Universidad Autónoma De Baja California

Facultad De Ciencias Marinas
Instituto De Investigaciones Oceanológicas

Patrones de variación genética y sistema de apareamiento de la ballena azul, *Balaenoptera musculus*, en el Golfo de California.

TESIS

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS

PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS EN ECOLOGIA MOLECULAR Y BIOTECNOLOGIA

PRESENTA

Paula Costa Urrutia

ENSENADA, BAJA CALIFORNIA, MEXICO

Abril del 2013

FACULTAD DE CIENCIAS MARINAS
INSTITUTO DE INVESTIGACIONES OCEANOLOGICAS
POSGRADO EN ECOLOGIA MOLECULAR Y BIOTECNOLOGIA

Patrones de variación genética y sistema de apareamiento de la ballena azul, *Balaenoptera musculus*, en el Golfo de California.

T E S I S

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS

PRESENTA

Paula Costa Urruria

Aprobada por:

Dra. Diane Gendron

Dra. Simona Sanvito

Dra. Yolanda Schramm

Dr. Luis Enríquez Paredes

Dr. Eduardo Santamaría del Angel

Dr. Francisco Correa Sandoval

Agradecimientos

La realización de este trabajo fue posible gracias a la colaboración de muchas personas que han participado en las distintas etapas de este proceso enriqueciendo y haciendo únicas cada una de ellas.

Parte fundamental de este proceso fueron mis orientadoras y directora académica de tesis. Le agradezco enormemente a mi orientadora, la Dra. Diane Gendron, por su confianza y dedicación en cada una de las etapas de este trabajo. Gracias Diane por compartir conmigo no sólo tu experiencia y tu conocimiento sobre las ballenas azules, gracias por compartir inolvidables momentos en el mar. A la Dra. Simona Sanvtio, orientadora de mi tesis, le agradezco por su invaluable apoyo, paciencia y gran contribución en este trabajo en cada una de sus etapas. A la Dra. Yolanda Schramm, le agradezco enormemente por compartir momentos de discusión académica, por escucharme cada vez que lo necesité y por hacer que mi adaptación, no sólo a mi nueva vida como estudiante de doctorado, sino también a mi nuevo país, fuera más fácil.

Agradezco enormemente a mi comité de tesis: Eduardo Santamaría-del Ángel, Francisco Correa y Luis Enríquez-Paredes por los aportes a este trabajo.

Quiero de expresar mi profundo agradecimiento a Filippo Galimberti colaboración y aportes a este trabajo.

Deseo agradecer a mis amigas y colegas que me han acompañado en este viaje desde el principio; Carolina Abud, Valentina Franco-Trecu, Paula Franco-Fraguas, Cecilia Pasadore, María Nube Szephegy, Federico Riet y Katerina Dimitriadis. Particularmente Carolina Abud y Esther Jiménez les agradezco muchísimo por sus valiosos aportes en la traducción de los artículos y edición de las figura respectivamente. A mi amiga Diana Kusters por recibirme en su casa desde el primer día que llegué a Ensenada, para luego hacer que se convirtiera en la mía también. A Yess Zea por su colaboración en la edición final de la tesis.

Mi agradecimiento y admiración a todo el Grupo de Ecología de Cetáceos de CICIMAR-IPN, compuesto por estudiantes y técnicos, los cuales han trabajado en la colecta de datos y comparación de fotos durante más de dos décadas. En especial a Azucena Ugalde Cruz por su trabajo con el catálogo de foto identificación de ballena azul. Un especial agradecimiento a Angélica Arce e Ivone Giffard por el apoyo y facilitación de las actividades administrativas relacionadas con mi tesis.

Un agradecimiento especial a los organismos e instituciones que apoyaron económicamente este trabajo; al Consejo Nacional de Ciencia y Tecnología por apoyar mi formación académica a través de la beca CONACyT. El trabajo de campo anual fue financiado por el Instituto Politécnico Nacional y el CONACyT (SEMARNAT 2002-C01-628). Asimismo, este trabajo fue parcialmente apoyado por la Universidad Autónoma de Baja California (Proyecto 12C-0351) y por la Sociedad Americana de Cetáceos de Monterey Bay, USA (081.209). Las muestras fueron recolectadas con permisos 071197,050199, DOO.02-5167, 0095, 8318, SGPA/DGVS/7000, 00624, 01641, 08021, 00506, 09761, 08796 y emitido por la Secretaría del Medio Ambiente de México y Recursos Naturales (SEMARNAT).

Finalmente, no tengo palabras de agradecimiento para mi familia y para el amor de mi vida Rafa por el apoyo incondicional y creer en el valor de la ciencia en todo momento.

Resumen General

La diferenciación de poblaciones es un aspecto clave en los procesos microevolutivos. Por otro lado, la identificación de unidades genéticas en ambientes sin barreras geográficas claramente definidas representa un desafío para la conservación y manejo de las especies silvestres. Aunque es aceptado que la estructura social y los sistemas de apareamiento influyen en la estructura poblacional, incluso en poblaciones con distribución continua, poco se conoce sobre aspectos sociales de la mayoría de los misticetos. En este trabajo se reunió información sobre foto-identificación obtenida a largo plazo de la ballena azul, Balaenoptera musculus, en el Golfo de California con información genética para investigar su estructura genética a escala fina, aspectos generales de la estructura social y asociaciones espaciales de parentesco. Para ello fueron analizados 177 individuos usando nueve loci de microsatélites a partir de biopsias de piel. Los análisis de estructura poblacional sugieren que las ballenas azules conforman una única unidad poblacional. Sin embargo, se encontró estructura a escala fina para las hembras utilizando análisis espaciales de clúster bayesiano. Esta estructura se vio apoyada por el hecho de que uno de los clústers presentó mayor nivel de parentesco en relación al otro, lo que sugiere una mayor fidelidad y filopatría por parte de las hembras a un área en particular. Los grupos de ballenas azules se caracterizaron por mostrar alta variación temporal en relación al tamaño y composición de los mismos. El bajo nivel de parentesco promedio observado en los grupos, sugiere que estos no son grupos familiares, esto se lo ha asociado en otras especies a un sistema de apareamiento promiscuo. Estas características constituyen el primer indicio que sugiere una dinámica de fusión-fisión. Los grupos de mayor tamaño y los que contienen pares madre-cría presentaron mayores niveles de parentesco, ambos podrína conferir una ventaja para la supervivencia de la cría Un análisis global de la distribución de la variabilidad genética en diferentes niveles inclusivos (nivel poblacional, sub-poblacional y social) sugiere que el nivel poblacional representa el nivel más homogéneo y estable. Variaciones en la dispersión de los machos podrían resultar en fluctuaciones en la heterocigosidad de la población entre temporadas reproductivas, sin embargo, es probable que a largo plazo resulte en un efecto homogeneizador a nivel poblacional. En cambio, el sesgo en la distribución de las hembras resulta en cierta heterogeneidad genética espacial a escala sub-poblacional. Probablemente, esto representa un nivel intermedio de estabilidad, dado que los movimientos de hembras y cambios en el sitio de nacimiento entre temporadas reproductivas pueden aumentar o diluir la intensidad de divergencia genética en este nivel. Por último, los grupos con un nivel de inestabilidad elevado, probablemente representando una alta dinámica fusión- fisión, corresponden al nivel más inclusivo e inestable en esta población, dado que la distribución de la variabilidad genética puede cambiar entre semanas o incluso días. Es probable que esta dinámica mantenga bajos niveles de divergencia a nivel poblacional. Este mecanismo evolutivo pudo haber contrarrestado la pérdida selectiva de la diversidad genética debido a la intensiva caza comercial dirigida hacia esta especie durante los últimos dos siglos.

General Abstract

Population differentiation is a key aspect of microevolutionary processes. Furthermore, identification of genetic units in environments without well-defined geographical barriers represents a challenge for conservation and wildlife management. Although it is accepted that social structure and mating system structure produce great impact in shaping fine-scale population structure even in taxa characterized by continuously distributed populations, little is known about the social aspects of most baleen whales. Here we combined long-term photo-identification data with genetic information to investigate fine-scale population structure, general aspects of social structure and spatial kinship associations of blue whales, Balaenoptera musculus, in the Gulf of California. We analyzed a total of 177 individuals using nine microsatellite loci. Fine-scale population analysis provided information that suggests that blue whales are part of a single population unit. This is supported by all analyses conducted on the complete data set: temporal grouping criteria, isolation by distance and Bayesian clustering approach. However, structure among females was found using spatial Bayesian clustering approach. This structure was supported by the finding that one of the clusters showed higher level of kinship than the other, which suggests high site fidelity and philopatry of some females to a particular area. Blue whales groups were characterized by high temporal variation in size and composition. Low average group relatedness suggests no family-breeding groups. This low relatedness within groups has been related in other cetaceans with promiscuous mating systems. These features are the first steps to suggest a fusion-fission dynamic to explain the blue whale social system. Bigger groups and groups including mother-calf pairs into them showed higher level of kinship, this may provide an advantage for calf survival. A global analysis of the distribution of genetic variability at different inclusive levels (population, subpopulation and social levels) suggests that population level represents the most homogenous and stable level, since it is the result of the interaction of the other more inclusive levels. Variations in male dispersal may result in population heterozygosity fluctuations among breeding seasons; however, due to gene flow it could have a long-term homogenizing effect at the population level. On the other hand, female distribution bias results in spatial genetic heterogeneity at subpopulation level. This probably represents an intermediate level of stability. Female movements and some changes in natal site across breeding season may either enhance or dilute the intensity of genetic divergence at this level. Finally, high fusion-fission group dynamics may represent the most inclusive and unstable level in this population, since the distribution of allele frequencies changes over weeks or even days. It is likely that this dynamic maintains low levels of divergence in the population. Thus may constitute an optimal evolutionary mechanism to respond to selective genetic diversity loss due to the intensive commercial hunting toward this species over the last two centuries.

Indice General

Listas de Tablas y Figuras

Artículo 1

Listas de Tablas y Figuras

Artículo 2

Listas de Figuras

Discusión General

Introducción General

Esta tesis trata sobre la estructura poblacional y social de la ballena azul, Balaenoptera musculus, analizando la variabilidad genética a dos escalas de trabajo: a nivel poblacional y a nivel de grupos sociales dentro la población muestreada. Este trabajo se enmarca en un creciente interés sobre el estudio de los niveles de estructuración en poblaciones naturales y los procesos evolutivos implicados. Este interés, se vió reforzado por el hecho de que la detección de unidades biológicas discretas se transformó en una herramienta básica para la conservación y el establecimiento de planes de manejo. Más recientemente, debido al incremento sobre el conocimiento de las estructuras sociales que poseen los cetáceos, ha venido creciendo el interés en comprender la relación entre la estructura social y la conformación de poblaciones discretas. El uso de aproximaciones moleculares junto con otras, (metales pesados, parásitos, isótopos estables) ha abierto nuevas oportunidades de avanzar e integrar datos ecológicos, comportamentales y genéticos, permitiendo mayor comprensión sobre la relación de estos procesos.

Esta introducción comienza por explicar factores que determinan e influyen sobre la estructura poblacional, social y los sistemas de apareamiento. Se ejemplifica con trabajos realizados en cetáceos y se termina resumiendo los antecedentes sobre la estructura poblacional y social de la ballena azul.

Estructura Poblacional

El estudio de diferenciación poblacional en ambientes sin barreras geográficas definidas es tema de controversia y gran interés tanto en biología evolutiva como en el manejo de las poblaciones silvestres (Parson et al. 2006, Waples & Gaggiotti 2006, Mendez et al. 2010, Sremba et al. 2012). Los mamíferos marinos, en especial los cetáceos, a pesar de tener amplia distribución y gran capacidad de dispersión, presentan estructuración poblacional al menos por parte de alguno de los dos sexos. Por otro lado, los estudios de estructura social y sistemas de apareamiento en organismos marinos son escasos, exceptuando el caso de los pinípedos comparados con la información proveniente de animales terrestres. Por lo que, generar información en este sentido contribuirá a comprender la evolución de los sistemas sociales y cómo estos influyen en la diferenciación poblacional.

La ballena azul se distribuye a lo largo de Pacifico Nororiental y en el Golfo de California. Un

estudio realizado con ADN mitocondrial (ADNmt) utilizando muestras provenientes de aguas mexicanas mostró la existencia de dos grupos de linajes maternos (Enríquez-Paredes 2005). Sin embargo, hasta el momento no se ha analizado la estructura poblacional utilizando un marcador nuclear que representa a toda la población, por lo que ésta aún se considera incierta. Por otro lado, dada la dificultad de estudiar el comportamiento de estos animales en el campo, el enfoque molecular parece ser un complemento ideal para contribuir a esclarecer las incertidumbres sobre la estructura social y sistema de apareamiento.

El análisis de la subdivisión poblacional es un tema central en la genética de poblaciones. La estructuración de las especies en unidades discretas es un importante factor influenciado por múltiples aspectos de la historia de vida de los organismos. Estas unidades pueden estar afectadas por diversos factores ecológicos, por lo tanto, el potencial de dispersión de los individuos no siempre predice la cantidad de flujo génico entre sus poblaciones. Para determinar la estructura poblacional a nivel molecular, es necesario comprender la distribución espacial de la variabilidad genética (Allendorf & Lukiart 2007). Uno de los desafíos principales en este análisis, es hallar la magnitud de flujo génico entre poblaciones (Slatkin 1995). En general, el flujo génico reduce las diferencias genéticas entre poblaciones, incrementando la variabilidad genética dentro de las poblaciones. Este es un componente fundamental en la estructuración dado que determina hasta qué punto las poblaciones evolucionarán como una única unidad o unidades independientes (Allendorf & Lukiart 2007).

Otra fuerza clave en la estructuración de las poblaciones es la deriva génica. Ésta es un cambio al azar en las frecuencias alélicas en poblaciones finitas. Dado que el efecto de la deriva es inversamente proporcional al tamaño poblacional, poblaciones aisladas y pequeñas son más susceptibles a los efectos de ésta. En consecuencia la tendencia resultante es la reducción de la heterocigosidad poblacional (Wright 1931).

En suma, en ausencia de selección natural, la divergencia genética entre poblaciones constituye un balance entre los efectos homogeneizadores del flujo génico y los efectos divergentes producidos por la deriva (Allendorf & Lukiart 2007). Esta interacción ha sido analizada a través del modelo de islas, introducido por Wrigth (1931). En este modelo, una serie de poblaciones de tamaño finito, las cuales intercambian migrantes y el número de migrantes alcanzan eventualmente un equilibrio, donde la variación total entre islas está determinada por la deriva (función del tamaño poblacional) y la migración.

En condiciones de equilibro, mencionadas en el párrafo anterior, es posible medir el flujo génico a partir del índice de fijación F_{ST} (Gillespie 1998). Este índice es comúnmente utilizado para estudiar la conectividad y patrones de flujo génico entre poblaciones (Wright 1931).

Los estadísticos F o índices de fijación fueron originalmente introducidos por Wrigth (1931, 1951) para cuantificar los efectos de la endogamia y subdivisión poblacional. El modelo de mutación en el cual se basa este índice se denomina Modelo de Alelos Infinitos (IAM, por sus siglas en ingles) el cual considera que cada mutación genera un nuevo alelo (a una tasa μ) que no estuvo presente anteriormente en la población. Alelos idénticos serían idénticos por descendencia, por lo tanto, este modelo no admite homoplasia (Kimura & Crow 1964, Balloux & Lugon-Moulin 2002).

Medir la subdivisión genética a través los estadísticos F presenta limitaciones cuando se utilizan loci de alta variabilidad como es el caso los microsatélites, en el cual no es conveniente considerar a la mutación como una fuerza poco probable. Los F_{ST} tienden a subestimar la divergencia entre subpoblaciones cuando la heterocigosidad en cada una de ellas es alta. Esto genera que la proporción de variación total distribuidas entre las poblaciones sea baja. Otra de las limitaciones que presenta es que el F_{ST} no considera la identidad de los alelos. Esta información es incorporada con un índice relacionado al F_{ST} denominado el R_{ST} (Allendorf & Lukiart 2007).

El R_{ST} incorpora información sobre el largo de los alelos de microsatélites y es definido como la proporción de la variación en cuanto a lo largo de los microsatélites debido a diferencias entre poblaciones. R_{ST} asume que cada mutación producirá un cambio en una unidad de repetición, ya sea una adición o una deleción, con igual probabilidad en las dos direcciones. Alelos similares en tamaño estrían más relacionados que alelos diferentes en tamaños (Allendorf & Lukiart 2007).

En otras palabras, si los alelos presentes en dos poblaciones difieren en pocos pasos podrían estar bajos los efectos homogeinizadores del flujo génico. En cambio, si difieren en varios pasos las poblaciones podrían experimentar acumulación de divergencia debida a un escaso o nulo flujo génico entre las mismas. Este modelo se denomina Modelo de Mutación por Pasos (SMM, por su sigla en inglés) (Kimura & Otha 1978) y permite homoplasia por lo que alelos iguales podrían ser iguales por estado y no por descendencia.

Es aceptado que el mecanismo de mutación de los microsatélites se ajusta mejor al SMM que al IAM, sin embargo, ningún modelo se ajusta perfectamente a todos los loci de microsatélites (Balloux & Lugon-

Moulin 2002). Si el mecanismo de mutación de los microsatélites considerados se ajusta estrictamente al SMM, es esperable que los valores de R_{ST} sean más elevados que los de F_{ST}. En cambio si no se ajustan a un modelo de mutación por pasos se espera que ambos estadísticos presenten valores similares.

Una cosa importante que resaltar es que ambos modelos (IAM y SMM) consideran un número infinito de estados alélicos posibles, sin embargo, al parecer el número de estados alélicos es finito, lo que limita el tamaño de los alelos en un intervalo dado. Si se considera un número de alelos posibles K en un locus dado, el modelo de mutación de los microsatélites se ajustaría mejor al Modelo de Alelos K (KAM, por su sigla en inglés). Este modelo considera K alelos posibles y la probabilidad de mutación es K-1 a una tasa $\mu/(K-1)$. Este modelo al igual que el SMM admite homoplasia. Es importante notar que el IAM es un caso particular de KAM cuando $K=\infty$ (Balloux & Lugon-Moulin 2002). En este caso, la diferencia en el largo de los microsatélites no reflejaría distancias genéticas entre los mismos y el R_{ST} podría subestimar la divergencia entre las poblaciones. Sin embargo, este efecto no sería tan importante en el F_{ST} (Balloux et al. 2000). Si bien existe mayor aceptación en que el R_{ST} se ajusta mejor al modelo de mutación de los microsatélites, es importante considerar los dos índices y tomar precauciones a la hora de la interpretación (Balloux & Lugon-Moulin 2002).

El enfoque mencionado, basado en los estadísticos F o también llamado enfoque basado en poblaciones, dado que requiere poblaciones predefinidas, constituye la base conceptual y estadística de la genética de poblaciones. Por lo tanto, es considerado el enfoque tradicional (Neigel 2002). Desde un punto de vista estadístico, este enfoque es robusto, pero una estratificación a priori puede tener poco o ningún sentido para ciertas poblaciones (Pritchard et al. 2000). Este enfoque puede complementarse con otros más recientemente desarrollados basados en individuos, los cuales tienen en cuenta la variación genética individual para asignar individuos a grupos poblacionales discretos. En otras palabras analizan la estructura poblacional de una muestra dada, sin la necesidad de realizar subdivisiones a priori. En los métodos más usados se encuentra el análisis de clúster Bayesiano o espacial (Guillot et al. 2005, Corander et al. 2008), el aislamiento por distancia (Hardy & Vekemans 2002, Peakall & Smouse 2006) y el análisis multivariado de autocorrelación (Peakall & Smouse 2006).

En particular, los métodos Bayesianos asignan probabilísticamente individuos a grupos en equilibrio Hardy-Weinberg. Más recientemente la inclusión de las coordenadas geográficas de cada individuo, como información a priori, ha resultado una herramienta interesante para detectar discontinuidades en el espacio.

Estos métodos han sido adoptados por un creciente número de investigadores en los últimos años, con lo que se han desarrollado un importante número de algoritmos y modelos (Corander et al. 2008, Guillot et al. 2005, Guillot et al. 2009, Tang et al. 2009). Además, varios estudios han demostrado que los modelos espaciales de clúster Bayesiano son una potente herramienta para detectar diferenciación genética críptica o baja entre poblaciones (Coulon et al. 2006, Fontaine et al. 2007, Holzer et al. 2009). Sin embargo, la robustez de los métodos bayesianos podría disminuir en algunos escenarios evolutivos particulares (Francois & Durand 2010), por ejemplo, cuando las poblaciones se encuentran bajo el patrón de aislamiento por distancia (es decir, aumento gradual de la distancia genética entre los individuos o subpoblaciones según la distancia geográfica) (Frantz et al. 2009, Safner et al. 2011).

Según el clásico modelo de islas, se considera que los migrantes son un grupo de individuos que se dispersan de forma aleatoria en cada generación, y que todos los individuos tienen la misma tendencia de dispersión y mismo éxito reproductivo (Hewitt & Bulit 1997). Al estudiar poblaciones naturales, se ha observado que en varios casos esto no sucede. Por ej., en poblaciones de mamíferos, es común observar sesgo en la dispersión entre sexos, siendo en general las hembras las que presentan filopatría al sitio natal (Greenwood 1980). Según la resolución del marcador utilizado, este sesgo en la dispersión puede arrojar distintos resultados en relación a los niveles de estructura poblacional (Hewitt & Bulit 1997).

En los ambientes marinos hay pocos límites físicos que puedan conducir a la diferenciación de especies que presenten alta capacidad de dispersión como es el caso de los cetáceos. Sin embargo, en general éstos presentan estructuración poblacional que posiblemente se debe a una combinación de factores, como la especialización comportamental por recursos locales y la estructura social (Hoelzel et al. 1998).

Algunos ejemplos de estructuración poblacional que reflejen la especialización por recursos locales se han reportado en el género Tursiops (Natoli et al. 2004, Parsons et al. 2006), ballenas piloto de aleta corta y larga (Globicephala macrorhynchus y G. melas, respectivamente) (Kasuya et al. 1998, Fullard et al. 2000), orcas (Orcinus orca) (Hoelzel et al. 1998) y cachalotes (Physeter macrocephalus) (Whitehead & Rendell 2004). Algunos ejemplos de la influencia de la estructura social sobre estructura poblacional se han reportado en orcas (Hoezel 1998), ballenas pilotos de aleta larga (Amos et al. 1993), delfín listado (Stenella coeruleoalba) (Gaspari et al. 2007) y toninas (Tursiops truncatus) (Lusseau et al. 2006).

En el caso particular de las ballenas, se reconoce que la fidelidad al sitio es transmitida vía materna. Las crías aprenden la ubicación del área de crianza y de alimentación en su primera migración. Esta fidelidad al sitio por parte de las hembras puede resultar en diferencias genéticas entre las poblaciones (o subpoblaciones) (Hoelzel 1998). Estructura genética poblacional utilizando ADNmt tanto entre sitos de crianza como de alimentación ha sido hallada en ballenas jorobadas (Megaptera novaeangliae) (Baker et al. 1998) y ballenas francas del norte (Eubalaena glacialis) (Shaeff et al. 1993). En un estudio realizado con ballena franca austral se encontró que valores isotópicos son más similares entre individuos que comparten el mismo haplotipo. Los autores sugieren que las crías aprenden la ubicación de las aéreas de alimentación por sus madres y además que esta herencia cultural se mantiene durante varias generaciones (Valenzuela et al. 2009).

Estructura Social

El objetivo de la ecología del comportamiento es analizar y entender el comportamiento como el resultado de la historia evolutiva de las especies. Para esto es importante comprender los eventos ocurridos a nivel poblacional o intra-poblacional y las características particulares de los entornos en los que ocurren determinados comportamientos (Danchin et al. 2008).

Por grupo se entiende al conjunto de individuos que presentan mayor proximidad dentro de las poblaciones, representando uno o varios niveles más inclusivos en el marco de la subdivisión poblacional. Si estos grupos son agrupaciones sociales, se esperaría que la interacción entre los mismos tenga consecuencias tanto para el individuo que realiza un comportamiento particular como para los demás miembros del grupo (Danchin et al. 2008). Asimismo, se esperaría que la selección juegue un rol diferenciando los distintos tipos de agregaciones de acuerdo a los costos reproductivos y los beneficios a nivel individual (Barnard 2004).

Uno de los desafíos más grandes que ha tenido tanto la etología clásica como la ecología del comportamiento es explicar el origen y mantenimiento de los comportamientos altruistas, en el marco de la Teoría neo-darwiniana o teoría Sintética de la evolución, en la cual la unidad de selección es el individuo. Cuando se observaba un comportamiento altruista, el cual reducía el éxito reproductivo del individuo que lo realizaba pero aumentaba el éxito de otros individuos en el grupo era incomprensible entender, para Darwin y los evolucionistas de la época, cómo había evolucionado el dicho comportamiento (Danchin et al.

2008). Una de las teorías que más aportó a este dilema fue la teoría de selección de parientes propuesta por Hamilton (1964), quien introduce a través de la misma, el concepto de eficacia biológica inclusiva (inclusive fitness). La selección de parientes se refiere a los cambios en las frecuencias alélicas a través de las generaciones que son debidos, al menos en parte, a interacciones entre individuos emparentados. El concepto de eficacia biológica inclusiva representa una ampliación del concepto clásico de eficacia biológica darwiniana. Hamilton (1964) en su formulación matemática añade un componente indirecto debido al éxito reproductivo de un individuo, obtenido a través de la interacción con parientes.

En este contexto, el desarrollo de la ecología molecular ha influenciado la forma de pensar y estudiar la ecología del comportamiento, permitiendo examinar las bases de la evolución social (Whitehead et al. 2000). El conocimiento de los patrones de parentesco dentro de un grupo permite conocer aspectos sobre el origen y estabilidad de los mismos, sobre el sistema de apareamiento de la población (Amos et al. 1993, Whitehead et al. 2000).

Los cetáceos son longevos por lo que presentan generaciones solapadas. Asimismo varias especies han mostrado tener un amplio repertorio de señales acústicas, así como alta filopatría al sitio natal, características que favorecen la evolución de las estructuras sociales diversas. En las especies de odontocetos que se han realizado mayor número de estudios tales como las delfines toninas (o nariz de botella), cachalotes, orcas y ballenas piloto se han observado grupos con elevada fusión-fisión, grupos matrilineales y grupos familiares estables respectivamente (Connor et al. 1996). Un caso extremo en relación a la estabilidad de los grupos se encontró en una población de orcas "residentes" del Pacífico Norte y de ballenas piloto donde los individuos tanto machos como hembras permanecen varios años en grupo natal (Amos et al. 1993). De forma contraria, un caso bien conocido en relación a la inestabilidad de los grupos la presentan los delfines tonina, los cuales presenten sociedad caracterizada por la fisión y fusión de grupos (Möller 2012). Las sociedades de intensa fisión-fusión están caracterizadas por cambios en la composición y tamaño del grupo. Estos cambios en la composición grupal pueden ser semanales, diarios o inclusive en horas (Connor et al. 2000). Más recientemente, se propuso el término dinámica de fisión-fusión, en lugar de sociedades fisión-fusión para caracterizar cualquiera sociedad por su intensidad en la fisión y fusión de grupos. Esta puede variar desde grupos estables, como grupos familiares, caracterizados por una baja dinámica de fisión-fusión a muy fluida caracterizada por la formación y partición de grupos de forma diaria o inclusive u horas (Aureli et al. 2008).

En conjunto, los cetáceos muestran varias formas de comportamiento social, incluyendo cuidado colectivo de las crías o asociaciones como hembras emparentadas con la madre. Tales comportamientos pueden involucrar formas de altruismo recíproco (Trivers 1972), favoreciendo la formación de grupos de cooperación tanto en la reproducción como en la alimentación.

Sistemas de apareamiento

Uno de los mayores avances conceptuales al estudio de los sistemas de apareamiento fue introducido por el trabajo de Emlen y Oring (1977). Según estos autores el sistema de apareamiento es el conjunto de estrategias comportamentales empleadas para la obtención de cópulas. Esto implica, el número de cópulas obtenidas, la estrategia de adquirir la pareja y los patrones de cuidado parental provisto por cada uno de los sexos. Estos factores estarían determinados por la proporción sexual operacional, la cual es definida como la relación promedio entre las hembras fertilizadas por los machos sexualmente activos. Esta relación está fuertemente influenciada por la distribución de los recursos o del sexo limitante. Este último es definido como aquel que tiene menor potencial reproductivo; generalmente las hembras, al menos en el caso de los mamíferos. En otras palabras, la proporción sexual operacional estaría influenciada por la sincronía reproductiva del sexo limitante y por las condiciones ambientales. Esto es lo que Emlen y Oring (1977) denominan el potencial poligínico del medio, lo que a grandes rasgos significa que en un ambiente dado deben de existir las condiciones necesarias para monopolizar hembras o los recursos necesarios para garantizar el éxito reproductivo de las mismas, lo que generaría un sesgo en el éxito reproductivo de los machos.

Más adelante Reynolds (1996) introduce el concepto de sistemas reproductivos donde se incluye la descripción de los comportamientos de apareamiento, se enfatiza en el cuidado parental de ambos sexos y la variación de estos aspectos a nivel individual.

Actualmente, al hablar de sistema de apareamiento se hace referencia a la manera en que individuos de una especie acceden a su pareja reproductiva, el número de parejas con la que interactúan, el tiempo de asociación entre éstas y la proporción de cuidado parental provisto por cada uno de los progenitores. En otras palabras, los sistemas de apareamiento pueden ser determinados por la habilidad de un sexo (usualmente los machos) en monopolizar cópulas del sexo opuesto, ya sea por asociación directa o control de recursos esenciales. En este contexto, el sexo con menor potencial reproductivo (en la mayoría de los especies las

hembras) es el recurso limitante para el otro sexo (Cezilly & Danchin 2008).

La teoría predice que la distribución de las hembras se ajustará a la disponibilidad de recursos, riesgo de depredación y zonas aptas para la reproducción, mientras que la distribución de los machos se ajusta a la distribución de las hembras. Este esquema, por demás simplificado, se complica cuando el macho invierte más allá de la producción de gametos, por ejemplo, proporcionando cuidado parental a las crías (Cezilly & Danchin 2008). A nivel general la probabilidad de que predomine un sistema de apareamiento u otro depende de múltiples factores tanto ecológicos (ej. distribución espacial de los recursos), fisiológicos (ej. sincronía en el estro de la hembras de una población) y la historia demográfica de las poblaciones (Elmen & Oring 1977, Brownell & Ralls 1986, Reynolds 1996, Wade & Suuster 2002, Weigman & Nguyen 2006). Las restricciones ecológicas impuestas por el medio, los factores demográficos que afectan directamente la proporción sexual operacional y la historia filogenética de la especie determinan si ambos padres cuidarán a las crías, sólo uno de ellos o ninguno.

Los sistemas de apareamiento se suelen clasificar de forma muy general en cuatro categorías principales basadas fundamentalmente en el número promedio de copulas de cada sexo, en una estación reproductiva (Cezilly & Danchin 2008). Estas categorías son poliginia, poliandria, monogamia y sistemas promiscuos (poliginandria). En varias especies los sistemas de apareamiento no son fijos, sino que pueden variar tanto entre como dentro de las poblaciones, probablemente debido a cambios en las condiciones ambientales, los cuales modificarían la proporción sexual operacional (Elmen & Oring 1977, Moblye & Jones 2009, Wilson 2009). Si bien la clasificación en las cuatro categorías antes mencionadas obscurece la dinámica y la complejidad de estos sistemas, engloba las generalidades de cada uno de ellos.

Los sistemas sociales y de apareamiento son mucho menos conocidos en el caso de los misticetos. Al parecer la unidad social básica mejor conocida es el par madre-cría. En varias especies se ha observado que estas díadas presentan segregación espacial dentro de las áreas de crianza, prefiriendo, en general, las zonas más costeras (Best 2000, Gendron 2002). Se han sugerido varias alternativas para explicar la segregación observada, como evitar depredadores, ocupar zonas de aguas calmas protegidas de vientos y mareas fuertes y evitar el acoso de los machos (Elwen & Best 2004). Si bien en la actualidad el tema está resuelto, las posibilidades mencionadas no son mutuamente excluyentes e integran las estrategias del cuidado parental por parte de hembras (Elwen & Best 2004). Al igual que en varias especies de mamíferos, el cuidado parental en misticetos parece ser exclusivamente materno (Berta & Sumich 1999, Clutton-Brock 1991) y en

las especies estudiadas, el sistema de apareamiento parece una combinación de bajo nivel de poliginia y/o promiscuidad (Berta & Sumich 1999, Cerchio et al. 2005, Frasier et al. 2007, Carrol et al. 2012).

En particular, la especie en la que se ha realizado mayor número de estudios en relación al sistema social y de apareamiento es la ballena jorobada. Éste último se caracteriza principalmente por formación de grupos de competencia e interacciones agresivas entre los machos, quienes emiten señales acústicas complejas denominadas cantos. Dada la ausencia de cuidado parental por parte de los machos y el bajo número de crías por machos detectado a través de los análisis de paternidad es probable que el sistema apareamiento oscile entre promiscuo y poliginia baja o moderada (Clapham 1996, Cerchio et al. 2005).

La ballena azul

La ballena azul (Balaenoptera musculus) es el animal de mayor tamaño, alcanzando en algunos casos los 30 m de longitud (Tomilin 1957), presenta distribución cosmopolita, tanto costera como oceánica.

Esta especie fue intensamente cazada con fines comerciales desde el siglo pasado. Se calcula que en el siglo XX se capturaron cerca de 6,000 ballenas en el Pacífico Norte, 7,000 en el Atlántico Norte y 330,000 en la Antártida (Donovan 1981). En 1966 la Comisión Ballenera Internacional declara prohibida la casa comercial de ballenas azules (Donovan 1981). Las estimaciones de abundancia indican que existen entre 7,690 y 8,750 ballenas azules en todo el mundo (Perry et al. 1999) y entre 2.000 y 3.000 en el Pacifico Norte (Calambokidis & Barlow 2004). Por esta razón se considera que la mayoría de sus poblaciones se encuentran amenazadas o en peligro de extinción (Reilly et al. 2008).

Como la mayoría de los misticetos, la ballena azul realiza migraciones entre las áreas de alimentación en zonas altamente productivas en altas latitudes, y las áreas de reproducción y cría, en latitudes medias y bajas (Tomilin 1957). Las hembras tienen una sola cría cada dos o tres años (Yochem & Leatherwood 1984), aunque existen registros de intervalos de hasta 7 años (Gendron 2002). Las crías al nacer alcanzan los 6-7 m de longitud (Yochem & Leatherwood 1984). El periodo de gestación es de 10 a 11 meses, la lactancia dura aproximadamente 7 meses y la madurez sexual se estimó entre los 5 y 10 año de edad (Lockyer 1984).

A pesar de que habita en todos los océanos, se reconocen tanto subespecies como stocks poblacionales (Leduc et al. 2007, Attard et al. 2012, Sembra et al. 2012). Si bien la clasificación es controversial, aún se reconocen tres áreas geográficas diferenciadas genéticamente en el hemisferio sur (LeDuc et al. 2007). Dichas áreas son el Pacífico Sudoriental, el Océano Indico y Antártica, cada una representada por una

subespecie, la ballena azul pigmea del Pacífico la pigmea del Indico (B. m. brevicauda) y la ballena azul verdadera (B. m. intermedia) respectivamente.

En particular en el Pacífico Norte con base en registros históricos, avistamientos recientes y estudios acústicos, se reconocen seis áreas importantes de agregación de ballena azul: 1) Sur de Japón; 2) Norte de Japón/Islas Kurils/Península de Kamchatka; 3) Islas Aleutianas; 4) zona sureste del Golfo de California; 5) California-México y 6) la zona del Domo de Costa Rica (Oshumi & Wada 1972, Reilly & Thayler 1990, Perry et al. 1999, Moore et al. 2002, Stattford et al. 2002). Hasta el momento, existe gran incertidumbre sobre los límites de estas agregaciones y sobre sus rutas migratorias, por lo cual la Comisión Ballenera Internacional reconoce al Pacífico Norte como un único stock (Donovan 1981).

Las ballenas azules del Pacífico Nororiental, constituyen el remanente poblacional más grande de la especie a nivel mundial y el único que muestra signos de recuperación (Perry et al. 1999). En relación a la estacionalidad de las agregaciones de la especie en el Pacifico Nororiental, se reconoce a las costas de California como la zona principal de agregación en verano (Calambokidis et al. 1990); la costa occidental de la Península de Baja California y el Golfo de California (Gendron 2002) así como el Domo de Costa Rica (Mate et al. 1999) como áreas de agregación invernales. A través del seguimiento satelital y de foto-identificación se han registrado movimientos entre estas áreas invernales y California (Calambokidis et al. 1990, Gendron 2002, Bailey et al. 2009).

En la porción sudoccidental del Golfo de California (Loreto-La Paz), donde se ha realizado un estudio a largo plazo de foto identificación, se observó alta variabilidad en la residencia estacional, desde más de 2 meses en individuos conocidos a sólo algunos días; mientras que el 70% de las ballenas fueron observadas una sola vez (Gendron 2002). Esto resulta en residencias largas, en áreas locales para algunas ballenas, lo que sugiere que dichas áreas pueden ser buenas zonas de alimentación en invierno, además de utilizarse como zona de crianza.

La presencia de crías de tamaños desde 7 a 13m así como la observación del epizoonte Xenobalanus adherido a las crías, sugieren que los partos probablemente ocurren poco antes del periodo de mayor frecuencia de avistamiento de las crías, entre enero y marzo (Gendron 2002). Esta información, en conjunto con el periodo de gestación que se ha estimado de entre 10 y 11 meses (Lockyer 1984) permite inferir que la cópula también puede estar ocurriendo entre marzo y junio, cuando una parte importante de la población

se encuentra dentro del Golfo de California (Gendron 2002).

La información existente sobre la estructura social y el sistema de apareamiento de la ballena azul es escasa. En un principio fue considerada una especie solitaria (Gambell 1976), pero estudios de comportamiento realizados en el Golfo de California sugieren la existencia de cierto nivel de estructura social (Martínez-Serrano 2005).

Por otro lado, estudios acústicos indican la existencia de señales acústicas emitidas exclusivamente por los machos y otras emitidas por ambos sexos, las cuales podría estar vinculadas al comportamiento reproductivo y comunicación e interacciones sociales, respectivamente. Estos últimos se detectaron a baja profundidad, donde la luminosidad también podría contribuir al reconocimiento de con-específicos (Oleson et al. 2007).

Estudios de comportamiento recientes realizados en el Golfo de California, han aportado evidencia de que algunas crías interactúan con otros individuos y no sólo con su madre (Gendon com. pers.). Si bien hasta el momento no se conoce el sexo ni la clase de edad del otro individuo, estas observaciones abren la puerta a múltiple interrogantes y perspectivas tanto en lo que respecta a la organización social como al sistema de apareamiento.

En relación al sistema de apareamiento, las ballenas azules poseen una baja proporción relativa entre el peso de los testículos y el resto de cuerpo, comparado con especies como la ballena franca (Eubalaena spp), la ballena boreal (Balaena mysticetus) y la ballena gris (Eschrichtius robustus) (Brownell & Ralls 1986). Los machos de la ballena franca poseen el peso relativo de testículos más alto que cualquier otro misticeto y es frecuente observar grupos de cortejo y cópula donde una hembra es rodeada por varios machos, por lo que se ha sugerido que la competencia espermática es la forma más probable de competencia entre machos de esta especie (Kraus & Hutch 2001). Las ballenas jorobadas (Megaptera novaeangliae) poseen similar peso relativo (al tamaño corporal) de los testículos, comparado con las ballenas azules, pero son bien conocidos los grupos de competencia entre machos con interacciones agresivas (Clapham 1996). En el caso de las ballenas azules, no se han observado grupos de apareamiento con comportamientos similares a los de la ballena franca o jorobada, lo que aunado al tamaño relativo de sus testículos, hacen de la espermática un mecanismo poco probable de competencia en esta especie (Brownell & Ralls 1986, Gendron 2002).

Esta tesis aborda, desde una perspectiva molecular, el análisis de la estructura poblacional y la

estructura de parentesco de grupos de las ballenas azules del Golfo de California. Para ello se utiliza la base de datos del CICIMAR, de identificación individual de ballena azul obtenida a lo largo de 20 años, en conjunto con información genética. Este estudio se divide en dos artículos. El primero titulado: Fine-scale population structure of blue whale wintering aggregations in the Gulf of California, publicado en PLoS ONE 8(3): e58315, doi:10.1371/journal.pone.0058315 en el corriente año (Costa-Urrutia et al. 2013). El segundo titulado: Spatial kin pattern of blue whales in the Gulf of California. En conjunto, ambos artículos analizan la distribución de la variabilidad genética a nivel poblacional y social respectivamente, con el objetivo de obtener conocimiento sobre mecanismos de evolución en cada uno de ellos y la interacción entre los mismo, brindando una visión integrada en dichos temas.

Referencias

Allendorf F, Luikart G. 2007. Conservation and populations genetics. Capitulo 9. Blackwell Publishin (Ed). 642pp.

Amos B, Schlotterer D, Tautz D. 1993. Social structure of pilot whales revealed by analytical DNA profiling. Science. 260:670-672.

Attard CRM, Beheregaray LB, Jenner KCS, Gil PC, Jenner MN, Morrice MG, Robertson KM, Möller LM. 2012. Hybridization of Southern Hemisphere blue whale subspecies and a sympatric area off Antarctica: impacts of whaling or climate change? Molecular Ecology. 21:5715-5727.

Bailey H, Mate BR, Palacios DM, Irvine L, Bograd SJ. Costa DP. 2009. Behavioural estimation of blue whale movements in the Northeast Pacific from state space model analysis of satellite tracks. Endangered Species Research. Doi: 10.3354/ esr00239.

Balloux F, Brunner H, Logon-Moulin N, Hausser J, Goudet J. 2000. Microsatelites can be misleading: an empirical simulation study. Evolution. 54:1414-1422.

Balloux F, Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology. 11:155-165.

Baker CS, Medrano-Gonzalez L, Calambokidis J. 1998. Population structure of nuclear and mitochondrial DNA variation among humpback whales in the north Pacific. Molecular Ecology. 7: 695-707.

Barnard C. 2004. Evolution and behavior. Capítulo 2. Animal behavior: mechanism, development, function and evolution. Peardon Education Limited (Ed). 725pp.

Berta A, Sumich JL. 1999. Mating, Breeding and Social organization: marine mammals: evolutionary biology. Academic Press (Ed). 364pp.

Best PB. 2000. Coastal distribution, movements and site fidelity of right whales, *Eubalaena australis*, off South Africa, 1969-1998. African Journal of Marine Science. 22: 43-55.

Brownell RL, Ralls K. 1986. Potential for sperm competition for baleen whale. Report to the International Whaling Commission. 8: 97-112.

Carroll EL, Childerhouse SJ, Christie M, Lavery S, Patenaude N, Alexander A, Constantine R, Steel D, Boren L, Baker CS. 2012. Paternity assignment and demographic closure in the New Zealand southern right whale. Molecular Ecology . 21: 3960-3973

Calambokidis J, Barlow J. 2004. Abundance of blue and humpback whale in the Eastern North pacific estimated by capture-recapture and line transect methods. Marine Mammal Science. 20:63-85.

Calambokidis J, Steiger GH, Cubbage JC, Balcom KC, Ewald C, Kruse S, Wells R, Sears R. 1990. Sightings and movements of blue whales off central California 1986-88 from photo-identification. 1990. Report to the International Whaling Commission. 12: 343:348.

Cerchio S, Jacobsen JK, Danielle, Cholewiak DM, Falcone EA, Merriwether DA. 2005. Paternity in humpbackwhales, *Megaptera novaeangliae*: assessing polygyny and skew in male reproductive success. Animal Behaviour. 70:267-277.

Cezilly F, Danchin E. 2008. Mating system and parental care. Cap 12. Behavioural Ecology. Oxford University Press (Ed). 874pp.

Clapham PJ. 1996. The social and reproductive biology of the humpback whale: an ecology perspective. Mammal Review. 26:27-49.

Clutton-Brock TH. 1989. Mammalian mating system. Proceeding of Royal Society of London. 235: 339-372.

Connor RC, Smolker RA, Richards AF. 1996. Patterns of female attractiveness in Indian Ocean in bottlenose dolphin. Behaviour. 133: 37-69.

Costa-Urrutia P, Sanvito S, Victoria-Cota N, Enriquez-Predes L Gendron D. 2013. Fine scale population structure of blue whale, *Balaenoptera muslucus*, in the Gulf of California. Plos One. 8(3): e58315. doi:10.1371.

Corander J, Marttinen P, Sirén J, Tang J. 2008. Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics. 9:539.

Coulon A, Guillot G, Cosson JF , Angibault JMA, Aulagnier S, Cargnelutti B, Galan M, Hewison AJM. 2006. Genetic structure is influenced by landscape features:empirical evidence from a roe deer population. Molecular Ecology. 15:1669-1679.

Danchin É, Cézilly F, Giraldeau LA 2008.Fundamental concepts in behavioural ecology. Capítulo 2. Behavioural ecology. Oxford University Press (Ed). 874pp.

Donovan GP. 1991. A review of IWC stock boundaries. Genetics ecology of whale and Dolphin. Hoelzel AR (Ed). Report to the International Whaling Commission. 13: 39-68.

Elwen SH, Best PB. 2004. Female southern right whale *Eubalaena australis:* are there reproductive benefits associated with their coastal distribution off South Africa? Marine Ecology Progress Series. 269: 289-295.

Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating system. Science. 197:215-223.

Enríquez-Paredes L. 2005. Identidad genética de la población de ballena azul, *Balenoptera musculus*, en el Pacifico Nororiental: agregaciones mexicanas. Tesis de Doctorado. UABC, Ensenada, Baja California México. 198pp.

Fontaine MC, Baird SJE, Piry S, Ray N, Tolley KA, Duke S, Birkun JrA, Ferreira M, Jauniaux T, Llavona A, Öztürk B, Öztürk AA, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson GA, Bouquegneau JM, Michaux JR. 2007. Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbor porpoises in Old World waters. BMC Biology. 5: 1-16

Francois O, Durand E. 2010. Spatially explicit Bayesian clustering models in population genetics. Molecular Ecology Resources. 10: 773–784.

Frantz AC, Cellina S, Krier A, Schley L, Burke T. 2009. Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? Journal of Applied Ecolology. 46: 493–505.

Frasier TR, Hamilton PK, Brown MW, Conger LA, Knowlton AR, Marx MK, Slay CK, Kraus SD, White BN. 2007. Patterns of male reproductive success in a highly promiscuous whale species: the endangered

North Atlantic right whale. Molecular Ecology.16:5277-5293.

Fullard KJ, Early G, Heide-Jørgensen MP, Bloch D, Rosing-Asvid A, Amos W. 2000. Population structure of long-finned pilot whales in the North Atlantic: a correlation of with a surface temperature? Molecular Ecology. 9:949-958.

Gambell R. 1976. World whale stock. Mammal Review. 6:41-53.

Gaspari S, Azzellino A, Airloldi S, Hoelzel AR. 2007. Social kin associations and genetics structuring of stripped dolphin populations, *Stenella coeruleoalba*, in the Mediterranean Sea. Molecular Ecology. 16: 2922-2933.

Gendron D. 2002. Ecología Poblacional de la ballena azul, *Balaenoptera musculus* de la Península de Baja California. Tesis de Doctorado. CICESE. Ensenada, Baja California México. 105pp.

Gillespie JH. 1998. Populations genetics. The Johns Hopkins University press (Ed). London, UK. 174pp.

Greenwood PJ. 1980. Mating system, philopatry and dispersal in birds and mammals. Journal of Animal Behaviour. 28:1140-1162.

Guillot G, Estoup A, Mortier F, Jean Francois Cosson J. 2005. A spatial statistical model for landscape genetics. The Genetics Society of America. 170: 1261–1280.

Guillot G, Leblois RL, Coulon A, Frantz AC 2009.Statistical methods in spatial genetics. Molecular Ecolology. 18: 4734-4756.

Hamilton WD. 1964. The genetical evolution of social behaviour I. Journal of Theoretical Biology. 7: 1-16.

Hardy O, Vekemans X. 2002. Spagedi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Molecular Ecology Notes. 2: 618-620.

Hewitt GM, Bultin RK. 1997. Cause and consequences of population structure. Capítulo 15. Behavioural Ecology: an evolutionary approache. Krebs JR, Davis NB (Eds). 455pp.

Hoelzel AR. 1998. Genetic structure of cetacean populations in simpatry, parapatry and mixed assemblages: implications for conservations and policy. The American Genetic Associations. 89:451-458.

Hoelzel AR, Dahlheim M, Stern SJ. 1998. Low genetic variation among killer whales, *Orcinus orca*, in

the Eastern North Pacific and genetic differentiation between foraging specialist. The Journal of Heredity. 89: 121-128.

Holzer B, Keller L, Chapuisat M. 2009. Genetic clusters and sex-biased gene flow in a unicolonial Formica ant. BMC Evolution Biology. 9:69

Kasuya T, Miyashita T, Kasamatsu F. 1998. Segregation of two form of the short-fine pilot whale off Pacific coast of Japan. Scientific Report of the Whales Research Institute. 30: 77-90.

Kimura M, Crow JE. 1964. The number of alleles that can be maintained in a finite population. Genetics. 49: 725-738.

Kimura M, Otha T. 1978. Stepwise mutation model and distribution of allelic frequencies in a finite population. Proceedings of the National Academy of the USA. 75: 2868-2872.

Kraus SD, Hatch JJ 2001 Mating starategies in the North Atlantic right whale. *Eubalaena australis*. Journal of Cetacean Research and Management. 2:237-244.

LeDuc RG, Dizon AE, Goto M, Pastene LA,Kato H, Nishiwaki S, LeDuc CA, Brownell RL. 2007. Patterns of genetic variation in Southern Hemisphere blue whales and use of assignment test to detect mixing on the feeding grounds. The Journal of Cetacean Research and Management. 9: 73-80.

Lockyer C. 1984. Review of baleen whale reproduction and implications for management. Report to the International Whaling Commission. 5:27-50.

Lusseau D, Wilson B, Hammond P, Grellier K, W. Durban JW, Parsons KM, Barton TR, Thompsom PM. 2006. Quantifying the influence of sociality on population structure in bottlenose dolphin. Journal of Animal Ecology. 75:14-24.

Martínez-Serrano I. 2005. Patrones de asociación entre individuos de ballena azul (*Balaenoptera musculus*) en el suroeste del Golfo de California, México. Tesis de Maestria. IPN-CICIMAR.95pp.

Mate BR, Lagerquist BA, Calambokidis J. 1999. Movements of north pacific blue whales during the feeding season off southern California and their southern fall migration. Marine Mammal Science. 15:1246–1257.

Méndez M, Rosembaum HC, Subramaniam A, Yackulic C, Bordino P. 2010. Isolation by environmental

distance in mobile marine species: molecular ecology of franciscana dolphins at their southern range. Molecular Ecology. 19: 2212-2228.

Mobly KB, Jones AG. 2009. Environmental demographic and genetic mating system variation among five geographically distinct dusky pipefish, *Syngnathus floryade*, populations. Molecular Ecology. 18:1476-1490.

More SE, Watkins WA, Daher MA, Davis JR, Dahlheim ME. 2002. Blue whale habitat association in the northwest Pacific: analysis of remotely sensed data using a Geographic Information System. Oceanogrhaphy 15:20 25.

Natoli A, Peddemors V, Hoelzel AR. 2004. Populations structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. Journal Evolution of Biology. 17:363-375.

Neigel JE. 2002. Is FST obsolete? Conservation Genetics. 3: 167-173.

Oleson EM, Calambokidis J, Burguess WC, MacDonald MA, LeDuc CA, Hildebrand JA. 2007. Behavioral context of call production by eastern North Pacific blue whale. Marine Ecology Progress Series. 330:269-284.

Oshumi S, Wada S. 1974. Status of whale stocks in the north Pacific. Report of the International Whaling Commission. 24: 114-126.

Parson N, Durban JW, Claridge DE, Herzing DL, Balcom KC, Noble LR. 2006. Population genetic structure costal bottlenose dolphin, *Tursiops truncates*, in the northern Bahamas. Marine Mammal Science. 22: 276-298.

Peakall R, Smouse PE. 2006. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288-295.

Perry SL, DeMaster DP, Silber G. 1999. The great whales: history and status of six species listed as endangered under the U.S. Endangered Species Act of 1973. Marine Fisheries Review. 61: 1-74.

Pritchard JK, Matthew P, Donnely P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Reilly SB, Bannister JL, Best PB, Brown M, Brownell Jr RL, Butterworth DS, Clapham PJ, Cooke J, Donovan GP UrbánJ, Zerbini AN. 2008. Balaenoptera musculus. En: IUCN 2010. IUCN Red List of

Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 12 December 2012.

Reilly SB, Thayer VG. 1990. Blue whales distribution in the Eastern Tropical Pacific. Marine Mamal Science. 6: 265-277.

Reynolds JD. 1996. Animal breeding system. Tree. 11:68-72.

Rowntree V, Payne R, Schell DM. 2001. Changing patterns of habitat use by southern right whales, *Eubalaena australis*, on their nursery ground at Península Valdés, Argentina, and their long range movements. The Journal of Cetacean Research and Management. 2: 133-143.

Safner T, Miller MP, McRae BH, Fortin MJ, Manel S. 2011. Comparison of Bayesian clustering and edge detection methods for inferring boundaries in landscape genetics. Journal of Molecular Science.12: 865-889.

Schaeff C, Kraus S, Brown MW, White B. 1993. Assessment of the population structure of western North Atlantic right whales, *Eubalaena glacialis*, based on sighting and mtDNA data. Canadian Journal of Zoology. 71: 339-345

Slatkin M. 1995. A measure of population subdivision based on microsatellites on allele frequencies. Genetics. 139:457-462.

Sremba AL, Hancock-Hanser B, Branch TA, LeDuc RL, Baker CS. 2012. Circumpolar diversity and geographic differentiation of mtDNA in the critically endangered Antarctic blue whale (*Balaenoptera musculus intermedia*). PLoS ONE. 7: e32579.

Sttaford KM, Bohnenstiehl DR, Tolstoy M, Chapp E, Mellinguer DK, Moore SE. 2004. Low-frequency whales sound record on hydrophones moored in eastern tropical Pacific. Journal of the Acoustic Society of America. 106:3687-3698.

Tang J, Hanage WP, Fraser C, Corander J. 2009. Identifying currents in the gene pool for bacterial populations using an integrative approach. PLoS Computation Biology. 5: e1000455.

Tomilin AG. 1957. Mammals of the U.R.R.S and adjacent countries. Volume IX. Cetacea. Nauk S.S.S.R, Moscu. 717pp.

Trivers RL. 1972. Parental investment and sexual selection. Sexual selection and the descent of man, 1871-1971. Campbell B (Ed).p136-179.

Valenzuela LO, Sironi M, Rowntree V, Seger J. 2009. Isotopic and genetic evidence for culturally inherited site fidelity to feeding ground in southern right whales, *Eubalaena australis*. Molecular Ecology. 18: 782-791.

Wade MJ, Shuster SM. 2002. The evolution of parental care in the context of sexual selection: A Critical Reassessment of Investment Theory. 160: 285-292.

Waples RS, Gaggiotti O. 2006.What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology. 15: 1419–39.

Weigmann DD, Nguyen T. 2006. Mating System and demographic constraint on the opportunity for sexual selection. Theoretical Population Biology. 69: 34-47.

Whitehead H, Christal J, Tyack PL. 2000. Studying cetacean social structure in space and time. Capítlulo3. Cetacean Society, Field Study of Dolphins and Whales. Mann J, Connor RC, Tyack P, H Whitehead H(Eds) 65-87.

Wilson AB. 2009. Opening Pandora's box: comparative studies of genetic mating system reveal reproductive complexity. Molecular Ecology. 18: 1307-1309.

Wrigth S. 1931. Evolution of Mendelian populations. Genetics. 16: 97-158.

Wrigth S. 1951. The genetically structure of population. Annual Eugenics. 15:323-354.

Yochem PK, Leatherwood S. 1985. Blue whales, *Balaenoptera musculus* (Linneaus, 1975). Handbook of marine mammals: the sirenians and baleen whales. SH Ridgewa, R Harrison (Eds).

Objetivo General

Determinar la variabilidad genética nuclear de la ballena azul (*Balanenopteta musculus*) en distintos niveles inclusivos en el Golfo de California.

Objetivos Específicos

1. Determinar la estructura genética poblacional de la ballena azul en el Gofo de California.

2. Determinar la estructura de grupo de la bzallena azul en el suroeste del Golfo de California.

3. Determinar la estructura espacial de parentesco de la ballena azul en el suroeste del Golfo de California.

.

Artículo Uno

Fine-scale population structure of blue whale wintering aggregations in the Gulf of California

Paula Costa-Urrutia[1]

paula.costa.urrutia@gmail.com

[1]Laboratorio de Ecología Molecular, Facultad de Ciencias Marinas, Universidad Autónoma de Baja California.

Simona Sanvito[1,2]

simo_esrg@eleseal.org

[1]Laboratorio de Ecología Molecular, Facultad de Ciencias Marinas, Universidad Autónoma de Baja California.

[2]Elephant Seal Research Group, Sea Lion Island, Falkland Islands

Nelva Victoria-Cota[3]

nelva.victoria@uabc.edu.mx

[3]Laboratorio de Ecología y Epidemiología Molecular, Escuela de Ciencias de la Salud, Universidad Autónoma de Baja California.

Luis Enríquez-Paredes[1]

lmenriquez@uabc.edu.mx

[1]Laboratorio de Ecología Molecular, Facultad de Ciencias Marinas, Universidad Autónoma de Baja California.

Diane Gendron[4]

dgendron@ipn.mx

[4]Laboratorio de Ecología de Cetáceos y Quelonios, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional.

Abstract

Population differentiation in environments without well-defined geographical barriers represents a challenge for wildlife management. Based on a comprehensive database of individual sighting records (1988-2009) of blue whales from the winter/calving Gulf of California, we assessed the fine-scale genetic and spatial structure of the population using individual-based approaches. Skin samples of 187 individuals were analyzed for nine microsatellite loci. A single population with no divergence among years and months and no isolation by distance (R_{xy}=0.1-0.001, $p>0.05$) were found. We ran two Bayesian clustering methods using Structure and Geneland softwares in two different ways: 1) a general analysis including all individuals in which a single cluster was identified with both softwares; 2) a specific analysis of females only in which two main clusters (Loreto Bay and northern areas, and San Jose-La Paz Bay area) were revealed by Geneland program. This study provides information indicating that blue whales wintering in the Gulf of California are part of a single population unit and showed a fine-scale structure among females, possibly associated with their high site fidelity, particularly when attending calves. It is likely that the loss of genetic variation is minimized by male mediated gene flow, which may reduce the genetic drift effect. Opportunities for kin selection may also influence calf survival and, in consequence, have a positive impact on population demography in this small and endangered population.

Keywords: Fine-scale population structure, Blue whale, winter/calving ground, Gulf of California

Introduction

Detecting population structure in taxa characterized by continuous distribution in environments without well-defined geographical barriers is one of the most challenging problems in population genetics. This applies to the conceptual definition of populations, but also represents a challenge for conservation and wildlife management. Following the evolutionary paradigm proposed by [1] we consider a population as "a group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member". However, fine-scale genetic structure may be a confounding factor if the population shows distinct levels of genetic stratification as heterogeneity are among adjacent breeding groups or spatial segregated kin selection [2].

Many aspects of life history, as well as several environmental factors, can influence the structure of populations and, therefore, the individual's dispersal potential alone does not always allow predictions about the gene flow among populations. This is especially true for cetaceans, for which movements are not limited by physical barriers. Although most cetacean species show wide distributions and high potential for dispersal, their populations usually exhibit some kind of structure [e.g.3, 4, 5, 6, 7], that may depend on the local distribution of resources and/or their social system [8].

Female philopatry and migration patterns are known to have a strong influence on population structure in large mammals [8,9], as observed in humpback whales, Megaptera novaeangliae [e.g. 10], northern and southern right whales Eubalaena glacialis and E. australis respectively [e.g. 11, 12, 13], grey whales, Eschrichtius robustus [14, 15], and sperm whales, Physeter macrocephalus, [16, 17]. Recently, maternal site fidelity was also suggested for the Antarctic blue whales (Balaenoptera musculus intermedia), that showed divergence in mitochondrial DNA and microsatellite markers among the six feeding grounds in the Antarctic [18]. In general, baleen whale females disperse less than males [8], but few studies addressed the fine-scale population structure, probably because of the time and effort required to obtain long-term serial records of individually recognized whales.

The blue whale (Balaenoptera musculus) is a cosmopolitan species, with both coastal and oceanic distribution. Like most baleen whales, this species migrates between feeding grounds, located in areas at high latitudes, and calving grounds at mid and low latitudes [19]. This species was one of the main targets of commercial whaling and, due to its reduced population size and slow recovery rate, it is still considered

endangered [20]. The largest known remnant population, between 2000 and 3000 blue whales [21], inhabits the eastern North Pacific. This population appears to be separated from populations in the central and western North Pacific, as suggested from differences in call types [22, 23]. The link between the blue whales sighted off California, their main feeding area in summer, and those in the Gulf of California (GC) in winter has long been demonstrated by photo-recapture evidences [24] and also well-illustrated through the movements of individuals tracked with satellite tags [25]. Around 300 blue whales are estimated to winter annually in the GC, and then migrate northwest along the Pacific coast of Baja California, following the seasonal shift of marine productivity around the peninsula [26]. Long-term surveys and photo-identification records in the southwestern GC showed seasonal residence periods that ranged widely from few days to over 70 days [26]. This study has also pointed out that the GC is an important calving and feeding ground for this population, thus making it a promising site where to study the fine-scale population structure of this species.

A molecular analysis of Southern Hemisphere blue whale feeding aggregations showed that three geographic areas (Southeast Pacific, Indian Ocean and Antarctica) were occupied by distinct populations, and in each of them no structure was found probably due to small sample sizes [27]. More recently, a low but significant divergent structure was found among the six feeding grounds in the Antarctic Ocean [18]. However, a larger sample size was used to analyze the Perth Canyon and Bonney Upwelling, two Australian feeding grounds, but no population structure was found [28].

In this paper we investigated the population structure of blue whales in the GC, the only studied calving ground for this species using an extensive data base of individual sighting histories obtained during the last 22 years [29]. Being interested only in the fine-scale structure of a small, localized, but continuously distributed aggregation, we selected microsatellites as the optimal marker to carry out our study [e.g. 30, 31]. We expected to find some structuring, at least among females, due to the high female site fidelity and philopatry found in calving grounds of other baleen whales [e.g. 8, 9, 15, 18], and to the observed bias toward females in the sex ratio of blue whales sampled in the GC (females/males=1.5/1) [26].

Methods

Study area

The GC is a narrow internal sea that separates Baja California Peninsula from the Mexican mainland, located between 23º to 31ºN and between 107º to 115ºW in the Northeast Pacific Ocean (Figure 1). It is 1,120 km long and 150 km wide on average, with an area of about 200,000 square kilometers [32], considered highly productive from December to June [33].

Sample collection

We conducted 2-6 day-long surveys between January and May 1988-2009, covering the area between the cities of La Paz and Loreto (Figure 1). Moreover, longer surveys were conducted covering the whole GC in 2005 and the Upper GC in 2006. Once a whale was sighted, the GPS positions were recorded and photographs for individual identification were taken [34]. We used the CICIMAR photo-ID catalogue categorized by the dorsal fin shapes and the body pigmentation patterns to identify the animals [29].

From 1997 to 2009 skin-blubber biopsies were collected with a similar system as described in Lambersten [35], using a dart of 7 mm of diameter and 40 mm long stainless steel core sampler with three internal, inward-facing barbs. The dart is screwed in the tip of an arrow designed with a stopper that limits the depth of penetration and makes the arrow rebound out with the sample in it. The arrow was fired from a 68kg crossbow at a distance of about 10 m from the whale and retrieved once it floated. Skin-blubber biopsies were extracted from the dart using sterilized stainless tweezers. In order to prevent contamination between samples and infection to the animals, the core sampler was sterilized before each biopsy attempt by immersion in a 50% chlorine solution, then transferred to a 70% ethanol solution, exposed for 10 s to a blowtorch flame and finally wrapped in aluminium foil.

We avoided duplicating biopsy samples within and between seasons by comparing newly photographed whale (digital camera viewing) with those included in a field catalogue of biopsied whales. The biopsies collected from 1997 to 2001 were preserved in 20% dimethylsulfoxide (DMSO) saturated with table salt [36], thereafter samples were immediately frozen in liquid nitrogen. It is important to clarify that biopsy-sampling procedure started in 1997, but several of the biopsied individuals in our data base have long sighting histories; thus all the sightings from each biopsied individual were used in this study.

Ethics Statement

Photo-identification and biopsy samples were collected under the annual research permits issued by the Secretaria de Medio Ambiente Recursos Naturales y Pesca (180796-213-03, 071197-213-03, DOO 750-00444/99, DOO.0-0095, DOO 02.-8318) from 1997 to 2001, and by the Dirección General de Vida Silvestre, Secretaria de Medio Ambiente y Recursos Naturales (SGPA/DGVS-7000, 00624, 01641, 00560, 12057, 08021, 00506, 09760) from 2002 to 2009, which now represents the only approved Government institution to issue research permits on endangered species in Mexico. By issuing these annual research permits they approved the number of blue whale skin-blubber biopsies collection for each year of the permit. Our institution does not have an Animal Ethics Committee; therefore no information is given on that aspect.

Sample processing and analysis

Total DNA was extracted from skin using the standardized protocol [37]. We carried out genotyping at 19 microsatellite loci on a subset of 92 samples to evaluate polymorphisms. We found low variability (two to four alleles) for nine microsatellites: AC45, CAAA74, AC82, GT122, GT129, GT227, CA141, GATA53 [38], DlrFC17 [39]. The 10 most polymorphic and informative loci were chosen for further analyses (Table 1) [40, 41, 42]. We performed PCR reactions with 30 ng of DNA template and the following reagent concentrations: 22 mM Tris-HCl pH 8.4, 55 mM KCl, 1.2 U DNA Taq Polymerase (Invitrogen™), 0.5 µM of each primer (forward primers were labeled with a fluorescent dye, 6-FAM®, VIC® or NED®, of Applied Biosystems), 0.2mM dNTPs and $MgCl_2$ concentration as specified in Table 1. . The following PCR cycling profile was used: initial denaturation step at 94ºC for 2 min, followed by 7 cycles consisting of denaturation at 96ºC for 20 sec, annealing at ATºC$_1$ (see Table 1) for 30 sec and extension at 72ºC for 30 sec, followed by 25 cycles of denaturation at 95ºC for 30 sec, annealing at ATºC$_2$ (see Table 1) for 30 sec and extension at 72ºC for 30 sec, and a final extension step at 72ºC for 15 min. We resolved PCR products on an ABI Prism 310 genetic analyzer using the GeneScan™ 600 LIZ® size standard (Applied Biosystems) and included non-template PCR controls to check for cross-contamination and replicates (nine percent of the samples) to evaluate the reproducibility and genotyping errors. We processed the raw ABI files with GeneMarker® 1.9 software (SoftGenetics) to obtain the genotypes, validated the allele binning with FlexiBin software [43], and checked the genotypes for null alleles and genotyping errors with Micro-checker v.4.0.7 [44]. The gender of each individual was identified by PCR amplification of ZFX and ZFY genes [45].

Data analysis

Heterozygosity, Hardy-Weinberg equilibrium and linkage disequilibrium. We calculated the number of alleles per locus, the observed (H_O) and the expected (H_E) heterozygosity and with Arlequin 3.1 [46]. We tested linkage disequilibrium (LD) between loci, deviations from Hardy-Weinberg equilibrium (HWE) for each locus, and global deviation from HWE for all loci, using the MCMC method (10000 iterations) [47] implemented in Genepop 4.0 [48]. We corrected the significance levels of HWE and LD test for multiple comparisons with sequential Bonferroni adjustments [49]. We calculated F_{IS} coefficient in Fstat 2.9.3.2 [50].

Temporal variation in population structure. Based on photo-recapture records, we defined groups (more than 10 individuals) according to field season when individuals were sighted (hereafter "years"), and to the month and year of sightings (hereafter "month-year"). As a result of this criterion, the 1997-2009 period for the "years" category, and the 1999-2009 period for the "month-year" category were analyzed (Table 2). Additionally, individuals were assigned to one of three categories based on the number of years (consecutive or not) in which we observed them ("sighting frequency groups"): i) occasional (observed only in one year), ii) frequent (two-four years), and iii) highly frequent (five years or more).

All above grouping criteria (years, month-years and sighting frequency) were tested for departure from our population sex ratio (see results) with chi-square test. We tested deviation from HWE and calculated the fixation indices- R_{ST} [51] and F_{ST} [52] of genetic differentiation between groups with Arlequin 3.1. Adjustments for multiple comparisons were made for dependent (Benjamini-Yekutieli, [53]) and independent samples (sequential Bonferroni's, [49]) for HWE, F_{ST} and R_{ST}. We used the correction for dependent samples [53] for year and month-year groups, since the same individual could be included in more than one group, and sequential Bonferroni's [49] adjustment for sighting frequency groups as they are independent groups.

Isolation by distance (IBD). To verify the presence of isolation by distance we carried out an individual-based Mantel test, both on the complete dataset (females and males pooled) and on females only, using the kinship coefficients R_{QG} [54] and R_{RL} [55] as measures of genetic distance. A geographic distance matrix was made based on geometric means of the GPS fixes, for individuals recorded in more than one position (68%), which can be considered a rough estimate of the center of individual activity [56]. The P-value for the correlation coefficient was calculated with a permutation test (10000 replicates) as implemented in

Genalex 6 [57].

Bayesian clustering. We carried out two Bayesian clustering analysis, both on the complete dataset (females and males pooled) and on females only. In the first, we used the admixture model with correlated allele frequency implemented in Structure 2.3 [58]. The number of clusters (K) ranged from 1 to 8, and for each K we run 10 independent MCMC runs with 10^6 iterations, following a burn-in period of 60000 iterations. The mean values of the 10 runs for each K were reported, and we chose the K value with the highest probability (Ln P(D)) as the best estimated number of clusters. In the second analysis, we used the mixture model with correlated allele frequency and free Voronoi tessellation [59] implemented in the R package Geneland [60]. This program uses geo-referenced multi-locus genotyped individuals to probabilistically assign them to a cluster. We carried out 25 independent runs using 10^6 iterations and K_{max}=10. Each set of 25 runs were ranked using the posterior probability of each, and we post-processed the best 10 runs. We obtained the K value from the mode value of these 10 runs, and reported the best posterior probabilities of this mode. For post-processing a burn-in period of 60000 iterations and a 800x200 pixel resolution for x and y axis were used respectively. The geo-referencing of each individual used in this analysis was the same as in the individual-IBD analysis (see above). The range of some blue whale movements can extend as much as the whole study area [26] and this complicates the estimation of the position uncertainty. To calculate the uncertainty in the positioning of individuals that was inputted in Geneland, we did a four-step analysis: 1) using the first daily positions of the individuals resighted between 1988- 2009; 2) estimating the 95% area of activity with the Kernel Density Estimator (KDE) [61] implemented in the Home Range Tool extension [62] of ArcGIS 9.3 (ESRI); 3) calculating the kernel bandwidth with the Least Square Cross Validation method; 4) using the square root of the mean individual KDE area as measure of uncertainty. We used the maximum number of locations per individuals that showed no positive correlation with the estimated size area. As a result, twenty individuals (n=17 females, n=3 males) were used to estimate the KDE individual utilization area. The number of locations of these individuals ranged between 11 and 44 per individual, and no linear correlation was found with the estimated KDE (Pearson=0.05, p=0.9). The mean $KDE_{95\%}$ was 3197.6 km^2, thus the uncertainty value used was 57 Km.

In order to verify if clusters obtained were artifacts of the models we analyzed HWE and divergence between them, using clusters with at least five individuals [59]. We calculated $R_{ST,}$ F_{ST} and F_{IS} between and within clusters respectively. For both models we performed 10 preliminary runs in order to know the maximum K

value and the length of the chain that should be used so as not to affect the results.

Results

Genetic variation

PCR conditions and statistics on the allelic variation of each locus are presented in Table 1. The locus GATA28 showed evidence for the presence of null alleles and we decided to exclude this locus from the analyses. Two loci departed from HWE, GATA98 and AC137 (Table 1), though there was no global deviation from HWE when all loci were considered (x^2=28.8, p=0.053). No pair of loci was in linkage disequilibrium (observed p=0.0024-0.9, sequential Boferroni p=0.0014, 36 test). The inbreeding coefficient was close to zero (F_{IS}=0.007, p=0.3), and the mean observed (H_O) and expected (H_E) heterozygosity were equal (H_O=0.74, H_E=0.74) (Table 1).

A total of 187 blue whale samples were analyzed for 9 microsatellite loci. We obtained a 100% match in genotyping of 5 duplicated samples that were classified as different individuals by photo-ID. These 10 samples were excluded and we carried out all further analyses on 177 whales (Females=99, Males=70, Not sexed=8). In accordance to a previous study [26], our blue whale sex ratio (females/males=1.41/1) was skewed towards females.

Temporal variation of the population structure

Years. The number of individuals observed ranged from 16, in 1998, to 61 in 2009 (Table 2). We found no deviation from the 1.41/1 (female/male) population sex ratio among years (Table 2). We found a significant deviation from HWE only in 2009 (observed p=0.0007, Benjamini-Yekutieli p=0.004, 13 test). No significant divergence was found among years (Range: R_{ST}=0-0.01, p> 0.05, F_{ST}=0-0.03, p> 0.05).

Months-year. This analysis involved 23 groups observed in February (n=10 groups), March (n=11) and April (n=2). We found a significant deviation from the 1.41/1 population sex ratio towards females in February (female/male=3/1; Table 2). A significant departure from HWE was found only in March-2009 group. Out of a total of 253 month-group pairwise comparisons, we found small but significant R_{ST} and F_{ST}

in nine comparisons, but not after we applied the Benjamini-Yekutieli correction (Table 2).

Sighting frequency. Sixty-nine individuals were sighted as occasional, 69 as frequent and 39 as highly frequent. We found no deviation from the 1.41/1 population sex ratio among groups, neither deviation from HWE after sequential Bonferroni correction, nor significant divergence among groups (Table 2). Since no evidence of genetic structure in a temporal basis was found, all samples were grouped for the spatial genetic analysis.

Spatial structure

Isolation by distance. No evidence of IBD was found in individual-based Mantel tests performed on the complete data set (Rxy_{QG}=0.01 p=0.3, Rxy_{RL}=0.01 p=0.17), nor in females only data set (Rxy_{QG}=0.05 p=0.24, Rxy_{RL}=0.05 p=0.5).

Bayesian clustering analysis. Using the admixture model with correlated allele frequencies implemented in Structure software, we found only one cluster (K=1) in both analyses (complete and females only data set , Figure 2). All individuals were admixed and assignment values were close to 0.5. This indicates the program is assigning individuals randomly to K populations, owing to the lack of underlying population structure [63]. Using the mixture model with correlated allele frequency and free Voronoi tessellation, implemented in Geneland, only one cluster for the complete dataset was found. In contrast, when the same model was run only for females, most individuals (96%) were assigned to two clusters with low but significant divergence (Table 3). Although one of the clusters is mainly represented in the central portion (green cluster, herein Loreto cluster) and the other in the south (yellow cluster, herein San Jose- La Paz cluster), no clear homogenous distribution of these clusters was found. Individuals from the yellow cluster (named San José-La Paz cluster) were also found in the northern GC, which suggests that individuals from both clusters do not show a complete segregation among these areas (Figure 3).

Discussion

In this study we investigated the fine-scale population structure of blue whales that winter in the GC. Our work provides information that suggests these blue whales are part of a single population unit. This is supported by all analyses; temporal grouping criteria, isolation by distance and Bayesian clustering approach, conducted on complete data sets. However, structure among females was found using the Geneland model.

The mean observed heterozygosity of blue whales in the GC (H_O=0.74) was similar to values reported for this species in the South Pacific (Ho=0.72) and the Antarctic (Ho=0.75) Oceans [27], and higher than those found in Australian aggregations (H_O=0.66 in Perth Canyon, Western Australia; Ho=0.59 in Bonney Upwelling, Southern Australia) [28]. Despite the smaller estimated blue whale population size in the GC (n=283, %CV=48.4) [26], the observed diversity and the inbreeding coefficient (F_{IS} close to zero) suggest a panmictic population.

The lack of a clear population structure for the whole data set found in this study agrees with the lack of structure found in blue whales of the southern hemisphere [28]. It also agrees with the results on bioacoustic studies that suggest the presence of a single population in the Northeast Pacific, extending from Vancouver to the Dome of Costa Rica, and possibly beyond Ecuador [22, 23]. Our analysis based on individual sighting frequencies also supports one Northeast Pacific genetic stock, since occasional individuals (sighted only one year) that possibly use other wintering grounds are not genetically divergent from the frequent (two-four years) and very frequent (five years or more) individuals. Movements of blue whales from California to the Costa Rica Dome during winter have been reported [25], but whales were also found year-round in that upwelling-enriched area [64]. Thus, some gene flow between the northern and southern Pacific blue whales is plausible. In this context, if some of our occasional individuals also use the Costa Rica Dome wintering area, they may guarantee enough gene flow in the Northeast Pacific population so as to result in a single homogenous genetic stock. Additionally, certain amount of gene flow further south could not be discarded.

Although we found no genetic divergence among individuals of different years and months-year, we observed some signs of a temporal segregation of females. Overall, there is a bias in the sex ratio in the GC toward females, which becomes stronger in February in all years analyzed (month-year group, Table 2). This higher proportion of females observed in February may depend on their reproductive status. Births

likely occur between January and March [26], and late-pregnant females could be the earliest individuals to reach the GC to give birth, producing the bias toward females at the beginning of the season. Segregating migration has been shown in humpback whales, although in that species late-pregnant females is the last class of individuals to arrive to the wintering ground [65]. Blue whales are using the GC also as a feeding ground, where they feed principally on the abundant euphausiid, Nyctiphanes simplex [66]. Lactating females are often emaciated [26] and likely have higher nutritional requirements. Therefore, pregnant females may match the birth period with the period of highest density of their main prey, which occurs in February-March [67].

Although we found no genetic sub-structuring of the population when analyzing the whole data set, we obtained two clusters when analyzing only females with Geneland. Differences in the results between the two Bayesian approaches cannot be explained straightforward, but it has been reported that admixture models fail to find clusters in shallow divergent populations [68], and especially the Structure model tends to gather individuals into the largest cluster, suggesting an unreal lack of structure [69]. By using a Bayesian model that takes into account spatial information, the analysis has proven to be more powerful [60]. Even though this approach looks promising, our result should be interpreted with caution. When applied to wild populations, Bayesian spatial clustering models may infer a wrong number of clusters [63, 68, 70, 71]. To reduce this risk we ruled out isolation by distance that may greatly affect Bayesian clustering inference [70]. We compared the genetic divergence and the inbreeding between and within the clusters respectively, to test if they were real or artifact, and found a small but significant divergence. Furthermore, we used an uncertainty value produced by the analysis of individual utilization area of blue whales in the GC.

As mentioned above, it has been reported that Structure models fail to find structure when F_{ST} values are low (F_{ST}<0.02) [63, 68]. According to this, Structure model fails to find a structure among the six Antarctic blue whale feeding grounds defined upon whaling records (F_{ST}=0.005 p=0.031) [18]. Thus, it is not surprising not to find any structure with Structure analysis under the low divergence among clusters found in this study (R_{ST}=0.01, p=0.01, F_{ST}=0.008, p=0.02). The spatial distribution of the genetic clusters was not completely homogeneous, as expected in very mobile animals, suggesting that the genetic segregation is only partially matched by ecological segregation. Moreover, the low values of R_{ST} and F_{ST} indicate that gene flow is maintained between clusters, allelic frequencies are correlated among them, and the recognition of clusters may be difficult [59, 72, 73]

The uncertainty in spatial position is an important parameter of the Geneland model, and allows to take into consideration both the measurement error (e.g., coordinates recorded with low precision), and the process error (e.g., very large range of movements). Our uncertainty value was calculated mainly from females' individual utilization areas and, therefore, the higher power in detecting clusters with data on females may just be an effect of the better representation of their spatial structure.

Fine-scale population structure has been reported in various mammalian species. In most cases, female natal philopatry generates aggregated grouping patterns, and this produced some level of genetic differentiation [2, 31, 74]. In a worldwide sperm whale population structure analysis [17] no microsatellite divergence was found at large spatial scale (oceans), but significant genetic heterogeneity was found between female social groups, suggesting the presence of greater relatedness within groups than between them, and a potential role of kin selection in sperm whale evolution. The blue whales social structure is poorly understood [26], but overall our results indicate 1) no temporal divergence but seasonal segregation among females, 2) no spatial divergence from the complete data set and the inbreeding coefficient close to zero and 3) no homogeneous distribution of Geneland clusters, which altogether may represent social aggregations. We suggest two complementary hypotheses to explain the blue whale female structure in the GC winter calving-feeding ground; one ecological, related to resource concentration, and a behavioural one, related to female social relationships.

From an ecological point of view, blue whale spatial distribution is linked to the distribution of euphausiids [75,76], their dominant prey [77]. Therefore, the whale distribution observed in the GC may be the result of feeding area choices as found in other areas [75, 76]. In the GC, up to 30% of the blue whales recorded during the aerial surveys were observed feeding close to the surface and/or defecating (used as indirect clue of feeding behaviour), revealing that feeding is an important activity for blue whales, including lactating females, in this calving ground [26]. Mother-calf pair feeding behaviour in a Chilean summer ground has also been reported [78]. The southwestern GC is characterized by the presence of several coastal islands, where dense daytime surface swarms of the euphausiid N. simplex have been reported [66]. In particular, the north of the Carmen Island in front of Loreto and the area between the islands of San Jose and Espiritu Santo north of La Paz (24°50'- 24°36') are characterized by the presence of a very high density of N. simplex during the winter [66, 79]. Each of these two areas corresponds to the spatial distribution of one of the two female genetic clusters. We hypothesized that, if there is a local segregation due to the

heterogeneous environment, this could lead to some segregation among blue whale females and hence to the observed genetic divergence.

From a behavioural point of view, blue whale female-calf pairs prefer coastal waters, more protected from the strong dominant North wind and swell [26]. This female preference has been reported also in southern right whales [e.g.80, 81, 82] and humpback whales [83, 84]. In mammals, including baleen whales, there is evidence of strong female site fidelity [e.g.13, 85] and resulting in genetic divergence such as in grey whale [14]; elephants [74], ungulates [2] and sea lions [86]. In our study, the majority of mother-calf pairs (67%, n=94) were sighted in San Jose-La Paz area (corresponding to the yellow cluster). Moreover, most (85% 17 out of 20) genotyped mother-calf pairs were observed in San Jose-La Paz area. Although mother-calf pairs are not restricted to this area, it appears to be more intensively used as a nursing area than Loreto area. In southern right whales (Eubalaena australis), mother-calf pair congregates in specific nursery grounds, and are segregated from males in winter grounds, possibly to reduce the occurrence of male harassment which can be fatal to the calves [80, 81, 87]. Contrasting with the right whales, foraging is an important activity for blue whales in the GC, thus mother-calf segregation could reduce not only male harassment, but also resource competition with males. If females that congregate in San Jose-La Paz area have a higher level of kinship than Loreto area, this could explain the divergence found between the two areas. Future kinship analysis among whale groups in each cluster may provide further insight into social structure and how it influences the population structure of blue whales in the GC. While it is known that some whales that use the GC in winter, are feeding off California in summer [24], fine-scale segregation among females has not been reported in this feeding ground. However, if site fidelity is a widespread behavior in female blue whales, segregation is likely to be observed in other areas, mainly among pregnant or late-nursing females in which energy loss should be minimized.

Female fine-scale genetic structure has potentially important consequences for conservation and management. Opportunities for kin selection may have a positive effect on calf survival. Blue whale females with calves, that need to implement efficient foraging strategies due to heavy energetic demands, may take advantage of the high seasonal productivity and calm waters of the southwestern GC islands, and share these areas with relatives. Mature females and calves survival has a dramatic impact on population demography, and, therefore, management policies tailored to the areas where mother-calf pairs are frequently sighted could be greatly beneficial for population viability. Moreover, female fine-scale genetic structure

may influence the rate at which genetic diversity is lost through genetic drift. Our results suggest that in blue whales, the loss of genetic variation may be minimized by male mediated gene flow. In this context, a socially structured population, with at least two main groups with high levels of heterozygosity, may lead to a reduction of inbreeding, and of the effects of genetic drift.

Acknowledgements

Paula Costa Urrutia thanks the Consejo Nacional de Ciencia y Tecnología for the scholarship CONACyT-67013. We are grateful to Yolanda Schramm, Filippo Galimberti, Eduardo Santamaría-del-Angel and Francisco Correa for their constant collaboration with this work. We are thankful to Carolina Abud and Esther Jiménez for their valuable suggestions on translation and figure editing, and to Gilles Guillot for his Geneland program assistance and his useful comments to this manuscript. We are indebted to Azucena Ugalde de la Cruz for the photographic comparison and to all the students and technicians from the Laboratorio de Ecología de Cetáceos y Quelonios at CICIMAR-IPN, who collaborated during the field work.

Referencias

1.Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419-39.

2. Coltman DW, Pilkington JG, Pemberton JM (2003) Fine-scale genetic structure in a free-living ungulate population. Mol Ecol 12: 733-742.

3 Andersen E, Born EW, Dietz R, Haug T, Øien N, Bendixen C (2003) Genetic population structure of minke whales Balaenoptera acutorostrata from Greenland, the North East Atlantic and the North Sea probably reflects different ecological regions. Mar Ecol Prog Ser 247: 263-280.

4 Bérubé M, Larsen F, Notarbartolo di Sciara G, Sears R, Aguilar A, Sigurjónsson J, Urban-Ramirez J, Dendanto D, Palsbøll PJ (1998) Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, Balaenoptera physalus (Linnaeus, 1758); analysis of mitochondrial and nuclear loci. Mol Ecol 7:585-599.

5 Escorza-Treviño S, Pastene LA, Dizon EA (2004) Molecular analysis of the truei and dalli morphotypes of dall's porpoise Phocoenoides dalli. J Mamm 85:347-355.

6 Mendez M, Rosembaum HC, Subramaniam A, Yackulic C, Bordino P (2010) Isolation by environmental distance in mobile marine species: molecular ecology of franciscana dolphins at their southern range. Mol Ecol 19: 2212-2228.

7 Patenaude NJ, Portway VA, Schaeff CM, Bannister JL, Best PB, et al. (2007) Mitochondrial DNA Diversity and Population Structure among Southern Right Whales (Eubalaena australis). J Hered 98: 147-157.

8 Hoelzel AR (1998) Genetic structure of cetacean populations in simpatry, parapatry and mixed assemblages: implications for conservations and policy. J Hered 89: 451-458.

9 Tiedemann R, Hardy O, Vekemans X, Milenkovitch M (2000) Higher impact of female than male migration on population structure in large mammals. Mol Ecol 9:1159–1163.

10 Rosenbaum HC, Pomilla C, Mendez M, Leslie MS, Best PB, et al. (2009) Population structure of humpback whales from their breeding grounds in the South Atlantic and Indian Oceans. PLoS ONE 4: e7318.

11 Schaeff C, Kraus S, Brown MW, White B (1993) Assessment of the population structure of western North Atlantic right whales (Eubalaena glacialis) based on sighting and mtDNA data. Can J Zool 71:339–345.

12 Carroll EL, Childerhouse SJ, Christie M Lavery S, Patenaude N, et al. (2012) Paternity assignment and demographic closure in the New Zealand southern right whale. Mol Ecol 21: 3960–3973.

13 Valenzuela LO, Sironi M, Rowntree V, Seger J (2009) Isotopic and Genetic evidence for culturally inherited site fidelity to feeding ground in southern right whales (Eubalaena australis). Mol Ecol 18: 782-791.

14 Goerlitz DS, Urbán J, Rojas-Bracho L, Belson M, Schaeff CM (2003) Mitochondrial DNA variation among Eastern North Pacific grey whales (Eschrichtius robustus) on winter breeding grounds in Baja California. Can J Zool 81: 1965–1972.

15 Frasier TR, Koroscil SM, White BN, Darling JD (2011) Assessment of population substructure in relation to summer feeding ground use in the eastern North Pacific gray whale. Endang Species Res 14: 39–48.

16 Lyrholm T, Gyllensten U (1998) Global matrilineal population structure in sperm whales as indicated by mitochondrial DNA sequences. Proc R Soc 265: 1679-1684.

17 Lyrholm T, Leimar O, Johanneson B, Gyllensten U (1999) Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. Proc R Soc 266: 347–354.

18 Sremba AL, Hancock-Hanser B, Branch TA, LeDuc RL, Baker CS (2012) Circumpolar Diversity and Geographic Differentiation of mtDNA in the Critically Endangered Antarctic Blue Whale (Balaenoptera musculus intermedia). PLoS ONE 7: e32579.

19 Lockyer C, Brown SG (1981) The migration of whales. In: Aidley DJ editors. Animal migration Society for Experimental Biology. Cambridge: Univ Press. pp. 105-137.

20 Reilly SB, Bannister JL, Best PB, Brown M, Brownell Jr RL, et al. (2008) Balaenoptera musculus. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 27 september 2011.

21 Calambokidis J, Barlow J (2004) Abundance of blue and humpback whale in the Eastern North pacific estimated by capture-recapture and line transect methods. Mar Mamm Sci 20: 63-85.

22 Stafford KM, Nieukirk SL, Fox CG (1999) Low-frequency whale sounds recorded on hydrophones moored in the Eastern Tropical Pacific. J Acoustic Soc America 106: 3687-3698.

23 Stafford KM, Nieukirk SL, Fox CG (2001) Geographical and seasonal variation of blue whale calls in the North Pacific. J Cetacean Res Manage 3: 65-76.

24 Calambokidis J, Steiger GH, Cubbage JC, Balcom KC, Ewald C, et al. (1990) Sightings and movements of blue whales off central California 1986-88 from photo-identification. Rep Int Whal Comm 12: 343-348.

25 Bailey H, Mate BR, Palacios DM, Irvine L, Bograd SJ, et al. (2009) Behavioural estimation of blue whale movements in the Northeast Pacific from state space model analysis of satellite tracks. Endang Species Res Preprint doi: 10.3354/esr00239

26 Gendron D (2002) Population Ecology of the Blue Whales, Balaenoptera musculus, of the Baja California Peninsula. Centro de Investigacion Cientifica y de Educacion Superior de Ensenada (CICESE), Mexico. Ph.D. Thesis

27 LeDuc RG, Dizon AE, Goto M, Pastene LA, Kato H, et al. (2007) Patterns of genetic variation in Southern Hemisphere blue whales and use of assignment test to detect mixing on the feeding grounds. J Cetacean Res Manage 9: 73-80.

28 Attard CRM, Beheregaray LB, Curt JC, Gill P, Jenner M, et al. (2010) Genetic diversity and structure of blue whales (Balaenoptera musculus) in Australian feeding aggregations. Conserv Genet 11: 2437-2441.

29 Gendron D, Ugalde De La Cruz A (2012) A new classification method to simplify blue whale photo-identification technique. J Cetacean Res Manage 12: 79-84.

30 Wang IJ (2009) Fine-scale population structure in a desert amphibian: landscape genetics of the black toad (Bufo exsul). Mol Ecol 18: 3847-3856.

31 Nussey DH, Coltman DW, Coulson T, Kruuk LEB, Donald A, et al. (2005) Rapidly declining fine-scale spatial genetic structure in female red deer. Mol Ecol 14: 3395–3405.

32 Roden GI (1964) Oceanographic aspects of the Gulf of California. In: van Andel TH, Shore J GG editors. Marine Geology of the Gulf of California.Tulsa: American Association of Petroleum Geologists Memoir. pp. 30-58.

33 Santamaría-del-Angel E, Alvarez-Borrego S, Müller-Karger F (1994) Gulf of California biogeographic regions based on coastal zone color scanner imagery. J Geoph Res 99:7411-7421.

34 Sears R, Williamson JM, Wenzel FW, Bérubé M , Gendron D, et al. (1990) Photographic identification of the blue whale (Balaenoptera musculus) in the Gulf of St. Lawrence, Canada. Rep Int Whal Comm 12: 335-342.

35 Lambertsen, RH (1987) A biopsy system for large whales and its use for cytogenetics. J. Mamm 68(2):443–445.

36 Amos W, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. Rep Int Whal Comm 13: 99–103.

37 Aljanabi SM, Martínez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-base techniques. Nuc Acid Res 25: 4692-4693.

38 Bérubé M, Rew MB, Skaug H, Jorgensen H, Robbins J, et al. (2005) Polymorphic microsatellite loci isolated from humpback whale, Megaptera novaeangliae and fin whale, Balaenoptera physalus. Conserv Genet 6: 631-636.

39 Buchanan FC, Friesen MK, Littlejohn RP, Clayton JW (1996) Microsatellites from the beluga whale (Delphinapterus leucas). Mol Ecol 5:571-575.

40 Palsbøll PJ, Berube M , Jørgensen H (1999) Multiple Levels of Single-Strand Slippage at Cetacean Tri- and Tetranucleotide Repeat Microsatellite Loci. Genetics 151: 285–296.

41 Bérubé M, Jørgensen H, McEwing R, Palsbøll PJ (2000) Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, Megaptera novaeangliae. Mol Ecol 9: 2181-2183.

42 Valsecchi E, Amos W (1996). Microsatellite markers for the study of cetacean populations. Mol Ecol 5: 151-156.

43 Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, et al. (2007) Automated binning of microsatellite alleles: problems and solutions. Mol Ecol Notes 7:10-14.

44 Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538.

45 Bérubé M, Palsbøll PJ (1996) Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Mol Ecol 5:283–287.

46 Excoffier L, Laval G, Schneider S (2005) Arlequin 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47-50.

47 Guo S, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

48 Raymond M, Rousset F (1995) Genpop (version 1.2): Populations genetics software for exact test and ecumenicist. J Hered 86: 248-249.

49 Rice WR (1989) Analyzing tables of statistical test. Evolution 43: 223-225.

50 Goudet J (1995) Fstat (version 1.2): a computer program to calculate F-statistics. J Hered 86: 485-486.

51 Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

52 Wright S (1978) Evolution of the genetics populations. London: University of Chicago Press. 590 p.

53 Benjamini Y, Yekutieli D (2001) The Control of the false discovery rate in multiple testing under dependency. Ann Statist 29:1165-1188.

54 Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258-275.

55 Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152:1753-1766.

56 Bekoff M, Mech LD (1984) Computer Simulation: Simulation analyses of space use: Home range estimates, variability, and sample size. Behav Res Methods Instrum Comput 16: 32-37.

57 Peakall R, Smouse PE (2006) Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6: 288-295.

58 Pritchard JK, Matthew S, Donnely P (2000). Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

59 Guillot G, Leblois RL, Coulon A, Frantz AC (2009) Statistical methods in spatial genetics. Mol Ecol 18: 4734-4756.

60 Guillot G, Estoup A, Mortier F, Cosson JF (2005) A Spatial statistical model for landscape genetics. Gene Soc Amer 170: 1261-1280.

61 Worton BJ (1989) Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70:164-168.

62 Rodgers AR, Carr AP, Smith L, Kie JG (2005) HRT: home range tools for ArcGIS. Ontario Ministry of Natural Resources Thunder Bay: Centre for Northern Forest Ecosystem Research.

63 Latch EK, Dharmarajan G, Glaubitz JC, Rhodes Jr OE (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conserv Genet 7:295–302.

64 Reilly SB, Thayer VG (1990). Blue whale (Balaenoptera musculus) distribution in the Eastern Tropical Pacific. Mar Mamm Sci 6: 265-277.

65 Clapham PJ (2000) The humpback whale. In: Mann J, Connor RC, Tyack P, Whitehead H, editors. Cetacean society: field study of dolphins and whales. Chicago: University of Chicago Press. pp. 173-196.

66 Gendron D (1992) Population structure of daytime surface swam of Nyctiphanes simplex (Crustacea: Euphausiacea) in the Gulf of California, Mexico. Mar Ecol Prog Ser 87:1-6.

67 Brinton E, Townsend AW (1980) Euphausiids in the Gulf of California- the 1957 Cruises. Calcofi Rep. 21: 212-232.

68 Francois O, Durand E (2010) Spatially explicit Bayesian clustering models in population genetics. Mol Ecol Res 10: 773-784.

69 Kalinowski ST (2011). The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity 106: 625–632.

70 Frantz AC, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? J App Ecol 46: 493-505.

71 Safner T, Miller MP, McRae BH, Fortin MJ, Manel S (2011) Comparison of Bayesian clustering and edge detection methods for inferring boundaries in landscape genetics. Int J Mol Sci 12: 865-889.

72 Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567-1587.

73 Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, et al.(2002) Genetic structure of human populations. Science 28:2381-2385.

74 Okello JBA, Wittemyer G, Rasmussen HB, Arctander P, Nyakaana S, et al. (2008) Effective population size dynamics reveal impacts of historic climatic events and recent anthropogenic pressure in African elephants. Mol Ecol 17: 3788-3799.

75 Branch TA, KM Stafford, Palacios DM, Allison C, Bannister JL et al. (2007). Past and present distribution, densities and movements of blue whales, Balaenoptera musculus, in the Southern Hemisphere and northern Indian Ocean. Mamm Rev 37:116-175.

76 Croll DA, Marinovic B, Benson S, Chavez FP, Black N, et al. (2005) From wind to whales: trophic links in a coastal upwelling system. Mar Ecol Prog Ser 289: 117-130.

77 Gaskin DE (1982) The ecology of whales and dolphins. London: Heinemann Educational Books. 459p.

78 Hucke-Gaete R, Osman LP, Moreno C, Findlay, KP, Ljungblad DK (2004) Discovery of a blue whale

feeding and nursing ground in southern Chile. Proc R Soc B 271: 170–S173.

79 De Silva-Davila R, Palomares-Gacria JR (1998). Unusual larval growth production of Nyctiphanes simplex in Bahia de la Paz, Baja California Sur, Mexico. J Crust Biol 18: 490-498.

80 Elwen SH, Best PB (2004a) Female southern right whale Eubalaena australis: Are there reproductive benefits associated with their coastal distribution off South Africa? Mar Ecol Prog Ser 269: 289-295.

81 Elwen SH, Best PB (2004b) Environmental factors influencing the distribution of southern right whales (Eubalaena australis) on the southern coast of South Africa I: broad scale patterns. Mar Mamm Sci 20: 567-582.

82 Thomas PO, Taber SM (1984) Mother-infant interaction and behavioral development in southern right whales, Eubalaena australis. Behaviour 88: 42-60.

83 Clapham PJ (1996) The social and reproductive biology of humpback whales: and ecological perspective. Mamm Rev 37:27-49.

84 Craig AS, Herman LM (1997) Sex differences in site fidelity and migration of humpback whales (Megaptera novaeangliae) to the Hawaiian Islands. Can J Zool 75:1923-1933.

85 Baker CS, Palumbi SR, Lambertsenm RH, Weinrich MT, Calambokidisa J, et al. (1990) The influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. Nature 344:238-240.

86 Campbell RA, Gales NJ, Lento GM, Baker CS (2008) Islands in the sea: extreme female natal site fidelity in the Australian sea lion, Neophoca cinerea. Proc R Soc Biol Lett 4: 139–142.

87 Payne R (1986) Long term behavioral studies of the southern right whale (Eubalaena australis). Rep Int Whal Commn 10: 161-67.

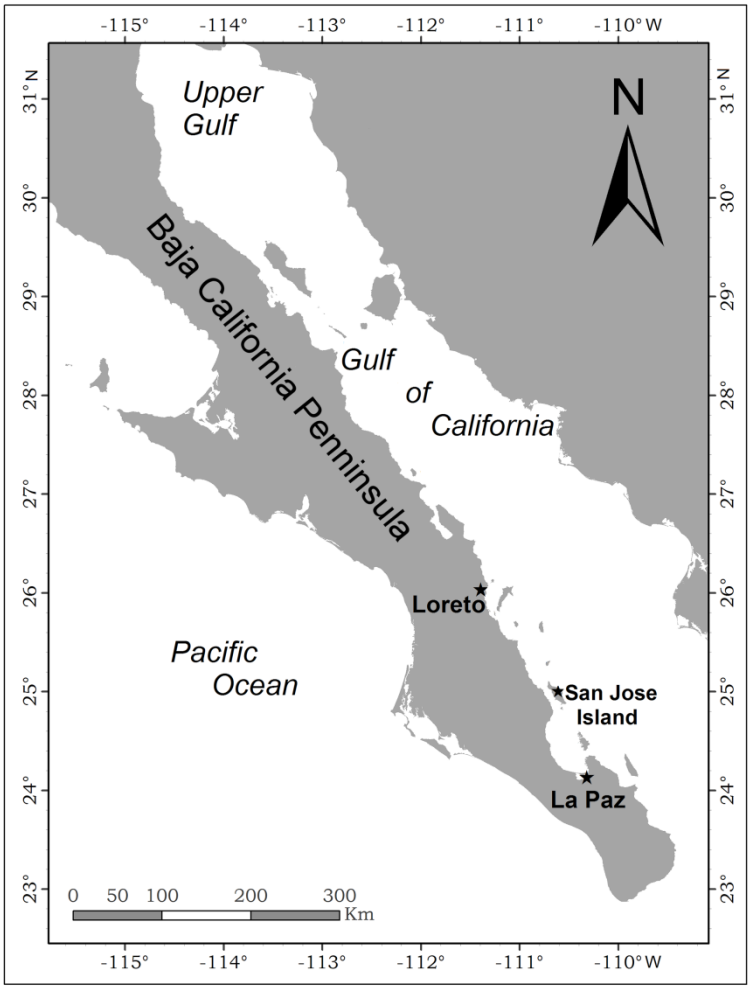


Figure 1. Map of the study area.

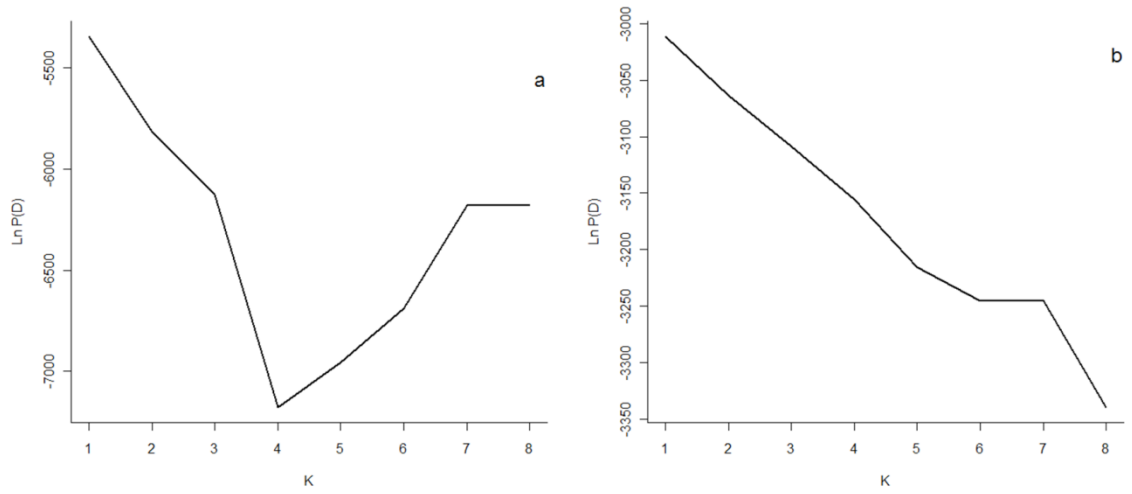


Figure 2. Results of the Structure model fitting. K=number of population. Ln P(D)=logarithm of the data probability obtained for complete (a) and female (b) data set. Highest posterior probability in both cases is for k=1.

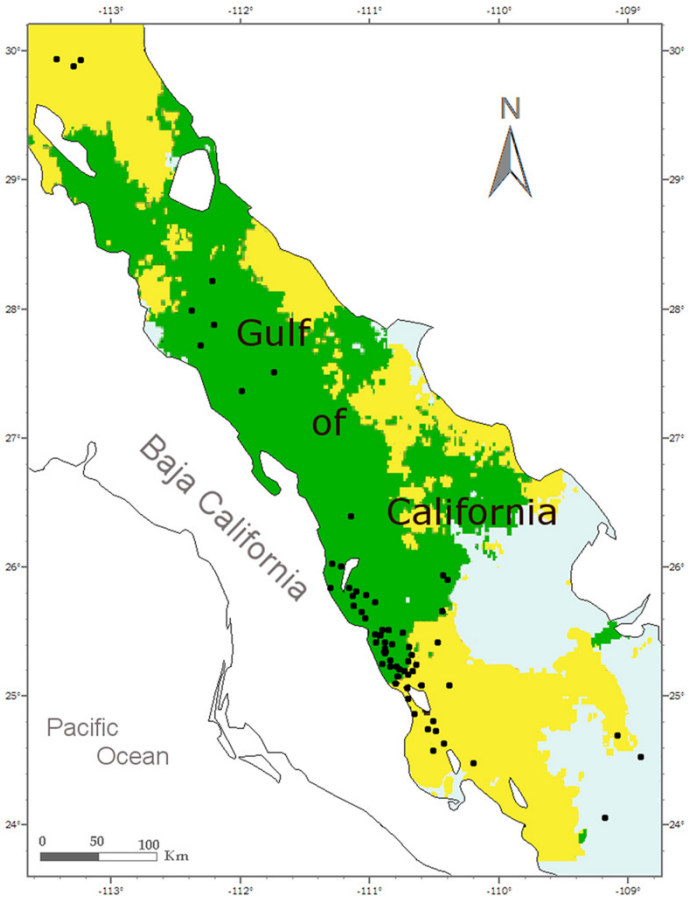


Figure 3. Map of the mode posterior probabilities obtained with the Geneland model. Estimated clusters of blue whales in the Gulf of California are shown in different colours. Green cluster represent Loreto cluster and yellow cluster represent San Jose- La Paz cluster. Dots represent the geographic centroid of individual female blue whale sightings.

Table 1. Statistics of the nine microsatellite loci. At=Annealing temperature (subscript $_1$ and $_2$ correspond to the first and second PCR cycle). Na=Number of alleles per locus, Size=observed range in fragment size in base pairs (bp), Observed (H_O) and Expected (H_E) heterozygosity per locus. H_O deficiency loci are highlight in bold ($p<0.05$). [a] 36 Palsbøll et al., 1999, [b] 34 Bérubé et al. 2005, [c] 37 Bérubé et al. 200, [d] 38 Valsecchi and Amos 1996.

Locus	$MgCl_2$ (mM)	AT_1/AT_2(°C)	Na	Size (bp)	H_O	H_E
GATA98[a]	4.7	51°/52°	9	74-120	**0.69**	**0.73**
GT541[b]	3.1	56°/55°	9	79-99	0.70	0.76
AC137[b]	3.1	55°/54°	9	91-119	**0.64**	**0.66**
GT023[c]	3.1	60°/58°	6	114-124	0.77	0.76
CA232[b]	3.1	56°/55°	8	142-168	0.65	0.65
AC087[b]	3.1	56°/55°	12	165-180	0.83	0.83
EV037[d]	3.9	51°/52°	8	172-194	0.58	0.58
GATA417[a]	3.1	56°/55°	13	174-226	0.83	0.83
CA234[b]	3.1	55°/54°	13	191-215	0.91	0.88
Mean			9.6		0.74	0.74
SD			2.4		0.03	0.09

Table 2. Blue whale grouping criteria for population structure testing at the temporal scale. Grouping criteria: see Methods. Groups=number of groups of the temporal structure analyses. Size=range, mode and standard deviations of groups. Sex ratio=sex ratio of the group compared to overall sex ratio (1.5:1) in the population. HWEL=Hardy-Weinberg equilibrium. R_{ST} and F_{ST}=range values of pairwise R_{ST} and F_{ST} comparisons among groups. Adjusted B and B-Y p-values refer to the adjusted p-values after sequential Bonferroni and Benjaminy-Yekutiely correction respectively. [a] denotes all p-values were >0.05. Significant results are in bold.

Grouping Criteria	Groups	Size	Sex ratio	HWE	R_{ST}, F_{ST}
Years	13	16-61 mode=39, SD=12	X^2=4.5, p=0.9, df=12	**2009 group: p=0.0014** Remaining Groups: p=0.3-0.008 adjusted B p=0.004, 13 test	R_{ST}=0-0.01, p> 0.05[a] F_{ST}=0-0.03, p> 0.05[a]
Month-year	23	10-36 mode=11, SD=8	**February:X^2=43.1, p=0.03, df=9** March: X^2=15, p=0.07, df=10 April: X^2=0.6, p=0.6, df=1	**March 2009:p=0.0018** Remaining Groups: p=0.3-0.008 adjusted B p=0.0022, 23 test	R_{ST}=0.0001-0.03, p=0.01-0.9 F_{ST}=0.0001-0.001, p=0.03-0.9 adjusted B-Yp=0.008
Sighting frequency	3	39-69 mode=39, SD=17	X^2=0.68, p=0.8, df=2	p=0.02-0.2, adjusted B p=0.01	R_{ST}= 0-0.001, p>0.05[a] F_{ST}= 0-0.0004, p>0.05[a]

Table 3. Results of the fitting of the Bayesian clustering model with Geneland. K= number of inferred clusters; in brackets are the number of K with significant R_{ST} and F_{IS} close to zero. Individual per K=number of individuals assigned to each K. R_{ST} and F_{ST}=pairwise divergence among K, F_{IS}=inbreeding coefficient per K. Significant divergence and inbreeding close to zero are in bold, p= p-value at 95% confidence.

Data set	K	Individuals per K	Divergence (R_{ST}, F_{ST})	Inbreeding (F_{IS})
Complete	3(1)	1=171,2=3,3=3		
Females	3(2)	1=66, 2=29, 3=4	**1-2: R_{ST}=0.01, p=0.01** **1-2: F_{ST}=0.008, p=0.02**	**1:F_{IS}=0.01, p=0.3** **2: F_{IS}=0.03, p=0.07**

Artículo Dos

Spatial kin pattern of blue whales in the Gulf of California

Abstract

Social structure and mating systems are challenging to determine in baleen whales, because it is difficult to collect data on social and mating interactions in these species. Here we combined long-term photo-identification data with genetic information to investigate kinship association and parentage of blue whales, Balaenoptera musculus, in the Gulf of California. We analyzed 172 individuals using nine microsatellite loci. In the study period 1995-2009 we observed 126 groups (2-12 whales), most of which included whales of both sexes (n=74). We found a strong temporal variation among group size and composition, and the same group was never observed more than once across surveys. Average group kinship was close to zero suggesting no family groups. Overall, these results suggest high fission-fusion group dynamics. Kin structure was analyzed performing autocorrelation analysis within two spatial females clusters, named San Jose-La Paz and Loreto. We found some evidence of genetic structure only in the short distance classes of the San Jose-La Paz cluster. Within group pairwise kinship analysis showed that most groups (58.7%) were composed of 1 to 7 kin-dyads. Bigger groups showed higher level of kinship and more mother-calf pairs. Female kin-dyads were found more frequently in groups with mother-calf pairs, whereas mixed kin-dyads were found more frequently in groups without mother-calf pairs. This higher level of kinship in larger groups may provide a forage advantage among kin-dyads minimizing resource competition among them. Finally, based on background and our overall results, we suggest two nonexclusive male mating tactic hypotheses that could operate in the Gulf of California, one regarding social interactions and the other one roving.

Keywords: kinship, social structure, Blue whale, winter/calving ground, Gulf of California

Introduction

Group cohesion, dispersal and recruitment pattern play an important role influencing social structure and mating systems (Sugg et al. 1996, Storz 1999) shaping the population structure (Ross 2001). Differences in the distribution of individuals may produce small-scale genetic heterogeneity, even in continue distributed populations, and may lead fine-scale population structure (Constasti et al. 2012).

Cetaceans are long-living species and thus have overlapping generations, as well as high female philopatry to the natal site, features that favor the evolution of social structure. Several species of toothed whales, such as bottlenose dolphins (Tursiops truncatus), sperm whales (Physeter macrocephalus), killer whales (Orcinus orca) and pilot whales (Globicephala melas) exhibit high social organization (Connor et al. 1998). In many species it has been observed female and male philopatry to the natal group (killer whales: Bigg et al. 1990, pilot whale: Amos et al. 1993), group cohesion favored by kin associations (killer whales: Bigg et al. 1990, Pilot et al. 2010; pilot whales: Amos et al. 1993, bottlenose dolphin: Wiszniewski et al. 2010, franciscana, Pontoporia blainvillei: Costa-Urrutia et al. 2012), alloparental care (sperm whale: Whitehead 1996, Gero et al. 2009), male coalitions and alliances among kin and non kin individuals, (bottlenose dolphins: Connor et al. 1992, Krutzen et al. 2003,Wiszniewski et al. 2012) and associations between kin or non kin females in the same reproductive state (bottlenose dolphins: Möller and Harcourt 2008). Such behaviors may imply kin selection, reciprocal altruism or tolerance (Trivers 1972), favoring the formation of cooperative groups for both reproduction and feeding.

However, the social and mating system in baleen whales remains unknown for most of the species. Exceptions are humpback (Megaptera novaeangliae) and right whales (Eubalaena sp) in which long-term research has revealed most of the current knowledge about baleen whales (humpback whale: Clapham 1996, Clapham 2000, Ersts and Rosenbaum 2003, Cerchio et al. 2005; northern right whales, Eubalaena glacialis: Kraus and Hatch 2001; southern right whales, Eubalaena australis: Rowntree et al. 2001, Elwen and Best 2004, Valenzuela et al. 2009). Most baleen whales are migratory species and exhibit a strong annual cycle; mating and calving is observed in tropical and subtropical waters in winter and spring, whereas feeding occurs in high productivity areas in high latitudes in summer (Berta and Sumich 1999). In general, the social organization of baleen whales lacks of cohesiveness (Clapham 2000); the basic social unit best known is the mother-calf pair. In some species it has been shown that these dyads exhibit spatial segregation within the calving-breeding grounds, preferring, in general, coastal areas (Elwen and Best 2004). As in

several mammalian species, parental care appears to be exclusively maternal (Clutton-Brock 1991, Berta and Sumich 1999) and species for which its mating system has been studied, appear to be polygamous and/or promiscuous (Cerchio et al. 2005, Frasier et al. 2007, Carrol et al. 2012).

The blue whale (Balaenoptera musculus) is the largest animal on earth, reaching 30 m of length (Tomilin 1957), and has a cosmopolitan distribution, both coastal and oceanic. This species was intensively hunted commercially since the last century, and currently is considered an endangered species by IUCN Red list (Reilly et al. 2008).

Until now, the Gulf of California in México is the only winter-spring ground intensively studied in which blue whales are frequently sighted (Gendron 2002, Costa-Urrutia et al. 2013). This area is used as calving and feeding ground by a population of about 300 blue whale individuals, mostly females (sex ratio near 1.5/1, Gendron 2002).

At first, the blue whale was considered a solitary species (Gambell 1976), but long term field surveys have shown group formation across a single day in small and enclosed local embayments, social interactions among individuals (Gendron per. comm), and that some calves interact with other individuals apart from their mothers (Gendron 2002). A recent study showed female fine-scale population structure. This structure could be the result of local adaptation to differences in resource concentration and/or differences related to female social relationships (Costa-Urrutia et al. 2013). This may generate nonrandom patterns of mating, dispersal and social structure (Amos et al. 1993, Archie et al 2008, Pilot et al. 2009).

The study of the social structure of the blue whales through direct observation is overly difficult, due to the life style of the species. Therefore, we combined a long-term data base of re-sights with genetic data from nuclear markers to analyze kin pattern and discussed some aspects of mating tactics of blue whales in the southwestern region of the Gulf of California. The main goals of our study were to: 1) test whether female clusters found Costa - Urrutia et al. (2013) have different kin structures, 2) analyze blue whale groups composition, and 3) analyze kinship within groups.

Methods

Study area and sample collection

The Gulf of California is a narrow sea that divides the Baja California Peninsula from the Mexican mainland, located between 23º to 31ºN and between 107º to 115ºW in the Northeast Pacific Ocean (Roden 1964). Intensive surveys were made in the southwestern region, along the coast from Loreto Bay (26º10´N-111º 03´W) to La Paz Bay (24º 05´N-109º 30´W), covering a distance of about 280 km (Figure 1), from 1988 to 2009. This area comprises the two female's clusters, Loreto and San José-La Paz (SJ-LP), found in Costa - Urrutia et al. (2013).

The survey protocol, including the collection of skin-blubber biopsies, is described in details in Costa - Urrutia et al. (2013). Briefly, once a whale was observed, geographic position and pictures for individual identification were taken (Sears et al. 1990). The photo-ID catalogue classification was based on dorsal fin shape and body pigmentation pattern (Gendron and Ugalde de la Cruz 2012). From 1997 to 2009 skin-blubber biopsies were collected from photo-identified whales only, to avoid duplicated biopsy samples. Biopsy samples collected from 1997 to 2001 were preserved in 20% dimethylsulfoxide (DMSO) saturated with table salt (Amos and Hoelzel 1991), while later samples were frozen in liquid nitrogen.

Aspects of blue whale breeding biology

Long-term photo identification analysis showed that blue whale females produce one calf every two to three years (Yochem and Leatherwood 1984, Gendron 2002) although there are records of calving intervals of up to 7 years (Gendron 2002). Calves at birth reach 6-7 m in length (Yochem and Leatherwood 1984) after a gestation period of 10 to 11 months and they are probably weaned at about seven months (Lockyer 1984). Sexual maturity is reached between 5 and 10 years of age (Lockyer 1984). Lactating females are observed between mid-February and April, with a peak in March, and the copulation period likely happens between March and June (Gendron 2002).

Definition of blue whale classes

Blue whales were categorized as adult, calves and immature depending on their age and/or reproductive status. Females were defined as adults when they had given birth at least once or were at least six years old, while adult males were defined as males 10 years old or older (Yochem and Leatherwood

1984). Calves of the year were defined as animals smaller than half of the length of an adult, as they are commonly defined in baleen whale field studies (Burnell 2001, Rowntree et al. 2001). Following this criterion if the adult in a calf-adult pair was a female and the pair war first order relative, then they were defined as mother–calf pair (see below). Finally, the immature (named juveniles) were between one and five or nine years old, respectively for females and males. Individuals were assigned to one of three categories based on the number of years (consecutive or not) in which they were observed: 39% (Females=19%, Males=20%) were transient (observed only in one year), 39% (Females=24%, Males=15%) were frequent (two-four years), and 22% (Females=15%, Males=7%) of whales were highly frequent (five years or more) (Costa-Urrutia et al. 2013).

Group and variables definition

Both aerial and long-term ship surveys (Gendron 2002) showed the tendency of blue whales to aggregate in the Gulf of California (i.e. long spaces in which just one or any whale were observed, followed by shorter spaces in which two or more whales were observed). Further, from ship surveys we observed group formation across a single day in small embayments and that individuals do not move longer (around 10 km) distances in a day. So we defined a group as 2 or more whales seen during one day with no more than 20 km between two individuals. The variables that we measured were group size (number of whales in the group), group composition (sexual proportion and age classes), group area, used as a rough measure of spatial proximity when three or more individuals were observed (calculated thorough Minimum Convex Polygon methods, using the Hawths Tool extension in ArcGIS 9.3, ESRI), locality (SJ-LP, 24º 05-25°30´N, and Loreto, 25°30-26º10, for which a genetic divergence was found in Costa - Urrutia et al (2013).

DNA extraction and genotyping

Total DNA was extracted using a protocol from Aljabani and Martinez (1997). Amplification by PCR was performed on 172 individuals (females: n=98, males: n=74) using the same protocol described in Costa et al. (2013). We used the nine most polymorphic microsatellite loci out of 19 loci previously tested on a subset of 92 blue whale individuals.All nine loci showed very low probability of genotyping error (Micro-Checker program 4.0.7; Van Oosterhout et al. 2004) and were under Hardy-Weinberg equilibrium (Global c^2test: c2=28.8, p=0.053, see Costa - Urrutia et al. 2013 for furthers details). Gender of each individual was identified by PCR amplification of ZFX and ZFY loci (Bérubé and Palsbøll 1996).

Data analysis

Female spatial structure

To identify if kinship influences the formation of the two female clusters found in the southwestern region (Loreto: n=61, SJ-LP: n=33) (Costa-Urrutia et al. 2013) a genetic autocorrelation analysis was performed in Genalex6 (Peakall and Smouse 2006). This program implements the method of Smouse and Peakall (1999), which provides a measure of the genetic similarity (rc) between pairs of individuals according to a specified number of distance classes (km) by averaging all loci. We used short distance classes of 5 km with the aim to detect kin associations in enclosed small embayments. Genalex program provides a measure of the genetic correlation (rc) according to distance classes averaging all loci. Autocorrelation value rc varies between -1 and 1, where rc=0 when lacking spatial structure. The significance of the analysis is evaluated by random permutations generating a 95% confidence interval, which are the boundaries of the null distribution of no autocorrelation (rc=0). Error bars around values of rc are generated by Bootstrap re-sampling method. Significant structure is considered in cases where the values of rc fall outside the confidence interval and error bars do not intercept the x-axis (Peakall and Smouse 2006).

Kinship analysis

The coefficient of relatedness (r), was calculated with the Coancestry 1.0 program (Wang 2011) which implements two likelihood (dyadic, Milligan 2003 and triadic, Wang 2007) and five moment estimators (Li et al. 1993, Queller and Goodnight 1989, Ritland 1996, Lynch and Ritland 1999, Wang 2002) to estimate the pairwise relatedness using their multi-locus genotypes. Several factors may affect the performances of these estimators, including uncertainty about the actual allele frequencies, sample size, number of polymorphic markers, and relatedness among dyads. Therefore, the use of simulated data to evaluate the suitability of each estimator to a particular data set is recommended (Van de Casteele et al. 2001, Wang 2011). Thus, we performed Monte Carlo simulations implemented in Coancestry 1.0 program (Wang 2011) to calculate correlation coefficients between the kinship estimators and true relatedness values (Weir et al. 2006, Wang 2011) in order to select the optimal relatedness estimator. True values means identical by descendent kinship coefficients values in a non-inbreeding population. These are 0.5 for first-order relatives (parents–offspring, and full siblings), 0.25 for second-order relatives (half siblings, grandsons and nephews), 0.125 for third-order relatives (cousins) and 0 for unrelated individuals. Genotyping error was set at 0.01. The two likelihood

estimators (dyadic, DydML and triadic, TrioML) yielded the highest correlation between true and simulated values (R=0.73, p=0.0001, Table 1). Correlations among estimators from empirical data were obtained, and the two likelihood estimators had a similar performance (R=0.97 p=0.0001, Table 1), so we selected the TrioML method because it incorporates a reference individual to obtain dyad relatedness estimations. The relatedness estimates are improved for dyads of closely related individuals because genes that are identical-in-state are less likely to be assumed as identical-by-descendants for the dyad (Wang 2007). Bootstrapping ($n=10^3$) were used to obtain 95% confidence intervals.

We validated our r estimation of female-calf pairs recorded in the field with the expected kinship coefficient value for first order relative (r=0.5) to avoid mother-calf pairs misclassification.

Groups

A generalized linear model (GLM) was used to model the relationship of the number of kin-dyads within groups with locality, treated as a nominal factor, and group size and sexual proportion, treated as continuous variables. The model was fitted assuming a Poisson error distribution and the canonical log link function, as kin-dyads was a count variable apparently showing a Poisson distribution (Lambda = 1.1, Chi-Square test: $\chi^2= 5$, df = 2, p=0.08).

Due to the fact that groups with mother-calf pairs tend to be larger than groups without them in some cetacean species (Connor et al. 2000), the presence of these pairs may influence both group size and number of kin-dyads apart from mother-calf. Thus, we ran a second complementary model in which we modeled the relationship of the presence of kin-dyads excluding mother-calf pairs with group size and the number of mother-calf pairs within groups. The model was fitted assuming Binomial distribution and logit link function, as the presence/absence of kin-dyads excluding mother-calf pairs showed a Geometric distribution (Poisson Lambda = 1.1, Chi-Square test=5, df=2, p=0.08) and was treated a binary response. The maximum likelihood criterion was used to select the best models. Normality of residuals was confirmed via quantile plots.

Comparisons among the type of kin-dyad measured as first, second and third order relatives among females, males and mixed groups were analyzed using the chi-square goodness of fit test.

Results

General characteristics of the groups

A total of 126 groups and 31 single individuals were observed in the period 1995-2009, which mean that near 80% of observation were classified as groups. The great majority were mixed groups (n=74) followed by female (n=29), followed by female-calf pairs (n=17) and male (n=6) groups. The observed median sex ratio (66:34 females/males) was different from the 50:50 expected sex ratio (Chi-square test: χ^2=2644.3, df=124, p=0.0001) and from the 60:40 population sex ratio (Chi-square test: χ^2=1635.1, df=124, p=0.0001). Groups varied in size (range=2-12, mean=3.8, median=3, sd=2), composition within or between weeks (Figure 2), and spatial proximity measured thorough the area (range=0.01-259.7 km^2, mean=28, median=8, sd=52.3).

Groups were observed in all sampled years (range=2-15, mean=8.3, median=9, sd=4.5) and months (March n=63, February n=44, April n=17, January n=3). Similar number of groups was found in both localities (Loreto: n=57, SJ-LP: n=68). Nearly 60% of the individuals were observed in two or more groups (Table 2).

Kinship in females clusters

The coefficient of relatedness (r) was close to zero, suggesting the lack of inbreeding at the population level (r=0.007, 95% CI: 0.004-0.010), as well as for each cluster: Loreto cluster (r=0.005, CI: 0.001-0.020) and SJ-LP cluster (r=0.030, CI: 0.010-0.060).

The autocorrelations between kinship and distance class indicated that females from Loreto cluster did not show any genetic autocorrelation (Figure 3a), while females from SJ-LP showed a positive genetic structure within the first 5 kms (Figure 3b).

Kinship in Groups

Although within group mean relatedness was close to zero (range: 0-0.13, mean=0.03, median=0, SD=0.03), most groups (n=74, 58.7%) included 1 to 7 kin-dyads (mean=2, median=1, SD=1.3), while the rest (n=52, 41.3%) were composed of unrelated individuals. These unrelated groups consisted of few individuals (range=2-6, mean=2.8, median=2.5, SD=1.1) and were more frequently observed at Loreto (n=33, 63%).

Regarding groups with kin-dyads, these were groups without mother-calf pairs (n=31, 42%), groups

with mother-calf pairs (n=25, 34%) and mother-calf pairs alone (n=18, 24%). These three types of groups were more frequently observed in SJ-LP locality, but when we compared the number of these groups between localities no significant differences were found (without mother-calf pair groups: SJ-LP=19, L=12; with mother-calf pair groups: SJ-LP=19, L=7, mother-calf pairs alone: SJ-LP=10, L=5, Kruskal-Wallis test: H=2.2, p=0.3).

We compared the proportion of the three types of kin dyads, first, second and third order relatives among mixed, female and male kin-dyads (total of 9 comparisons) between groups with and without mother-calf pairs. While groups with mother-calf pairs showed that most kin-dyads were second and third order relatives female-dyads (chi-square test χ^2=22.1, df=8, p=0.0001, Figure 4a), groups without mother-calf pairs showed that most kin-dyads were second order relative mixed-dyads, followed by third order relatives female-dyads (chi-square test χ^2=18.3, df=8, p=0.0001, Figure 4b). Among groups with mother-calf pairs we found six types of kin-dyads (Figure 5). The highest frequency was found to be females related with a mother followed by females related with a calf but differences were not significant (chi-square test χ^2=0.9, df=5, p=0.3

GLM showed that the number of kin-dyads was mainly explained by group size and locality. While group size showed a positive effect, locality showed a negative effect over Loreto, indicating that this locality showed lower number of kin-dyads within groups compared to SJ-LP (Table 3). Complementary to the first model, the second model showed that the presence of kin-dyads that were not mother-calf pairs were explained by both number of mother-calf pairs within group and group size (Table 4).

Discussion

The blue whales in the Gulf of California showed a strong temporal variation in group size and composition, and no equal groups were found across surveys. Although variation in spatial proximity was observed, most groups of three or more individuals were found to occupy an area of 8km^2 or less. This probably represents the most common spatial proximity within group. Twenty four percent of individuals were seen in five or more groups, this percentage is not surprising since 22% of whales were classified as highly frequent (sighted five years or more). Thus, it is possible that these whales have the potential to form preferential association among them. These observations could be compared with the fluid small delphinids societies (Möller 2012). Bottlenose dolphins is the best-studied cetacean for its accessibility to near shore waters, in which the fusion-fission grouping pattern is well described in several populations and isdefined as individuals associating in small groups, which change composition within a day or even hours (Connor et al. 2000). Regarding females, bottlenose dolphin have a large network of associations; while some females show a preferential association among them, other do not associate preferentially to a particular band (Connor et al. 2000). More recently, the term fission-fusion dynamics, instead of fission-fusion societies, was proposed to characterize any societies by its degree of fission-fusion dynamics, which can vary from highly cohesive with stable group membership (low fission-fusion) to highly fluid with either relatively stable or flexible subgroup membership (high fission-fusion) (Aureli et al. 2008).

There are some environmental, behavioral and kinship features that support the comparison between blue whale social dynamics and small dolphin, particularly bottlenose dolphins. Regarding environmental factors, the Gulf of California calving ground is a high productivity area that also represents a feeding ground for blue whales. This type of habitat is more comparable to dolphin habitat, in which rearing offspring, reproductive and feeding behaviors are commonly observed in the same area (Connor et al. 2000), than to the well-studied right and humpback whales which do not feed in their calving grounds (Berta and Sumich 1999). Thus, dolphins and blue whales have in common resource competition in calving ground. This, and the high fluctuation in spatial and temporal distribution of food resources characteristic to marine environment could lead to some convergence in the general aspects of their societies.

Regarding behavioral features, changes in group size and composition on a weekly basis resemble the dolphin fluid society (Connor et al. 2000, Möller 2012). Bottlenose dolphins' association index and network analysis showed that some part of the population, both females and males, associate preferentially

among each other. Unfortunately, we were unable to perform confident association index analysis due to low number of groups and associations, but our preliminary results suggest that some part of the population (highly frequent individuals) may not associate randomly. Probably, future index association analysis using a bigger dataset will bring more light on blue whales sociality.

Regarding kinship features, average relatedness in blue whales groups was close to zero, similarly to what is found in most dolphin groups (Möller 2012), and contrary, for example, from killer whales, in which stable family-matrilineal groups are well known (Whitehead et al. 2000). In this context it is expected that mother-calf pairs may be the most cohesive family group representing the most inclusive social level. Low average group relatedness in delphinids has been associated to promiscuous mating system (Möller 2012). In blue whales, like in bottlenose dolphins, sexual behavior is hard to distinguish from social behavior (Connor et al. 2000, Gendron per. comm.). Thus promiscuous mating system o low polygyny may the probably mating system in blue whales.

Together the features described above are the first steps to suggest high fission-fusion dynamics (Connor et al. 2000, Aureli et al. 2008) to explain, at least in part, the blue whale social system.

Female kinship structure

Different kinship structures between female clusters was supported by our autocorrelation results; while kinship appears not to play a role in Loreto, the pairwise relatedness within SJ-LP suggests that kin pattern shapes female fine-scale population structure in this locality. This structure is supported by two other results. First, most mother-calf pairs within groups and alone were found more frequently in SJ-LP, suggesting spatial segregation. Second, we found that Loreto showed lower number of kin-dyads within groups than SJ-LP

Further, females (but also males) were found more frequently related to the mother or the calf in most groups with mother-calf pairs. These results explain the higher number of kin dyads found in SJ-LP and illustrate a case in which social structure shapes fine-scale population structure.

Mother-calf pairs segregation has been reported in several species because of differences in resources needs (Grevy zebra, Equus grevyi, Ginsberg and Rubenstein 1990), habitat preferences (in the case of baleen whales: shallow and sheltered waters, bowhead whales, Balaena mysticetus: Heide-Jørgensen et al. 2010, right whales: Elwen and Best 2004, humpback whales: Clapham 2000) or male harassment avoidance

which could be harmful for calves (right whales: Elwen and Best 2004). As we mentioned before, foraging is an important activity for blue whales in this calving ground (Gendron 1992, 2002). Because of energy demands, lactating females' foraging strategies need to be extremely efficient, and this could be obtained by sharing the foraging among relatives, or leaving calves attended by kins. This agrees with field observations of calves spending time with a third individual, when the mother is not observed, and is probably feeding. Thus, blue whales mother-calf segregation and association with kins could provide a forging advantage and an increase in calves survival.V

Groups kinship structure

One or more kin-dyads were frequently found within blue whales groups, but there were also unrelated groups. These results suggest that both kin and non kin associations could be important in this population.

The higher number of kin-dyads was observed in bigger groups and in groups with mother-calf pairs. The presence of mother-calf pairs in bigger groups has been reported for bottlenose dolphins and has been associated to calf protection and survival (Connor et al. 2000). In the case of blue whales, which are virtually under no predation pressure, bigger groups could be consequences of rich food patches, so that higher levels number of kin dyads may provide a forage advantage in term of inclusive fitness by minimizing intense resource competition among kin pairs

Aggregating in big groups of individuals was suggested as a solution for large-bodied primates who face a relatively low risk of predation, in order to cope more efficiently with patchily distributed and temporally varying food sources (Milton 1984, Dunbar 1988, Klein and Klein 1977). In this context, mother-calf pairs alone or in some cases with a third relative may spread out from each other when resources are scarce to reduce feeding competition and aggregate to forage together when resources are plentiful.

Females and mixed kin-dyads were the most frequent kin-dyads within groups. Although the presence of many female's kin associations was expected due to females high site fidelity compared to males (Gendron 2002), the high frequency of mixed kin-dyad was not expected. Females associations are common in group-living taxa, in which females spend their lives in the company of female relatives (e.g. elephants, Loxodonta Africana: Archie et al. 2006, bottlenose dolphin: Möller et al. 2006, Wiszniewski et al. 2010, baboons, Papio cynocephalus: Silk et al. 2006, sperm whale; Gero et al. 2008). The reproductive success of females is limited primarily by nutritional constraints, and female kin association probably confers foraging

benefits. The blue whales seem to follow the rule, suggesting that females may be increasing their control over food by associating with each other.

Mixed kin associations are less common and have been documented in few vertebrates including some cetacean and primate species (e.g. pilot whales: Amos et al. 1993, bonobos, Pan Paniscus: Hohmann et al. 1999, killer whales: Baird and Dill 2000, bootlenose dolphin: Wiszniewski et al. 2010). It has been suggested that mixed kin pairs provide opportunities for developing kin relationships. Males may obtain inclusive fitness benefits if increased cooperation with kin results in increased survival of their siblings (Connor et al. 2000). Since our groups consist of both related and unrelated mature females, males may also benefit by associating with relative females, if they gain familiarity and/or evaluation with potential mating partners, as suggested for bottlenose dolphins (Möller et al. 2001). Although here we are unable to explain the mechanism or process that generate a positive kin selection pressure, inclusive fitness gain among females or mixed pairs may not be discarded.

Male mating tactics hypotheses

Resource competition is probably a strong selective pressure for blue whale males in the Gulf of California, since this is both a breeding and foraging ground. This may result in a mating system more condition dependent (e.g. resource availability, social interactions among kins or non kins, population density) than in other whales for which resource competition could be discarded in a mating-calving ground, such as humpback and southern right whales. Based on background information and our overall results, we suggest two nonexclusive male mating tactic hypotheses that could operate in the Gulf of California: social interactions which include courtship and roving.

First, we may roughly discard sperm competition (Brownell and Ralls 1986). One of the conditions for sperm competition is that females have access to multiple mates. In this context there may be a selection pressure that favors the increase in testicular size and therefore sperm production (Parker 1970). Blue whales have a low relative proportion between the weight of the testes and body weight compared to right whales (Eubalaena sp), (Brownell and Ralls 1986) in which sperm competition is the most likely way of male competition (Kraus and Hutch, 2001, Frasier et al. 2007). Second, we have not observed yet mating groups as described in humpback whales (Megaptera novaeangliae) in which groups with males competing with aggressive interactions are well known (Clapham et al. 1992), or in right whales mating groups, in which a

female may be surrounded by several males (Kraus and Hutch 2001, Best et al. 2003).

Males social interacions hypothesis. As discussed above, the presence of mature males in groups with predominance of females may be beneficial for a given female. Male courtship behaviours may include some degree of calf care, as suggested in some primates (Garber 1998). It could also reduce the harassment of other males with lower reproductive potential as proposed for dall porpoise (Phocoenoides dalli, Willis and Dill 2006); in both cases, female fitness would be increased. Also, inverted sexual dimorphism could make social interactions suitable as courtship tactics since blue whales are probably unable to force copulation

Roving hypothesis. An opportunistic mating system in which some males just rove among groups and mate with each and every female that they are able to find. This is a common male mating tactic for species where females and other resources are widely distributed, which results in males that rove searching for estrous females (Clutton-Brcok 1989). Among cetaceans this tactic was reported for sperm whales (Whitehead 1994) and bottlenose dolphins (Connor et al. 2000).

It has been suggested that high fission-fusion dynamics create opportunities for transient individuals because it is impossible to monitor new individuals and each individual behavior while the group is dispersed (Enquist and Leimar 1993, Aureli et al. 2008). The fact that our population is represented by 40% transient whales, suggests that high fission-fusion dynamic may provide social opportunities for both males and females due to interactions with unknown individuals that may be common in this type of society. In the context of male mating tactics, both social interactions and roving could be two widespread strategies for both frequent and transient males in a high fission-fusion dynamics.

Overall promiscuous mating system could fit with both, the two proposed male mating tactics and the high fission-fusion dynamics.

To our best knowledge here we provided for the first time insights about the social system and mating tactics of blue whales, and any balenopteride. Although most aspects of the social and mating system remains unknown, the results obtained here showed a more complex social system than previously thought.

Referencias

Aljanabi SM, Martínez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-base techniques. Nucleic Acids Research. 25: 4692-4693.

Amos W, Hoelzel AR. 1991. Long-term preservation of whale skin for DNA analysis. Report of the International Whaling Commission.13: 99-103.

Amos B, Schlotterer C, Tautz D. 1993. Social structure of pilot whales revealed by analytical DNA profiling. Science. 260:670-672.

Archie EA, Moss CJ, Alberts SC. 2006. The ties that bind: genetic relatedness predicts the fission and fusion of social groups in wild African elephants. Proceedings of the Royal Society B. 273: 513-522.

Archie EA, Maldonado JE Hollister-Smith JE, Poole JH, Moss CJ, Fleischer RC, Alberts SC. 2008. Fine-scale population genetic structure in a fission–fusion society. Molecular Ecology. 17:2666-2679.

Aureli F, Schaffner CM, Boesch C, Bearder SK, Call J, Chapman CA, Connor R, Di Fiore A, Dunbar RIM, Henzi SP, Holekamp K, Korstjens AH, Layton R, Lee P, Lehmann J, Manson JH, Ramos-Fernandez G, Strier KB, van Schaik CP. 2008. Fission-fusion Dynamics: new research frameworks. Current Anthropology. 49: 627-654.

Baird RW, Dill LM. 2000. The killer-whale-foraging specializations and group hunting. In: Cetacean Societies: Field Studies of Dolphins and Whales. (Mann J, Connor RC,. Tyack PL, Whitehead H Eds, pp127-153. Chicago: University of Chicago Press.

Berta A, Sumich JL. 1999. Mating, Breeding and Social organization. In: Marine Mammals: Evolutionary Biology. Academic Press Ed, pp 335-359.San Diego, California, USA.

Bérubé M, Palsbøll PJ. 1996. Identification of sex in Cetaceans by multiplexing with three ZFX and ZFY specific primers. Molecular Ecology. 5:283–287.

Best PB, Schaeff CM, Reeb D, Palsbøll P. 2003. Composition and possible functions of social groupings of

southern right whales in South African waters. Behaviour. 140: 1469-1494.

Bigg MA, Olesiuk PK, Ellis GM, Ford JKB, Balcom KB. 1990. Social organization and genealogy of resident killer whales, Orcinus orca, in the coastal waters of British Columbia and Washington State. Report of the International Whaling Commission. 12: 383-406.

Brownell RL, Ralls K. 1986. Potential for sperm competition for baleen whale. Report of the International Whaling Commission. 8: 97-112.

Carroll EL, Childerhouse SJ, Christie M, Lavery S, Patenaude N, Alexander A, Constantine R, Steel D, Boren L, Baker CS. 2012. Paternity assignment and demographic closure in the New Zealand southern right whale. Molecular Ecology. 21: 3960-3973

Cerchio S, Jacobsen JK, Cholewiak DM, Falcone EA, Merriwether DA. 2005. Paternity in humpbackwhales, Megaptera novaeangliae: assessing polygyny and skew in male reproductive success. Animal Behaviour. 70:267-277.

Clapham PJ, Palsboll P, Mattila DK, Vásquez O. 1992. Composition and dynamics of humpback whales competitive group in the West Indies. Behaviour. 122: 182-194.

Clapham PJ.1996. The social and reproductive biology of the humpback whale: An ecology perspective. Mammal Review. 26:27-49.

Clapham PJ. 2000. The humpback whale. In Cetacean society: field study of dolphins and whales. Mann J, Connor RC, Tyack P, Whitehead H, Eds, pp. 173-196. Chicago: University of Chicago Press.

Clutton-Brock TH. 1991. The evolution of sex differences and the consequences of

polygyny in mammals. In: The Development and Integration of Behaviour: Essays

in Honour of Robert Hinde. Bateson P Ed,, pp229–253. Cambridge: Cambridge University Press.

Coltman DW, Pilkington JG, Pemberton JM. 2003. Fine-scale population genetic structure in a free-living ungulate population. Molecular Ecology. 12:733-742.

Connor RC, Smolker RA, Richards FA. 1992. Two levels of alliance formation among bottlenose dolphin, Tursiops sp. Proceedings of the Natural Academy of Science. 89: 987-990.

Connor RC, Mann J, Tyack PL, Whitehead H. 1998. Social evolution in toothed whales. Trends in Ecology and Evolution. 13: 228-232.

Connor RC. 2000. Group living in whales and dolphins. In: Cetacean Societies: Field Studies of Dolphins and Whales. Mann J, Connor RC, Tyack PL, Whitehead H Eds, pp199-218. Chicago: University of Chicago Press.

Connor RC, Wells RS, Mann J, Read AJ. 2000. The bottlenose dolphin: social relationships in a fission-fusion society. In: Cetacean Societies: Field Studies of Dolphins and Whales (Mann J, Connor RC,Tyack PL, Whitehead H Eds, pp 91-126. Chicago: University of Chicago Press.

Contasti AL, Tissier EJ, Johnstone JF, McLoughlin PD. 2012. Explaining spatial heterogeneity in population dynamics and genetics from spatial variation in resources for a large herbivore. Plos One. 7(10): e47858. doi:10.1371/journal.pone.0047858

Costa-Urrutia P, Abud C, Secchi ER, Lessa EP. 2012. Population genetic structure and social kin associations of franciscana dolphin, Pontoporia blainvillei. Journal of Heredity. 103: 92-102.

Costa-Urrutia P, Sanvito S, Victoria-Cota N, Enríquez-Predes L, Gendron D. 2013. Fine scale population structure of blue whale, Balaenoptera muslucus, in the Gulf of California. Plos One. 8(3): e58315. doi:10.1371/journal.pone.0058315.

Elwen SH, Best PB. 2004. Female southern right whale Eubalaena australis: are there reproductive benefits associated with their coastal distribution off South Africa? Marine Ecology Progress Series. 269: 289-295.

Enquist M, Leimar O. 1993. The evolution of cooperation in mobile organisms. Animal Behaviour. 45:747–757.

Ersts PJ, Rosenbaum HC. 2003. Habitat preference reflects social organization of humpback whales (Megaptera novaeangliae) on a wintering ground. Journal Zoology of London. 260: 337–45.

Dunbar RIM. 1988. Primate social systems. Ithaca: Cornell University Press.

Frasier TR, Hamilton PK, Brown MW, Conger LA, Knowlton AR, Marx MK, Slay CK, Kraus SD, White BN. 2007. Patterns of male reproductive success in a highly promiscuous whale species: the endangered

North Atlantic right whale. Molecular Ecology.16:5277-5293.

Gambell R. 1976. World Whale stock. Mammal Review. 6:41:53.

Garber PA. 1997. One for all and breeding for one: cooperation and competition as a Tamarin reproductive strategy. Evolutionay Anthropology. 5:187-199.

Gendron D. 1992. Population structure of daytime surface swam of Nyctiphanes simplex (Crustacea: Euphausiacea) in the Gulf of California, Mexico. Marine Ecology Progress Series. 87:1-6.

Gendron D. 2002. Ecología Poblacional de la ballena azul, Balaenoptera musculus de la Península de Baja California. Tesis de Doctorado. CICESE. Ensenada, Baja California México. 105pp.

Gendron D, Ugalde De La Cruz A. 2012. A new classification method to simplify blue whale photo-identification technique. Journal of Cetacean Research and Management. 12: 79-84.

Gero S, Engelhaupt D, Rendell L, Whitehead H. 2009. Who Cares? Between-group variation in alloparental caregiving in sperm whales. Behavioral Ecology. 20:838–843.

Gero S, Engelhaupt D, Whitehead H. 2008. Heterogeneous social associations within a sperm whale, Physeter macrocephalus, unit reflect pairwise relatedness. Behavioral Ecology and Sociobiology. 63: 143-151.

Ginsberg JR, Rubenstein DI. 1990. Sperm competition and variation in zebra mating-behavior. Behavioural Ecology and Sociobiology. 26:427-434.

Heide-Jørgensen MP, Laidre KL, Wiig Ø, Postma L, Dueck L, Bachmann L. 2010. Large-scale sexual segregation of bowhead whales. Endangered Species Research. 13: 73-78.

Hohmann G, Gerloff U, Tautz D, Fruth B. 1999. Social bonds and genetic ties: kinship, association and affiliation in a community of bonobos, Pan paniscus. Behaviour, 136:1219-1235.

Klein LL, Klein DJ. 1977. Feeding behavior of the Colombian spider monkey, Ateles belzebuth. In Primate ecology: Studies of feeding and ranging behaviour in lemurs, monkeys, and apes. Clutton-Brock TH Ed , pp153–181. London: Academic Press.

Kraus SD, Hatch JJ. 2001. Mating strategies in the North Atlantic right whale, Eubalaena australis. Journal

of Cetacean Research and Management. 2:237-244.

Krützen M, Sherwin WB, Connor RC, Barré LM, Van de Casteele T, Mann J, Brooks R. 2003. Contrasting relatedness patterns in bottlenose dolphins (Tursiops sp) with different alliance strategies. Preceding of Royal Society. 270:497-502.

Li CC, Weeks DE, Chakravarti A. 1993. Similarity of DNA fingerprints due to chance and relatedness. Human Heredity. 43: 45–52.

Lockyer C. 1984. Review of baleen whale reproduction and implications for management. Report of the International Whaling Commission. 5:27:50.

Lynch M, Ritland K. 1999. Estimation of pairwise relatedness with molecular markers. Genetics. 152: 1753-1766.

Milligan BG. 2003. Maximum-likelihood estimation of relatedness. Genetics. 163: 1153-1167.

Milton K. 1984. Habitat, diet, and activity patterns of free ranging woolly spider monkeys (Brachyteles arachnoides E Geoffroy 1806). International Journal of Primatology 5:

491-514.

Möller LM, Beheregaray LB, Allen SJ, Harcourt RG. 2006. Association patterns and kinship in female Indo-Pacific bottlenose dolphins, Tursiops aduncus, of southeastern Australia. Behavioral Ecology and Sociobiology. 61:109-177.

Möller LM, Harcourt RG. 2008. Shared reproductive state enhances female associations in dolphins. Research Letters in Ecology. doi:10.1155/2008/498390article ID 498390.

Möller LM. 2012. Sociogenetic structure, kin associations and bonding in delphinids. Molecular Ecology. 21:745–764.

Nussey DH, Coltman DW, Coulson T, Kruuk LEB,. Donald A, Morris SJ, Clutton-Brock TH, Pemberton J. 2005. Rapidly declining fine-scale spatial genetic structure in female red deer. Molecular Ecology. 14: 3395-3405.

Parker GA. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews.

45: 525–567.

Peakall R, Smouse PE. 2006. Genalex 6: genetic analysis in Excel. Population genetic software or teaching and research. Molecular Ecology Notes. 6: 288–295.

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

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. Evolution. 43: 258-275.

Reilly SB, Bannister JL, Best PB, Brown M, Brownell Jr RL, Butterworth DS, Clapham PJ, Cooke J, Donovan GP UrbánJ, Zerbini AN. 2008. Balaenoptera musculus. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 04 December 2012.

Ritland K.1996. Estimators for pairwise relatedness and individual inbreeding coefficients. Genetical Research. 67:175-185.

Roden GI. 1964. Oceanographic aspects of the Gulf of California. In: Marine Geology of the Gulf of California. van Andel TH, Shore J GG Eds. . pp30-58. American Association of Petroleum Geologists Memoir.

Ross KG. 2001. Molecular Ecology of social behaviour: analysis of breeding system and genetic structure. Molecular Ecology. 10:265-284.

Rowntree V, Payne R, Schell DM. 2001. Changing patterns of habitat use by southern right whales, Eubalaena australis, on their nursery ground at Península Valdés, Argentina, and their long range movements. The Journal of Cetacean Research and Management. 2: 133-143.

Sears R, Williamson JM, Wenzel FW, Bérubé M , Gendron D, Jones PW. 1990. Photographic identification of the blue whale, Balaenoptera musculus, in the Gulf of St. Lawrence, Canada. Report of the International Whaling Commission. 12: 335-342.

Silk JB, Altmann J, Alberts SC. 2006. Social relationships among adult female baboons, (Papio cynocephalus.) I. Variation in the strength of social bonds. Behavioral Ecology and Sociobiology. 61:183-195.

Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of multi-allele and multi-locus genetic

microstructure. Heredity. 82:561-573.

Storz JF. 1999. Genetic consequences of mammalian social structure. Journal of Mammalogy. 80:553-569.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL. 1996. Population genetics meets behavioral ecology. Trends in Ecology and Evolution. 11:338-342.

Sundaresan SR, Fischhoff IR, Dushoff J, Rubenstein DI. 2007. Network metrics reveal differences in social organization between two fission-fusion species, Grevy's zebra and onager. Oecologia.151:140-149

Tomilin AG. 1957. Mammals of the URRS and adjacent countries. Volume IX. Cetacea. Nauk SSSR, Moscu. 717pp.

Trivers RL. 1972. Parental investment and sexual selection. In Sexual selection and the descent of Man, 1871-197. Campbell B Ed. pp136-179Adeline, Chicago, USA.

Valenzuela LO, Sironi M, Rowntree V, Seger J. 2009. Isotopic and genetic evidence for culturally inherited site fidelity to feeding ground in southern right whales, Eubalaena australis. Molecular Ecology. 18: 782-791.

Van de Casteele T, Galbusera P, Matthysen E. 2001. A comparison of microsatellite-based pairwise relatedness estimators. Molecular Ecology. 10: 1539-1549.

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes. 4: 535-538.

Wang J. 2002. An estimator for pairwise relatedness using molecular markers. Genetics: 160: 1203-1215.

Wang J. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetical Research. 89: 135-153.

Wang J. 2011. Coancestry: a program for simulating, estimating and analyzing relatedness and inbreeding coefficients. Molecular Ecology. 11:141-145.

Weir BS, Anderson AD, Hepler AB. 2006. Genetic relatedness analysis: modern data and new challenges. Genetics. 7:771-780.

Whitehead H. 1996. Babysitting, dive synchrony, and indications of alloparental care in sperm whales.

Behavioral Ecology and Sociobiology. 38:237-244.

Whitehead H, Christal J, Tyack PL. 2000. Studying cetacean social structure in space and time. In Cetacean Society, Field Study of Dolphins and Whales. Mann J, Connor RC, P. Tyack P, Whitehead H Eds. pp65-87.Chicago, USA.

Whitehead H, Weilgart L. 2000. The sperm whales: social females and roving male. In Cetacean Society, Field Study of Dolphins and Whales. Mann J, Connor RC, Tyack P, Whitehead H Eds, pp154-172. Chicago: University of Chicago Press.

Willis PM, Dill LM. 2007. Mate guarding in male Dall's porpoises (Phocoenoides dalli). Ethology. 113:587-597.

Wiszniewski J, Corrigan S, Beheregaray LB, Möller LM. 2012. Male reproductive success increases with alliance size in Indo-Pacific bottlenose dolphins, Tursiops aduncus. Journal of Animal Ecology. 81:423-431.

Wiszniewski J, Lusseau D, Möller LM. 2010. Female bisexual kinship ties maintain social cohesion in a dolphin network. Animal Behaviour. 80: 895–904

Yochem PK, Leatherwood S. 1985. Blue whales, Balaenoptera musculus (Linneaus, 1975) Handbook of marine mammals: the sirenians and baleen whales. Ridgeway H, Harrison R Eds, pp.93-240Academic Press, London.

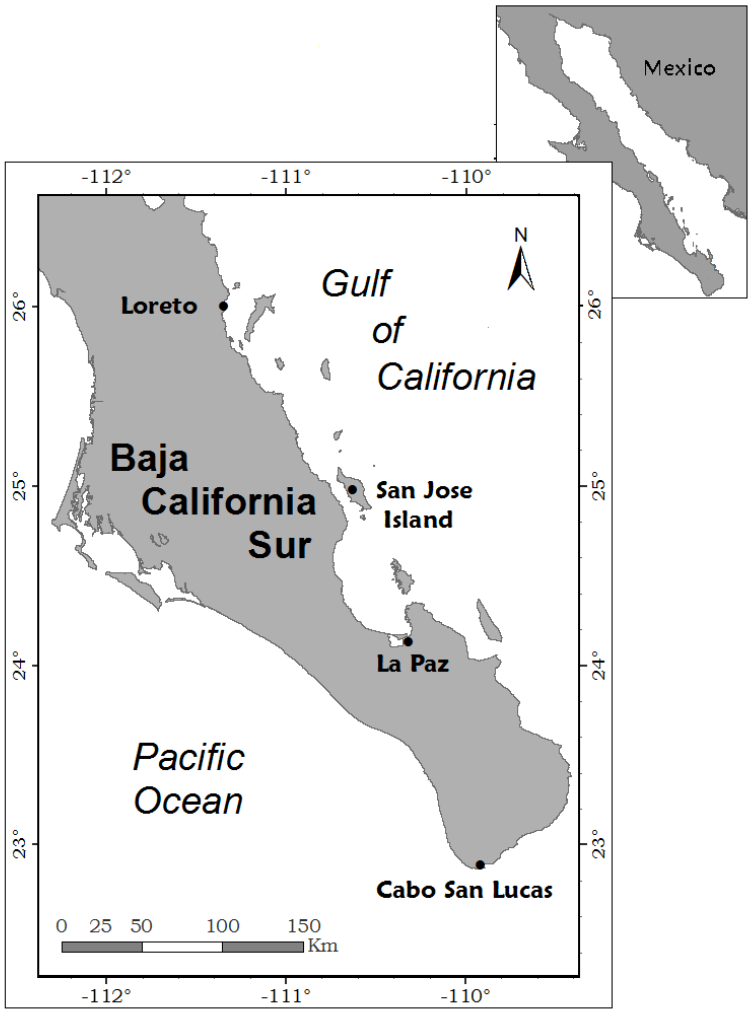
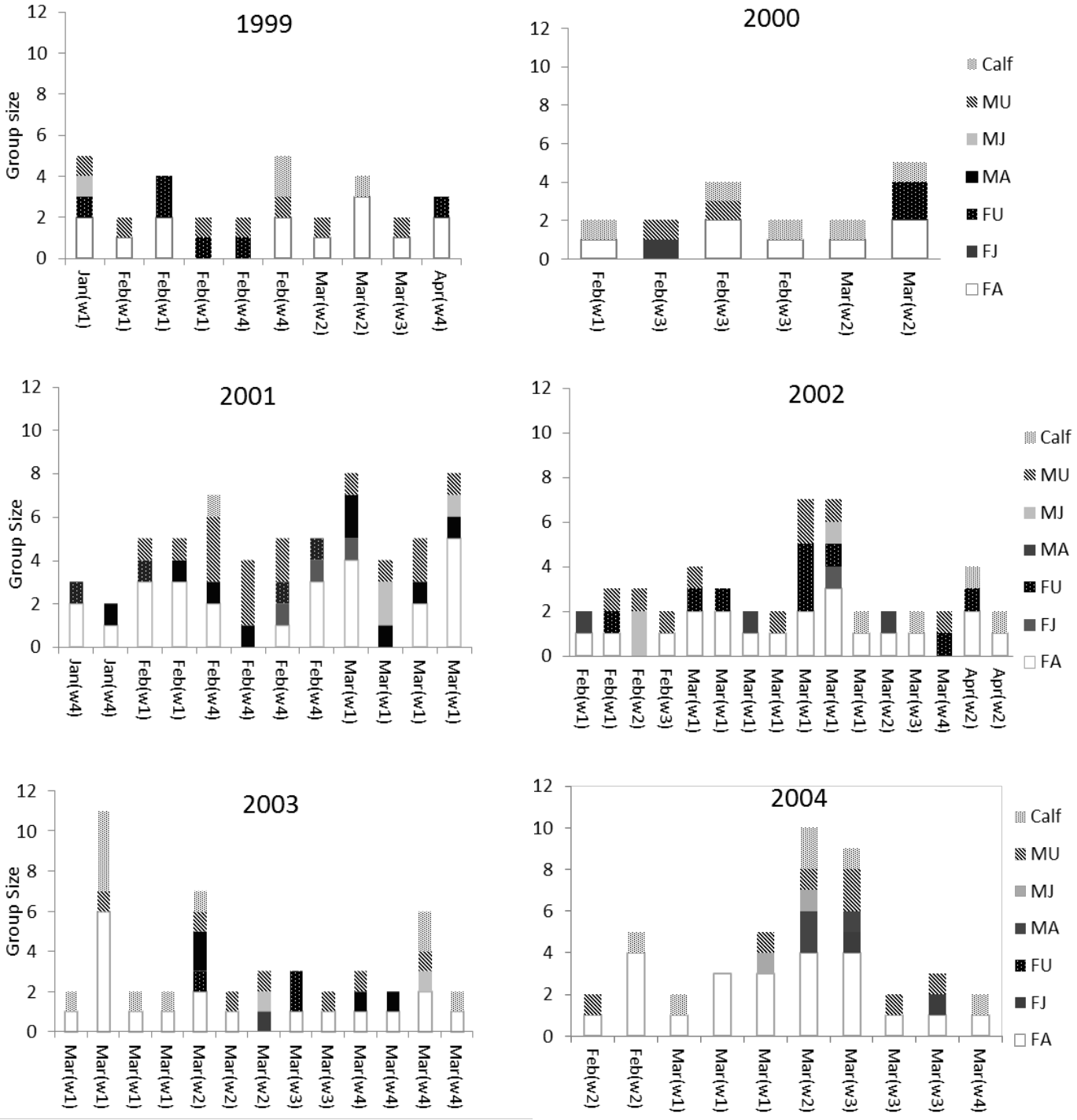


Figure1. Map of the study area.


1999
Group size
Jan(w1)
Feb(w1)
Feb(w1)
Feb(w1)
Feb(w4)
Feb(w4)
Mar(w2)
Mar(w2)
Mar(w3)
Apr(w4)
2000
Feb(w1)
Feb(w3)
Feb(w3)
Feb(w3)
Mar(w2)
Mar(w2)
Calf
MU
MJ
MA
FU
FJ
FA
2001
Group Size
Jan(w4)
Jan(w4)
Feb(w1)
Feb(w1)
Feb(w4)
Feb(w4)
Feb(w4)
Feb(w4)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w1)
2002
Feb(w1)
Feb(w1)
Feb(w2)
Feb(w3)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w2)
Mar(w3)
Mar(w4)
Apr(w2)
Apr(w2)
Calf
MU
MJ
MA
FU
FJ
FA
2003
Group Size
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w2)
Mar(w2)
Mar(w2)
Mar(w3)
Mar(w3)
Mar(w4)
Mar(w4)
Mar(w4)
Mar(w4)
Mar(w4)
2004
Feb(w2)
Feb(w2)
Mar(w1)
Mar(w1)
Mar(w1)
Mar(w2)
Mar(w3)
Mar(w3)
Mar(w3)
Mar(w4)
Calf
MU
MJ
MA
FU
FJ
FA

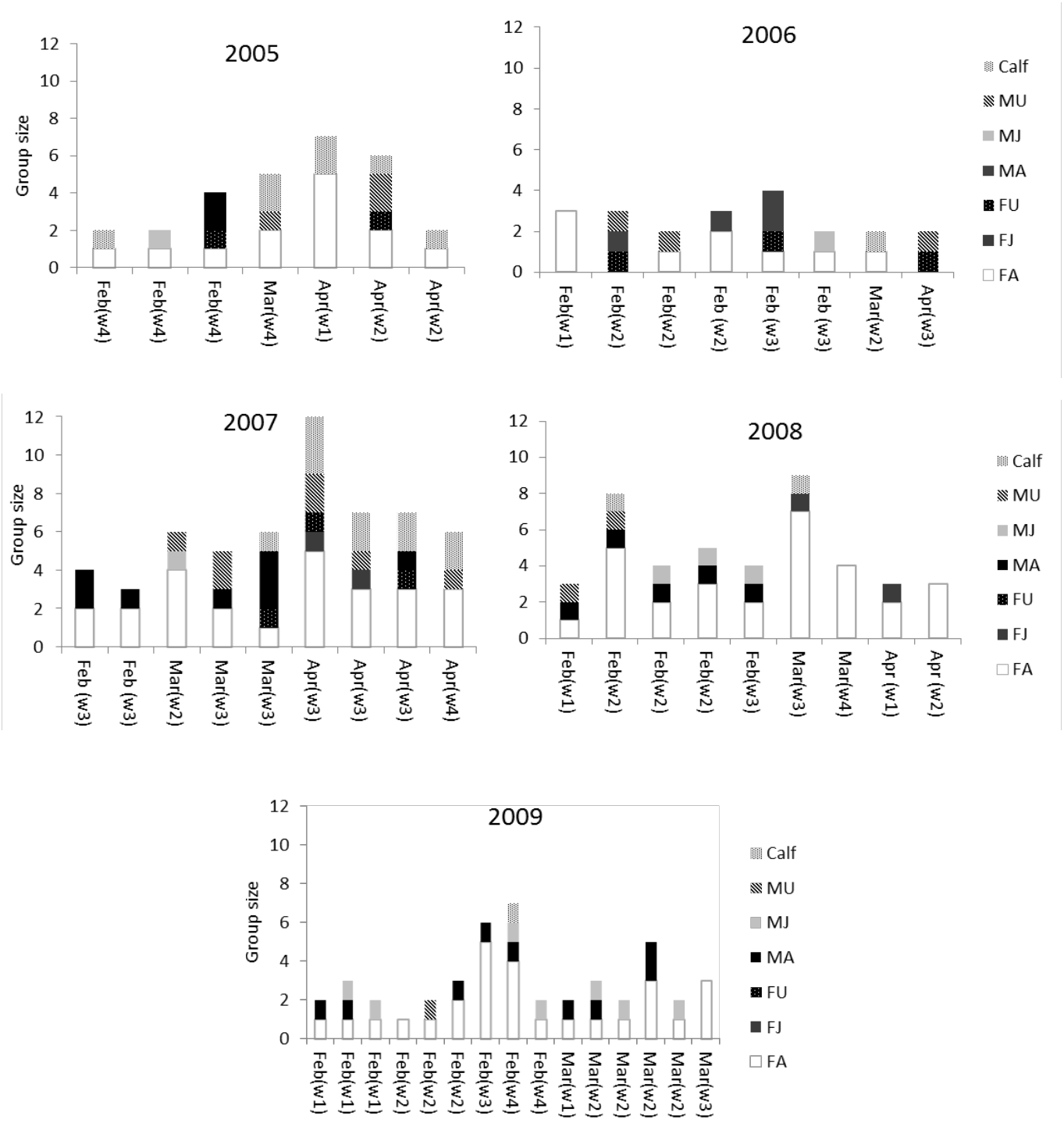


Figure 2.Yearly group size and group composition of the 126 blue whale groups over 1999-2009. FA=Adult female, FJ=Juvenile female, FU= Unknown age female, M=Adult male, MJ=juvenile, MU= Unknown age male. w1,2,3,4= First, second, third and fourth week of the month. Due to the small number of groups in the period 1995-1998 for practical purpose only 1999-2009 period is presented.

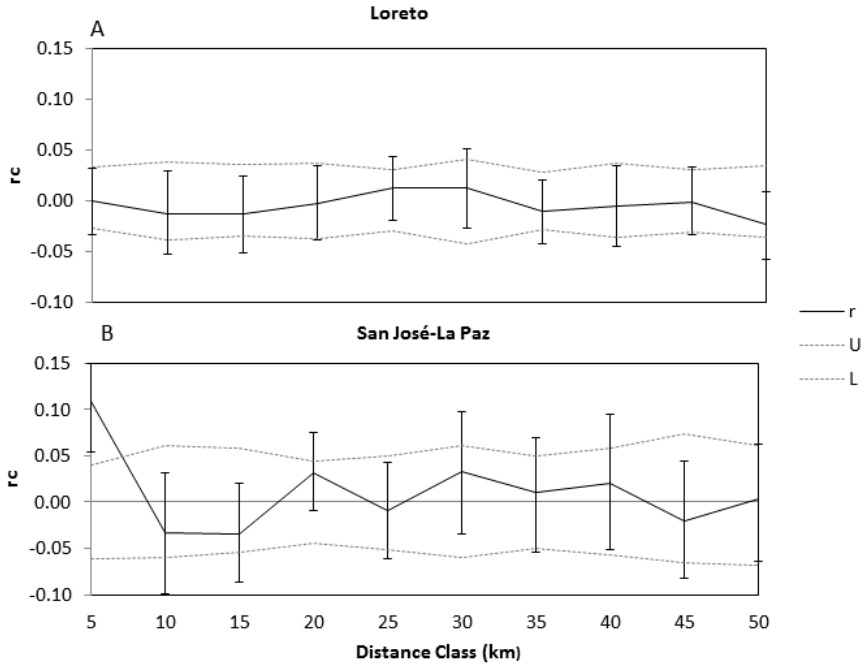


Figure 3. Spatial autocorrelation as a function of genetic distance (r) to distance classes (km). A) and B) shows the fine-scale structure of Loreto and San José-La Paz clusters respectively. U and L correspond to upper and lower limits of the confidence interval at 95% around the null hypothesis of absence of spatial structure (rc= 0). The error bars (95% confidence) around the rc value were generated through Bootstrap re-sampling method.

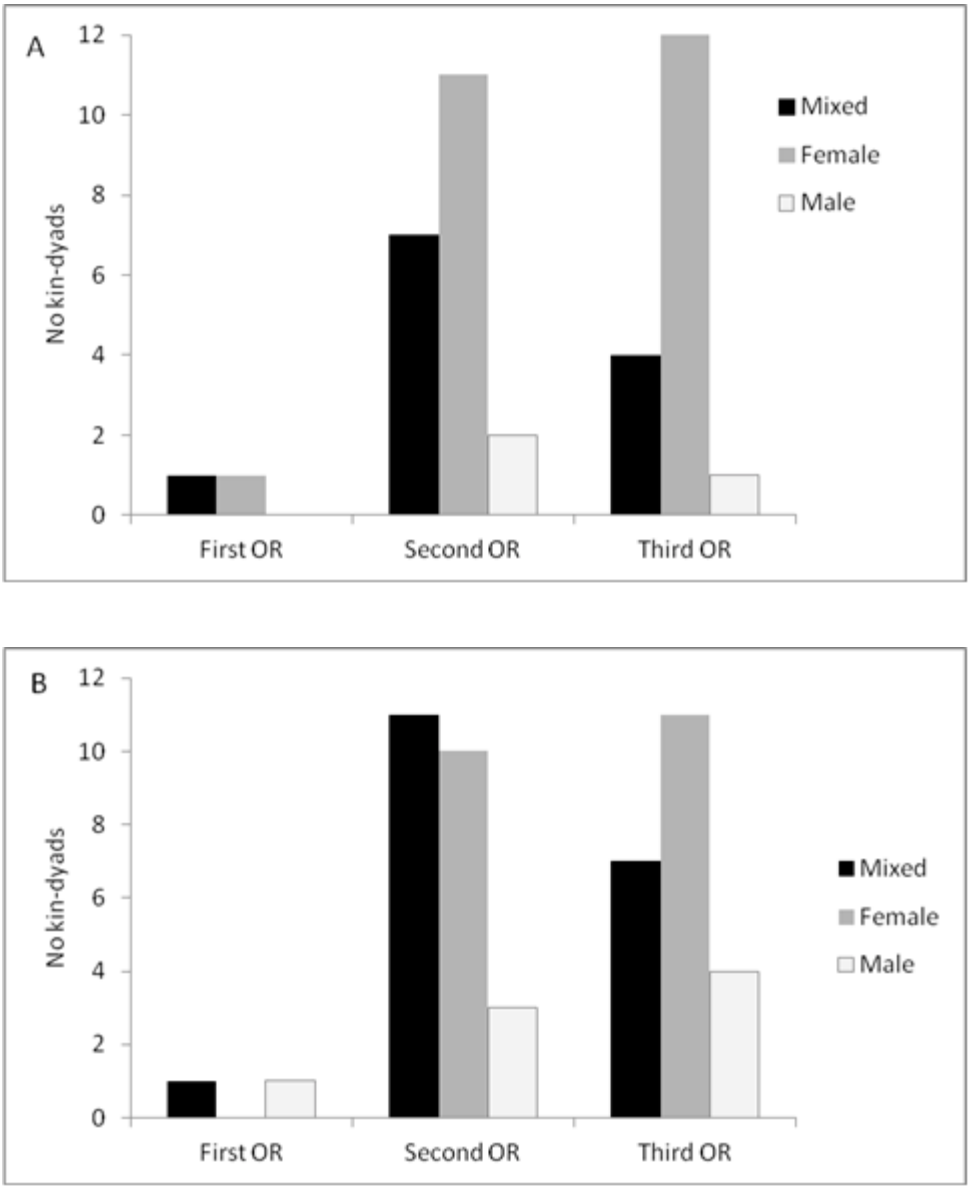


Figure 4. First, second and third order of relatives (OR) kin-dyads, among females, males and mixed kin-dyads within groups with A) mother-calf pairs, B) without mother calf-pairs.

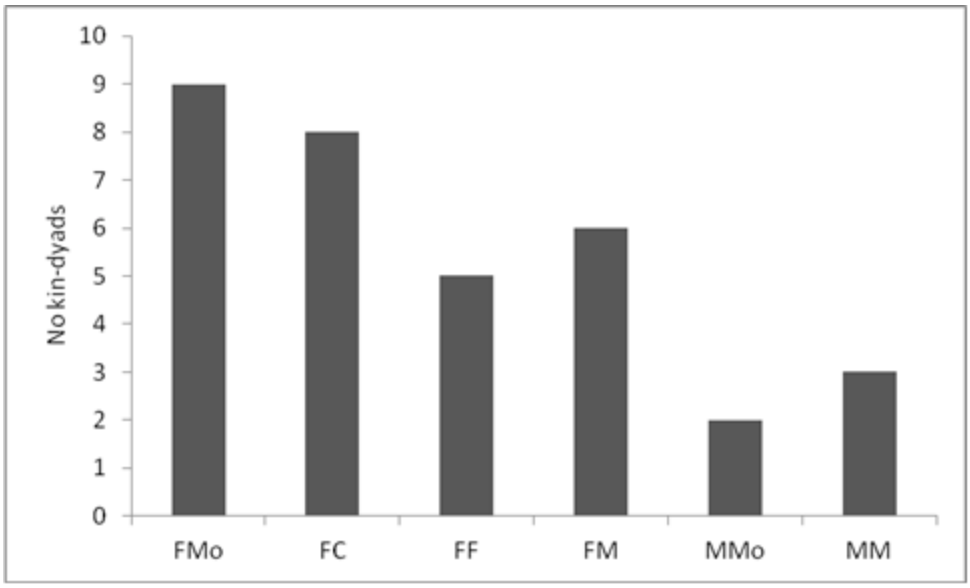


Figure 5. Types of kin-dyads in groups with mother-calf pairs. F= Female; M=Male; C=Calf; Mo=Mother. i.e. FMo=Female-Mother kin dyad.

Table 1. Correlation values from simulated data using the 7 kinship coefficients and the true value (expected kinship values in non-inbreeding population) are shown in the bottom row. Correlation values from empirical data among the 7 kinship coefficient are shown in the rest of the rows. In bold are highlighted the highest values. All $p<0.005$.

	TrioML	Wang	Lynch-Li	Lynch-Rd	Ritland	QG	DyadML	TrueValue
TrioML								
Wang	0.72							
LynchLi	0.71	0.91						
LynchRd	0.80	0.72	0.70					
Ritland	0.71	0.62	0.65	0.91				
QuellerGt	0.73	0.83	0.92	0.75	0.63			
DyadML	**0.97**	0.71	0.90	0.82	0.73	0.73		
TrueValue	**0.73**	0.70	0.68	0.58	0.36	0.70	**0.73**	

Table 2. Percentage of whales observed in different groups.

% of whales	Number of groups
40	1
21	2
6	3
9	4
9	5
3	6
3	7
2	8
2	10
2	11
1	12
1	13
1	14
1	15
1	16

Table 3. GLM results. A) The best model selected by Maximum likelihood criterion. B) Significant variables of GLM with respect to the number of kin-dyads within groups. df =degree of freedom, Score= likelihood sore, SE=Standar Error, L and U CL95%= Lower and Upper 95%confidence limit.

A	Variables			df	Socre	p-value
Group Size	Sexual Proportion	Locality		3	145,2	0,001
B	Level of effect	Estimate	SE	L CL 95%	U CL 95%	p-value
Intercept		-1,40	0,35	-2,10	-0,70	0,001
Group Size		0,26	0,03	0,20	0,32	0,001
Locality	Loreto	-0,28	0,10	-0,48	-0,09	0,004

Table 4. GLM results. A) The best model selected by Maximun likelihood criterion. B) Significant variables of GLM with respect to the presence of kin-dyads that were not mother-calf pairs within groups. df =degree of freedom. Score= likelihood sore, SE=Standar Error, L and U CL95%= Lower and Upper 95%confidence limit. MC= number of mother calf pairs within group.

A	Variables		df	Score	p
	Group Size	MC	2	37,31	0,001
B	Estimate	SE	L CL95%	U CL95%	p
Intercept					
Group Size	3,26	0,59	2,10	4,43	0,001
MC	0,85	0,16	1,18	0,51	0,001

Discusión General

El comportamiento de dispersión juega un papel crítico en múltiples escalas en la ecología animal (Sugg et al. 1996, Storz 1999). Actualmente es aceptado que las diferencias en la distribución de los individuos pueden conducir a heterogeneidad espacial a pequeña escala en una población con distribución continua. Cuando esto ocurre, es posible detectar estructura genética a escala fina (Constasti et al. 2012).

En general el grado de subdivisión genética en especies estructuradas socialmente depende del efecto de la combinación de tres factores principales: el sistema de apareamiento, la dispersión y la estabilidad de los grupos (Storz 1999). Para determinar el rol de la estructura social sobre los cambios microevolutivos es importante determinar el efecto de estos factores en su conjunto y por separado.

En esta sección se proponen dos modelos que intentan explicar la distribución y variabilidad genética de las ballenas azules en el Golfo de California. El primer modelo se relaciona con la distribución de individuos y de la variabilidad genética, mientras el segundo modelo se relaciona con los mecanismos de evolución poblacional y social que pueden haber moldeado la variabilidad genética actual de esta población. Para ello, primero se analizarán brevemente los tres niveles inclusivos de variabilidad genética que fueron abordados en el presente trabajo: i) escala poblacional, ii) escala sub-poblacional, iii) escala social y por último el rol del sistema de apareamiento en estos niveles.

Escala poblacional

Al analizar todos los individuos muestreados de la población de ballena azul del Golfo de California (hembras y machos) la variabilidad genética resultó lo suficientemente homogénea como para considerar un único stock poblacional (Costa-Urrutia et al. 2013).

Escala subpoblacional

Nuestros resultados sugieren que la estructura a nivel sub-poblacional está influenciada por la distribución de las hembras, quienes mostraron un cierto grado de heterogeneidad genética entre dos áreas contiguas. Es probable que uno de los factores más influyentes de esta estructura sea el comportamiento

social de las madres con cría (Costa-Urrutia et al. 2013). Estas pueden hallarse solas o en grupos, sin embargo al integrar grupos, éstos, resultaron ser los de mayor tamaño y poseer mayor nivel de parentesco. Asimismo estos grupos fueron encontrados con mayor frecuencia en el área de San José-La Paz. Esto sugiere que el sesgo en la distribución espacial de dichos pares estaría influenciando la estructura genética a nivel sub-poblacional (Costa-Urrutia et al. 2013).

Nuestros resultados también sugieren que los machos serían los mayores responsables de la homogenización genética a nivel poblacional. Esta sugerencia es apoyada por las siguientes observaciones: i) no se observó divergencia genética al incorporar los machos en el análisis, y ii) machos con edad reproductiva fueron observados en grupos con y sin madres con cría, sugiriendo que tampoco presentan algún sesgo en la distribución espacial.

Esta estructura encontrada coincide con el modelo de Tiedemann et al. (2000), en el cual propone que la estructura poblacional en grandes mamíferos está fuertemente influenciada por la migración de las hembras más que la migración de los machos. En el caso de los misticetos es aceptado que las hembras con crías presentan mayor filopatría en comparación con los machos, sin embargo, se han observado hembras con cría en distintas temporadas en San José-La Paz y Loreto respectivamente. Asimismo se detectaron movimientos de madres con cría entre estas localidades en la misma temporada. Estas hembras se caracterizaron por ser de las hembras más avistadas y con mayor número de crías a lo largo del período de estudio. Esto sugiere un patrón de distribución flexible, al menos para las hembras que han adquirido un mayor conocimiento del área. Sin embargo, es probable que la continuidad de los estudio permitan detectar mayor número y más detalle de movimientos de individuos avistados en menor frecuencia. Estos movimientos entre áreas podrían verse reflejados en el bajo valor de divergencia entre las hembras de ambas localidades.

Escala social

La estructura social de la ballena azul parece caracterizarse por una dinámica de alta fusión-fisión. Esta dinámica se caracteriza por cambios tanto en el tamaño como composición de los grupos, dichos cambios en la conformación de los grupos pueden ser a escala diaria o semanal (Aureli et al. 2008). Esta dinámica podría considerarse como un proceso contínuo de formación de nuevos grupos, lo que inevitablemente produce cambios a nivel microevolutivo, pero que no necesariamente debe verse

reflejado en una marcada divergencia entre grupos (Möller 2012).

Desde el punto de vista genético, la dinámica fusión-fisión podría verse como el proceso de muestreo y re-muestro (con reposición) de las variantes alélicas de los grupos parentales. Dependiendo del parentesco de los individuos de los grupos éstos podrían elevar o disminuir el grado de divergencia genética entre los distintos grupos (Slatkin 1997, Storz 1999). Por ejemplo, en una estructura social donde los grupos se forman a partir de la fisión de grupos cohesivos con alto nivel de parentesco (ej. grupos de orcas, los cuales presentan alta filopatría por parte de ambos sexos), dichos grupos presentarán alto nivel de parentesco. Si las crías de las hembras o las hembras reproductivas no se dispersan, aumentará la divergencia entre los grupos de la población. Por el contrario, si los individuos formadores de nuevos grupos provienen de grupos no emparentados disminuirá la divergencia entre estos. En una dinámica de alta fusión-fisión, el proceso de formación y partición de grupos es particularmente acelerado, lo que disminuye considerablemente la formación de grupos familiares estables con altos niveles de coancestría. En este contexto, es de esperarse que esta dinámica diluya la heterogeneidad genética a nivel poblacional. Esta hipótesis es apoyada por los bajos niveles de parentesco observados dentro de los grupos de ballena azul, que coincide con lo observado en varias especies de delfines las cuales presentan este tipo de dinámica social (Möller 2012).

Sistema de apareamiento

Desde el punto de vista genético el tipo de sistemas de apareamiento predominante en la población refleja, de forma general, qué proporción de genes se trasmitirá de una generación a la siguiente. Estos genes se distribuirán por toda la población y por esto se puede considerar que los sistemas de apareamiento influyen en todos los niveles de variabilidad genética existentes en una población determinada. Por ejemplo, en un sistema de elevada poliginia una pequeña fracción de machos trasmitirá sus genes a la siguiente generación. Para que la poliginia ocurra, un cierto número de machos debe de monopolizar la mayoría de las cópulas y por lo tanto, dejar la mayor parte de la descendencia (Emlen & Oring 1977, Greenwood 1980, Storz 1999). En este contexto el tipo de grupo social que predomine en la población tendrá algún grado de parentesco dado que la mayoría de las cría descenderán del mismo padre. Dependiendo del

grado de parentesco entre las hembras reproductoras del grupo, el parentesco promedio del mismo podría oscilar entre niveles de tercer orden (r~0.125), en el caso de hembras no emparentadas, de segundo orden (r~0.25) si éstas tienen algún grado de parentesco, o inclusive primer orden (r~0.5), si existe una o dos hembras reproductivas emparentadas en el grupo. Nuestros resultados mostraron grupos con promedios de parentesco más bajos que de tercer orden, lo que sugieren que el sistema de apareamiento podría ser promiscuo (o de baja poliginia). Esto significa que las crías descenderán de un mayor número de machos en comparación con un sistema de poliginia elevada. Esto ocasiona un aumento en la variabilidad genética a nivel poblacional y una disminución del nivel de parentesco dentro de los grupos.

De forma complementaria a lo propuesto en el párrafo anterior, existen factores ecológicos y comportamentales que apoyan la hipótesis propuesta. Para que un sistema poligínico pueda establecerse en una población, debería al menos en teoría, existir lo que se denomina el potencial poligínico del medio. Esto, a nivel general, significa que en un ambiente dado deben de existir las condiciones necesarias para monopolizar hembras o los recursos necesarios para garantizar el éxito reproductivo de las mismas (Emlen & Oring 1977).

En los ambientes marinos, existe una elevada fluctuación a nivel espacial y temporal de la distribución de los recursos alimentarios, por lo que ha sido tradicionalmente considerado que la monopolización de recursos por parte de los machos es una estrategia muy poco probable (Clapham 1996). Por esta razón y por el potencial de movimientos que poseen los cetáceos, la monopolización de hembras tampoco parece ser una estrategia probable, al menos para la mayoría de las especies. Esto aunado a la propia dinámica de alta fusión-fisión propuesta en este trabajo disminuiría considerablemente la probabilidad de monopolizar hembras. Este contexto ecológico y comportamental no sustentaría elevados niveles de poliginia para la población de ballenas azules. En este sentido un sistema promiscuo mantendría bajos niveles de parentesco dentro de los grupos, bajos niveles de divergencia entre los mismos y por lo tanto, sería un factor de dilución de subdivisión poblacional. Este sistema de apareamiento concuerda con lo propuesto tanto para otras especies de ballenas como de delfines (Cerchio et al. 2005, Frasier et al. 2007, Carrol et al. 2012, Möller 2012).

El primer modelo sobre distribución de la variabilidad genética se esquematiza en la Figura 1. Este modelo propone que la población de ballenas azules está conformada por niveles inclusivos de variabilidad genética y, a su vez, que la estabilidad de dicha variabilidad disminuye conforme aumenta el nivel de inclusión. El tamaño del guión representa la estabilidad en los distintos niveles.

De afuera hacia adentro, el círculo externo representa la población, indicando la variabilidad genética lo suficientemente homogénea y estable como para englobar a los individuos dentro de un una población discreta. El círculo siguiente (representado por guion largo) muestra la distribución de los machos simbolizando el efecto homogeneizador de la frecuencia alélica a nivel poblacional.

Los círculos negros y grises mostrados por guiones de tamaño intermedio muestran la distribución de las hembras con cría y hembras sin cría, respetivamente, representando heterogeneidad genética y al mismo tiempo flexibilidad en la discontinuidad genética a nivel sub-poblacional (Costa-Urrutia et al 2013). Dicha flexibilidad podría ser el resultado de movimientos de hembras con cría y sin cría entre ambas zonas (Costa-Urrutia et al 2013), como ha sido observado en otros misticetos (Rowntree et al. 2001). Esto podría modificar el nivel de divergencia y los límites de discontinuidad genética a nivel sub poblacional, tanto dentro como entre temporadas.

Los círculos punteados, muestran la subdivisión espacial más interna e inestable dentro de la población, representando la distribución de la variabilidad genética aportada por los grupos con una dinámica de alta fusión-fisión (Artículo 2, Figura 2). En este nivel la distribución de la variabilidad genética es esperable que varíe entre semana o días. De forma general este modelo sugiere que tanto la adaptación local a un entorno particular como la estructura social influyen en la estructura de la población. Ambas características podrían reflejar divergencia reciente o el equilibrio entre diferenciación local por parte de las hembras y flujo génico mediado por machos.

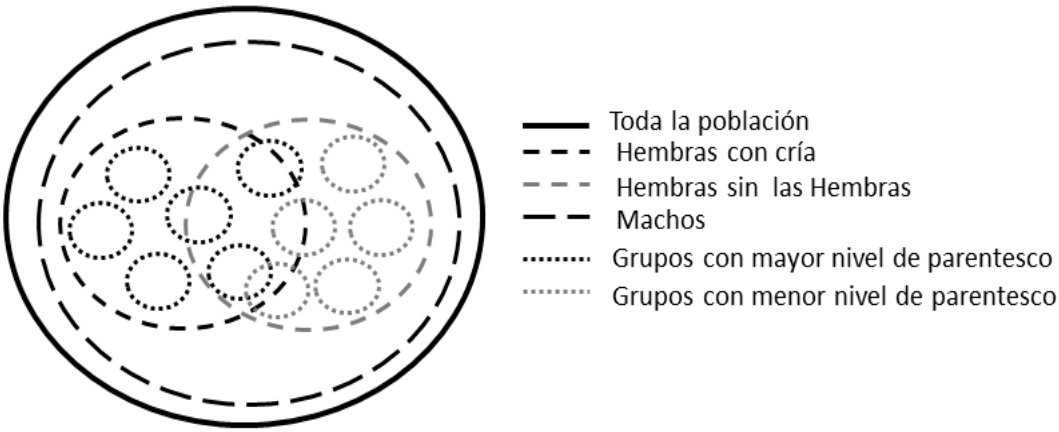


Figura 1. Distribución de la variabilidad genética de las ballenas azules en el Golfo de California.

El segundo modelo es un modelo evolutivo simple para las ballenas azules en el Golfo de California el cual se esquematiza en la Figura 2. Este modelo se basa en la información conjunta obtenida a nivel filogenético con ADN mitocondrial (Enríquez-Paredes 2005) y a nivel poblacional y social obtenida con microsatélites (Costa-Urrutia et al. 2103, Artículo 2). Estos estudios en su conjunto permiten proponer un modelo de evolución de la distribución de variabilidad genética representada en distintas escalas temporales y espaciales. La escala a nivel temporal puede verse reflejada por el uso de distintos marcadores moleculares. Dadas las diferencias en las tasas de mutación de los marcadores mitocondriales con respecto a los microsatélites, estos dos marcadores reflejan distintos tiempos evolutivos: 10^6 y 10^3 millones de años, respectivamente (Avise 2000).

Los análisis a nivel filogenético indican la presencia de dos clados maternos. El clado I agrupa la mayor parte de los linajes comunes, representando cerca del 80% de la muestras. El clado II agrupa uno de los linajes comunes y varios de baja frecuencia representando el 16% de los individuos analizados. Esto generó la hipótesis que el linaje del clado I posee carácter ancestral con la subsecuente incorporación de linajes pertenecientes del clado II. La información en su conjunto llevó al autor a sugerir un escenario evolutivo de flujo génico recurrente para las agregaciones mexicanas de ballena azul (Enríquez-Paredes 2005). No se observó segregación temporal o espacial por parte de los grupos mitocondriales, por lo que fue

propuesto que estos caldos se mezclaron una vez que colonizaron el Pacífico Nororiental y comenzaron a usar el Golfo de California. Esto sugiere que los grupos de linajes maternos no representan poblaciones discretas en un tiempo evolutivo reciente (Enríquez-Paredes 2005). Esta hipótesis es apoyada por el hecho de que los clústers espaciales de hembras hallados con microsatélites no presentan correspondencia con los clados mitocondriales. Por lo que es probable que los clados maternos representan huellas ancestrales de la probable conformación poblacional, pero que efectivamente no funcionan como poblaciones discretas en un escenario evolutivo reciente.

En este punto es válido preguntarse cuáles fueron los factores que motivaron la mezcla de los clados y la distribución actual de la variabilidad genética. Es probable que la estructura social haya jugado un papel importante en la mezcla de linajes maternos y al mismo tiempo en mantener bajos niveles de endogamia en la población. Más arriba fue propuesto que la dinámica de alta fusión-fisión diluye los niveles de co-ancestría en comparación a los que podría presentar una población con un sistema social caracterizado por grupos familiares estables, o sea una dinámica de baja fusión-fission. Esto es particularmente apoyado por el hecho que los grupos fueron conformados por linajes maternos de ambos clados (datos no mostrados). Esto nos permite situarnos en el siguiente escenario teórico para proponer un modelo de evolución social y asimismo cómo este influyó en los niveles actuales de diversidad genética.

Supongamos que los nuevos grupos se habrían formado a partir del muestro aleatorio de ambos grupos de linajes maternos. Esta conformación de nuevos grupos conduciría a la reducción de la endogamia a nivel poblacional y al aumento de variación genética dentro de cada grupo. Extendiendo este escenario a una constante dinámica de formación y partición de grupos por fusión-fisión, resultaría en elevados niveles de heterocigosidad dentro de los mismos. En este contexto, una población socialmente estructurada por grupos con altos niveles de heterocigosidad, conducirá potencialmente a una reducción de la endogamia, contrarrestando así los efectos de la deriva génica en una población pequeña como es el caso de las ballenas azules en el Golfo de California. Esta dinámica poblacional podría constituir una ventaja adaptativa para responder a la pérdida de variabilidad genética que puede haber sufrido la población debido a la intensa captura comercial desde el siglo XIX.

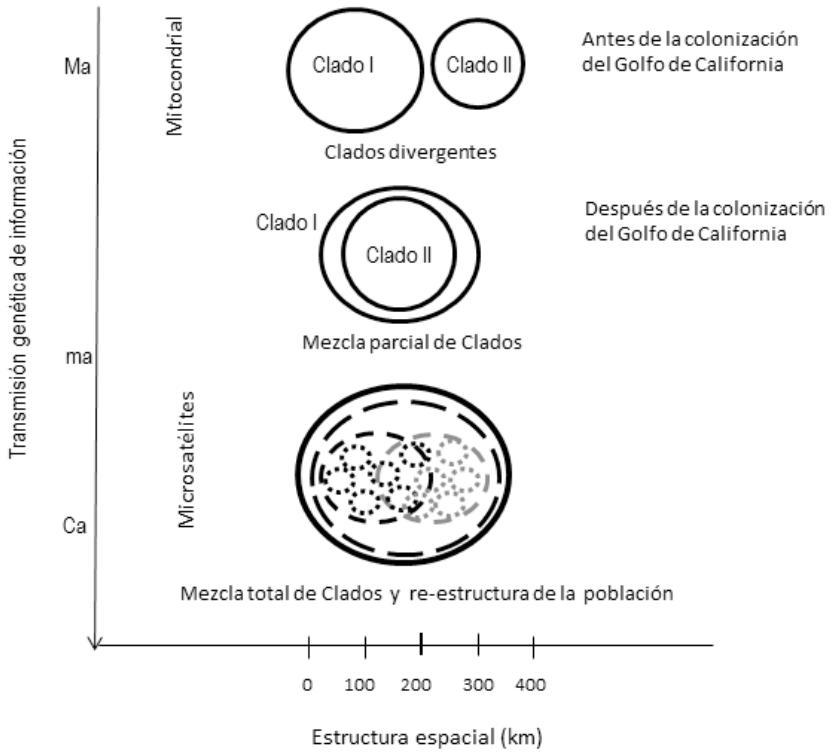


Figura 2. Modelo evolutivo de las ballenas azules en el Golfo de California. Ma=millones de años, ma=miles de años, Ca=cientos de años. Clado I y II representan los clados mitocondriales. Los círculos en el nivel inferior de la figura se explican en la Figura 1. La escala de la estructura espacial simplemente representa la escala a la cual la estructura de hembras fue observada y sólo corresponde para el periodo después de la colonización del Golfo de California.

Referencias

Archie EA, Maldonado JE Hollister, Smith JA, Poole JH, Moss CJ, Fleischer RC, Alberts SC. 2008. Fine-scale population genetic structure in a fission–fusion society. Molecular Ecology. 17:2666–2679.

Avise JC, 2000. Phylogeography: The History and Formation of Species. Harvard University Press (Ed). 447pp

Best PB, Payne R Rowntree V, Palazzo JT, Both MD. 1993. Long-range movements of South Atlantic right whales, *Eubalaena australis*. Marine Mammal Science. 9: 227-34.

Carroll EL, Childerhouse SJ, Christie M, Lavery S, Patenaude N, Alexander A, Constantine R, Steel D, Boren L, Baker CS. 2012. Paternity assignment and demographic closure in the New Zealand southern right whale. Molecular Ecology . 21: 3960-3973.

Cerchio S, Jacobsen JK, Danielle, Cholewiak DM, Falcone EA, Merriwether DA. 2005. Paternity in humpbackwhales, *Megaptera novaeangliae*: assessing polygyny and skew in male reproductive success. Animal Behaviour. 70:267–277.

Clapham PJ.1996. The social and reproductive biology of the humpback whale: An ecology perspective. Mammal Review. 26:27-49.

Coltman DW, Pilkington JG, Pemberton JM. 2003. Fine-scale population genetic structure in a free-living ungulate population. Molecular Ecology. 12:733-742.

Connor RC, Wells RS, Mann J, Read AJ. 2000. The bottlenose dolphin: social relationships in a fission-fusion society. Capítulo 4. Cetacean Societies: Field Studies of Dolphins and Whales. Mann J, Connor RC, Tyack PL, Whitehead H (Eds). 433pp.

Contasti AL, Tissier EJ, Johnstone JF, McLoughlin PD. 2012. Explaining spatial heterogeneity in population dynamics and genetics from spatial variation in resources for a large herbivore. Plos One. 7: e47858.

Costa-Urrutia P, Sanvito S, Victoria-Cota N, Enriquez-Predes L Gendron D. 2013. Fine scale population structure of blue whale, *Balaenoptera muslucus*, in the Gulf of California. Plos One. 8(3): e58315.

doi:10.1371.

Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating system. Science. 197:215-223.

Enríquez-Paredes L. 2005. Identidad genética de la boblación de ballena azul, *Balenoptera musculus*, en el Pacifico Nororiental: agregaciones mexicanas. Tesis de Doctorado. UABC. 198pp.

Frasier TR, Hamilton PK, Brown MW, Conger LA, Knowlton AR, Marx MK, Slay CK, Kraus SD, White BN. 2007. Patterns of male reproductive success in a highly promiscuous whale species: the endangered North Atlantic right whale. Molecular Ecology.16:5277-5293.

Greenwood PJ. 1980. Mating system, philopatry and dispersal in birds and mammals. Journal of Animal Behaviour. 28:1140-1162.

Mirimin L, Miller R, Dillane1 E, Berrow SD, Ingram S, Cross1 T, Rogan E. 2010. Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters. Animal Conservation. Doi:10.1111/j.1469-1.

Möller LM. 2012. Sociogenetic structure, kin associations and bonding in delphinids**.** Molecular Ecology. 21:745-764.

Nussey DH, Coltman DW, Coulson TN , Kruuk LEB,. Donald A, Morris SJ, Clutton-Brock TH, Pemberton J. 2005. Rapidly declining fine-scale spatial genetic structure in female red deer. Molecular Ecology. 14: 3395-3405.

Slatkin 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theoretical Population Biology. 12:253-262.

Storz J. 1999. Genetic consequences of mammalian social structure. Journal of Mammalogy. 80:553-569.

Sugg DWCR, Dobson FS, Hooglang JL. 1996. Population genetics meets behavioral ecology. Trends in Ecology and Evolution. 11:338-342.

Tiedemann R, Hardy O, Vekemans X, Milenkovitch M. 2000. Higher impact of female than male migration on population structure in large mammals. Molelular Ecolology. 9:1159-1163.