, and found evidence for two ecological types (fish eating/mammal eating) similar to what has been observed in the Pacific and Antarctic. Genetically, the fish eating whales from Norway and Iceland formed a genetically distinct grouping based on both mtDNA control region (1000bp) sequences and microsatellite variation. Other groups of populations, particularly from Gibraltar and the Canary Islands, also clearly formed discrete populations based on the microsatellite variation, but clustered with other groups (Pacific offshores, Antarctic type A) in the mtDNA tree.

Parsons *et al.* (2013) conducted a study of population structure of a large (462) sample of resident and transient killer whales from the Gulf of Alaska, the Aleutian Islands and the Sea of Okhotsk. The focus of the study was primarily on elucidating population structure within each ecotype, but the study is also the largest study to date (in terms of whales and loci) of nuclear genetic variation between the resident and transient ecotypes. Using two different assignment methods, all samples with sufficient data (n> 20 loci) assigned unambiguously to their known ecotype. When individuals with greater levels of missing data were included, a single individual (missing data at 15/27 loci) assigned to the 'incorrect' ecotype at a low level of

confidence (0.54). These results, combined with the lack of any shared mtDNA haplotypes, led the authors to conclude that there is at most negligible gene flow between the two ecotypes.

Study[1]	Geographic focus	Number of samples	Type of data
Hoelzel *et al.* (2007), Pilot *et al.* (2010)	North Pacific plus Iceland	203	Microsatellites (16), mtDNA control region (~1000 bp)
LeDuc *et al.* (2008)	Antarctic (with comparison to published data in Pacific and Atlantic)	80	mtDNA control region (~1000 bp)
Foote *et al.* (2009)	North Atlantic	125	mtDNA control region (partial)
Morin *et al.* (2010), Foote *et al.* (2011b), Foote *et al.* (2011a)	North Pacific, North Atlantic, Antarctic, some tropical	143	mtDNA full genome (~16,390 bp)
Foote *et al.* (2011c)	North Atlantic (with comparison to published data in Pacific and Antarctic)	85	mtDNA control region and full genomes; microsatellites (17)
Ford *et al.* (2011b)	Southern Residents, North Pacific	78	Microsatellites (26)
Parsons *et al.* (2013)	North Pacific	462	mtDNA control region (~1000 bp); microsatellites (27)

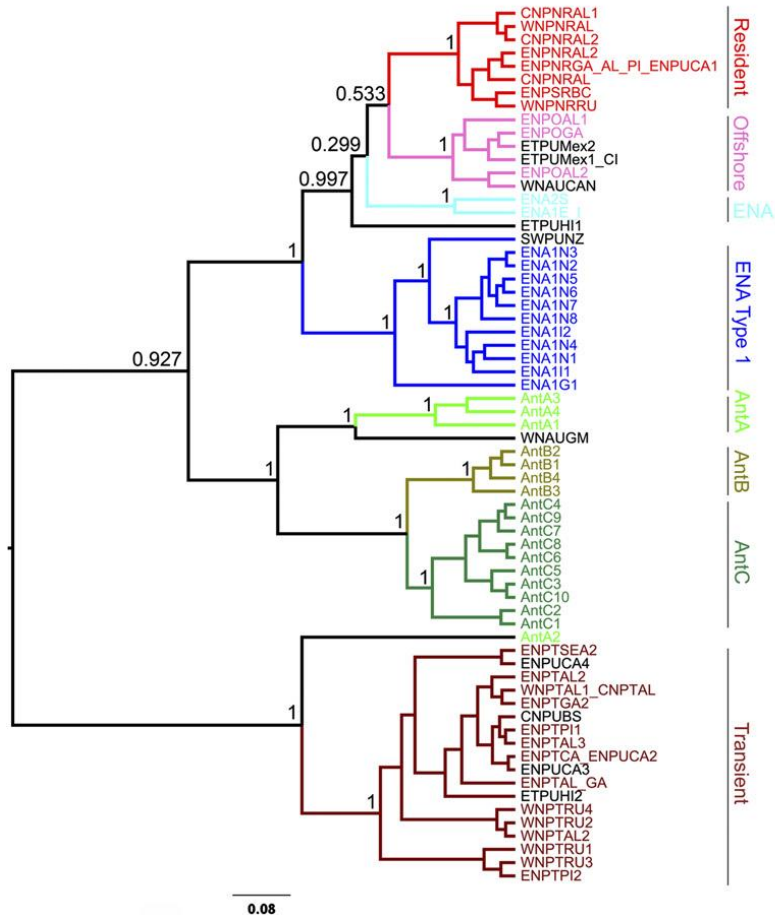[1]Separate papers based on largely the same data are grouped.

Figure 2 -- Whole mitochondrial genome phylogeny of 66 unique killer whale haplotypes. Posterior probabilities are indicated for nodes of interest. Whales of known type are indicated in color, and those of unknown type are in black type. Reproduced from Morin *et al.* (2010).

Summary, genetics

Our understanding of killer whale population structure has improved considerably since 2004, due both to analysis of new samples, larger numbers of nuclear loci, and the collection of full mitogenome data. At least at the high latitude areas examined, the full mitogenome trees are much more geographically and ecotypically structured than was true of the control region trees available in 2004. The genetic studies published since 2004 also clearly support earlier suggestions of differentiation between some of the Antarctic ecotypes.

Despite the greater resolution provided by the mitogenome data compared to that of only the control region sequences, the total depth of the mitochondrial phylogeny within *O. orcus* remains relatively shallow compared to the levels of divergence typically observed between mammalian sister species. For example, Johns and Avise (1998), Avise *et al.* (1998) and Baker and Bradley (2006) have reviewed divergence at the mitochondrial cytochrome-b gene for a large number of mammal

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sister species, and levels of divergence are typically >5%, although some are much lower. The divergence between resident and transient killer whales is ~0.4% (based on sequences from Morin *et al.* (2010)), suggesting that if the ecotypes are species they are relatively young species. The relatively shallow divergence could also be consistent with incipient speciation (Riesch *et al.* 2012), or with subspecies (Reeves *et al.* 2004).

Evaluating variation at multiple nuclear genes is also important for gaining a full understanding of population structure, both to reduce the stochastic noise associated with inference at a single locus such as mtDNA and to ensure that population processes mediated by male gene flow are evaluated.

Studies of nuclear variation published since 2004 have provided results consistent with what was available to the 2004 BRT, albeit with considerable improvements in terms of numbers of samples and loci analyzed. In 2013, as in 2004, all published studies of killer whale population structure that use nuclear loci have utilized microsatellites, although the number of loci has increased from 17 (Hoelzel 2004) to 27 in the most recent study (Parsons *et al.* 2013). The studies that have most directly attempted to estimate rates of gene flow among populations using nuclear loci (Hoelzel *et al.* 2007, Pilot *et al.* 2010), estimate no contemporary gene flow between the North Pacific residents and either transients or offshores, and at most very little contemporary gene flow between transients and offshores. The most sophisticated estimates of historical gene flow (the Ima2-based estimates from Hoelzel *et al.* 2007) are all <1 migrant/generation among the Pacific ecotypes. The largest available study of microsatellite variation among North Pacific killer whales (Parsons *et al.* 2013) also found no evidence for contemporary gene flow between residents and transients. Estimates of rates of gene flow within the North Pacific resident populations vary somewhat, but most analyses indicate little gene flow, particularly into or out of the more southern populations. All of these results continue to strongly support the 2004 BRT's conclusion that there is a "… high degree of contemporary reproductive isolation among eastern North Pacific killer whale populations" (Krahn *et al.* 2004, p. 16).

Our understanding of killer whale population structure outside of the North Pacific has also progressed considerably since 2004. Studies of variation among killer whale groups in the Antarctic (Leduc *et al.* 2008; Morin *et al.* 2010) in particular have confirmed the presence of distinct groups that correspond to the ecological/morphological groups previously identified (Pitman *et al.* 2003; Pitman *et al.* 2007). Population structure in the Atlantic is also starting to be elucidated (Foote *et al.* 2009), as is the relationship between the North Atlantic and North Pacific (Foote *et al.* 2011b).

Despite this considerable progress, it is also clear that a full understanding of killer whale structure at a global scale remains incomplete. There have been no published genetic studies focusing on samples from tropical areas (although Morin *et al.* 2010 included some tropical samples), and large portions of the killer whale's range,

including the coasts of South America, Africa, Australia, and eastern North America, remain essentially unanalyzed.

In addition to the lack of sampling in some areas, the issue of the evolutionary age of the Pacific ecotypes and of killer whale populations worldwide remains somewhat uncertain and subject to varying estimates. Based on the low levels of mtDNA control region divergence, Hoelzel *et al.* (2002) hypothesized that killer whales globally experienced a population bottleneck 145,000 to 210,000 years ago. By fitting population genetic models to microsatellite and mtDNA control region data, Hoelzel *et al.* (2007) estimated the divergence time between the Pacific ecotypes at 20,000-30,000 years ago. In contrast, Morin *et al.* (2010) estimated the time to the most recent common ancestor of the killer whale mitogenomes that characterize the ecotypes to be 170,000 to 700,000 years ago, implying a much deeper divergence time than had been estimated previously. However, Hoelzel *et al.* (2007) estimated divergence times between populations, whereas Morin *et al.* (2010) estimated divergence time among gene sequences and these estimates are not expected to be the same (topic reviewed by Edwards *et al.* 2000). Hoelzel *et al.*'s estimate was based on a much smaller mtDNA segment than the Morin *et al.* estimate, but Hoelzel's estimate also included information from nuclear loci. In addition, all of these estimates are sensitive to the estimated or assumed mutation rate, which differed between the studies. It is therefore not immediately obvious which of these estimates is more reflective of the true evolutionary age of the ecotypes, or even that these estimates are necessarily inconsistent with each other. Additional nuclear sequence data is likely to improve the precision of the estimated divergence times.

Review papers

Riesch *et al.* (2012) and Foote (2012) recently reviewed evidence for ongoing ecological speciation among killer whale ecotypes. Riesch *et al.* focus particularly on the role that cultural factors might play in promoting ecological divergence and reproductive isolation. Both reviews conclude that most if not all of the behavioral, ecological and perhaps even some of the morphological (e.g., size) differences between the North Pacific ecotypes are likely to be non-heritable, culturally transmitted traits. Riesch *et al.* concluded that the reproductive and social isolation observed among ecotypes is largely culturally based, and there is no evidence for either pre or post-zygotic reproductive incompatibility. Ultimately, Riesch *et al.* concluded that there is not sufficient evidence to conclude that the ecotypes are currently separate species or subspecies, but rather that "We could be witnessing the early stages of an adaptive radiation of killer whales, whereby a variety of incipient species are beginning to exploit diverse ecological niches, or conversely, we could be looking at an old and continuing process by which new ecotypes periodically form and become extinct again." Foote (2012) evaluates much of the same information, and concludes that it is very hard to prove conclusively from field data alone that the specific process of ecological speciation (Schluter 2001; Schluter 2009) is occurring in killer whales or any "non-model" organism. Foote suggests

that genome scans, by identifying specific functional genes subject to natural selection, might be a fruitful way to evaluate the causes of divergence in such systems. de Bruyn *et al.* (2013) recently reviewed information on Southern hemisphere killer whales both in Antarctica and in temperate latitudes and concluded that there is relatively little information on the social structure and ecology of killer whales in this region and that firm designation of ecotypes outside of the North Pacific may be premature.

Summary and Conclusions

Determination of the Taxon

Based on several lines of evidence, including differences in morphology, behavior, diet and feeding ecology, acoustical dialects and practices, and both mtDNA and nuclear DNA variation, the 2004 BRT concluded (with some uncertainty) that the North Pacific resident killer whales were a subspecies of *O. orca* distinct from the sympatric transient whales (Krahn *et al.* 2004, p. 40-41). With somewhat less confidence, the BRT also concluded that the North Pacific resident subspecies consisted of only the North Pacific residents, and did not include killer whales of the offshore ecotype or fish-eating killer whales from elsewhere in the world.

After reviewing information in the petition, the public comments, and the scientific literature published in the nine years since the 2004 status review, we found no new information that would likely lead to a different conclusion from that of the 2004 BRT. In particular, all of the new genetic data and analyses published since 2004 (Table 2), including the Pilot *et al.* (2010) paper discussed extensively by the petition, are either consistent with or strengthen the 2004 BRT's conclusion that there is a high degree of contemporary reproductive isolation among the North Pacific killer whale ecotypes. No genetic analysis published since the 2004 status review has indicated a higher level of interbreeding among the ecotypes than was indicated by the analyses considered by the 2004 BRT.

In addition to new genetic analyses, the studies on feeding ecology and diet published since 2004 are also generally consistent with or strengthen the 2004 BRT's conclusions that the ecotypes differ in diet and feeding ecology. The one new study that touches indirectly on morphological differences between the ecotypes (Zerbini *et al.* 2007) supports the 2004 BRT's conclusion (based on earlier literature) that the ecotypes can be morphologically differentiated. No new information on acoustics or behavior contradicts the conclusions of the 2004 BRT. Recent observations (NWFSC unpublished data) indicate that offshores consume at least some Chinook salmon, but stable isotope and tooth wear data also indicate substantial dietary differences. The petition discusses numerous questions regarding the morphological, behavioral and ecological data cited by the 2004 BRT,

but does not raise issues not already discussed by the BRT or the 2004 Taxonomic workshop.

A broader scientific consensus regarding whether the North Pacific ecotypes are a subspecies of *O. orca* remains mixed, as was the case at the time of the 2004 BRT (Krahn *et al.* 2004; Reeves *et al.* 2004). Some experts have suggested that the ecotypes clearly meet criteria for subspecies or species designation (Morin *et al.* 2010), and at least one scientific society (the Society for Marine Mammalogy) now formally recognizes North Pacific residents and transients as subspecies (Committee on Taxonomy 2012). Other experts are less certain that either species or subspecies status is currently appropriate, based on some estimates of non-zero male mediated gene flow among ecotypes (Hoelzel public comments; de Bruyn *et al.* 2013; Riesch *et al.* 2012). Some of this lack of consensus appears to be related to differing conceptions of subspecies definitions rather than substantial disagreement about the biological differences characterizing the ecotypes.

Although the 2004 BRT concluded that the North Pacific resident killer whales meet the criteria for being a subspecies, the BRT expressed some uncertainty about whether to also include Pacific offshores, tropical Pacific killer whales, and by extension perhaps also Atlantic fish-eating killer whales in this subspecies as well (Krahn *et al.* 2004, pp. 40-41). The data available since 2004 tend to strengthen the BRT's conclusion that the North Pacific resident killer whales are taxonomically distinct from the sympatric offshores and allopatric populations of killer whales in the tropics and Atlantic. In particular, Morin *et al.* (2010) found that the North Pacific residents form a monophyletic mtDNA clade distinct from offshores, Atlantic whales and the limited number of Pacific tropical whales included in the study (Figure 1). Estimated rates of gene flow between residents and Atlantic populations differ greatly between studies, but generally suggest that such gene flow is occurring on evolutionary rather than ecological time scales. The fact that the three Pacific ecotypes retain their genetic and ecological distinctiveness when in sympatry also strongly suggests they are currently on divergent evolutionary trajectories. Nonetheless, as was the case in 2004 clearly demarcating the phylogenetic boundaries of the resident taxon remains somewhat uncertain and the rationale for taxonomically distinguishing the residents from the offshores and from fish eating whales in the Atlantic appears somewhat less compelling than taxonomically distinguishing transients from other North Pacific killer whales.

Taken together, however, the best available information clearly strengthens the lines of evidence cited by the 2004 BRT (Krahn *et al.* 2004) to support the designation of the North Pacific resident and transient killer whales as an unnamed subspecies of *O. orca.*

Determination of the DPS

As of December 31, 2012, the SRKW population consisted of 84 individuals divided into three pods (26 in J, 19 in K, and 39 in L) (Center for Whale Research and NWFSC unpublished data). An additional captive animal originating from the SRKW population and with a genotype consistent with a southern resident origin (Hoelzel *et al.* 2007; Hoelzel pers. com.), "Lolita", has resided at the Miami Seaquarium since her capture in August of 1970 (Hoyt 1981). Lolita's original pod is not known with certainty, but her acoustic calls are typical of L pod (Ford 1987; Candice Emmons, personal communication).

The 2004 BRT concluded that there was strong evidence that the SRKW are discrete as defined by the 1996 DPS policy, citing significant genetic differentiation, separate demographic trajectories, differences in core and summer range, and behavioral differences with other resident populations (Krahn *et al.* 2004, p 44). The BRT was less certain that the SRKW met the DPS policy's criteria for significance, but concluded (by a 2-to-1 margin) that they did, citing differences in ecological setting, range, marked differences in genetic variation, and potential cultural differences.

The new information subsequent to 2004 is consistent with and generally strengthens the conclusion that the SRKW are a discrete population within the North Pacific resident taxon. In particular, recent genetic studies all indicate that SRKW are significantly differentiated from other resident populations. New information on the winter range of SRKW provides for a considerably more complete picture than was available in 2004, and continues to indicate that the SRKW (particularly L and K pods) have a winter and summer range distinct from other resident populations, although it does overlap substantially with the northern resident population. A recent analytical comparison of demographic rates found significant differences in both survival and fecundity rates between the southern resident population and the northern resident population, providing further evidence of demographic discreteness (Ward *et al.* 2013). In short, as in 2004 all the available information clearly indicates that the southern residents are a distinct population.

Compared to 2004, new information related to the significance of the SRKW to the North Pacific resident taxon provides a somewhat more nuanced picture. Each of the factors listed by the 2004 BRT in support of the significance criteria is discussed below with reference to new information.

Ecological setting and range – The 2004 BRT noted that the southern residents appeared to occupy a distinct ecological setting, being the only North Pacific resident population to spend substantial time in the California Current ecosystem and having a diet somewhat different from other resident populations, particularly those in Alaska. The BRT also cited the possibility that the southern residents historically utilized the large runs of salmon to the Sacramento and Columbia River as a major source of prey. With regard to range, the BRT noted that the southern residents were the only resident population to be observed to spend time in Puget

Sound and off the coasts of Washington, Oregon and California and that if they were to go extinct this would result in a significant gap/reduction in the resident's range.

New information since 2004 generally continues to support most of these conclusions, but also challenges some of them. In particular, new information on the coastal distribution of the southern and northern resident populations confirms that the southern residents spend substantial time in coastal areas of Washington, Oregon and California and utilize salmon returns to these areas (NWFSC unpublished data). However, there is also new information indicating that the Northern Resident population may also spend more time off the Washington coast than was previously believed (Riera *et al.* 2011; NWFSC unpublished data), and the known northern range of the southern residents is now Chatham Strait in SE Alaska based on photographs taken in 2007 (John Ford, DFO, pers. com). In addition, diet information on the Alaskan resident populations indicates that some of these populations also consume salmon, although not the Chinook salmon that dominate the southern and northern resident diets (Saulitis *et al.* 2000). Updated diet data from the southern and northern resident populations confirms that these two populations have very similar diets and consume many of the same salmon stocks (Ford *et al.* 2010; Hanson *et al.* 2010). Overall, the southern residents remain unique in occupying the most southern part of the resident's range, and are clearly occupying a somewhat different ecological setting from populations in Alaska and further west around the Pacific Rim. The southern portion of the southern resident's range is also quite distinct from that of the northern resident population, but the southern and northern residents clearly share a similar ecological setting throughout much of their range.

Genetic differentiation – Genetic data available since 2004 confirms or strengthens the conclusions that the southern resident population is genetically differentiated from other resident populations. In particular, there are no new data to change the 2004 BRT's conclusions that the southern resident population differs markedly from other North Pacific resident populations at both nuclear and mitochondrial genes.

Behavioral and cultural diversity – The 2004 BRT noted several instances of known and apparent cultural differentiation among resident killer whale populations, and hypothesized, based on studies in other long-lived mammals, that such diversity could be important for the survival of the North Pacific resident taxon as a whole. Since 2004, several studies have contributed further information to this topic. For example, Ward *et al.* (Ward *et al.* 2013; 2011) found significant differences in survival among the three southern resident pods and between the southern and northern resident populations. These differences are likely related to differences in diet and habitat use, both of which appear to be culturally determined. Riesch *et al.* (2012) and Foote (2012) reviewed cultural differences, particularly acoustic behavior and prey preferences, among killer whale populations and ecotypes, and concluded that such cultural differences may be leading to reproductive isolation and subsequent ecological speciation. On the whole, therefore, the available data

appear consistent with the BRT's conclusion that such cultural differences may be important factors in the overall viability of the resident killer whale taxon.

Overall, new information on genetics and behavioral and culture diversity available since 2004 is consistent with or strengthens the 2004 BRT's conclusion that the southern resident killer whale population meets the significance criteria of the DPS policy. New information on ecological setting and range tends to weaken the 2004 BRT's conclusion somewhat, as it indicates greater overlap in range or diet with other resident and offshore populations than was previously believed. Overall, the new information available since 2004 appears consistent with the 2004 BRT's conclusion that southern resident killer whales are likely to be a DPS of the unnamed North Pacific resident subspecies.

Appendix – Review of specific points made in the petition

Workshop on Cetacean Taxonomy

p. 14 – "No experts in the field of cetacean taxonomy were included to inform the workshop participants." The list of participants is in Appendix 1 of workshop report (Reeves *et al.* 2004). It contains multiple experts on cetacean taxonomy, such John Heyning, Marilyn Dahlheim, William Perrin, and James Mead. In the paragraph preceding the sentence quoted above, the petition references papers by Perrin, Heyning and Dahlheim as authoritative on killer whale taxonomy.

p. 14 – 17 – In summarizing the Cetacean Taxonomy workshop, the petition fails to mention that among the workshop's conclusions was that "Overall, a majority of participants felt that Resident- and Transient-type killer whales in the ENP [Eastern North Pacific] probably merited species or sub-species status." (Reeves *et al.* 2004 pp. 5 and 72).

p. 17 – "Most importantly of all, the workshop contained the following: [C]onsideration of whether to add the 'southern resident' killer whales of the eastern North Pacific to the U.S. Endangered Species List hinged on poorly understood evolutionary relationships between this population and killer whales globally (LJ/04/KW10). In the absence of a fundamental understanding and agreement on the number of species and subspecies of killer whales, consensus could not be reached on whether this whale population was significant to the taxon to which it belongs."

The petitioners present this statement as a conclusion of the workshop. However, the text quoted appears in the first page of the workshop report and is referring to the inability of the 2002 BRT (Krahn *et al.* 2002) to reach a consensus on killer whale taxonomy. In other words, this statement is describing the motivation for the workshop, not the workshop's conclusion.

p. 17-18 – The discussion of the 2006 listing fails to cite the BRT reports (Krahn *et al.* 2002, 2004) and the discussions therein regarding killer whale taxonomy and population structure.

Scientific basis for identification of subspecies

p. 26 – "Contradicting the scientific consensus in the cetacean's [sic] workshop, and without any support from the broader taxonomic community, the Service unilaterally created a killer whale subspecies – the North Pacific residents – based apparently on geographic distribution."

This statement is misleading. With regard to killer whale taxonomy, the taxonomy workshop report stated: "Overall, a majority of participants felt that the Resident- and Transient-type killer whales in the ENP probably merit at least species or subspecies status." (Reeves *et al.* 2004, p. 72). In addition, the BRT report discusses multiple lines of evidence both for and against sub-species, and clearly does not rely solely on geography (Krahn *et al.* 2004).

p. 26, 27 – The petition notes that NMFS has not provided a Latin trinomial for the hypothesized North Pacific Resident sub-species, and suggests that "… the Service has chosen to ignore 275 years of biological classification and taxonomic nomenclatural convention…". The issue of nomenclature was in fact explicitly discussed in the BRT report, which noted that all the biological issues surrounding the subspecies will need to be resolved before the nomenclature can be settled (Krahn *et al.* 2004, p. 18). In addition, the Cetacean Taxonomy Workshop report contains a section that specifically discusses unnamed subspecies, noting several examples and concluding that "Designation of unnamed subspecies can provide a mechanism for allowing recognition of highly differentiated forms without having to wait until its nomenclature is settled." (Reeves *et al.* 2004, p. 8). The Society for Marine Mammalogy also recognizes the residents and transients as unnamed subspecies of *O. orca* (Committee on Taxonomy 2012).

Genetic data

The petition relies heavily on a recent paper, Pilot *et al.* (2010), that uses a variety of analyses to estimate rates of interbreeding among groups of killer whales (see section above for a summary of this paper). Much of the petition's discussion of this paper is misleading, misrepresenting both the results of the Pilot *et al.* study and how these results combine with the results of other studies to provide a more complete description of killer whale population structure.

p. 29 – "Pilot *et al.* (2010) reported that comparative assessments of kinship, parentage, and dispersal reveal high levels of kinship and male-mediated gene flow within local populations, including among ecotypes that are highly divergent within the mtDNA phylogeny."

Using the parentage and assignment methods the petition appears to prefer, Pilot *et al.* found a single putative instance of interbreeding (gene flow) between whales from different the Pacific ecotypes – an offshore whale that genetically assigned to the transient ecotype (Pilot *et al.* 2010 Appendix S3). They found no instances of putative interbreeding between the residents and transients or residents and offshores. We therefore disagree with petition's conclusion that Pilot *et al.* (2010) found "high levels" of male mediated gene flow among ecotypes. Another, larger study (in terms of whales sampled and loci genotyped) found no instances of interbreeding among ecotypes (Parsons *et al.* 2013).

p. 29 – "In contrast to the Service's insistence that its speculative unnamed North Pacific resident subspecies (and Southern Resident DPS) are genetically isolated, Pilot *et al.* (2010) show that they are not."

The 2004 BRT did not claim that the ecotypes were completely isolated, merely that there was a "... high degree of contemporary reproductive isolation..." (Krahn *et al.* 2004 p. 16). The petition's claims to the contrary, the Pilot *et al.* (2010) results show that there is at most rare and episodic contemporary gene flow between the transient and offshore ecotypes and no evidence of contemporary gene flow between the resident and offshore ecotypes or the resident and transient ecotypes. Using model fitting methods to estimate historical gene flow, Pilot *et al.* (2010) estimate that there has been low (generally < 1%) rates of gene flow among the ecotypes historically (see Table 5 of Pilot *et al.* 2010). These rates are consistent with the BRT's interpretation of a high degree of reproductive isolation, and are also consistent with the information available to the 2004 BRT when it made its evaluation (see Tables 4 and 5 of Hoelzel 2004).

p. 30 – "The significance of the findings of Pilot *et al.* (2010) is threefold. First, they demonstrate with data that social interactions among killer whale pods do occur in the wild and they occur more frequently than has been reported (i. e., many interactions are simply "missed" by human observers who cannot watch a vast area of ocean to take note of killer whale pod interactions, 24 hours a day, 7 days a week, year round)."

Actually, Pilot *et al.* (2010) only studied patterns of genetic data, and contained no data or analysis of social interactions.

p. 30 – "The genetic data provide evidence that these inter-pod social interactions occur, and that they can and do result in mating among individuals in different pods, including mating among individuals of different ecotypes (i.e., between resident and transient killer whales)."

As we explain above, Pilot *et al.* (2010) found no direct evidence at all of mating between resident and transient killer whales (see Appendix S3 of Pilot *et al.*), and their indirect (model fitting) methods indicated that rates of gene flow between residents and transients were less than one half a percent (Table 5 of Pilot *et al.*). Pilot *et al.* did find somewhat higher rates of gene flow among resident populations (ie, within the resident ecotype), but even these were very low for all pairs of populations except between Russia and the Bering Sea and Bering Sea and Alaska: "In residents, very high gene flow rates were revealed from RU to BS (0.28) and from BS to AR (0.14), and much lower rates (ranging from 0.005 to 0.024) between other pairs of resident populations." (p. 26).

p. 33 – "Therefore, if only mtDNA is considered in an analysis, the loss of mtDNA variation in populations (also referred to as lineage sorting) can give an erroneous

appearance of populations (and putative species) being genetically isolated because they are trying to maintain taxonomic differences (i.e., Morin *et al.* 2010) while at the same time ecotypes and populations are not isolated for nuclear genetic variation. This is precisely the case with killer whales, a fact the Service did not acknowledge in its 2005 listing of the killer whale DPS, or in its 2011 status review of the population."

There are multiple inaccuracies with this statement and the discussion of mtDNA patterns that surrounds it in the Petition. First, the BRT explicitly discussed the strengths and limitations of mitochondrial (maternal) and nuclear genetic markers (see pp. 22-23 of Krahn *et al.* 2002 and p. 16 of Krahn *et al.* 2004). Second, the statement seems to imply that North Pacific killer whales ecotypes and populations are not strongly differentiated at nuclear loci. This is simply not correct: Hoelzel *et al.* (2007), Pilot *et al.* (2010), Morin *et al.* (2010), and Parson *et al.* (2013) all describe patterns of microsatellite (nuclear) variation among populations, and all find significant levels of divergence consistent with generally low rates of gene flow (typically < 1 migrant/generation among ecotypes and very much less for some analyses). A preliminary version of one of these analyses (Hoelzel 2004) was discussed extensively by the 2004 BRT (Krahn *et al.* 2004 pp. 11-13).

With regard to 'lineage sorting' of mtDNA, this phenomena was explicitly considered by the BRT (see Krahn *et al.* 2002 p. 23 paragraph 3), who ultimately concluded that much of mtDNA variation among populations was in fact random and due to stochastic events. That conclusion, although reasonable at the time, must now be updated based on the new whole mitogenome data of Morin *et al.* (2010), which shows that when whole mitogenomes are considered patterns of mtDNA variation among killer whales are not at all random but instead are very highly correlated with ecotype. This new result, combined with the new nuclear data reported in the same paper and by Hoelzel *et al.* (2007), Pilot *et al.* (2010) and Parsons *et al.* (2013), in fact strengthens the original conclusion of the BRT that North Pacific killer whale ecotypes are highly reproductively isolated from each other.

p. 34 – "Thus, outbreeding occurs (particularly those in different ecotypes) but is limited by the frequency of interactions in the ocean, rather than by killer whales trying to maintain taxonomic or population isolation."

The implication that the only factor limiting interbreeding between resident killer whales and transient killer whales is infrequent opportunity for interactions in the ocean is not consistent with the available data. For example, both residents and transients are frequently observed in the Salish Sea, often on the same day and in the same general location but have never been observed to interact or socialize (Baird 2000). The ocean is indeed vast, but the resident and transient ecotypes have a primarily coastal distribution, have a long distance means of potentially locating each other through their acoustic calls, and are frequently sighted in the same general vicinity by human observers (see e.g. Table 2 of Zerbini *et al.* 2007). It

therefore seems highly implausible that only lack of random encounters is limiting gene flow between ecotypes.

p. 35 – "Thus, the Service has erroneously attributed the patterns of genetic variation and behavior between ecotypes to genetic differences, when learned behaviors are responsible for these ecotypes."

It seems reasonable to conclude that "patterns of genetic variation" have a genetic basis. With regard to the behavioral and ecological differences among the ecotypes, the BRT never concluded that these traits were genetically based. For example, the 2004 BRT report summarized arguments for and against multiple species of North Pacific killer whales, and in the "Arguments for a single species" section noted: "Foraging specializations and other behavioral characteristics such as distinct vocalizations may be learned and therefore are not good indicators of species status (Barrett-Lennard and Heise 2004)." The BRT did consider the ecological, social and foraging differences among the ecotypes as one of several lines of evidence for subspecies status (Krahn *et al.* 2004, p. 39-40), but never claimed that these were genetically based characteristics. In discussing the factors leading to the conclusion that the southern resident killer whales are a DPS, the BRT discussed ecological setting, range, genetic differentiation, and behavioral and cultural diversity (Krahn *et al.* 2004 p. 44-45). In other words, in its DPS determination the BRT stated explicitly that it was considering behavioral and cultural factors in addition to genetic variation in assessing DPS status, consistent with USFWS and NMFS policy on DPS determination.

p. 36 – "In sum, there is no competent genetic evidence to support the designation of the North Pacific resident whale population as a subspecies."

At a minimum, this is a debatable point. Rates of contemporary gene flow have been estimated as zero between the residents and either the transient and offshore ecotypes (Pilot *et al.* 2010, Ford *et al.* 2011, Parsons *et al.* 2013). The three ecotypes can be unambiguously identified using either mtDNA or nuclear genetic data (Morin *et al.* 2010, Parsons *et al.* 2013) or photographs (Zerbini *et al.* 2007). These genetic differences are maintained in sympatry, a factor even biologists concerned about taxonomic inflation view as important evidence of taxonomic distinctiveness (Zachos *et al.* 2013). There is no question that there is some uncertainty regarding the taxonomic status of the North Pacific ecotypes and that it is possible for reasonable experts to come to somewhat different conclusions (see pp. 41 and 45 of Krahn *et al.* 2004, for example). But to conclude that there is "no competent genetic evidence" is inconsistent with the available information.

Morphological data

p. 36 - 38 – "The Service fails to distinguish the difference between variation that is primarily due to environmental influences on development, such as body size, and variation that has a genetic basis." "In the listing decision, references to morphological differences that distinguish ecotypes are based upon studies that are anecdotal, qualitative, or pseudo-quantitative in nature (Baird & Stacey 1988; Baird 2000). There are no data to substantiate objectively actual distribution of these traits in the wild. There are no data to support the genetic basis for variation in these traits (e.g., body size, which is primarily influenced by environment rather than genetics in most mammals). Further, there are no data to support the presumption that the morphological differences in question have any functional significance (i.e., they confer a survival advantage to an ecotype). The Service's key morphological "evidence" to describe three ecotypes of killer whales in the 2005 listing rule is subjective, or involves incomplete qualitative comparisons, or both (Table 1)."

In fact, the 2004 BRT noted similar points in evaluating the morphological data (see Krahn *et al.* p. 38), and with the exception of the saddle patch pigmentation trait never claimed that the morphological differences among the ecotypes were necessarily genetically based or proven to be adaptive. Indeed, the criteria for subspecies designation suggested by Reeves *et al.* (2004) and used by the BRT do not require that morphological variation be proven to either genetically based or adaptive in order for it be used as one of several factors to delineate subspecies. It is also important to note that at the time of the status reviews in 2002-2004 (and even now) relatively little data were available for offshore killer whales.

Nonetheless, we agree with the petitioners that much of the information on morphological variation within and among the North Pacific ecotypes is qualitative in nature and would benefit from additional quantitative analysis. It is important to note, however, that the qualitative differences among the ecotypes that have been described are based on decades of field observations by biologists who have spent their entire careers studying killer whales. The BRT therefore felt comfortable including these descriptions as one of several lines of information related to potential taxonomic status. Subsequent to the 2004 BRT report, the analyses of Zerbini *et al.* (2007) indicates that at least when comparing multiple individuals of each ecotype the groups can be reliably distinguished on the basis of morphology.

p. 39 – "Saddle patches are another morphological trait used to treat the North Pacific resident whale population as a separate subspecies. Yet again there is substantial overlap among ecotypes, and the categories of patterns have been described differently by different authors. Evans *et al.* (1984) described three patterns, while Baird and Stacey (1988) described five. As shown in the line drawings from each paper on the following page (Evans *et al.* 1984; Baird and Stacey 1988), there is no overlap in the patterns, yet the Service relied on this subjective classification in its listing decision even in the absence of supporting data such as field notes, photographs, or measurements. Finally, the Service did not acknowledge another source of error in classifying saddle patch patterns: saddle

patches are not always symmetrical. Therefore, different classifications can be obtained depending upon which side of the killer whale is photographed, leading to erroneous assignments."

The BRT did not use or cite the Evans *et al.* (1984) study, which was focused on patterns of killer whale pigmentation at a global scale and did not include ecotype information. The Baird and Stacey (1988) paper clearly cites the sources of the photographs they analyzed, which are from readily available publications. The publication also clearly stated that only photographs of the left side of the whales were used. The Petition speculates that right-hand-side photographs may produce different results, but provides no analysis to back up this statement.

p. 42 – "The Service fails to recognize the evolutionarily more parsimonious explanation that the behavioral traits it uses to distinguish among supposed subspecies or ecotypes are learned rather than the result of genetic differences."

As was noted above, the BRT reports never concluded that variation in vocalization or behavioral traits is genetically based.

p. 42-42 – "In a recent paper, Rehn *et al.* (2010) reported that a killer whale vocalization associated with high arousal behaviors is common to all killer whales and does not vary regardless of pod, ecotype, or location in the Pacific. Thus, this innate behavior is consistent with the killer whale's current classification as a single species"

The experimental design of the Rehn *et al.* (2010) paper was to examine isolated, non-interacting, groups of killer whales in order to find common and thus presumably innate call types. While the finding of such a call type certainly is consistent with the known evolutionarily recent common ancestry of the ecotypes, it is not strong evidence that they belong to a single species. Indeed, the Pacific ecotypes and killer whales worldwide share a great many traits due to common ancestry. For that matter, they share a great many traits in common with other delphinids. However, simply sharing traits is obviously not strong evidence that two putative taxa are conspecific or are not reproductively isolated. Humans and chimpanzees, for example, share ~99% of their genomes (Mikkelsen *et al.* 2005), but few would argue that they are not distinct species.

p. 48 – "An unbiased method would have used DNA amplification primers and reaction conditions capable of detecting types of potential prey other than just fish (i.e., marine mammals, birds, and squid). Such a method would use a pair of conserved DNA amplification primers for animals (i.e., 16sRNA), or combinations of primers that would amplify fish, marine mammals, birds, and squid, followed by application of culture independent methods (e.g., PCR, cloning of PCR products, and sequencing of the clone library). That would provide DNA sequences from virtually all animal DNAs in a sample, even if they are at low frequency. This method is widely

used in microbial genomics and forensics, and is needed to detect total diversity of the prey items in the sample (Hugenholtz *et al.* 1998)."

The petition is correct that primers used in the Hanson *et al.* (2010) study were designed specifically to detect fish prey. This was in part to avoid amplifying DNA from the killer whales being sampled. However, another study (Ford *et al.* 2011b) did use 16s ribosomal DNA primers to obtain PCR amplicons from ~200 killer whale fecal samples collected from the southern resident population, including many of the same samples used in the Hanson (2010) study. These primers have been demonstrated to amplify both harbor seal and harbor porpoise, two common marine mammals preyed upon by transient killer whales. In controlled experiments in which harbor seal or harbor porpoise DNA was mixed with killer whale DNA and amplified and sequenced using these primers, the harbor porpoise and harbor seal sequences were readily detectable, along with that of killer whale. Using the same primers and methods, marine mammal sequences (other than killer whale) were not detected in any of the >200 fecal samples collected from the field (Hempelmann 2012).

References

Avise, J., and R. Ball, 1990 Principles of genealogical concordance in species concepts and biological taxonomy. Oxford surveys in evolutionary biology **7:** 45-67.

Avise, J. C., D. Walker and G. C. Johns, 1998 Speciation durations and Pleistocene effects on vertebrate phylogeography. Proceedings of the Royal Society B-Biological Sciences **265:** 1707-1712.

Baird, R. W., 2000 The killer whale -- foraging specializations and group hunting, pp. 127-153 in *Cetacean societies: field studies of dolphins and whales*, edited by J. Mann, R. C. Connor, P. L. Tyack and H. Whitehead. University of Chicago Press, Chicago.

Baird, R. W., and P. J. Stacey, 1988 VARIATION IN SADDLE PATCH PIGMENTATION IN POPULATIONS OF KILLER WHALES (ORCINUS-ORCA) FROM BRITISH-COLUMBIA, ALASKA, AND WASHINGTON STATE. Canadian Journal of Zoology-Revue Canadienne De Zoologie **66:** 2582-2585.

Baker, R. J., and R. D. Bradley, 2006 Speciation in mammals and the genetic species concept. Journal of Mammalogy **87:** 643-662.

Beck, S., S. Kuningas, R. Esteban and A. D. Foote, 2011 The influence of ecology on sociality in the killer whale (Orcinus orca). Behavioral Ecology.

Berzin, A. A., and V. L. Vladimirov, 1983 A NEW SPECIES OF KILLER WHALE (CETACEA, DELPHINIDAE) FROM THE ANTARCTIC WATERS. Zoologichesky Zhurnal **62:** 287-295.

Committee on Taxonomy, 2012 List of marine mammal species and subspecies. Society for Marine Mammology, http://www.marinemammalscience.org, consulted on April 25, 2013.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace and R. K. Wayne, 2000 Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution **15:** 290-295.

Dahlheim, M. E., A. Schulman-Janiger, N. Black, R. Ternullo, D. Ellifrit *et al.*, 2008 Eastern temperate North Pacific offshore killer whales (Orcinus orca): Occurrence, movements, and insights into feeding ecology. Marine Mammal Science **24:** 719-729.

Dahlheim, M. E., and P. A. White, 2010 Ecological aspects of transient killer whales Orcinus orca as predators in southeastern Alaska. Wildlife Biology **16:** 308-322.

de Bruyn, P. J. N., C. A. Tosh and A. Terauds, 2013 Killer whale ecotypes: is there a global model? Biological Reviews **88:** 62-80.

Deecke, V. B., J. K. B. Ford and P. J. B. Slater, 2005 The vocal behaviour of mammal-eating killer whales: communicating with costly calls. Animal Behaviour **69:** 395-405.

Deecke, V. B., M. Nykanen, A. D. Foote and V. M. Janik, 2011 Vocal behaviour and feeding ecology of killer whales Orcinus orca around Shetland, UK. Aquatic Biology **13:** 79-U186.

Deecke, V. B., P. J. B. Slater and J. K. B. Ford, 2002 Selective habituation shapes acoustic predator recognition in harbour seals. Nature **420:** 171-173.

Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. Demaster and J. Sisson, 1992 Rethinking the stock concept: a phylogeographic approach. Conservation Biology **6:** 24-36.

Edwards, S. V., and P. Beerli, 2000 Perspective: Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution **54:** 1839-1854.

Foote, A. D., 2012 Investigating ecological speciation in non-model organisms: a case study of killer whale ecotypes. Evolutionary Ecology Research **14:** 447-465.

Foote, A. D., P. A. Morin, J. W. Durban, R. L. Pitman, P. Wade *et al.*, 2011a Positive selection on the killer whale mitogenome. Biology Letters **7:** 116-118.

Foote, A. D., P. A. Morin, J. W. Durban, E. Willerslev, L. Orlando *et al.*, 2011b Out of the Pacific and Back Again: Insights into the Matrilineal History of Pacific Killer Whale Ecotypes. PLoS ONE **6**.

Foote, A. D., J. Newton, S. B. Piertney, E. Willerslev and M. T. P. Gilbert, 2009 Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations. Molecular Ecology **18:** 5207-5217.

Foote, A. D., J. T. Vilstrup, R. de Stephanis, P. Verborgh, S. C. A. Nielsen *et al.*, 2011c Genetic differentiation among North Atlantic killer whale populations. Molecular Ecology **20:** 629-641.

Ford, J., and G. Ellis, 2006 Selective foraging by fish-eating killer whales *Orcinus orca* in British Columbia. Marine Ecology Progress Series **316:** 185–199.

Ford, J. K. B., G. M. Ellis and K. C. Balcomb, 2000 *Killer whales: The natural history and genealogy of Orcinus orca in British Columbia and Washington State. Second edition.* . UBC Press, Vancouver, BC, Canada.

Ford, J. K. B., G. M. Ellis, C. O. Matkin, M. H. Wetklo, L. G. Barrett-Lennard *et al.*, 2011a Shark predation and tooth wear in a population of northeastern Pacific killer whales. Aquatic Biology **11:** 213-224.

Ford, J. K. B., B. M. Wright, G. M. Ellis and J. R. Candy, 2010 Chinook salmon predation by resident killer whales: seasonal and regional selectivity, stock identity of prey, and consumption rates. DFO Can. Sci. Advis. Sec. Res. Doc. 2009/101. iv + 43 p.

Ford, M. J., 2003 Conservation Units and Preserving Diversity in *Salmonid perspectives on evolution*, edited by A. Hendry. Oxford University Press, Oxford.

Ford, M. J., M. B. Hanson, J. A. Hempelmann, K. L. Ayres, C. K. Emmons *et al.*, 2011b Inferred Paternity and Male Reproductive Success in a Killer Whale (Orcinus orca) Population. Journal of Heredity **102:** 537-553.

Fraser, D. J., and L. Bernatchez, 2001 Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology **10:** 2741-2752.

Haig, S. M., E. A. Beever, S. M. Chambers, H. M. Draheim, B. D. Dugger *et al.*, 2006 Taxonomic considerations in listing subspecies under the US Endangered Species Act. Conservation Biology **20:** 1584-1594.

Hanson, M., R. Baird, J. Ford, J. Hempelmann-Halos, D. Van Doornik *et al.*, 2010 Species and stock identification of prey consumed by endangered southern resident killer whales in their summer range. Endangered Species Research **11:** 69-82.

Hempelmann, J., 2012 Memorandum from Jennifer Hemplemann to Michael Ford. November 20, 2012 (via email).

Herman, D. P., D. G. Burrows, P. R. Wade, J. W. Durban, C. O. Matkin *et al.*, 2005 Feeding ecology of eastern North Pacific killer whales, Orcinus orca, from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Marine Ecology Progress Series **302:** 275-291.

Hey, J., R. S. Waples, M. L. Arnold, R. K. Butlin and R. G. Harrison, 2003 Understanding and confronting species uncertainty in biology and conservation. Trends in Ecology & Evolution **18:** 597-603.

Heyning, J., and M. Dahlheim, 1988 Orcinus orca. Mammalian Species **304:** 1-9.

Hilborn, R., S. Cox, F. Gulland, D. Hankin, N. Hobbs *et al.*, 2012 The Effects of Salmon Fisheries on Southern Resident Killer Whales: Final Report of the Independent Science Panel. Prepared with the assistance of D.R. Marmorek and A.W. Hall, ESSA Technologies Ltd., Vancouver, B.C. for National Marine Fisheries Service (Seattle. WA) and Fisheries and Oceans Canada (Vancouver. BC). xv + 61 pp. + Appendices. .

Hoelzel, A. R., 2004 Report on killer whale population genetics for the BRT review on the status of the Southern Resident population. Unpubl. report to BRT. (Available from L. Barre, NOAA Fisheries, NWR, 7600 Sand Point Way NE, Seattle, WA 98115.), pp.

Hoelzel, A. R., J. Hey, M. E. Dahlheim, C. Nicholson, V. Burkanov *et al.*, 2007 Evolution of population structure in a highly social top predator, the killer whale. Molecular Biology and Evolution **24:** 1407-1415.

Hoelzel, A. R., A. Natoli, M. E. Dahlheim, C. Olavarria, R. W. Baird *et al.*, 2002 Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history. Proceedings of the Royal Society of London, Series B **269:** 1467-1473.

Hoyt, E., 1981 *The whale called killer*. E.P. Dutton, New York.

Johns, G. C., and J. C. Avise, 1998 A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. Molecular Biology and Evolution **15:** 1481-1490.

Krahn, M. M., M. J. Ford, W. F. Perrin, P. R. Wade, R. P. Angliss *et al.*, 2004 2004 Status review of Southern Resident Killer Whales (*Orcinus orca*) under the Endangered Species Act, pp. 73 in *NOAA Technical Memorandum*. Northwest Fisheries Science Center, Seattle.

Krahn, M. M., D. P. Herman, C. O. Matkin, J. W. Durban, L. Barrett-Lennard *et al.*, 2007 Use of chemical tracers in assessing the diet and foraging regions of eastern North Pacific killer whales. Marine Environmental Research **63:** 91-114.

Krahn, M. M., P. R. Wade, S. T. Kalinowski, M. E. Dahlheim, B. L. Taylor *et al.*, 2002 Status Review of Southern Resident killer whales (Orcinus orca) under the Endangered Species Act. NOAA Technical Memorandum NMFS-NWFSC-54. 133pp.

LeDuc, R., and B. Taylor, 2004 Revision of meeting document LJ/04/KW7, Mitochondrial sequence variation in North Pacific killer whales, Workshop on Shortcomings of Cetacean Taxonomy in Relation to Needs of Conservation and Management, April 30–May 2, 2004, La Jolla, California. Unpubl. report to BRT. (Available from L. Barre, NOAA Fisheries, NWR, 7600 Sand Point Way NE, Seattle, WA 98115.).

Leduc, R. G., K. M. Robertson and R. L. Pitman, 2008 Mitochondrial sequence divergence among Antarctic killer whale ecotypes is consistent with multiple species. Biology Letters **4:** 426-429.

Mallet, J., 1995 A SPECIES DEFINITION FOR THE MODERN SYNTHESIS. Trends in Ecology & Evolution **10:** 294-299.

McElhany, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright and E. P. Bjorkstedt, 2000 Viable Salmonid Populations and the Recovery of Evolutionarily Significant Units. NOAA Technical Memorandum NMFS-NWFSC **42:** 156p.

Mikhalev, Y., M. ivashin, V. Savusin and F. Zelenaya, 1981 The distribution and biology of killer whales in the Southern Hemisphere. Report of the International Whaling Commission **1:** 551-566.

Mikkelsen, T. S., L. W. Hillier, E. E. Eichler, M. C. Zody, D. B. Jaffe *et al.*, 2005 Initial sequence of the chimpanzee genome and comparison with the human genome. Nature **437:** 69-87.

Morin, P. A., F. I. Archer, A. D. Foote, J. Vilstrup, E. E. Allen *et al.*, 2010 Complete mitochondrial genome phylogeographic analysis of killer whales (Orcinus orca) indicates multiple species. Genome Research **20:** 908-916.

Moritz, C., 1994 Defining 'evolutionarily significant units' for conservation. Trends in Ecology and Evolution **9:** 373-375.

NMFS, 2002 Endangered and Threatened Wildlife and Plants: 12-Month Finding for a Petition To List Southern Resident Killer Whales as Threatened or Endangered Under the Endangered Species Act (ESA). Federal Register **67:** 44133-44138.

NMFS, 2013 Effects of Salmon Fisheries on Southern Resident Killer Whales. http://www.nwr.noaa.gov/protected_species/marine_mammals/cetaceans_whales_dolphins_porpoise/toothed_whales/killer_whales/southern_resident_killer_whale/effects_of_salmon_fisheries_on_southern_resident_killer_whales.html, pp.

Olson, P. A., P. Ensor and S. Kuningas, 2012 Observations of killer whales off East Antarctica, 82-95E, in 2009. Journal of Ceacean Research and Management **12:** 61-64.

Parsons, K., J. Durban, A. Burdin, V. Burkanov, R. Pitman *et al.*, 2013 Geographic patterns of genetic differentiation among killer whales in the northern Pacific. Journal of Heredity **doi: 10.1093/jhered/est037**.

Pilot, M., M. E. Dahlheim and A. R. Hoelzel, 2010 Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (Orcinus orca). Journal of Evolutionary Biology **23:** 20-31.

Pitman, R., and P. Ensor, 2003 Three forms of killer whales (*Orcinus orca*) in Antarctic waters. Journal of Ceacean Research and Management **5:** 131-139.

Pitman, R. L., and J. W. Durban, 2012 Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (Orcinus orca), type B, in Antarctic Peninsula waters. Marine Mammal Science **28:** 16-36.

Pitman, R. L., J. W. Durban, M. Greenfelder, C. Guinet, M. Jorgensen *et al.*, 2011 Observations of a distinctive morphotype of killer whale (Orcinus orca), type D, from subantarctic waters. Polar Biology **34:** 303-306.

Pitman, R. L., W. L. Perryman, D. Leroi and E. Eilers, 2007 A dwarf form of killer whale in Antarctica. Journal of Mammalogy **88:** 43-48.

Reeves, R., W. Perrin, B. Taylor, C. Baker and S. Mnesnick, 2004 Report of the workshop on shortcomings of cetacean taxonomy in relation to needs of conservation and management, April 30-May2, 2004 La Jolla, California. . NOAA-TM-NMFS-SWFSC-363**:** 94pp.

Rice, D. W., 1998 *Marine Mammals of the World. Systematics and Distribution.* Allen Press, Lawrence, KS.

Riera, A., J. K. B. Ford, J. Hildebrand and N. Chapman, 2011 Acoustic monitoring of killer whale populations off the west coast of Vancouver Island. Journal of the Acoustic Society of America **129:** 2607.

Riesch, R., L. G. Barrett-Lennard, G. M. Ellis, J. K. B. Ford and V. B. Deecke, 2012 Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? Biological Journal of the Linnean Society **106:** 1-17.

Saulitis, E., C. Matkin, L. Barrett-Lennard, K. Heise and G. Ellis, 2000 FORAGING STRATEGIES OF SYMPATRIC KILLER WHALE (ORCINUS ORCA) POPULATIONS IN PRINCE WILLIAM SOUND, ALASKA. Marine Mammal Science **16:** 94-109.

Schluter, D., 2001 Ecology and the origin of species. Trends in Ecology & Evolution **16:** 372-380.

Schluter, D., 2009 Evidence for Ecological Speciation and Its Alternative. Science **323:** 737-741.

USFWS, and NMFS, 1996 Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register **61:** 4722.

Waples, R. S., 1991 Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Marine Fisheries Review **53:** 11-22.

Ward, E., M. Ford, R. Kope, J. Ford, A. Velez-Espino *et al.*, 2013 Estimating the impacts of Chinook salmon abundance and prey removal by ocean fishing on Southern Resident killer whale population dynamics. NOAA Technical Memorandum NMFS-NWFSC **in prep**.

Ward, E. J., B. X. Semmens, E. E. Holmes and K. C. Balcomb, 2011 Effects of Multiple Levels of Social Organization on Survival and Abundance. Conservation Biology **25:** 350-355.

Zachos, F. E., M. Apollonio, E. V. Barmann, M. Festa-Bianchet, U. Gohlich *et al.*, 2013 Species inflation and taxonomic artefacts-A critical comment on recent trends in mammalian classification. Mammalian Biology **78:** 1-6.

Zerbini, A. N., J. M. Waite, J. W. Durban, R. LeDuc, M. E. Dahlheim *et al.*, 2007 Estimating abundance of killer whales in the nearshore waters of the Gulf of

Alaska and Aleutian Islands using line-transect sampling. Marine Biology **150:** 1033-1045.