

APPENDIX 6

Report of the Working Group on Killer Whales as a Case Study

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SUMMARY

The overall goal of the killer whale workshop was to summarize the current state of knowledge concerning killer whale biology and taxonomy. Killer whales are among the most widely distributed of all mammals. Density of killer whales increases with latitude and is strongly associated with oceanic productivity. Currently, all killer whales are considered to be a single, cosmopolitan species, although this designation has been increasingly questioned in recent years. In the eastern North Pacific (ENP), three types are currently recognized: so-called “Residents” (which feed on fish), “Transients” (which feed on mammals) and “Offshores” (which may feed largely on fish).² The three types are distinguishable (in some cases unequivocally) through differences in many characteristics, including morphology, genetics, group size, social behavior, foraging specialization, range, dispersal, and call repertoires. Of the three putative types, the Offshore animals are the least well understood. Existing evidence suggests that there is no switching between ecotypes (although at present there is no way to determine whether this might happen over a timeframe of decades or longer).

In Antarctica, there is good evidence for the existence of three types which are also more or less distinguishable on the basis of morphology, genetics and ecology: Type A (a circumpolar specialist eating primarily minke whales), Type B (a mammal eater-taking mainly seals), and Type C (a fish-eater found primarily in eastern Antarctica).

² The working group acknowledged the long-held recognition among killer whale researchers that the terms “Resident”, “Transient” and “Offshore” are misleading in that they do not strictly reflect the complex and sometimes overlapping habits, movements and distribution of the animals in these ecotypes. However, pending nomenclatural clarification (which is a recommendation of this working group), the terms have been retained here to reflect their continued wide use.

Genetic analysis of mitochondrial DNA shows low diversity, no consistent worldwide geographical pattern, and no consistent correlation between mtDNA lineage and ecotype. The data are consistent with a fairly recent divergence of ecotypes, while lack of regional variation may reflect the matrilineal expansion of local populations. The distributional pattern of haplotypes implies that divisions in the mitochondrial phylogeny may have arisen as differentiation between ocean basins. There are fixed differences in the mtDNA control region between putative populations (among Resident populations, between Residents and Transients in the northeastern Pacific, and among geographical populations). Among the three Antarctic types, B and C are closely related, while Type A animals exhibited fixed differences from B and C whales.

Nuclear data do not discriminate unequivocally among the various killer whale ecotypes. For example, the Resident ecotype in the North Pacific shows the same genetic distance between the Kamchatka Peninsula (Russia) and Washington State (U.S.A.) populations (same mtDNA type) as between Resident and Transient types in the ENP.

Whether the different ecotypes of killer whales constitute a single species, multiple species or subspecies is not yet resolved. While there are clearly different ecotypes, it is not clear whether these represent a plastic and ephemeral response to changing habitat conditions, or the beginning of an irreversible process of speciation. Single-species advocates believe the genetic data are not strong enough to argue for long-term reproductive isolation, while proponents of the multiple-species view disagree; the latter contend that there are potentially two or three species of killer whales in Antarctica, and perhaps others in the North Pacific. The majority view among workshop participants was that multiple species probably exist at least in Antarctica, and that this might well be confirmed in the future, but that current data are limited (absent or insufficiently quantified) and therefore that separation at the species level is at this point premature. The working group agreed that it was not possible at this point to comment meaningfully on the relationship between Antarctic and ENP killer whales on the basis of existing data, except to note convergent similarities in summer feeding preferences be-

tween Antarctic Type B and ENP Transients, and Antarctic Type C and ENP Residents.

A majority of participants felt that Resident- and Transient-type killer whales in the ENP probably merited species or subspecies status. The relationship of Offshore-type killer whales to Residents and Transients (or any other killer whales in the world) is not clear.

INTRODUCTION

The working group met on April 30 and May 1, 2004 at the Southwest Fisheries Science Center in La Jolla, California. Clapham served as rapporteur. The principal goals of the working group were to review existing information on the characteristics, ecology and behavior of killer whales worldwide and attempt to resolve biological relationships within the genus *Orcinus* at all levels from species to local breeding populations. The hierarchy of biological diversity within the nominal species *Orcinus orca* can be partitioned in many ways to identify subspecific conservation units. These latter designations may be guided or dictated by legal mandates and may include (but are not necessarily limited to) Evolutionarily Significant Units (ESUs), Distinct Population Segments (DPSs), and stocks. The specific objectives were to:

- 1) review and summarize the current status of killer whale taxonomy at the level of species and subspecies; and

- 2) in light of the results from that endeavor, review biological relationships among different groups of North Pacific killer whales, focusing both on relationships among different groups of "Residents" and on relationships of Residents to other groups ("Offshores," "Transients").

It was expected that those exercises would be informed by the more general results and discussions that emerged from the symposium (Appendix 4) and the workshop as a whole.

This working group report is intended to summarize the existing state of the science on: a) global killer whale biology and taxonomy, b) biological relationships between North Pacific Residents and other groups of killer whales worldwide, and c) biological relationships between Puget Sound Southern Residents and other North Pacific Residents. The report identifies areas of scientific agreement as well as disagreement and suggests future work that could help resolve remaining uncertainties. A draft agenda circulated before the meeting proved inadequate to the aims and needs of the participants, and the report therefore follows instead the course of the discussions as they developed.

Background

Linnaeus (1758) described the species *Orcinus orca*, and the most recent formal taxonomic syntheses (Rice 1998; Baker et al. 2003) still considers all killer whales to be a single, cosmopolitan species. Killer whales are the most widely distributed marine mammal (Leatherwood and Dahlheim 1978; Heyning and Dahlheim 1988) and (except perhaps for *Homo sapiens*, *Rattus norvegicus*, and *Mus musculus*) may be the world's most widely distributed mammalian species. Hoelzel *et al.* (2002) analyzed samples of killer whales collected from selected locations around the world and found relatively low levels of genetic diversity at mtDNA and microsatellite loci; many local populations exhibited no mtDNA variation. In contrast to patterns seen in other cetacean species, they found little correspondence between geographical populations and genetic distance, and some mtDNA haplotypes were found in individuals from distant geographical regions. They suggested that these results could most plausibly be explained by a species-level bottleneck (perhaps in the later stages of the Pleistocene), followed by post-bottleneck expansion and local divergence among different matrilineal lines. One limitation of the study by Hoelzel *et al.* was that although the samples included individuals from around the world, coverage was limited in some areas and for some morphological/life history types.

Some other lines of evidence suggest that the nominal species *Orcinus orca* may actually include multiple species. Soviet scientists (Mikhalev *et al.* 1981; Berzin and Vladimirov 1983) proposed two new species of dwarf killer whales from Antarctica, based on samples collected over a 20-year period. However, as discussed by Pitman and Ensor (2003), the paucity of descriptive detail and lack of a designated holotype in the former paper and the subsequent loss of type material designated in the second paper make it difficult to determine the validity of their proposals. Recently, Pitman and Ensor (2003) summarized existing information on Antarctic killer whales and concluded that, in addition to *Orcinus orca*, two different types of killer whales occur in Antarctic waters. It is unclear whether the two new types correspond to the species proposed by the Soviet scientists. The three types are distinguished by morphological and behavioral characteristics, and Pitman and Ensor (2003) suggested that they likely represent separate species *sensu* the Biological Species Concept. They cautioned, however, that this hypothesis should be tested with more information, particularly genetic data. Neither of the two new Antarctic forms was represented in Hoelzel *et al.*'s (2002) recent world-wide analysis of killer whale mtDNA and microsatellites.

SUMMARY OF KNOWLEDGE CONCERNING KILLER WHALES WORLDWIDE

Global overview

An invited paper by Barrett-Lennard and Heise (LJ/04/KW1) summarized influences of ecology, cultural traditions and social organization on population structure and systematics of killer whales worldwide. The paper (1) described the population complexity of *Orcinus orca* in the northeastern Pacific, (2) speculated about its causes and genetic consequences, (3) discussed evidence (or lack thereof) for population structure in the rest of the species' range, and (4) discussed the implications of known patterns of population structure for systematics of the species worldwide.

The paper included a short history of killer whale studies in the northeastern Pacific, including Michael Bigg's discovery of the Resident (fish-eating) and Transient (marine mammal-eating) ecotypes. At the present time, there is evidence for at least three populations of Residents, three populations of Transients, and at least one population of an "Offshore" type that also appears to eat fish. Genetically, these populations are diverged from each other at mitochondrial and nuclear microsatellite loci. The authors concluded that, based on observation and genetic information, the ecotypes appear to be largely reproductively isolated from each other.

The mechanism of isolation is speculated to be at least partially xenophobia (avoidance of non-population members), mediated by population-specific calls. The mechanism of new population formation is speculated to be allopatric or sympatric group fission, a process that may lead to highly structured, perhaps ephemeral populations; this may be at least a partial explanation for the low genetic diversity of the species worldwide.

The paper next discussed the evidence for population structure or ecological partitioning outside the northeastern Pacific. Areas with some evidence for genetically, behaviorally, or morphologically differentiated groups include the eastern tropical Pacific, the Russian far east, Norway, New Zealand and Antarctica. None of these areas is as well studied as the northeastern Pacific, however. The authors speculated that in areas of high marine productivity killer whale populations become more specialized to particular prey items, promoting the formation of populations and ecotypes.

With regard to systematics, the authors suggested that revisions should be based upon the identification of lineages that are relatively deep and upon ecological similarities. They noted that this pattern had not yet been reported in any published studies. Instead, the known patterns are consistent with either of two

other scenarios: a recent adaptive radiation into a variety of new niches, or a species propensity to live in small xenophobic and ephemeral populations. If the former is correct, at least some of the currently diverged forms are probably incipient species and systematic revision may be useful. If the latter is correct, most forms are probably ephemeral and there is little to be gained in according them species status. The authors suggested that until the divergence process in killer whales is better understood the taxonomy should not be formally revised and killer whales should instead be described in terms of a species complex.

A recent review of killer whale abundance and density estimates worldwide (Forney and Wade, in review) found that density clearly increases with latitude (very low densities in tropical and mid-latitude waters, increasing densities above about 40-50 degrees, and densities peaking above 60 degrees). Given the reluctance of survey vessels to enter the ice in Antarctic waters, it is likely that existing data actually under-represent the true density of killer whales in the highest-latitude areas. Killer whale density was also strongly correlated with oceanic productivity as reflected in remotely sensed chlorophyll- α measurements.

Little is known about the densities of Offshore-type killer whales in most areas of the world, and observations by Pitman and others suggest that these animals are more common than may be thought; it should be noted that existing survey effort is heavily biased toward coastal areas. The often poor definition of the saddle patch in killer whales found in lower latitudes and the fact that animals with distinct saddles (as is typical of higher-latitude animals) are not observed there argues against seasonal migration of animals from high latitudes into warmer waters. However, the poorly defined saddle patch is not a universal characteristic, and the presence of southern elephant seal remains in the stomach of a killer whale (ecotype uncertain) taken by whalers off Durban (South Africa) indicates some migratory movement from at least the sub-Antarctic to temperate latitudes.

The working group noted that the current distribution of killer whales worldwide might not be representative of historic distribution, given the significant changes in the abundance and distribution of prey species (due to human exploitation or environmental changes). Furthermore, high directed takes of killer whales occurred in some locations (e.g. Japan, Norway, Iceland, Antarctica and Puget Sound), and frequent shooting by whalers of killer whales scavenging around large whale carcasses in at least some areas (notably in Antarctica); some populations may not have recovered from these large losses.

Summary of data relating to multiple ecotypes

There is clear evidence for the existence of multiple ecotypes with sympatric or parapatric distributions in the North Pacific and Antarctica. There is some anecdotal and other evidence that distinct ecotypes also exist in Norway and New Zealand, but currently available data are inconclusive. The suggestion has been made that the radiation of different ecotypes is more likely to have occurred in high-latitude areas characterized by high productivity (e.g. the north-eastern Pacific and the Antarctic); however, it would be worthwhile to investigate this further in unstudied areas with similar oceanographic characteristics (e.g. the Benguela Current region off South Africa).

Antarctica

According to Pitman and Ensor (2003), three types of killer whales have been documented in Antarctica that do not strictly correspond to the three ecotypes found in the Eastern North Pacific (ENP) and Alaska. Type C is a fish-eater found primarily in eastern Antarctica, similar to the Resident-type in the ENP. Type B is a mammal feeder taking mainly seals, and is thus similar to the ENP Transient-type; its distribution appears to be circumpolar. However, the Antarctic Type A is an open-ocean specialist on minke whales (also with a circumpolar distribution), and thus is not similar to the Offshore type in the ENP. However, it should be noted that categorization of feeding preferences was based upon observations made in a single Antarctic summer. These observations correspond roughly to descriptions by Soviet scientists (Berzin and Vladimirov 1983), whose data are very problematic in their selection and presentation and were analyzed without regard to possible biases; nonetheless, this earlier work stimulated the more recent observations by Pitman and Ensor (2003).

On current, rather limited evidence, there do not appear to be intermediate forms among the three Antarctic types, nor does it appear that any of the three represent an intermediate type between the other two. Observations made to date have not provided any evidence for intermediate forms. Animals with some of the morphological features of Types B and C have been observed outside Antarctica (e.g. off New Zealand and the Falkland Islands), but it is not known whether these few records are indicative of migration or occasional/extra-limital occurrence, or indeed whether the whales concerned were truly the Antarctic forms.

In summary, the evidence for reproductive isolation among the three Antarctic forms includes different morphology (color patterns and size), food specializations, habitat preferences and genetic groupings (see below). In addition, there appear to be no inter-

mediate forms. One alternate view is that some or all of the three types might have arrived in Antarctica quite recently but there has not yet been sufficient time for measurable gene flow to occur. Another view is that the data (from Antarctica and from killer whale types worldwide) are currently inconclusive, with multiple (and sometimes poorly quantified) morphotypes, global distribution of some haplotypes, and insufficient information on gene flow; in this view, it would be premature to assign separate species status to different forms. More detailed discussion on the latter is given below.

Eastern North Pacific (Mexico to the Bering Sea)

J. Ford summarized characteristics of killer whales (with an emphasis on acoustic differences) from long-term, dedicated studies off the western coast of North America and the implications of this knowledge for population and social structure (LJ/04/KW4, KW5). Individual killer whales have repertoires of discrete (or stereotyped) calls. These calls are culturally inherited (indicating vocal learning), stable for more than one generation (about 25 years), and differ within and among populations. Such repertoires were first described in the northeastern Pacific, but similar regional distinctive repertoires have also been documented off Norway, Iceland, New Zealand, Russia and Patagonia.

Resident-type killer whales from the ENP live in highly stable groups based upon matriline. Individuals stay in their group for life, with no dispersal having been detected. New groups are created by matrilineal fission, either gradually or quite abruptly upon the death of the senior female. Residents are typically found in clusters of 3-5 matriline, and clusters mix to form what are termed "communities." Each pod (closely related matriline that travel together) emits about a dozen calls, shared by all pod members. All call types are used in all behavioral contexts. Calls are shared by some but not all pods in the community. Shared calls differ structurally at the level of the pod and matriline (= "dialects"). Pods that share calls form what is termed a clan. Thus, call-sharing closely reflects matrilineal heritage, and vocal similarity indicates relatedness. Acoustic distance is correlated with genetic distance in clans, and the probability of mating increases with acoustic distance; this appears to be an outbreeding mechanism. Clans may have had allopatric or sympatric origins, or both.

In the ENP, several Resident-type clans are currently recognized: Southern Residents (1 clan of about 83 whales, all sharing the same mtDNA haplotype), Northern Residents (3 clans, about 204 whales, all sharing the same mtDNA haplotype), and Southern Alaskan Residents (2 clans, one of which has

about 190 whales and has the Northern Resident haplotype, and another of about 50 whales with the Southern Resident haplotype).

In contrast to Residents, Transient-type killer whales are wide-ranging and are found sporadically in any specific area, with less seasonality of occurrence. There is a variable social structure: some matrilineal groups are stable, while others exhibit dispersal (Baird and Whitehead 2000). Like Residents, however, Transients form communities. Transients are acoustically cryptic; call repertoires are very stable, and these differ among the three documented Transient communities. Community-specific calls may correspond to mtDNA differences.

Offshore killer whales constitute the third "type", although there was some disagreement as to whether "Offshores" should be considered a separate population of "Residents." Offshores travel in large groups (50-75 whales) and are generally smaller animals with more rounded dorsal fins than those of Residents and Transients. The feeding ecology of Offshores is poorly known. They are found mostly on the outer continental shelf, but will visit inshore waters. The social structure is also unknown. Acoustically, Offshores have a rich repertoire of calls, with what appears to be extensive sharing among groups. They are acoustically quite distinct from Residents and Transients.

In addition, there is evidence for the existence of groups of killer whales that do not fit any of the three types above. The so-called "LA pod" (13 whales) and the Mexican "A" community (50+ whales) both appear to be acoustically different from Residents, Transients and Offshores.

Overall, in Ford's view the evidence suggests that killer whale acoustic repertoires are cultural traditions that define identity and promote cohesion, but that on larger scales can also serve as an isolating mechanism that drives divergence among populations. Worldwide, one can think of killer whale communities as a mosaic of acoustically discrete and socially isolated populations that may not in themselves be ESUs, but that might instead be considered "culturally significant units."

Wade summarized the results of surveys conducted in Alaskan and Bering Sea waters. Animals observed during these surveys were assigned to ecotype through photographs showing distinct morphological differences, and through genetic analysis of biopsy samples. All three killer whale ecotypes were present in the Aleutians and the Bering Sea. Many Resident-type animals were observed around Kodiak Island and the Aleutians, but few in between. Transients were observed off the Alaska Peninsula and in the Aleutians but much less commonly to the east toward Kodiak. Abundance estimates derived from line-

transect surveys for the area from Kodiak to the central Aleutians were: Residents 1675 whales (95% CI = 1166-2404); and Transients 543 whales (95% CI = 172-1712). There is evidence from genetic data that the central Aleutians region (notably in the area of Samalga Pass) represents a mixing zone for different populations; this is consistent with local oceanography and with known distribution breaks observed in other marine and avian species. Northern Resident haplotypes occur primarily to the east of the Pass, while haplotypes sampled on the western side are primarily of the Southern Resident type.

Dahlheim summarized what was known about the range and movement patterns of killer whales in the eastern North Pacific. Ranges of southern, northern and Alaskan Resident killer whales show some degree of overlap toward the extreme ends of their known ranges (and occasionally within the main portion of the range); however, while animals from different populations are sometimes seen in the same area, they do not appear to mix. A similar pattern of overlap is observed in Transient-type whales. Sasha Burdin has collected biopsy samples of killer whales off Kamchatka, and these are currently being analyzed.

Offshore killer whales appear to be capable of much more extensive movements, with matches recorded between the Bering Sea and locations as far afield as British Columbia and California. There is some evidence for a seasonal basis to these movements; for example, sightings of Offshores off California occur primarily in winter.

Mesnick summarized recent genetic insights into the social structure of killer whales observed in offshore waters, using samples obtained from the Eastern Tropical Pacific (LJ/04/KW9). Both mtDNA and microsatellites were used. Six of nine sampled groups had a single (Offshore) haplotype. Two other groups had at least two haplotypes, and one had at least three (not all groups were completely sampled). Mesnick cautioned that it was not clear what constitutes a "group," and that some samples may therefore have come from multiple (mixed) groups. Haplotypes in these groups were either the known Offshore type, or new types that were unassignable to ecotype; however, in an analysis the latter grouped more closely with known Resident types. One of the haplotypes (from a whale off Hawaii) had previously been recorded from a Type-A Antarctic whale. Overall, the mtDNA data show the existence of multiple haplotypes (and therefore matrilineal groups) in killer whale groups in offshore waters. The preliminary microsatellite data showed a high degree of within-group relatedness (many shared alleles); in some cases, even animals with different mtDNA haplotypes were closely related, possibly suggesting shared

paternities. A far lower rate of shared alleles was observed between groups. Mesnick cautioned that the data were preliminary, and that additional analyses involving more markers were required.

Summary of differences between Resident and Transient-type killer whales in the eastern North Pacific

Resident- and Transient-type whales in the ENP exhibit measurable differences in morphology (different dorsal fin and saddle patch shapes, Baird and Stacey 1988), group size (modal differences, with Transient groups being smaller, Baird and Dill 1996), dispersal (none for Residents versus some for Transients), range and distribution, contaminant burdens (generally higher in Transients) and acoustic repertoires and call types. Five known Transient mtDNA haplotypes were associated with Transient-type animals in all existing samples; these haplotypes do not appear in any other animals worldwide (although related haplotypes do).

Offshore-type killer whales are also different from Residents and Transients in several characteristics: morphology (the dorsal fin represents a third type in shape, and the saddle patch is more like that of Residents, though not identical and frequently much less defined); body size (generally smaller with less sexual size dimorphism), group size (probably larger but not certain), range (probably larger), dispersal (unknown), and contaminant burden (more similar to that of Resident-type whales). Only a single mtDNA haplotype has been assigned to confirmed Offshore-type animals, and is closer to the haplotypes of Residents than Transients. The acoustic characteristics of Offshore whales, and their relationship to Residents and Transients, are unclear.

Do killer whales ever switch ecotypes?

The question of whether killer whales of a particular ecotype ever switch to radically different prey at various times is important given the use of differing ecotypes as partial evidence in arguments for speciation. Soviet whalers operating in the Southern Ocean suggested the possibility that the same killer whale groups were exploiting very different prey (i.e. both fish and marine mammals) at different times, perhaps depending on seasonal availability, but the reliability of these reports is uncertain. In the ENP, data from field observations and from fatty acid analysis support the idea that the diet is consistent year-round (e.g., Resident-type whales always feed on fish and perhaps squid, with no indication that they take marine mammals). Stable isotope analyses (A. Abend and G. Worthy, pers. comm.) suggest trophic-level differences in prey between Resident- and Transient-type killer whales in this region, although since skin samples were used for those analyses, in-

terpretation is constrained by the fast turnover of this tissue (and thus of the isotopic signatures therein).

Contaminant levels and patterns of detected congeners differ between Transient- and Resident-type killer whales along the western coast of North America, and also markedly by area. Mammalian prey of killer whales can metabolize organochlorine contaminants, which fish cannot; as a result, the pattern of contaminants in mammalian prey are passed on to Transient-type killer whales, and are distinguishable in contaminant profiles of the latter relative to those of Resident-type (fish-eating) animals. Because individual killer whales have not been repeatedly sampled, contaminant analysis cannot currently be used to assess whether prey switching occurs.

As noted above, vocalization patterns are quite different between Transient and Resident-type killer whales, which is consistent with different foraging patterns. Specifically, fish-eating whales are much more vocal than mammal-eating whales, which is presumed to relate to the greater ability of mammals to recognize (through hearing) imminent predation. In some cases (e.g. Norwegian killer whales, which appear to be herring specialists), sound may be used as a herding strategy. The lack of observed changes in vocalization patterns over the year in groups of one ecotype provides further support for the idea that there is no major prey-switching on a seasonal basis.

Overall, available evidence strongly points to a lack of dietary overlap between the fish-eating and mammal-eating types. However, it must be recognized that we do not know whether prey switching occurs on a much longer timescale (e.g. decades). Stable isotope analysis of museum specimens and of annual biopsies of individuals of known ecotype might provide insights into this question.

Genetic studies: implications for ecotype and population differentiation

In terms of phylogenetics, the relationship of killer whales to other cetaceans is not entirely clear. A cytochrome b analysis (LeDuc et al. 1999) did not show a clear relationship between this taxon and other cetaceans; Orcaella was the closest species, but the link was rather weakly supported. The fossil record for killer whales is poor, but one specimen dating to about 5 MYA is clearly a killer whale of some kind.

Hoelzel and colleagues (Hoelzel et al. 2002, Hoelzel unpublished data) analyzed samples from numerous locations worldwide including the ENP (Resident, Transient and Offshore types), the western North Pacific (Russia), the eastern and western North Atlantic, Argentina, New Zealand and Antarctica (a single Type A animal). Killer whale mtDNA (control region) exhibits low diversity and no consistent geo-

graphical pattern worldwide, which may be due to a bottleneck occurring some 150,000-300,000 years ago. Lack of regional variation may be due to the strictly matrilineal expansion of local populations.

Some working group participants expressed doubt that a species with such a widespread distribution could have undergone a bottleneck; interestingly, low diversity is also characteristic of some other abundant, cosmopolitan species (e.g. sperm whales and humans). It has also been suggested that a bottleneck may have occurred in sperm whales, but this suggestion raised extensive discussion in the literature (Whitehead 1998 and responses). Although humans have relatively low mtDNA diversity given their current distribution and population size, the absolute level of diversity is higher than in killer whales. The ratio between census population size and effective population size in killer whales is not clear, but is a factor that should be considered in such analyses.

Hoelzel's analysis of killer whales samples from the North Pacific indicated that all individuals in a single local community had the same mtDNA genotype, but some of the same types were found in different, widely separated communities (e.g. from Washington State and Russia). Resident and Offshore types are closely related in terms of mtDNA, and quite distinct from Transient types. Overall, Hoelzel considered that the genetic data provided no reason to reclassify the ecotypes as different species. There are fixed differences at three sites (sequence length = 995bp) between Resident and Transient-type whales for mtDNA control region lineages, although these lineages are defined by few haplotypes (2 and 5, respectively). Overall, these findings, in association with data on cultural and foraging differences (recognizable differences between types) could be explained by a pattern of fairly shallow (i.e. recent) divergence of matrilineages that invaded an area and subsequently underwent fission. Whether this is the beginning of a process of speciation, or an equilibrium process (with ongoing but low levels of gene flow), is not clear. It is possible that multiple ecotypes have evolved on multiple occasions over the evolutionary history of killer whales.

Microsatellite analysis showed significant differences among virtually all populations sampled, although the results could be heavily influenced by small effective population sizes in combination with kinship. Gene flow analyses of microsatellite data among Alaskan Transients, Alaskan Residents and Southern Residents produced results ranging from one to seven migrants per generation (a generation is estimated to represent 25 years). In light of the assumptions underlying the analytical method used, as well as the fact that the results are at odds with current knowledge (notably regarding lack of observed

dispersal between Residents and Transients), some participants expressed skepticism regarding the reliability of these estimates. One general difficulty is that the moderate levels of nuclear genetic differentiation can be explained either by an equilibrium model involving low levels of ongoing gene flow, or by an isolation model involving recent divergence and no current gene flow. The methods available to date cannot reliably distinguish between these hypotheses, but new models (e.g. as described by Hey et al. – 2004) may be able to in the future.

Hoelzel presented an additional analysis using the program STRUCTURE, which attempts to determine the number of gene pools in a mixed sample and assign individuals to the most likely gene pool. This analysis produced results generally consistent with currently hypothesized population structure in the North Pacific, but also identified some individuals that may be recent migrants. One of these might be explained by a sample taken in a probable mixing zone of two populations in the Bering Sea. Some members felt that another potential "migrant" may have been an artifact of the fact that there are likely unsampled populations in the North Pacific. Although the analysis was run allowing for the existence of one or more unsampled populations, the potential effect of unsampled populations on individual assignments is difficult to evaluate.

LeDuc and Taylor presented mtDNA control region sequence haplotypes from mid and low latitudes in the North Pacific, adding these to new and published data from higher latitudes and from other regions (LJ/04/KW7). The sequences from animals sampled in temperate and tropical waters, together with samples from Antarctica, added considerably to the number of haplotypes recorded globally from killer whales. The previously reported distinction between so-called "Resident" and "Transient" haplotypes was still maintained in a global phylogeny, but was not congruent with patterns of known ecotypes. However, distributional patterns of the haplotypes suggest that the Atlantic basin carries only haplotypes from the side of the phylogeny that contains the "Resident" and "Offshore" types, referred to as the "R" clade (in contrast to the "T" clade). Such a result implies that this division in the mitochondrial phylogeny may have arisen as differentiation between ocean basins. The observed haplotype distribution, together with the latitudinal limits of known fish specialists in the Northern Hemisphere, leads to a hypothesis of killer whale biogeography and evolution. Specifically, Northern and Southern Residents are hypothesized to be recent arrivals to the North Pacific from the North Atlantic, with subsequent character displacement leading to the ecological specialization seen today between fish and mammal specialists in the North

Pacific. Habitat differences in lower latitudes suggest that killer whales in these areas tend to be generalists. The possibilities of comparable specialization in other regions, as well as the possibility of a *rassenkreis* (species circle), cannot be ruled out.

In the Pacific, the largest gap in current sampling (relative to the distribution of killer whale sightings) is off Central America and northern South America. Also, given that the deep-water zone between 40 and 50 degrees North is known to be a high-productivity area and has not been covered well by sighting surveys, it is possible that killer whales exist there, and samples from that area would be very useful.

LeDuc and Pitman summarized mtDNA analyses of 49 samples from Antarctica (LJ/04/KW8). They believed the results supported the contention that two or three species of killer whales exist in that region. The 49 samples yielded 16 haplotypes. The Type B and C killer whales were closely related and constituted what appeared to be a monophyletic clade, although it was noted that firm conclusions could not be drawn until the tree was rooted. There were fixed differences between Type A whales and the other two types, indicating female reproductive isolation and thus (in the authors' view) a situation consistent with separate species status. Evidence for reproductive isolation between Types B and C was weaker, although the authors noted that, unless intermediate types exist, the existing data were suggestive of separate species status under the Biological Species Concept. This might also be consistent with marked differences in morphology and prey preferences between these two forms.

Some other participants disagreed with LeDuc and Pitman's conclusions, citing similar-level genetic differences among populations of single species of terrestrial taxa (e.g., jackals, ravens), as well as similarities with some human populations. It was noted that the existence of different ecotypes in killer whales, showing very short mtDNA branch lengths, is not dissimilar to some other taxa (e.g. wolves), and that it is possible that these forms evolve repeatedly and quite quickly over evolutionary time. This apparent plasticity suggests that such forms could be relatively ephemeral in evolutionary time. In wolves, animals raised in certain habitats and feeding on certain prey tend to colonize similar habitats when they disperse; this tendency appears to be the major axis of genetic differentiation in wolves, and it does not mean that different ecotypes represent different species. African canids such as wild dog packs in Kruger National Park show high F_{st} values (e.g. 0.25, as large or larger than values found between killer whale ecotypes), but dispersal is mediated by kinship (when packs break up they form new groups based on same-sex siblings); these are not unlike clans in killer

whales. Some participants disagreed with this argument, and suggested that the degree of foraging specialization in killer whales is fundamentally greater than in other mammals. Mayr (1996) stated, "...there are no niches of mammal species that would be suitable for sympatric speciation"; however, this was not the case with killer whales, in which Baird et al. (1992) suggested disruptive selection for prey type as a potential mechanism for sympatric divergence.

As noted above, Hoelzel's estimates for divergence of ecotypes worldwide, following a possible bottleneck in the late Pleistocene, was on the order of 150,000 years, based upon mtDNA control region data. M. Ford addressed the question of divergence time of Southern Resident killer whales using simple models applied to published genetic data (mtDNA and microsatellites) (LJ/04/KW6). Both types of markers are consistent with a broad range of divergence times, from hundreds to hundreds of thousands of years, depending on the assumptions. Assuming a complete isolation model, the microsatellite data suggest a divergence time of less than 3000 years, but even a small amount of migration would make the data consistent with an ancient divergence. The mtDNA data clearly indicate that female gene flow does not occur regularly even at low levels, but this does not rule out the possibility of male-mediated gene flow. Like the microsatellite data, the mtDNA results are consistent with a very broad range of divergence times, depending on the assumptions made. For example, estimates of divergence time are affected by values used for effective population size. In addition, some of the haplotypes might be ancient, predating any divergence. Overall, the genetic data on killer whales are open to several interpretations regarding the date of the divergence and the nature of subsequent radiation. For example, the broad distribution of some haplotypes worldwide could reflect stochastic events following an ancient divergence and population expansion, while the much tighter genetic pattern observed in the Antarctic Types B and C suggests a single founder event.

Some participants felt it was important to recognize that genetic data reveal patterns on a long-term historical timescale but that, in contrast, we are attempting to interpret these differences in light of present-day observations. Such interpretations are questionable given the fact that the ecosystem was very different hundreds of years ago. It could be speculated that the existence of much larger fish prior to over-exploitation by human fisheries meant that killer whales now feeding on large mammalian prey were once exploiting fish. However, there is obviously no way at present to assess the likelihood of this.

Other participants noted that, although "lost" ecotypes in killer whales might eventually be replaced

by reappearance of the type in question, this would likely not occur on a human management timeframe (decades or centuries), and therefore such replacement should not be anticipated as a the basis for present-day management.

Other information relating to ecotypes

Fung presented data to address the question of whether different foraging specializations (ecotypes) were reflected in anatomical differences (Fung 2004). Various measurements of skulls and associated features tentatively suggested anatomical differences between Residents and Transients. For example, the data could be taken as suggesting that Transients have larger skulls and more massive jaws, possibly indicating the development of more powerful musculature for dealing with larger (mammalian) prey. However, this was based upon small sample sizes with little associated information about the animals concerned; thus it was not clear whether age and size differences in the samples could account for these (and similar) results. Genetic analysis of the sampled specimens should be undertaken to determine their ecotype; some of this work is currently underway.

Pitman noted a Soviet observation that fish-eating killer whales in Antarctica supposedly had significantly smaller flukes than killer whales preying upon mammals. Aerial photogrammetry in this and other areas would be a useful way to assess whether such morphological differences truly exist among ecotypes.

Sexual size dimorphism in killer whales may be more related to foraging specialization (i.e. different roles within a group) than to the mating system. Data on testis size in mature male killer whales are sparse, which is regrettable given that this information might be very informative with regard to the mating system; different mating systems can be reliable indicators of reproductive isolation among species.

Are different killer whale ecotypes separate species?

The working group recognized at the outset that there was no way to select an approach to species designation in killer whales that would be consistent with such designations for all other taxa, since there is no consistency in this regard across the animal kingdom (including in cetacean taxonomy).

In general, the participants recognized that it was not currently possible to discriminate between a single species that was capable of plastic, ephemeral adaptations (i.e. foraging specializations = ecotypes) and the early stages of a true speciation event (i.e. adaptive radiation on an irreversible evolutionary path).

It could be argued that killer whales are not typical mammals. For example, they constitute a rare mam-

malian example of sympatric divergence of different types, and possess a complex social system that likely promotes isolation among groups. Looking to taxa that are ecologically or culturally similar might represent a way forward, but such taxa were not identified. It could also be argued that considerable value should be placed on the cultural uniqueness of local killer whale groups, and that this should be given strong consideration in conservation. However, an alternative view is that cultural traditions are ephemeral and are therefore relatively unimportant for consideration with respect to species-level questions.

One approach to assessing species status would be to take all of the available information on killer whales, including data on morphology, acoustics, distribution and genetics (etc), and test it against criteria for different species concepts. This approach might well yield different conclusions depending on the concept used.

The working group also recognized that, in general, significant and reliably quantified morphological differences between groups of animals provide strong evidence for separate species, and that such differences may or may not be accompanied by genetic differentiation. Similarly, cryptic species (those showing no obvious morphological differences but which are genetically and/or ecologically distinct) may exist. Whether either of these cases applies to killer whales is the topic of much debate.

It was suggested that our inability to entirely delineate differences among types of killer whales should not stop us from defining reproductive isolation in cases where it clearly exists. However, there is currently no agreement on this issue.

Several participants felt strongly that the way in which taxonomic classification is conducted should not be changed to suit management purposes. It should continue to attempt to classify taxa based upon objective scientific observations rather than political considerations relating to conservation needs.

Summary of arguments

Overall, a number of divergent views on how to classify killer whales were expressed. Arguments for *Orcinus orca* being a single species, several species or several subspecies can be summarized as follows (caveats to some of the supporting contentions are noted in earlier sections of this report).

Single-species argument -- The proponents of the idea that killer whales constitute a single global species argued as follows:

- The mtDNA data could be explained by remnant variation from an ancient divergence, and these data therefore are not very

informative in regard to higher-level structures. There is evidence that some populations are extended family units that were founded relatively recently from an oceanic population, when the current habitat became available (post-glaciation). The mtDNA data do not identify different lineages that correlate to ecotypes; instead, they suggest that trophic specialization may have evolved multiple times within the genus.

- The nuclear data do not discriminate unequivocally among ecotypes. For example, the Resident ecotype in the North Pacific shows the same genetic distance between the whale off Kamchatka (Russia and) Washington State (same mtDNA type), as between Resident and Transients in the ENP.
- Genetic data can be taken as indicative of a fairly recent divergence. This could mean that we are witnessing the early stages of a unique divergence, with insufficient time having elapsed to establish strong genetic differences. Alternatively, the ecotypes may be ephemeral phenomena that appear and disappear over relatively short evolutionary timescales.
- Foraging specializations and acoustic repertoires are likely learned and therefore might be ephemeral, and as such they are not good indicators of species difference.
- Morphological differences are observed in other taxa that are not necessarily considered to indicate separate species (although not so much in mammals).
- While there are some intriguing and suggestive observations on possible morphological differences among killer whale ecotypes, little quantification of these purported differences exists at present, and until better data become available it is premature to conclude that multiple species exist.

Multiple-species argument -- The proponents of the idea that killer whales constitute multiple species argued as follows:

- A congruence exists between patterns of genetic variation, acoustics, color patterns and ecological specialization between Residents and Transients, at least in the eastern North Pacific.
- Within those groups, mtDNA haplotypes are shared across a geographic range, but there is no evidence of movement across ecological space (i.e. no Transient animal has been observed to switch to the Resident ecotype,

or vice versa). Haplotype sharing can be historical and does not necessarily imply recent movement.

- The variation within ecotypes does not negate the differences among them.
- The mtDNA data, as well as the lack of interaction among Residents and Transients in the North Pacific, provide evidence for female philopatry. It is unclear how other killer whales elsewhere should be placed relative to these groups.
- The data on morphology and genetics, and on trophic and habitat specialization (and the absence of intermediate forms) in Antarctic killer whales suggests that there are at least two, and possibly three species in this region. Types B and C are separate from other types elsewhere in the world, while Type A may or may not be the same species as the ENP Transient type.
- Overall, in the above view there may be four species: ENP Residents, ENP Transients (possibly including Antarctic A), Antarctic B/C (possibly two species, with the caveat that the separation of these types is currently based upon morphology, not genetics).

Subspecies argument -- Discussion of whether different types of killer whales represent subspecies became mired in disagreements about the validity of the subspecies as a taxonomic unit, and how it could be defined as something different from a discrete population.

A brief review in the working group of sub-species designations in odontocetes indicated that these have usually been based upon morphological differences with known or presumed genetic differentiation. Most such designations have involved allopatric or parapatric groups; in the latter, there are sometimes intermediate forms in areas of overlap. In some cases (e.g. inshore/offshore bottlenose dolphins), no subspecies designation has been made despite the existence of evidence that is consistent with that used for recognizing other cetacean subspecies.

Some participants felt that it was not wise to discuss subspecies without an explicit definition of the term, and no agreement could be reached on such a definition. A further complication was the uncertainty among participants regarding the conventions of zoological nomenclature conventions, specifically how subspecies trinomials would be assigned to (for example) ENP Residents and Transients, and how these ecotypes relate to the original designation of *Orcinus orca*.

Synthesis -- Overall, participants were divided over whether or not different killer whale ecotypes constituted separate species. The range of views can be broadly characterized as follows:

- Of the 17 participants in the working group, six felt that there was only a single species of killer whale worldwide, and that this designation will remain valid when additional data become available.
- Only one participant felt that multiple species definitely exist and that existing data support this.
- The majority of participants (ten) felt that multiple species probably exist, at least in Antarctica, and that this might well be confirmed in the future, but that current data are limited (absent or insufficiently quantified) and therefore that full species designation is at this point premature. In some cases, subspecies or ESU designations might be more appropriate until better data become available.
- The working group agreed that it was not possible at this point to comment meaningfully on the relationship between Antarctic and ENP killer whales on the basis of existing data, except to reiterate nominal similarities between Antarctic Type B and ENP Transients, and Antarctic Type C and ENP Residents.
- All 11 participants who felt that there are (or probably are) multiple species of killer whales based this belief upon the Biological Species Concept. Those who supported only one species based this opinion on either the Biological Species Concept, the Phylogenetic Species Concept, or both; some of this latter group felt that subspecies designations might be appropriate for the ENP, Antarctica, or both. Others in the same group felt that subspecies designations were based largely upon geographic distinctions and thus were not appropriate for use in killer whales.
- Overall, a majority of participants felt that Resident- and Transient-type killer whales in the ENP probably merited species or subspecies status.
- The relationship of Offshore-type killer whales to Residents and Transients (or any other killer whales in the world) is not clear.
- Insufficient time was available to consider in more detail proposed agenda items related to Puget Sound Southern Residents, their historic population size and distribution, and

their relationships to other ENP killer whales.

FUTURE WORK

The following topics for future work were considered high priorities to help resolve outstanding taxonomic issues (note that these are not listed in order of importance):

- The current nomenclature systems for ecotypes (“Residents,” “Transients,” etc.) and for haplotypes are very confusing, especially to those who do not work directly with killer whales, and a new standardized system should be developed to clarify the situation. E-mail correspondence groups were formed under Baird (ecotypes) and J. Ford (haplotypes) for this purpose.
- Microsatellite data should be obtained for all ENP whales, and pedigrees should be established.
- Genetic researchers should coordinate their activities, pool samples, and standardize analyses.
- Additional analyses should be conducted using stable isotopes and fatty acids to elucidate dietary preferences of different ecotypes.
- Morphological differences among ecotypes should be better quantified, using oblique photographs, aerial photogrammetry, or other methods.
- Better information should be obtained on reproduction and mating systems, using recently developed methods for assessing pregnancy from skin biopsies, as well as anatomical examination of dead animals, or other techniques.
- Biopsies should be obtained from killer whale calves for paternity analyses.
- Additional genetic markers (e.g., Y chromosome, other autosomals) should be developed.
- The effect of kinship on genetic analyses should be assessed.
- Biopsy sampling by different individuals should be coordinated to standardize collection and preservation techniques and thus maximize the use of each sample for multiple analyses. Similarly, protocols for necropsy of stranded animals should be widely distributed so that the maximum amount of information can be obtained from each recorded death.

- Additional acoustic sampling from other populations is needed.
- Greater genetic sampling within populations would give a better idea of how genetic variation has changed over time. This should include sampling of Southern Residents, with priority given to the oldest animals so that they are sampled before they die.
- Greater sampling of different populations worldwide is needed.

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