

**Symposium on the results of the SPLASH humpback whale  
study**

**Final Report and Recommendations**

**11 October 2009  
Quebec City, Canada**

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Commission on Environmental Cooperation  
Hawaii Humpback Whale National Marine Sanctuary

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**SPLASH Symposium Schedule - 11 October 2009**  
**Quebec Convention Center Room 303AB**

<b>Time</b>	<b>General section</b>	<b>Presenter</b>	<b>Topic</b>
8:00	Meet/introductions	David Mattila / John Calambokidis	Meet/introductions
8:20	General overview	John Calambokidis	Intro to SPLASH and overview of findings
8:40	General overview	Erin Falcone	SPLASH data management and visual health assessment
9:00	Sample analyses	Bree Witteveen	Stable Isotope results from SPLASH
9:20	Sample analyses/impacts	Cris Elfes / Paul Wade	Analysis of contaminants in SPLASH samples
9:40	Impacts	Jooke Robbins	Entanglement scarring and implications
10:00	Break		
10:20	Impacts	Ed Lyman	Gear entangling N. Pacific humpbacks during SPLASH years
10:40	Regional -feeding	Jan Straley	Gulf of AK inshore-offshore movement
11:00	Regional -feeding	Heather Riley	Regional analysis of humpbacks in the E. Aleutians
11:20	Regional -feeding	John Calambokidis	Regional examination of humpback whales off the US West Coast incl. long term trends
11:40	Regional -feeding	John Ford	British Columbia humpback whales
12:00	Lunch		
13:00	Regional -feeding	Sasha Burdin	Humpback whales in summering areas in the Russian Far East
13:20	Regional-Wintering	Jorge Urban	Overview of Mexico, Ursula presenting at Biennial
13:40	Regional-Wintering	Jeff Jacobsen	Humpback whales of the Revillagigedos
14:00	Regional-Wintering	Manami Yamaguchi	Humpback whales in Asia
14:20	Other Oceans	Rochelle Constantine	S Pacific humpback whale studies
14:40	Other Oceans	Phil Clapham	N. Atlantic humpback whales: MONAH/YONAH
15:00	Break		
15:30	Population and Management units	Jay Barlow	SimSPLASH and abundance estimates
15:45	Population and Management units	Paul Wade	Alternate abundance estimates from MARK
16:00	Population and Management units	Debbie Steele	Migratory interchange and sex specific estimates of abundance based on genotypes

16:15	Population and Management units	Scott Baker	Genetic differentiation of eco-stocks and breeding-stocks in North Pacific humpback whales
16:30	Population and Management units	Barb Taylor	Management units
16:45	Population and Management units	Panel (Barlow, Wade, Baker, Taylor, Urban, and Ford)	Management units, downlisting (IUCN,ESA), conservation issues, long term access and use of SPLASH information
17:15	Wrap up		Wrap up -concluding remarks

## Attendees/Participants

Close to 150 people attended the Symposium. The list below shows those who registered either before or at the Symposium but does not include all participants:

Adam Pack	Jean-Luc Tison	Phil Clapham
Alecia Van Atta	Jeff Jacobsen	Phil Hammond
Alison Agness	Jennifer L. McGee	Philippe Bouchet
Alyson Fleming	Jennifer Tackaberry	Richard M Pace, III
Amy Sloan	Jim Borrowman	Rochelle Constantine
Andrea Bendlin	John Calambokidis	Sally Mizroch
Astrid Frisch	John Ford	Sasha Burdin
Barbara Taylor	John Hildebrand	Sergio Martinez Aguilar
Bree Witteveen	Jooke Robbins	Teri Rowles
C Scott Baker	Jorge Urban	Theresa Kirchner
Carolyn Kraft	Julia Goss	Thomas Norris
Carrie Hubard	Julie Rivers	Ursula Gonzalez
Charles J. Rennie III	Kagari Aoki	Wendy Szaniszlo
Chiara Giulia Bertulli	Kate Swails	Yoko MITANI
Chris Gabrielle	Katherine Ralls	Yulia Ivashchenko
Christian Ramp	Keiko Sekiguchi	Paul Wade
Christie McMillan	Kristy Beard	Allan Ligon
Claire GARRIGUE	Laetitia Georgina Schmid	Naomi McIntosh
Craig Hayslip	Laura Ganley	Kate Wynne
Craig Matkin	Linda Nichol	Mina Innes
Cristiane Elfes	lori beraha	Beth Goodwin
Danielle Cholewiak	Lou Herman	Sara Celano
David Mattila	Maita Moura	Jen Jackson
Debbie Steele	Manami Yamaguchi	Morten Tange Olsen
Dee Allen	Mark Fraker	Gaby Serra-Valente
Diane Alps	Mary Grady	Hannah Herman
Edward Lyman	Mason T Weinrich	Bob Brownell
Elia Herman	Maude Tremblay	Olgavon Ziegesar
Ellen Chenoweth	Megan Kessler	Patricia Naessig
Erin Falcone	Michelle Yuen	Michael Johns
Ester Quintana	Monica DeAngelis	Kim Valentine
Frederick Wenzel	Morgan Richie	Fred Sharpe
Graemme Ellis	Nancy Black	Adam U
Heather Riley	Nic Dedeluk	Sandra Pompa
Ivan Chan	Nicole Brandt	Siri Hakala
Jackie Hildering	Paula Olson	Kristin Rasmussen
Jan Straley	Paulina Godoy	Haruna Okabe
Jay Barlow	Petra Reimann	Amy Hapeman

## **Extended Abstracts of SPLASH Symposium presentations**

## Introduction to SPLASH and overview of findings

Presented by John Calambokidis, Cascadia Research, 218½ W 4<sup>th</sup> Ave., Olympia, WA 98501

SPLASH (Structure of Populations, Levels of Abundance and Status of Humpbacks) represents one of the largest international collaborative studies of any whale population ever conducted. It was designed to determine the abundance, trends, movements, and population structure of humpback whales throughout the North Pacific and to examine human impacts on this population (Calambokidis et al. 2008). This study involved over 50 research groups and more than 400 researchers in 10 countries. It was supported by a number of agencies and organizations including the National Marine Fisheries Service, the National Marine Sanctuary Program, National Fish and Wildlife Foundation, Pacific Life Foundation, Department of Fisheries and Oceans Canada, and Commission for Environmental Cooperation with additional support from a number of other organizations and governments for effort in specific regions.

Field efforts were conducted on all known winter breeding regions for humpback whales in the North Pacific during three seasons (2004, 2005, 2006) and all known summer feeding areas during two seasons (2004, 2005). A total of 18,469 quality fluke identification photographs were taken during over 27,000 approaches of humpback whales. After reconciling all within and cross-regional matches, a total of 7,971 unique individuals were cataloged in SPLASH. A total of 6,178 tissue samples were also collected for genetic studies of population structure, with fairly even representation of wintering and feeding areas.

Migratory movements and population structure of humpback whales in the North Pacific were found to be more complex than had been previously described; a high degree of structure, however, was also apparent (Figure 1). Migrations between feeding and wintering areas were documented based on 873 whales that were seen on both a wintering and feeding areas. The overall pattern showed that coastal areas of western (Asia) and eastern (mainland Mexico and Central America) North Pacific were the primary wintering areas for coastal feeding aggregations in the western and eastern North Pacific. The wintering areas off Hawaii and the Revillagigedos were the primary wintering regions for the more central latitude feeding areas.

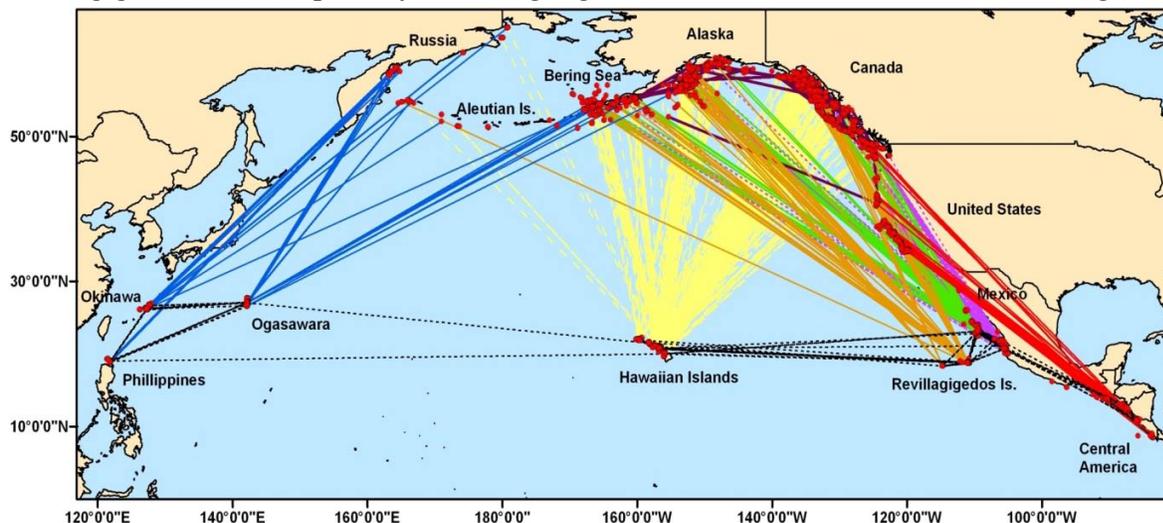


Figure 1. Connections between resightings of identified humpback whales in the SPLASH study color coded by wintering area.

Even though the SPLASH study collected data from all known wintering and feeding areas for humpback whales in the North Pacific, the SPLASH data did suggest the likely existence of missing wintering areas that have not been previously described. Humpback whales that feed off the Aleutians and in the Bering Sea were not well represented on any of the sampled wintering areas and must be going to one or more unsampled winter locations. Thus, it is likely that SPLASH has revealed a new breeding ground for humpback whales. While it would be logical to assume that this region would be located in the eastern central North Pacific, the complexities of the migratory pathways revealed here indicate that this is not certain.

Individual whales showed high rates of return to specific wintering and feeding areas, suggesting strong site fidelity to both habitats. Interchange of whales between feeding areas both within and between seasons was unusual, and all but a few of these were between adjacent areas. Similarly, whales tended to return to the wintering region they had inhabited previously, although the geographic scale of this varied by region. Whales showed frequent interchange among areas within the Hawaiian Islands but only rarely switched between broader regions. Some wintering areas that were sampled, especially Ogasawara and Baja Mexico, appeared to be transitory areas rather than migratory destinations. These findings are consistent with preliminary analyses of the genetic structure population showing a high degree of maternally-directed fidelity to both breeding and feeding grounds but a complex relationship between seasonal habitats.

Using several methods, the abundance of humpback whales was estimated to be about 20,000 for the entire North Pacific, and these and other new estimates of abundance are discussed in more detail in some of the later talks in this Symposium. Over 50% of this population was estimated to winter in Hawaiian waters with large numbers also inhabiting Mexican waters. The abundance estimates of humpback whales wintering in Asia and Central America were fairly low (1,000 or less). Among feeding areas, regional estimates differed greatly among models. Average estimates of abundance ranged from about 100-700 for Russia, 6,000-14,000 for the Bering Sea and Aleutians, 3,000-5,000 each for the Gulf of Alaska and the combined Southeast Alaska and Northern British Columbia area, 200-400 for Southern British Columbia-Northern Washington, and 1,400-1,700 for California-Oregon.

*Table 1. Estimates of annual increases in humpback whale abundance based on comparison to previous estimates and those with similar methods (Calambokidis et al. 1997, 2001).*

Region/basis	Previous		Current		Yr span	Annual incr.
	Yr	Estimate	Yr	Estimate		
<b>Total N Pacific estimates</b>						
Hilborn best NPAC to best SPLASH	1991-93	9,819	2004-06	18,307	13	4.9%
Rice to best SPLASH Hilborn	1966	1,400	2004-06	18,307	39	6.8%
<b>Hawaii estimates</b>						
Adj. year Petersen NPAC to SPLASH	1991-93	3,556	2004-06	7,120	13	5.5%
Hilborn – Wint/Feed NPAC-SPLASH	1991-93	3,760	2004-06	8,034	13	6.0%
Petersen using SEAK marks	1991-93	5,151	2004-06	10,425	13	5.6%
<b>Asia</b>						
Adj year Petersen NPAC to SPLASH	1991-93	405	2004-06	943	13	6.7%

The SPLASH estimate represents a dramatic increase in abundance from other post-whaling estimates for the overall North Pacific, yet is consistent with a moderate rate of

recovery for a depleted population. Comparison of the SPLASH estimate of 18,302 for all feeding and wintering areas to the estimate of 9,819 obtained for 1991-93 in a previous study suggests a 4.9% annual increase over this 13-year period. Going back to the estimate of 1,400 whales at the end of whaling for humpbacks in 1966, a 6.8% annual increase over the 39-year period would be required to reach the current SPLASH abundance. For Hawaii, three methods were used to compare estimates to determine trends since the early 1990s and yielded very similar annual rate of increase from 5.5 to 6.0%.

While the overall humpback whale abundance and trends in the North Pacific are encouraging, some areas should be of concern, especially Asia. The western-most feeding and wintering areas were distinct from the rest of the North Pacific with a very low level of interchange between Asian wintering or feeding areas and those in the central and eastern North Pacific. Abundance estimates in this area are low (below historical levels based on the number taken in this region) and whales along the Asian coast appear to be subject to a high level of incidental mortality.

### **References cited**

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- Calambokidis, J., E.A. Falcone, , T.J. Quinn, A.M. Burdin, P.J. Clapham, J.K.B. Ford, C.M. Gabriele, R. LeDuc, D. Mattila, L. Rojas-Bracho, J.M. Straley, B.L. Taylor, J. Urbán R., D. Weller, B.H. Witteveen, M. Yamaguchi, A. Bendlin, D. Camacho, K. Flynn , A. Havron, J. Huggins, N. Maloney, J. Barlow, and P.R. Wade. 2008. SPLASH: Structure of Populations, Levels of Abundance and Status of Humpback Whales in the North Pacific. Final report for Contract AB133F-03-RP-00078 prepared by Cascadia Research for U.S. Dept of Commerce. May 2008.

## **Flukes, flanks and the SPLASH Central Matching Office: How your data became SPLASH data, where it is now, and where it is headed**

Presented by Erin Falcone, Cascadia Research, 218½ W 4<sup>th</sup> Ave., Olympia, WA 98501

Cascadia Research Collective acted as the central coordinating body for processing, archiving, and analysis of all sighting and photographic data collected under the SPLASH program. Given that SPLASH relied heavily on the collaboration of existing researchers within each sampling region, there was a strong interest to allow for the maximum flexibility in the data collection protocols to avoid compromising existing projects while still ensuring complete and consistent data collection for accomplishing the study objectives. This in turn required a similar degree of flexibility on the part of Cascadia and the regional coordinators for reconciling the wide variety of data formats which were received following each season. Beyond the initial importation of data, Cascadia also needed to develop efficient and effective means of managing increasingly large collections of digital images, comparing the many collections, and estimating any error rate these processes might have introduced into the final results. This abstract summarizes the methodology developed by Cascadia for accomplishing these tasks, the present status of the collection, and plans underway for future use and accessibility. It also includes a brief summary of the use of SPLASH flank photographs for monitoring population level trends in health and human injury.

SPLASH requested a comprehensive suite of information from participants, including data related to effort, overall group composition and behavior, individual behavior and photo-identification, and tissue sample collection. Data collected in the field was compiled first at the regional level by regional coordinators, who combined most within-region submissions into a more unified format, and who also conducted a preliminary reconciliation of identification photos from that region and season, prior to providing the data (both digital and raw copies of field notes) and photos (a digital or printed regional catalog and complete archive of all images in jpg format) to Cascadia. Cascadia then imported data from each region into a relational database in Microsoft Access 2003 format, comprised of six primary data tables and two supplementary data tables for further processing and cross-regional reconciliation (Figure 1). Regional coordinators also sent all tissue samples to the Southwest Fisheries Science Center in La Jolla, CA for archiving a distribution for subsequent analyses.

SPLASH began just as most research groups were making the conversion from film to digital photography, so techniques for managing large digital collections were still relatively new. Cascadia opted for the use of ACDSee photo management software, in part for its tools for managing metadata associated with images but also because it provided a useful plug-in that allowed for custom printing of images and associated metadata without sequential editing and resaving original files. An early decision was made to conduct all matching with printed photographs rather than digital images, as it allowed for more standardized viewing and editing of images and eliminated variation associated with monitor resolution, and matchers were familiar with matching in this format and found it produced less eye strain.

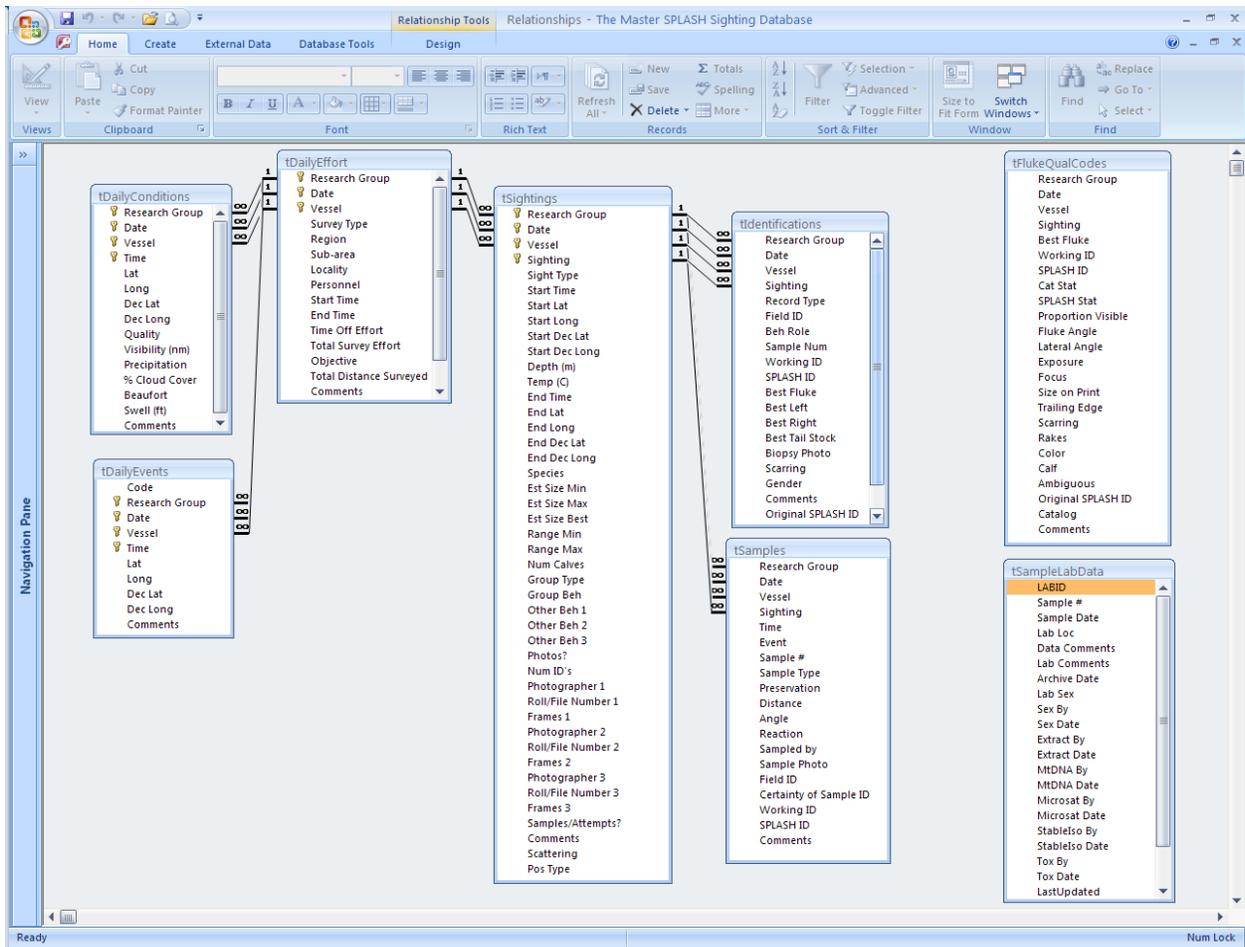


Figure 1. Screen shot of the data fields and table structure of the SPLASH database.

Once best-of-sighting fluke identification photos had been printed, they underwent comprehensive quality and characteristic (color and distinctiveness) coding. Those whales exceeding specific quality criteria were then compiled into a regional/seasonal catalog and assigned a SPLASH ID number following a system that indicated the year and region when the ID was assigned, and this ID was updated into all occurrences of the whale in the database. While it is often referred to singly, the SPLASH catalog actually consists of 24 separate regional-seasonal collections which allowed for multiple simultaneous comparisons to be conducted, and prioritized by known regional match rates.

It was evident early in the study that the SPLASH collection was going to be prohibitively large to allow for a complete duplicate reconciliation. Even a single match of every single fluke against every other would have precluded the allotted time for completing the study, so several protocols were developed to expedite matching. These included restricting the number of color categories a fluke was compared against, and also the removal of a whale from active seasonal comparison once it was identified in an earlier catalog. These steps, coupled with the prioritization of comparisons between high match rate collections (e.g. the first comparisons were always between the same region in a previous season) both increased the chances of finding a given whale quickly and reduced the total number of whales it had to be checked against. Because it was accepted that these steps had the potential to introduce a degree of error into the

final results, a series of additional steps were also taken to quantify this error rate, and also to refine protocols during the comparison accordingly. These included selective duplicate comparisons each season, and also the introduction of 266 known matches into the final SPLASH season (Winter 2006), such that the error rate within this collection could be determined, and factors affecting match success could be assessed. In the end the overall missed match rate within the collection was estimated at less than 10%, a level that was deemed acceptable for analyses based on the results.

Today the SPLASH collection exists with each whale represented by its best of season image in each catalog where it existed in the primary reconciliation, although all whales are assigned to the single lowest SPLASH ID number it first received. A fully reconciled dataset, including all field data, match data, and reconciled sample data is maintained by Cascadia. Results from subsequent analyses, including sex and haplotype from tissue samples, are being incorporated into this database as they become available. The single best fluke photos of all individuals in the SPLASH catalog have also been compiled into a complete digital catalog, and techniques are under development for managing future comparisons within this collection, and also making this available to SPLASH participants and potentially the public in the coming years.

While the primary objectives of SPLASH relied on fluke photographs, the study also requested that contributors collect flank photographs of whales encountered. A preliminary study was conducted by Cascadia in which approximately 3,000 flank photographs from animals with a SPLASH ID were scored for quality and the following visible characteristics, mostly related to health and human impacts: evidence of injury and possible cause, overall body and skin condition, pigmentation patterns, prevalence and nature of lesions, dorsal fin damage, scarring on the body, and the prevalence of “pocks”, “bumps”, rake marks, and barnacles. Analyses suggested several population-level trends were evident even in this limited sample of flanks, including detectable decline/improvement in average body condition of whales expected with fasting and migration, and regional variation in several of the scored features. Although limited in sample size, the analysis also suggested that whales at continental feedings areas (Russia and the US West Coast) are subject to higher rates of serious injury. This analysis shows promise as a tool for population level health and injury assessment, and will be further developed into a manuscript incorporating a larger sample size of scored flanks in the coming year.

# Using stable isotopes to assess population structure and feeding ecology of North Pacific humpback whales

Presented by Bree Witteveen, University of Central Florida; University of Alaska Fairbanks

In recent years, the use of stable isotope analysis has proved a valuable technique for providing information on trophic position, diet, and feeding origins of migratory animals. Isotopes are atoms of the same element with different atomic weights. The utility of stable isotopes stems from the fractionation of the heavy to light isotopes which allow the isotopic signature of a food source to be reflected in a consumer's tissue in a predictable manner. With respect to ecological studies, stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios are most commonly analyzed. Rates of fractionation are small for carbon making  $\delta^{13}\text{C}$  a good predictor of sources of primary productivity and origins of feeding. In contrast, fractionation in  $\delta^{15}\text{N}$  is larger and thus provides a measure of relative trophic position. In this study, skin samples collected from all known feeding and breeding areas as a part of SPLASH were analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and used to increase understanding of the population structure and feeding ecology of North Pacific humpback whales.

Part one of the study addressed the hypothesis that the stable isotope ratios of foraging humpback whales reflect location of feeding through two objectives: 1) describe distinct feeding groups of humpback whales through analysis of geographic variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and 2) use classification tree analysis to develop a predictive model to assign individuals to their foraging origins based on the observed variation.

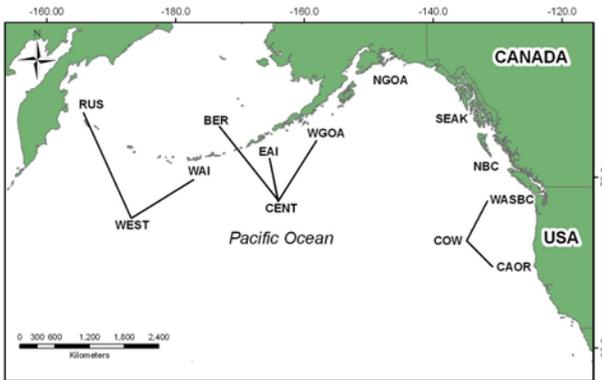


Figure 1: Map of the North Pacific Ocean showing the 10 sampling regions of the SPLASH project. Lines drawn from sampling regions indicate consolidated feeding groups. Sampling locations are also shown (x).

Table 1. Classification results produced by classification tree analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) as predicting variables for humpback whale skin collected from six feeding groups.

Feeding Group	Predicted Feeding Group						Total	% Correct
	WEST	CENT	NGOA	SEAK	NBC	COW		
WEST	28	14	12	26	0	1	81	35%
CENT	19	218	17	15	12	1	282	77%
NGOA	1	50	66	47	16	18	198	33%
SEAK	3	28	24	150	8	14	227	66%
NBC	2	44	21	35	25	8	135	19%
COW	1	3	18	14	3	142	181	78%
							Overall	57%

Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were calculated from skin samples ( $n = 1105$ ) collected across 10 feeding regions. Based on geographic considerations and results of initial statistical analyses, the 10 feeding regions were combined into six feeding groups (Fig 1). Analysis of variance (ANOVA) showed that the feeding groups were significant different from one another for both isotope ratios ( $F_{5,1098} = 102.9, p < 0.001$  for  $\delta^{13}\text{C}$  and

$F_{5,1099} = 130.0, p < 0.001$  for  $\delta^{15}\text{N}$ ). The classification tree using both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as parameters was able to assign individual feeding whales to their correct feeding location with 57% accuracy (Table 1). Classification accuracy ranged from a low of 19% for the northern British Columbia (NBC) group to a high of 78% for the California, Oregon, and

Washington (COW) group. Accuracy based on random assignment to feeding groups was only 17%, meaning the classification tree was 3.4 times better at assignment than chance. Misclassification was most often to a neighboring feeding group. Reasons for misclassification may include similarities in latitude or longitude of collection site, feeding groups that are more transitional areas and not distinct feeding areas, and the low sample size of the western most feeding grounds.

The migratory movements of humpback whales were explored in the second part of this study through the hypothesis that the stable isotope ratios in tissues assimilated while on feeding

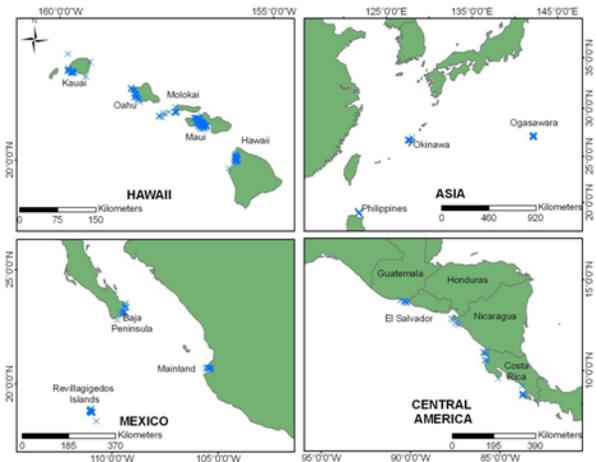


Figure 2: Maps of breeding areas within each of the 4 SPLASH sampling regions. Locations of sample collections (x) are also shown

groups are retained while on breeding grounds and enable North Pacific humpback whales sampled on breeding grounds to be assigned to a specific feeding group. This hypothesis was address through two objectives. The objectives were to 1) analyze the stable isotope ratios of individual humpback whales sampled on both their feeding and breeding grounds and 2) use the classification tree developed from the feeding analysis to assign breeding animals to a feeding group. Stable carbon and nitrogen values were calculated from skin samples ( $n = 597$ ) from four breeding regions in the North Pacific (Fig 2). ANOVAs showed that both isotope ratios were significant different between

these regions ( $F_{7,589} = 34.9, p < 0.001$  for  $\delta^{13}\text{C}$  and  $F_{7,589} = 18.9, p < 0.001$  for  $\delta^{15}\text{N}$ ). A total of 46 individuals were sampled on both their feeding and breeding habitat. Linear regression between their feeding and breeding isotope signatures were significant (Fig 3) while pair-wise t-tests were not ( $t_{45} = 1.41, p = 0.17$  for  $\delta^{15}\text{N}$ ;  $t_{45} = -1.15, p = 0.26$  for  $\delta^{13}\text{C}$ ). Together these results support the assumption that the stable isotope ratios remain relatively unchanged on breeding grounds and allowed for the application of the classification tree model. Assignment of breeding whales to feeding groups using the classification tree showed regional patterns of movement with the western-most breeding grounds assigned with a greater frequency to the western-most feeding groups and assignment of the more eastern breeding groups to the COW feeding group (Fig 4). Patterns were quite similar to those resulting from SPLASH matching of fluke photographs.

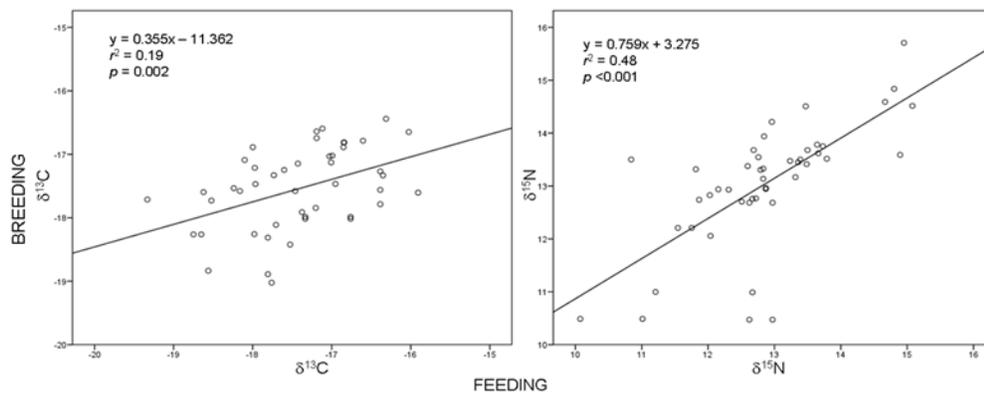


Figure 3: Relationships between feeding and breeding values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for 46 ind. humpback whales sampled in both breeding and feeding habitats. Each point represents a single animal.

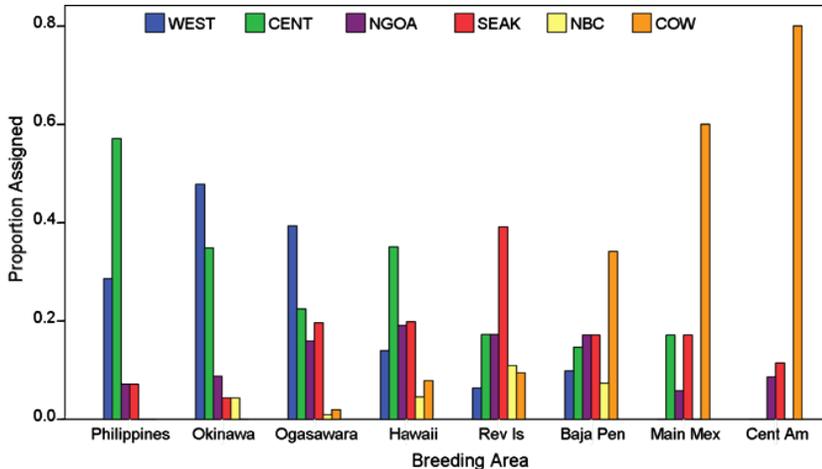


Figure 4. Distribution of breeding area animals assigned to feeding groups based on: (a) classification tree analysis of stable isotope ratios and (b) SPLASH photographic matching. Numbers indicate the total number of photographic matches made for each breeding area

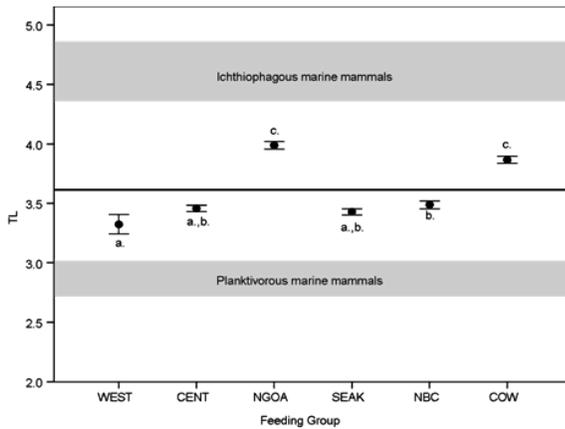


Figure 5: Mean ( $\pm$  S.E.) TL for each of the six feeding groups of North Pacific humpback whales. The solid black line represents the overall mean values for all groups. Shaded regions represent the range in trophic levels for strictly fish eating (4.4 to 4.8) and strictly plankton eating (2.8 to 3.0) marine mammals.

The third and final hypothesis to be addressed stated that  $\delta^{15}\text{N}$  reflects relative trophic position and can be used to compare foraging habits between feeding groups. The mean trophic level of the six feeding groups was calculated as  $2 + (\delta^{15}\text{N}_{\text{humpbackwhale}} - \delta^{15}\text{N}_{1^\circ\text{consumer}})/2.4$ , where 2 is the trophic level of regional primary consumers and 2.4 is the average enrichment in  $\delta^{15}\text{N}$  per trophic level for marine mammals.

Primary consumers were collected from the geographic region of each feeding group and

included copepods, scallops, and mussels. The mean trophic level of North Pacific humpback whales was  $3.6 (\pm 0.02)$  supporting long-standing assumptions that humpback whales are generalist predators (Fig 5). The north Gulf of Alaska (NGOA) and COW feeding groups showed the highest trophic levels, suggesting the diets in these regions are higher in fish. In contrast, trophic level in the WEST was lower indicating a diet higher in zooplankton.

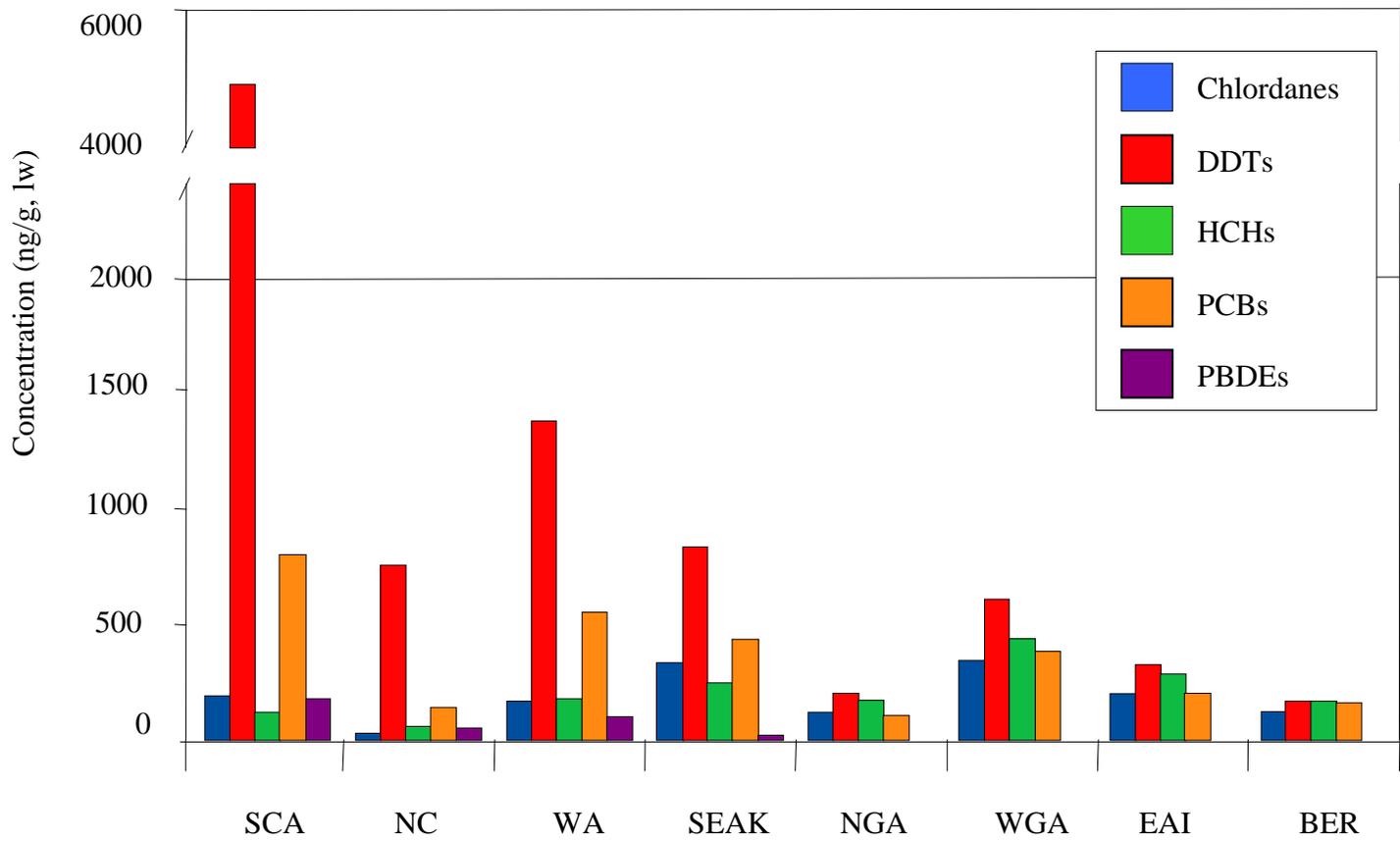
Stable isotope techniques commonly used to describe components of the life history of migratory animals were successfully applied and showed the utility and benefits of such techniques for studying humpback whale ecology.

## **Persistent organic pollutant levels in North Pacific humpback whale feeding areas.**

Presented by Cristiane T. Elfes, Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St, Box 355020, Seattle, WA 98195, USA Email: celfes@u.washington.edu

Seasonal feeding behavior and high fidelity to feeding areas allow humpback whales (*Megaptera novaeangliae*) to be used as biological indicators of regional contamination. Limited information is available on humpback whale contaminant levels, particularly on a large scale. Biopsy blubber samples from male individuals (n=67) were collected through SPLASH, a multi-national research project, in eight North Pacific feeding grounds. Persistent organic pollutants (POPs) were measured in the samples and used to assess contaminant distribution throughout the feeding areas (Figure 1), as well as to investigate the potential for health impacts on the study populations. Concentrations of polychlorinated biphenyls (PCBs), dichloro diphenyl trichloroethanes (DDTs), and polybrominated diphenyl ethers (PBDEs) were more prevalent along the U.S. West Coast, with highest concentrations detected in southern California and Washington whales. A different pattern was observed for chlordanes and hexachlorocyclohexanes (HCHs), with highest concentrations detected in the western Gulf of Alaska whales and those from other high latitude regions, including southeast Alaska and eastern Aleutian Islands. PBDEs were not detected in remote areas, but were found in whales feeding closer to industrialized centers. Concentrations of DDTs were exceptionally high in humpback blubber samples collected off southern California and levels decreased in samples collected to the north and west along the Pacific Rim. When northern and southern California regions were compared, mean concentrations of all POP classes were significantly higher in southern California whales. This difference was most significant for DDTs, with mean concentrations in southern California samples more than six times those of northern California, likely the result of historic discharge of DDTs off Palos Verdes Peninsula. Blubber from humpbacks from the western Gulf of Alaska (Shumagin Islands) consistently showed higher POP levels than found for whales from neighboring areas (northern Gulf of Alaska and eastern Aleutian Islands). It is unclear whether local sources, or other factors, such as a higher proportion of older animals, may have contributed to these differences. In general, contaminant levels in humpback whales were comparable to other mysticetes, and lower than those found in odontocete cetaceans and pinnipeds. Whereas these concentrations likely do not represent a significant conservation threat, levels of some contaminants, particularly DDTs in southern California may warrant further study.

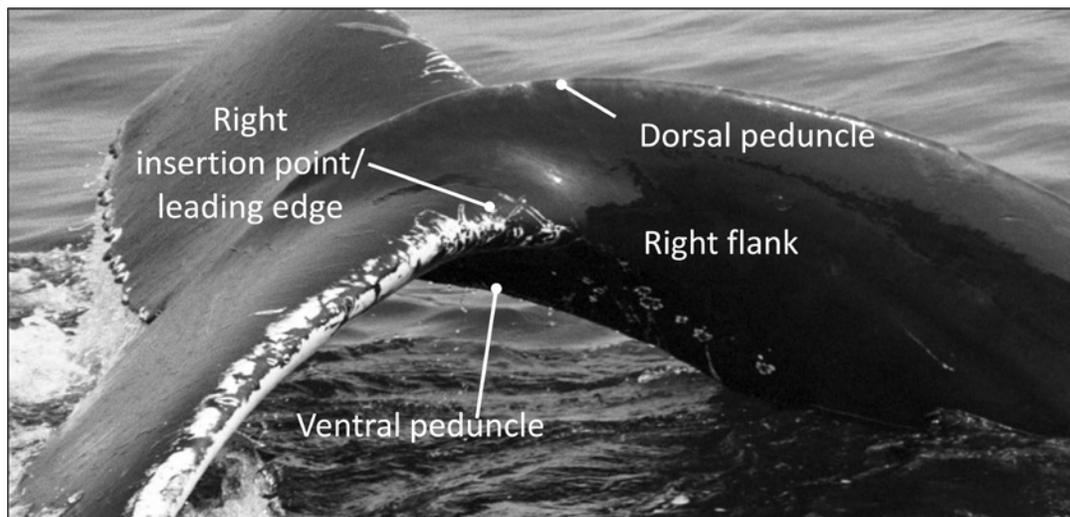
Figure 1. Geometric mean concentrations (ng/g, lipid weight) of contaminants by feeding region.



## Entanglement Scarring on North Pacific Humpback Whales

Presenter: Jooke Robbins, Provincetown Center for Coastal Studies

Entanglement in fishing gear is a documented source of injury and mortality to humpback whales in the North Pacific. However, the frequency of events, areas of concern and impacts to populations are not yet known. Systematic sampling and scar interpretation were performed as part of the SPLASH project to provide insight into these issues across the North Pacific Ocean. Participants were instructed to photograph the posterior tailstock/leading edges of the flukes whenever they were in proper orientation (Figure 1), without regard for apparent injuries. Images were obtained opportunistically, secondary to photo-identification and biopsy sampling priorities. Nearly 4,500 images of potential relevance were obtained and subsequently screened for use in this study. Of these, 20% (911) were considered optimum quality angle, distance, focus and lighting for scar analysis. Images were examined for evidence of scars or injuries that were consistent with wrapping around at least two of six coded features (Figure 1). Recent (unhealed) injuries were observed in all regions studied. Sample sizes were not adequate to rigorously compare all feeding areas, but results indicated lower entanglement frequency at the Bering Sea versus Southeast Alaska and Northern British Columbia, with the Northern Gulf of Alaska as intermediary (Figure 2). Hawaii was not significantly different from other breeding grounds, although Asia had a lower frequency of entanglement injuries than Mexico (Figure 3). Overall, results were consistent with higher entanglement rates in coastal feeding areas, and particularly in the Eastern North Pacific. With further development, scar-based studies of survivors may also provide insight into entanglement mortalities which are otherwise missed by this method. This was the first effort to study large whale entanglement across an ocean basin. The results confirm entanglement to be a wide-spread issue, with coastal areas particular priorities for further research. Local monitoring programs should consider continuing to collect these data to build upon the SPLASH baseline data set.



*Figure 1: Example of preferred image for scar-based inference, showing four of six coding areas.*

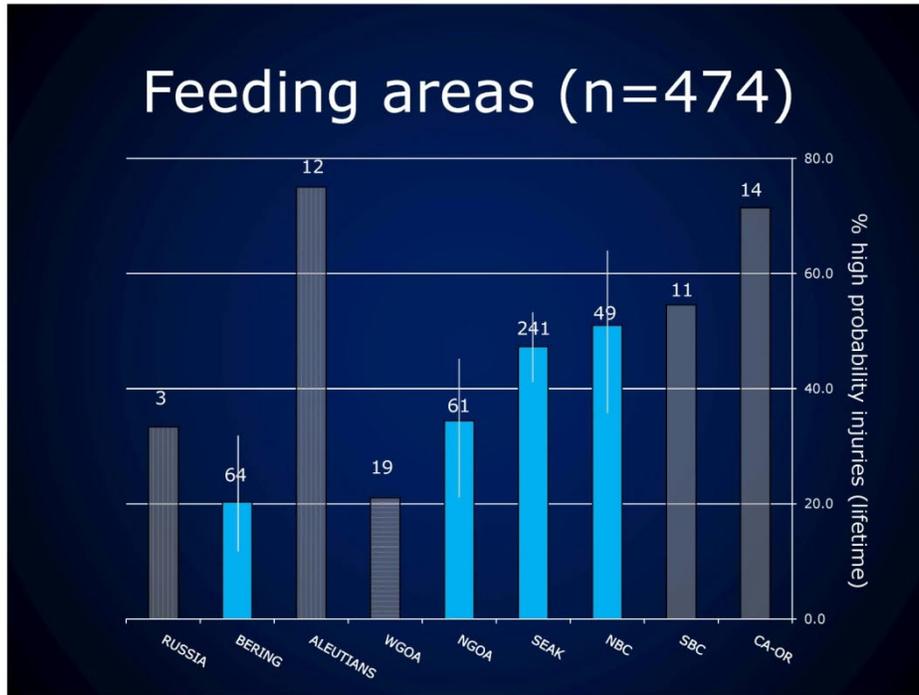


Figure 2: Frequency of high probability entanglement injuries across North Pacific feeding areas (bars). Sample sizes shown in white. Blue bars with 95% confidence intervals (vertical lines) were areas with sample sizes adequate for regional comparisons.

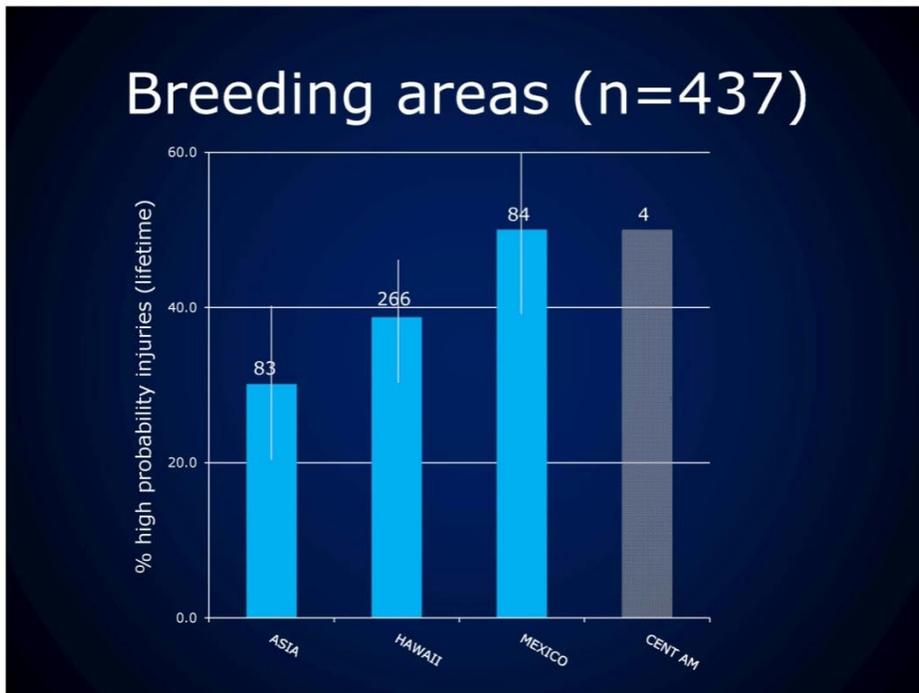


Figure 3: Frequency of high probability entanglement injuries across North Pacific breeding grounds (bars). Sample sizes shown in white. Blue bars with 95% confidence intervals (vertical lines) were areas with sample sizes adequate for regional comparisons.

## **A preliminary investigation of gear entangling humpback whales, *Megaptera novaeagliae*, in the North Pacific**

Presented by: Ed Lyman, Hawaiian Islands Humpback Whale National Marine Sanctuary

Many countries rely heavily on opportunistic reports of large whale entanglements for assessing by-catch and for management of populations. While past studies of some regions have shown that approximately 50% of these reports could not be confirmed (Lyman et al, 2007), the other half were confirmed and represented valuable information. Here we take a preliminary look at the confirmed reports to get an idea of the frequency of entanglements and the types of gear found entangling humpback whales (*Megaptera novaeagliae*) within much of the western North Pacific. Johnson and others (2005) performed a similar, and more in-depth, investigation on the gear entangling humpback and northern right whales in the western North Atlantic. They found that confirmed reports in which gear was identified provided a great deal of insight on humpback and right whale entanglements in the region.

Confirmed humpback whale entanglement reports and identifiable gear types were compiled from Alaska (AK), British Columbia (BC), Washington (WA), Oregon (OR), California (CA), and Hawaii (HI) between 2001 and October, 2009; thus covering a great deal of the North Pacific over a significant amount of time. In addition, if data allowed, reports were analyzed for gear source (set location), entanglement duration (based on when gear was last tended), and lethality of gear.

*Table 1: Confirmed reports of entangled humpback whales over region*

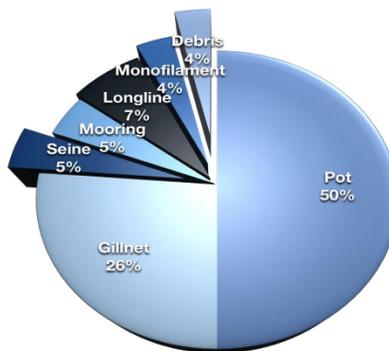
	<b>HI</b>	<b>AK</b>	<b>BC</b>	<b>OR/ WA</b>	<b>CA</b>	<b>Total</b>
<b>2001</b>	3	6	0	0	1	10
<b>2002</b>	6	6	2	0	0	14
<b>2003</b>	4	4	1	0	4	13
<b>2004</b>	1	8	4	0	1	14
<b>2005</b>	6	16	4	0	3	29
<b>2006</b>	10	15	2	1	4	32
<b>2007</b>	7	10	4	1	4	26
<b>2008</b>	8	7	3	1	2	21
<b>2009</b>	7	6	9	0	1	23
<b>Total</b>	52	78	29	3	20	182
<b>Avg/ yr.</b>	5.8	8.7	3.2	0.3	2.2	20.2

For the area and time covered, 182 reports of entangled humpbacks were confirmed. Table 1 shows the breakdown by region and year. From these reports, 40% of the gear recovered and/or documented entangling humpback whales was unidentified (Table 2). Johnson and others (2005) found that 20% of the gear entangling humpback and right whales in the western North Atlantic could not be identified. However, of the gear identified, most (76%) reported entangling humpback whales in the western North Pacific involved passively set, fixed-fishing gear, like pots (traps) and gillnets (Diagram 1). Similar results were found for the investigation of gear entangling right and humpback whales in the western North Atlantic, where 89% of the gear was found to be passively set, fixed-fishing gear (Johnson et al, 2005).

Table 2: Gear types from confirmed humpback whale entanglement reports

	HI	AK	BC	OR/ WA	CA	Total
<b>Pot</b>	13	20	11	2	9	55
<b>Gillnet</b>	0	18	8	0	3	29
<b>Seine</b>	0	4	1	0	0	5
<b>Mooring</b>	2	0	3	0	0	5
<b>Longline</b>	5	2	1	0	0	8
<b>Monofilament</b>	3	1	0	0	0	4
<b>Debris</b>	3	1	0	0	0	4
<b>Unknown</b>	26	32	5	1	8	72
<b>Total</b>	52	78	29	3	20	182
<b>Total Known</b>	26	46	24	2	12	110
<b>% Known</b>	50.0%	59.0%	82.8%	66.7%	60.0%	60.4%

Diagram 1: Proportion of known gear types



Some of the gear found entangling humpback whales was carried over long distances. For instance, gear recovered and/ or documented from 8 entangled humpbacks within the Hawaii breeding/ calving grounds was set off Alaska or British Columbia. Seven of these reports were pot gear, including crab, fish, and shrimp fisheries. The average minimum straight-line distance the gear was carried was 2,175 nm. The longest distance, in which the exact location of the set was known, was a straight-line distance of 2,450 nm. It involved shrimp pot gear set near Wrangell, AK, which was later removed, from the animal off Maui, HI. These distances are much greater than those found in the western North Atlantic, but effort differed. Whereas a great deal of effort has recently been carried out in the Hawaii calving and breeding grounds, less effort, and thus fewer reports, have been received for entanglements in the breeding and calving grounds in the North Atlantic.

Sample sizes were small in determining the duration of entanglement (N=3) for gear carried between Alaska and Hawaii, but for those known, the average maximum duration was 47 days. The minimum possible duration was 30 days for the humpback whale carrying gear from Wrangell, AK, to Maui, HI. Average minimum speed using these values was 2.2 knots. Gillnet entanglements represented nearly 30% of confirmed reports where the gear was identified. Alaska had the highest incidence of gillnet entanglements at 39% (n=46), with British Columbia at 33% (n=24) and California at 25% (n=12). Hawaii and the northwest US (OR and WA) had no confirmed reports of humpback whales entangled in gillnets during the period. For

the Alaska region, more reports of humpback whales entangled in gillnet involved mortality (17%, N=18) compared to reports of entanglement in pot gear involving mortality (0%, N=20). Five reports of humpback whales reported entangled off Hawaii were confirmed as carrying local pot gear. However, there were no confirmed reports of humpback whales in Alaska carrying identified gear from Hawaii.

Six (6) percent (11 of 182) of all confirmed reports of entangled humpback whales involved mortality. The most lethal gear based on reports received and ability to confirm gear type was seine gear. Sixty percent (60%) of reported and confirmed seine entanglements involved mortality. Of these, 80% reported involvement of juvenile animals. Gillnet and pond gear were the next deadliest gear types. However, sample sizes were small across all gear types. Age class of individuals reported entangled was known in 64% of cases (118 of 182). The majority of cases/ reports (47%, n=56) involved juveniles. Of the juvenile entanglements 7% (n=4) involved mortality. However, results may represent minimums, since identity of juveniles was based almost entirely on size, rather than known age. The higher percentage of juveniles may reflect inexperience?

Results rely heavily on filtering the opportunistic reports and many other variables that affect our ability to quantify entanglement threat. These variables include: small sample sizes, the nature of the gear, and how gear might affect the animal. For instance, how identifiable is the gear over time? What is the impact of the gear on the animal? Gear that is less identifiable over time, or more immediately lethal will likely be under-represented in reports. So, more effort needs to be invested in gathering information on large whale entanglement threat. While much can be garnered from the filtering of opportunistic reports, more emphasis needs to be placed on more dedicated and directed efforts to obtain information on the large whale by-catch issue.

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### **Acknowledgements and disclaimer:**

Marine mammal response networks in the United States and Canada, including NOAA Fisheries Protected Resources Divisions in Alaska, the Northwest and Southwest regions, and the Pacific Islands region, and Canada's Department of Fisheries and Oceans, collected data. These networks are staffed and supported by many individuals, institutions, and agencies. Much of the information they collected was obtained from opportunistic platforms and voluntary reporting of dead and distressed animals. It is unknown to what extent all incidents are reported. As a result, absence of incidents at any location does not demonstrate absence of a threat in the report's timeframe.

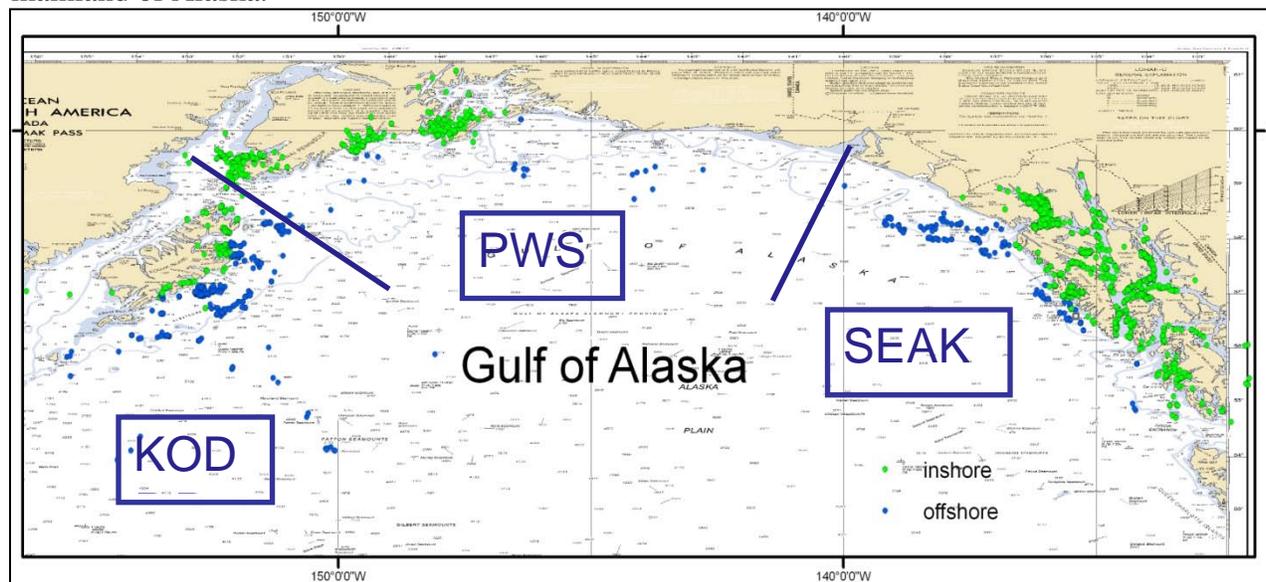
## **Inshore and offshore movement of humpback whales in the Gulf of Alaska: Are offshore whales different from their coastal neighbors?**

Presented by Jan Straley, Univ. of Alaska Southeast, Sitka, AK

Collaborators: Bree Witteveen, Scott Baker, Erin Falcone, Debbie Steel, Olga von Ziegesar, Ellen Chenoweth Jay Barlow, Kelly Robertson, Christine Gabriele, Janet Neilson, Craig Matkin, Paul Wade and Beth Goodwin.

Humpback whales have been studied in the coastal waters of the Gulf of Alaska (GOA) since the late 1960s but offshore data is scarce. Difficulty in working offshore in small vessels and limited funding for humpback research precluded data collection despite reports of large numbers of whales reported in offshore waters in the GOA by fishermen and others. Fortunately, private and federal funding provided SPLASH the opportunity for large ship surveys to collect humpback whale data offshore during 2004 and 2005.

The GOA study area was divided up into three primary areas: Kodiak (KOD), Prince William Sound (PWS) and southeastern Alaska (SEAK) (Fig. 1). The boundary dividing KOD and PWS was just north of Kodiak Island and the boundary dividing PWS and SEAK was at Cape Suckling. Each area was further divided between offshore and inshore making a total of six sub areas. Offshore was defined as the waters beyond the 50 fathom contour along the perimeter of the GOA. Inshore was defined as waters from the 50 fathom contour to the mainland of Alaska.



*Figure 1. Three inshore areas and three offshore study areas in the Gulf of Alaska (GOA). Blue dots represent offshore whale sightings and green dots represent inshore sightings.*

Multiple methodologies were used to characterize humpback whales in the GOA: 1) photo identification of individuals provided overall numbers of whales sighted 2) sightings of whales seen two or more times documented movement across or within areas and 3) biopsy skin samples determined mtDNA haplotype, sex and trophic level. Photo identification data were from SPLASH surveys during 2004 and 2005. Sex and mtDNA haplotype data were analyzed for KOD, offshore PWS and SEAK from samples collected during SPLASH in 2004 only.

Inshore PWS samples were collected by SPLASH in 2004 and supplemented with samples collected by von Ziegesar, Goodwin and Baker in 2002 and analyzed by Baker and Steel. SPLASH sex data was supplemented with known sexes of individual whales from regional KOD and SEAK databases based on genetic data only. Pairwise  $\chi^2$  analysis was used to differentiate haplotypes and sex by area ( $\alpha=0.05$ ). Trophic level data was a subset of the data presented by BW at this symposium and analyzed on a finer geographic scale for this presentation. Trophic level analysis used stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) adjusted for  $\delta^{15}\text{N}$  of regional of primary consumers and determined if whales were primarily fish or zooplankton feeders. Differences in trophic levels were determined through Tukey-Kramer HSD ( $\alpha=0.05$ ) and sized for all differences among the means.

**Sightings:** The overall number of humpback whale sightings was 4,585 with 3,537 seen inshore and 1,058 seen offshore. These sightings represented 2,136 unique individuals with twice as many whales sighted inshore than offshore (Table 1). A total of 748 whales were seen at least twice inshore, with only 185 seen at least twice offshore, while 109 whales were seen in both inshore and offshore areas (Table 2).

Table 1. Number of unique whales seen in the GOA, 2004-05.

Area	# whales
Inshore only	1,348
Offshore only	679
Both areas	109
Total	2,136

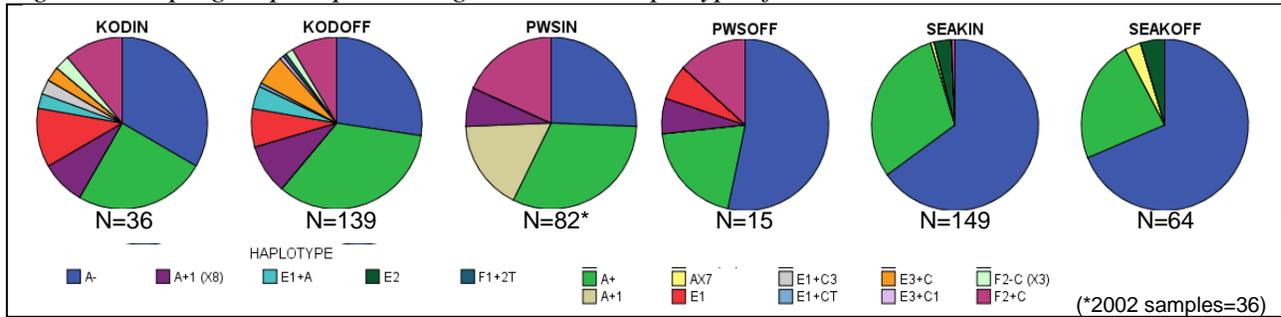
Table 2. Movement of the 109 whales seen both inshore and offshore in the GOA, 2004-05.

		Inshore				
Offshore	Area	KOD	PWS	SEAK	Total	
	KOD	35	3	1	39	
	PWS	15	12	6	33	
	SEAK	2	0	35	37	
	Total	52	15	42	109	

**Movement:** Of the total number of unique whales sighted in KOD (N=752), PWS (N=384) and SEAK (N=1149), 7%, 9% and 4%, respectively, were seen both an inshore and offshore subarea. This movement was primarily aligned with the adjacent offshore or inshore areas for KOD and SEAK (Table 2). However, movement within the PWS primary area was not as clear, with 12 whales sighted in both inshore and offshore PWS areas and 15 whales seen both offshore PWS and inshore KOD. This movement may simply reflect whales in transit migrating through the offshore PWS waters to reach inshore KOD. Observed movement between other non adjacent inshore and offshore primary areas was minimal. No whale was sighted in three or more areas.

**mtDNA:** GOA humpback whales (N=485) are represented by 15 mitochondrial (mtDNA) haplotypes (Figure 2). The frequency of haplotypes present within each area is defined as a haplogroup. SEAK inshore and offshore haplogroups were not significantly different from one another but were significantly different than all other subareas. KOD inshore, offshore and offshore PWS were not significantly different from each other. Inshore PWS was significantly different than all other subareas.

Figure 2. Haplogroups representing 15 mtDNA haplotypes found in the six subareas in the GOA.



**Sex:** Sexes and sex ratios were determined for 568 whales with more males found in KOD and more females found in PWS (Table 3). However, sex ratios were not significantly different from any other area and the overall sex ratio was 1:1.

Table 3. Sexes and sex ratios of humpback whales sighted in six subareas of the GOA, 2004-05.

Sex/Area	KOD IN	KOD OFF	PWS IN	PWS OFF	SEAK IN	SEAK OFF	total
Male	23	80	35	6	116	33	293
Female	18	59	43	10	110	35	275
M:F	1.3	1.4	0.8	0.6	1.1	0.9	1.1

**Trophic level:** Trophic level for the six subareas ranged from a high of 4.4 for inshore PWS indicating primarily a diet of fish, possibly herring or capelin, to a low of 3.4 offshore SEAK indicating a diet dominated by zooplankton, likely euphausiids (Table 4). Trophic levels differences for the other areas were less distinct.

Table 4. Trophic level pairwise comparisons using Tukey-Kramer HSD. Subareas not connected by the same letter are significantly different.

Area	Mean	Prey
PWS Inshore	4.397	Fish ↓ Zooplankton
KOD Inshore	4.017	
PWS Offshore	3.865	
KOD Offshore	3.809	
SEAK Inshore	3.440	
SEAK Offshore	3.382	

In summary, comparative analysis from multiple methodologies resulted in defining the connections and difference between whales present offshore and inshore in the GOA. These data showed whales in the offshore waters of the GOA are aligned with their nearest coastal neighbor for KOD and SEAK. Clearly, whales present in offshore and inshore SEAK should be considered together as one feeding aggregation, as well as whales present offshore and inshore KOD. PWS inshore whales appear to be distinct genetically and trophically, feeding at a significantly higher trophic level from whales present in all other areas. The relationships between KOD subareas and offshore PWS remain nebulous. Movement data showed connections between KOD and PWS however these data are difficult to interpret because whales may have been sampled while on migration to or from the breeding area. Further resolution of differentiation among subareas for KOD and PWS would be enhanced from increasing the sample size for sightings and tissue samples. This could be accomplished by analyzing the remaining 285 unprocessed SPLASH samples from the northern GOA, particularly for offshore

PWS, and supplementing with additional surveys to fill in geographic gaps. Reassessment of the definition used for offshore may resolve issues for the KOD areas, as well. Timing of movement among areas will help resolve the relationships among the whales sighted in offshore PWS and KOD and will further our understanding of the feeding ecology of the whales present in the GOA.

## Humpback Whale (*Megaptera novaeangliae*) Numbers on their Summer Feeding Grounds of the Eastern Aleutian Islands

Presented by: Heather Riley, M.S. student, University of Alaska Fairbanks

Humpback whales have been studied since the early 70's in nearshore regions of their North Pacific summer feeding grounds in southeastern Alaska and Prince William Sound. In 2001, research extended further west to the Shumagin Islands and into the Eastern Aleutians (Witteveen et al. 2004). SPLASH has provided data to add a better understanding about the whales feeding in coastal waters west of the Shumagin Islands into the Eastern Aleutians.

The objectives of this study were to answer the following questions:

- 1.) To what extent do humpback whales exhibit site fidelity on the summer feeding grounds in the Eastern Aleutian Islands?
- 2.) Can site fidelity be used as a proxy to define a distinct feeding area?
- 3.) How many humpback whales are using the summer feeding grounds in the Eastern Aleutians?

The eastern Aleutian Islands are defined in this study as the islands located between Unimak Pass and Samalga Pass. These islands border the Bering Sea to the northwest and the Pacific Ocean to the southeast. This study primarily focused on sightings that occurred in the nearshore waters of the Bering Sea.

The feeding area described in this study has yielded 1,985 photographs of animals collected between the summers of 2001 through 2006 by the following 4 different research groups: North Gulf Oceanic Society, National Marine Mammal Lab, Southwest Fisheries Science Center, and Cascadia Research Collective (Table 1). All photos were rated good, fair, and poor based on photo quality and only the good and fair photos were used for this study. Each photo was then assigned a fluke distinctiveness rating of low (1), medium (2), or high (3) based on the individual flukes' trailing edge (presence of one or more notches), pattern (identifiable scars), color type, and overall distinctiveness (McSweeney et al. 2007). All photos were then grouped according to fluke color and then matched. Of the 1,985 identification photographs submitted by the 4 research groups, 1,441 were selected for comparison and these resulted in 802 distinct individuals.

*Table 1. Humpback whale summer study months by contributors for the Eastern Aleutians summer feeding grounds, 2001-2006. \*\*\*denotes SPLASH funded years*

Study Group	2001	2002	2003	2004***	2005***	2006
NGOS	Aug	Jun-Aug	Jun-Aug	Jun-Sep	Jul-Aug	Jul-Aug
SWFSC				Aug-Sep		
NMML				Jun-Aug	June-Sep	
CRC					Aug-Sep	

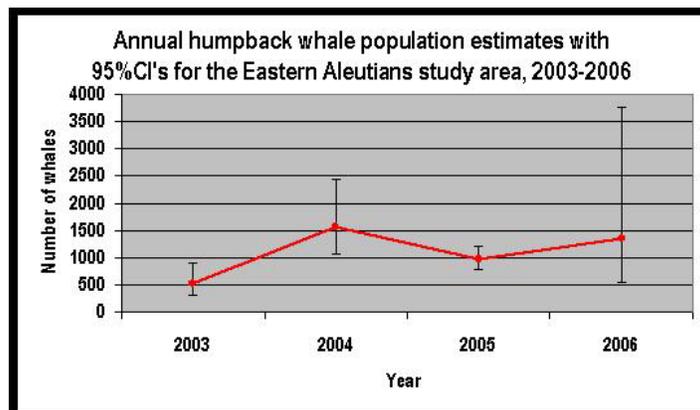
Site fidelity is the trend for individuals to return to the same area over and over. Humpback whales have exhibited fidelity to specific foraging areas in Alaska such as Kodiak and Southeast Alaska. To investigate if site fidelity exists in the Eastern Aleutian summer feeding ground an identity matrix was created. Site fidelity was determined to be 22.57% between 2001 and 2006 (Table 2). This was considered 'moderate' site fidelity when compared with other humpback

whale feeding grounds. This may provide some evidence that the Eastern Aleutians could be a distinct feeding aggregation for humpback whales, however other parameters such as genetics and/or photo ID comparisons to neighboring regions would give us a more detailed understanding.

*Table 2. Humpback whale sighting matrix for the Eastern Aleutian study area, 2001-2006.*

Time of Last Capture	Time of Recapture:						Total # Whales (sum newly captured) minimal estimate
	2001	2002	2003	2004	2005	2006	
2001	0	0	2	0	0	1	
2002	0	0	3	5	11	3	
2003	0	0	0	16	34	10	
2004	0	0	0	0	50	17	
2005	0	0	0	0	0	29	
Recaptured	0	0	5	21	95	60	181
Newly Captured	7	50	111	247	291	96	802
Total Captured	7	50	116	268	386	149	976
Total % Recaptured	22.57%						

Capture-recapture techniques have been used to estimate abundance of humpback whales in Alaskan waters including northern Southeast Alaska (Straley et al. 2008) as well as Kodiak and the Shumagin Islands in western Alaska (Witteveen et al. 2004). Program MARK was used to perform a mark recapture analysis and therefore provide a yearly estimate of the number of humpback whales using this area. A Huggins Full Closed Capture with Heterogeneity dot model was chosen over other models. Data from 2001 and 2002 was excluded from the analysis due to sample size. Time (t), effort (e), and heterogeneity (h) were the biologically reasonable variables tested in each model. Fluke distinctiveness (FLDI) was also added to each model as a covariate. The (Akaike’s Information Criterion)  $AIC_c$  was used to rank models and the  $\Delta AIC_c$  values were used for overall model support. Figure 1 graphs the results from the top model that included (t) and (e) with the covariate FLDI.



*Figure 1. Eastern Aleutian summer humpback whale population estimates using Huggins closed capture with Heterogeneity from Program MARK, 2003-2006.*

This model reported an estimate of between approximately 500 to 1600 animals in Eastern Aleutian summer humpback whale population. It is important to examine the following

violations to the assumptions made by the closed capture population analysis: Calves were not excluded from this study which violates independence (probability of capture is not exclusive from their mother) and the assumption that markings will not change between capture sessions. Inherently, heterogeneity will always be present in animal studies and therefore an animal's behavior may change between capture sessions (trap happy/trap shy). Sampling effort was not consistent which has an effect on capture probability. Lastly, temporary immigration and/or emigration from study area was not controlled for.

To improve population estimates and reduce biases/violations, I suggest that coverage be extended to the north/northeast end of the study area into Bristol Bay and that open population models should also be considered. Describing and comparing habitat utilization in the Eastern Aleutians with other humpback whale summer feeding grounds will also lead to a better understanding of the humpback whales in the Eastern Aleutians.

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## Regional examination of humpback whales feeding off the US West Coast including long-term trends

Presented by John Calambokidis, Cascadia Research, 218½ W 4<sup>th</sup> Ave., Olympia, WA

The US West Coast was one of the SPLASH feeding area regions where long-term photographic identification has been conducted since 1986 by Cascadia Research (Calambokidis et al. 1990, 2009, Calambokidis and Barlow 2004). This presentation was designed to put the SPLASH results for the US West Coast into a long-term context. Photographic identifications of humpback whales along the US West Coast has been conducted annually since 1986 from small boats operating along different portions of the coast. Since 1991, field effort has been conducted along a broad set of regions along the US West Coast. The primary estimates of estimating abundance for this region has been using inter-year Petersen mark-recapture estimates using adjacent years as the two samples. These have yielded very consistent results generally in good agreement with those from line-transect estimates (Calambokidis and Barlow 2004).

High rates of interchange have been documented among many areas primarily as a function of distance apart (Calambokidis et al. 1996). Off the US West Coast, there appears to be two distinct feeding aggregations with little interchange between them. While there is not a defined border between these aggregations, lower densities near the Oregon-Washington border make this a good location to divide these two aggregations. Haplotypes were found to change dramatically (Figure 1) and also changes in winter migratory destinations occurs in this area.

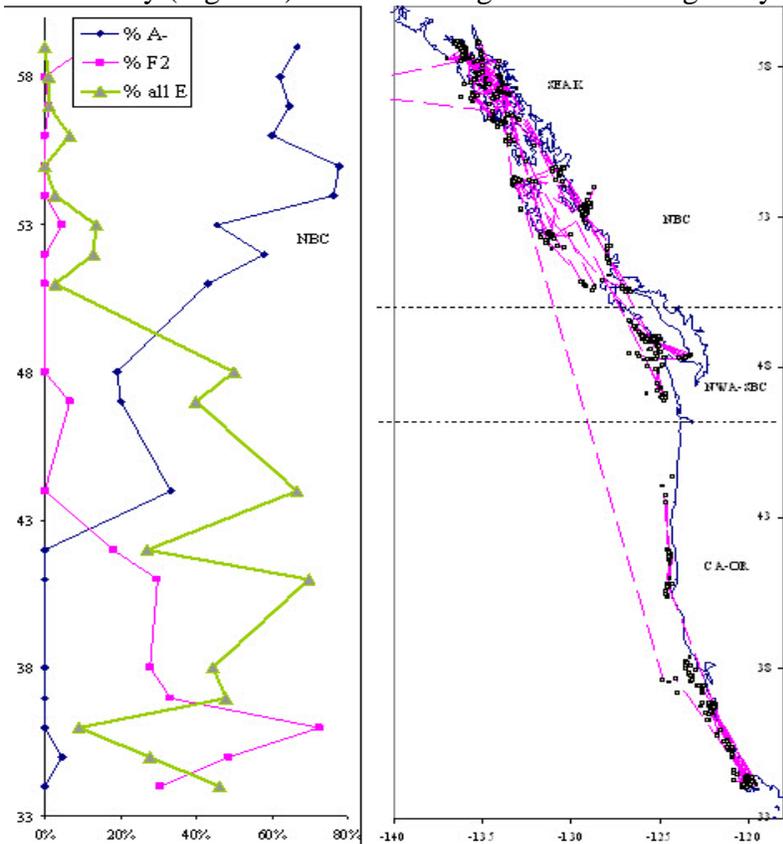


Figure 1. Humpback whale mtDNA haplotype proportions by latitude from California to SE Alaska (data from Baker et al. 1990, 1998, unpublished data, Calambokidis 2009).

Abundance estimates for the larger concentration off California and Oregon, has been increasing at about 7-8% per year since the early 1990s when the more complete surveys began (Figure 2). There was an apparent drop in estimates of abundance in the late 1990s but a number of years of strong annual growth has brought the population sharply up. Estimates of abundance have become more challenging as the samples obtained represent a smaller and smaller proportion of this increasing population.

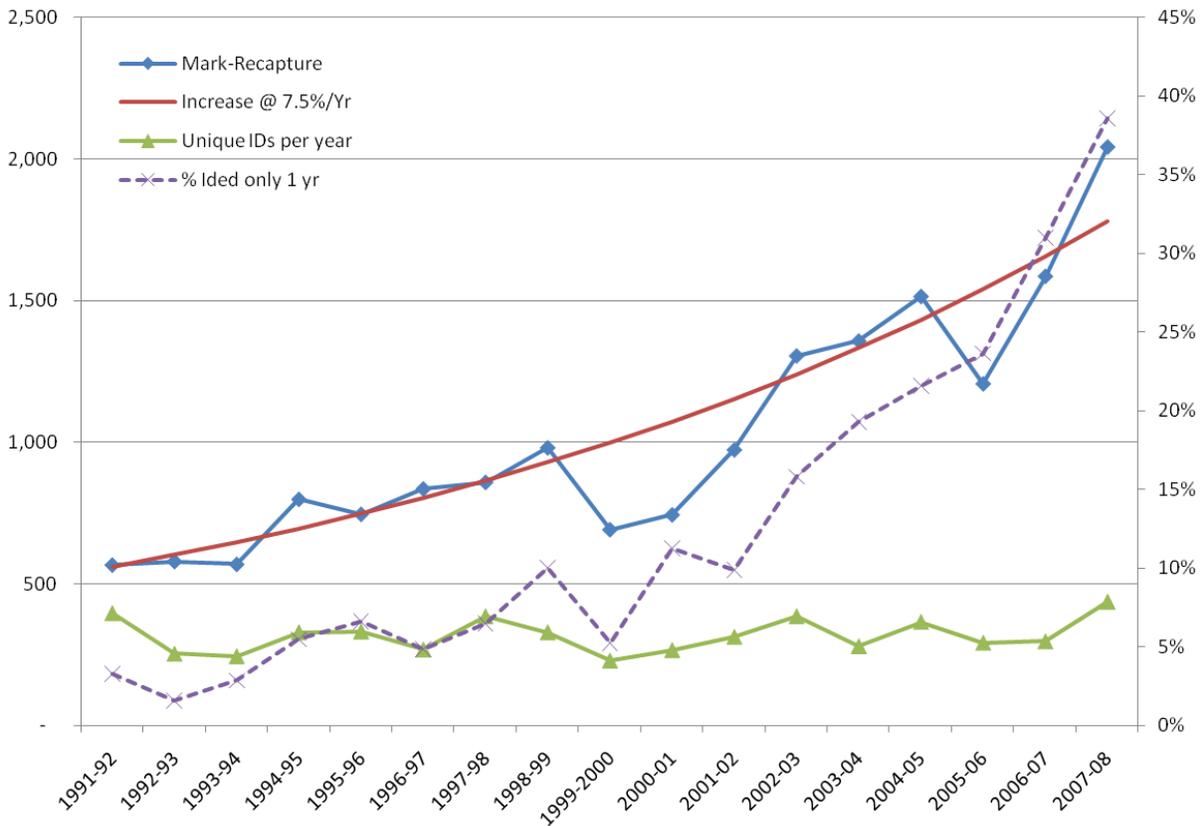


Figure 2. Abundance estimates of humpback whales from mark-recapture off California and Oregon through 2008 based on Petersen mark-recapture estimates using adjacent years as samples. Left axis also shows proportion of identified animals not seen previously and the proportion seen only one year. From Calambokidis (2009).

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## Site fidelity and population structure of humpback whales in British Columbia.

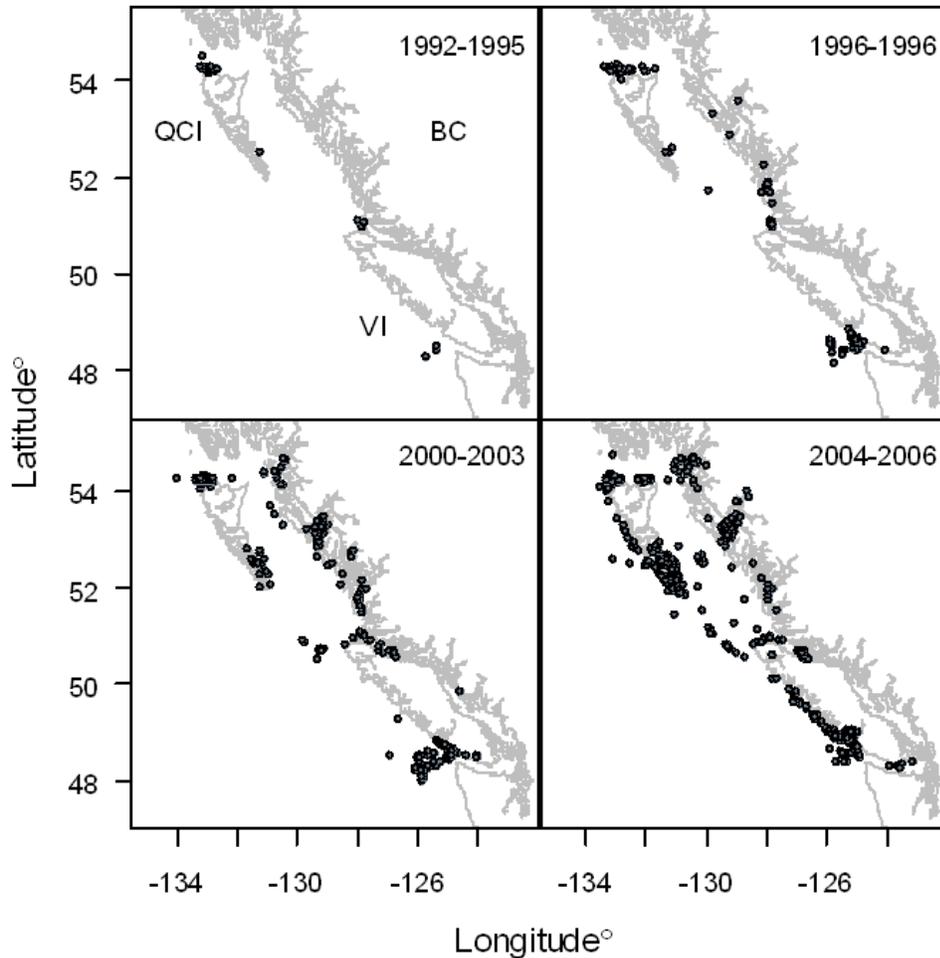
Presented by Andrea L. Rambeau<sup>1</sup> and John K.B. Ford<sup>2</sup>

1. Department of Zoology, University of British Columbia, Vancouver, B.C., Canada
2. Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, B.C., Canada

In this presentation, we describe studies of humpback whales in waters of British Columbia (BC), Canada, aimed at determining abundance levels and trends, site fidelity and distribution patterns with respect to breeding ground affiliation. Although SPLASH analyses utilized photo-identification data collected in BC during 2004-05, these were split geographically, combining northern BC humpbacks with Southeast Alaska, and southern BC humpbacks with Washington State. This regional subdivision was made since previous studies had shown that a high proportion of humpbacks off southern Vancouver Island mixed with those in neighboring western Washington, and together these whales showed a strong affiliation with breeding grounds off mainland Mexico (Calambokidis et al. 1996, 1997). Similarly, considerable mixing had been observed among whales off northern BC and Southeast Alaska, and these together showed a strong affiliation with Hawaiian breeding grounds. In order to estimate population abundance and trends in Canadian waters, as required for recovery planning under Canada's Species at Risk Act, photo-identification data collected for SPLASH in BC during 2004-05 were combined with other data in the region between 1992 and 2006. This dataset was also used to determine the level of site fidelity to feeding grounds within BC waters and whether a sharp demarcation existed between with humpbacks off southern Vancouver Island and northern BC waters.

Our 16-year dataset (1992-2006) contained 8,785 humpback whale photo-identifications collected throughout BC waters, representing 1,986 unique individuals. To estimate population abundance, we examined the results of several capture-recapture models to assess which provided the least biased estimates for our dataset, which contained geographically and temporally heterogeneous capture probabilities as a result of uneven sampling effort (Figure 1). We concluded that estimates (and 95% confidence intervals) predicted by a Sampling Importance Resampling (SIR) Jolly-Seber model provided the most reasonable results, with an estimated abundance of 2145 whales (1970-2331) in 2006, an annual survival rate of 97.6% (96.0-99.2%), and a population growth rate of 4.1% per year (3.2-5.0%).

Comparing photographs between years revealed that 872 (44%) of the identified whales were seen in BC in more than one year, with the greatest number of sightings occurring from May to October, and peaking in September. Maximum distance between sightings of the same individual across years ranged from 0.41 km to 842 km, and showed strong site fidelity within a median distance of 75 km. We examined how much small-scale mixing and movement there was north-south within the province by setting all whales observed north of 54° as a northern 'sub-population'. We then compared this sub-population to all whales south of 54°, by observing if and how matching rate declined to the south in 0.5° latitudinal bins. Inter-matching of whales within BC decreased as a function of increasing north-south distance (Figure 2). Thirty-two percent of the whales seen above 54° were re-sighted above 54° in later years, yet matching rate decreased linearly, the further away from 54° that the comparison was made.



*Figure 1. Locations of photo-identified humpback whales, showing variation in spatial effort from 1992 to 2006 in British Columbia (BC), Canada. Years are pooled for ease of viewing only. QCI = Queen Charlotte Islands, VI = Vancouver Island*

Overall, humpback whales in BC showed a strong pattern of high site fidelity to small, localized areas with occasional longer distance movements. Despite not finding an absolute demarcation or boundary for humpback whales within BC, the clustering of sightings is consistent with the hypothesis that BC may be comprised of two loosely distinct populations to the north and south. Future genetic work could help elucidate this picture and resolve whether either of these BC clusterings are extensions of the central or eastern North Pacific stocks of humpback whales. Stock structure of humpback whales in British Columbia is highly complex and site fidelity in particular should be considered in future management of this population.

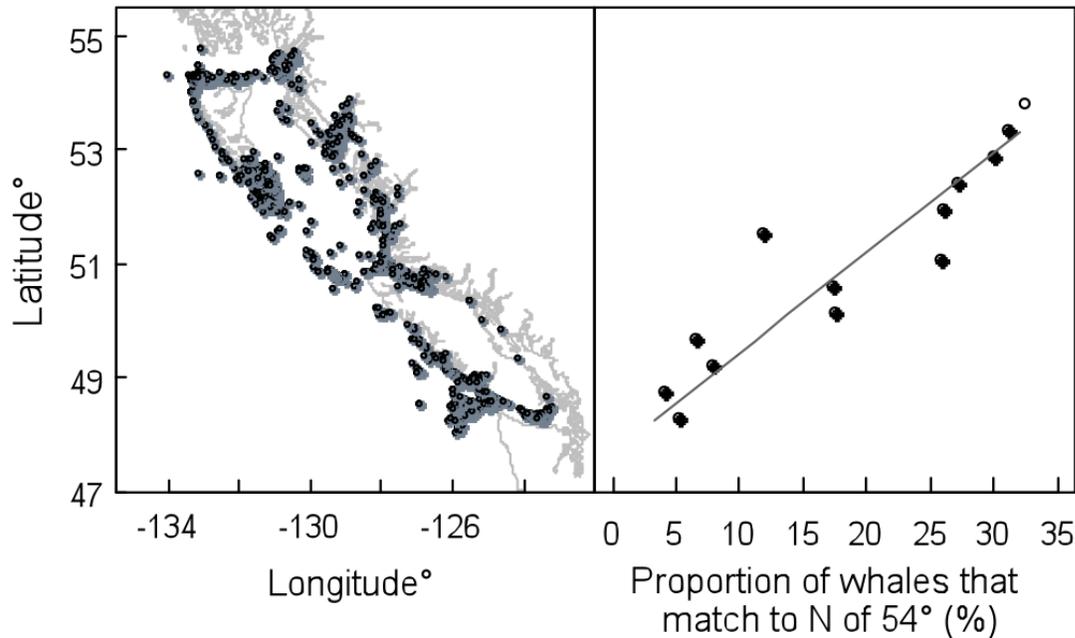


Figure 2. Proportion of humpback whales observed in  $0.5^\circ$  bins below specified latitude that match to whales observed above  $54^\circ$  in British Columbia between 1992 and 2007. The independent categorical variable (latitude) is plotted on the y-axis here for ease of viewing against regional map only. Open circle is proportion of whales sighted above  $54^\circ$  that were re-sighted in multiple years.

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## Humpback whales in summering areas in the Russian Far East

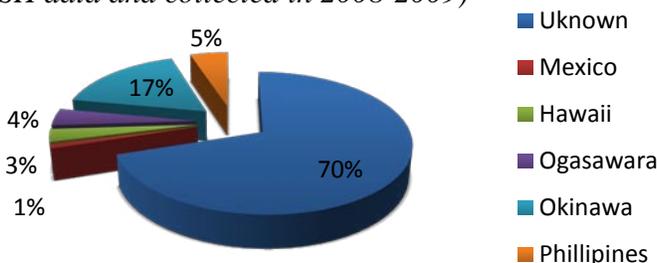
Presented by Alexander Burdin, UAF, Kamchatka Branch of Pacific Institute of Geography, RAS.

Despite of the long history of humpback whale research in the Pacific Ocean, there was a serious gap of information about this species for the western part of the Northern Pacific, both from breeding and feeding areas. To collect information about distribution, abundance, genetic composition and feeding areas of humpback whales in the Russia Far East Seas we conducted two SPLASH surveys in Russian waters: 1) in 2004 (18 July- 17 August) covering the area from Petropavlovsk-Kamchatsky to Anadyr Gulf and 2) in 2005 (17 July-14 August) along Eastern Kamchatka, Koryak Coast, Anadyr Gulf and Chukotka peninsula up to Bering Strait. Study of humpbacks was continued in Russia Far East as a land based survey in 2008 and 2009 on the Bering and Karaginsky Islands.

As a result of the 2004-2005 SPLASH surveys, a photo ID catalog of humpback whales in the feeding areas of the western Bering Sea was started. About 70 biopsy samples were collected in two years in Russia Far East for the genetic and biochemistry analysis.

According to the limited data collected in 2004-2009, humpback whales feeding areas in the Russian waters in the western Bering Sea are important for whales from all breeding aggregations, but should be the main destination for the whales from the Asian stock, mostly from Okinawa. Based on match analysis between breeding and feeding areas (Calambokidis et al., 2008; our data), the origin of 70% of humpback whales identified in Western Bering Sea is unknown, but 17% of humpbacks matched in Russian waters, was from Okinawa (Fig.1)

*Fig.1 Composition of humpback whales on the feeding areas in the Western Bering sea (based on 2004-05 SPLASH data and collected in 2008-2009)*



In total 112 individual humpback whales were positively identified in Russia waters in 2004-2005 in SPLASH cruises (Calambokidis et al., 2008). Additional research effort on humpback whales after the SPLASH cruises in 2006-2009 increased the number of identified whales up to 222 and new matches between breeding and feeding areas were found. These follow up studies in multiple years in two locations (Bering and Karaginsky Islands) demonstrate different patterns of whale resightings. In 2008-2009 in Karaginsky Island 50% of humpbacks had been seen in previous years, and in Bering Island only 3.3% (Table 1). The possible explanation of differences in humpbacks resighting rate and site fidelity in two feeding areas can be:

- 1) Whales with different breeding area origins preferred different feeding areas. For example whales from Okinawa more often observed feeding in Karaginsky Gulf than other areas (Table 2).
- 2) Stronger site fidelity of feeding near Karaginsky Island compare with Commander Island.

- 3) Larger numbers of whales feeding near Commander Islands than in other areas. Between 60 whales identified near Bering Island in 6 surveys in August-September 2009, 48 whales (67%) have been seen only once.
- 4) Seasonal changes in humpback whale distribution and movement between feeding areas.

*Table 1. Identification and resighting of humpback whales in two subarea in the Russia FE (Bering Island and Karaginsky Island) in 2004-2009*

<b>Location</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>
Bering Identified/resighted	11/0	7/1	0	0	13/0	60/2
Karaginsky Identified/resighted	38/1	29/10	0	0	10/5	14/7

*Table 2. Humpbacks from Okinawa observed in two locations in Western Bering Sea*

	<b>2004</b>	<b>2005</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>
Bering Isl.	1	1		2	1
Karaginsky Isl.	11	11	0	3	2
Anadyr gulf		1			
Kamchatka East Coast			1		2
Koryak Coast		1			

As a result of the SPLASH cruises conducted in 2004-2005, three main areas were recognized as humpback whales feeding areas: Karaginsky Gulf, Anadyrsky Gulf, and Commander Islands. Eastern Kamchatka and Koryak Coast were recognized as transit areas where no significant concentration of whales was found. Since then we have identified two additional areas of concentration that can be important feeding areas for humpback whales: Chukchi Sea and Eastern part of Okhotsk Sea.

Despite humpback whales being included in the IUCN “RED DATA BOOK” for the Russian Federation, little effort has been done to study and protect these whales, and little funding exists for regular whale research in Russian waters except opportunistic information off Kamchatka and Commanders. Several areas may be important for conservation including the western Bering Sea because this feeding ground is visited by whales from all breeding locations and also areas off Kamchatka which are a key feeding area with a high proportion of whales coming from the Asian wintering areas which appear at highest risk.

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## **SPLASH: Overview for Mexico.**

Presented by Jorge Urbán R. and Ursula González-Peral, Universidad Autónoma de Baja California Sur La Paz, B.C.S. 23081 México

Humpback whales wintering in the Mexican Pacific aggregate along the southern coasts of Baja California Peninsula (BC); the mainland coasts of Mexico, particularly in Bahía de Banderas (ML); and the Revillagigedo Archipelago (AR). To identify the relationship among these three aggregations we used 2639 photo-identified individuals (790 in 2004, 912 in 2005 and 937 in 2006) and 1114 skin samples collected as part of the Structure of Populations, Levels of Abundance and Status of Humpbacks (SPLASH) sampling effort. Movements of 70 photo-id whales within the same winter season indicate that humpback whales in the Mexican Pacific are very dynamic, they present movements among the three winter aggregations, being the highest number of recaptures between Baja California and Mainland (67.1%), followed by AR-BC (24.3%) and AR-ML (8.6%) (Figure 1).

Site fidelity (recaptures between years) was highest in AR (22.4%) followed by ML (21.6%) and BC (4.9%). 35 whales were seen the three winter seasons in AR, 20 in ML and 0 in BC (Table 1).

The overall proportion male/female based on 1088 skin samples was 2.33:1. AR showed the highest ratio (3.33:1, N=434), followed by ML (2.63:1, N=323) and BC (2.04:1, N=331). Movements among aggregations in the same season showed a higher ratio of males between ML-BC (7:1, N=24), followed by AR-BC (2.5:1, N=14) and AR-ML (1:2, N=3). Based on 674pb of mitochondrial control region sequence from 1114 humpback whale skin samples (341 from BC, 331 from ML, and 442 from AR), 27 haplotypes were found in the three winter aggregations of the Mexican Pacific. The humpback whales of Baja California present the highest diversity of haplotypes in all the North Pacific. A pair-wise  $F_{st}$  values showing significant differences among all three aggregations in the Mexican Pacific (BC-ML  $F_{st}$  0.0058, BC-AR  $F_{st}$  0.0074, and ML-AR  $F_{st}$  0.0258,  $p < 0.001$ ) (Figure 2).

We conclude that, although the complexity of the different approaches, there are at least two population units in the Mexican Pacific, AR and ML, which should be managed according to their different conservation pressures. The third aggregation, Baja California, represents a management challenge due its characteristics of both wintering aggregation and transit area for whales from different aggregations.

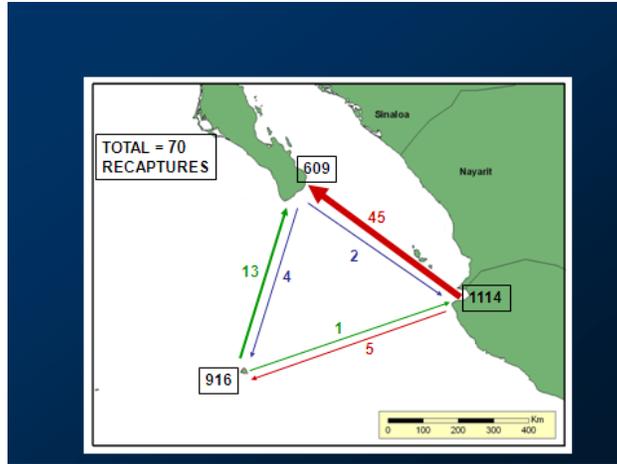


Figure 1. Movements of 70 photo-id whales within the same winter season

FIDELITY		
Recaptures in the same winter aggregation in different years.		
BAJA	2004 (223) – 2005 (247) = 14 (6.2%)	- Fidelity
	2005 (247) – 2006 (139) = 9 (3.6%)	
	2004 (223) – 2006 (139) = 11 (4.9%)	
➤ 0 whales photographed the three years		
MAINLAND	2004 (219) – 2005 (399) = 48 (21.9%)	+ Fidelity
	2005 (399) – 2006 (496) = 80 (20.0%)	
	2004 (219) – 2006 (496) = 50 (22.8%)	
➤ 20 whales photographed the three years		
REVILLA	2004 (348) – 2005 (266) = 76 (21.8%)	+ Fidelity
	2005 (266) – 2006 (302) = 63 (23.7%)	
	2004 (348) – 2006 (302) = 76 (21.8%)	
➤ 35 whales photographed the three years		

Table 1. Fidelity of the humpback whales to the three Mexican wintering aggregations.



Figure 2. Fst values showing significant differences among all three aggregations in the Mexican Pacific

## **SPLASH and the Archipiélago Revillagigedo 1996-2006**

Presented by Jeff K Jacobsen, Humboldt State University, Department of Biological Sciences  
1 Harpst St., Arcata, CA 95521 Email: [jkjacobsen@reninet.com](mailto:jkjacobsen@reninet.com)

The purpose of this presentation was to explore how results from previous humpback whale research at the Archipiélago Revillagigedo, México from 1996-2003 interrelate with the results of SPLASH. Due to an unusually high degree of site fidelity at Revillagigedo relative to other North Pacific breeding areas, detailed sighting information exists for many individuals within the population, such as sex, reproductive status, time of arrival, and duration of residency across multiple years. SPLASH has greatly increased the number of known migratory destinations of Revillagigedo individuals, especially to feeding areas, which were largely unknown.

Research on humpback whales at the Archipiélago Revillagigedo began in 1986 at Isla Socorro, the largest of the three major islands in the chain. The first full three-month field season was in 1991, and consistent effort was maintained at Isla Socorro from 1996 to 2001 and included several seasons at Isla Clarión, the second largest and only other habitable island, 200 nmi to the west. No effort occurred during the 2002 season, however effort resumed in 2003 and expanded from 2004-2006 with the participation of the SPLASH project. This presentation focused on the years 1996-2003 for comparisons with the SPLASH years.

Table summarizes recapture characteristics at Revillagigedo from 1996-2006. Around 50% of individuals identified within any given season were sighted on more than one day, providing the opportunity to accumulate data on sighting rates, durations of stay, and dates of arrival for many individuals. Most of this population has been sexed by behavior and/or by genetic analysis of skin biopsy samples. We've observed previously that males were sighted in significantly more years than females, on more days within each season, and had greater overall durations of stay (1996-2001,  $p < 0.01$  Aspin-Welch unequal variance t-test).

*Table 1. Sighting characteristics (number of unique individuals, number identified on more than one day during season, number identified in previous years, and cumulative rate of recapture) of humpback whales at Archipelago Revillagigedo, 1996-2006.*

<b>Year</b>	<b># IDs</b>	<b>Identified &gt; 1 Day</b>	<b>Previous Years</b>	<b>Rate of Recapture</b>
1996	193	76 (0.39)	0	0
1997	286	158 (0.55)	61	0.21
1998	184	100 (0.54)	80	0.43
1999	256	149 (0.58)	141	0.55
2000	323	205 (0.63)	174	0.54
2001	333	220 (0.66)	190	0.57
2003	206	107 (0.54)	112	0.54
2004	337	206 (0.61)	140	0.58
2005	242	123 (0.51)	170	0.70
2006	301	178 (0.59)	214	0.71
Total	1287			

A total of 137 migratory recaptures of 135 individuals were observed during SPLASH. The Northern Gulf of Alaska (NGOA) was the most frequently observed migratory destination (Table 2). Of the recaptures to other wintering areas it is noteworthy that the same (low) number occurred between Revillagigedo and Hawaii as between Revillagigedo and mainland Mexico, despite the ten-fold difference in distance between them. To investigate whether there is variation in migratory destination associated with a whale's long-term sighting history at Revillagigedo, we compared the migratory destinations of individuals that had been seen at Socorro from 1996-2001 versus those seen only during the SPLASH years, 2004-2006. Although sample sizes were low, there was no significant difference in destinations for individuals with and without a long-term history at Revillagigedo (Chi-square=8.625, df=9, p = 0.473).

*Table 2. Migratory destination and history at Revillagigedo. The number of recaptures at three breeding and seven feeding areas of Revillagigedo individuals identified during SPLASH (2004-2006, N=562) that also had been identified during 1996-2001 (N=266) and those new to the SPLASH years (N=288).*

<b>Region</b>	<b>1996-2001</b>	<b>NEW SPLASH</b>	<b>1996-2001</b>	<b>NEW SPLASH</b>
MX-BC	12	10	19%	13%
MX-ML	6	8	10%	11%
Hawaii	5	9	8%	12%
Russia-CI	0	1	0%	1%
Bering	3	8	5%	11%
WGOA	7	6	11%	8%
NGOA	23	20	37%	27%
SEAK	4	5	6%	7%
NBC	1	7	2%	9%
NWA-SBC	1	1	2%	1%
Total	62	75		

In a comparison of SPLASH migratory destinations based on within-season recapture characteristics from 2004-2006, we found no significant difference between Socorro and Clarion (Chi-square = 4.32, df = 3, p = 0.220), nor in the total number of days sighted at Revillagigedo each year (Kruskal-Wallis one-way ANOVA). However, a better indication of seasonal breeding area residency is the number of days spanned by the first and last sightings during a season, or duration of stay. We did find a significant difference in duration of stay between whales migrating to NGOA and to SEAK-NBC (Table 3, Kruskal-Wallis one-way ANOVA) – suggesting whales that reside longest at Revillagigedo tend to migrate more to NGOA.

We then refined this comparison to the date individuals were first sighted at Revillagigedo, an possible indicator of when they arrived at the breeding area, and found significant differences between areas (Table 4, p<0.098, One-way ANOVA). The trend was for whales from western feeding areas to arrive before those from the eastern feeding areas, which may be due to the differences in the onset of winter.

*Table 3. Feeding area migratory destination and residency at Revillagigedo. The number of recaptures at four feeding areas of Revillagigedo individuals identified during SPLASH (2004-2006) and number of days spanned by their first and last sightings within a season.*

<b>Region</b>	<b>A.R. Whales</b>	<b>Median Duration</b>	<b>Min Duration</b>	<b>Max Duration</b>
Bering	11	4.0	2	25
WGOA	15	9.0	2	34
NGOA	53	10.0	2	61
SEAK-NBC	20	5.5	3	23
Total	99			

*Table 4. Feeding area migratory destination and date of first sighting at Revillagigedo. The number of recaptures at four feeding areas of Revillagigedo individuals identified during SPLASH (2004-2006) and the average first date of first arrival at Revillagigedo for these individuals.*

<b>Region</b>	<b>A.R. Whales</b>	<b>Mean Julian</b>	<b>Mean Date</b>
Bering	11	55.2	24-Feb
WGOA	15	55.6	25-Feb
NGOA	53	59.1	28-Feb
SEAK-NBC	20	69.7	10-Mar
Total	99		

## **Conclusion**

SPLASH contributed significantly to our understanding of where Revillagigedo whales migrate to feed. Combining our detailed sighting data with these migratory data has indicated differential seasonal residency times may exist for whales coming from different feeding areas, and feeding area may also influence time of arrival. These results can all be greatly improved in a complete comparison of the Revillagigedo historical collection against SPLASH.

## Humpback whales in Asia

Presented by Manami Yamaguchi, Ogasawara Marine Center, Byobudani, Chichijima, Ogasawara-mura, Tokyo 100-21, Japan

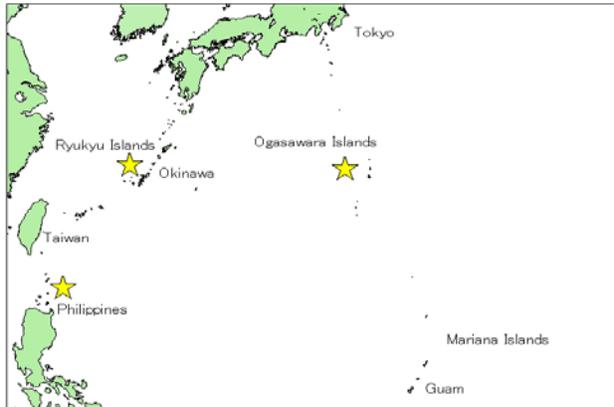
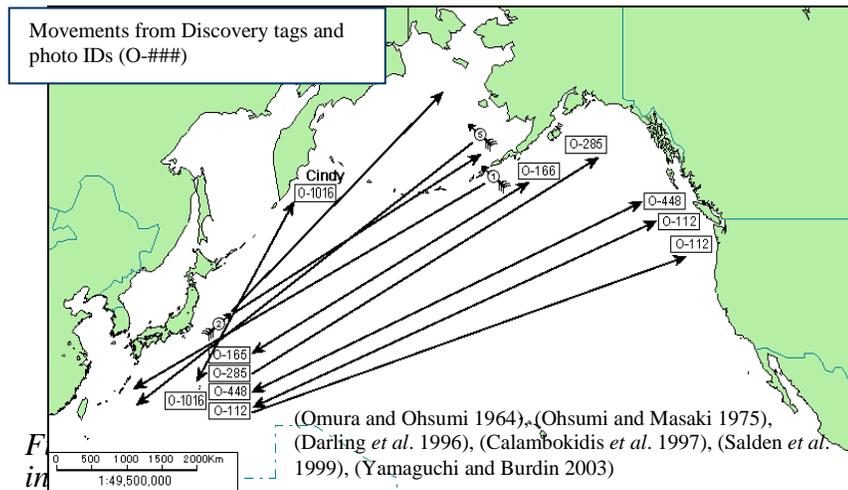


Fig. 1. SPLASH study areas in the western North Pacific

most of the year. This regularity of re-sighted numbers to date suggests the distribution and total population size is not huge. And it is believed the whales are using these areas as a continuous habitat throughout the winter months.

Humpback whales have been studied in nearshore waters off the Ogasawara Islands since 1987, the Ryukyu Islands (Okinawa) since 1989 and the Babuyan Islands, Philippines since 1999 (Fig.1). Whales are distributed densely within each area along the chain of islands and they appear to make repeated local movements among the area within a given season.

In Ogasawara, a total of 1,438 individual whales were identified in the 1987-2008 period. In Okinawa, a total of 829 individual whales were identified in the 1989-2008 period. In both areas, the re-sighted numbers outnumbered the newly identified numbers



The interchange of whales among these three Asian areas occurred even within the same season (Yamaguchi *et al.* 1995, Yamaguchi *et al.* 2002). Although they seemed to have favorite areas such as “home ground”, these regions were not considered three completely separated regional populations.

Before SPLASH there were only a few connections to show the feeding grounds for whales in Asia (Fig 2).

## SPLASH

Although the sample sizes are comparatively small compared to other breeding areas such as Mexico and Hawaii, the international cooperation provided by SPLASH revealed many new facts. While there is still some complexity to consider in each breeding ground (Mexico, Hawaii and Asia) a high ratio of interchange was seen within the three areas of Asia sampled (Ogasawara, Okinawa and the Philippines, 1,600-2,300km apart)(Table 1). Among the three principal breeding grounds, two whales were seen in both Asia (one each in Ogasawara and Philippines) and Hawaii. Although these are not direct interchange within a season, there is possible gene flow between Hawaii and Asia.

Table 1. Interchange among wintering areas 2004-2006. Numbers along the diagonal show the total number of unique identifications within that area and numbers along upper right portion of the matrix show number of individuals seen in multiple areas. From Calambokidis *et al.* (2008).

Area	Asia-PHI	Asia-OK	Asia-OG	HI	MX-REV	MX-Baja	MX-ML	Cent Am
Philippines	77	5	5	1	0	0	0	0
Okinawa		215	10	0	0	0	0	0
Ogasawara			294	1	0	0	0	0
Hawaii				2317	14	2	1	0
MX-REV					562	22	14	0
MX-Baja						406	66	2
MX-ML							690	9
Cent Am								105

Among three Asian breeding areas, less than 10% of the identified whales had also been seen on a feeding ground and this was less than 5% for Ogasawara (Table 2). This suggests that some of the feeding areas for some of the Ogasawara were under sampled in SPLASH.

Table 2. Identifications and connections made between wintering areas (columns) and feeding areas (rows). Seasons are pooled for both wintering and feeding areas. Sum reflects the total of the whales matching between areas and overall reflects the number of individuals matching to any area (where the same whale matched to multiple areas). From Calambokidis *et al.* (2008).

Region	Daily IDs	Asia-PHI	Asia-OK	Asia-OG	Hawaii	MX-REV	MX-Baja	MX-ML	Cent Am	Sum	Overall	
	Unique	77	215	294	2317	562	406	690	105			
Russia	128	102	6	14	5	4	1	0	0	0	30	29
Aleutians	64	63	0	1	0	4	0	2	0	0	7	7
Bering	728	491	0	1	5	44	11	8	11	0	80	77
WGOA	516	301	0	0	2	26	13	7	4	0	52	51
NGOA	1792	1038	0	0	1	124	44	20	21	0	210	200
SEAK	2382	1115	0	0	0	215	9	3	8	0	235	235
NBC	1183	583	0	0	0	99	8	5	4	0	116	114
NWA-SBC	380	207	0	0	0	20	2	8	22	3	55	53
CA-OR	881	525	0	0	0	0	0	20	97	26	143	133
Sum			6	16	13	536	88	73	167	29	928	
Overall matches			6	16	13	516	87	70	164	29		873

The Asian humpback population has been considered as one population so called “Asian stock”. However it seems to be more complex and has some interchange with other areas (Table 3). A high proportion of the whales that feed off Kamchatka matched to the Asian wintering areas while those from the Commanders and the Gulf of Anadyr had more varied destinations like those from other feeding areas in the Aleutians and Bering Sea (Table 3). The whales that breed in Ogasawara were identified in Kamchatka where other whales from Asia were found and also in Bering where whales from Hawaii feed. Also there is evidence to show the genetic difference between Ogasawara and Okinawa. Additionally, there is the possibility of existence of an undiscovered breeding grounds for the whales feed in the Commander and Aleutian Islands and in the Bering Sea (Calambokidis *et al.* 2008). Further investigation to understand the complexity of Asian sub-regional areas is required.

According to the whaling record, in addition to these Asian breeding areas, Taiwan, Hainan and Mariana Islands were also considered to be breeding areas in Asia. Currently, there are sightings by residents and whale watching activities and some investigation on humpback whales in some of these areas. And for the feeding ground, also the same activities are happening in

northern Japan around Krill Islands and Hokkaido. More information will be expected for better understandings of whales in Asia in the near future.

*Table 3. Summary of migratory destinations of humpback whales from Russian waters showing differences by sub-area and similarities to the Aleutian and Bering areas in US waters. From Calambokidis et al. (2008).*

	IDs	Asia-PHI 77	Asia-OK 215	Asia-OG 294	Hawaii 2317	MX 1558	Any area	%
Gulf of Anadyr	27	0	1	0	3	0	4	15%
Kamchatka	58	6	13	4	0	0	22	38%
Commanders	17	0	0	1	1	1	3	18%
All Russia	102	6	14	5	4	1	29	28%
Aleutians	63	0	1	0	4	0	7	11%
Bering	491	0	1	5	44	27	77	16%

### SPLASH Training Program

SPLASH has provided the great opportunities to learn more of new skills in training program for better sampling. The program was supported by the Hawaiian Islands Humpback Whale National Marine Sanctuary and operated by Coastal Studies in Maine, US. People from Japan, Mexico and Hawaii participated in this program to learn biopsy sampling, tissue treating and field recording methods.



### SPLASH symposium in Tokyo

In June 2005, the SPLASH symposium was held in Tokyo to report what SPLASH is and its progress. Presenters were four from the U.S., one from Mexico, one from Philippines and five from Japan. About 100 people are participated.



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## **Humpback whales of the South Pacific: an overview of results from the South Pacific Whale Research Consortium**

Presented by Rochelle Constantine on behalf of the South Pacific Whale Research Consortium, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand. r.constantine@auckland.ac.nz

Over 200,000 humpback whales were hunted in the Southern Ocean during the 20<sup>th</sup> Century (Clapham & Baker 2001). A total of 55,522 whales were taken in the Antarctic feeding grounds Areas V and VI and along their migration paths past east Australia and New Zealand. This total number of catches peaked in the 1959-60 summer followed by a crash of the humpback whale stocks in South Pacific region. In response to monitoring the recovery of the South Pacific humpback whales on their breeding grounds, the South Pacific Whale Research Consortium was formed in 1999. This is an international collective of researchers focusing on the whales of Oceania and their links to east Australia. Photo-identification, genetic sampling and acoustics have been the main research focus but in recent years satellite tags have been used to track movements of individual whales from breeding grounds to feeding areas (Clapham et al. 2008, Hauser et al. in press). The South Pacific, stretching from Australia to South America, is a vast and complex region with poor infrastructure for scientific research. Our primary effort has been in four regions of current or historical concentration; New Caledonia, Tonga, Cook Islands and French Polynesia, but has included some effort in Vanuatu, Fiji, New Zealand, Samoa, American Samoa and Niue.

### **Movements between regions**

A photo-ID catalogue of individual whales seen in Oceania between 1999-2004 (n = 672 individuals) was matched to a catalogue from east Australia (EA) (n = 1242 individuals) resulting in only seven matches; four between EA and New Caledonia and three between EA and New Zealand (Garrigue et al. In Press). Over the same period of time 28 movements were reported throughout Oceania (Garrigue et al. in review). Similar results for interchange between Oceania regions were reported using genotype matching (SPWRC 2007). These findings are not surprising as the South Pacific is a complex area with many atolls, islands and seamounts that connect regions, but the interchange is low overall and this is clear with genetic differentiation found on the breeding grounds between the main study areas (Olavarria et al. 2007). Ongoing research on humpback whale song (conducted by the University of Queensland) throughout the South Pacific shows that song spreads from west to east and changes in song structure occur at a variable rate (Garland et al. 2009).

### **Population estimates – preliminary results**

The 2004 abundance estimates for EA and Oceania indicated considerable difference in the recovery rate for these two populations. The EA population estimate using a land-based census was 7090 ( $\pm$  660) (Noad et al. 2006) and for Oceania the preliminary estimate using photo-ID and genotyping was 2392 ( $\pm$  275) (SPWRC 2007) whales.

### **Comprehensive Assessment – preliminary results**

Consortium members have been involved in completing the Comprehensive Assessment of Humpback Whales in the Southern Hemisphere, now underway by the Scientific Committee of the International Whaling Commission. The goal is to assess current abundance and model

recovery in relationship to historical catch records and the estimated pre-exploitation abundance. A two-stock Bayesian Logistic ‘HITTER’ model has been used to reconstruct the population recovery for EA and Oceania separately taking into account the distribution of catches, including the Soviet illegal catches, and the differences in apparent rates of recovery (Jackson et al. 2006, 2008). The model is still under development and will be updated with new estimates of abundance from both photo-ID and microsatellite genotypes. However, preliminary results of the assessment indicate that, prior to exploitation, the combined EA and Oceania populations were considerably larger than previously estimated and that the Oceania component has been slow to recovery and remains considerably more depleted. Based on these preliminary results the International Union for the Conservation of Nature (IUCN) has listed the Oceania population as ‘endangered’ (Childerhouse et al. 2008).

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## **North Atlantic humpback whales: The YONAH and MONAH projects**

Presented by Phil Clapham, Cetacean Assessment and Ecology Program, National Marine Mammal Laboratory, Seattle, WA 98115

### **Years of the North Atlantic Humpback (YONAH, 1992-93)**

By the late 1980's, a considerable amount of information had been gathered from photo-identification studies regarding the abundance and population structure of North Atlantic humpback whales. However, existing abundance estimates were old and suffered from bias relating to use of different methods and platforms in the various areas where whales had been sampled. Furthermore, although it was clear that whales from all the western North Atlantic feeding grounds migrated to the Dominican Republic, there had been little sampling in the central and eastern portions of this ocean (notably Iceland and Norway).

In response to these gaps, a two-year ocean-basin-wide photographic and biopsy survey (Year of the North Atlantic Humpback, or YONAH) (Smith *et al.*, 1999) was conducted in 1992 and 1993. Using a combination of photo-identification and biopsy-based genetic sampling, the YONAH project involved scientists from seven countries and covered much of the range of North Atlantic humpback whales, from the West Indies to the Arctic. This included all known feeding grounds (the Gulf of Maine, Newfoundland/Labrador, the Gulf of St Lawrence, West Greenland, Iceland and Norway); Silver Bank and Samaná Bay (Dominican Republic) were the focus of the breeding ground portion of this landmark study. As a result of this extensive and broad geographic coverage in sampling, YONAH provided the most comprehensive and reliable population assessment to date.

Consistent spatial and seasonal effort and a standardized sampling protocol significantly reduced sampling bias. Nearly three thousand individuals were photographed, and just over two thousand were biopsied. The resulting population estimates of 10,400 (95% CI = 8,000-13,600 from biopsy data) and 11,570 (95% CI = 10,290 to 13,390, Stevick *et al.*, 2003) were much larger than the estimates from the 1980's and likely reflected a combination of population growth and less bias. Palsboll *et al.* (1997) used the biopsies from the YONAH study to produce the first mark-recapture abundance estimate based on microsatellite data. Analysis of the breeding ground genetic samples produced significantly different population estimates for females (2,804 95% CI = 1,776-4,463 ) and males (4894, 95%CI = 3,374-7,123), and the total population estimate derived from breeding ground photographic and genetic tagging data alone is significantly lower than the ocean-wide estimate. These differences could be a reflection of sampling bias due to sex-based differences in behavioral class habitat preference, and/or migrational timing.

YONAH confirmed the extensive mixing in Dominican waters of whales from all western North Atlantic feeding grounds, and additionally documented matches to both Iceland and Norway. Although there were indications from genetic data of the existence of one or more additional breeding areas, the YONAH data further reinforced the importance of Dominican habitats to the entire North Atlantic population.

Ultimately, the YONAH project proved that a study on such a broad spatial scale, while

logistically complex, produces a more reliable and comprehensive dataset than multiple small-scale surveys. Advancements in genetic tagging techniques allow for a deeper understanding of population composition, and future data obtained from genetic tags can potentially reveal evolutionary, demographic and behavioral trends that could not previously be addressed. YONAH has subsequently been seen as a model for other large-scale studies, notably the Structure of Populations Levels of Abundance and Status of Humpbacks (SPLASH) project in the North Pacific.

### **More North Atlantic Humpbacks (MONAH, 2004-05)**

In 2002, there was interest within the U.S. government in conducting a further review of North Atlantic humpbacks to determine their status relative to the U.S. Endangered Species Act. By this time, the estimates of abundance produced by YONAH were ten years old, and there was a need to establish whether the population growth documented by Stevick *et al.* (2003) had continued. Consequently, a follow-up study to YONAH, More North Atlantic Humpbacks (MONAH), was initiated. Because funding was too limited to repeat the full range of sampling undertaken in YONAH, it was decided to conduct the work on Silver Bank. This was because of the importance of this habitat, the large number of humpbacks found there, and the area's known status as a mixing ground for whales from high-latitude feeding areas. MONAH sampling was conducted over two two-month winter field seasons in 2004 and 2005. Unlike in YONAH, biopsy sampling for genotyping was the priority over photo-identification, although fluke photos were of course taken whenever possible. MONAH was immensely successful, with 2,753 biopsies obtained over the two winters; a summary of these, by the class of individual whales, is given in Table 1 below.

Genetic analyses from MONAH samples are currently underway, and their completion (which was delayed by lack of funding) is expected by the middle of 2010. The results will be used to generate a male-specific estimate of abundance based upon genotyping. The male estimate from YONAH was approximately half the total population estimate derived from photo-identification samples in both breeding and feeding areas; thus it is considered more reliable than a two-sex estimate from the breeding range.

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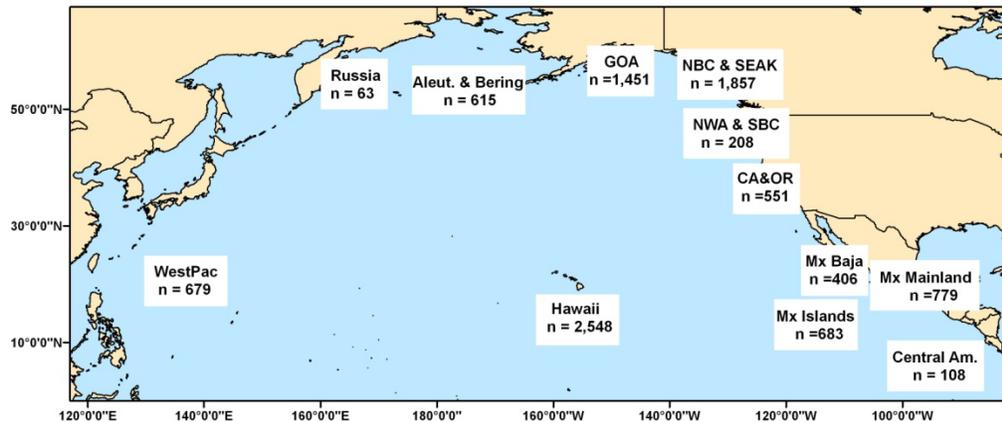
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# Humpback whale abundance in the North Pacific estimated by photographic capture-recapture with bias correction from simulation studies

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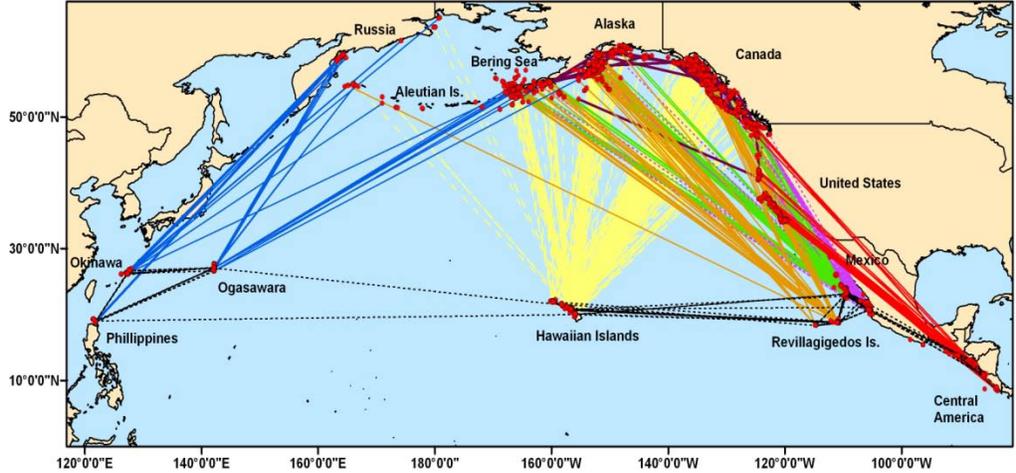
Collaborators: John Calambokidis, Erin A. Falcone, C. Scott Baker, Alexander M. Burdin, Phillip J. Clapham, John K.B. Ford, Christine M. Gabriele, Richard LeDuc, David K. Mattila, Terrance J. Quinn, Lorenzo Rojas-Bracho, Janice M. Straley, Barbara L. Taylor, Jorge Urban R., Paul Wade, David Weller, Briana Witteveen, Manami Yamaguchi

Numerous line-transect and photographic capture-recapture studies have shown that local populations of North Pacific humpback whales (*Megaptera novaeangliae*) are growing. However, the last estimate of abundance for the entire ocean basin (~8,000) was made based on data collected more than 15 years ago from a limited portion of their range. We use over 18,000 fluke identification photographs collected in 2004–2006 to estimate the abundance of humpback whales in the entire North Pacific Basin. Fluke photographs were collected by over 400 researchers in all known feeding areas from Russia to California and in all known wintering areas from Okinawa & the Philippines to Central America & Mexico as part of the Structure of Populations, Levels of Abundance and Status of Humpbacks (SPLASH) sampling effort (Fig 1).



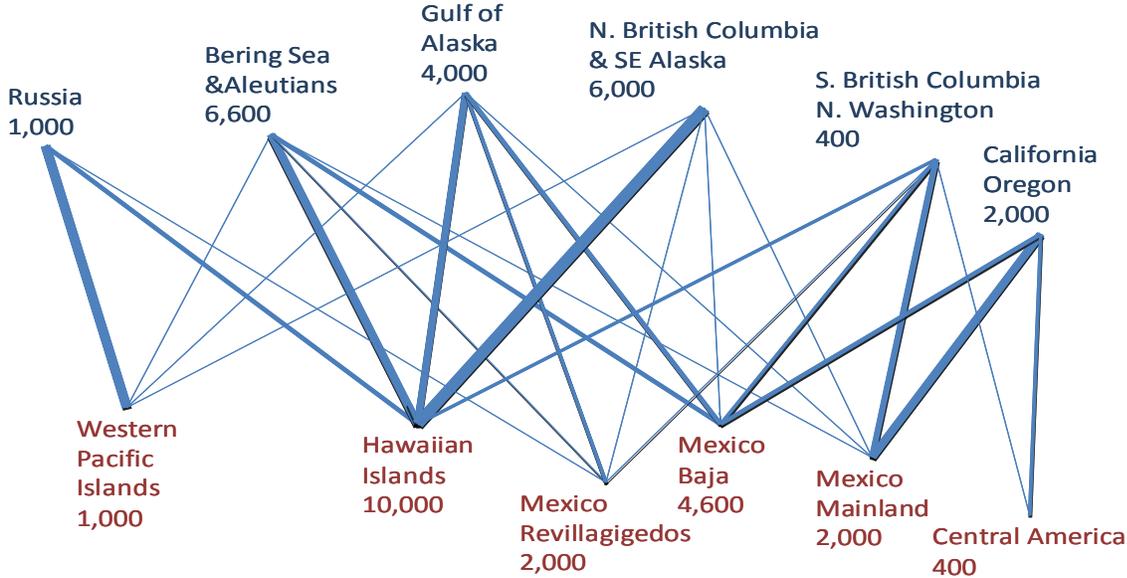
**Figure 1.** Numbers of high-quality identification photographs obtained during the 2004-06 SPLASH sampling in six breeding areas and 6 feeding areas.

Based on a comparison of all winter identifications to all summer identifications and matches (Fig. 2), the Chapman-Petersen estimate of abundance is 21,808 (CV=0.04).



**Figure 2.** Photographic matches to feeding areas are shown for breeding areas in Asia (blue), Hawaii (yellow), Revillagigedos Islands (brown), Baja California (green), mainland Mexico (lavender) and Central America (red). Matches are also shown between breeding areas (dotted black) and feeding areas (purple).

A simulation program called SimSPLASH was written to evaluate potential biases in the Chapman-Petersen estimate of North Pacific abundance given that not all assumptions of this method are likely to be met. The simulation was based on a population of 20,000 humpback whales distributed between six feeding and six breeding areas (Figure 3). The movements of animals between these areas were designed to mimic what is known about actual humpback whale migrations in the North Pacific.



**Figure 3.** Regional abundances in each of the six feeding and six breeding areas used in the SimSPLASH simulation study. The thickness of the lines indicate the relative magnitude of the movements between pairs of feeding and breeding areas.

The simulation study identified significant biases in this estimate from violations of the closed population assumption (+5.3%), exclusion of calves (-10.3%), failure to achieve random geographic sampling (+1.5%), and missed matches (+9.8%). Sex-biased sampling favoring males in wintering areas does not add significant bias if both sexes were proportionately sampled in the feeding areas. The bias-corrected estimate is 20,800 after accounting for a net positive bias of 4.8%. This estimate is likely to be lower than the true abundance due to two additional sources of bias: individual heterogeneity in the probability of being sampled (un-quantified) and the likely existence of an unknown and un-sampled wintering area (-7.2%). Additional research is needed to quantify individual heterogeneity and to find this mysterious wintering area. Clearly the population in the North Pacific has continued to increase and is now greater than some estimates of pre-whaling abundance.

## **Preliminary SPLASH feeding and winter areas abundance estimates.**

Presented by Paul R. Wade, Cetacean Assessment and Ecology Program, National Marine Mammal Laboratory, Seattle, WA 98115

Contributors: Barlow, Jay; Calambokidis, John; Falcone, Erin A; Baker, C. Scott; Burdin, Alexander M; Clapham, Phillip J; Ford, John K B; Gabrielle, Christine M; LeDuc, Richard; Mattila, David K; Quinn, Terrance J; Rojas-Bracho, Lorenzo; Straley, Janice M; Taylor, Barbara L; Urban R., Jorge; Weller, David; Witteveen, Briana; Yamaguchi, Manami

The goal of this effort was to apply area-specific models of abundance available through the program MARK to the SPLASH data for comparison to the results of the Hilborn multistrata models conducted previously (Calambokidis et al. 2008).

The models tested were spatially explicit and was based on 6 feeding areas (Russia, Aleutian/Bering, Gulf of Alaska, SE Alaska/N BC, S BC/WA, California/Oregon) and 6 wintering areas (Asia, Hawaii, Baja Mexico, Revillagigedos Mexico, mainland Mexico, and Central America). For each area the following parameters were evaluated: 1) Capture probability (p) both stratum specific (12 parameters) and stratum and year specific (24 parameters), 2) Survival probability (S) both stratum specific but also fixed at 0.96 for all areas, and Movement probabilities (Psi) describing the proportion of animals (sums to 1 for all areas) moving from a specific winter to each summer area and reverse. It was assumed that all whales are in one of the areas each season and that all whales leave winter areas in summer and vice versa.

In general, estimates from the multistrata model for wintering areas agreed fairly well for with those from the Hilborn models especially for Asia and Hawaii (Figure 1). Mexico poses challenges because of the problematic status of Baja which appears to be a mixing ground for animals from other wintering areas. Estimates for Central America were dramatically higher than from the Hilborn method. Results for individual summer areas generally agreed with the Markov-based Hilborn estimates (Figure 2) but deviated from those from the non-markov Hilborn for the Aleutian-Bering Sea area. The Aleutian-Bering Sea region had a relatively small sample size and a low proportion of matches to any wintering area which caused problems for all models. Overall, the sum of either the individual wintering or summering area estimates totaled slightly less than the 20,800 estimate found in the pooled abundance estimates (see Barlow this report) but was consistent with the overall totals found from the Hilborn estimates. Additional tests are planned including assigning an unobserved wintering area and excluding Baja Mexico as an independent region (due to the mixing with other areas).

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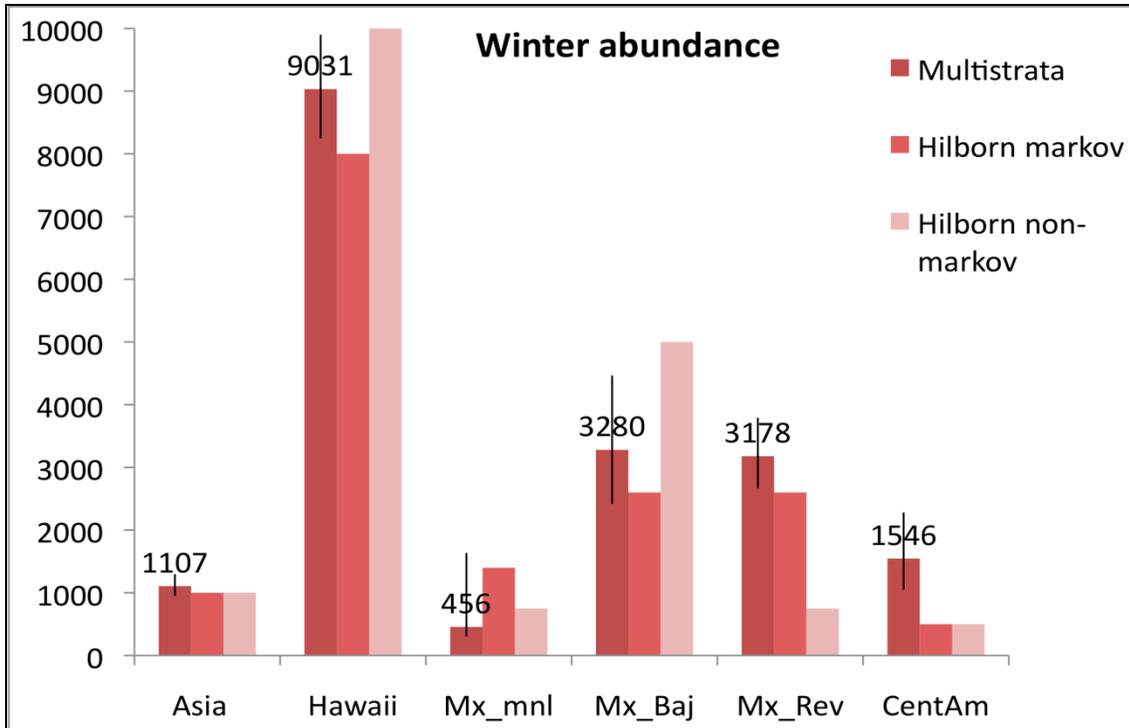


Figure 1. Summary of estimates of abundance by wintering area for the multistrata model reported here in comparison to estimates previously reported from Hilborn models (Calambokidis et al. 2008).

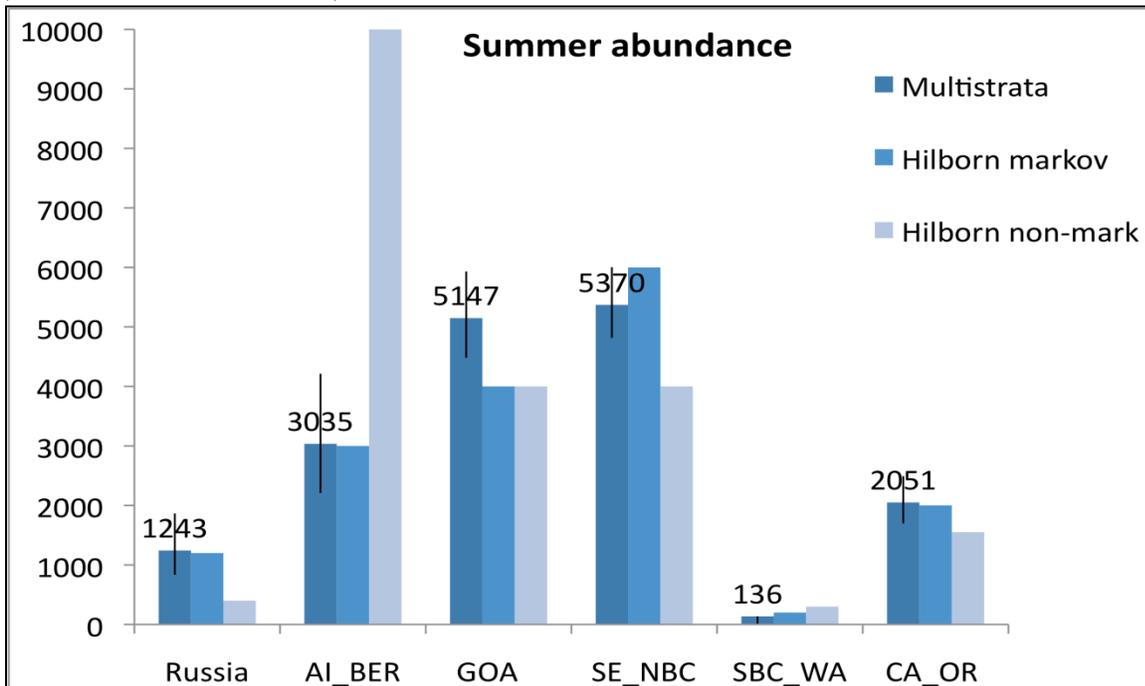


Figure 2. Summary of estimates of abundance by summering area for the multistrata model reported here in comparison to estimates previously reported from Hilborn models (Calambokidis et al. 2008).

## ***geneSPLASH*: migratory interchange and sex-specific estimates of abundance based on genotype matching.**

Presented by Debbie Steel and C. Scott Baker, Marine Mammal Institute and Department of Fisheries & Wildlife, Oregon State University, 2030 SE Marine Science Dr, Newport, OR 97365

Here we report an initial survey of migratory interchange between North Pacific feeding and breeding areas and an initial ocean basin abundance estimate based on genotype matching. A total of 2,188 samples collected from 10 feeding regions (Russia, Western Aleutians, Bering, Eastern Aleutians, Western Gulf of Alaska, Northern Gulf of Alaska, South East Alaska, Northern British Columbia, Southern British Columbia/Washington and California/Oregon) and 8 breeding grounds (Philippines, Ogasawara, Okinawa, Hawaii, Offshore Mexico, Baja California, Mainland Mexico and Central America) were chosen for this genetic survey. Total genomic DNA was extracted either at South West Fisheries Science Center or at Oregon State University. Up to 10 microsatellite loci were amplified for each sample using previously published primers (Ev14, Ev37, Ev96 Valsecchi and Amos 1996; GATA417, GATA28 Palsbøll *et al.* 1997; rw48, rw4-10 Waldick *et al.* 1999; GT211, GT23, GT575 Bérubé *et al.* 2000). All samples were amplified and sequenced for 680 bp of mitochondrial control region and genetically sexed following protocols described in Olavarria *et al.* (2007), giving a per sample genotype of 11 nuclear loci and 1 mitochondrial loci. As a measure of quality control (QC), samples with fewer than 8 microsatellite loci successfully genotyped were removed from the dataset leaving a total of 2,090 QC samples. Unique genotypes within each region were resolved with the program CERVUS (Marshall *et al.* 1998) requiring an exact match of at least 8 microsatellite loci, supported by sex and control haplotype where available.

Under these criteria a total of 1,040 unique genotypes were resolved from the feeding regions and 845 unique genotypes from the breeding grounds (Table 1). These unique genotypes were compared between regions to identify 51 cases of movement, 33 of which were between the seasonal habitats, 4 were between neighboring feeding regions and the remaining 14 were between neighboring breeding grounds giving a total of 1,834 unique individuals in the dataset. These patterns of individual movement are similar to that seen with the photo-identification dataset, with the central North Pacific feeding regions identified as an area of mixing for all breeding grounds. Initial abundance estimates, using genotype recaptures between seasonal habitats (i.e. all feeding to all breeding), are also concordant with estimates calculated from photo-id data. Interestingly the sex-specific estimates of abundance differed from each other, contrary to the assumed 1:1 sex ratio on the feeding grounds. This is possibly being driven by a female bias in the current analyzed sample from the Bering Sea and Western Gulf of Alaska regions (see Table 1).

We conducted a preliminary investigation into the utility of assignment testing for evaluating the strength of regional connections. This analysis showed some promise as more samples than expected by chance were reassigned to the breeding ground they were sampled in and patterns of assignment between feeding regions and breeding grounds were similar to that indicated from individual movement. Overall we confirmed the potential for individual based analyses of regional interchange and sex-specific estimates of abundance. We also indicated potential for population assignment where records of individual interchange are lacking. With analysis of more samples and more microsatellite loci we hope to improve our understanding of individual

movement, improve our abundance estimates, increase the specificity of assignment testing and conduct paternity and kinship analyses.

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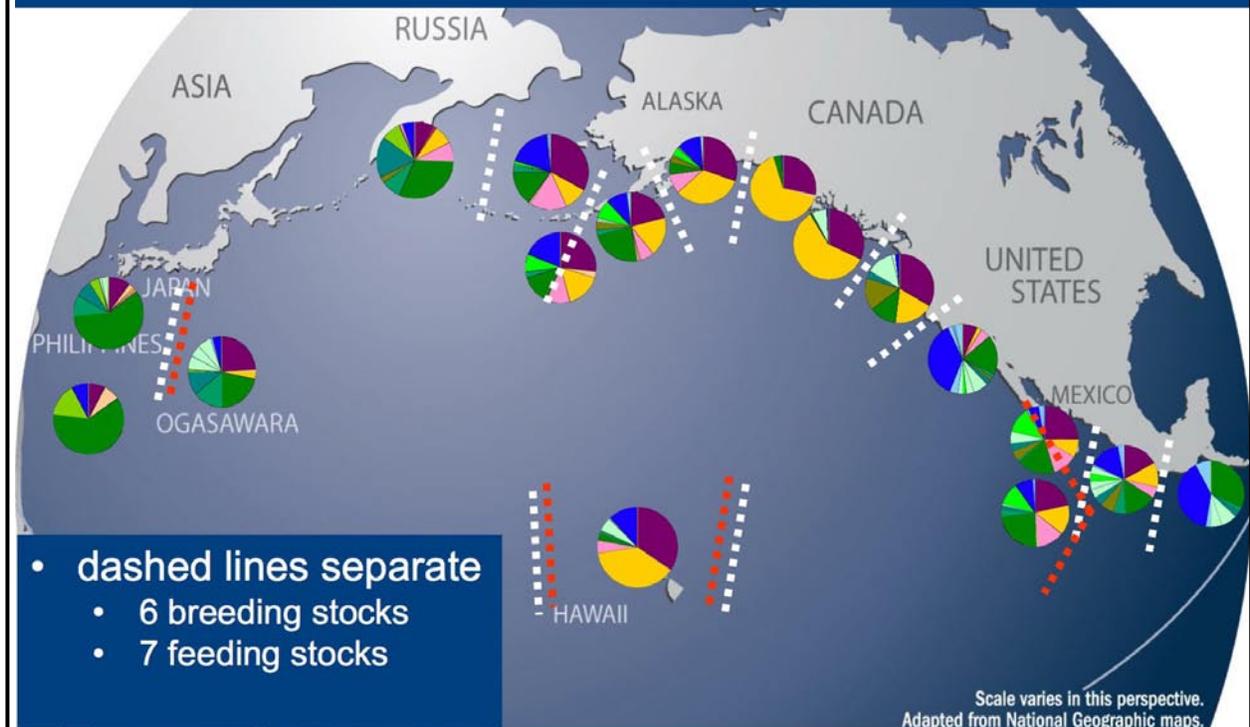
## ***geneSPLASH*: genetic differentiation of ‘ecostocks’ and ‘breeding stocks’ in North Pacific humpback whales**

Presented by: C. Scott Baker and Debbie Steel, Marine Mammal Institute and Department of Fisheries & Wildlife, Oregon State University, 2030 SE Marine Science Dr, Newport, OR 97365

The population structure of humpback whales in the North Pacific is complex, with strong maternally directed fidelity to feeding regions and geographic isolation of breeding regions. Given this complexity, it is possible that ecological units or ‘ecostocks’ could be defined by differences in the maternally inherited mitochondrial (mt) DNA and that significant reproductive units or ‘breeding stocks’ could be defined by differences in nuclear (nu) DNA. Here we report on initial results of a comprehensive, ocean-wide survey of mtDNA (control region sequences) and nuDNA (microsatellite loci) diversity using  $n = 2,188$  samples collected from 10 feeding and 8 breeding regions by *SPLASH*, primarily in the winter and summer of 2004. We first used microsatellite genotyping of 10 loci to identify replicate samples within regions and matches between regions. After review for quality control and removal of replicate samples, we identified  $n = 1,834$  regional individuals, 33 of which demonstrated migratory movement between feeding and breeding regions based on genotype matching (see Steel and Baker ‘*geneSPLASH*: migratory interchange and sex specific estimates of abundance based on genotype matching’).

Significant differences in frequencies of mtDNA haplotypes supported the delineation of 7 ecostocks among the 10 feeding regions (overall  $F_{ST} = 0.179$ ), corresponding to Russia, Bering Sea, western Gulf of Alaska, northern Gulf of Alaska, southeastern Alaska/northern British Columbia, southern British Columbia/Washington and California/ Oregon (Figure 1). Significant differences in allele frequencies of microsatellite loci delineated 5 ‘breeding stocks’ among the 8 breeding regions (overall normalized  $F'_{ST} = 0.034$ ), corresponding to Okinawa/Philippines, Ogasawara, Hawaii, offshore Mexico, and mainland Mexico/Central America. Significant differences in frequencies of mtDNA supported further subdivision between mainland Mexico and Central America. Baja California was intermediate between offshore Mexico and mainland Mexico for both mt and nuDNA markers, suggesting mixing of breeding stocks during migration. The complexity of the Asian breeding regions requires further investigation, given differences between Okinawa and Ogasawara and the absence of samples from coastal Asia (e.g., Korea). This initial comprehensive analysis of both mtDNA haplotype and nuDNA allele frequencies confirms the potential to define Distinct Population Segments based on patterns of reproductive isolation, as well as maternal fidelity.

## Eco-stocks and breeding-stocks of humpback whales in the North Pacific



**Figure 1:** The genetic differentiation of ‘ecostock’ (based on mtDNA haplotype frequencies) and ‘breeding stocks’ (based on nuclear DNA microsatellites and mtDNA) for humpback whales in the North Pacific. Dashed white lines separate ecostocks and breeding stocks with significant differences in mtDNA haplotype frequencies. Dashed red lines separate breeding stocks with significant differences in microsatellite allele frequencies

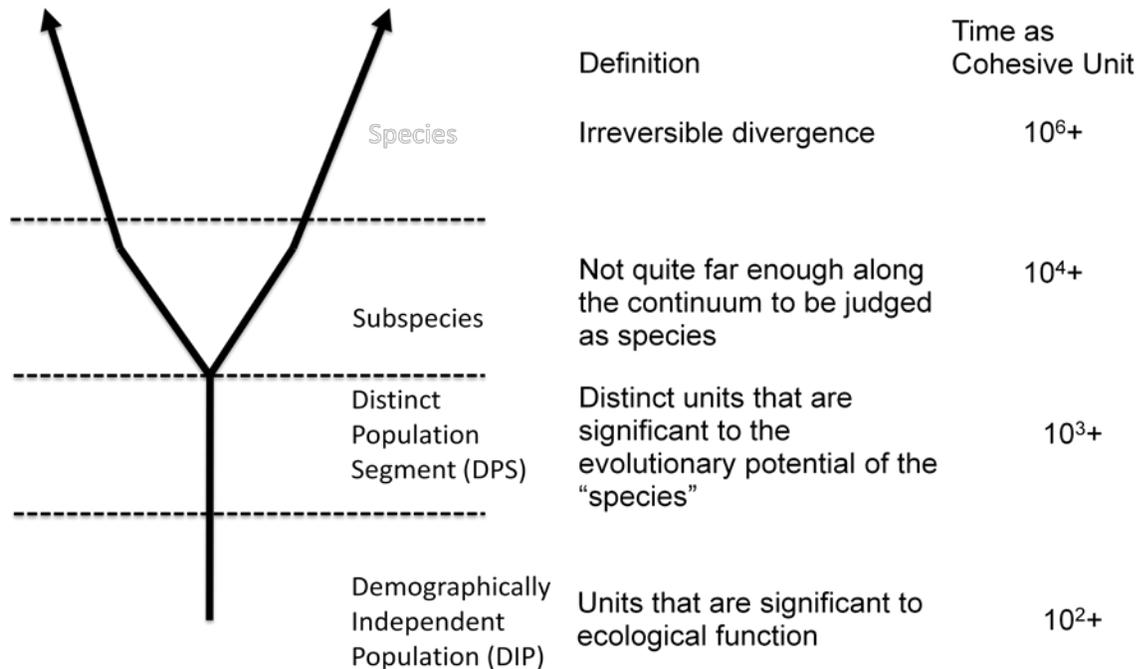
## Units to Conserve relevant to humpback whale conservation

Barbara L. Taylor, Southwest Fisheries Science Center, NOAA Fisheries

Potential management changes for humpback whales based on interpreting SPLASH results will be enhanced by understanding the different levels of population structure recognized by different management constructs. Here I review general background of population structure and several different management systems that relate to humpback whales: the IUCN redlist criteria, U.S. Endangered Species Act, Canada’s Species at Risk Act, International Whaling Commission, and U.S. Marine Mammal Protection Act.

### General introduction to structure

Evolution is a continuous process often represented by trees with time running from the trunk to the tips of branches and major branch splits representing genetically independent lineages. The simplified diagram below represents different levels of population structure. At the top we see two lineages that have diverged into separate species. Species are defined as having irreversibly diverged and are cohesive units roughly on the order of at least hundreds of thousands of years. Subspecies are not quite far enough along the continuum to be judged as species and have been cohesive units for on the order of tens of thousands of years. No humpback whale subspecies are recognized, so we are for the most part looking at lower levels of structure. The next level down generally refers to Units that are significant to the evolutionary potential of the species and these units are cohesive on the order of thousands of years. These units go by several names, described below. The lowest level of structure is units that are significant to ecological function and are cohesive on the order of hundreds of years.



For humpback whales we are concerned using SPLASH data to refine units for the lower two levels. Let me first discuss the level of evolutionary significance. The US Endangered Species

Act protects units and calls them Distinct Population Segments or DPSs. To qualify as a DPS the unit must sequentially pass three tests. The first test is discreteness of the population relative to the rest of the “species”. Under the ESA a “species” is either a species or a subspecies. If the segment under consideration has a trinomial (3 Latin names), then it would be considered relative to that subspecies. Since there are no subspecies for humpback whales, the test questions will be relative to the global distribution. To pass the discreteness test, you must be markedly separated by one of the following four factors: physical, physiological, ecological or behavioral. Note that genetics is not mentioned, though it has been used here and it certainly seems like a useful way to describe discreteness. Some have argued that using genetics here is using the same data twice since genetics is explicitly mentioned on the second test: significance.

The significance test is met when a segment is biologically or ecologically significant to the “species” by meeting any one of the following criteria: 1) persistence in an ecological setting unusual or unique to the taxon, 2) loss of the segment would result in a significant gap in the range of the taxon, 3) the segment differs markedly from other segments in genetic characteristics. Note that in this case, genetics is assumed to link to biological or ecological significance. One could argue that frequency differences or even no shared haplotypes for neutral markers is not sufficient here. On the other hand, there is nothing in either criteria that suggests that evidence of a different evolutionary pathway is required. Overall, the flavor of the DPS is that it is a unit that is important to the evolutionary potential of the species. It aims at maintaining healthy diversity. It wants to avoid preservation under the ESA of segments like the squirrels of Central Park in New York City. The last criterion of conservation status doesn't pertain to this talk but I show it because it does relate to the panel discussion. Under the ESA there are no quantitative criteria that define what a “threatened” or “endangered” species is and the argument is made on a case-by-case basis often in the Recovery Plan.

Canada's Species at Risk Act differs in several ways not least of which is by having quantitative criteria based on the IUCN redlist criteria. This Act considers a species to be a species, but allowances are made for the subspecific level through Designatable Units (DUs). Subspecies are DUs but you can also be a DU by passing criteria very similar to the DPS criteria. The Canadians have, however, been much more specific with respect to genetics. For example, the criteria for discrete are: 1) Evidence of genetic distinctiveness including, but not limited to, inherited traits (e.g. morphology, life history, behaviour) and/or neutral genetic markers (e.g. allozymes, DNA microsatellites, DNA restriction fragment length polymorphisms (RFLPs), DNA sequences), 2) natural barrier to gene flow, and 3) occupation of differing eco-geographic region. The criteria for Significant are: 1) differs markedly from others in genetic characteristics thought to reflect relatively deep intraspecific phylogenetic divergence<sup>1</sup>, 2) persistence in unique ecological setting conducive to local adaptation, or 3) loss would result in a significant gap in the range of the wildlife species in Canada.

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<sup>1</sup> Such differences would typically be manifested as qualitative genetic differences at relatively slow-evolving markers (e.g. fixed differences in mitochondrial or nuclear DNA sequences or fixed differences in alleles at multiple nuclear loci). Quantitative (frequency) differences of shared alleles, especially for rapidly-evolving markers such as microsatellites, generally would not be sufficient to meet this criterion.

The IUCN oddly uses “population” to mean “species”. They have only one other Unit, the subpopulation, which is geographically or otherwise distinct groups in the (global) population between which there is little demographic or genetic exchange (typically one successful migrant individual or gamete per year or less; IUCN 2001); a subpopulation may or may not be restricted to a region.

The U.S. has one more level of protection under the Marine Mammal Protection Act. The primary objective of this Act is to maintain “population stocks” as functioning elements of their ecosystem. A population stock is a demographically independent population where internal population dynamics are far more important to maintaining status the external dynamics (immigration).

The North Pacific illustrates structure at several scales relating to different management goals. Feeding grounds are quite separate and can be considered to be DIPs (and therefore treated separately under the MMPA). This is similar to the North Atlantic. Breeding grounds are also quite separate and may constitute different DPS status. However, some feeding grounds have mixed stocks from breeding grounds (Kodiak as an example) while other feeding grounds (like California) are connected only to Mexican/Central American breeding grounds. This biology makes categorization of “units” according to the different goals expressed in laws challenging to implement.

Finally we come to the International Whaling Commission. Historically the IWC managed based on large geographic sectors that were supposedly connected to whale movements. The new approach seems to be case specific. The overall idea is to construct a management model where the allowed kills are robust to plausible scenarios of stock structure. As far as I know, the IWC has still not defined what exactly it intends to conserve so the arguments continue to be influenced by who is at the table.

## **SPLASH Symposium Panel discussion summary**

Notes from Erin Falcone, Cascadia Research, Olympia, WA

Following the scheduled presentations a panel was convened to allow for open discussion among the Steering Committee members and symposium participants. The discussion began with general remarks by several panel members and then proceeded into a question and answer period. Most comments related to the conservation implications of the SPLASH results. Paul Wade opened the discussion by noting that given the complexity of stock structure, any future management decisions would need to address both feeding and breeding stocks to be effective. Barlow followed this by noting that distinct mark-recapture estimates should be derived from both the photographic and genetic datasets to provide measures of heterogeneity inherent in each. Baker added to this comment, clarifying that at this point the photographic and genetic datasets have not been fully reconciled, but that a 60% overlap in individuals between the two is expected. He also offered that his lab at OSU is willing to provide supplementary genetic data back to sample contributors as it becomes available, although there may need to be an accompanying proposal for sample use.

**Questions to the panel follow, in Q and A format:**

**Q1. (Directed to Barb Taylor) What are the management implications of delisting North Pacific humpbacks under the ESA, given they will continue to receive protection under the MMPA?**

A1. Assuming the MMPA was truly functional as intended, there would be no risk to delisting humpbacks in US waters in theory. However, the ESA provides for much stronger management “teeth” than does the MMPA, therefore a species receives more benefit from ESA listing than MMPA protection alone. Further, the MMPA may not protect species as effectively from indirect harm as ESA listing can.

**Q2. (Directed to Scott Baker) Were there significant pairwise differences in Fst scores presented between regions?**

A2. Yes, and delineation between regions depicted in slides reflects where these Fst scores suggest samples were distinct. These scores have not tested for variation related to distance.

**Q3. The 1991 recovery plan for humpbacks dictated a doubling of the population size within 20 years. What is considered the baseline population size for the North Pacific? Based on this, has it doubled yet?**

A3. The population estimate derived in from the NPAC retrospective study using data from 1991-1993 is considered the best baseline currently available. Using this, the results of SPLASH indicate that the population appears to have doubled. One key analysis that remains to be done is to subsample the SPLASH dataset to mirror the regional samples included in the NPAC study for a more accurate comparison between the two. John Calambokidis added that using both the NPAC results as well as an earlier rough estimate by Rice suggest the population has been growing at an overall rate of 4-6% throughout the period of protection.

**Q4: Is anything known regarding the actual carrying capacity of the ocean for this species? Is anyone, such as the IWC, looking at this?**

A4. We can only assume that the population was at carrying capacity prior to exploitation, and while difficult these estimates are being attempted for several populations using early whaling records where available.

**Q5. The SPLASH photo-ID results suggest less discrete populations units than do the genetics. Why this and what are the consequences of this difference for managing this population, given variation in standards for defining management units?**

A5. Genetics are more likely to reflect true long-term site fidelity, where photo-ID picks up the whales on “vacation” (i.e. temporarily outside their normal feeding/breeding area) in a given sample. Dispersal is a poor indicator of gene flow in some places, and whales have to actually reproduce during an excursion outside their normal range for this movement to be reflected genetically, therefore genetics generally provide a more conservative estimate of differentiation within a population. It’s complicated in a small dataset, but likely fairly static on average through greater periods of time: for example, areas with long term historical datasets show genetic stability in feeding areas. An audience member also added that whaling and discovery tag data (though samples are small) often suggest patterns very different from what we perceive from photo-ID.

**Q6. Why not delist the overall North Pacific humpback whale population from ESA and instead list Distinct Population Segments (DPS) corresponding to feeding areas under the MMPA?**

A6. To delist a species or population we must ensure that whatever depleted it initially is no longer a threat, so a decision to delist would have to assume the whaling ban would persist. Another issue is that the habitat the species relies on must be considered stable, and many organizations are likely to fight against delisting based on our general lack of knowledge about the actual carrying capacity for this population and the unknown effects climate change may present to the continued stability of their habitat.

**Q7. SPLASH results suggest Asia is home to a small, heavily depleted population segment that is not recovering as well as others in the North Pacific. Is it possible to separate the Asian stock before delisting the rest?**

A7. Central America may also represent a fragile population segment. In general, these questions require a better review of catch records before delisting any population segments is considered, as there are still too many unknowns. Both Asia and Central America also appear to be subject to higher risks, which will make delisting very difficult. Paul Wade also noted what is likely the most effective designation for these two population segments is IUCN listing, since both spend so much time outside US borders.

**Q8. What units, Fst or other another statistic, should be used to formally define these segments? What program was used for genetic differentiation in the results presented?**

A8. The program Gene Class was used, and results of Fst and exact tests are a robust means of addressing these types of questions at this point. Genetic results also tend to indicate larger trends than photo-ID can as they provide an additional measure of relatedness among sampled individuals.

**Comment from audience (Brownell): Whales were originally listed under the ESA due to the ineffectiveness of IWC at managing their decline. There is currently effort underway to uplist the western North Pacific stock under the IUCN, which if successful may prevent the US from downlisting under the ESA, given an agreement for protection on the IUCN level.**

**Q9: What is the current status of the ESA downlisting process for this population?**

A9. A five-year status review is overdue for humpbacks, so this needs to happen. New information is being compiled now, and early next year a message of intent to form review team should be posted. This team will recommend what DPS should be identified and protected based

on global information and status. This recommendation then goes to US Dept of Commerce for public review, then ultimately implementation. A decision to downlist would have to be attempted following this step of designating new DPS. John Ford adds that in Canada the government doesn't dictate management units, rather the COSEWIC marine mammal subcommittee does. In Canada the Atlantic population has already been downlisted, and the Pacific population is about to go under review in light of the SPLASH results. It is early in the process, however, as they need to assess if more than one management unit is present in the Canadian Pacific, and if so whether the units face different threats.

**Q10. Is there coordination between the Canadian and US governments for listing species given proximity?**

A10. With Southern Resident Killer Whales there has been coordination in listing within both countries, though no formal process exists for doing so. Canada listed Southern Resident Killer Whales first though, and has been making sure standards agree across the border. Given frequent use of this border region, humpbacks would likely also benefit from such a coordinated process. On a related note, Mexico is in process of making humpbacks their second PACE listing (a new process similar to the US ESA, the Vaquita was the first listed species). At this point the process is not well-developed, and very subject to political cycles. Interest in listing humpbacks was driven by visible impacts on this species as well as the growing whale watch industry. It is unclear if the Mexican government will treat inshore/offshore population segments differently under listing.

**Closing Comment: The SPLASH Steering Committee will be developing recommendations following this symposium for all management concerns in all regions.**

## **Non-research benefits of the SPLASH Project**

Prepared by David Mattila, Hawaiian Islands Humpback Whale National Marine Sanctuary, 726 South Kihei Road, Kihei, Hawaii 96753

The SPLASH project has produced a number of important results and discoveries, and its results will continue to come out in scientific publications over the next several years. However the project can also take credit for a number of other accomplishments that may not be documented elsewhere, and so we do so here. Some of these were planned or at least foreseen, however, others were completely unexpected products of this large, international effort.

As with many large research projects, the institutions and individuals involved created opportunities for students to participate and learn, both in the field and in the lab. But this was taken a step further by NOAA-HIHWNMS and its partners PCCS and UABCS, who were able to support several students and researchers from the Asian, Hawaiian and Mexican breeding grounds to participate in research cruises each summer in the Gulf of Maine. On these cruises they learned photo-ID and data management, scar and health imaging, and biopsy sampling techniques. Many of these trainees went on to lead their SPLASH teams in the winter.

Several SPLASH scientists were able to participate in a symposium in Tokyo, organized and hosted by the SPLASH Asia coordinator. This successful event provided an opportunity for the project, and humpback whales, to gain visibility with the Japanese public and University students in particular.

The relatively new emphasis that SPLASH placed on humpback whale entanglement, and the communication that the project fostered, led to several opportunities for outreach and training on this human impact issue. These included numerous trainings, and ultimately the initiation of a coordinated disentanglement network, along the Alaska and US West Coast. In addition, two entanglement seminars and disentanglement trainings were conducted in Mexico (Pt. Vallarta and La Paz), with the support of CEC representatives in both countries.

The training and experience accumulated during the SPLASH project in Russia and Central America, has led to continued humpback whale research by local scientists. These regions had no modern history of conducting this type of work, but there now exists a dedicated and passionate local humpback researchers in each.

The results of the SPLASH project showed new linkages between humpback populations, along with cementing previously known ones. This connectivity, along with the international cooperation and communication that the project engendered between the hundreds of participants, help to highlight the commonality between the ten countries involved. Several other international collaborations arose from the environment thus created. For instance, NOAA's HIHWNMS hosted the first international conference on marine mammal protected areas in Maui, Hawaii, using the connectivity of migratory cetaceans to inspire its primary theme of "networking". This conference inspired several initiatives, including investigating the establishment of bilateral agreements between the Hawaiian humpback whale Sanctuary and marine protected areas housing the same humpback whales in other habitats and countries in the North Pacific.

# **SPLASH Steering Committee Research and Management Recommendations**

Based on SPLASH Symposium discussion and Steering Com. Meeting, October 2009

Following the Symposium the SPLASH Steering Committee met to both review the results of the Symposium and consider future research and management recommendations including those that were discussed at the Symposium.

## **Research Recommendations**

- 1 All unanalyzed SPLASH biopsy samples should be analyzed for DNA to further elucidate patterns of stock structure and to provide sex determination of identified animals. (a rough estimate of \$300-400K was given for these analyses).
- 2 Further genetic studies are needed to better understand the relationship between North Pacific humpback whales and Southern Hemisphere populations. It is recommended that further samples be obtained from Central America, including Colombia, and that full mtDNA genome analyses be undertaken to determine with better resolution the S Hemisphere haplotypes in the N Pacific population.
- 3 Review taxonomic status of North Pacific humpbacks relative to global populations including whether the North Pacific population constitutes a separate population, subspecies or other taxonomic unit.
- 4 Analysis is needed of existing additional samples and photo-identifications for Russia waters and any other areas that were undersampled and now have IDs, e.g. Guatemala.
- 5 Given apparent rates of entanglement in fishing gear, we recommend that there be an increased and coordinated effort to document gear types involved in entanglement incidents throughout the range of North Pacific humpback whales. Further scarring data should be collected in areas where there are currently insufficient samples.
- 6 The results of SPLASH should be re-evaluated as potential major habitat changes associated with climate change develop in the future.
- 7 Pregnancy rates of humpback whales should be evaluated by hormonal assays using existing SPLASH samples.
- 8 Given the probable existence of an unknown breeding area in the North Pacific, efforts should be made to identify this area and to obtain a larger sample from its primary feeding area the western Aleutian Islands .
- 9 New approaches to investigate the age structure of the North Pacific population song SPLASH samples be encouraged.
- 10 As a result of SPLASH, the humpback whale is now a particularly well-studied species that can and should be used as an example to better understand the population structure of other baleen whale species.
- 11 Given the success of SPLASH, it is recommended that similar cooperative and collaborative approaches be applied to assessments of other large whale populations.

## **Management Recommendations**

- 1 Support designation of western Pacific humpback whales as a distinct subpopulation under IUCN. Further research (see above) is recommended to determine whether the Central American humpbacks are sufficiently distinct to warrant similar status.
- 2 Due to high site fidelity of humpback whales on their summer feeding grounds in the North Pacific, which was revealed by SPLASH genetic and photo-ID, it is recommended that management of humpback whales be based primarily on units defined by feeding grounds (e.g., as is already being done for humpback whales in the Gulf of Maine). Conservation issues on wintering grounds should be managed regionally, but should also be taken into account in management of the animals' feeding ground affiliation.
- 3 Recognizing that Russian waters appear to contain a small feeding aggregation that may contain significant subunits and is linked to a small, genetically distinct breeding area, it is recommended that this area be considered of special conservation concern.
- 4 SPLASH evidence revealed that there are three distinct humpback whale breeding areas within Mexico with unique compositions and properties, and efforts within Mexico to protect these areas should be supported.

## **SPLASH Legacy**

A commitment has been made by the SC to make data public by end of 2011. We also have a commitment to provide a certain amount of SPLASH data available on the Pacific Life-sponsored web site. This would include making IDs available for matching, without providing associated data, until end of 2011. Applicants would receive data provided that they agree to a set of conditions, including appropriate acknowledgements, consideration of co-authorship for significant contributions of data where warranted.