Universidad de Huelva

Departamento de Biología Ambiental y Salud Pública

Ontogenia del comportamiento del lince ibérico (*Lynx pardinus*) en cautividad

Memoria para optar al grado de doctor
presentada por:

Francisco Javier Yerga Rufo

Fecha de lectura: 15 de enero de 2016

Bajo la dirección de los doctores:

Javier Calzada Samperio

Xavier Manteca Vilanova

Huelva, 2016

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RESUMEN

El lince ibérico es una de las especies de mamífero más amenazada del mundo. Su cría en cautividad es una de las medidas puestas en marcha para la conservación de esta especie. Esta Tesis Doctoral tiene como objetivo describir la ontogenia física y del comportamiento de los cachorros de lince ibérico nacidos en cautividad, y conocer los factores que van a influir en su desarrollo, para mejorar el manejo de la especie en cautividad. Se realizó el seguimiento del comportamiento de los cachorros de lince ibérico nacidos en los centros de cría "El Acebuche" (Huelva, España) y "La Olivilla" (Jaén, España) entre los años 2005 y 2013, mediante un sistema de video vigilancia remota que permitía obtener los registros del comportamiento evitando las alteraciones derivadas de la presencia del observador cerca de los animales.

El lince ibérico es una especie semi-altricial. Los cachorros nacieron en el interior de las parideras y no eran capaces de caminar, su desarrollo sensorial era relativamente atrasado y dependían de la madre para alimentarse. Durante el primer mes de vida, se produjo la maduración sensorial y motora, lo que permitió a los cachorros abandonar la paridera, propiciando los principales cambios en el comportamiento. El crecimiento de los cachorros se ajustó a la función de von Bertalanffy. Los machos crecieron más rápido que las hembras, de forma que eran un 8% más grandes de adultos; mientras que el método de crianza (criados con sus madres/crianza artificial) modificó la tasa de crecimiento, pero no el tamaño de adulto. La lactancia ocupó gran parte del tiempo de los cachorros durante las primeras semanas de vida, pero decreció rápidamente conforme crecieron. El destete, periodo en el que se alimentan tanto de comida sólida como de leche materna, ocurrió durante el último tercio del total del periodo lactante, por lo que el papel principal de la lactancia en el lince ibérico parece ser la nutrición de los cachorros. Una vez fuera de la paridera, la actividad diaria se incrementó hasta el tercer mes de vida, a partir del cual permaneció constante en torno al 50%. El patrón circadiano bimodal con máximos en los crepúsculos surgió en el segundo mes de vida y quedó completamente establecido a partir del tercer mes.

El incremento en la actividad diaria vino acompañado de la aparición de nuevos comportamientos como el juego y la depredación. Se pudieron observar cuatro categorías de juego en los cachorros de lince ibérico. El juego social fue el primero en aparecer al mes de vida y ocupó la mayor parte del tiempo de juego de las crías. Los

juegos de tipo locomotor se vieron por primera vez a los 55 días, los juegos con objetos a los 59 días y los juegos con la presa a los 61 días. El juego alcanzó su máxima prevalencia entre las semanas 11 y 14, descendiendo posteriormente, aunque sin desaparecer por completo al final del estudio. Por su parte, el proceso de aprendizaje de la caza comenzó al mes de vida, cuando la madre les llevó una presa a sus cachorros por primera vez. Las primeras etapas del aprendizaje dependieron de la iniciativa materna, que les facilitó la tarea de ingestión y captura de la presa, hasta que las crías eran capaces de cazar sin ayuda a las 15 semanas. La secuencia básica del etograma de la depredación estuvo constituida por la búsqueda, el ataque, la captura, el mordisco, el traslado de la presa y la ingesta; viéndose alterada en ocasiones por juegos o peleas. Durante el ataque, los cachorros emplearon cuatro técnicas de caza, en orden de prevalencia: acoso, rececho, acecho y persecución. El éxito total de captura fue del 53%.

A partir de los resultados obtenidos se estima que la duración de las cuatro etapas del desarrollo en especies altriciales para el lince ibérico es: periodo neonatal (semanas 0 a 3); periodo de transición (semanas 4 y 5), periodo de socialización (semanas 6 a 15) y periodo juvenil (semana 16 hasta la maduración sexual).

SUMMARY

The Iberian lynx is one of the most endangered mammal species in the world. The captive breeding is one of the main measures implemented for the conservation of this species. This thesis aims to describe the physical and behavioral ontogeny of Iberian lynx cubs born in captivity and to know the factors that may influence its development, in order to improve the husbandry of the species in captivity. It was tracked the behavior of the Iberian lynx cubs born in the Iberian Lynx Breeding Centers of "El Acebuche" (Huelva, Spain) and "La Olivilla" (Jaen, Spain) between 2005 and 2013, by a video surveillance system, that allowed the remote data collection, avoiding the disturbances arising from the presence of the observer close to the animals.

The Iberian lynx is a semi-altricial species. The cubs were born inside a den and were not able to walk, its sensory development was relatively backward and they were dependent on the mother for feeding. During the first month of life, there was the sensory and motor maturation, allowing the cubs to leave the den, leading to the major changes in behavior. The growth of the cubs fitted to von Bertalanffy function. Males grew faster than females, so they were an 8% larger in adulthood; whereas the rearing method (mother vs hand-reared) modified the growth rate, but not the adult body mass. The lactation occupied much of the time of the cubs during the first weeks of life, but it decreased rapidly as grew. The weaning period, in which the cubs feed both on solid food and on milk, occurred during the last third of lactation period, so the main role of lactation in the Iberian lynx seems to be the nutrition of the cubs. Once out of the den, daily activity increased until the third month of life, after which it remained constant at around 50%. The bimodal circadian pattern with peaks in the twilights emerged in the second month of life and was fully established after the third month.

The increase in daily activity was accompanied by the emergence of new behaviors like play and predation. It was observed four categories of play in the Iberian lynx cubs. The social play was the first in appear at one month old and occupied most of the playtime of the cubs. The locomotor play was observed for the first time at 55 days, the object play at 59 days and the predatory play at 61 days. The play behavior reached its highest prevalence between weeks 11 and 14. Then decreased, but it did not disappear completely at seven months old. Meanwhile, the process of learning to hunt began when the cubs were one month old and the mother took them a prey for the first time. The early stages of learning depended on maternal initiative, which facilitated the tasks of ingestion and capture the prey, until the cubs were able to hunt without help at 15 weeks. The basic sequence of predatory ethogram consisted on search,

attack, capture, bite, move the prey and eat; and sometimes was disrupted by play bouts or fights. During the attack, the cubs used four hunting techniques, in order of prevalence: harassment, ambushing, stalking and persecution. The hunting success was 53%.

According to the results of the thesis, it is estimated that the duration of the four periods of development in altricial species for the Iberian lynx is: neonatal period (weeks 1-3); transition period (weeks 4 and 5), socialization period (weeks 6-15) and juvenile period (week 16 to sexual maturation).

INTRODUCCIÓN

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1.- Justificación-Estructura de la Tesis Doctoral

La diversidad de vida en la Tierra se encuentra en un periodo de crisis sin precedentes. Nuestro entorno se modifica de forma intensa a un ritmo muy elevado. La pérdida de hábitat, la fragmentación de las poblaciones, la introducción de especies exóticas, la sobreexplotación de los recursos y el cambio climático están haciendo que muchas poblaciones se reduzcan y se extingan las especies silvestres de tal modo, que se está modificando el equilibrio de los ecosistemas y se están perdiendo los recursos que sostienen a la humanidad (Chapin et al., 2000; Balvanera et al., 2006; Cardinale et al., 2012). La velocidad a la que ocurren estos cambios es tan alta que muchos hablan de que estamos sumidos en una sexta extinción masiva (Barnosky et al., 2011). Desde el siglo XIX, la desaparición de especies se ha incrementado. Los datos apuntan a que la velocidad de extinción de especies es de 100 a 1000 veces superior a la tasa normal (De Vos et al., 2015). Por ejemplo, según recoge la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza (UICN), el 13% de las aves, el 26% de los mamíferos, el 41% de los anfibios y el 33% de los corales están en peligro de desaparecer (Hoffmann et al., 2010; IUCN, 2015).

En respuesta a esta situación, y debido a la preocupación de muchos biólogos por la pérdida de hábitat y la desaparición de especies, surgió hace unas cuatro décadas una nueva disciplina del conocimiento: la Biología de la Conservación. Los biólogos de la conservación buscan mantener tres aspectos importantes de la vida en la Tierra: la diversidad biológica; la composición, estructura y funcionamiento de los ecosistemas; y su resiliencia y capacidad de persistir en el tiempo (Callicott et al. 1999). De esta forma, se trata de aplicar los conocimientos generados en estudios científicos para llevar a cabo programas de protección y restauración de la naturaleza, así como apoyar el desarrollo de políticas de conservación del medio ambiente (Soulé & Wilcox, 1980).

La biología de la conservación aborda la temática desde una perspectiva multidisciplinar, combinando principios de la genética, la ecología o la biología de las poblaciones, además de conceptos pertenecientes a las ciencias sociales, en relación a la interacción del hombre y su sociedad con el entorno que lo rodea (Groom et al., 2006). Más recientemente, su ámbito se ha ampliado para dar cabida a nuevos puntos de vista que puedan ayudar a la consecución de sus objetivos. Una de estas materias que tratan de acercarse a los principios de la conservación es la etología. Estas dos disciplinas han estado tradicionalmente separadas, como se evidencia en la dificultad que existía para encontrar estudios que aplicasen el conocimiento del comportamiento animal a la conservación de las especies (Gosling & Sutherland, 2000). Por ejemplo, sólo 9 de las 577 comunicaciones presentadas al International Ethological Conference de 1997 mostraban interés en la resolución de problemas de conservación (Sutherland, 1998). A pesar de esto, a mediados de la década de los 90, ya había comenzado a vislumbrarse un cambio de tendencia. Diversos autores pusieron de manifiesto la necesidad de aunar las dos disciplinas y el potencial que las investigaciones sobre el comportamiento pueden tener en la conservación de las especies (Kleiman, 1992; Clemmons & Buchholz, 1997; Linklater, 2004; Berger-Tal et al., 2011). Con el tiempo, se han identificado numerosas áreas en las que el estudio del comportamiento de una especie, puede contribuir a la planificación de las medidas de gestión y conservación. Por ejemplo, estudiar el sistema de reproducción de una especie ayuda a estimar si la endogamia puede llegar a suponer un problema y cuál sería la mejor forma de gestionar sus poblaciones para evitarlo (Caro, 2007).

Muchas de las primeras conexiones entre la conservación y el comportamiento surgieron en instituciones zoológicas y centros de cría (Caro, 2007; Miller et al., 2013). Uno de las medidas más habituales en conservación de las especies es su cría en cautividad. Para que estas actuaciones tengan éxito, es necesario conocer con profundidad la biología y la etología de las especies, para así conseguir una mejor adaptación a la cautividad y que la reproducción sea un éxito. Por ejemplo, se ha estudiado que el tamaño del área de campeo de los carnívoros está relacionado con el desarrollo de comportamientos estereotípicos que, a su vez, afectan a la reproducción en cautividad (Moberg, 2000; Shepherdson et al., 2004). Para algunas especies, como el oso polar (*Ursus maritimus)*, cuyos territorios son extraordinariamente grandes, no se recomienda la cría en cautividad como medida de conservación, de forma que se pueden dirigir los esfuerzos hacia otras actuaciones con mayor perspectiva de éxito (Clubb & Mason, 2003).

El marco teórico de la etología se articula alrededor de las cuatro preguntas de Tinbergen (Tinbergen, 1963): (1) Causa - ¿Cuáles son los mecanismos que desencadenan la conducta?; (2) Función - ¿Cuál es el valor adaptativo de la conducta para la supervivencia?; (3) Ontogenia - ¿Cómo se desarrolla la conducta durante la vida del animal?; y (4) Filogenia - ¿Cómo ha evolucionado la conducta? De estas ramas de la etología, la ontogenia se ha desvelado como una parte fundamental en el estudio del comportamiento de las especies cautivas. El mantenimiento de animales en cautividad puede tener consecuencias indeseadas para su comportamiento (Wallace, 2000; McDougall et al., 2006). Por ejemplo, es frecuente que la cría de aves en cautividad se realice mediante crianza artificial. Se comprobó que aquellos pollos criados por humanos sufren un proceso de impronta que más adelante provoca anomalías en el desarrollo de comportamientos sociales con sus conespecíficos, dificultando la reproducción. El uso de marionetas o disfraces en las tareas de crianza como la alimentación disminuyen la impronta con los humanos (Valutis & Marzluff, 1999; Kreger et al., 2004). Otro ejemplo de la importancia del estudio de la ontogenia para la cría en cautividad y la conservación lo encontramos en Vargas & Anderson (1996). Estos investigadores mantuvieron ejemplares de turón de patas negras (*Mustela nigripes*; una especie de mustélido americano muy amenazado) en cautividad para su cría y posterior reintroducción. En sus investigaciones mostraron que la preferencia de presa en las crías no es innata, si no que se adquiere por exposición durante un periodo crítico del desarrollo. De esta forma, para incrementar las probabilidades de éxito de la reintroducción, las crías debían tener contacto con la presa más habitual de la especie durante este periodo crítico. Como consecuencia de estos resultados, se realizaron cambios en el manejo de los animales para que tuvieran acceso a perritos de las praderas (*Cynomis spp*), su presa principal, durante su desarrollo y hasta su suelta (Vargas & Anderson, 1996). En definitiva, el estudio de la ontogenia de las especies aporta una serie de conocimientos que pueden contribuir a las actuaciones de conservación de las especies y, muy concretamente, son especialmente útiles en la cría de ejemplares en cautividad para reintroducción, ya que permiten ajustar el manejo con el objetivo de compensar los efectos negativos de la cautividad sobre el comportamiento natural y preparar a los ejemplares de forma adecuada para formar parte de proyectos de conservación.

Este trabajo de Tesis Doctoral trata de estudiar la ontogenia del comportamiento del lince ibérico (*Lynx pardinus)*, criado en cautividad. Esta especie se encuentra en peligro de extinción y su cría en cautividad es una de las medidas puestas en marcha para luchar contra la desaparición de la especie. Se pretende conocer factores claves de su desarrollo comportamental que sirvan para afinar el manejo de estos animales en cautividad y para que su futura reintroducción en la naturaleza sea lo más efectiva posible, y con ello se puedan crear nuevas poblaciones de linces en libertad, paliando la delicada situación de la especie.

La presente memoria se estructura con una primera sección a modo de **Introducción**. En esta sección se presenta y justifica el proyecto; se reseñan los principios generales de la ontogenia del comportamiento; se describen los aspectos principales de la biología, de la ecología, del comportamiento y del estado de conservación del lince ibérico; se explica, de forma general, la metodología empleada durante el estudio (instalaciones, protocolo de manejo, métodos de muestreo y análisis estadístico); y, por último, se presentan los objetivos de la Tesis.

Los Capítulos I-V comprenden el cuerpo principal de la Tesis. El **Capítulo I** trata sobre el desarrollo físico y las curvas de crecimiento. En el **Capítulo II** se describe el comportamiento lactante de los cachorros. El **Capítulo III** aborda la ontogenia de la actividad diaria y los ritmos circadianos. La ontogenia del comportamiento de juego se trata en el **Capítulo IV**. Por último, en el **Capítulo V** se describe la ontogenia del comportamiento depredador. Cada uno de estos capítulos se articula alrededor de un resumen, una breve introducción, una metodología específica, unos resultados y su discusión.

Para finalizar, se presenta una **Discusión general** de los resultados obtenidos en los capítulos anteriores y una última sección que resume las **Conclusiones** del proyecto de Tesis Doctoral.

2.- Ontogenia del comportamiento

2.1.- Ontogenia del comportamiento: mecanismos generadores del cambio

Todos los organismos existentes se encuentran sometidos a un continuo cambio desde su concepción hasta su muerte. La ontogenia trata de describir y estudiar qué cambios se producen a lo largo de la vida de un individuo, cuáles son los mecanismos que los desencadenan y qué factores influyen en este proceso. En el caso de los animales, estos cambios se producen principalmente a tres niveles: fisiológico, morfológico y comportamental.

A nivel fisiológico, los cambios más evidentes están relacionados con la secreción de hormonas (Bernstein et al., 2008). Por ejemplo, cuando un individuo alcanza la pubertad, se produce la activación de las gónadas y la secreción de las hormonas sexuales. Estas hormonas van a desencadenar una serie de modificaciones en el organismo, diferentes en función del sexo (Breedlove, 1992; Drickamer & Vessey, 1992), como la aparición de caracteres sexuales secundarios o la intensificación de la territorialidad y la competencia por el sexo opuesto (Nelson, 2005).

La magnitud de los cambios morfológicos es muy variada en la naturaleza. En algunas especies, como la mayoría de los vertebrados, los recién nacidos son morfológicamente muy similares a los adultos y los cambios que experimentan están más relacionados con un incremento del tamaño (Colmenares & Gómez, 1994). Sin embargo, en especies con fases larvarias, como los anfibios, los cambios de forma que sufre un individuo a través de las diferentes etapas que atraviesa son muy drásticos (Duellman & Trueb, 1994).

Por último, el tercer nivel de cambios se produce en el comportamiento del individuo. El comportamiento presenta una gran flexibilidad y capacidad de adaptación al entorno (Dugatkin, 2009). Estos cambios ocurren desde la etapa prenatal (Bekoff, 1981; Oppenheim & Haverkamp, 1986), aunque es en el periodo postnatal que transcurre desde el nacimiento hasta la madurez sexual, cuando se producen la mayoría de las modificaciones (Immelmann, 1980). De esta forma, el comportamiento de las crías es muy diferente al de los adultos. Se han sugerido dos motivos para explicar estas diferencias. Por un lado, los individuos jóvenes están sometidos a una presión selectiva diferente a la de los adultos (Bateson, 1981). Por ejemplo, el desarrollo de un comportamiento lactante adecuado en mamíferos o la adaptación a hábitats acuáticos en la fase larvaria de los anfibios. Por otra parte, muchos comportamientos de adultos necesitan de un periodo de aprendizaje y maduración durante el periodo postnatal (Vincent & Bekoff, 1978; Seyfarth & Cheney, 1980; Chivers et al., 1996).

Tradicionalmente, se ha considerado que la causa última de los cambios en el comportamiento durante la vida de un organismo se basa en el concepto dicotómico de innato/aprendido. Los comportamientos innatos serían aquellos programados en los genes, inamovibles e independiente de los factores ambientales (Staddon, 1983). Por su parte, los comportamientos aprendidos serían aquellos que resultan de la experiencia, el aprendizaje y la interacción del individuo con su entorno (Staddon, 1983). En la actualidad, se ha demostrado que el comportamiento es el resultado de la interacción entre la información codificada en los genes y la exposición a los factores ambientales. Un ejemplo de esta interacción es la epigenética, una rama reciente de la genética que estudia los factores no genéticos que intervienen en la ontogenia y en la regulación de la expresión genética (Berger et al., 2009). Se ha comprobado que las crías de rata que han sido acicaladas con mayor frecuencia por la madre durante las primeras etapas del desarrollo responden de manera más ajustada a las situaciones de estrés. Esto se debe a que el lamido de la madre desencadena la expresión de unos genes que actúan en la regulación de esta respuesta (Meaney, 2001).

En la práctica, existen tres mecanismos fundamentales que van a caracterizar el proceso de la ontogenia del comportamiento: los genes, los procesos de maduración y el aprendizaje. La cuestión de cómo los genes afectan al desarrollo del comportamiento ha sido uno de los focos principales de la etología. Los genes contribuyen a que el desarrollo del comportamiento siga unos patrones preorganizados específicos para cada categoría taxonómica (Colmenares & Gómez, 1994). La información contenida en el genoma va a permanecer invariable durante toda la vida del individuo. Aunque es difícil cuantificar el papel de los genes en el comportamiento (Gottlieb, 1998; Whalstein, 1999), sí se ha podido estimar, mediante el concepto de heredabilidad, cuál es su influencia en la variabilidad de un determinado rasgo de comportamiento. Este concepto refleja una estimación de la variación de un comportamiento que se debe a la herencia genética, frente a la fracción debida a factores ambientales (Wray & Visscher, 2008). Por ejemplo, se conoce que el carácter del miedo en el perro tiene una heredabilidad de 0.4-0.5 (Manteca, 2003).

La maduración está constituida por el conjunto de procesos de desarrollo típico de una especie (Oyama, 1982). Se caracteriza por ser altamente predecible, pero no depende exclusivamente de los genes, si no que la interacción con el entorno va a influir en este proceso ontogenético. Estos procesos de maduración tienen una especial influencia en las primeras etapas tras el nacimiento. Por ejemplo, la maduración del sistema nervioso central es clave en el proceso de ontogénesis de las crías, sobre todo en las especies altriciales, que presentan un grado de desarrollo sensorial y motor bajo al nacer (Manteca, 2003).

El último motor de la ontogénesis es el aprendizaje. El aprendizaje genera cambios relativamente permanentes en el comportamiento de un individuo como resultado de la experiencia (Shettleworth, 2010). Se pueden distinguir dos categorías básicas de aprendizaje: el individual y el social. El aprendizaje individual involucra únicamente al individuo sujeto al proceso de aprendizaje y a los estímulos externos que generan la experiencia causante de éste (Shettleworth, 2010). Los dos mecanismos más simples de aprendizaje individual son la habituación y la sensibilización. En el primer caso, se produce una disminución en el nivel de respuesta debido a una repetición del estímulo (Bouton, 2007); el segundo consiste en la reacción contraria: el nivel de respuesta se incrementa conforme se somete al individuo al mismo estímulo (Shettleworth, 2010).

En el condicionamiento clásico y en el operante, el aprendizaje implica un proceso cognitivo más complejo. El condicionamiento clásico se basa en la asociación de dos estímulos: uno importante para la supervivencia y otro que no lo es, pero señala la aparición de un estímulo importante (Domjan, 2005). El ejemplo más conocido es el experimento llevado a cabo por Pavlov (Pavlov, 1927), en el que, repetidamente, se hacía sonar un timbre a la vez que se le proporcionaba alimento a un perro, que iniciaba la salivación. Con el tiempo, el perro asoció la llegada de la comida con el timbre, de forma que cuando se hacía sonar el timbre, el perro salivaba. El condicionamiento operante o instrumental funciona de una manera similar, aunque en este caso la asociación se produce entre una respuesta voluntaria del animal (un determinado comportamiento) y una consecuencia relevante para el individuo (Skinner, 1938).

Todos estos mecanismos de aprendizaje tienen una ventaja adaptativa para el animal: la habituación permite obviar aquellos estímulos que no son imprescindibles para la supervivencia, la sensibilización permite responder de manera rápida a estímulos importantes, el condicionamiento clásico resulta útil para anticiparse a un acontecimiento y el condicionamiento operante permite al animal ajustar su conducta y conseguir que aparezcan o desaparezcan determinadas consecuencias (Colmenares & Gómez, 1994).

El segundo gran bloque de mecanismos de aprendizaje es de tipo social. Se trata de la forma más compleja de aprendizaje y no se ha observado en todas las especies. El aprendizaje social se basa en el beneficio que reporta la experiencia de otro individuo (Shettleworth, 2010). Esto es especialmente útil para las crías que deben aprender comportamientos típicos de los adultos, como los comportamientos sociales o de alimentación. Por ejemplo, se ha demostrado ampliamente que en el proceso de aprendizaje de la depredación, la observación de otros individuos cazando, especialmente si es la madre, mejora las habilidades depredadoras de la cría (Polsky, 1975). La principal ventaja del aprendizaje social radica en que es una manera muy eficiente de transmitir información de unos individuos a otros. Además, lo aprendido de esta manera no desaparece con la muerte del individuo, de forma que el desarrollo de un comportamiento importante de cara a la supervivencia es fácilmente transmisible de una generación a otra de una misma población o especie. Por ello, a este tipo de aprendizaje también se le denomina transmisión cultural (Zentall & Galef, 1988; Laland & Janik, 2006).

Un ejemplo de la capacidad de transmisión del aprendizaje social se comprobó en los años 1940 en el Reino Unido. Allí, observaron que algunos herrerillos comunes (*Cyanistes caeruleus)* eran capaces de abrir botellas de leche. Poco tiempo después, prácticamente toda la población de la especie en el Reino Unido había adquirido esa habilidad. Fisher and Hinde (1949) argumentaron que lo más probable es que algún individuo aprendiera por azar a abrir la botella y el resto, por imitación, adquiriera ese comportamiento, extendiéndose así por toda la población.

2.2.- Precocidad frente a altricialidad

Como hemos visto anteriormente, en la etapa postnatal se producen gran cantidad de cambios físicos y de comportamiento en un periodo de tiempo relativamente corto. Las presiones evolutivas para mejorar el éxito reproductor han llevado a la aparición de dos estrategias en función del grado de desarrollo que presentan los cachorros al nacer.

Se cree que la condición original en los mamíferos era la altricialidad (Hopson, 1973; Case, 1978). Los recién nacidos de las especies altriciales presentan un desarrollo motor y sensorial relativamente atrasados. Se caracterizan por presentar los ojos y el canal auditivo cerrados, el pelaje está ausente, su capacidad locomotora es muy limitada y no son capaces de termorregular por sí mismos (Thompson et al., 2010). Este grado de desarrollo bajo no les permite proveerse de su propia comida, por lo que dependen completamente de la madre para su nutrición (Hayssen, 1993). Por su parte, las especies precoces se caracterizan porque las crías nacen con un grado de desarrollo elevado. Los sentidos son completamente funcionales y tienen una capacidad locomotora plena, siendo capaces de caminar y correr poco después del nacimiento. El pelaje está completo y pueden regular su temperatura por sí mismos (Thompson et al., 2010). Además, aunque se alimenten principalmente de leche materna, la comida sólida aporta una parte significativa de la dieta desde el inicio de la etapa postnatal (Hayssen, 1993). La altricialidad y la precocidad son los dos extremos de un fenómeno que, en la práctica, no se presenta como una dicotomía (Zeveloff & Boyce, 1986). En muchas especies de mamíferos, las crías nacen con algún grado intermedio de desarrollo. En estos casos, se suele hablar de especies semi-altriciales o semi-precoces, según se asemejen las características del neonato a una condición u otra.

La mayoría de las especies de mamífero son altriciales (Derrickson, 1992). La distribución de las especies precoces es desigual en los distintos órdenes de mamíferos. Por ejemplo, los carnívoros son principalmente altriciales, los artiodáctilos son esencialmente precoces y, en los roedores, hay una gran variedad entre ambas condiciones (Derrickson, 1992). Esta diseminación de la precocidad indica que ha surgido de forma recurrente a lo largo de la evolución debiendo aportar, por tanto, algún beneficio adaptativo para la supervivencia. Se ha sugerido que la disponibilidad de recursos (Daly, 1975; Neal, 1984), una baja disponibilidad de agua (Layne, 1986) o un incremento de las interacciones sociales (Gould, 1975) han podido favorecer la precocialidad. El estado de desarrollo al nacer también se ha relacionado con otras características biológicas. Por ejemplo, los recién nacidos de las especies precoces tienen un mayor tamaño relativo (Martin & MacLarnon, 1985), el periodo de gestación es más largo (Zeveloff & Boyce, 1980) y el tamaño de camada es menor (Eisenberg, 1981).

2.3.- Etapas del desarrollo postnatal en especies altriciales.

El desarrollo físico y del comportamiento en el periodo postnatal de las especies altriciales es un proceso continuo en el que se conjugan diversos factores que determinan el ritmo de los cambios. Aun así, se suelen distinguir cuatro periodos: neonatal, de transición, de socialización y juvenil (Manteca, 2003). La longitud de cada una de estas etapas varía entre las distintas especies. Esta división ha sido estudiada especialmente en animales domésticos (perros y gatos) o de laboratorio (ratas), por la facilidad que supone el seguimiento exhaustivo de sus crías.

El primer periodo en el que se divide el desarrollo de las crías altriciales tras el nacimiento es el neonatal. Durante esta etapa, tanto el sistema locomotor como los órganos de los sentidos de la vista y el oído no están completamente desarrollados (Markwell and Thorne, 1987). Así, su capacidad de interacción con el entorno es muy limitada todavía y la supervivencia del cachorro depende completamente de la madre. Su alimentación se basa exclusivamente en la leche materna (Martin, 1986) y la eliminación de orina y heces es un reflejo que depende de la estimulación táctil de la zona perineal por parte de la madre (Fox, 1970). Además, estos cachorros no son capaces de termorregular, por lo que necesitan del calor proporcionado por la madre. Para esto, presentan un reflejo de termotropismo positivo (Bateson, 2000). Cuando la madre está fuera de la madriguera, los cachorros suelen juntarse para ayudarse mutuamente a mantener la temperatura (“huddling”; Bautista et al., 2008). El comportamiento del cachorro se limita prácticamente a dormir y mamar. Por ejemplo, el comportamiento lactante ocupa el 30% del tiempo en los cachorros de perro (Grant, 1987).

El final del periodo neonatal y el inicio del periodo de transición vienen marcados por un rápido desarrollo sensorial y locomotor (Manteca, 2003). Los ojos comienzan a abrirse (Braastad & Heggelund, 1984), a la vez que el cachorro empieza a responder a estímulos auditivos (Olmstead & Villablanca, 1980). Además, se pueden observar algunos patrones locomotores rudimentarios. Estos cambios tienen como consecuencia una mayor capacidad de la cría para interaccionar con su entorno, lo que se refleja en el inicio de conductas exploratorias y la exhibición de los primeros intentos de juego (Markwell & Thorne, 1987).

El inicio del periodo de socialización es variable en función del ritmo de desarrollo del cachorro a nivel sensorial y locomotor. Una vez que el cachorro muestra patrones locomotores avanzados y los órganos sensoriales están suficientemente desarrollados, comienzan la mayor parte de los cambios en el comportamiento: el tiempo que dedican los cachorros a explorar sufre un aumento marcado (Manteca, 2003) y se observan los primeros comportamientos sociales típicos de los adultos, como pueden ser el establecimiento de relaciones de dominancia (Wright, 1980). Se produce un incremento de la capacidad del cachorro para recibir estímulos del medio y para aprender. En esta fase, las diferentes experiencias pueden afectar permanentemente al desarrollo del cerebro (Byers, 1998; Siviy, 1998). La existencia de este periodo sensible para el aprendizaje del cachorro tiene consecuencias prácticas en la cría en cautividad. Por ejemplo, es en este periodo cuando las crías deben aprender a seleccionar aquellas especies adecuadas para su consumo (Apfelbach, 1986). El aprendizaje es posible durante toda la vida del individuo, pero en la edad adulta estos procesos son más lentos y difíciles (Fox, 1971).

El periodo juvenil es la última etapa en el desarrollo de las crías altriciales. La mayoría de los cambios del comportamiento ya se han producido (Manteca, 2003). Durante este periodo, se perfeccionan las habilidades motoras y los comportamientos aprendidos durante las etapas anteriores, mientras que aquellos comportamientos más relacionados con el aprendizaje, como la exploración o el juego, disminuyen. Con el tiempo, el etograma del cachorro se asemeja cada vez más al de un adulto. Este periodo finaliza cuando el animal alcanza la madurez sexual (Markwell & Thorne, 1987).

2.4.- Ontogenia del comportamiento en félidos

Los félidos son una familia del orden *Carnivora* que se distribuye, de forma natural, prácticamente por todo el planeta, exceptuando la Antártida, la mayor parte de Oceanía y algunas otras islas como Madagascar (Nowell et al., 1996). Sin embargo, el gato doméstico ha sido introducido casi en cualquier lugar donde existen asentamientos humanos. Actualmente se reconocen 18 géneros y 36 especies de félidos (Nowell & Jackson, 1996).

Excepto los leones (*Panthera leo*), los guepardos (*Acinonyx jubatus)* machos y algunas poblaciones silvestres de gato doméstico, los félidos son mayoritariamente solitarios (Sunquist & Sunquist, 2002). Su morfología los convierte en los cazadores más especializados entre los carnívoros, siendo capaces de abatir presas de gran tamaño, incluso varias veces más grandes que ellos (Walker et al., 1983). Son depredadores ágiles y rápidos, que suelen cazar de noche, aunque la máxima actividad la suelen desarrollar en los crepúsculos (Boorer & Warner, 1970). Su órgano sensorial más desarrollado es la vista, adaptada a funcionar durante todo el día, independientemente de la cantidad de luz (Sunquist & Sunquist, 2002).

Una característica común a todos los félidos es que las crías son altriciales al nacer (Grzimek, 2003). Los recién nacidos normalmente son ciegos y sordos, sin capacidad de locomoción y necesitan de la protección de la madre para sobrevivir (Sunquist & Sunquist, 2002). Nacen cubiertos de un pelaje suave llamado lanugo que les ayuda a mantener la temperatura corporal, ya que no son capaces de termorregular por sí mismos (Anderson, 1983). Las hembras suelen dar a luz en madrigueras, en fisuras de rocas o troncos huecos, donde las crías permanecen ocultas hasta que el sistema locomotor está suficientemente desarrollado para caminar (Grzimek, 2003).

Al tratarse de especies altriciales, los primeros cambios que experimentan las crías derivan de la maduración motora y sensorial. La variabilidad intraespecífica en la edad a la que ocurren estos primeros cambios es muy alta, aunque en general, las especies del género *Panthera* abren los ojos y son capaces de caminar antes que los félidos más pequeños, como los de los géneros *Lynx* o *Felis (*Hemmer*,* 1979*)*. En el gato doméstico, se ha comprobado que la edad de apertura de ojos depende de factores como el padre, la exposición a la luz, el sexo y la edad de la madre: los cachorros hembras criados en zonas oscuras y de madres jóvenes abren los ojos antes (Braastad & Heggelund, 1984).

La capacidad de caminar y el desarrollo sensorial se asocian con el abandono de la seguridad de la paridera y la aparición de nuevos comportamientos que necesitan patrones motores avanzados, como los sociales, el juego y la depredación (Manteca, 2003). Como casi todas las especies de félidos son solitarias, la variedad de comportamientos sociales que deben aprender las crías es limitada. Las interacciones están generalmente asociadas a la adquisición y defensa de un territorio y a la reproducción. Estas interacciones se realizan mediante comunicación olfativa (marcaje con orina y heces), visual (posturas con diferentes significados) y auditiva (vocalizaciones diversas) (Beaver, 2003).

El juego de los félidos puede ser de tipo social, locomotor o con objetos (Barret & Bateson, 1978). El juego social es el que ocupa la mayor parte del juego total de los cachorros y suele ocurrir entre hermanos de camada, mientras que el juego con la madre suele ser escaso (Mendl, 1986). Sin embargo, las madres con camadas de un cachorro compensan la ausencia de hermanos para el juego con un incremento en el juego con su cría (Mendl, 1988). Por lo general, en los carnívoros el primer juego en aparecer es el juego social, mientras que el juego locomotor y el juego con objetos aparecen posteriormente (Bekoff, 1974, Drea et al., 1996). La función del juego en los félidos, como en otras especies, no está claramente definida. Se ha sugerido que el juego con objetos sirve para practicar la caza en guepardos (Martin and Caro, 1985), que el juego locomotor sirve para ejercitar y mejorar las habilidades motoras como la coordinación (Byers and Walker, 1995) y que el juego social ayuda a la creación de vínculos sociales y mejora las habilidades relacionadas con el comportamiento agonístico, la depredación y la reproducción (Bekoff, 2007). Sin embargo, en los leones, Schaller (1972) observó que la forma más habitual del juego social, la lucha, raramente se usaba de adultos, mientras que el acecho, un componente importante de la caza, era observado con poca frecuencia durante el juego. En cualquier caso, dada la ubicuidad del comportamiento en los mamíferos y los costes asociados que conlleva, como el energético, es indudable la importancia del juego en el desarrollo de los félidos.

Los cachorros son dependientes de la madre también para la alimentación. Aunque es difícil observar la lactancia en los félidos, se ha comprobado que ocupa una parte muy elevada del tiempo durante los primeros días (Schneirla et al., 1963). La madre suele ser la responsable de iniciar la lactancia en las primeras semanas tras el parto (Martin, 1986). A medida que los cachorros crecen, tienen mayor iniciativa y son ellos los que se acercan a la madre para mamar, aunque el tiempo que dedican a lactar decrece exponencialmente (Schneirla et al., 1963). En los leones, existe cierta colaboración entre las distintas hembras del grupo para la cría, ya que las hembras toleran que los cachorros de otras madres puedan mamar de ellas (Pusey & Packer, 1994). Las primeras relaciones de dominancia aparecen asociadas al periodo lactante. En el gato doméstico se ha comprobado que los cachorros desarrollan preferencia de mama, es decir, aquellos cachorros dominantes utilizan en exclusividad las mamas que más leche aportan (Ewer, 1959).

La edad de comienzo del destete varía entre las distintas especies y está vinculado a la aparición de la dentición decidua. Generalmente ocurre entre el mes y los dos meses de vida, aunque en los leones y tigres comienza alrededor de los 100 días (Sunquist & Sunquist, 2002). La lactancia desaparece progresivamente, pudiendo alargarse hasta los 6-8 meses en las especies de gran tamaño (Sunquist & Sunquist, 2002). Aun cuando los cachorros están destetados, siguen dependiendo de la madre para alimentarse, ya que el proceso de aprendizaje de la depredación es complejo y necesitan mejorar sus habilidades locomotoras. En este aprendizaje, la madre juega un papel fundamental. La primera experiencia de las cría con la depredación ocurre cuando las madres les llevan una presa, lo cual ha sido observado en diferentes especies de félidos: gatos domésticos (Ewer, 1969); leones (Schenkel, 1966); guepardos (Kruuk & Turner, 1967) o tigres (Schaller, 1967). Una vez que han adquirido suficiente competencia locomotora, los cachorros comienzan a acompañar a la madre en la caza. Por ejemplo, los guepardos pueden acompañar a la madre a partir de los 2 meses, aunque es a partir de los 4 meses cuando lo hacen en la mayoría de las ocasiones en las que la madre sale a cazar (Caro, 1994). Al principio, los cachorros se limitan a observar a la madre desde una distancia prudente. Conforme crecen, la madre les permite tener un mayor peso en la secuencia de la caza, hasta que son capaces de matar una presa por primera vez. La edad a la que capturan la presa por primera vez es muy variable entre las distintas especies: los gatos domésticos pueden matar a las 5 semanas, los caracales a los 3 meses, los guepardos y leopardos a los 8 meses y los linces canadienses a los 9 o 10 meses (Sunquist & Sunquist, 2002).

El desarrollo de la depredación en felinos depende de componentes innatos y aprendidos. Parece que la experiencia previa tiene poca influencia en la persecución y captura de la presa. Según Eaton (1974), el acecho, la persecución, el derribo de la presa y la orientación del bocado son innatos en el guepardo, mientras que el aprendizaje sería especialmente importante en el reconocimiento, la muerte y la ingestión de la presa. En cualquier caso, diferentes estudios en gatos han mostrado que aquellos individuos criados en compañía de conespecíficos adultos y, especialmente la madre, presentan una mayor tasa de captura y de otros comportamientos predatorios, tanto de cachorros como de adultos (Kuo, 1930; Caro, 1980).

Una vez las crías son capaces de cazar por sí mismas y adquieren cada vez mayor independencia de la madre y los hermanos, comienzan el proceso de dispersión y búsqueda de un territorio propio. Aunque la edad de independencia es muy variable entre las diferentes especies, muchos felinos la alcanzan a los 18 meses (Sunquist & Sunquist, 2002). Los leones representan la principal excepción: las hembras permanecen en el grupo cuidando de las crías de otros miembros de la manada (Patterson, 2007). Las especies más pequeñas de félidos alcanzan la madurez sexual en menos de un año, mientras que en los de mayor tamaño puede retrasarse hasta los dos años de vida (Grzimek, 2003).

El estudio de la ontogenia en los félidos presenta muchas dificultades. El hecho de que las crías permanezcan durante sus primeros días o semanas en el interior de la paridera dificulta evaluar los cambios que se producen (McGuire et al., 2011). En este sentido, la cría en cautividad se ha convertido en una gran oportunidad para conocer más detalles sobre las primeras etapas del desarrollo (McVittie, 1978). La facilidad de criar y observar la ontogenia temprana en el gato doméstico ha hecho que sea uno de los ejemplos de desarrollo temprano en especies altriciales mejor conocido (Bateson, 2000).

En las primeras semanas de vida de los gatos, la interacción con el medio se basa en estímulos olfativos, térmicos y táctiles (Rosenblatt, 1976). El sentido del oído, aunque está presente a los pocos días de nacer -son capaces de reaccionar a sonidos a partir de los cinco días-, se desarrolla por completo al mes de vida (Olmstead & Villablanca, 1980). Los ojos permanecen cerrados hasta los 7-10 días, cuando comienza el proceso de apertura (Villablanca & Olmstead, 1979). A partir de la tercera semana, la vista es fundamental para guiar el comportamiento (Norton, 1974). Sin embargo, no alcanza su máximo desarrollo hasta los tres o cuatro meses (Ikeda, 1979).

Los cachorros pueden regular su propia temperatura a partir de la tercera semana (Jensen et al., 1980) y los patrones de sueño-vigilia se asemejan a los de los adultos a los dos meses (McGinty et al., 1977). Son capaces de moverse de forma rudimentaria a las dos semanas de nacer (Moelk, 1979). A partir de la quinta semana pueden correr y, antes de los dos meses, usan todas las formas de locomoción de los adultos (Peters, 1983). El periodo de lactancia estricto dura unas tres semanas (Martin, 1986), aunque la dependencia nutricional completa se alarga hasta las cinco semanas, cuando empiezan a cazar presas por sí mismos (Baerends-van Roon & Baerends, 1979). Son completamente independientes para alimentarse a partir de las 7 semanas, cuando cesa la lactancia (Martin, 1986). La eliminación de heces y orinas ya no depende de la madre a partir de las cinco semanas de vida (Fox, 1970).

El juego social aparece a las cuatro semanas de vida (West, 1974; Barrett & Bateson, 1978), teniendo una prevalencia alta hasta las semanas 12-14, a partir de las que comienza un leve descenso (West, 1974; Caro, 1981). El juego con objetos se desarrolla algo más tarde que el social, a las 7-8 semanas (Barret & Bateson, 1978), a la vez que se observa por primera vez el juego locomotor (Martin & Bateson, 1985). La ontogenia en el gato, por tanto, se caracteriza por una primera fase, de corta duración, de maduración sensorial y locomotora, seguida por numerosos cambios en el comportamiento durante los dos primeros meses, de forma que el gato es completamente independiente alrededor de los tres meses de vida.

3.- El lince ibérico: una especia amenazada

3.1.- Biología, ecología y comportamiento

El lince ibérico *(Lynx pardinus)* es una especie de felino endémica de la Península Ibérica. Mayor que un gato doméstico y del tamaño de un perro mediano, presenta un aspecto estilizado, con las extremidades largas, la cabeza pequeña y la cola muy corta (Rodríguez, 2007). Los linces ibéricos son de color leonado, con manchas negras de tamaño variable que van desde un moteado fino sin patrón definido hasta individuos con manchas de gran tamaño, que llegan a alinearse a lo largo del cuello y la zona dorsal, existiendo multitud de patrones intermedios (Beltrán & Delibes, 1993). El pelaje ventral es de color blanco sin manchas. Varias de las características morfológicas más llamativas de la especie las comparte con las otras especies de linces. En los laterales de la cara posee unos mechones de pelo a modo de patillas (Rodríguez, 2007), que despliega en situaciones de estrés, miedo o excitación. En el extremo superior de la oreja presenta un mechón de pelo, llamado pincel, de color negro (Rodríguez, 2007). La parte trasera de la oreja es negra, con un ocelo en la base de color blanco. La cola es muy corta y terminada con una mancha de color negro (Figura 1).

FIGURA 1. Imagen de un lince ibérico. (Programa de conservación Exsitu/T. Rivas)

Es una especie dimórfica (Beltrán & Delibes, 1993). La longitud cabeza-cuerpo en los machos es de 74-87 cm, la altura en la cruz de 41-55 cm y su peso de 7.5-15.9kg. Las hembras son más pequeñas, con una longitud cabeza-cuerpo de 69-78cm, una altura en la cruz de 36-47cm y un peso entre 6.1 y 12.4kg (Rodríguez, 2007).

Según Johnson et al. (2006), el género *Lynx* tuvo su origen cuando se separó respecto del ocelote (género *Leopardus*) y del puma (*Puma concolor*) hace aproximadamente 8.0-6.7 millones de años. El origen geográfico de los linces no se conoce con seguridad, aunque la hipótesis más aceptada indica que se originó en Norteamérica, expandiéndose a través del Estrecho de Bering hasta Eurasia (Johnson et al., 2006). El proceso evolutivo condujo a los predecesores de los linces ibéricos a adaptarse a una alimentación basada en el conejo y a la vida en el monte mediterráneo, y ello dio lugar a un proceso de especiación que lo diferenció del lince boreal (*Lynx lynx)*, su congénere filogenéticamente más cercano, que está adaptado a vivir en bosques y a alimentarse de ungulados (Nowell et al., 1996).

Históricamente, la distribución del lince ibérico comprendía toda la Península Ibérica y el sureste de Francia (Rodríguez & Delibes, 1990). El norte de la Península Ibérica también fue habitado por el lince boreal (Clavero & Delibes, 2013; Rodríguez-Varela et al., 2015). Se cree que la distribución del lince ibérico y del lince boreal pudo alternarse en el norte de la Península y el sur de Francia (Sommer & Benecke 2006), de forma que, en periodos más fríos, las poblaciones de lince boreal avanzaran hacia el sur, mientras que en periodos cálidos las de lince ibérico ocuparan territorios más septentrionales (Rodríguez et al., 2011).

A principios del siglo XX, las poblaciones del norte de la Península comenzaron a disminuir, aunque seguía siendo abundante en el centro y en el sur (Cabrera, 1914). A mediados del siglo, las poblaciones remanentes se distribuían por el Sistema Central, Montes de Toledo y la Sierra de San Pedro, Sierra Morena y el litoral de la provincia de Huelva (Rodríguez & Delibes, 1990). El declive continuó hasta principios del siglo XXI, desapareciendo probablemente de Portugal en los años 1990 (Sarmento et al., 2009) y restringiéndose en España a dos poblaciones reproductoras: Andújar-Cardeña en Sierra Morena, y Doñana-Aljarafe, en el entorno del Espacio Natural Protegido de Doñana (Guzmán et al., 2005). En 2002 se alcanzó el mínimo poblacional de la especie, 94 animales. En la última década, los programas de conservación puestos en marcha han incrementado el número de ejemplares en libertad hasta los 327 individuos (Iberlince LIFE Project, 2014b).

Dentro de su área de distribución, el lince ibérico prefiere áreas con alta densidad de matorral mediterráneo (Palomares et al., 2000), aunque también son adecuadas zonas rocosas con matorral (Fernández et al., 2006). La máxima densidad de linces se suele encontrar donde el hábitat configura un mosaico de arbustos de gran tamaño, agrupados en manchas separadas por pastizales (Palomares, 2001; Fernández et al., 2003). Tratan de evitar hábitats abiertos como marismas o cultivos, así como plantaciones forestales (Palomares et al., 1991). Se les suele encontrar en altitudes de entre 400m y 1300m (Rodríguez & Delibes, 1992)

El patrón de uso del hábitat está relacionado con factores ambientales y con las diferencias estacionales en la disponibilidad de comida (Beltrán & Delibes, 1991; Palomares et al., 1991). En Doñana más del 90% de las localizaciones de descanso diurnas se encuentran en hábitats arbustivos densos (Beltrán et al., 1987). Para la depredación, prefieren pastizales abiertos y el ecotono entre bosques y matorrales, donde el conejo es más abundante (Beltrán, 1991).

Los linces son principalmente crepusculares y nocturnos (Calzada, 2000), aunque hay diferencias individuales y estacionales importantes. Durante el verano son principalmente nocturnos, con los niveles más altos de actividad durante los crepúsculos, momento en el que se trasladan de las zonas de descanso a las de alimentación. Durante el invierno, es más fácil observarlos durante el día y el patrón se hace más irregular, sobre todo en los juveniles (Beltrán & Delibes, 1994). En general, los patrones de actividad del lince están muy sincronizados con los de su presa principal, el conejo (*Oryctolagus cuniculus*; Monterroso et al., 2013).

El conejo constituye entre el 85% y el 99% de la dieta del lince ibérico (Delibes, 1980; Palomares et al., 2001). Este porcentaje decrece ligeramente durante el invierno, coincidiendo con los periodos de mínimo anual de las poblaciones de conejo. En cambio, se observa un incremento de la depredación sobre crías y juveniles de ciervos y gamos (Delibes, 1980; Beltrán et al., 1985). Algunos roedores y aves también pueden formar parte de la dieta del lince (Delibes, 1980). La forma de matar la presa es similar a la de otros felinos. En las raras ocasiones en las que cazan presas grandes, como los ciervos, las matan propinándoles uno o varios bocados en la garganta, rompiéndoles la laringe y provocando la muerte de la presa por asfixia (Beltrán et al., 1985). Por el contrario, a su presa principal, el conejo, los matan mordiéndoles en la base del cráneo o en la nuca, provocando una dislocación cervical y dañando la espina dorsal (Sunquist & Sunquist, 2002). Los requerimientos energéticos del lince se han estimado en aproximadamente un conejo al día (Aldama et al., 1991). Suelen cazar los conejos al rececho, permaneciendo ocultos hasta que el conejo se encuentra a una corta distancia, o esperando el paso de conejos camuflados en los arbustos (Rodríguez, 2004)

El lince ibérico es un animal solitario y territorial (Ferreras et al., 1997). El solapamiento de territorios entre animales del mismo sexo es muy bajo, aunque el territorio de un macho suele solaparse ampliamente con el de una hembra y parcialmente con el resto de hembras vecinas (Ferreras et al., 1997). Esto sugiere que el lince ibérico presenta un sistema de reproducción monógamo, aunque con cierta tendencia a la poliginia en algunos individuos (Ferreras et al., 1997). El tamaño medio del área de campeo se estima en 10.3km^2 para los machos y en 8.7 km^2 para las hembras, aunque puede variar entre los 4 y los 30km^2 en función del sexo y de la densidad de conejos (Ferreras et. al, 1997; Palomares, 2001; Palomares et al., 2001). Por ejemplo, en las hembras se observa una reducción ostensible del área de campeo durante la época de cría. La ocupación de un territorio es marcada mediante heces y orina (Robinson & Delibes, 1988). El establecimiento del primer territorio propio para ambos sexos ocurre alrededor de los 2 años, coincidiendo con el inicio de la madurez reproductiva (Aldama, 1993). Sin embargo, la adquisición de un territorio óptimo para la cría se retrasa hasta los 4-7 años para los machos y hasta los 3-7 años para las hembras, probablemente debido a la escasez de territorios aptos para la reproducción (Ferreras et al., 1997).

La dificultad para adquirir un territorio reproductor implica que las hembras suelen reproducirse por primera vez entre los 3 y los 9 años de vida, aunque a partir de los dos años sean maduras sexualmente (Palomares et al., 2005). No todas las hembras se reproducen cada año, siguiendo el patrón observado en otros felinos territoriales (Palomares et al., 2005). La reproducción es estacional: el celo tiene lugar durante el invierno, principalmente en los meses de enero y febrero (Ferreras et al., 2004). Las hembras tienen ovulación inducida, es decir, no ovulan en el caso de que no haya monta por parte del macho (Göritz et al., 2009) El periodo de celo se prolonga alrededor de tres días con un promedio de 28 cópulas y la gestación dura entre 63 y 66 días (Vargas et al., 2009). La mayoría de los partos ocurren entre marzo y abril, aunque se han observado nacimientos más tardíos (Fernández et al., 2002; Palomares et al., 2005). Las hembras suelen parir en los huecos de árboles viejos y de gran tamaño (Fernández & Palomares, 2000) o en grandes berrocales de granito (Simón et al., 2012). El tamaño de camada habitual es de tres cachorros, aunque es frecuente observar camadas de entre 2 y 4 individuos (Palomares et al., 2005), aunque en cautividad han nacido camadas de 1 y 5 individuos.

Las crías son consideradas semi-altriciales al nacer (Fernández et al., 2002). Su desarrollo físico y su ontogenia temprana son poco conocidos dada la dificultad de realizar estudios de los recién nacidos en libertad. En cautividad, se conoce que los cachorros de lince exhiben un comportamiento agresivo que desemboca en un episodio de peleas entre hermanos de camada entre las 6 y las 8 semanas de vida (Antonevich et al., 2009). El periodo agresivo puede durar desde unas pocas horas hasta varios días. La madre suele intervenir separando a los cachorros y manteniéndolos alejados para evitar nueva agresiones. En casos extremos, las heridas infligidas (a veces por la propia madre en su intento de separarlos) han ocasionado la muerte de un cachorro. Aún no se conoce que mecanismo desencadena estas peleas, aunque se ha descartado que la disponibilidad de alimento, el sexo del cachorro o el tamaño de la camada influyan en el inicio de las agresiones (Antonevich et al., 2009). Las peleas se han descrito también en otras dos especies del género *Lynx:* el lince rojo *(Lynx rufus)* y el lince boreal *(Lynx Lynx*; Antonevich & Naidenko, 2013). Su función adaptativa no se ha determinado aún, aunque se ha sugerido que se establece una jerarquía en la camada al final del periodo agresivo (Sokolov et al., 1994).

Los juveniles permanecen en el territorio natal hasta la dispersión, que se produce cuando los linces tienen entre uno y dos años de edad (Ferreras et al., 2004). Los machos suelen recorrer una mayor distancia que las hembras, que a menudo heredan el territorio de la madre. La etapa dispersiva dura unos 6 meses de media y se establecen a una distancia de entre 10 y 21km del área natal (Ferreras et al., 2004)

La longevidad del lince ibérico es poco conocida, aunque se estima que un cráneo de un lince de la colección de la Estación Biológica de Doñana tenía 14 años (Zapata et al., 1997). En cautividad, el ejemplar más longevo murió con 20 años.

3.2.- Estado de conservación. Amenazas y medidas de conservación.

El lince ibérico es uno de los mamíferos más amenazados del mundo. Está catalogado como “En peligro” por la IUCN (Rodríguez & Calzada, 2015) debido al escaso número de individuos adultos y a la existencia de tan solo dos poblaciones reproductoras. La escasez de conejos de monte en la Península, diezmado por las enfermedades (Villafuerte et al., 1995; Villafuerte & Delibes-Mateos 2007); la destrucción, alteración y fragmentación de su hábitat, el monte mediterráneo (Rodríguez & Delibes, 2003); y otros factores como la caza furtiva y los atropellos (Rodríguez & Delibes, 2004; Ferreras et al., 2010; Iberlince LIFE Project, 2014a) son las causas que han conducido a la especie hasta su mal estado de conservación actual (Calzada et al., 2007; Rodríguez & Calzada, 2015).

Debido a la situación en la que se encuentra la especie, están en marcha diversos planes de conservación con medidas de actuación tanto en su hábitat como en cautividad. Desde 1999 hay una "Estrategia Nacional para la Conservación del Lince Ibérico (*Lynx pardinus*) en España" que establece las bases generales para la conservación de la especie en el país (Dirección General de Conservación de la Naturaleza, 1999; Dirección General de Medio Natural y Política Forestal, 2009). Además, las Comunidades Autónomas de Andalucía, Castilla La Mancha y Extremadura cuentan con los pertinentes Planes de Recuperación (Calzada et al., 2010) y desde 2001, hay un Plan de Acción para la Cría en Cautividad del Lince Ibérico, que se convirtió en un más amplio Programa de Conservación Ex-Situ del Lince Ibérico, integrado dentro de la Estrategia Nacional y que corre a cargo del Ministerio de Medio Ambiente y la Junta de Andalucía.

Por su parte, la Unión Europea ha venido financiando diferentes proyectos de conservación, la mayoría de escasa cuantía económica, en España y Portugal a través de los fondos LIFE desde principios de los años 90. Aunque, sin duda, los trabajos de gestión que han supuesto un cambio significativo en la tendencia poblacional del lince son los liderados por la Comunidad Autónoma de Andalucía desde 2002, también cofinanciados con fondos LIFE, y que aglutinan el 82% de la inversión realizada en la conservación del lince a través de estos fondos (Calzada, 2013). Las actuaciones principales que se han adoptado en estos proyectos son el manejo para la mejora del hábitat, encaminado a incrementar la disponibilidad de alimento, madrigueras y de agua; la puesta en marcha de programas de educación entre la población local para disminuir la mortalidad asociada a factores antrópicos, como la caza furtiva o el trampeo ilegal; el vallado y, por otra parte, la construcción de pasos seguros de carreteras y otras infraestructuras de transporte, para disminuir el impacto de la fragmentación del hábitat y de los atropellos; la realización de convenios con propietarios de fincas para la adecuación de éstas para la conservación del lince; la traslocación de individuos para el refuerzo genético de las poblaciones; y la reintroducción de ejemplares procedentes de la cautividad para reforzar las poblaciones existentes y crear otras nuevas en el área de distribución histórica de la especie (Simón et al., 2012).

El éxito de las medidas de conservación, tanto exsitu como insitu, ha conseguido revertir la tendencia decreciente de las poblaciones de la especie de forma que desde 2002 la población está creciendo. El número de individuos se ha incrementado desde los 94 en 2002 hasta los 327 en el censo de 2014, acompañado de un incremento de la superficie ocupada (Iberlince LIFE Project, 2014b). Este aumento de la población se debe tanto a una recuperación de las dos poblaciones existentes como a la creación de nuevos núcleos mediante traslocación y reintroducción. Las dos primeras zonas de reintroducción, Guadalmellato (Córdoba) y Guarrizas (Jaén), se localizan en Sierra Morena, cerca de las poblaciones ya existentes, creando una metapoblación en la que ya se han observado los primeros intercambios de lince entre los diferentes núcleos. En la actualidad, con la colaboración de los gobiernos de Portugal y de las regiones de Extremadura y Castilla-La Mancha, se han preparado nuevas zonas de reintroducción en sus territorios, con las primeras sueltas en 2014, habiéndose registrado incluso el nacimiento en libertad de dos camadas procedentes de animales reintroducidos en Extremadura en 2015.

3.3.- Programa de Conservación Exsitu del Lince Ibérico

En el documento de aprobación de la “Estrategia Nacional para la Conservación del Lince Ibérico” en 1999, se manifestó la necesidad de poner en marcha medidas de conservación exsitu, que sirvieran de apoyo a las actuaciones sobre el hábitat y las poblaciones de conejo que se planeaban realizar. Así, la Comisión Nacional para la Conservación de la Naturaleza aprobó en 2001 el “Plan de Acción para la Cría en Cautividad del Lince Ibérico”. Este documento sentó las bases para la creación del Programa de Conservación Exsitu del Lince Ibérico, a cargo de la Dirección General de Biodiversidad del Ministerio de Medio Ambiente, en colaboración con la Junta de Andalucía. En los años posteriores, se creó una Comisión Multilateral para coordinar las actuaciones del Programa de Cría, dando cabida a las diferentes administraciones que entraron a formar parte del programa: los gobiernos de España y Portugal, junto con los gobiernos autonómicos de Andalucía, Extremadura y Castilla-La Mancha.

El Plan de Acción ha sufrido diversas modificaciones desde su redacción, siendo la última actualización en el año 2007. Este documento recogía los dos objetivos principales del Programa de Cría en Cautividad: el mantenimiento de una población en cautividad viable desde un punto de vista sanitario, genético y demográfico y la preparación de animales para la reintroducción al medio natural.

En cuanto al primer objetivo, la meta se fijó en la conservación del 85% de la diversidad genética de la especie en la población cautiva durante 30 años. Para ello, se planteó la necesidad de albergar un mínimo de 60 ejemplares, 30 machos y 30 hembras. Se estableció un calendario de actuación con el número de individuos salvajes que deberían incorporarse cada año al Programa para el periodo 2004-2009; así como la necesidad de incorporar, a partir de 2010, al menos un ejemplar salvaje cada dos años, de entre aquellos ingresados en centros de recuperación, para asegurar la diversidad genética a largo plazo. El objetivo del tamaño poblacional se alcanzó en el año 2010, cumpliendo así las previsiones de crecimiento demográfico de la población cautiva (Figura 2).

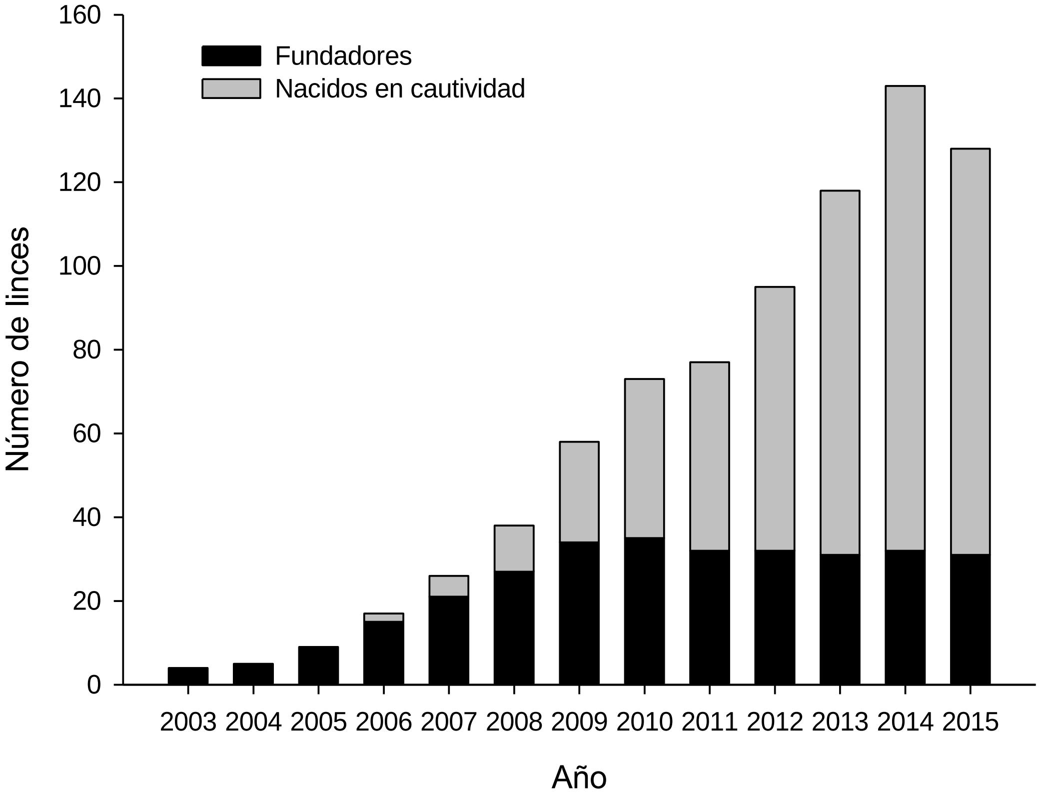

FIGURA 2. Evolución de la población de lince ibérico del Programa de Cría en Cautividad entre los años 2003 y 2015.

El segundo objetivo consistía en la preparación de ejemplares nacidos en cautividad para ser reintroducidos al medio natural. Así, de manera coordinada con las medidas de conservación insitu, se pretendía reforzar las poblaciones de lince ibérico existentes y crear nuevas poblaciones en aquellas áreas de la distribución histórica donde hubiera desaparecido recientemente. Los animales destinados a la reintroducción debían ser adecuados desde un punto de vista sanitario, etológico, genético y reproductor, para incrementar su probabilidad de supervivencia en el medio natural. En el año 2010, se realizó la primera reintroducción de dos hembras nacidas en el Centro de Cría del Lince Ibérico (CCLI) “La Olivilla” en Guarrizas (Jaén). En los años posteriores, un total de 100 cachorros nacidos en los distintos centros de cría del Programa han sido liberados en las diferentes áreas preparadas para la reintroducción en Andalucía, Extremadura, Castilla La-Mancha y Portugal.

La población cautiva del lince ibérico se encuentra repartida entre cuatro centros de cría exclusivos para el lince ibérico y un centro asociado. El primer centro en comenzar a funcionar fue el Centro de Cría del Lince Ibérico “El Acebuche” en el ENP de Doñana (Huelva), bajo la gestión del Ministerio de Medio Ambiente. El primer macho reproductor procedente del medio silvestre fue capturado en Diciembre del año 2003, uniéndose a un grupo de 4 hembras que ya estaban en cautividad en este centro y en el año 2005 se consiguió el nacimiento de la primera camada en cautividad. En 2007 se inauguró el Centro de Cría de “La Olivilla” (Jaén), gestionado por la Junta de Andalucía. En 2009, el Programa de Cría adquiere un carácter internacional al comenzar a funcionar el Centro Nacional de Reproduçao do Lince Iberico en Silves (Portugal), gestionado por el gobierno portugués. El último centro se inauguró en 2011 en Zarza de Granadilla (Cáceres), también dependiente del Ministerio de Medio Ambiente. Además, el programa de cría cuenta con un centro asociado, el Zoobotánico de Jerez de la Frontera (Cádiz), perteneciente al Ayuntamiento de esta localidad. Los animales albergados en los distintos centros de cría se manejan como si se tratara de una sola población. Así, teniendo en cuenta criterios genéticos, cada año se produce el traslado de varios ejemplares entre los distintos centros. De esta forma, se evita el empobrecimiento de la diversidad genética de los animales nacidos en cautividad a largo plazo.

4.- Metodología general

4.1.- Funcionamiento de los Centros de Cría del Programa de Conservación Exsitu

El funcionamiento de los diferentes centros de cría que forman parte del Programa de Conservación Exsitu se rige por unas pautas similares. Así, las instalaciones, el sistema de video vigilancia y los protocolos de manejo empleados son comunes a todos los centros, aunque se adaptan posteriormente a las necesidades y características singulares de cada uno. La información recogida en esta sección se ha extraído del Manual de Manejo del Lince Ibérico en Cautividad (Rivas et al., 2015).

4.1.1.- Instalaciones de los Centros de Cría

Los centros de cría cuentan con una gran variedad de instalaciones, que se pueden dividir en dos grandes grupos, según se destinen a albergar animales o a su uso por parte del personal de los centros.

Los recintos de cría albergan a la mayor parte de los animales de un centro. Suelen disponerse a lo largo de dos filas enfrentadas, con 4 u 8 recintos en cada lado, separados por un pasillo central, por donde se produce el tránsito diario del cuidador durante las tareas de limpieza y manejo. El conjunto de los recintos está separado del exterior por un valla perimetral que se encuentra a una distancia de 7m de los recintos, creando una zona de amortiguación que evita el contacto con fauna salvaje y permite contener posibles fugas. Generalmente, los recintos se encuentran situados en una zona tranquila, a 150-200m del resto de edificios del centro, para disminuir las molestias debidas al trabajo diario y al tránsito de coches.

El tamaño de los recintos varía entre 550m^2 y 1000m^2. Están rodeados por una vallado perimetral de 3.5m que cuenta, en la parte superior, con un pastor eléctrico y un voladizo hacia el interior, para evitar posibles fugas. El vallado está formado por una malla de simple torsión plastificada de 5cm de luz. La zona inferior cuenta, además, con una malla electro-soldada con una luz de 1cm para evitar el contacto directo entre animales o entre el animal y el cuidador. El techo de muchas de las instalaciones también se encuentra cubierto por malla metálica. Todos los recintos están provistos de cámaras fijas y móviles y micrófonos, que permiten el seguimiento exhaustivo de los animales (ver sección 4.1.2. para más detalles). Por lo general, cada recinto alberga un solo individuo, excepto en la época de celo o en el caso de las madres con sus camadas. La distribución de los animales se realiza de modo que no haya dos individuos del mismo sexo en instalaciones contiguas, para evitar conflictos.

Cada recinto de cría se divide en varias zonas diferentes. El área de campeo comprende la mayor parte del área exterior de la instalación. Se trata de una zona abierta, cubierta con sustrato natural (arena y/o rocas) y una cobertura de vegetación arbórea y arbustiva similar a la que se encuentra en el hábitat natural de la especie. Cuenta con un sistema de riego para el mantenimiento óptimo de la vegetación. Además, está enriquecida con diversas estructuras naturales o artificiales, como troncos o repisas elevadas, que incrementan el espacio efectivo de la instalación. Los bebederos simulan charcas naturales y el llenado/vaciado está controlado desde el exterior del recinto (Figura 3).

FIGURA 3. Área de campeo enriquecida de un recinto de cría. (Programa de conservación Exsitu/T. Rivas)

Anexa al área de campeo, se encuentra la zona de manejo. Esta zona se emplea para encerrar a los animales durante las labores rutinarias de limpieza y mantenimiento del área de campeo y para realizar un seguimiento más exhaustivo de animales enfermos. Tiene un tamaño de 225m^2 y también está enriquecida con repisas y techados para ofrecer refugios a los animales.

En un lateral de la zona de manejo o del área de campeo, hay una habitación paridera realizada en obra, de unos 4m^2 y una altura de 3.5m, que utilizan los animales para el refugio y descanso. En el interior hay repisas a diferentes alturas y un cajón empleado por las hembras para dar a luz. Además, para aumentar la oferta de lugares donde parir y permitir que las hembras elijan el lugar donde se encuentren más cómodas, también puede encontrarse una paridera naturalizada en el área de campeo o en la zona de manejo (Figura 4). Estas parideras están realizadas en madera y miden aproximadamente 50x50x100cm. Están forradas por dentro con materiales naturales como el corcho, y cuentan con una cámara propia para permitir el seguimiento de los animales en su interior.

FIGURA 4. Paridera naturalizada en el área de campeo de un recinto de cría. (Programa de Conservación Exsitu/T. Rivas)

Entre dos instalaciones contiguas, se encuentran unos pasillos de separación realizados con malla metálica, que permiten el movimiento de individuos entre estos recintos y que se usan, principalmente, para la socialización entre ejemplares, ya que permiten diferentes grados de acercamiento. La comunicación entre instalaciones enfrentadas se realiza mediante túneles desmontables de malla metálica que se fijan al suelo mediante tensores.

En el exterior de los recintos se encuentran otras instalaciones como torres de observación, para la vigilancia de los animales sobre el terreno; un almacén con el material necesario para la rutina diaria; y un vestuario, que sirve como barrera de bioseguridad, donde se procede al cambio de ropa y a la desinfección antes de entrar a las instalaciones.

Además de los recintos de cría, hay otras instalaciones en los centros que pueden albergar animales de forma temporal. El edificio de crianza artificial de cachorros está dotado con el equipamiento necesario para la cría de animales huérfanos o abandonados: incubadoras, cocina, vestuario que actúa como barrera de bioseguridad y dos recintos anexos, con una parte interior y otra exterior, para cachorros de mayor edad. Las cuarentenas albergan animales procedentes del campo y sirven para controlar el riesgo de introducir enfermedades en la población cautiva. Suelen situarse lejos del resto de instalaciones del centro y están compuestas por módulos independientes dotados de un vestuario, un recinto cubierto y otro exterior. El suelo está cubierto de un material de fácil limpieza y desinfección. Las instalaciones para presas vivas albergan principalmente conejos (tanto doméstico como silvestre), perdices y codornices, en jaulas, para alimentar a los linces. Están diseñadas para adecuarse a las condiciones óptimas que necesitan estas especies durante la estancia en los centros. Incluyen pequeñas instalaciones donde los conejos domésticos puedan vivir varios días en unas condiciones más silvestres para aumentar su capacidad de huida y refugio frente al lince.

El resto de instalaciones están orientadas para su uso por parte del personal de los centros de cría: oficinas, sala de video vigilancia, clínica/laboratorio, cocina, viviendas para estancias programadas y vivienda para el responsable del centro

4.1.2.- Sistema de video vigilancia

El sistema de video vigilancia instalado en los distintos centros de cría tiene tres objetivos principales: el seguimiento y la observación de los animales, la realización de estudios etológicos y el apoyo a programas de sensibilización de la población.

El seguimiento de los animales se efectúa las 24 horas del día mediante un equipo mixto compuesto por video vigilantes profesionales y voluntarios. De forma general, se realiza un muestreo puntual durante todo el año, en el que se registra, una vez cada hora, la actividad del animal y el comportamiento que esté mostrando, además de otras variables como la climatología o la ubicación del ejemplar. Las categorías de comportamiento anotadas están estandarizadas para todos los centros de cría. Como complemento a este muestreo puntual, a lo largo del año se obtienen diferentes datos de comportamientos específicos para cada momento de la temporada: cópulas, partos, lactancia, preparación para la reintroducción…

La gran cantidad de datos que se registran a diario en los centros de cría, junto con los muestreos puestos en marcha para estudios específicos, convierten al sistema de video vigilancia en una herramienta vital para la realización de estudios que permitan mejorar el conocimiento que se tiene sobre la etología del lince ibérico. La principal ventaja de este sistema es que se evitan las molestias y las modificaciones que se causan habitualmente en el comportamiento de los animales debidos a la presencia del observador cerca de ellos. El seguimiento exhaustivo que se realiza también sirve de apoyo a la gestión diaria de los centros. Por ejemplo, permite la detección de anomalías en el comportamiento o patologías, el seguimiento de animales enfermos o el apoyo a actuaciones específicas de manejo, como las capturas.

Por último, el sistema de video vigilancia permite la distribución de imágenes de los animales albergados en los centros, que se emplean para dar a conocer la biología y el comportamiento de esta especie, colaborando en la sensibilización de la población con respecto a la problemática de la conservación del lince.

La parte principal de la infraestructura del sistema de video vigilancia está constituida por las cámaras, que pueden ser de dos tipo: fijas o móviles (cámaras DOMO). El número de cámaras instaladas en cada recinto depende del tamaño y de las necesidades del centro de cría. Por lo general, las cámaras fijas son de pequeño tamaño y están diseñadas para enfocar a corta distancia, por lo que suelen instalarse en lugares pequeños como las parideras o la zona de manejo. Las cámaras DOMO están motorizadas y dotadas de zoom, permitiendo su manejo desde la sala de control para realizar un mejor seguimiento de los animales. Suelen colocarse en el área de campeo de las instalaciones, existiendo 2 ó 3 cámaras para cubrir todos los ángulos muertos. Las cámaras fijas cuentan con iluminación infrarroja mediante leds, mientras que las cámaras DOMO necesitan una iluminación de infrarrojos externa que sirva de apoyo. El empleo de luz infrarroja permite el seguimiento de los animales durante la noche, sin alterar su fotoperiodo. Las imágenes obtenidas por las cámaras se visualizan desde la sala de videovigilancia a través de una serie de videograbadores que permiten mover las cámaras DOMO de forma remota y que almacenan todas las imágenes. Además de las cámaras, los recintos cuentan con micrófonos repartidos por las instalaciones que permiten registrar los diferentes tipos de vocalizaciones de los linces.

4.1.3.- Protocolos de manejo de los animales

El manejo de los animales albergados en los centros de cría varía en función del destino del animal y de la época del año. Por un lado, el manejo para los animales destinados a la cría en cautividad debe conseguir un equilibrio entre la ausencia de estrés por la presencia del hombre y de las condiciones de la cautividad, y el mantenimiento de los comportamientos naturales de la especie. Por otra parte, los animales que están destinados a la reintroducción requieren de un manejo específico para prepararlos adecuadamente y mejorar su probabilidad de supervivencia en el medio natural. En general, se emplean técnicas poco intrusivas, para favorecer la tranquilidad de los animales, incrementar la probabilidad de reproducción y evitar la impronta con los humanos.

Un aspecto imprescindible a tener en cuenta en el manejo diario para el funcionamiento de un centro de cría es la bioseguridad. El riesgo de transmisión de patógenos es alto al mantener un gran número de animales en un espacio relativamente reducido. Por ello, existen medidas preventivas que se basan principalmente en la colocación de pediluvios con desinfectantes en la entrada de las instalaciones de los animales y el uso de ropa exclusiva para el interior de los recintos.

La rutina diaria de los centros de cría consiste en la recogida de muestras, la limpieza y el mantenimiento de las instalaciones y la alimentación. La dieta que se emplea trata de imitar la que tienen los linces en libertad. Así, la alimentación básica para una semana consistiría en tres días de conejo vivo, dos días de conejo muerto, un día de ternera o ave (perdiz, codorniz o pollo) y un día de ayuno. Acostumbrar a los animales a comer carne muerta facilita algunas tareas, como el suministro de medicación sin necesidad de anestesiar al animal. En el caso de los animales destinados a la reintroducción, no se les ofrece carne muerta para evitar que se acostumbren a carroñar.

Para cumplir con el objetivo de que los animales se encuentren en un entorno libre de estrés, el entrenamiento es una de las herramientas más útiles. Por un lado, aumenta el vínculo entre cuidador y animal, permitiendo que las tareas rutinarias no supongan un estrés para el animal. Además, evita el uso de método invasivos. Por ejemplo, el entrenamiento veterinario para la realización de radiografías o el pesaje en los túneles de comunicación evita que los animales tengan que ser capturados y sedados. Además del entrenamiento, en los centros de cría se han implantado programas de enriquecimiento ambiental, con el fin de mejorar el bienestar de los animales en la cautividad. Estos programas se basan en un mayor enriquecimiento de la instalación, tanto física como sensorial, mediante la introducción o modificación del mobiliario, el uso de olores, ruidos… Así, se disminuye la aparición de efectos no deseados por la falta de bienestar, como la inapetencia por el alimento o los comportamientos estereotípicos.

Manejo durante la cría

El manejo de las hembras gestante se basa principalmente en mantener la mayor tranquilidad posible en los recintos. Conforme se acerca el día estimado para el parto, la video vigilancia se intensifica y se minimizan las tareas en torno al recinto de la hembra. En la primera semana tras el parto, se evita entrar en esa instalación, además de agilizar el resto de tareas para que los cuidadores permanezcan el menor tiempo posible en la zona de los recintos de cría.

El único contacto de los cachorros con el personal del centro ocurre a las 4, 8 y 12 semanas, cuando son chequeados, desparasitados y vacunados si procede. En estos chequeos, los cachorros de camadas múltiples son marcados mediante pequeños rapados de pelo en diferentes lugares, para poder diferenciarlos individualmente. Para la rutina diaria de limpieza y mantenimiento, la madre es separada en la zona de manejo durante el menor tiempo posible, mientras que los cachorros suelen permanecer refugiados en algunas de las parideras. La bioseguridad es un aspecto esencial en las primeras semanas de vida de los cachorros, debido al escaso desarrollo del sistema inmune que presentan durante este periodo. Así, se utilizan mono y calzas para entrar en estos recintos.

La alimentación se modifica para cubrir las mayores necesidades energéticas de las hembras gestantes y lactantes. Durante la gestación, se incrementa la cantidad de alimento ofrecido en función del aumento de peso que se observe en la hembra, para evitar un sobrepeso que dificulte el parto. En los 12 últimos días de la gestación y durante el periodo lactante, se le ofrece alimento “ad libitum”. Conforme los cachorros crecen y empiezan a ingerir comida sólida, se incrementa la cantidad de conejo ofrecido hasta llegar a un conejo por individuo en la instalación al día cuando los cachorros cazan por sí mismos.

Durante el periodo agresivo que atraviesan las crías entre las 6 y 8 semanas de vida, el manejo realizado se ha ido modificando con el tiempo. En los primeros años del programa de cría, los cuidadores intervenían separando los cachorros. Sin embargo, esta interrupción del conflicto antes de su finalización prolongaba de forma excesiva este periodo de agresividad. En la actualidad, se parte de la premisa de la no intervención, excepto en casos puntuales en los que se valore, a través del sistema de video vigilancia, que la actuación de la madre o la agresividad de los cachorros pueda poner en peligro la vida de alguna de las crías.

Los cachorros se mantienen en el mismo recinto de la madre hasta los 7-9 meses, cuando son separados para permitir a la hembra participar en la siguiente temporada reproductora y disminuir la carga de animales en un mismo recinto.

En algunas ocasiones, los cachorros recién nacidos son abandonados por sus madres y tienen que ser criados de forma artificial. Sin embargo, este método conlleva una serie de alteraciones en el desarrollo del comportamiento del cachorro, especialmente en aquellos derivados de la socialización con individuos de su propia especie. Por ello, hay varias alternativas que se tratan de poner en marcha, en función de la situación, para evitar una crianza llevada a cabo completamente por el personal de los centros.

La adopción por parte de otra hembra se suele realizar en los casos en los que el comportamiento maternal anómalo de la madre sea la causa del abandono o la retirada del cachorro. Siempre que el cachorro esté sano, se trata de insertarlo en la camada de otra hembra que haya parido en una fecha similar. Si se observa que un cachorro está en mal estado, pero el comportamiento de la madre es adecuado, se intenta retirarlo para recuperarlo lo antes posible y volver a insertarlo en su camada. Si no es posible una de las técnicas anteriores, se realiza una crianza mixta. Los cachorros siguen siendo alimentados artificialmente, pero son mantenidos en la misma instalación donde se encuentra la madre, u otro adulto compatible, durante varias horas al día, para que se socialicen con otro lince. Cuando los cachorros están algo más desarrollados y son más independientes, se les mantiene en una instalación anexa al de un adulto. El destete y el desarrollo de la caza en estos cachorros se organizan intentando imitar, en la medida posible, el proceso natural en la especie.

Manejo de reintroducción

Uno de los dos objetivos principales del Programa de Conservación Exsitu del Lince Ibérico es la preparación de ejemplares nacidos en cautividad para su reintroducción al medio natural. Para que se pueda alcanzar este objetivo es necesario un manejo específico que prepare a estos animales para la liberación. Este manejo se basa en el fomento del comportamiento depredador, de los comportamientos huidizos frente al hombre y de otros comportamientos básicos para la especie como la exploración, el juego o la competencia intraespecífica en la camada.

Una vez que los cachorros han comenzado a cazar por sí mismos, comienza a aplicarse el protocolo de alimentación para la reintroducción. Este protocolo se basa en un incremento de la proporción de cantidad de conejo silvestre ofrecido, para aumentar la dificultad de la caza. Además, se elabora un calendario semanal de alimentación para cada camada, en el que se especifican los días y las horas a las que la comida va a estar disponible. El horario se establece de forma aleatoria, para evitar la existencia de rutinas de alimentación, pero teniendo en cuenta los periodos diarios de máxima actividad de los linces. La cantidad de comida que se les ofrece es variable en función de la semana, aunque tiende a ser menor conforme los cachorros crecen. Así, pueden llegar a tener hasta dos días de ayuno a la semana, repartidos de forma aleatoria.

En los centros de cría existen dos sistemas distintos de aporte indirecto de la presa, cuyos objetivos son evitar que los linces asocien la comida con el cuidador, aumentar la impredecibilidad en la aparición de la presa y aportar estructuras para que los conejos puedan refugiarse. Uno de los sistemas se basa en la construcción de una red subterránea de tubos y arquetas, a modo de majanos. Los conejos son introducidos por uno de los tubos, que tiene salida al exterior de la instalación. Los majanos del interior se encuentran naturalizados para dificultar la depredación y ofrecer mayor refugio a los conejos. El otro sistema consiste en la instalación de dispensadores automáticos. Estas estructuras naturalizadas se colocan junto a la malla perimetral del recinto. Desde el exterior de la instalación, se puede dejar la presa en un cajón que queda fuera del alcance de los linces. Los dispensadores cuentan con un temporizador programable que, a la hora programada, deja pasar a los conejos hacia un majano enterrado. Este majano puede tener salida directa al área de campeo de la instalación o estar conectado a un sistema de tubos y arquetas subterráneos. Ambos sistemas para proporcionar la presa se complementan con la existencia de vivares o refugios repartidos por toda la instalación para dificultar la depredación.

Además de las técnicas empleadas para evitar la asociación del cuidador con la comida, se realiza un manejo específico para fomentar las conductas huidizas respecto a los humanos. Este manejo consiste en que, si se consigue separar a la madre de las crías, un cuidador entra en el recinto haciendo ruidos molestos y persiguiendo a los cachorros. Así se consigue reforzar negativamente la presencia del hombre. En cuanto un cachorro encuentra un refugio adecuado o muestra algún comportamiento de huida, los ruidos y la persecución cesan para reforzar estas respuestas. Este manejo se realiza esporádicamente para evitar la habituación del cachorro. Las instalaciones cuentan, además, con barreras visuales colocadas adecuadamente para permitir a los linces esconderse de los humanos.

Por último, en los centros de cría se trata de fomentar los comportamientos sociales de los cachorros destinados a reintroducción. Este manejo radica principalmente en disminuir el aporte de alimento para favorecer la competencia intraespecífica entre hermanos de camada y el establecimiento de jerarquías. Es importante realizar un seguimiento exhaustivo de la condición corporal de los animales mientras se aplique este manejo. La frecuencia de las agresiones o la presencia de restos de comida permiten ajustar la cantidad de alimento que se les proporciona. Además, se permite el contacto visual con otros adultos y camadas, para facilitar el aprendizaje y desarrollo del lenguaje corporal típico de la especie.

4.2.- Metodología de muestreo y análisis estadístico

En este apartado se pretende resumir de manera general la metodología empleada para la recolección de los datos de la Tesis Doctoral, así como presentar una síntesis del análisis estadístico utilizado. Ambos aspectos se explican con más detalle en la sección "Material and Methods" de los Capítulos I-V.

Metodología general de muestreo

Para los tres primeros capítulos (desarrollo físico y crecimiento, comportamiento lactante y ontogenia de la actividad), se emplearon las metodologías de muestreo ya implementadas en los centros de cría. Se consideró que eran adecuadas para el registro sistemático de la información, y dada la dificultad de obtener un elevado número de datos debido al escaso número de cachorros nacidos cada año, se optó por no modificarla para poder aprovechar los registros recogidos en años anteriores al inicio de este proyecto. Así, se incrementa la fuerza de los resultados con un mayor tamaño muestral.

Los datos sobre el desarrollo físico se obtuvieron mediante el seguimiento diario de los cachorros, a través del sistema de videovigilancia para los cachorros criados con sus madres, o mediante observación directa en el caso de cachorros criados artificialmente. La base de datos de pesos para las curvas de crecimiento se construyó tras una revisión profunda de los archivos, tanto digitales como físicos, de lo Centro de Cría "El Acebuche".

El seguimiento del comportamiento lactante se realizó también mediante las cámaras instaladas en las parideras y en el área de campeo de los recintos. Dada la necesidad de un seguimiento continuo de los cachorros para la gestión de los centros de cría, los datos de lactancia se registraban las 24 horas del día desde el nacimiento hasta que los cachorros dejaban de mamar.

Los registros de la actividad de los cachorros se integraban en el muestreo puntual rutinario de los centros de cría, lo que permitió obtener datos diariamente sobre la actividad diaria y los ritmos circadianos desde el nacimiento hasta que los cachorros eran separados de la madre.

Para los capítulos sobre la ontogenia del comportamiento de juego y del comportamiento depredador, se diseñó una metodología de muestreo específica para la Tesis Doctoral. El seguimiento del juego se realizó a diario, registrando cada camada durante 15 minutos dos veces al día, coincidiendo con el amanecer y el atardecer, cuando los cachorros estaban activos y exhibían este comportamiento. Las grabaciones se realizaron desde el nacimiento, para registrar la edad a la que se iniciaba el juego; hasta que los cachorros eran separados de la madre. Los videos eran almacenados y posteriormente revisados para conocer la frecuencia de cada tipo de juego mediante un muestreo puntual.

Los hitos del comportamiento depredador se registraron mediante el seguimiento 24horas de los cachorros a través del sistema de video vigilancia. Para establecer el etograma de la depredación, se realizó una grabación semanal de un episodio de caza de cada cachorro desde que se aplicó el protocolo de manejo para animales destinados a la reintroducción hasta que los cachorros fueron separados de la madre.

Análisis estadístico

Uno de los objetivos principales de la tesis, además de describir cómo se desarrolla el comportamiento de los cachorros de lince ibérico, era conocer qué factores podían influir en el curso de este desarrollo. El análisis estadístico se realizó con el software R (R Core Team, 2015) y se basó en el uso de modelos mixtos lineales generalizados (GLMM). Este método permite analizar datos longitudinales en los que existen varias medidas para cada individuo y estudiar cómo influyen diferentes variables en la respuesta que se analiza, incluida el tiempo, que actúa como covariable (Landau & Everitt, 2004). Para estudiar las curvas de crecimiento tuvo que emplearse un modelo mixto no lineal (NLMM), ya que el crecimiento en mamíferos sigue una curva sigmoidea (Zullinger et al., 1984). En cualquier caso, los NLMM funcionan de manera similar a los GLMM.

El análisis de los ritmos circadianos requirió el uso de estadística circular, que se emplea habitualmente en el estudio de los ritmos biológicos (Batschelet, 1981). Este conjunto de método de análisis se aplica a aquellos datos que pueden representarse en un círculo, es decir, se trata de variables en las que dos valores coinciden en un mismo punto, como por ejemplo las 0h y las 24h de un día (Lahoz-Beltrán, 1994).

5.- Objetivos

Los objetivos de este proyecto de Tesis Doctoral son los siguientes:

1.- Conocer el curso del desarrollo físico en las primeras etapas postnatales en los cachorros de lince ibérico, determinar los parámetros que definen su curva de crecimiento y los factores que influyen en ella.

2.- Describir el comportamiento lactante del lince ibérico, determinar la duración de los periodos de lactancia estricta y de destete, el tiempo que dedican a lactar los cachorros en función de su edad y los factores que lo afectan; y establecer el rol principal de la lactancia en la especie.

3.- Caracterizar la ontogenia de la actividad diaria y el ritmo circadiano en el lince ibérico y comparar el patrón circadiano de los cachorros destinados a reintroducción con lo observado en la especie en su hábitat.

4.- Describir el comportamiento de juego en el lince ibérico, determinar a qué edad se inicia, analizar el tiempo que dedican a jugar los cachorros y los factores que influyen en él.

5.- Caracterizar la ontogenia de la depredación en el lince ibérico, determinar el papel de la madre en el aprendizaje de la caza y establecer el etograma de la caza en cachorros destinados a la reintroducción.

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CAPÍTULO I. Early Development and Growth in Captive-Born Iberian lynx (*Lynx pardinus*)

Yerga J, Calzada J, Manteca X, Vargas A, Rivas A, (2014). Early development and growth in captive-born Iberian Lynx (*Lynx pardinus*). *Zoo Biology*, 33: 381-387.

Abstract

Knowledge on the growth and early development patterns of endangered species can become a useful conservation tool because it may allow detecting anomalous growth in newborns, both in captivity breeding and in the wild. We studied the growth and early development of 40 Iberian lynx (*Lynx pardinus*) cubs belonging to 21 litters born in captivity between 2005 and 2012 at "El Acebuche" Iberian Lynx Breeding Centre. This is the first study on growth in this critically endangered species. The Iberian lynx cubs were not fully developed at birth. During the first 3 weeks of life, cubs underwent many of the physical changes that allowed them to improve their interaction with the environment, such as the opening of eye and auditory channels, teeth eruptions, and the ability to walk. When the cubs were 1 month old, they were ready to leave the den and develop new behaviors such as the exploration of their environment, play, or hunt. Three different models had been fitted to the body mass growth of the Iberian lynx. The von Bertalanffy curve provided the best fit. The asymptotic adult mass was the only parameter that differed between males and females (males being 8% larger), due to the higher growth rate of males. The adult weight of hand-reared cubs (i.e., those abandoned at born) did not differ from that of cubs reared by their mothers. Both growth and development showed differences from other lynx species.

Keywords: early development; growth; Iberian lynx; sex; rearing method.

INTRODUCTION

Growth is a key parameter in the life history of a species. It is a complex phenomenon, which involves changes in body form, metabolism, and body processes. Growth can be an important source of variation of individual fitness (Rodel et al., 2008) and can affect the animal's health (Birgersson & Ekvall, 1997) and survival probability (Wauters et al., 1993). In precocial mammals, as ungulates, the newborn have their senses of sight and hearing fully developed, and they are capable of walking almost immediately after birth. On the other hand, the cubs of altricial species are born blind, with poorly developed hearing and motor coordination. Thus, altricial species require care for a longer amount of time (Thompson et al., 2010). The occurrence of developmental landmarks such as the opening of eye and auditory channels, the ability to walk or the eruption of teeth determine the cub’s ability to interact with the environment and to develop the behaviors required to survive in adulthood (Bateson, 2000).

In mammals, the body mass growth pattern follows a non-linear sigmoidal curve, both for altricial and precocial species (Zullinger et al., 1984). Different models have been employed to describe the increase in body mass, allowing the comparison between individuals, populations or species (Zullinger et al., 1984; Gaillard et al., 1997). In the past, it was usual to run a non-linear regression on growth data (Smuts et al., 1980), but this did not account for variability due to repeated measures of different individuals or to analyze the effects of other variables that may affect growth. The later development of hierarchical non-linear mixed models allowed accounting for these sources of variability (Pinheiro & Bates, 2000; Griffiths & Brook, 2005; English et al., 2012).

The aim of this paper is to describe the growth and development of one of the most endangered mammal species of the world, the Iberian lynx (*Lynx pardinus*). Very little is known about the growth of Iberian lynx cubs, since access to wild litters is very limited. So far the only available published data refer to the growth of a few hand-reared individuals (Rivas et al., 2009). The Iberian lynx is considered as a semialtricial species (Fernández et al., 2002). It is sexually dimorphic, with males being larger and heavier than females when they are adults (Beltrán & Delibes, 1993). It is a food specialist, being rabbits (*Oryctolagus cuniculus*) its main prey (Delibes, 1980).

The knowledge of the growth and early development of a species is a useful tool in conservation programs of endangered animals (Laurenson, 1993; Wielebnowski, 1998). This allows the detection of anomalous growth in newborns or to analyze differences between cubs raised under different conditions, both in captivity and in the wild.

MATERIAL AND METHODS

We analyzed the growth and development patterns of 40 newborn Iberian lynx (henceforth simply lynx). They were born between 2005 and 2012 in 21 litters from eight different mothers. Animals belonged to the Iberian Lynx Captive Breeding Programme (henceforth ILCBP; Vargas et al., 2008) and all of them were born in “El

Acebuche” Iberian Lynx Breeding Centre (37°00' N 6°33' W), in Doñana National Park. The ILCBP began in 2003 with the aim to breed healthy Iberian lynx, from an ethological, reproductive and genetic perspective, suitable for reintroduction in the wild (Vargas et al., 2008).

To achieve the ILCBP objectives, the cubs are intended to be raised by their mothers and the contact with humans is minimized to avoid disturbances and imprinting of the young. However, mothers sometimes abandon their offspring (Vargas et al., 2009) and abandoned lynx cubs must be hand-reared. Therefore, we considered two rearing groups (hand or mother-reared) to analyze growth and development.

Mother-reared cubs fed only on milk until 8 weeks old. After then, mothers gradually introduce the cubs to solid food and lactation is reduced. During lactation, the mother was fed ad libitum with live rabbits. Once young were fully weaned, the amount of food provided to the family group was progressively increased until the cubs were able to hunt (in week 14 approximately). From then on, one rabbit per animal and day was provided. Twice a month food was enriched with live partridges or quails.

Abandoned cubs were at first instance housed in an incubator and latter moved to a room with controlled temperature and humidity when they gained mobility. During the first 3 days, the cubs were fed every 3 hr with a milk substitute (KMR®, PetAg, Inc., Hampshire, IL, USA; BabyCat Milk®, Mars, Inc., McLean, VA, USA; or LactadietGatos®, OPKO Health, Inc., Miami, FL, USA). Following the patterns of mother-reared cubs, from the second month the number of milk feedings progressively decreased and solid food started to be provided. Hand-reared cubs were weaned at the same age as mother-reared ones, and when they were able to hunt, they were moved to a enclosure similar to those of the other families. From then on, the feeding protocol was the same as in mother-reared cubs. Other issues related to hand-rearing of Iberian Lynx cubs are widely explained in Rivas et al. (2009).

Early Development Sampling

Developmental status of 31 cubs (12 of them hand-reared) of Iberian lynx was recorded daily from birth to 2 months old. Mother-reared cubs were tracked through a remote video surveillance system. All lynx enclosures, including dens, were equipped with fixed and dome cameras with infrared lights that allowed around-the-clock monitoring of the cubs, minimizing human disturbances. Data from hand-reared cubs were taken through direct examination. Developmental landmarks recorded were: ear rising, eye opening, auditory channel opening, walking ability, teeth eruptions, claw retraction, and downy fur lost. We calculated the mean age (in days) and its standard error and age range at which each of these landmarks was achieved.

Body Mass Sampling

We repeatedly measured the body mass of 33 Iberian lynx cubs (22 mother-reared, 9 males and 13 females; and 11 hand-reared, 9 males and 2 females) until adulthood. Overall, we made 1,333 measurements covering between the birthday and 3.5 years old. The time between measurements was variable depending on the rearing method. Mother-reared cubs were weighed fewer times (mean=12, range=4-34), because the priority was not to disturb the animals. Hand-reared cubs were weighed almost daily until they were placed in the enclosures. From then on they were weighed as often as mother-reared ones. On average, hand-reared cubs were weighed 97 times (range=56-159).

Growth Modeling

Three different previously employed growth models for mammals were fitted to the data (Zullinger et al., 1984):

(a) von Bertalanffy: $M(t) = A\left\{1 - {}^{1}\!/_{3}\, e^{-K(t-I)}\right\}^{3}$

(b) Gompertz: $M(t) = Ae^{-e^{-K(t-I)}}$

(c) Logistic: $M(t) = A\left\{e^{-K(t-1)} + 1\right\}^{-1}$;

where M(t)=mass (g) at age t, A=asymptotic adult mass (g), K=growth rate constant (day^{-1}), I=age at the inflection point (day).

We fitted weight measurements through non-linear mixed effects models, in order to account for the non independence and repeated nature of measurements. This method allows to estimate the parameters for each individual level random effects. The models were fitted following Pinheiro & Bates (2000). While building the models, we found a strong negative correlation (above 0.9 in absolute value) between two parameters, K and I. Individual random effects over K were thus removed in order to avoid convergence errors due to over-parameterization of the model. Once the estimates of the parameters for the three models had been obtained, we chose the most accurate growth model based on Akaike's Information Criterion (AIC). The subsequent analyses employed only the model with the best fit. However, parameters estimates of the three models are provided.

Then, sex and rearing method were included as fixed factors to assess their influence on growth. The effects of these factors over K were again removed to avoid overparameterization. Once the model was ran, all non-significant (P>0.05) parameter-factor interactions were removed to simplify it and to obtain the final model. Statistical analysis was made with R 2.15.1, using the "nlme" library (Pinheiro et al., 2010).

RESULTS

Early Development

Iberian lynx cubs were born helpless and dependent on maternal care. They were not able to walk and the senses of sight and hearing were not developed. Although they were covered with downy fur, they were unable to thermoregulate by themselves (Rivas et al., 2009). They were born without teeth and with unsheathed and non-retractile claws.

During the first 2 weeks of life, there were no external noticeable changes in the development of Iberian lynx cubs. The first developmental landmark observed was the starting of the raising of ears, which happened on average 12 days after birth (range: 6-22). The age at eye opening was highly variable among cubs, with the onset at 14.6 days old (8-19) and the end at 19 (14-24). At 19 days old (8-24), cubs were observed to stand for the first time, but were still unable to walk. The auditory channels opened at day 20 (18-23) and then they began to respond to sound stimuli. Teeth eruption began on the third week. The first teeth to appear (17 days; 14-19) were the upper canines, followed by lower ones at (19 days; 16-24). Upper and lower incisors appeared at the age of 19-20 days (16-26). The claws were sheathed at 21 (8-32) days old. Cubs started losing the downy fur progressively and it was completely disappeared from the head at 18 (11-35) days old.

TABLE 1. Mean age, standard error, range, and sample size for the occurrence of developmental landmarks in the Iberian lynx cubs

Landmark		x ± SE	Range	N
Ear				
Half-folded ears		11.84 ± 0.941	6-22	25
Fully-raised ears		32.27 ± 0,46	25-38	28
Opened auditory channel		20.4 ± 1.12	18-23	5
Eyes				
Half-opened eyes		14.61 ± 0.46	8-19	26
Full-opened eyes		19.16 ± 0.43	14-24	31
Teeth				
Canines				
	Upper	16.73 ± 0.45	14-19	11
	Lower	18.7 ± 0.7	16-24	10
Incisors				
	Upper	19.5 ± 1.21	16-26	8
	Lower	19.89 ± 0.89	16-24	9
Premolars				
	Upper	38.2 ± 1.32	33-40	5
	Lower	37 ± 2	33-39	3
Motor skills				
Standing		18.79 ± 0.65	8-24	28
Walking		27.66 ± 1.02	17-37	29
Claws				
Sheathed claw		20.88 ± 2.91	8-32	8
Retractile		25.43 ± 1.78	20-32	7
Downy fur				
Missing on head		18.27 ± 1.71	11-35	15
Missing on limbs		22.87 ± 0.96	15-29	15

The downy fur was continuously reduced, disappearing from limbs at 23 (15-29) days old. At day 25 (20-32) days claws were retractable and at 28 (17-37) days old the cubs were able to walk. The latest changes that occurred were the complete raising of the ears, at 32 (25-38) days old, and the eruption of the premolars, at the age of 37-38 (33-40) days. The mean age, standard error, range and sample size for each landmark are summarized in Table 1.

Growth Curves

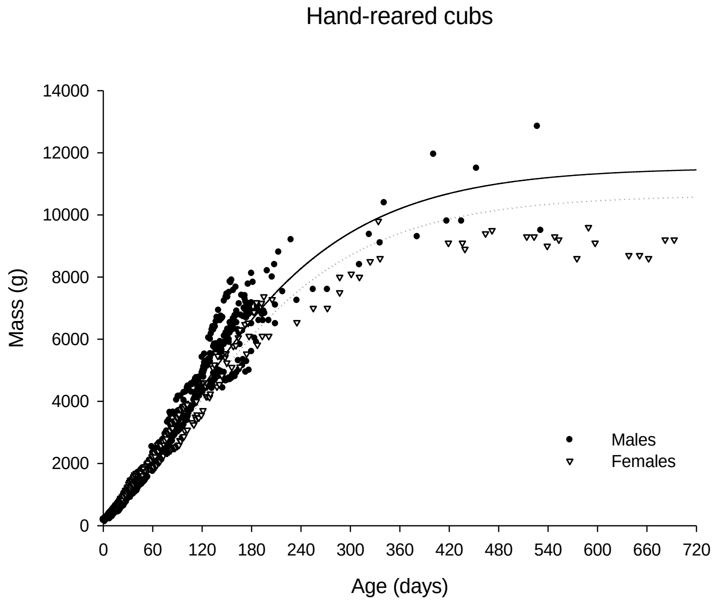
The von Bertalanffy model provided the highest asymptotic adult mass (A=11,123.28 g) and the lowest age at the inflection point (I=92.99 days) and growth rate constant (K=0.008). The logistic model estimated the lowest asymptotic adult mass (A=9,874.37 g) and the highest age at inflection point (I=135.01 days) and constant growth rate (K=0.019), and the Gompertz model estimated intermediate values for all parameters of the three models (A=10,804.7, I=109.96, K=0.011). The von Bertalanffy growth model was the best for describing growth in Iberian lynx based on the AIC (von Bertalanffy: 18,689.51; Gompertz: 18,850.43; logistic: 19,674.56).

TABLE 2. Parameters estimations of the final von Bertalanffy model for Iberian lynx growth, differentiated by sex, and rearing method.

		A	SE	K	SE	I	SE	N
Males								
	Mother-reared	11,526.21	349.85	0.008	0.0001	89.804	15.582	9
	Hand-reared	11,526.21	349.85	0.008	0.0001	95.098	21.164	11
Females								
	Mother-reared	10,655.614	2,822.943	0.008	0.0001	89.804	15.582	13
	Hand-reared	10,655.614	2,822.943	0.008	0.0001	95.098	21.164	2

Parameters: A, asymptotic adult mass (g); K, growth rate constant (day^{-1}); I, age at the inflection point (day). N is the sample size of each group.

When sex and rearing method were included in the model as fixed factors, the only significant effect was those of sex on asymptotic adult mass (t=2.54, P=0.011, df=1,294) and rearing method on the age at the inflection point (t=-2.36, P=0.018, df=1,294; Table 2, Figure 1).



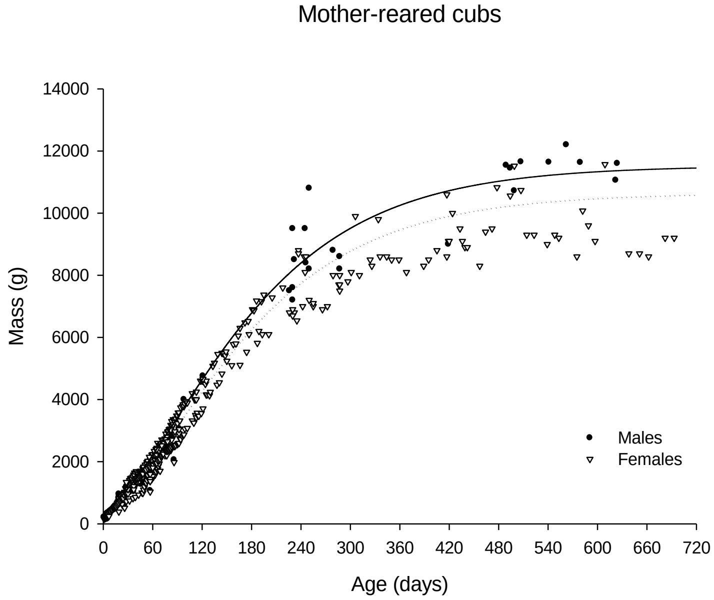


FIGURE 1. Body mass of the Iberian lynx cubs born in the ILCBP with age and growth curves estimated by the von Bertalanffy model, differentiated by sex, and rearing method.

The model estimated a birth weight slightly higher (253.4±12.5 g; range=125-446) than the those measured for eleven neonates (Table 3). The measured birth weight represented 1.48±0.61% of adult weight. According to the estimation of the model, at day 90 males had attained 3.2 and 3.4 kg and females 2.9 and 3.1 kg -hand and mother-reared cubs, respectively- the 27±9% of the adult mass. Mother-reared cubs reached 50% of adult body mass at 155 days old and 95% at 467 days old, while hand-reared cubs attained these body masses at 150 and 462 days old. The highest rate of increase was reached at the age of 90 and 95, mother and hand-reared, respectively (40.98 g/day for males and 37.89 g/day for females), after which growth rate decreased exponentially until the asymptotic mass was reached (Figure 2). Before reaching the maximum growth rate, the hand-reared cubs increased faster than the mother-reared ones. Then, the trend was reversed and the cubs raised with their mothers were the ones that grew faster. After the first month of life, the growth rate of males was higher than females regardless of the rearing method.

TABLE 3. Body mass (mean ± SE) of the Iberian lynx born in the ILCBP at birth, at 30, 60, 90 days old and adult mass.

		Birth mass		**30 days**		**60 days**		**90 days**		**Asymptotic mass**	
Males		X ± SE	N	X ± Se	N	X ± SE	N	X ± SE	N	X ± SE	N
	Mother-reared	215 ± 5	2	778 ± 132	3	1,616 ± 106	8	2,964 ± 180	7	11,300 ± 240	2
	Hand-reared	189 ± 7.3	6	1,092 ± 41	9	2,131 ± 82	9	3,386 ± 125	9	11,567 ± 595	6
Females											
	Mother-reared	157	1	1,087 ± 110	5	1,605 ± 94	11	2,932 ± 169	7	10,495 ± 450	4
	Hand-reared	187 ± 3	2	1,187 ± 73	2	2,075 ± 165	2	3,020 ± 460	2	10,150 ± 750	2

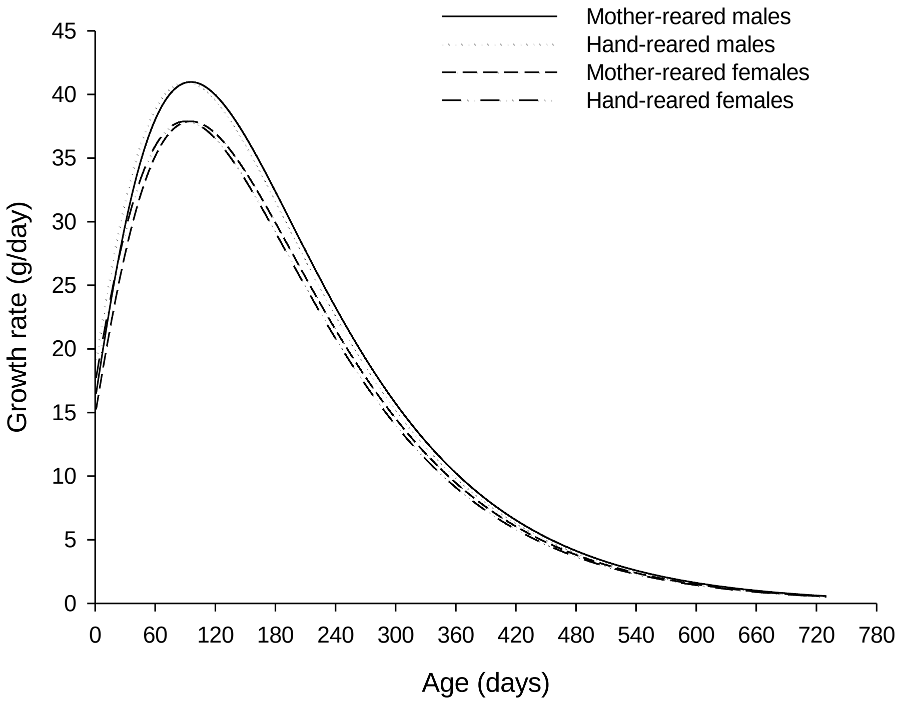


FIGURE 2. Growth rate (g/day) of the Iberian lynx depending on sex and rearing method according to the results of the von Bertalanffy model fit.

DISCUSSION

Early Development

Iberian lynx is a semi-altricial species that has not completed physical, sensory and locomotor development at birth. Therefore, cubs are completely dependent on maternal care after birth. The changes experienced during the first 2 months of life allowed the cubs to acquire the minimal physical skills necessary to begin to interact with the environment.

The third week of life is very important in the development of Iberian lynx cubs, because it is in that moment when eyes and the auditory channels are opened and cubs are able to walk for the first time. Cubs are able to leave the den and explore their environment when they are 1 month old, then starting the period when most of the changes in their behavior occur -for example, they start to play with their siblings and mother, learn to hunt, and gradually establish the typical circadian rhythm of adults.

Iberian lynx cubs open their eyes later than most of the felid species. This is more remarkable when comparing with the other species of the *Lynx* genus, in which the cubs open the eyes at 6 days (*L. rufus*; Hemmer, 1979), 13 days (*L. lynx*; Hemmer, 1979), and 14 days old (*L. canadensis*; Sunquist & Sunquist, 2002). The age at which a species open their eyes may be related to the lighting conditions of the places where the species give birth to their cubs (Deag et al., 1988). The Iberian lynx lives in an environment where the light is very intense throughout the year—in the southwest of the Iberian Peninsula-and usually give birth in bushes or in shallow dens (Fernández et al., 2002), so this delay in opening the eyes could be the result of an adaptation to their environment.

Regarding dentition, the canines erupted before the incisors, and premolars were the last teeth to appear. The eruption sequence for deciduous dentition was different from what has been reported in other felines, in which incisors appear the first, followed by canines and premolars (McClure et al., 1973; Jackson et al., 1988). In none of the Iberian Lynx cubs studied by us did the incisors erupt before the canines, although sometimes both had appeared in the same day.

Growth Curves

The von Bertalanffy curve provided the best fit for the Iberian lynx growth, followed by Gompertz and logistic models. According to Childerhouse et al. (2010), the Gompertz model has been employed especially in those works interested in comparing growth curves between different species because it provides a good fit for a large number of mammals' species. The von Bertalanffy models tend to overestimate the birth weight (Zullinger et al., 1984), as was the case of our Iberian lynx dataset. This overestimation could be the consequence of having a small sample size in early ages (Zullinger et al., 1984), in our case because most of the cubs were mother-reared.

As suspected for a bigger species, *L. lynx* give birth to cubs heavier than Iberian Lynx (245-430 g; Sunquist & Sunquist, 2002). However, *L. rufus* (280-340 g; Lariviere & Walton, 1997) and *L. canadensis* (197-211 g; Saunders, 1964) also give birth to heavier cubs, although adult body masses are similar to that of the Iberian Lynx (Saunders, 1961; Petraborg & Gunvalson, 1962). Growth rate increased during the period the cubs are dependent on their mother for food (lactation, weaning, and first solid food intakes). The inflection point of growth, marking its slowdown, occurred when the cubs started to hunt by themselves, at 3 months old.

There are three main factors that drives to a sexual size dimorphism: differences between sexes in birth mass (Badyaev, 2002), in growth rate (Andersson, 1994) or in the length of the growth period (Stamps & Krishnan, 1997). In the Iberian lynx, the asymptotic adult mass was the only parameter of the curve that was different between males and females -males being 8% larger-, and dimorphism was due to the higher growth rate of males. Similar results were obtained for the *L. rufus* (Crowe, 1975). However, in Eurasian lynx, it seems that the difference between males and females adult body mass are because of males have a longer growth period (Naidenko, 2006). The differentiation in size between sexes in the Iberian lynx occurs gradually along the two first years of life. In fact, it is difficult to distinguish the sex of an individual lynx by its size before adulthood -as it has been reported in the wild; Beltrán & Delibes (1993)-.

An inadequate feeding protocol can slow down the growth and increase the mortality of hand-reared cubs (Beekman et al., 1999; MacRae et al., 2011). Our results showed that the hand-reared cubs grew faster during the artificial lactation and slower when they started to eat by themselves—without the help of a mother—. In any case, the adult weight was not influenced by the rearing method with the feeding protocols followed in "El Acebuche" Iberian Lynx Captive Breeding Center, which can be considered an indicator of its suitability.

CONCLUSIONS

1. Iberian lynx is a semi-altricial species. The cubs are not fully developed at birth.
2. The cubs suffer major physical changes in the first month of life.
3. von Bertalanffy model provides the best fit to the growth of this species.
4. Males grow faster than females and they are larger when they reach adulthood.
5. Rearing method affected the growth rate of the lynx cubs but not the adult body mass.

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CAPÍTULO II. Lactation and Suckling Behavior in the Iberian Lynx

Yerga J, Calzada J, Manteca X, Herrera I, Vargas A, Rivas A. Lactation and suckling behavior in the Iberian lynx. Submitted to *Zoo Biology*.

Abstract

Understanding the behavior of endangered species is crucial to improve the management tools to breed animals in captivity and, thus, to increase the success of ex-situ conservation programs. In this study, we monitored suckling behavior of 26 cubs born between 2008 and 2012 at "El Acebuche" Iberian Lynx Breeding Centre. The cubs devoted 251 ± 19.7 min (mean ± SE) to lactation on the day of birth, while mothers spent 426 ± 27 min (mean ± SE) nursing their offspring. The time cubs spent suckling decreased exponentially as they grown, until they were fully weaned at 65 ± 2.6 days. The onset of weaning (first intake of solid food) occurred at 54 ± 1.35 days (mean ± SE). Thus, the strict lactation period occupied most of the overall lactation period. Both suckling and maternal behavior were affected by litter size. In twins and triplets, the competition between siblings caused a decrease in the time spent suckling, in spite of the mothers spending more time nursing their young. Finally, no significant differences were found in time spent suckling between littermates or depending on the sex of the cub. Lactation appeared to play a key role in the nutrition of the Iberian lynx and should therefore be conveniently managed in captive breeding programs of this threatened species.

Keywords: captivity, litter size, maternal behavior, sex.

INTRODUCTION

Lactation is the key defining characteristic of mammals. It has several functions besides providing nutrition and immune protection to the young after birth (Hausberger, 2007; Chucri et al., 2010). Moreover, the role of lactation is not uniform in all mammalian species and the composition of milk varies according to the predominant role that lactation plays in the development of the offspring (Hayssen, 1993). For example, in precocial, monotocous and social species -such as most of the ungulates- the nutritional role of the milk has a minor importance -precocial newborns can feed on solid food early after birth- and lactation brings its greater benefits in maintaining contact between mother and the young and to learn the social behaviors that are necessary for group living (Hayssen, 1993). On the other hand, in altricial and polytocous species, it prevails the energetic role of the milk (Hayssen, 1993), especially in carnivores, in which the young are not able to hunt until they reach to advanced stages of development, so their nutrition after birth is highly dependent on the milk intake.

On the other hand, in polytocous species, lactation is the first competitive situation that a cub faces from birth. Competition during lactation is strongly related to litter size. It has been shown that an increment in litter size causes a higher competence between siblings in the access to the teats, which sometimes can result in an increased mortality in newborns (Andersen et al., 2011).

Lactation also greatly changes the physiology and behavior of the females of mammals. Maternal behavior is modified to get the energetic requirement that females needs to care and feed its litter, and these requirements are according to the size of the offspring (Guerra & Nunes, 2001). The mother has to devote more time in feeding and drinking herself (Priestnall, 1972), and/or it spends more time nursing the litter to offset the effect of larger litters (Schneirla et al., 1963; Caro, 1994).

After a period of strict lactation, growing mammals begin to ingest solid food while they are still being feed with milk. This is the weaning process (Martin, 1984), that ends once the cubs have finished lactation altogether. Weaning involves many changes in physiology, behavior and nutrition of the young (Galef, 1981). The digestive system of the young has to adapt to the new diet and, in some cases, they must learn to behave in a new way to get the food, as the predators that must learn how to hunt (Langer, 2003) and gradually, the independence of the cubs from the mother increase (Martin, 1986).

Little is known about suckling behavior and the development of the critically endangered Iberian lynx (*Lynx pardinus)*. Iberian lynx females in the wild give birth between March and April (Palomares et al., 2005). Mean litter size is three but could range from one to four (Fernandez & Palomares, 2000). Kittens are semi-altricial (Yerga et al., 2014). They are born with the body covered with downy fur, with the senses of hearing and sight undeveloped and unable to walk. Lynx females raise their cubs on their own (Ferreras et al., 1997), until the cubs disperse. The Iberian lynx is a trophic specialist that during the adult stage feeds preying on rabbits (*Oryctolagus cuniculus*; Delibes, 1980).

Studies on lactation of wild species are limited because of the difficulties to record this behavior without disturbing the mother and cubs, and especially when studying carnivores in their habitat, because they generally give birth in dens where the cubs are hidden (McGuire et al., 2011). The Iberian lynx is one of the rarest cats in the world, with only 305 individuals in wild (Simón, 2012). Due to the threatened status of Iberian lynx populations, it was necessary to start a Captive Breeding Program to support in-situ conservation efforts (Vargas et al., 2008). Keeping Iberian lynx in captivity facilitates the study of this species to improve the understanding of its behavior.

In this paper we aim to describe the suckling behavior in this species, to determine the duration of strict lactation and weaning periods, to know how sex and litter size affect the time spent suckling by the cubs, to study variations in maternal behavior depending on the litter size and to explore the differences in suckling behavior between littermates. We expect to establish the role of lactation in this species to increase the understanding of the suckling behavior and improve the captive management of this endangered species. The results can serve as a basic knowledge that can help for early detections of possible anomalies in suckling behavior, that may be indicators of disease or starvation and that could jeopardize the survival of cubs in captivity.

MATERIAL AND METHODS

The suckling behavior of 26 captive-born Iberian lynx cubs was studied at “El Acebuche” Iberian Lynx Breeding Centre (37º0’ N 6º33’ W) in Doñana National Park, Spain. Cubs were born between 2008 and 2012 belonging to 13 litters and six different mothers. The study included litters of one (n=4), two (n=5) and three (n=4) cubs.

Each mother with its litter was housed in a separate enclosure of 550 m^2. The facilities included two dens and a main outdoor area enriched with Mediterranean vegetation, resting structures, visual barriers and other elements similar to those that can be found in their natural habitat. Behavioral recordings were made through a video-surveillance system. There were six cameras in each enclosure, two dome robotized cameras in the outdoor area, and four fixed cameras in the dens. The cameras were operated from a room located 150 m away from the enclosures. This system allowed for recording lynx behavior without disturbing the animals.

Cub behavior was recorded continuously (Altmann, 1974) and time spent suckling each day (in minutes) was obtained for the entire lactation period. Time spent suckling was recorded as time elapsed from the moment a cub attached the nipple until it finished suckling by separating its mouth from the nipple. Maternal investment along lactation was registered as time spent nursing by a mother each day in minutes. The lactation period was considered the time period between birth and the first day that cubs did not suckle, and the weaning period was defined as the time between first intakes of solid food until the end of the lactation period. A total of 7192 hours of video were recorded for this study.

The video-surveillance system did not allow the correct individual identification of cubs by the pattern of spots during the first month of life because they are born covered with a uniform gray, downy fur without the typical spots of the species (Yerga et al., 2014), so the cubs had to be marked with a dye (Nyanzol D Powder®, Greenville Colorants, L.L.C., Jersey City, NJ, USA). The marking was performed at 3 weeks of life to prevent disturbances to the mother and reduce the probability of abandoning the cubs. Thus, it was impossible to discriminate between siblings in multiple litters during the first three weeks of life. Consequently, the time that each cub spent suckling each day during the first three weeks of life was calculated as the addition of the total time that all cubs of a litter spent suckling, divided by the number of cubs. At three weeks old, cubs were marked and sexed and from then on suckling behavior of each cub could be individualized until the end of the study.

A linear mixed model was conducted to analyze the development of time spent suckling over lactation period and the influence of litter size. Age and litter size were included as fixed factors and individual as a random factor to account for non-independence data from the same cub. Subsequently, gender was added in the model as a fixed factor to evaluate its effect. In this case, only data from the cubs that could be individualized was included (i. e., from the third week of life onwards, see above).

We developed another model to evaluate the maternal investment in lactation and the effect of litter size on the time the mothers spent nursing their litters. In this model, cubs age and litter size were included as fixed factors and mother plus year as random factors. All linear mixed models were performed with the "lme4" package in R 2.15.1 (R Development Core Team, 2008). Tukey *post-hoc* tests were conducted when it was necessary to assess differences between litter sizes using "multcomp" package of R Software. The dependent variable was log-transformed to meet normality criteria. Finally, a non-parametric Kruskal-Wallis test was conducted in four multiple litters to assess the existence of differences between siblings. The other multiple litters were not marked and sexed during lactation to avoid abandonments and thus could not be analyzed. The α-value was 0.05 in all cases.

RESULTS

Iberian lynx offspring began to suckle right after birth, even before the birth of the next sibling in multiple litters. Cubs moved themselves up to the mother's nipples. The role of the mother was limited to adopt a position that facilitated the access to the nipples and to keep the cubs close to her, if accidentally one walked away. When cubs were suckling, they often performed a massage on the breast area with the front paws. In multiple litters, it was common to have more than one cub suckling at the same time. However, a strong competition was observed between siblings few days after birth. This was not due to an insufficient number of nipples, since lynx females of lynx have four pairs.

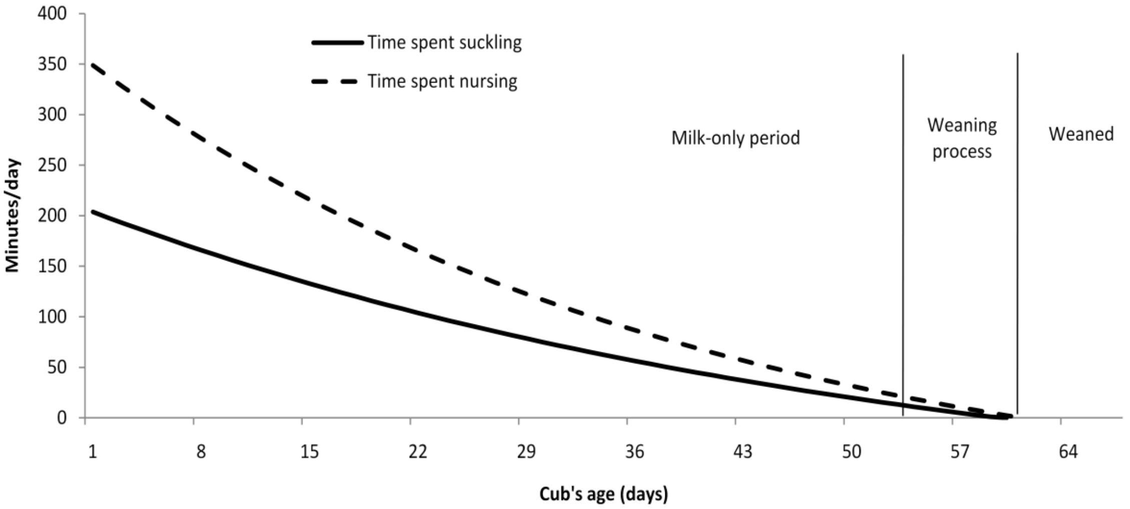

FIGURE 1. Time that a cub and a mother devoted to suckle and nursing, respectively, each day from birth until weaning.

The linear mixed model showed that age influenced the time the cubs spent suckling (t=-37.744, p <0.01). From birth until the onset of the weaning, the Iberian lynx cubs spent 8.49 ± 0.86% of the time on feeding. During the first day of life, they were suckling 251 ± 19.7 min (mean ± SE). The amount of time decreased exponentially as cubs grew (Figure 1). Time suckling was reduced by 50% at day 23 and by 75% at day

41. The beginning of the weaning period (defined here as the moment in which the cubs ate solid food for the first time; see above) happened at 54 ± 1.35 days (mean ± SE). At this age, cubs spent 26 ± 7 min on lactation.

Lactation ended at 65 ± 2.6 days, although occasionally we could observe isolated attempts to nurse up to six months of age. The gestation period for Iberian lynx was 64.15 ± 0.3 days (mean ± SE), so the lactation period comprised 50% of the total time of maternal investment (gestation + lactation).

Litter size also affected lactation significantly (Figure 2). Singletons spent more time suckling than twins (z = 2.999, p <0.01) or triplets (z = 3.712, p <0.01). However, there was no difference between the time spent suckling for cubs in litters of twins and triplets (z = 1.616, p = 0.224). There was no difference in time spent suckling between males and females (t = 0.023, p = 0.98), at least from the third week of age onwards (once cubs could be individualized). Finally, there were no significant differences in the time that the cubs of the same litter spent suckling (Table 1).

TABLE 1. Differences in time spent suckling among littermates (Kruskal-Wallis test).

Mother	Year	Litter size	H	Df	p
Aura	2008	2	0.1023	1	0.749
Saliega	2009	3	4.145	2	0.126
Adelfa	2009	3	2.241	2	0.326
Aura	2009	2	0.0312	1	0.860

The time that mothers invested on lactation was highly dependent on the time elapsed after birth (t = -33.865, p <0.01). The mothers spent 426 ± 27 min (mean ± SE) nursing cubs during the first day after birth. Thereafter, they spent less time as cubs grew older (Figure 1), decreasing in a similar way as the suckling behavior of cubs. Time spent nursing was also affected by litter size. When the litter consisted of a single cub, the mothers spent less time than if the litter had two (z = 2.743, p = 0.016) or three (z = 3.543, p <0.01) cubs (Figure 2). Meanwhile, mothers with twins and triplets spent equal time nursing their litters (z = 2.241, p = 0.06).

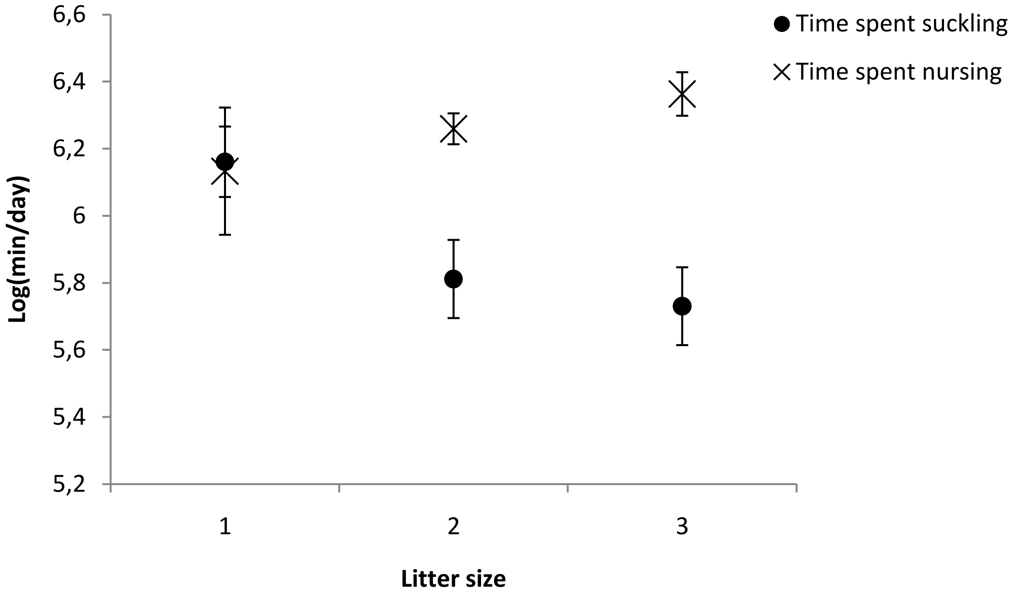

FIGURE 2. Time spent suckling and time spent nursing depending on litter size. Error bars represent SE of the estimation.

DISCUSSION

The high time spent on suckling by Iberian lynx cubs right after birth (up to four hours) reflects the fundamental role that lactation has during the first hours of life in this species. On the early hours of lactation the Iberian lynx, as many other mammals, ingest the colostrum, a form of milk produced in late pregnancy that contains important nutrients and other substances such as antibodies that are necessary for the protection of newborns (Chucri et al., 2010). Malnutrition in early lactation of felids can lead to alterations in brain development and to the exhibition of anomalous behavior in later stages of ontogeny (Bateson, 2000).

The time Iberian lynx spent suckling from birth to weaning is low compared to the domestic cat, which can spend up to 35 % of the time lactating up until the onset of the weaning period (Schneirla et al., 1963). This difference may be due to several factors such as differences in physiology or in the milk composition, although the data from this study do not let to draw any further conclusions.

The decrease in time spent suckling that we observe in Iberian lynx cubs coincides with that observed in other feline species (Pusey & Packer, 1994). Although cubs were completely dependent on milk as the main source of nourishment until the second month of age, they spent less time lactating as age increased, which also coincided with their greatest growth spurt (Yerga et al., 2014). This might seem contradictory, and we speculate that, perhaps, lynx cubs are more efficient with age when they are suckling, and they can ingest more milk in less time. Moreover, it may also be that the milk becomes more nutritious as the lactation period progresses, as has been observed in the domestic cat (Adkins et al., 1997).

The weaning process began on day 54, when the cubs made the first intake of solid food, and by day 65, the cubs were fully weaned. According with Hayssen (1993), in polytocous, altricial species with a short lactation period (less than 50 days), the first intake of solid food often occurs closer to weaning age. In the Iberian lynx, a polytocous but semialtricial species, lactation lasted a bit longer. The first intake of solid food occurred at the last third of the lactation period, i.e. the strict lactation period is proportionally larger suggesting that milk has an essential nutritional role in this species, and opposed to what happens in precocial species. The length of the lactation period is highly correlated with body size in mammals (Hayssen, 1993). Although this correlation could not be fully demonstrated in the *Felidae* family (Hayssen, 1993), it appears to apply to the *Lynx* genus, because the two species that are similar in size to the Iberian lynx, the bobcat (*L. rufus*) and the Canada lynx (*L. canadensis*) wean their offspring at 3 months of age, while in the Eurasian lynx (*L. lynx*), a larger species, cubs are weaned at five or six months of age (Sunquist & Sunquist, 2002).

Litter size is one of the main factors that affect suckling behavior in mammals, causing a drop in the time that cubs spend suckling as litter size increases. This is likely due to a combination of factors, such as competition between siblings and the difficulty of the mother to provide enough milk in some cases (Elwood & Broom, 1978; Mendl 1988; Hinch, 1989). In the Iberian lynx there was a decrease in the time cubs spent lactating in relationship to litter size. The differences appeared only between singletons and litters with more than one cub. Thus, the key seems to be the competition that is set when there is more than one cub in the litter. This competition does not seem to be a result of an insufficient number of nipples in females, as litters rarely have more than 3-4 cubs and females have 4 pairs of nipples, but it appears to be due to competition to monopolize a specific nipple. In the Eurasian lynx, it has been shown that the most dominant cubs tend to monopolize hind nipples, which are known to usually contain higher amounts of milk (Antonevich et al., 2013).

According to Clutton-Brock et al. (1981) and Lee & Moss (1989), often there are differences in the way of lactating between sexes in polygynous species, in which males must compete for females, and a larger size in adulthood could provide an important advantage. But this pattern does not fit the case of the Iberian lynx. Although the Iberian lynx is a sexually dimorphic species, with males larger than females (Yerga et al., 2014), males and females lactated equally. In the Iberian lynx both sexes must compete with other individuals of the same sex during adulthood to achieve and maintain a territory for breeding (Ferreras et al., 1997). Thus, maybe the adaptive pressure to grow occurs with equal force in both sexes, and that would explain the lack of differences in suckling behavior. In addition, size differentiation between sexes in the Iberian lynx occurs gradually. Males have a growth rate consistently greater than females, but during lactation, the weight difference between sexes is not significant (Yerga et al., 2014).

There are not differences in the time spent suckling between littermates. In the Iberian lynx, the cubs go through an aggressive period at seven week of age (Antonevich et al., 2009). During this aggressive period the siblings fight harshly and the first hierarchical relationships are established in the litter. These fights occur shortly before weaning so during most of the milk-only period there is no hierarchy established in the litter that can cause differences in the time that cubs engaged in suckling.

From the perspective of the mother, reproduction implies a very high energetic requirement, which begins from pregnancy and ends once the cubs are weaned. Lactation usually occupies approximately 50% of the total period of maternal investment (Hayssen, 1993), which coincides with that observed in the Iberian lynx. The energetic requirements of lactation for the mother are heavily influenced by litter size, as milk production increases when the litter is larger (Deag et al., 2000). The behavioral response of mothers to different litter size appears to vary between cat species. In lions, the females devote equal time to nurse their offspring regardless of litter size (Pusey & Packer, 1994), but if has been observed that in the domestic cat, the mothers of larger litters devote more time to nurse their kittens (Schneirla et al., 1963). In the Iberian lynx, females spent more time nursing their young when they have two or three cubs, although cubs in multiple litters suckled for less time. One possible explanation is that mothers increase their investment in time nursing the cubs when litter size is larger, but they are not able to fully offset the effect of competition between siblings when accessing nipples.

CONCLUSIONS

1. The lactation occupies much of the time of the cubs after birth, but decreases exponentially as they grow.
2. The main role of lactation in the Iberian lynx is to meet the nutritional needs of the cubs during the first weeks after birth.
3. The intake of solid food begins in the last third of lactation period, i.e. near weaning, so the milk-only period, in which the cubs were fully dependent on milk, takes up most of the total period of lactation.
4. Both suckling and maternal behavior were highly affected by litter size.

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CAPÍTULO III. Ontogeny of Daily Activity and Circadian Rhythm in the Iberian Lynx (*Lynx pardinus)*

Yerga J, Calzada J, Manteca X, Vargas A, Pérez MJ, Palomares F, Rivas A, (2015). Ontogeny of daily activity and circadian rhythm in the Iberian Lynx (*Lynx pardinus*). *Applied Animal Behaviour Science*, 169: 62-68.

Abstract

The aim of this paper is to describe the ontogeny of the circadian activity rhythms in captive-born Iberian lynx (*Lynx pardinus*) from birth to subadult age and to verify if they develop a bimodal circadian pattern similar to the one they show in the wild. The amount of daily activity and the circadian rhythmicity of 61 Iberian lynx (48 mother-raised and 13 hand-reared) cubs were studied in two breeding centers. During the first month of life, the cubs were active 30% of the day. Activity increased up to 50% during the following two weeks, and then it remained constant until the end of the lynx physical development. The location of the breeding center affected the amount of daily activity of the cubs probably as result of different climatic conditions. Once the lynx cubs had completed their development, there was no difference in the time spent active between wild and captive lynx. Newborns had a constant level of activity throughout the day (24 h) during the first month of life. During the second month, once they had developed sufficiently to leave the den, a crepuscular bimodal circadian rhythm started to emerge. This pattern was completely defined by the third month of life. The overlap in circadian activity was over 0.88 when comparing the sexes, breeding centers and rearing methods. However, there was a significant difference between mother-raised and hand-reared cubs -the latter being more active in the morning-, as well as between breeding centers -again due to climate differences. Finally, an overlap of 0.85 in circadian activity pattern was observed between wild and captive lynx, although the wild animals were more active at night.

Keywords: circadian rhythm, daily activity, Iberian Lynx, ontogeny, reintroduction.

INTRODUCTION

Circadian rhythmicity is present in various physiological mechanisms of animals. It affects sleeping and feeding patterns (Borbély & Neuhaus, 1978), as well as body temperature, brain activity and the production of hormones (Albrecht & Eichele, 2003). These rhythms have an endogenous time period, called free-running period, which lasts about 24 h, but they can be modified by external environmental factors (Aton et al., 2004). Daylight is the main zeitgeber or synchronizer of circadian rhythmicity (Kavanau & Ramos, 1975). Within these rhythms, the circadian rhythm of activity is one of the main adaptations of species to their environment (Daan & Aschoff, 1975). In predators, interspecific relationships between them and their prey play a fundamental role in the modeling of the activity rhythms (Halle, 2000; Lima, 2002), given that most predators must adapt their activity to that of their prey to improve their predatory efficiency (Monterroso et al., 2013). However, circadian activity rhythms can be modified by the combination of various environmental factors such as light (Pittendrigh, 1993) and temperature (Palkova et al., 1999; Kolbe & Squires, 2007).

Wild animals living in captivity often suffer disturbances in both their behavior and their activity rhythms (Berger, 2011). The captive environment may be very different to the wild one. The lack of space and natural stimuli, as well as captive husbandry practices, may hamper the development of the normal behavior of the species. A familiar example is provided by the artificially altered endogenous activity rhythms of carnivores as a result of the way they are fed, namely with dead meat (i.e. they cannot develop hunting behaviors) and on a fixed time schedule during the working hours of the keepers (Shepherdson et al., 1993). The difficulties in developing natural behaviors in captivity may lead to a reduction in animal welfare and to an increase in the prevalence of diseases (Munson et al., 2005) and stereotypical behaviors (Shepherdson et al., 1993; Carlstead, 1996), such as pacing (Clubb and Vickery, 2006). Preventing these negative effects is always important for ethical reasons, but even more so when the goal of breeding animals in captivity is to reintroduce them into the wild. Animals that are being breed for reintroduction programs should be housed in well-enriched and spacious enclosures and husbandry practices must strive to prevent the modification of circadian cycles of activity, as released animals must have their activity pattern synchronized with that of species in the wild. And in the case of predators that actively chase their prey, they have to be able to hunt, and particularly during the time of day that prey are active, i.e. available, in the wild (Eriksen et al., 2011).

The Iberian lynx, *Lynx pardinus*, is a wild felid species that is currently being breed for reintroduction in Spain and Portugal. The objective of the Iberian Lynx Captive Breeding Program (hereafter ILCBP) is to breed physically and behaviorally healthy lynx, which can reinforce wild populations (Vargas et al., 2009). The ILCBP lynx intended for reintroduction are subadults (8–20 months). The Iberian lynx is a specialist predator whose circadian rhythm is synchronized with the activity of its main prey, the rabbit (*Oryctolagus cuniculus*; Delibes, 1980). Both rabbits and lynx are active primarily at night and during twilights (Beltrán, 1988; Jilge & Hudson, 2001).

The Iberian lynx is a semi-altricial species whose young are born with poorly developed sensory and locomotor systems (Yerga et al., 2014). The pattern of circadian activity is not usually present in altricial species at birth (Ibuka, 1984; Kowalska et al., 2010). Rhythms typically appear days or weeks after birth, once the cubs leave the den and are exposed to local environmental factors such as the daylight cycle and temperature. Furthermore, mothers can act as synchronizers of the circadian behavioral rhythm of their young (Favreau et al., 2009).

The aim of this paper is to describe the ontogeny of the circadian activity rhythms in Iberian lynx born as part of the ILCBP from birth to subadult age and to verify if they develop a bimodal circadian pattern similar to the one they will need in the wild.

MATERIAL AND METHODS

The activity of 61 Iberian lynx (36 males and 25 females) born in two specific breeding centers, “El Acebuche” (hereafter EA, 37º03’00”N 6º33’17’’W) and “La Olivilla” (hereafter LO, 38º20’37’’N 3º33’33’’W), both located in Andalusia, Spain, was studied. The cubs were born between 2008 and 2011 and derive from 28 litters from 13 different mothers.

Housing conditions and protocols for handling animals were similar in the two breeding centers. Each mother and its litter were housed in a big and enriched enclosure. The size of the enclosures varied between the breeding centers (550 m^2 in EA and 1000 m^2 in LO). In both breeding centers, the facilities included a main outdoor area enriched with the typical vegetation of the Mediterranean habitat, sleeping structures, two or three dens, burrows where rabbits were released (see below), and visual barriers that allowed the lynx to hide from the view of keepers. The husbandry of the animals was planned so as to minimize disturbances to the animals and to avoid imprinting on humans as far as possible. Sometimes cubs were abandoned by their mothers and had to be hand-reared (13 cubs in the study). Those hand-reared cubs were kept in an incubator room during the first month of life. Later these animals were transferred to the enclosures and housed alone (i.e. without their mothers and brothers), where they were bottle fed for one additional month. At two months of age the cubs were weaned, and from that age onwards both mother-reared and hand-reared cubs were fed live wild rabbits. In order to stimulate behaviors similar to those exhibited by lynx in the wild, the animals were fed, as far as possible, with wild rabbits released in the burrows throughout the day.

Sampling Method

The activity of the cubs was recorded by a remote video surveillance system. It was considered that an animal was active when it was awake. The enclosures had 2–3 mobile cameras and an additional fixed camera in each den. All cameras were equipped with infrared lights that allowed the monitoring of the cubs 24 h a day. The video surveillance system was continuously operated (24 h) by trained observers from a room located 150 m away from the enclosures. Thus, it was possible to record activity throughout the day without disturbing the lynx.

The activity of the cubs was tracked between birth and 7 months of age using a point sampling method. The interval of recordings was predefined. The activity of each cub was recorded every 20 min in 2008 and 2009, and every 60 in 2010 and 2011, 24h a day, three to seven days a week. In total, 139,897 activity records were obtained, and the activity of each cub was documented between 168 and 216 times per week. The potential bias due to the amount of activity records obtained by lynx in a time interval was considered for the analysis of the activity.

In order to establish whether the amount of time that the captive young lynx remained active, and the activity pattern were similar to that observed in nature, the activity of captive lynx was compared with the activity of young and subadult lynx monitored in a long-term study on the ecology of the Iberian lynx performed in the north of Doñana National Park between 1993 and 1996 (Palomares et al., 2001), 15 km from the EA breeding center. In that study, 26 lynx were caught and radio tracked an average of 274±60 days, of which 18 (10 females and 8 males) were non-adult lynx. All lynx were fitted with 150–151 MHz radio-collars (Wildlife Materials, Inc., Carbondale, Illinois) which contained tip switches to monitor activity. The activity of each lynx was recorded an average of 10 times a week (range 1–59), including day and night locations. In addition, 11 of these animals were followed in 20 intensive 24 h monitoring sessions, where the animals were located and their activity recorded once per hour, beginning at 12:00 h of a given day and ending at 12:00 h of the next day (see details in Palomares et al., 2001).

Statistical Analysis

Daily activity

The daily activity (estimated as the proportion of daily records where the lynx were active) was calculated and the influence of age (in weeks), sex, rearing method (hand or mother-reared) and breeding center (EA, LO) was evaluated through a generalized linear mixed model (GLMM). This method is suitable for repeated measures data and provides estimates of the parameters for each level of the random effects. Sex, rearing method, breeding center and age were included as fixed factors and the individual lynx and the number of activity records registered by lynx as random factors to account for the non-independence of the data from the same animal and the variations in the number of records obtained by lynx in a time interval. A model for each of the possible combinations of fixed effects was constructed. The set of candidate models were ordered according to the AIC value. Analysis was performed using the 'lme4' package of R software (R Development Core Team, 2008). Finally, an ANOVA-test was performed to assess differences in the daily activity of the captive-born cubs and non-adult wild lynx. Normality was tested before performing the analysis. In all cases, α-value was 0.05.

Circadian rhythm

The circadian activity rhythm of each lynx cub, estimated as the proportion of active records in every hour in each month, was calculated. It was verified that the number of activity records registered per animals did not alter the proportion of activity in this analysis (Mardia-Watson-Wheeler test, W= 5.0968, df = 2, p = 0.078). The effect of age (in months), sex, breeding center, rearing method and birthplace (captivity or wild) on the circadian rhythms of the cubs was evaluated. Pairwise comparisons of the activity rhythms between sexes, breeding centers, rearing methods and birthplace were performed by estimating the overlap coefficient Δ_4, as suggested by Ridout and Linkie (2009) for large sample sizes. This overlap coefficient, which can have values between 0 (no overlap) and 1 (complete overlap), was obtained by taking the minimum of activity of the two groups at each time of day. The accuracy in the estimation of Δ_4 was obtained by calculating the standard deviation of 500 bootstrap samples.

However, the overlap coefficient is solely descriptive, and thus it does not provide a threshold that can determine the existence of significant differences between the compared groups. Thus, a Mardia-Watson-Wheeler test (MWW test; Batschelet,

1981) was performed to compare the distribution of circadian activity. This test combines the activity data and orders them in increasing angles, so that they are distributed interspersed around the daily cycle, and a uniform, or circular rank, score is then calculated. If the distribution of samples is the same, the resulting vector will be short and similar. Significant differences between the lengths of the vectors lead to a high value of the statistic W and the rejection of the null hypothesis that the distributions are equal (Kovach, 2011). All analyzes were performed using R (R Development Core Team, 2008) software, including the package 'circular' for the MWW test.

RESULTS

Daily activity

During the first five weeks of age, while the cubs remain in the den, they were active around 30% of the day (Figure 1). Afterwards, between the sixth and the ninth week of life, there was a progressive increase in daily activity from 30% to 50%. And finally, daily activity remained at 50% from the ninth week of age to the end of the study (at seven months old). A slight decrease in the amount of activity occurred between weeks 21 and 25 (during the summer, Figure 1).

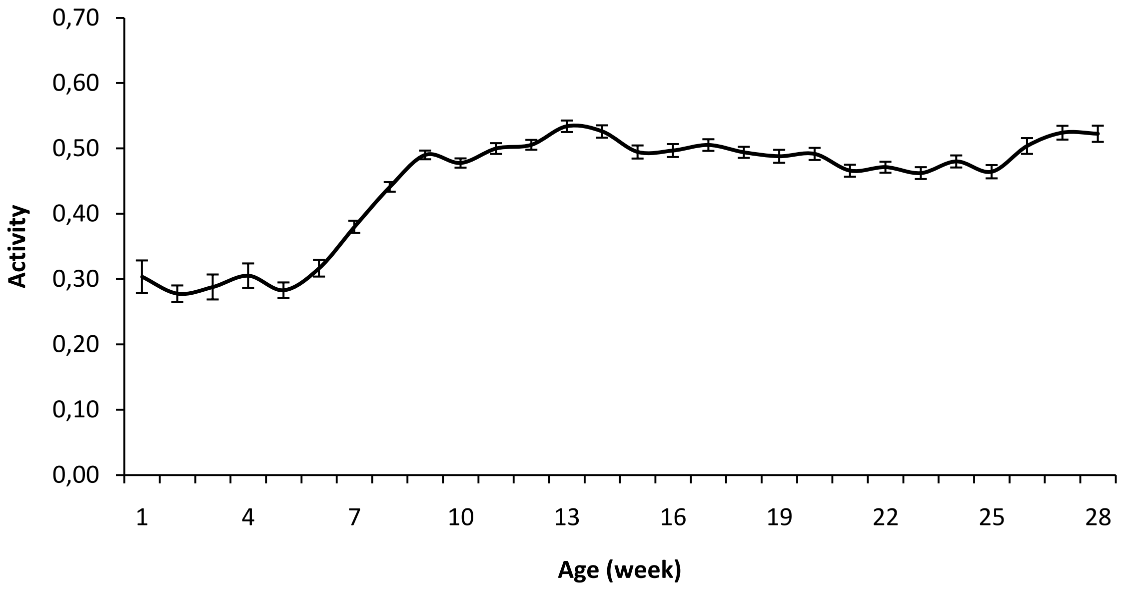

FIGURE 1. Daily activity (proportion of daily records where the lynx were active, mean ± SE) of the Iberian lynx cubs born in captivity between birth and seven months old.

The model that best explained (i.e. had the lowest AICc) the daily activity of the Iberian lynx cubs included age and breeding center as explanatory variables (Table 1). The daily activity of lynx changed significantly with the age ($z = 10.926$, $p < 0.01$), and it was different for each breeding center ($z = 5.663$, $p < 0.01$). Cubs born in LO displayed less activity than those born in EA (5 ± 1%; mean ± SE). No significant differences between mother-reared and hand-reared cubs were found ($z = 1.353$, $p = 0.202$), nor between males and females ($z = 0.594$, $p = 0.745$). Daily activity of wild lynx was 45 ± 5%. Only the daily activity of cubs over 9 weeks was used to assess differences with the daily activity of wild animals. No significant differences were found comparing the daily activity of captive and wild lynx (ANOVA, $F = 0.94$, $p = 0.336$).

TABLE 1. Comparison of the different models for describing the ontogeny of the daily activity of Iberian lynx cubs.

Model	df	logLik	AICc	ΔAICc	W
A + BrC	5	2,959.81	-5,909.61	0	0.94
A + BrC + ReM	6	2,957.22	-5,902.42	7.19	0.03
A	4	2,954.91	-5,901.81	7.8	0.02
A + BrC +S	6	2,956.34	-5,900.66	8.95	0.01
Null model	3	2,902.44	-5,798.88	110.73	0

A is the age, BrC is the breeding center, ReM is the rearing method and S is the sex. Degrees of freedom (df), log-likelihood (logLik), AICc (corrected Akaike's Information Criterion), AICc (difference between the AICc of a model and the lowest value of AICc for the set of models) and Akaike weight (W) for each model is provided. The table includes those models in which AICc < 10 and the null model.

Circadian Rhythm

The evolution of the circadian pattern of lynx cubs born in captivity is summarized in Figure 2. During the first month of life, the activity of cubs was quite uniform throughout the 24 h day, varying between 19 ± 3.2% at 20:00 h and 39 ± 6.2% at 12:00 h. In the second month of life, a bimodal pattern emerged. This coincided with the first time the cubs left the den (at 33 ± 0.3 days old, mean ± SE). Activity increased at both dawn and dusk with peaks at 6:00 h (58 ± 2.4%) and 19:00 h (73 ± 2.9%). In the evening, the activity peak was shorter and higher than at dawn, when activity lasted longer but never surpassed 60%. From the third to the seventh month of life, the bimodal activity pattern was fully established. The maximum activity peak at dawn and at dusk exceeded 80% (83 ± 2.2% between 4:00 h and 5:00 h and 85 ± 1.5% between 18:00 h and 19:00 h, respectively) and activity at midday was reduced (below 22% from 12:00 h to 14:00 h).

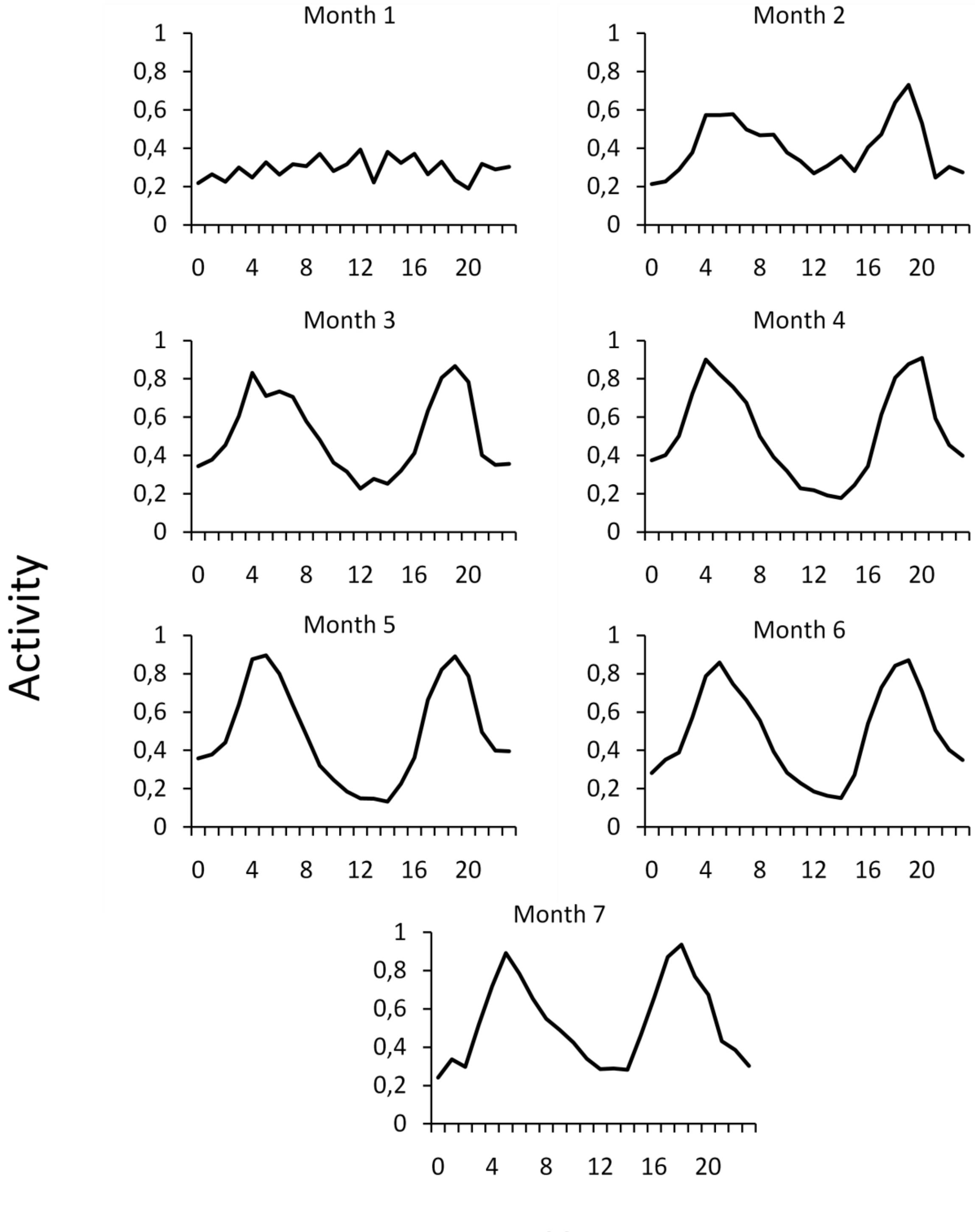

FIGURE 2. Circadian activity pattern of Iberian lynx cubs born in captivity during the first seven months of life. Activity is presented as the proportion of records cubs were active for each hourly interval.

Males and females lynx exhibited a similar circadian rhythm pattern (Figure 3a), with a Δ_4 overlap coefficient of 0.96 ± 0.006. The MWW test showed no significant differences in the distribution of activity between sexes throughout the day (W= 1.93, p = 0.381). On the other hand, there were significant differences in the activity circadian pattern depending on the rearing method (MWW test; W= 43.73, p < 0.01) and the breeding center (MWW test; W= 10.92, p < 0.01), although both Δ_4 overlap coefficients were high (0.88 ± 0.016; 0.90 ± 0.015, respectively). At night, hand-reared cubs exhibited less activity than those reared by mothers. At dawn, the latter developed their maximum activity at 4:00 h, while hand-reared cubs had their maximum at 6:00 h. During midday, the activity of mother-reared cubs decreased quickly and stayed below the activity of hand-reared cubs until the dusk peak of activity, in which the groups displayed a similar level of activity (Figure 3b). The activity of the cubs born in LO was less than that of EA cubs for much of the day (from 9:00 h to 24:00 h; Figure 3c).

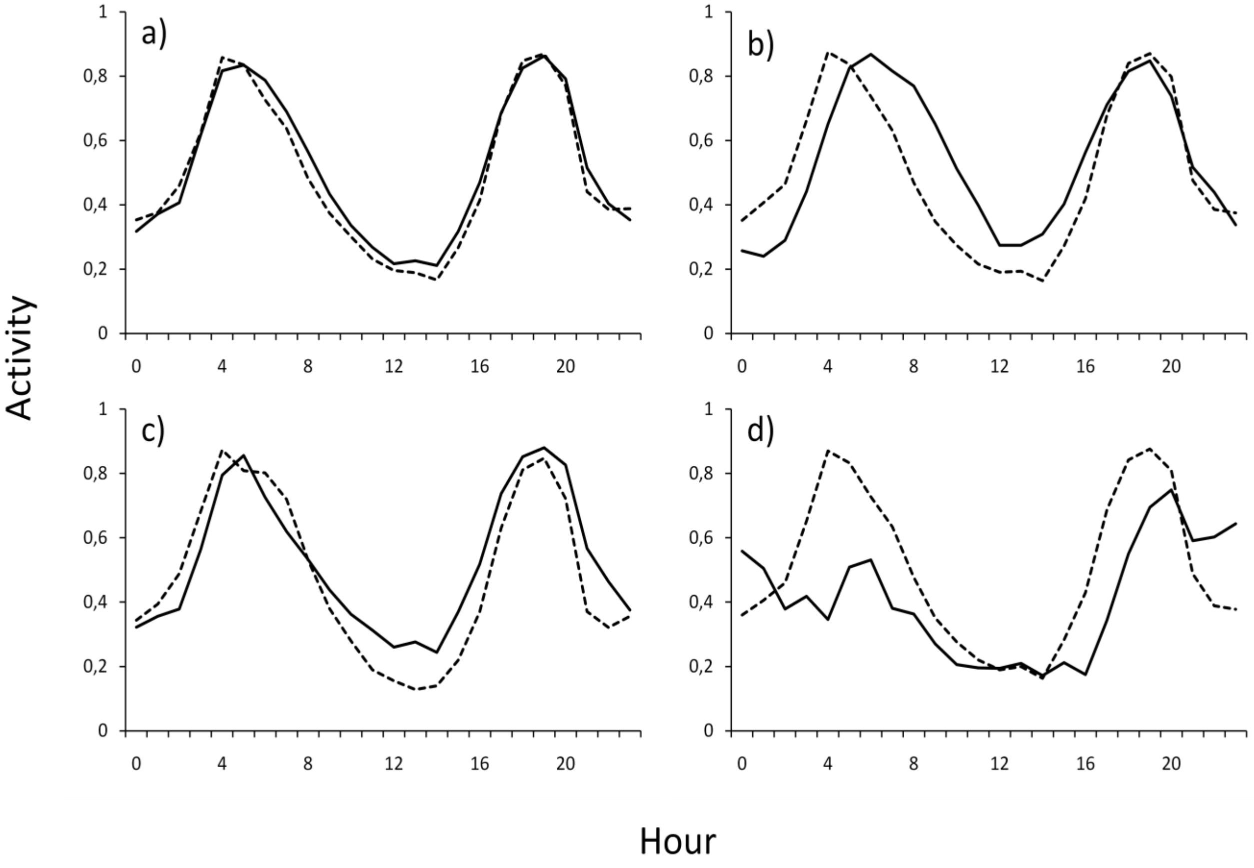

FIGURE 3. Pairwise comparison of circadian activity rhythms. Activity is presented as the ratio of time that the animals were active: (a) males (solid line) vs. females (dashed line), (b) hand-reared (solid line) vs. mother-reared (dashed line), (c) EA (solid line) vs. LO (dashed line), (d) wild (solid line) vs. captive (dashed line).

Only the activity pattern of mother-reared cubs was compared with the circadian rhythm of non-adult wild lynx (Figure 3d), because hand-reared cubs were not intended for reintroduction. Wild animals were more active at night than captive animals (around 60% between 21:00 h and 1:00 h). After that, the activity of the wild lynx decreased, until it increased again just before the dawn. This peak reached 53% of activity at 6:00 h, being much lower than the maximum observed in captive animals. During midday activity remained low in both groups (about 20%). During the dusk, captive animals began to increase their activity earlier than wild animals and they reached a higher maximum value. However, the peak of activity was also observable in wild animals. The maximum was reached at 20:00 h with 75% of activity. The Δ_4 overlap coefficient was 0.85 ± 0.016, but the difference was significant (MWW test, $W= 30.09$, $p < 0.01$).

DISCUSSION

Daily Activity

Daily activity of captive-born Iberian lynx cubs varied during their development. Changes in Iberian lynx activity occurred in a similar manner to that observed in the domestic cat (Hoppenbrouwers & Sterman, 1975), although cats develop slightly faster. This variation was closely related to the sensory and locomotor development of cubs and with their ability to interact with their environment. During the first month of life, while the cubs remained in the den, they were active 30% of the day. During this time, the sensory and locomotor development of lynx cubs was low (Yerga et al., 2014), so their ability to respond to external stimuli was diminished. During this first month of life, nursing was the only activity that cubs displayed, as they remained asleep the rest of the day.

A second period in the ontogeny of the activity of the lynx, between the six and the eight weeks of age, can be described. The beginning of this period coincided with the first explorations outside the den. At this age the cubs had reached a weight of 1 kg and were able to walk, and the senses of sight and hearing were fully developed (Yerga et al., 2014). Cubs began to exhibit new behaviors, like playing and exploring. The time that they spent engaging in these new behaviors led to an increase in activity from 30% to 50%. A third period in the ontogeny of the activity of the Iberian lynx cubs began at 9 weeks of age. Cubs honed their motor skills, spent more time playing and exploring the environment, lactation ended and they learned to hunt. The activity of the cubs then remained around 50% for a number of weeks. The slight decrease of activity observed between weeks 21 and 25, which correspond to the warmest period of the summer, was probably due to an increase in temperature that typically leads to a decrease in the activity of lynx (Beltrán & Delibes, 1994; McNab, 1970).

Even though hand-reared cubs were raised in a very different environment during the first month of life and they were subsequently housed alone, their level of activity was similar to that of cubs reared with their mother and siblings. We did not study whether there are specific differences during development in the type of behaviors they performed by the cubs when they are active, but from the second month of life, we would expect hand-reared and mother-reared cubs to show a different ethogram, as the former cannot perform some behaviors (e.g. social interactions). It would be necessary to study more thoroughly the behavior of both groups to assess how hand-reared cubs modify their time budget.

The effect of sex on daily activity has been studied in adult mammals, but not in cubs. It seems that differences in daily activity between sexes are related to variations throughout the year that depend largely on differences in behavior between breeding and non-breeding seasons (Schmidt & Nakanishi, 2009). Therefore, it is expected that this effect does not occur in animals that have not reached sexual maturity. In the Iberian lynx, adult males are slightly more active than females, both in captivity (Penabad et al., 2012) and in the wild (Beltrán, 1988), but the differences are not significant. The animals in our study were juveniles, in which sex specific behaviors are still not observed, thus consistent with the absence of differences in daily activity.

The differences in daily activity between the breeding centers are probably related to the climate of each site. Cubs reared in LO spent less time active than those born in EA as the average daily and maximum temperatures are higher in LO during summer (García, 2011).

Oftentimes, captive felids are generally less active than their wild conspecifics, which is probably due to the fact that the lack of stimuli in the enclosures does not allow them to carry out all of the typical behaviors of the species (Weller & Bennett, 2001). However, the activity of captive Iberian lynx, once it was stabilized after 9 weeks, was similar to that observed in the free ranging Iberian lynx. Anyway, a further study would be necessary to know if the type of behavior they perform when they are active are similar in both groups.

Circadian Rhythm

The development phases of the circadian pattern in the captive-born Iberian lynx cubs coincided with the development of the amount of daily activity. The lack of a defined pattern of activity in newborns has been widely observed in other altricial species (Ibuka, 1984). The appearance of overt rhythms depends on the developmental stage (Vargas & Anderson, 1998; Weinert, 2005). Iberian lynx cubs had not developed their sensory and locomotor systems and the main zeitgebers, namely light and temperature, did not have any influence inside the dens. In addition, mothers nursed the cubs regularly for 24 h a day, so that the activity of the cubs was almost constant throughout the day. Once the cubs were out of the den, they were subjected to the environmental factors that regulate the circadian cycle and they gradually established the characteristic bimodal pattern of the species.

The overlap of circadian rhythms obtained in pairwise comparisons by sex, rearing method and breeding center were very high and the bimodal pattern of circadian activity was maintained in all groups. However, some significant differences were observed. The peak of maximum activity at dawn occurred two hours later in hand-reared cubs than in mother-reared ones. This may be due to the fact that the hand-reared cubs had imprinted on humans. During the morning, keepers performed the tasks of cleaning and maintenance in the area where the enclosures were located. Their presence was a positive stimulus for these cubs, so hand-reared cubs spent more time active trying to interact with the keepers. On the other hand, mother-raised cubs had little contact with keepers during development, so humans were a negative or at least neutral stimulus, and during the morning these cubs were resting. As occurred with daily activity, the effect of the breeding center is likely to be related to the higher temperatures recorded at LO (García, 2011), which resulted in the lower activity, from midday until night, of cubs born at this center.

The comparison of activity patterns between wild and captive lynx has some limitations. The way the data was recorded for the two groups is markedly different, the records were made in different years with different weather conditions, and also the wild lynx had to adapt their activity to a changing and less predictable environment, especially concerning the availability of food. Wild lynx were more active at night, as are rabbits (Monterroso et al., 2013), their preferred prey. Despite all this, there was a bimodal pattern in wild and captive lynx, both exhibiting peaks of activity at dawn and at dusk. This is consistent with numerous studies which have shown that the circadian rhythm is regulated by an endogenous component (Aschoff, 1960; Minors & Waterhouse, 1984), apart from environmental factors.

Many studies have found that captive animals often develop a modified circadian cycle and exhibit less activity than those who live in the wild (Weller and Bennett, 2001; Berger, 2011). However, our results showed that, once the lynx cubs had completed their development, the time they remained active and the daily distribution of this activity over a 24 h period was similar to that observed in free-living adults. This is probably related to the type of husbandry and the size and level of enrichment of the enclosures. The husbandry of the animals was minimized to reduce the influence of human presence on their behavior. The enclosures far exceeded the minimum size recommended by the Association of Zoos and Aquariums (AZA), which is 8 m^2 for small felids between 10 and 20 kg (Mellen, 1997). Furthermore, it has been shown that the degree of enrichment is closely related to the prospect of developing a wide variety of natural behaviors and therefore increases the activity and welfare of animals in captivity (Bashaw et al., 2003; Young, 2003). The ILCBP enclosures included a wide variety of vegetation, vertical structures, visual barriers and burrows where prey could hide, and as a result hunting was more difficult and the lynx had to perform various behaviors, such as stalking. These actions appear to be essential to ensure that the captive-born Iberian lynx at the ILCBP are suitable, from the point of view of circadian activity rhythms, to contribute to the reintroduction projects of the species in the wild.

CONCLUSIONS

1. The daily activity of the cubs is similar to that of adults after the third month of life.
2. All cubs exhibited a bimodal circadian rhythm with peaks of activity in the twilights.
3. Cubs intended for reintroduction showed a similar circadian pattern to that observed in wild lynx.

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Yerga J, Calzada J, Manteca X, Vargas A, Rivas A, (2014). Early development and growth in captive-born Iberian lynx (*Lynx pardinus*). *Zoo Biology*, 33:381-387.

CAPÍTULO IV. Ontogeny of Play Behavior in the Iberian Lynx

Yerga J, Calzada J, Manteca X, Vargas A, Pérez MJ, Rivas A. Ontogeny of play behavior in the Iberian lynx. *Manuscript in preparation.*

Abstract

The aim of this paper is to describe the ontogeny of play behavior in the Iberian lynx (*Lynx pardinus*). The play behavior of 30 cubs born between 2009 and 2011 in the Iberian Lynx Breeding Centers "El Acebuche" and "La Olivilla" was tracked. The age of onset and time devoted to each category of play, including social, locomotor, object and predatory, was recorded. Social play behavior, which was influenced by litter size and sex, was the first to start at 29 ± 1 days of age. Locomotor play began at 55 ± 2 days, play with objects at 59 ± 2 days and predatory plays at 61 ± 2 days. From the commencement of play behavior until seven months of age, the cubs spent 16% of the time playing. Social play accounted for 73.5% of the total time spent playing and reached its highest prevalence in week 13 (21.3 ± 3.6%). Locomotor play constituted 9.6% and peaked at week 8 (5.97 ± 1.4%). Play with objects represented only 3.5% of the total playtime, with a maximum in week 12 (1.7 ± 0.6%). Predatory play accounted for 13.3% of the total playtime and had its greatest importance between weeks 11 and 19 (2.7 ± 0.8% - 6.6 ± 1.8%). Sex and litter size did not influence the total playing time or time spent in each category of play.

Keywords: cub, Iberian lynx, ontogeny, play behavior.

INTRODUCTION

Play behavior is one of the least studied subjects in ethology (Dugatkin, 2009), and until relatively recently (Bekoff & Byers, 1998; Burghardt, 2005) there has been no theoretical basis for this behavior. Bekoff & Byers (1981) defined play behavior as any motor activity, with no apparent function for the observer, that uses motor patterns typical of other behaviors, but which are modified in structure and/or sequence. Play activities that are focused on other living beings, involve moving or using inanimate objects are referred to as social, locomotor and object play, respectively. According to some proposed hypotheses, every play type corresponds to a different function and has a different effect on the ontogeny of young and, subsequently, each type of play commences at a different age (Gentry, 1974; Burghardt, 1998).

Play with objects is associated with learning and can benefit cubs both in the short and long term. For example, cheetahs use objects to practice hunting (Martin &

Caro, 1985), and "predatory play" makes them better hunters when mature (Caro, 1995). Locomotor play provides exercise and locomotor training to acquire the skills they will need later (Byers & Walker, 1995), as well as a better knowledge of the terrain, providing immediate benefits. Finally, the benefits of social play are many: it allows the construction of long-lasting social ties; provides physical skills related to fighting, hunting and breeding; and promotes the development of cognitive skills (Bekoff, 2007).

A typical category of social play, especially in carnivorous mammals, is "play fighting." According to Biben (1998), play fighting has several benefits: it teaches individuals how to be flexible in their behavior -as a very common occurrence in play fighting bouts is that playmates switch roles, e.g. the dominant individual assumes the subordinate role during the play fight - and how to gauge the intention of others (actual fights are dangerous and play fighting allows animals to comprehend the intention of others, which will be very useful in adult life).

Therefore, from a functional point of view, play behavior is considered to allow animals to develop the physical and mental skills needed to cope with unexpected situations in which they experience a loss of control. Thanks to the versatility of movements that can be used to recover from unforeseen shocks (such as loss of balance or falls), play behavior increases and improves the ability of animals to cope with emotionally stressful and unexpected situations (Spinka et al., 2001).

The development and prevalence of play behavior is not the same in all species of mammals. In general, they are influenced by some socio-ecological and life-history characteristics. For example, social species tend to devote more time to playing, than solitary species, especially to social play (Lewis, 2000), since they must develop more complex social skills to maintain group cohesion. Moreover, the ontogeny of play in newborns is affected by the degree of development of offspring at birth. In precocious species, the first play bouts can be observed just after birth, while in altricial species, play behavior appears later, after the cubs have developed their senses and their motor skills have improved. Also, the order in which the different categories of play are displayed also varies. Generally, bouts of locomotor play precede social play in primates (Fairbanks, 2000) and ungulates (Berger, 1980; Haynes, 1997), but in carnivores, social play commences before locomotor play (Bekoff, 1974, Drea et al., 1996). Object play seems to be the last type of play behavior to be exhibited in all groups.

The Iberian lynx (*Lynx pardinus*) is a threatened species, listed as "endangered" by the IUCN (Rodríguez & Calzada, 2015), and it is the most endangered cat species in the world. For this reason, in 2002 a captive breeding program was launched with the aim of raising suitable lynx to be released into the wild and assisting in-situ conservation efforts of the species. The Iberian lynx is a solitary and generally monogamous species (Ferreras et al., 1997), in which there is sexual dimorphism, with males slightly larger than females (Yerga et al., 2014). Births take place between March and May. Average litter size is 3 cubs (2-4; Palomares et al., 2005). The young are semi-altricial: the senses of sight and hearing are not fully developed at birth, and they are not able to walk (Yerga et al., 2014). During the early stages of development, the cubs go through a period, between 6 and 8 weeks of life, in which agonistic fights occur between littermates (Antonevich et al., 2009). These fights, which have also been observed in other lynx species, appear to be related to the hierarchical structure of the litter and the growth rate of the cubs (Sokolov et al., 1994; Naidenko & Antonevich, 2009).

Play prepares cubs to develop other behaviors and to deal with situations that may occur during both the juvenile stage and adulthood. Undoubtedly, increasing the versatility of movements can help improve the skills of an animal in certain behaviors like predation or social relations, including agonistic ones, which may be important for the adaptation of a lynx to life in the wild. Hence, it is necessary to know how play behavior develops in cubs born in captivity and, subsequently, released into the wild. In addition, play behavior is used as an indicator of welfare in captivity, as it only occurs in a non-threatening context (threats being defined not only as the risk of predation, but any stress such as malnutrition); and thus the characterization of play behavior also has a practical interest in the management of captive animals.

The objectives of this paper are to describe play behavior in the Iberian lynx, determine the age of onset of play behavior and the different types of play, analyze how time spent playing varies throughout development and examine the effect of sex and litter size on the ontogeny of play.

MATERIAL AND METHODS

The behavior of 30 Iberian lynx cubs (17 males and 13 females) born between 2009 and 2011 was recorded at two centers dedicated exclusively to the breeding of the Iberian lynx: "El Acebuche" (37°03'00'' N 6º33'17'' W) and "La Olivilla" (38º20'37'' N 3º33'33'' W), both in Andalusia, Spain. The cubs belonged to 12 different litters (7 litters of two cubs, 4 litters of three cubs and a single litter of four cubs). Each mother with its litter was housed in separate facilities. The enclosures varied between the 550 m^2 and 1000 m^2 and were enriched with typical Mediterranean trees and scrubs, along with visual barriers that allowed the animals to hide from humans, elevated wooden structures on which to climb or rest, several dens and shelters for prey species.

The behavior of the cubs was recorded using a remote video surveillance system. Each enclosure had 2-3 mobile cameras and several fixed cameras inside the dens, so it was possible to observe lynx cubs wherever they were. All cameras were equipped with infrared lights to allow the monitoring of the animals even at night. The video surveillance system was controlled 24 hours a day from a control room located 150 m away from the enclosures. Thus, it was possible to observe the behavior of the cubs while avoiding any possible disturbance caused by the presence of an observer in the enclosures. In fact, the entire animal husbandry procedure was developed so as to minimize human presence in the enclosures so that the lynx were reared in the most natural way, to diminish the risk of abandonment of the offspring by their mothers and to avoid cubs imprinting on their keepers (see details in Vargas et al., 2009).

The cubs were tracked from birth to 28 weeks of age. The behaviors of each animal were recorded for 15 minutes, twice a day (at dawn and dusk, coinciding with periods of cub activity; Yerga et al., 2015b), for 3-5 days per week. In total, we obtained 530 hours of video recordings. The collection of play data was carried out by reviewing the recordings, using an instantaneous sampling method with a sampling interval of 1 min (Altmann, 1974).

To identify which behaviors could be specifically assigned to play, the five criteria proposed by Burghardt (2005) were used. A behavior was considered play if it 1) had a limited immediate function, 2) had an endogenous component, 3) differed temporally and structurally from the behavior from which it derives, 4) was repetitive, and 5) took place in a relaxed and non-threatening context. For this study, play behavior was classified into four categories (Table 1).

TABLE 1. Categories of play and description of each one.

Category of play	Description
Social	Those play behaviors directed toward another individual. They can involve two or more cubs or a cub with its mother. It can be subdivided into two categories: play fighting or chasing.
Locomotor	A lone individual performs intense and vigorous movements, both horizontally and vertically, such as running, jumping or climbing. Animals can use various structures, such as rocks or tree trunks, to practice this type of play.
Object	Manipulation of objects with the paws or mouth by hitting, biting or throwing. The objects are usually items from their natural environment such as stones, pine cones, leaves, sticks or wood.
Predatory	Play aimed toward prey. It can be subdivided into two main categories: live-prey play, consisting of fighting with or chasing prey; dead-prey play, consisting of the manipulation of prey with the paws or mouth, lifting or throwing it.

Statistical analysis

Mean age (± SE) at which each category of play was observed for the first time was calculated. To check the effect of sex and litter size on the age of onset of each type of play, a two-way ANOVA for each play category was conducted. For those cases in which litter size had a significant effect, a post-hoc (Tukey test) analysis was performed to assess the differences between litter sizes. Prior to the analysis, normality of the data was checked. An α-value of 0.05 was used.

The proportion of time each cub spent playing every week, differentiated by play category, was calculated. A generalized linear mixed model (GLMM) was performed for the total playtime and for the time playing in each category in order to assess the effect of sex and litter size on the time cubs spent playing during their development. Sex and litter size were included as fixed factors. Additionally, week and squared-week were added. The latter term was included in order to properly model the changes in time devoted to play in relation to age, as a linear relationship between the dependent variables and the age of the cub was not observed. The individual cub, the mother and the breeding center were included as random factors. The dependent variables had to be square-root transformed to meet normality criteria. An α-value of 0.05 was employed.

RESULTS

Onset of play behavior

The Iberian lynx cubs exhibited four types of play behavior (Figure 1). The first category of play performed by all the cubs studied without exception was social play, which began at 29 ± 1 days of age (mean ± SE, range = 18-39, n = 20). The remaining play types were recorded for the first time around a month later; locomotor play at 55 ± 2 days (mean ± SE, range = 49-80, n = 19), object play at 59 ± 2 days (mean ± SE, range = 42-88, n = 18), and predatory play at 61 ± 2 days old (mean ± SE, range = 48-84, n = 18).

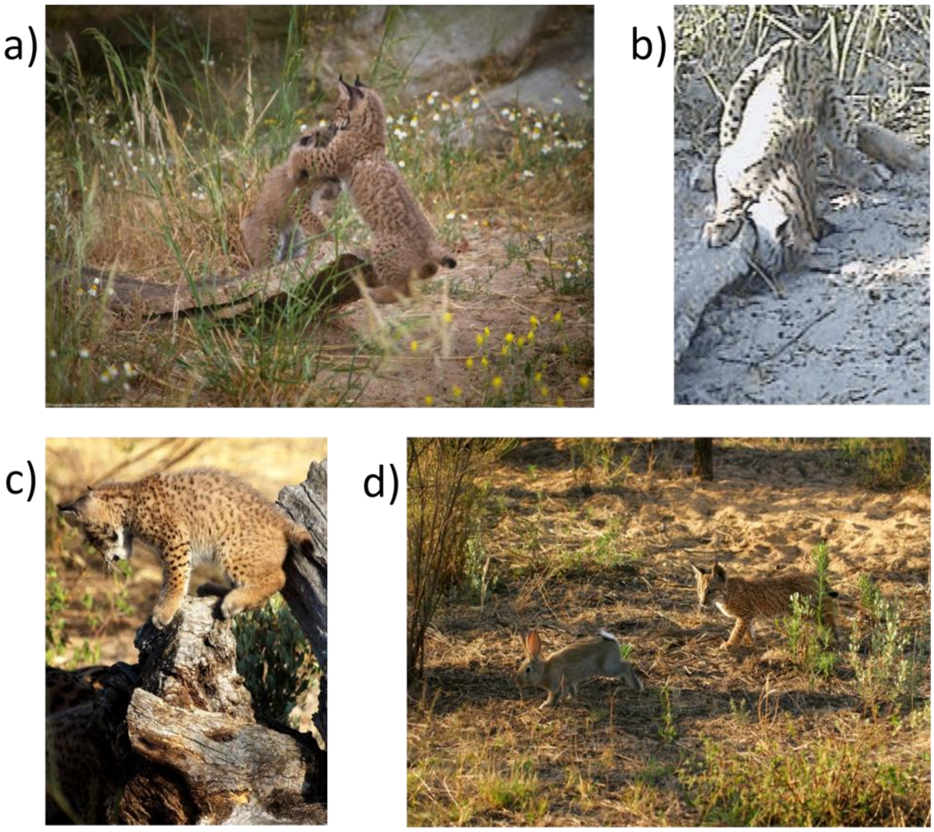

FIGURE 1. The four categories of play observed in the Iberian lynx: a) Social play, b) Object play, c) Locomotor play and d) Predatory play (Iberian Lynx Captive Breeding Programme/T. Rivas)

Sex and litter size affected the age at which social play began. Females began to play 3.6 days earlier than males (F = 4.554, df = 1, p = 0.049), while triplets began 6.2 days before twins (F = 5.427, df = 2, p = 0.015). Sex had no influence on the age at which the other form of plays started (locomotor play: F = 2.619, df = 1, p = 0.126; object play: F = 2.731, df = 1, p = 0.121; predatory play: F = 4.256, df = 1, p = 0.058). Litter size did not affect the age at which locomotor play (F = 0.361, df = 2, p = 0.703) and object play (F = 0.079, df = 2, p = 0.925) were initiated. However, twins began predatory play 14.6 days earlier than triplets (F = 6.995, df = 2, p <0.01). The lynx cubs of the only litter of four siblings that were studied began to practice the different types of play as follows: social play began 2.1 days before and predatory play 3.8 days later than in triplets.

Time budget of play throughout development

Between five weeks and seven months of age, lynx cubs expended an average of 16% of their time during dawn and dusk playing. During the first four weeks of life, play bouts were not observed, although in two litters social play was observed at 18 and 25 days, respectively. At week 4, the time spent playing was only 0.34 ± 0.19%. During week 5, the cubs started to engage in social play and the time spent playing per day increased to 6.6 ± 0.79%. This increment continued until 11 weeks of age. Between 11 and 14 weeks, the cubs spent about 28% of the time playing, with a maximum at 12 weeks old (29.1 ± 2.72%). At the age of 15 weeks, the time that the cubs devoted to play started to decrease progressively until they were 28 weeks old, the age at which this study terminated. However, play behavior did not completely disappeared at 28 weeks old, when the cubs still devoted 4% of the time to playing (Figure 2). There were no significant differences in the time spent playing by sex (t = -0.899, p = 0.38) or litter size (t = 1.132, p = 0.268).

Social play was the most frequent type of play engaged in by the Iberian lynx cubs. Seventy-three and a half percent of play time was dedicated to social play. Two main types of play within this category were discernible: play fighting and chasing. Play fighting consisted of the simulation of real fights, usually with one of the cubs lying on its back in a submissive position and the other located in a dominant position over it. Sometimes the cubs stood up, balancing on their hind legs, to slap one another. In chase play, one of the cubs tried to catch the other. If the cub succeeded, they often moved away from each other to start a new pursuit. Social play prevalence peaked at 13 weeks of age (21.3 ± 3.6%, mean ± SE), and thereafter began to decline until the end of the study, in week 28, when the cubs spent 3.82 ± 1.2% of the time in social play (Figure 2). Males and females spent equal time in social play (t = 1.371, p = 0.185) and no significant differences were found in the time each cub spent in social play depending on the size of the litter (t=1.096, p=0.278).

Locomotor play represented 9.6% of the total playtime during the development of the cubs. In the Iberian lynx this type of play consisted mainly of running, jumping or climbing, using different elements that were used to enrich the enclosures. The first episodes of locomotor play were observed at 7 weeks of age. This type of play peaked one week later, at 5.97 ± 1.4%, and subsequently decreased and stabilized around 1-3.5% up to week 19. From 19 to 28 weeks old the cubs spent less than 1% engaging in locomotor play (Figure 2). As with social play, locomotor play was not significantly influenced by the sex of the cub (t = 0.948, p = 0.344) or litter size (t = -0.027, p = 0.978).

Lynx cubs invested 3.5% of their playtime playing with objects. This was the type of play engaged in the least. Lynx cubs manipulated different elements that were in the enclosure, mainly corks and pine cones, for object play. Manipulation was performed by launching the objects into the air or by biting them. This type of play started in week 7. Object play did not occupy more than 2% of the time of the cubs, remaining fairly stable throughout development. The highest prevalence was reached in week 12 at 1.7 ± 0.6% (Figure 2). Neither sex (t = -0.237, p = 0.816) nor litter size (t = -1.044, p = 0.309) influenced the time spent by cubs playing with objects.

The onset of predatory play was the latest in the Iberian lynx cubs, but it was the second most practiced category of play. This type of play occupied 13.3% of the time devoted to play. The cubs engaged in predatory play with both live and dead prey. Chasing prey was the most frequent form of predatory play, although the lynx also performed simulated fights with the prey. When playing with dead prey, the action was similar to object play. The lynx cubs bit and threw dead rabbits into the air. The time spent by the cubs engaging in this play category increased starting in week 9 and ranged from 2.7 ± 0.8% to 6.6 ± 1.8% between weeks 11 and 19 (Figure 2). Predatory play disappeared completely at 26 weeks of age. No significant differences were found in the time spent playing with prey by sex (t = -1.241, p = 0.228) or litter size (t = 0.497, p = 0.624).

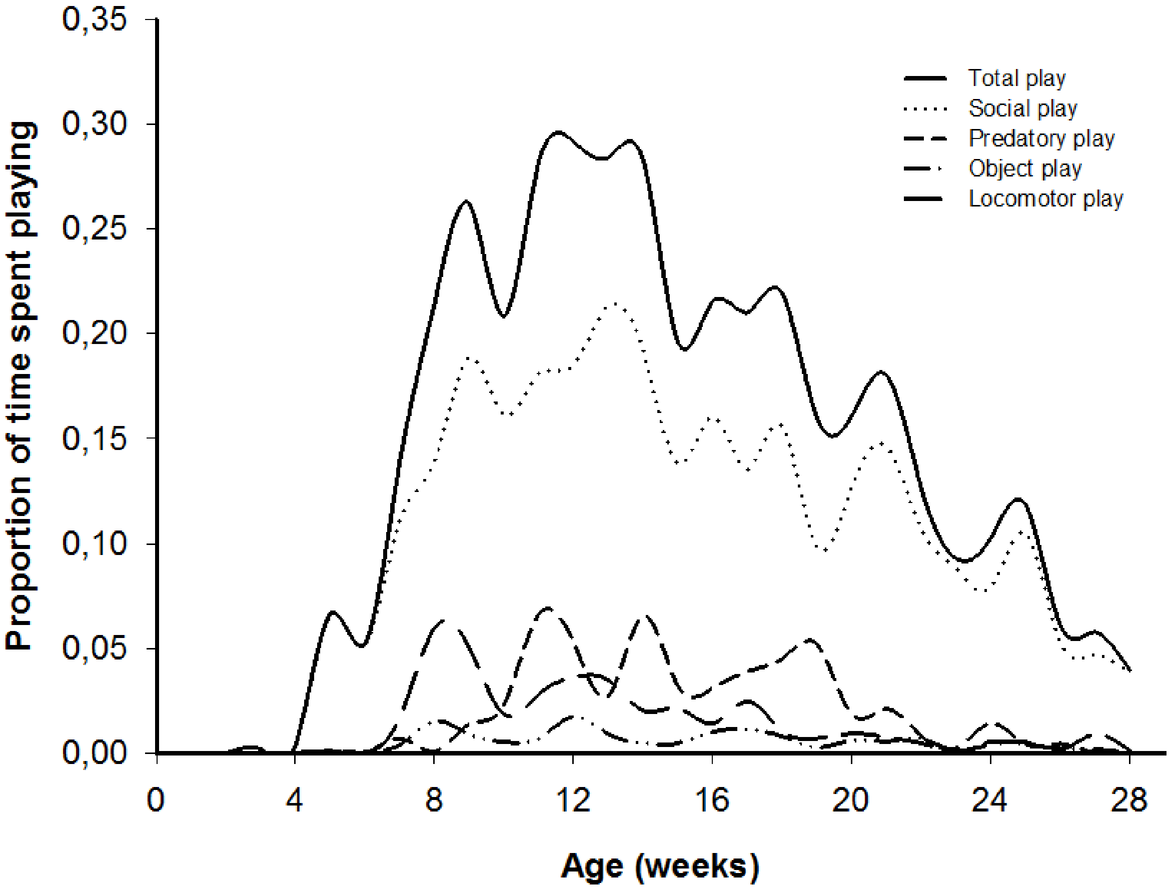

FIGURE 2. Proportion of time spent playing overall and in each play category (social, predatory, locomotor and object) by the Iberian lynx cubs from birth to 28 weeks old.

DISCUSSION

The Iberian lynx is a semi-altricial species (Yerga et al., 2014). Newborns have a relatively delayed sensory and locomotor development, so that they are not able to see, hear or walk. Hence their ability to interact with the environment, and thus play, is very limited. The degree of development of the offspring at birth has been associated with the onset of playing behavior. It is known that in precocial species play bouts start soon after birth (Waring, 1983; Drea et al, 1996), whereas in altricial species play is delayed until young are sufficiently developed (Fagen, 1976). Such is the case for the Iberian lynx: the first play behaviors, social ones, were observed from the first month of life, just as cubs develop the functional use of the sense of sight and hearing and they are able to walk (Yerga et al, 2014.)

The order in which the different types of play behaviors appear during development seems to depend on the short-term benefits they can provide. Thus, in ungulates and primates the onset of locomotor play is commonly first, which improves the flight response of young (Burghardt, 2005) and increases the likelihood of survival. In some carnivores, such as the hyena (*Crocuta crocuta*), red fox (*Vulpes vulpes*) and coyote (*Canis latrans*), the first play behaviors that cubs usually exhibit are social ones (Bekoff, 1974; Drea et al., 1996). In these species, the offspring develop agonistic behaviors very early in order to establish the hierarchy of the litter. In the Iberian lynx, the early onset of social play may be related to the occurrence of an aggressive period between 6 and 8 weeks of life (Antonevich et al., 2009), so that these play behaviors can be used to improve the skills related to real fights and to the display of social behaviors. Following fighting, object and locomotor play behaviors appeared when the cubs began to show more interest in exploring their environment. The onset of predatory play during the weaning period, which occurs between 8 and 10 weeks of age (Yerga et al., 2015a), suggests that it provides some immediate benefit in the process of learning to hunt; however, a link between predatory play and hunting success has not been found (Martin & Caro, 1985).

Iberian lynx females started to engage in social play before males. There is a scarcity of literature about the differences in the onset of social play between sexes. One possibility is that females gain some benefit for the agonistic period, as more time playing can counter the greater aggressiveness that males generally present (Antonevich et al., 2009). Triplets started social play earlier, but object play later. One may expect that the potential number of playmates can affect the ontogeny of social play, so that a bigger litter size can increase the likelihood that a cub starts social play and induces siblings to begin playing too.

Some studies have shown that motor patterns associated with play behavior can have permanent effects on brain structure and that play reaches its highest prevalence during periods of great brain development, thus maximizing their profits (Byers & Walker, 1995). After reaching this maximum, the time that cubs spend playing progressively decreases until it almost disappears in adulthood -in many species, adults have been observed playing, but always sporadically (Pellis, 1991; Walker & Byers, 1991)-. The ontogeny of Iberian lynx cubs observed in this study also followed this common pattern. Once the cubs were sufficiently developed, play behavior became increasingly important, with peak prevalence between weeks 11 and 14. From that age onwards playtime decreased, but nonetheless at the end of the study the cubs were still playing. It seems that Iberian lynx adults, like those of other species, also exhibit play behavior (Aldama & Delibes, 1991). Each category of play has a different rate of development within a species (Spinka et al., 2001), as observed for the Iberian lynx. These disparities in the age at which each type of play reaches its maximum prevalence may be related to the different roles they have and the short-term profits that the exhibition of each form of play provides in certain periods of development.

Most studies have shown that males display higher rates of play, show different motor patterns and tend to play more roughly than females (Symons, 1978; Pusey, 1990). It has been suggested that variations in play behavior based on sex should be more evident in those species that are sexually dimorphic as, in these species, females usually mature earlier (Smale et al., 1995) -i.e., their developmental period is shorter- and this could affect the ontogeny of their play behavior. These differences are often more evident in the later stages of development, especially in polygynous species, probably due to a divergence between the behavior of males and females as they approach adulthood. Despite being a sexually dimorphic species, Iberian lynx males and females spent the same amount of time playing overall, as well as in each category of play. However, we were not able to verify if the way in which males play is different from that of females or if there is a preference for same-sex playmates, as has been demonstrated in other species (Cheney, 1978; Watson 1993).

Social species spend more time playing than solitary species (Lewis, 2000). In primates, species that live in larger groups spend more time in social play and total time playing (Lewis, 2003). These individuals must learn specific social skills through social play to establish the complex relationships that enable the maintenance of a cohesive group. In carnivores, there are preliminary results that suggest a positive, although non-significant, correlation between group size and social play (Lewis, 2003). For solitary species such as the Iberian lynx, group size during the juvenile period depends solely on the size of the litter. In this species, the number of siblings did not influence the time cubs spent playing. It is likely that litter size does not influence the time spent playing, at least when the size of the litter is very small.

CONCLUSIONS

1. Social play is the first form of play that was observed in the Iberian lynx cubs, followed by locomotor, object and predatory play.

2. Sex affect the age of onset of the social play and litter size influences the beginning of the social and predatory play.
3. Social play takes up most of the time devoted to the play behavior.
4. The play behavior reaches its highest prevalence between weeks 11 and 14, and do not disappear altogether until the seven months old. Each type of play peaks at different ages.
5. Neither sex nor litter size affected the time spent playing.

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CAPÍTULO V. Development of predatory behavior in captive Iberian lynx cubs destined for reintroduction.

Yerga J, Calzada J, Manteca X, Vargas A, Pérez MJ, Rivas A. Development of predatory behavior in captive Iberian lynx (*Lynx pardinus)* cubs destined for reintroduction. *Manuscript in preparation.*

Abstract

This paper aims to describe the ontogeny of hunting behavior of 42 Iberian lynx cubs (*Lynx pardinus*), as well as the predatory ethogram of 10 cubs destined for reintroduction, all born in captivity between 2008 and 2013 in the Iberian Lynx Breeding Centers of "El Acebuche" and "La Olivilla". The learning of predatory behavior began when the mother took dead prey to the cubs at 30 ± 1.6 days of age. At 37 ± 1.8 days the cubs came into direct contact with prey for the first time. At 53 ± 1.3 days, they ate a rabbit opened by the mother and at 59 ± 2.1 days they were able to open rabbit skins and eat for themselves. Play with dead prey began at 62 ± 2.6 days of age and that with live prey at 69 ± 2.2 days. When the cubs were 80 ± 5.7 days old, they could kill prey wounded by their mother and they killed without any help at 103 ± 5.6 days of age. Sex did not influence the age at which the milestones occurred. The basic sequence of the predatory ethogram consisted of search, attack, capture, bite, move with the prey, and eat, which was modified or interrupted by bouts of play behavior, aggressions or abandonment of the hunt. During an attack, four hunting techniques were observed: harassment was the most frequently used (71% of the episodes), followed by ambushing (49%), stalking (22%) and persecution (18%). Fifty-three percent of the hunting episodes were successful and neither age nor gender nor mother influenced it.

INTRODUCTION

Hunting is the key behavior that defines predators. Whether a predator bases its technique on stalking or persecution, predation is a complex behavior involving the development of highly advanced motor patterns (Vargas & Anderson, 1999). The expression of predatory behavior has both innate and learned components (Leyhausen, 1973; Caro, 1994; Holekamp, 1997). On the one hand, stalking, persecution, knocking over, and orientation of the bite are known to be innate in some species of carnivores, such as cheetahs (*Acinonyx jubatus*; Eaton 1974). In some rodents, capture latency can be genetically selected (Polsky, 1975). On the other hand, advanced predatory skills depend largely on learning during the early ontogeny of behavior. For example, Vargas & Anderson (1999) found that juvenile black-footed ferrets (*Mustela nigripes*) that had the opportunity to hunt during early development had greater hunting success than those that had been fed with dead prey when they were cubs.

Previous experience with prey is not the only factor influencing learning. Socially isolated individuals in captivity have worse predatory skills, both quantitative (efficiency; Kuo, 1960) and qualitative (poorly-coordinated killing response; Myer, 1969). Hence the importance that observational learning has on the ontogeny of hunting behavior has been highlighted. In animals that remain socially isolated during early development and have no opportunity to hunt, predation efficiency when they are adults is higher if they were able to observe other individuals of their species hunting (Pion, 1969; Johnson et al., 1972). More specifically, observing the hunting behavior of their mother improves the predatory skills of cubs (Caro, 1980b). In the domestic cat, kittens exposed to prey in the presence of their mother have a higher rate of capture, both when they are young (Kuo, 1930) and as adults (Caro, 1980b).

When the goal of maintaining wild animals in captivity is breeding and reintroduction, it is necessary to pay special attention to the preservation of the natural behavior of the species (Kleiman, 1989; Wallace, 2000). In the case of carnivorous mammals, the development of a suitable predatory behavior is essential, since one of the most frequent causes of failure in reintroduction programs is starvation (Jule et al., 2008). However, the conditions imposed by captivity largely impede the normal display of predatory behavior. Many predators are fed with dead meat, which prevents the development of this behavior, and when it is possible to bring them live prey they are often provided at fixed schedules and locations, so predation is very predictable (Shepherdson et al., 1993). Moreover, the space limitation of enclosures hampers the development of the complete sequence of predatory behavior (Lindburg, 1988)

The inability to exhibit hunting behavior has also been related to the welfare of predators in captivity. Previous research has found that, in felids, the enrichment of enclosures that encourages the development of hunting behaviors increases animal welfare (Bashaw et al., 2003; Skibiel et al., 2007), whereas those individuals that cannot hunt develop a greater number of stereotypical behaviors, which affects their psychological and physical well-being, jeopardizing the chances of survival in the wild (Vickery & Mason, 2003).

The Iberian lynx (*Lynx pardinus*) is the world’s most endangered felid (Nowell & Jackson, 1996) and one of the most endangered mammals in the world. It is listed as "Endangered" by IUCN (Rodríguez & Calzada, 2015), since there are only about 300 individuals left in the wild (Iberlince LIFE Project, 2014). The loss and fragmentation of habitat, poaching and the decline of rabbit populations (*Oryctolagus cunniculus*), its main prey, are the most important causes of the decline of the species (Delibes, 1980;

Rodríguez & Delibes, 2004). Given this situation, in 2002 a captive breeding program was launched with two objectives: to maintain a lynx population in captivity to prevent species extinction and to help preserve its genetic diversity, and to produce suitable individuals for reintroduction and the reinforcement of wild populations (Vargas et al., 2009).

The Iberian Lynx Captive Breeding Programme (ILCBP) has implemented a protocol for preparing animals for reintroduction. This protocol is based on the promotion of predatory behavior, as the Iberian lynx is a strict predator (Delibes, 1980; Palomares et al., 2001), so that the development of hunting is essential for successful reintroduction. The aim of this study is to establish whether the management of lynx in captivity following this protocol allows cubs to develop an appropriate pattern of hunting, to determine the age at which the different milestones in the process of ontogeny of predation occur and to describe the predatory ethogram of the cubs.

MATERIAL AND METHODS

The predatory behavior of 42 Iberian lynx cubs (24 males and 18 females) born between 2008 and 2013 in the Iberian lynx breeding centers of "El Acebuche", Huelva, Spain (37º03'00"N 6º33'17"W) and "La Olivilla", Jaen, Spain (38º20'37"N 3º33'33"W) was studied. Each litter, with its mother, was housed in a separate enclosure. The size of these facilities varied between 550m^2 and 1000m^2. The enclosures were enriched with vegetation typical of the Mediterranean scrub, the main habitat of the Iberian lynx (Palomares et al., 2000), as well as several vertical structures on which to rest, visual barriers to hide from humans and burrows for prey.

The tracking of predatory behavior was performed by the use of a video surveillance system. Each enclosure had two mobile cameras and four fixed cameras, all equipped with infrared lights to allow night vision. The cameras were controlled 24 hours a day from a room located 150m from the enclosures. This method allowed the observation of behavior while avoiding any disturbance to the animals caused by the presence of an observer.

Starting at birth and continuing throughout the nursing period, the lynx cubs were fed "ad libitum" with live rabbit. The cubs always had access to the rabbits, so that weaning and learning to hunt were not affected by the availability of prey and occurred naturally (Rivas et al., 2015).

To study the process of learning to hunt, the age at which the following predatory behavior milestones occurred for the first time was recorded: presentation of dead prey by the mother; direct contact with prey; consumption of prey prepared by the mother (with an opening in the skin); consumption of intact, dead prey; play with dead prey; play with live prey; killing live but injured (by the mother) prey; and killing live and intact prey. The mean age (± SE) of each of the milestones was calculated. A Student t-test was performed to analyze the effect of sex on the age of occurrence of the milestones.

Once the predatory behavior was established, the ethogram of hunting behavior of 10 cubs born in the "El Acebuche" breeding center was studied. The lynx were fed following the protocol established by the Breeding Programme for the preparation of animals for reintroduction (Rivas et al., 2015). Under this protocol, live rabbits were released into the enclosures through an automatic dispenser (Figure 1). These dispensers, which are placed next to the outer mesh of each facility, have a drawer opening outward in which the keepers deposit the rabbits, so that they do not need to go into the enclosures. Each dispenser has a timer that was programmed for around dawn or dusk, when lynx are most active (Yerga et al., 2015), which released the rabbits into an underground burrow with access to the inside of the enclosure. The hour at which the timer was programmed randomly varied every day. Thus, the randomness in the availability of prey was increased and the likelihood that the animals associated food with the arrival of keepers was decreased. For each cub, a weekly recording between 4 and 7 months of life was performed. Each hunting episode began once the rabbits were available at the facility, that is, at the scheduled hour of the timer, and it ended when the cub killed the prey or one hour elapsed. The behaviors recorded for the lynx predation ethogram were: search, cub observes and explores the enclosure until it locates a prey; attack, cub uses a hunting technique in order to catch the prey; capture, cub grasps the prey between its teeth; bite, cub bites the prey, killing it; move prey, cub moves with prey, whether alive or dead, in the mouth; eat, cub ingests the prey either captured by itself or another individual (their mother or a sibling); live prey play, cub plays with a live prey, chasing it, without intending to kill it; dead prey play, cub plays with an already dead prey, hitting and throwing it into the air; aggression, cub becomes involved in a fight, either as the aggressor or the object of an attack;

abandon, cub loses interest in the hunt and leaves the episode. In total, 85 hunting episodes were recorded.

FIGURE 1. Automatic dispenser to feed the lynx with live rabbits (Iberian Lynx Captive Breeding Programme/T. Rivas)

The predation ethogram was constructed by estimating the transition frequencies (the probability that a following behavior will be entered once the preceding state has begun) between behaviors (Haccou & Mellis 1992). These frequencies were calculated as the number of transitions from each behavior to each of the other behaviors, divided by the total number of occurrences of the preceding behavior (MacNulty et al., 2007). A generalized linear mixed model (GLMM) was calculated to study the effect of age (in months), sex of the cub, and the mother on hunting success during the study period. The individual was included as a random factor to account for the non-independent and repeated individual measures data.

RESULTS

The first step in learning to hunt occurred at 30 ± 1.6 days of age (mean ± SE), when the mother took dead prey to the cubs for the first time. At this age, the cubs did not show much interest in the prey and merely observed it. A week later, at 37 ± 1.8

days, the first direct contact with dead prey occurred; the cubs approached the rabbit on its own initiative and explored the prey by sniffing and hitting it with the front paws. Interactions with dead prey became more frequent and intense as the cubs grew, until they ate rabbit for the first time at 53 ± 1.3 days of age. During this early phase, the cubs needed the help of their mother to eat. Thus, the mother made a prior opening in the skin on the side of the rabbit to facilitate the task of ingestion. A week later (59 ± 2.1 days) the cubs were able to open the rabbit skin by themselves to feed on it.

At two months old (62 ± 2.6 days) the cubs began to display a new behavior: playing with dead rabbits. This consisted of hitting, biting or throwing prey, which was sometimes followed by consumption. A week later, at 69 ± 2.2 days, the cubs were able to play with live prey. The lynx cubs tried to stalk and chase prey, without attempting to kill it. In multiple litters, several cubs participated in these games at the same time. It was also common to see the cubs tap the prey to encourage it to escape in order to chase it. Play with live prey usually culminated in the mother killing the prey. At 80 ± 5.7 days old, the Iberian lynx cubs were able to kill for the first time a rabbit wounded previously by its mother. Finally, at 103 ± 5.6 days of age, lynx cubs were able to hunt rabbits without any help from their mother. These first hunts were quite lengthy, with several chases and interspersed play bouts. In Table 1, mean age, standard error, range and sample size for each of the milestones is summarized. The sex of the cub did not influence the age at which the milestones occurred (Table 1).

TABLE 1. Mean age, standard error, range and sample size for the occurrence of each predatory milestone for the Iberian lynx cubs. The results of the Student t-test for sex are also included.

Milestones	Mean age	SE	Range	N	Sex effect
Presentation of dead prey	30	1.6	18-56	39	--
Direct contact with prey	37	1.8	16-45	16	t=0.689; df=14; p=0.502
Consumption of prey prepared by the mother	53	1.3	37-72	36	t=-0.745; df=34; p=0.461
Consumption of intact, dead prey	59	2.1	41-73	15	t=0.672; df=13; p=0.513
Play with dead prey	62	2.6	53-73	7	t=-1.144; df=5; p=0.305
Play with live prey	69	2.2	43-89	31	t=-0.093; df=29; p=0.926
Kill live but injured prey (by the mother)	80	5.7	64-111	7	t=0.588; df=5; p=0.582
Kill live and intact prey	103	5.6	58-195	30	t=1.277; df=28; p=0.212

With hunting behavior completely set, we studied the predatory ethogram of the lynx cubs. In Table 2, the transition frequencies between hunting behaviors are shown. The first step in hunting was searching for prey and approaching the place in which the rabbits were located. Usually the cubs hunted alone, although, as rabbits tended to be concentrated in certain burrows, it was common to see the cubs trying to hunt side by side. Although the rabbits were hidden, the lynx found them quickly given the small size of the facility, and thus the search phase was rather brief, usually only a few seconds in duration. Once they located prey, the lynx began an attack in 93% of cases. In the remaining episodes, the cubs played with the live or dead rabbits (4.7%) or abandoned the hunt (2.4%).

TABLE 2. Transition frequencies between hunting behaviors.

Preceding behavior	**Following behavior**									
	Abandon	*Aggression*	*Attack*	*Bite*	*Search*	*Capture*	*Eat*	*Dead-prey play*	*Live-prey play*	*Move prey*
Aggression	0.833	0	0	0	0	0.167	0	0	0	0
Attack	0.226	0.045	0	0	0.331	0.301	0	0	0.097	0
Bite	0.1	0.02	0	0	0	0	0.38	0.04	0	0.46
Search	0.024	0	0.929	0	0	0	0	0.008	0.039	0
Capture	0	0	0	0.706	0	0	0	0	0	0.294
Dead-prey play	0	0	0.25	0	0	0	0.75	0	0	0
Live-prey play	0.381	0	0.19	0	0.048	0.381	0	0	0	0
Move prey	0.026	0.026	0	0.368	0	0	0.526	0.027	0.027	0

The lynx cubs used four different attack techniques: stalking, ambushing, pursuing and harassing (see descriptions in Table 3; Figure 2). Harassing was the most frequently used technique, which occurred in 71% of the episodes. Ambushing was observed in 49% of hunting bouts, while stalking and pursuing techniques were used less often (22% and 18%, respectively). Once the attack phase concluded, the cubs captured rabbits in 30.1% of cases, but 9.8% of the time, instead of capturing prey, they began playing with it. In the event of losing the prey, the cubs searched again (33.1% of the episodes) or abandoned the hunt (22.6%).

TABLE 3. Hunting techniques used by Iberian lynx cubs during an attack.

Hunting technique	Description
Harrassing	The prey remains sheltered inside the burrow and the lynx actively tries to capture it inside the burrow or to force it out, introducing its claws, jumping over the burrow or digging next to the entrance of the shelter to get inside.
Ambushing	The lynx remains close (less than 5 m) to the opening of a burrow and waits until the prey appears from the inside the burrow.
Stalking	The lynx observes the prey and makes a stealthy approach toward it. It can make small breaks in its movement as it approaches the prey.
Pursuing	The lynx sees the prey and runs toward it or behind it along the enclosure.

Capture followed an attack. The cubs invariably captured prey (n = 44) by the neck, except on a single occasion in which it was captured by the back. Fifty-three percent of hunting episodes were successful, and success increased from the fifth to the seventh month of life. However, the GLMM showed that none of the analyzed factors explain the variability in hunting success: age (t = 1.137, p = 0.268); sex (t = 0.543, p = 0.593) and mother (t = 0.945, p = 0.355). After capture, the cubs killed the rabbit by giving a bite to the neck (70.6%) and then moved with the prey to eat elsewhere. In all other cases, after the capture, the cubs moved with the live prey (29.4%) and then killed it and ate elsewhere.

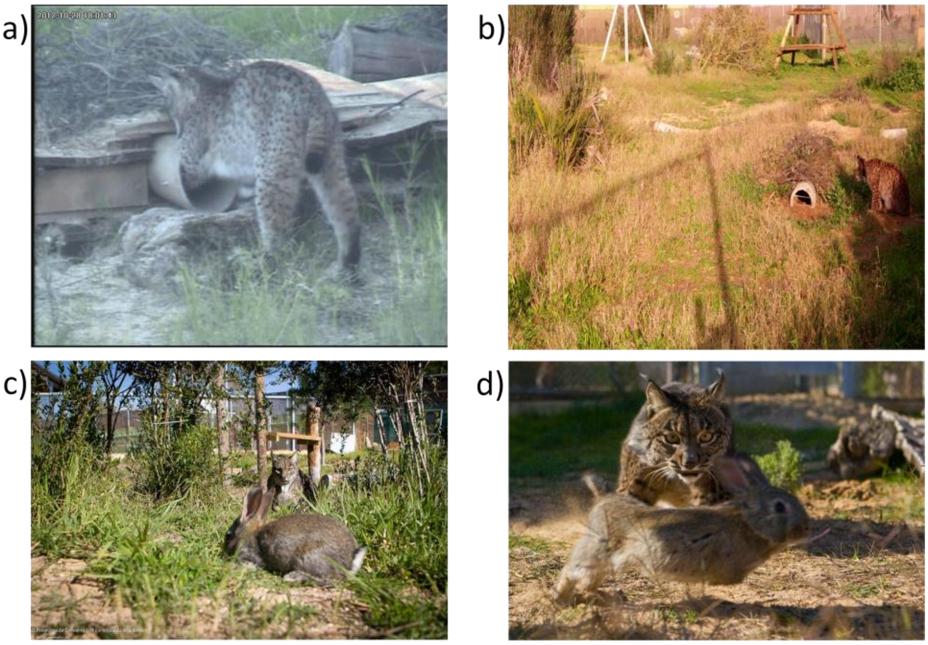

FIGURE 2. The four hunting techniques in the Iberian lynx: a) Harrassing, b) Ambushing, c) Stalking and d) Pursuing.

This basic sequence was at times altered by play with live or dead prey, fights or abandonment due to disinterest in the prey (Figure 3). The most common play type was that with live prey (21% of the episodes), which was generally observed after an attack. After playing with live rabbits, in 62% of cases the lynx continued the hunting process from one of its steps, namely search, attack and/or capture; and 38% of the time they abandoned the hunt. On the other hand, play with dead prey was only observed in 4.7% of the episodes, especially after biting or moving with the prey, and was largely followed by ingestion of the rabbit (75%). Fights between littermates or between a mother and a cub were uncommon, as they were observed in only 7% of hunting episodes. Typically, these aggressions occurred after an attack (4.5%), after biting a prey (2%) or after moving with the rabbit (2.6%), and usually led to abandonment of hunting (83% of the times).

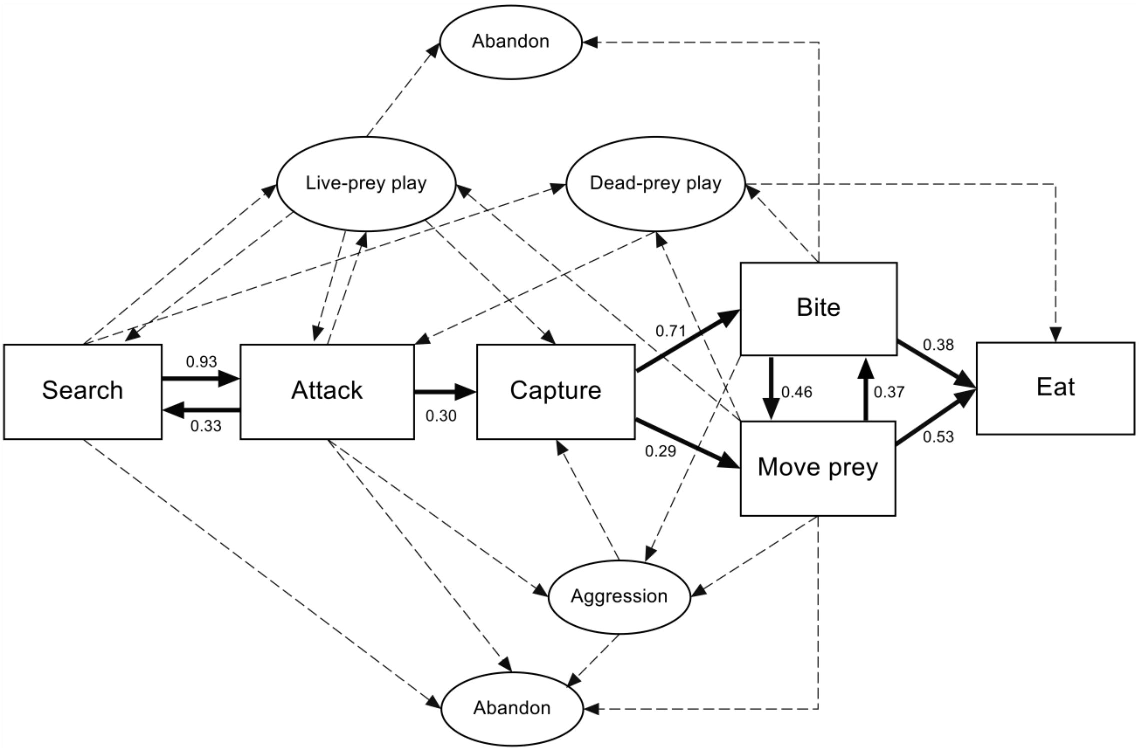

FIGURE 3: hunting ethogram of Iberian lynx cubs. The solid arrows are used for transitions of the main sequence of hunting. The numbers indicate the probability of occurrence of each behavior, according to the preceding behavior. Only those probabilities referred to the main sequence are included. The dashed arrows are used for transitions that involve any of the behaviors that modify or disrupt the main sequence of predation.

DISCUSSION

The ontogeny of predatory behavior in Iberian lynx cubs occurs gradually and the mother plays a fundamental role. The young of Iberian lynx are semi-altricial at birth. Newborns have very limited sensorial and locomotor development: they are not able to see, hear or walk, and as a result they remain hidden inside the den where mothers give birth (Yerga et al., 2014). Their ability to interact with the environment is thus conditioned by their state of physical development. When the cubs are one month old, they are able to walk, the senses of hearing and sight are already functional, and the deciduous canines and incisors erupt (Yerga et al., 2014). It is at this time when the first steps in the learning process of hunting occur: the presentation of dead prey that mothers bring to cubs, until the cubs begin (a week later) to interact with the dead rabbit.

During these early stages of development of the predatory behavior of the Iberian lynx, the role of the mother is essential and suggests that observational learning is very important in this species, as observed in other felids (John et al., 1968). In many species of felids, the first contact of the young with prey occurs as a result of mothers bringing dead prey to their cubs, even when they are still in the den (Schenkel, 1966; Kruuk & Turner, 1967; Schaller, 1967; Ewer, 1969). The presentation of dead prey by the mother occurred in the Iberian lynx at a similar age to that observed for the domestic cat (Caro, 1980a), but much earlier than that reported for the bobcat (*Lynx rufus*), in which mothers bring prey to their young starting the second month of life (Scott, 1976). The interest exhibited by Iberian lynx cubs in their prey progressively increased at the same time they were improving their predatory skills, until they gradually completed a pattern of behavior which allowed them to hunt rabbits for themselves. The age at which the different milestones were observed seems to be determined by factors related to both the degree of physical development of the cubs and the behavior of the mothers, especially during the early stages of ontogeny.

As the Iberian lynx cubs were acquiring skills for hunting, the role of their mothers was diminishing. The same pattern has been observed in the domestic cat, where mothers spend less time hunting for their kittens as the latter grow and develop new motor patterns and hunting skills (Caro, 1980a). In any case, the dependence on their mother for feeding did not end when the cubs were eating rabbit. With the first intake of meat the weaning period starts, which in the Iberian lynx extends between 8 and 10 weeks of life (Yerga et al., 2014). During this period, the lynx alternated between solid food and lactation, as solid food was provided by the female until the lynx cubs were able to hunt for themselves, at 12 weeks old. Iberian lynx in captivity begin to hunt at a similar age to wild bobcat cubs, which begin to accompany their mothers hunting between 3 and 5 months of age (Sunquist & Sunquist, 2002), but well before the Canadian lynx (*Lynx canadensis*), in which cubs do not participate in hunting before 7 months old (Parker et al., 1983). It is likely that conditions in captivity encourage faster development than in wild animals, since it is easier to capture prey; but the fact that Iberian lynx cubs are able to hunt at only three months of age indicates that the protocol applied for training is suitable for the development of this species.

Once Iberian lynx cubs acquire sufficient skills to hunt for themselves, the sequence of hunting behavior resembles the patterns described for other species (Lindburg, 1988). Before seizing prey, it is necessary to locate and approach it (Lindburg, 1988). In the wild, this phase can take a long time and the ability to find prey is essential for survival (Schaller, 1967; Eaton, 1974), but in captivity the search period is usually very brief, given the ease of locating prey in a confined space. The protocol followed by the ILCBP for the preparation of specimens for reintroduction is an attempt to complicate the search and localization of rabbits, although this phase is still short. The four lynx species share similar characteristics when attacking prey: they mainly use stalking or ambushing techniques, while observations of long persecutions are very rare (Sunquist & Sunquist, 2002). However, in this work, the most frequently recorded technique was harassing. This may be due to the fact that the captive rabbits had few hiding places and these were known to the lynx, and thus when they wanted to hunt, they went directly into the burrows to force the rabbits out. This was facilitated in multiple litters because more siblings can better control the different exits of a den at the same time.

More than half of the hunting episodes ended with the capture of prey. In almost all cases, prey was caught by the neck. This is consistent with the usual pattern followed by cats when they hunt small prey: biting the neck and killing it by breaking the spine (Leyhausen, 1965). Hunting success for all lynx species is highly variable depending on the density and type of prey. The hunting success rate for small prey (rodents and lagomorphs) and a solitary hunter usually ranges between 9 and 37.5% (Marshall & Jenkins, 1966; Haglund, 1966; O'Donoghue et al., 1998). In the Canadian lynx, hunting in family groups increases success to 55% (Parker, 1981). The success rate in Iberian lynx cubs was quite high compared to this data, which may be due to the combined effect of captivity and group size. Hunting success increased from the fifth to the sixth month of life, which is consistent with reports indicating that experience is a key factor to the optimal development of predatory behavior (Ewer, 1969; Fox, 1971).

The Iberian lynx cubs did not eat rabbits in the same place in which they were hunted, rather they generally moved toward the limits of the enclosure. This behavior has been extensively described in felids (Schaller, 1972). Iberian lynx raised in captivity can perceive their siblings and even their mother as competitors. This may explain the movement of prey to the farthest corner of the enclosure, and the position usually adopted by lynx to eat rabbit, in which lynx keep their back toward the mesh of the enclosure so that they can watch for the arrival of another individual. Sometimes fighting was observed between siblings or between a mother and a cub after capturing prey. However, the frequency of fights was low, which may be explained by the existence of hierarchical relationships among siblings (García-Rodríguez et al., 2012), since it has been shown that dominance relationships contribute to a decrease in agonistic behavior in mammal groups (Halle, 1964; Southwick, 1964, 1967).

Play with live or dead rabbits has been observed at different stages of the predatory ethogram of Iberian lynx cubs. Although the function of play has not been clearly established (Dugatkin, 2009), its prevalence during the juvenile period suggests that it should play a main role in learning. This behavior has been widely observed in many species of cats, both domestic and wild (Leyhausen, 1979; Caro, 1994). For example, a juvenile and an adult Iberian lynx were observed playing with a rabbit in the wild, which they did not ultimately kill (Aldama & Delibes, 1991). It has been suggested that play only occurs in contexts in which the individual is stress-free (Burghardt, 2005), and thus the existence of play in Iberian lynx cubs may indicate a high degree of well-being. However, it has also been suggested that play with prey increases in times of food abundance (Biben, 1979). Thus, tracking the frequency of play can serve as an indicator to control various aspects of the husbandry and welfare of captive lynx.

In the context of a captive breeding program aimed at reintroducing the lynx, the animals must be adequately prepared to hunt rabbits. The protocol of the ILCBP for the preparation of lynx for reintroduction seems to allow suitable development of hunting behavior and the establishment of a proper predatory ethogram. The presence of the mother which allows for observational learning seems to be useful for the development of predatory skills, and thus we recommend that the animals to be reintroduced continue to be raised in this way; that is,in the company of their mothers and siblings. Moreover, an improvement in the enrichment of the enclosures that makes the rabbits be harder to find, may help the lynx cubs more actively practice stalking and ambushing, the most common hunting techniques in wild lynx (Rodríguez, 2004).

CONCLUSIONS

1. The start of learning of predation is conditioned by the degree of development of the cubs at birth.
2. The mother plays a major role in encouraging the development of predatory behavior in its offspring, especially in the early stages of learning.
3. The ethogram of predatory behavior consists mainly of search, attack, capture, bite, the move of the prey and eat. This basic sequence is modified by play bouts or fights.
4. The lynx employ four hunting techniques. Harassment is the most used, followed by ambushing, stalking and pursuing.
5. The hunting success is 53%.

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DISCUSIÓN GENERAL Y CONCLUSIONES

DISCUSIÓN GENERAL

Los resultados de este estudio han servido para conocer cómo se desarrollan, a nivel físico y etológico, los cachorros de lince ibérico que nacen en cautividad. Las crías de esta especie nacen con los sistemas locomotor y sensorial poco desarrollados. La vista y el oído no son funcionales al principio, ya que los ojos y los canales auditivos están cerrados, y su capacidad locomotora está muy limitada, no pueden mantenerse en pie. Los cachorros presentan una capa de lanugo temporal tras el parto que les ayuda a termorregular. Nacen sin dientes y dependen exclusivamente de la leche materna para alimentarse. Comparando con otras especies de su género, el peso al nacer de los cachorros de lince ibérico es bajo en relación con su peso adulto (Saunders, 1961; Saunders, 1964; Petraborg & Gunvalsson, 1962; Lariviere & Walton, 1997; Sunquist & Sunquist, 2002).

Según Derrickson (1992), el grado de desarrollo de los cachorros al nacer puede medirse en base a la independencia que presentan en cuatro áreas: nutrición, locomoción, capacidad sensorial y termorregulación. Los cachorros de lince ibérico son prácticamente altriciales, dado que no son capaces de moverse, no tienen todos los sentidos desarrollados y dependen nutricionalmente de la madre. Sin embargo, sí que presentan una capa de pelo al nacer que les permite tener cierta capacidad de termorregulación, por lo que podrían definirse como semi-altriciales.

Después del nacimiento, como ocurre en las especies altriciales, los cachorros sufren multitud de cambios físicos y de comportamiento hasta que alcanzan la edad adulta (Markwell & Thorne, 1987). En base a los resultados obtenidos, se pueden definir cuatro etapas de desarrollo en el lince ibérico que son similares a las que se suelen describir en la ontogenia de otras especies altriciales: neonatal, de transición, de socialización y juvenil (Manteca, 2003).

Periodo neonatal (semanas 1 a 3)

Durante la primera etapa, la neonatal, lactar es el principal comportamiento del cachorro. Ocupa hasta el 16% de su tiempo y decrece de forma exponencial conforme el cachorro crece. El tiempo de lactancia supone algo más de la mitad del tiempo que permanecen activos, teniendo en cuenta que la actividad durante las primeras semanas se sitúa alrededor del 30%. Lo mismo se ha observado en el perro y en el gato (Grant, 1987; Bateson, 2000).

La actividad durante el periodo neonatal no presenta un patrón circadiano definido, tal y como ha sido observado en especies altriciales (Ibuka, 1984). La causa debe residir en el escaso desarrollo sensorial que tienen los cachorros y en que permanecen dentro de sus madrigueras (cuevas o truecas de árboles; Fernández & Palomares, 2000), aislados de los factores ambientales que sincronizan los ritmos circadianos: la luz y la temperatura (Kavanau and Ramos, 1975; Kolbe, 2007). Sin embargo, en algunas especies de mamíferos se ha comprobado que los ritmos circadianos de los neonatos son dependientes del ritmo circadiano de la madre (Favreau et al., 2009). En nuestro estudio este efecto no se ha observado, lo que puede deberse a que la madre, en condiciones de cautividad, está con los cachorros durante muchas horas al día, porque precisa de poco tiempo para satisfacer sus necesidades de alimentación, de defensa del territorio y de eliminación.

El final de la tercera semana es clave para la maduración motora y sensorial de los linces y marca el fin del periodo neonatal. Los caninos aparecen entre los 17 y los 19 días y los incisivos a los 19-20 días. Con 18 días el lanugo está ausente de la cabeza, lo que indica que su capacidad de termorregulación va aumentando. Abren los ojos a los 19 días, tarde comparándolo con la mayoría de los felinos (Hemmer, 1979). Con esta misma edad los cachorros comienzan a mostrar patrones motores muy rudimentarios, son apenas capaces de mantenerse en pie sobre las cuatro patas. A los 20 días el canal auditivo está abierto y son capaces de responder a estímulos sonoros. La maduración sensorial y motora del lince ibérico parece ser más lenta que en el perro y en el gato, en los que la etapa neonatal dura dos semanas aproximadamente (Markwell & Thorne, 1987; Manteca, 2003). Con respecto a otros félidos, es muy similar a lo que se ha observado en otras especies de linces (Hemmer, 1979) y más lenta que en el resto de las especies de la familia (independientemente del tamaño), exceptuando el género *Leopardus* en el que la maduración de sus crías es especialmente lenta (Hemmer, 1979; Sunquist & Sunquist, 2002).

Periodo de transición (semanas 4 a 5)

El periodo de transición se caracteriza por un rápido desarrollo sensorial y motor. Aunque los ojos y los canales auditivos estén abiertos desde la tercera semana y el cachorro tenga cierta capacidad para responder a estímulos visuales y sonoros, es probable que, como se ha observado en otras especies, no sean completamente funcionales. En el gato el desarrollo de la vista y el oído continúa durante las semanas posteriores a la apertura de ojos y oídos. Los gatos responden a los sonidos a partir de los cinco días y tienen los ojos abiertos a la semana de vida, pero no alcanzan la capacidad auditiva y visual de un adulto hasta el mes y los tres o cuatro meses de edad, respectivamente (Ikeda, 1979; Villablanca & Olmstead, 1979; Olmstead & Villablanca, 1980). Así, durante el periodo de transición, estos órganos sensoriales continúan inmersos en un proceso de maduración. A nivel motor los cachorros van adquiriendo mayor capacidad conforme crecen hasta que a los 28 días son capaces de caminar de forma rudimentaria.

Con el desarrollo sensorial y locomotor aparecen nuevos comportamientos. A los 29 días se observa a los cachorros realizar por primera vez un juego de tipo social, a los 30 días la madre les lleva una presa por primera vez y a los 33 días comienzan a salir de la madriguera. Todo esto indica que los cachorros son más capaces de interaccionar con el entorno, por lo que se puede decir que al final de la quinta semana finaliza el periodo de transición y comienza el inicio del periodo de socialización.

Periodo de socialización (semanas 6 a 15)

El periodo de socialización, que en el lince ibérico se inicia al comienzo de la sexta semana, es la etapa en la que se producen más cambios en el comportamiento de los cachorros de los mamíferos (Manteca, 2003). La exploración de los alrededores se incrementa y aparecen nuevos comportamientos de juego y de depredación. Por ejemplo, los cachorros muestran un mayor interés por los conejos y entran en contacto directo con ellos por primera vez.

Durante la mayor parte del segundo mes de vida no se observan grandes cambios en el comportamiento, excepto que los cachorros atraviesan un periodo de agresividad (semanas 6 a 8) en el que se producen violentas peleas entre hermanos. Estas peleas pueden durar desde pocas horas hasta varios días y en ocasiones llevan a la muerte de unos de los cachorros (Antonevich, 2009). No deja de sorprender que este periodo agonístico ocurra tan pronto (casi) como es posible. Unas semanas antes no eran capaces ya no de pelear, sino ni siquiera de estar de pie ni de morder. Además de esto, los únicos cambios comportamentales apreciables son un incremento del juego de tipo social (la única forma de juego que exhiben los cachorros a esta edad), de la actividad diaria y la aparición de un incipiente patrón circadiano bimodal en la actividad.

Las primeras salidas de parideras son claves para la ontogenia de la actividad y los patrones circadianos de los cachorros. La actividad diaria se incrementa progresivamente hasta la semana ocho. Desde este momento hasta el final de su desarrollo los cachorros permanecen activos alrededor del 50% del tiempo. Este valor coincide con lo observado en linces adultos en libertad y en otras especies de felinos (Beltrán, 1988; Crawshaw, 1995; McCarthy et al., 2005: Schmidt et al., 2009). Poco a poco se va estableciendo un patrón circadiano bimodal en torno a los crepúsculos. Los cachorros ya están fuera de la paridera y sometidos a los factores ambientales que rigen el establecimiento de los ritmos circadianos: luz y temperatura. Así, en el segundo mes, se observa un incremento de la actividad al anochecer y al amanecer, y a partir del tercer mes el patrón crepuscular bimodal se establece definitivamente y ya no sufre modificaciones durante el resto de desarrollo (similar al de los linces en libertad; Calzada, 2000).

Tras el periodo agonístico aparecen nuevos comportamientos de juego y de depredación. Los juegos locomotor, con objetos y con la presa surgen entre las semanas 8 y 9. El juego durante el segundo y el tercer mes de vida se incrementa de forma muy acentuada. Se ha sugerido que este incremento está relacionado con la gran capacidad de aprendizaje que tienen los cachorros de especies altriciales durante este periodo y con los efectos que los patrones motores asociados al juego pueden tener en el desarrollo cerebral, de forma que es en este periodo cuando el juego aportaría su mayor beneficio (Byers, 1998; Siviy, 1998).

A la vez que se desarrolla el comportamiento de juego se suceden cambios en la alimentación. En la octava semana los cachorros ingieren carne sólida por primera vez y se inicia el proceso de destete (Martin, 1984). El cambio de dieta involucra cambios fisiológicos (porque el sistema digestivo debe adaptarse a alimentos nuevos; Galef, 1981) y comportamentales. La relación materno-filial se modifica por el aumento de la independencia de los cachorros (Martin, 1986), aunque la independencia completa no llega ni siquiera cuando finaliza el destete (semana 10), ya que durante varias semanas más los cachorros no son capaces de matar por sí mismos y siguen dependiendo de su madre. En la semana 12 adquieren la habilidad suficiente para matar una presa pero no para cazar. Necesitan todavía de la ayuda de la madre que hiere a los conejos previamente y así facilita la tarea a sus cachorros.

En la semana 15 de vida se produce el último hito importante en la ontogenia depredadora del lince ibérico: los cachorros cazan y matan los conejos por sí mismos sin ningún tipo de ayuda materna. Una semana más tarde el tiempo que los cachorros dedican a jugar comienza a bajar. Se puede decir que al final de la semana 15 finaliza el periodo de socialización y comienza el periodo juvenil.

El periodo de socialización es fundamental para la cría en cautividad de la especie, especialmente cuando se pretende criar linces física y etológicamente sanos para su reintroducción. La capacidad de aprendizaje y de recibir nuevos estímulos es muy elevada durante esta etapa del desarrollo del cachorro, por lo que es conveniente que se críen en contacto con sus hermanos y su madre y que tengan la oportunidad de exponerse a aquellos estímulos que vayan a encontrarse posteriormente en su medio, en especial que puedan practicar y ver practicar la caza. En esta etapa deben aprender a identificar sus presas, por lo que, además de criarlos en lugares con conejos, deben tener contacto también con otras presas menos habituales de la especie como las aves. Por el contrario, el contacto con los cuidadores debe ser minimizado especialmente durante este periodo porque hay una mayor probabilidad de socialización (impronta) con el hombre. Es por tanto recomendable que los cachorros que se críen para ser reintroducidos al medio natural eviten el contacto con humanos y sus infraestructuras, disminuyendo de este modo el riesgo de mortalidad por causa antrópica, la principal causa de mortalidad en los proyectos de reintroducción de los carnívoros (Jule et al., 2008).

Periodo juvenil (semana 16 hasta la madurez sexual)

A partir de la semana 16 los cachorros ya exhiben todos los comportamientos típicos de la especie. El comportamiento de los cachorros se asemeja progresivamente al de los adultos. Disminuye el tiempo que dedican a jugar y perfeccionan su comportamiento depredador. Los linces alcanzan el peso adulto a los 16 meses aproximadamente, pocos meses antes de alcanzar la madurez sexual, marcando así el final del periodo juvenil.

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CONCLUSIONES

1. El lince ibérico es una especie semi-altricial. El sistema sensorial y motor de los cachorros está poco desarrollado al nacer. Los principales cambios en la maduración de estos sistemas ocurren durante el primer mes de vida.

2. Machos y hembras de linces crecen de modo diferente. Es una especie dimórfica en la que los machos crecen más rápido y se hacen más grandes que las hembras. El método de crianza, con sus madres o a biberón, modificó la tasa de crecimiento pero no el tamaño de adulto.

3. La lactancia ocupa gran parte del tiempo del cachorro tras el nacimiento. Aunque fue descendiendo exponencialmente conforme crecieron, el periodo de dependencia completa de la leche materna ocupó más de dos tercios del periodo total de lactancia, lo que indica que el rol principal de la lactancia en el lince ibérico es satisfacer el requerimiento nutricional de los cachorros.

4. El tamaño de la camada influye en el tiempo que dedican los cachorros a lactar. Los cachorros de camadas múltiples dedicaron menos tiempo a mamar, a pesar de que las madres de estas camadas invirtieron más tiempo a amamantar a sus crías.

5. El lince es una especie crepuscular. El ritmo circadiano bimodal con los máximos de actividad en los crepúsculos comenzó a observarse en el segundo mes y quedó completamente establecido a partir del tercer mes. Este patrón es similar al observado en la especie en libertad.

6. Al igual que en otros carnívoros el juego social es el primer tipo de juego que practican los linces. Además exhibieron otros tres tipos de juegos: locomotor, con objetos y con la presa.

7. Los linces invierten mucho tiempo jugando durante su desarrollo y la mayor parte de este tiempo está dedicada al juego social. El tiempo que dedicaron a jugar se fue incrementando progresivamente desde su comienzo en la semana 5 hasta la semana 14. A partir de entonces descendió sin acabar de desaparecer al final del estudio (7 meses de edad). El juego social ocupó la

mayor parte del tiempo de juego seguido del juego con la presa, del juego locomotor y del juego con objetos. Cada tipo de juego alcanzó su máxima prevalencia a distintas edades.

8. La caza es un comportamiento que se aprende de forma progresiva. Durante el aprendizaje de la depredación se observaron varios pasos intermedios y la madre jugó un papel fundamental especialmente durante las primeras etapas del aprendizaje.

9. Los linces cautivos son capaces de cazar siguiendo un patrón adecuado. La secuencia principal de depredación estuvo constituida por la búsqueda, el ataque, la captura, el mordisco, el traslado de la presa y la ingesta. Esta secuencia se vio modificada o interrumpida en ocasiones por la pérdida de interés en la presa, juegos o peleas. El acoso fue la técnica de caza más empleada, seguido del rececho, el acecho y la persecución.

10. A la vista de todos los resultados obtenidos, es posible distinguir cuatro periodos en el desarrollo físico y del comportamiento de los linces: periodo neonatal (semanas 1 a 3), periodo de transición (semanas 4 y 5), periodo de socialización (semanas 6 a 15) y periodo juvenil (semana 16 hasta la maduración sexual).