UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

GENETIC DIVERSITY OF THE CALIFORNIA SEA LION ASSESSED BY PEDIGREE ANALYSIS

JOANA ISABEL ALMEIDA CARRIÇO

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Doutor Luís Lavadinho Telo da Gama

TUTORA:
Dra. Sónia Paula de Almeida e Silva Pinto Matias

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Genetic Diversity of the California Sea Lion assessed by pedigree analysis

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Acknowledgements

Abstract

Genetic Diversity of the California Sea Lion Assessed by Pedigree Analysis

The genetic diversity and population structure of the California sea lion (*Zalophus californianus*) were assessed based on the pedigree information registered in the European studbook, currently under the management of the Lisbon Zoo, as an EEP programme. Records collected from 1930 until 2020 were used for this analysis, including a total of 1998 individuals, 477 of which represent the current living population.

To our knowledge, this is the most comprehensive and up-to-date analysis for this species, assessing the evolution of genetic diversity, inbreeding and relationships, and the genetic contribution of founders, using conventional pedigree analysis procedures and additional software tools commonly used in domestic species, such as ENDOG.

The average equivalent complete generations were 1.54 (±1.20) per animal for the overall population. However, there has been an increase in pedigree depth throughout the years, with the reference population reaching 3.37 (±0.76) complete generations, where all animals had maternal grandparents known and nearly 85% to 90% had paternal grandparents known. The whole population had a generation interval of 11.52 (±4.70) years, however the mean ages at which individuals had their progeny has been increasing, particularly in the last decade. Regarding the number of offspring for each parent, dams had an average of 4.05 (±3.16) pups and sires 7.97 (±8.57) pups.

The overall mean inbreeding was 2.03% (±5.92), though throughout the years, this has been increasing, with the reference population reaching 3.34% (±6.08). The rate of increase in inbreeding was around 1.6% per generation. The number of inbred animals has also been growing, representing 16.87% and 52.94% of the total and reference populations, respectively. However, the average inbreeding coefficient, of the inbred individuals has shown a significant decrease, with the value for the reference population being nearly half that of the overall population. The mean coancestry among individuals from the same institution of birth, was 0.10, and from different institutions was 0.01, providing the opportunity to maintain inbreeding under control by planned exchange of breeders between institutions. The number of founders contributing to 50% of the gene pool was 36 and 12, for the total and reference populations, respectively, with an effective number of founders of 96 and 34 in the two populations. The effective population size, for the whole population was 36. It is important to maintain a sound program aimed at the genetic management of the population, including management of selection and mating decisions, to avoid further losses of genetic diversity.

Keywords: sea lion; captive population; studbook; genetic diversity; pedigree analysis

Resumo

Diversidade genética do Leão-marinho Californiano avaliada através da análise do pedigree

A diversidade genética e a estrutura da população de leões-marinhos Californianos (*Zalophus californianus*) foi avaliada com base nos registos de pedigree do studbook Europeu, sob a gestão do Jardim Zoológico de Lisboa, como um programa EEP. Os registos, recolhidos desde 1930 até 2020, que foram utilizados para esta análise, incluem um total de 1998 indivíduos, 477 dos quais representam a população viva atual.

Para nosso conhecimento, esta é a análise mais compreensiva e atualizada desta espécie, que avalia a evolução da diversidade genética, consanguinidade, parentescos, e contribuição genética de fundadores, usando métodos convencionais de análise de pedigree e ferramentas de software adicional, tipicamente usadas para espécies domésticas, como o ENDOG.

A média de gerações completas equivalentes foi de 1.54 (±1.20) por animal, para a população total. No entanto, tem havido um aumento na profundidade do pedigree, ao longo dos anos, com a população de referência a alcançar as 3.37 (±0.76) gerações completas, e onde todos os animais tinham avós maternos conhecidos e 85% a 90% tinham avós paternos conhecidos. A população total teve um intervalo de geração de 11.52 (±4.70) anos, no entanto a média das idades às quais os indivíduos tiveram filhos tem vindo a aumentar, particularmente na última década. Em relação ao número de filhos por progenitor, para as fêmeas foi 4.05 (±3.16) crias e para os machos 7.97 (±8.57) crias.

A consanguinidade média geral foi de 2.03% (±5.92), embora ao longo dos anos, tenha vindo a aumentar, com a população de referência a alcançar um valor de 3.34% (±6.08). A taxa de aumento da consanguinidade foi de 1.6% por geração. O número de animais consanguíneos tem vindo a aumentar, representando 16.87% e 52.94% das populações total e de referência, respetivamente. No entanto, a média do coeficiente de consanguinidade, dos consanguíneos, mostra uma diminuição significativa, com o valor da população de referência a ser metade do da população total. O relacionamento médio, nos indivíduos da mesma instituição de nascimento foi de 0.10 e de diferentes instituições de 0.01, proporcionando a oportunidade de manter a consanguinidade sob controle por meio da troca planeada entre as instituições. O número de fundadores que contribuíram para 50% do pool genético foi de 36 e 12, para as populações totais e de referência, respetivamente, com um número efetivo de fundadores de 96 e 34, nas duas populações. O tamanho efetivo da população total foi de 36. É importante manter um programa apontado para a gestão genética da população, incluindo o maneio da seleção e decisões de acasalamento, para evitar maiores perdas de diversidade genética.

Palavras-chave: leão-marinho; população em cativeiro; studbook; diversidade genética; análise de pedigree

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List of Abbreviations, Acronyms and Symbols

% – Percentage

AFLP – Amplified Fragment Length Polymorphism

AR – Average Relatedness

AZA – American Zoo and Aquarium Association

CFC – Coancestry, Inbreeding (F) and Contribution

CPSG – Conservation Planning Specialist Group

CSL – California Sea Lion

DDT – Dichlorodiphenyltrichloroethane

DNA – Deoxyribonucleic Acid

EAZA – European Association of Zoos and Aquarium

EEP – EAZA's Ex-situ Programme

ESB – European Studbook

F – Inbreeding Coefficient

f_a – Effective Number of Ancestors

FAO – Food and Agriculture Organization

f_e – Effective Number of Founders

f_g – Founder Genome Equivalent

GCI – Genetic Conservation Index

GD – Gene Diversity

GI – Generation Length

GSD – Genotypic Sex Determination

GSL – Galápagos Sea Lion

ID – Identification Number

IUCN – International Union for Conservation of Nature

JSL – Japanese Sea Lion

LC – Least Concern

LR – Lower Risk

LTF – Lost to Follow Up

LTMP – Long Term Management Plan

mtDNA – Mitochondrial DNA

mya – Million Years Ago

N – Number of Individuals

N_e – Effective Population Size

No. – Number

NOAA – National Oceanic and Atmospheric Administration

OPA – One Plan Approach

PCB – Polychlorinated Biphenyl

PMx – Population Modelling

RCP – Regional Collection Plan

SAS – Statistical Analysis Plan

SDE – Standard Deviation Error

SPARKS – Single Population Animal Records Keeping Software

SNP – Single-nucleotide polymorphism

SSC – Species Survival Commission

STD – Studbook

TAG – Taxon Advisory Group

WAZA – World Association of Zoos and Aquarium

ZIMS – Zoological Information Management Software

1. INTERNSHIP PERIOD

The candidate's internship period took place, in part, at the Lisbon Zoo. Given that the pedigree data used in this study originated from a studbook currently being managed by this institution, it was thought to be important for the candidate, to spend a period of time at said organization. Unfortunately, due to the current pandemic, this time had to be cut short, with the remainder of the internship being conducted through video calls.

This period at the Lisbon Zoo, was mostly spent with Dra. Sónia Matias, who is the coordinator of European Breeding Programme for the California sea lion (CSL), as well as a member of the Taxon Advisory Group (TAG), specialized on marine mammals. The purpose was for the candidate to better understand the responsibilities of a studbook coordinator, as well as work with the current database for this species. During this time, the candidate was allowed to help in the organization and treatment of the records, and was able to acquire a better understanding of the current software (ZIMS) in use at the institution, so that it could be later compared to the ones used for this dissertation.

After this initial period, the remainder of the time was spent analysing and structuring the data, with the supervision of Professor Dr Luís Telo da Gama (Faculty of Veterinary Medicine, University of Lisbon).

2. INTRODUCTION

The fast expansion of the human population, and the inefficient use of the planet's natural resources, results in a strong impact on biodiversity which, consequently, has been decreasing at a startling rate (Lisbon Zoo, 2020). The world continues experiencing a rapid loss of entire populations and species with many of the existing populations undergoing significant declines in both size and world distribution. An increasing number of species can, therefore, be expected to require a more intensive management, in order to maintain their long-term persistence on this planet (EAZA, 2020).

Zoos, aquariums, parks, and wildlife reserves now, more than ever, play a crucial role in the survival of these endangered species. They contribute and collaborate to the conservation of species and their habitats (Lisbon Zoo, 2020). So, an effective integration of planning, as well as an optimal use of the very limited resources available is essential in order to achieve the global biodiversity targets that have been agreed upon (Byers et al., 2013).

And it is in these institutions, with the use of studbooks and their analysis, that much of this management is achieved. Traditionally studbooks were used for domestic species, to facilitate the selection of a suitable breeding animal, in order to improve a certain characteristic, thus aiding in their management. These were therefore the foundations on which studbooks of endangered species were established (Glatston, 1986; Princée, 2016).

By 1966, studbooks for wild species were officially recognised and endorsed by the International Union of Directors of Zoological Gardens and the Zoo Liaison Committee, and from then on began to expand. By 1969, 21 studbooks were planned or in existence, and only continued to increase from there (Glatston, 1986). By 1977, studies on zoo populations showed the negative impact that inbreeding could have, and by 1980 new management guidelines were introduced, involving a pro-active approach in conducting demographic and genetic analyses on studbooks. This resulted in conservation genetics being "introduced" to the zoo world during this time (Princée, 2016), with several authors stressing the importance of genetic diversity to long-term captive breeding and conservation projects (Glatston, 1986).

Therefore, the concept of conservation based on number of breeding animals was no longer considered sufficient, and it was recommended to maintain at least 90% of the original (wild) genetic variation in captive populations over a period of 200 years. Thus, minimising genetic loss due to inbreeding and genetic drift, required an approach based on demographic and genetic management. And so, by 2011 the number of international and regional studbooks had extended to 190 and 1,350, respectively (Princée, 2016).

And so, it was recognized that the analyses of pedigree information, for a certain species, could be of great use to monitor the evolution of its genetic diversity and population structure over time (Piccoli et al., 2014). And thus, the vast amount of relevant information that the analysis of the data, provided by a studbook, can bring has been described by several

authors. It ranges from the possibility of choosing breeding pairings, that can be made to obtain the maximum outbreeding; to their use for the identification of hereditary defects and deducing the pattern of their inheritance; as well as to improve husbandry, by the comparison of the diverse conditions and climates that the different institutions have, etc. (Glatston, 1986).

Ballou et al. (2010) estimate that previous studies have shown that genetic management, based on pedigree estimates of genetic diversity, tend to perform better at maintaining genetic diversity than molecular-based methods.

Specifically, the California sea lion studbook is believed to have evolved from a Dutch national studbook that was established in the early 1980s (Princée, 2016).

History describes seals, sea lions, and walruses as, probably, the first marine mammals to be held in captivity. Most of these species, started to appear in exhibits, in the late 1800s and early 1900s (Perrin, Wursig & Thewissen, 2008). Sea lions, in general, are a very common and widespread animal in many zoos, aquaria, and marine parks, and are often included in public shows. These exhibits and shows are among the most popular zoo attractions (Seal Conservation Society, 2011). In fact, the California sea lion (*Zalophus californianus*) has now become the most common of these to be held in captivity, most likely due to its ability to successfully breed, as well as its receptivity to training and performing (Reeves & Mead, 1999). These animals are intelligent, have a strong sensory capacity and are usually highly trainable. With this training being commonly used in a lot of institutions, as a way to create a routine human contact that will then facilitate medical examinations and treatments (Seal Conservation Society, 2011).

Throughout the years, husbandry in captivity has developed, and different strategies have been acquired in order to recreate an environment as similar as possible to the wild, with a growing effort to create enriched environments to improve the well-being by alleviating boredom and stereotypic behaviours. It has been shown that California sea lions kept in captivity can live longer than their counterpart in the wild (Seal Conservation Society, 2011). Another benefit of captivity has been shown by a study by the American Zoo and Aquarium Association (AZA), where Falk et al. (2007) found that zoos and aquariums do actually have a long-term positive impact on visitors' attitudes towards the animals. As well as the fact that captive individuals represent a valuable research prospect, which increases the understanding of the species.

3. LITERATURE REVIEW

3.1. Captive Species' Management and Conservation

The Lisbon Zoo collaborates with a number of institutions, universities, and national and international schools, in a wide range of diverse fields like Biology, Ethology and Veterinary Medicine, and it actively participates in scientific research projects (Lisbon Zoo, 2020). One of these collaborations is with EAZA (European Association of Zoos and Aquaria), whose mission is to facilitate the co-operation within the European zoo and aquarium community, and to contribute to scientific research as well as to maintain the conservation of global biodiversity (WAZA, 2020).

To help create a more cohesive approach to conservation, the Conservation Planning Specialist Group (CPSG) of the Species Survival Commission (SSC) of the International Union for Conservation of Nature (IUCN) has created the "One Plan Approach" (OPA) (EAZA, 2020). This initiative is based on "the joint development of management strategies and conservation actions for all populations, of a species, by all responsible parties, to produce a single, comprehensive conservation plan". The major obstacle to this approach is that conservation planning has traditionally followed two parallel but separate tracks. One for the wild population and the other for the captive managed one. However, this One Plan Approach has defined population management, through the bridging of wild and intensively managed conditions, which will then allow for the progression of conservation towards a fully conserved status (Byers et al., 2013).

The assessment of threats to the wild population and the evaluation of possible strategies, to address said threats, are some of the benefits towards the wild population, which are achieved by the captive population's management, and therefore might promote conservation for the wild individuals. In turn, the wild populations will improve the long-term viability of the captive ones, by the possibility of supplying genetic founders that can or must be removed from the wild, such as excess offspring, injured individuals that cannot be released or non-viable population fragments (Byers et al., 2013). At the same time the IUCN published the "Guidelines on the Use of Ex situ Management for Species Conservation", which was created to help determine if, when and how ex situ management would be a valuable element of the overall conservation strategy (EAZA, 2020).

Ex situ conservation involves the measures adopted for the conservation of a species, that are outside of their natural habitat and under human care. The survival of these very often depends on keeping them in captivity, so that healthy and genetically viable populations are established. Hence, the collaboration between institutions and the appropriate population management is essential for the survival of the species (Lisbon Zoo, 2020).

Therefore, EAZA's Population Management Structure centres on three main pillars: 1) Regional Collection plans (RCP), 2) EAZA Ex situ programme (EEP) application and 3) Long

term management plans (EAZA, 2020). This new EAZA ex situ Programme Structure was developed so that Taxon Advisory Groups (TAG), which are comprised by specialists who dedicate themselves to specific groups of animals, develop the RCPs that describe what species they are recommended for, and why and how to best manage them, always following the IUCN guidelines for the Ex-situ Program. These collection plans also suggest the species that should be put under additional protection through other conservation programs (Lisbon Zoo, 2020). This also allows for the Long Term Management Plans (LTMPs) to, in more detail, investigate which genetic and demographic goals are best linked to the assigned roles and the situational circumstances of the taxon (EAZA, 2020).

EAZA Ex situ programmes (EEP) are aimed at conserving healthy populations of animals in captivity. Therefore, the acquisition of new animals and the regular exchange of animals between and beyond EAZA members is essential when creating a sustainably and healthy population, both demographically and genetically. For these exchanges, all animals in the EAZA collection should come from a trustworthy source and accompanied by all relevant legislative paperwork. Preferably, institutions should only obtain animals born in captivity, which is achieved best by direct zoo to zoo contact. Transfers should always be made via the relevant coordinator of that specific breeding programme to make sure they contribute to the overall goals and roles of the respective EEP (EAZA, 2020).

The European Studbook (ESB) is a less intensive programme than the EEP, where a studbook keeper is responsible for a specific ESB which collects all the data for births, deaths, affiliation, etc., from the members of EAZA that keep said species (EAZA, 2020). In other words, this studbook is a document similar to a genealogy book, that provides information on the number of captive individuals, who their progenitors are, their offspring and their location (Lisbon Zoo, 2020). The data is then entered in specialized computer software programs, that allow the keeper to carry out analysis of the population of that species. As a result, other EAZA institutions are able to ask the studbook keeper for relevant information on the species. At the same time, the keeper can evaluate if the programme is doing well, and if not, propose a more rigid management in order to maintain a healthy population in the long term. In this situation, the keeper may then suggest that the species be considered for an EEP (EAZA, 2020).

The Lisbon Zoo started its participation in the EEPs in early 1990, with four programs and is currently taking part in five TAGs and sixty-four EEPs. It also participates in forty-four European studbooks and forty-eight international ones (Lisbon Zoo, 2020).

For the management of EEPs and the sharing of knowledge, Species360 ZIMS (Zoological Information Management Software) is used. This is a fully web-based application that connects to a global database, that enables a real-time management of captive animal records (EAZA, 2020). More than 1,000 aquariums and wildlife institutions in 96 countries use this software, which currently has records on more than 22,000 species and 10,000,000

individual animals. This results in the world's largest set of data for ex situ populations of wildlife. ZIMS has replaced previous software like SPARKS and PopLink, and contrary to these, it notifies records updates, suggests animals, among other data quality control tools. It also allows for the integration with PMx, which then enables keepers to conduct population analysis and conservation management (Species360, 2020).

The EAZA Marine Mammal TAG oversees the running of four EEPs, one of which is for the California sea lion (*Zalophus californianus*). This along with one other have only recently been upgraded from ESBs to EEPs. One of the reasons for this update was that, somewhat recently, there has been an issue with commercial activities where sea lions are being sold abroad in large numbers, and therefore disappearing from the studbooks (EAZA, 2020).

3.2.   Taxonomy of the California Sea Lion

The species *Zalophus californianus* (Lesson, 1828) is part of the *Otariidae* Family, *Carnivora* Order, *Mammalia* Class, *Chordata* Phylum and *Animalia* Kingdom (IUCN, 2015). Jefferson, Webber & Pitman (2015) also refer that it belongs in *Pinnipedia*, which includes seals, sea lions, and walruses. *Pinnipedia* was traditionally considered a suborder, but currently ranks as undetermined. However, these authors use this nomenclature when wanting to refer to these three groups in general. This suborder's evolution has been packed with controversy, conventionally being thought to be of a diphyletic origin, with walruses and eared seals (including the now CSL) evolving from ursid (bear-like) ancestors, and true seals originating from mustelid (otter-like) ancestors. Though, currently it is believed that they have a monophyletic origin, with an aquatic carnivore (likely an ursid) to be its ancestor 30-35 million years ago in the North Pacific (Jefferson et al.,2008).

 As stated by Wolf, J.B., Tautz, D. & Trillmich, F. (2007) it is widely believed that "bad taxonomy can kill". In other words, Berta & Churchill (2011), refer that an accurate taxonomic framework is essential for studies of biogeography, ecology, and conservation of any taxonomic group. Many decisions are made based on species-level taxonomic groups, making subspecies or populations, normally, less focused on. This lack of recognition can cause an inaccurate assessment of their biodiversity hotspots, which results in a failure to provide protection for important foraging and breeding locations. A possible example of these consequences would be the extinction of the, at the time not considered species, Japanese sea lion, with its last credible sighting in the late 1950's (Wolf, J.B., Tautz, D. & Trillmich, F., 2007).

The IUCN (2015) notes that California sea lions were once considered a subspecies of *Zalophus californianus,* the *Z. californianus californianus.* However, this was then disproved, with Berta & Churchill (2011) summarizing some of these findings, citing that Sivertsen (1953, 1954) described several of the morphological differences between the California sea lion of

the North Pacific (*Z. c. californianus*) and that of the Galapagos Islands (*Z. c. wollebaeki*). Then by Itoo (1985), who suggested that *Z. c. japonicus* (Japanese sea lion) might be a distinct species, through the analysis of their cranial morphology. And then, following these, in 1988, Rice considered that all three, *Z. californianus*, *Z. japonicus* (Peters, 1866), and *Z. wollebaeki* (Sivertsen, 1953), were distinct species. Throughout the years, other authors continued to confirm the separation of all three species (IUCN, 2015).

Wolf, J.B., Tautz, D. & Trillmich, F. (2007), based on molecular data (mtDNA and nuclear microsatellites and SNPs), suggested that the Galapagos sea lion and the CSL were indeed two distinct genetic entities, that deserved species status (Figure 1). They also found that this was partly supported by other behavioural studies.

Legend:
GSL – Galápagos sea lion;
JSL – Japanese sea lion;
CSL – California sea lion; With geographically different populations: Gulf of California (light grey), Monterey Bay (dark grey), Channel Islands (black).

Figure 1 - Distance based phylogenetic network showing relationships within the genus *Zalophus*. (Adapted from Wolf, Toutz & Trillmich, 2007).

As previously stated, Berta & Churchill (2011) recapped these authors' findings as well as later ones, like Sakahira & Nimi (2007) who were able to extract ancient DNA, from skeletal remains of the Japanese sea lions, that supported that *Z. japonicus* and *Z. californianus* were indeed a separate species. These authors further suggested that the JSL were, ancestrally, more closely related to the California sea lion and that the two diverged only 2.2 mya versus the 2.5 mya reported by Wolf, J.B., Tautz, D. & Trillmich, F. (2007) as the divergence time, of the CSL and GSL. Moreover Dasmahapatra et al. (2009) using Amplified fragment length polymorphism (AFLP nuclear gene) markers and Schramm et al. (2009) using mtDNA provided evidence supporting the elevation of *Z. c. californianus* and *Z. c. wollebaeki* to species level (Berta & Churchill, 2011).

Regarding the macroscopic differences between the genus *Zalophus*, Galapagos sea lions are smaller than California sea lions and appear to be much less sexually dimorphic (Perrin, W. F., B. G. Wursig, and J. G. M. Thewissen, 2008).

Currently, there are no confirmed subspecies of the California sea lion, however mitochondrial DNA analysis defined five genetically distinct geographic populations: Pacific temperate (waters off the US), Pacific subtropical (waters of the Pacific coast of Baja California, Mexico), and the southern, central and northern portions of the Gulf of California (Jefferson, Webber & Pitman, 2015).

3.3. Wild Population

3.3.1. Geographical Distribution

The wild populations of California sea lions can, mainly, be found on islands from the Los Islotes in Baja California Sur, to the Channel Islands in southern California (IUCN, 2015). Jefferson, Webber & Pitman (2015) relate that most rookeries (terrestrial sites used for mating, giving birth, and resting) are south of Point Conception, Southern California and that many of the islands free of predators and human occupation are very often used as haul-out sites. These sites are mainly used during fall and winter when predominately adult and sub-adult males move north to Oregon (USA) and British Columbia (Canada) (IUCN, 2015). Haul-out areas can go as far south as off the coast of Mazatlán and the Tres Marías Islands (Mexico). To the north, they reach all the way through the Gulf of Alaska (Jefferson, Webber & Pitman, 2015; IUCN, 2015). The overall global distribution of this species is shown in Figure 2.
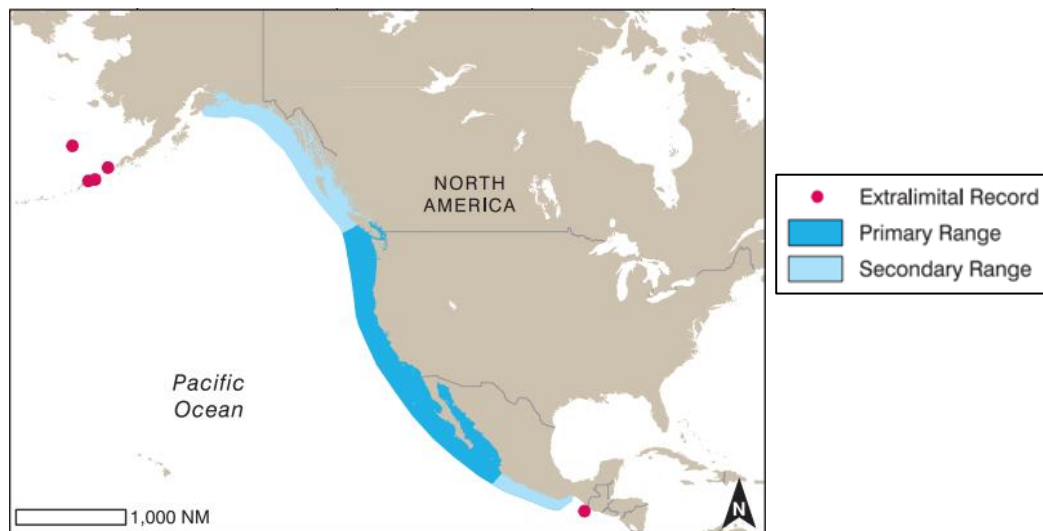
Figure 2 - Global distribution of the wild population of *Zalophus californianus*. (Adapted from Jefferson, Webber & Pitman, 2015).

There have been sightings, predominantly of male individuals, between 2005 and 2013, that reach even farther, and as far south as Punta San Pedrillo (Costa Rica). And as far east as the coast of the Yamsky Islands in the Okhotsk Sea (Russia), in 2007, 2008 and 2011. During the 1980's females tended to stay south of Point Conception, California, but are now

reoccupying former breeding sites in northern California (IUCN, 2015). However, most rookeries are still south of this area (Jefferson, Webber & Pitman, 2015). Nevertheless, they are known to keep to their rookeries, and based on estimates, females disperse on average 6.75 times less than males (IUCN, 2015). Perrin, Wursig, & Thewissen (2008), agree that female and immature individuals can disperse from their breeding islands, but do not migrate as broadly as males.

Additionally, all through this geographical range, California sea lions are known to be found in bays, harbours, and river mouths, as well as haul-out on boat docks and buoys (Jefferson, Webber & Pitman, 1994; 2015).

3.3.2. Population's History

Jefferson, Webber & Pitman (2015) state that California sea lions were, historically, important to Native American people in Southern California and the Channel Islands, prior to the arrival of Europeans. However, during the 19th and early 20th centuries the exploitation of these animals caused a decrease of the population, though not as severe as other pinniped species (IUCN, 2015). Even so, Jefferson, Webber & Pitman (2015) declare that the population may have been reduced to as few as 1,500 by the end of this period. However, in 1964, it was estimated that the size of the breeding population consisted of approximately 35,000 individuals (IUCN, 2015).

In the United States, the protection of these animals begun in the mid-20th century and was then solidified with the U.S. Marine Mammal Protection Act of 1972, with similar laws in Mexico being drawn. These entail that at coastal sites, and most offshore islands, the tourism is strictly regulated, with countries monitoring the California sea lion populations, regularly. These measures provided the momentum necessary for the recovery of this species (Jefferson, Webber & Pitman, 2015; IUCN, 2015). The IUCN (2015 citing Schramm et al. 2009) as mentioned earlier, identified 5 geographic populations, based on mitochondrial DNA. Combining with nuclear DNA information, the population structure in Mexico suggests a pattern of isolation by distance that resulted in at least three units (IUCN 2015 cited González-Suárez et al., 2009), with the species not showing any sign of a genetic variability decrease (IUCN, 2015).

Since not all age and sex classes are on shore at the same time, and pups, are the only age class that are permanently on land for the duration of the reproduction season, it is not possible to directly count the number of individuals, to determine the total population size. Hence, this value is estimated based on the number of births and the proportion of pups in the population following the methods developed by Boveng (1998) and Lowry et al. (1992) (IUCN, 2015). Perrin, Wursig & Thewissen (2008), reported that a 1992 census of the Gulf of California, revealed an estimate of 31,000 individuals. And a 2000 census of the Mexican

Pacific coast estimated a population of about 75,000 – 87,000 California sea lions. By 2007, the US population was thought to be around 141,842 individuals, with an annual pup production of 40,000 – 50,000, and a maximum population growth rate of 6.52%, with a total California sea lion population of about 248,000, throughout the world (Perrin, Wursig & Thewissen, 2008). With other authors reporting that at the end of 2014, the estimated population was around 350,000 – 387,646 individuals, in both the US and Mexico. With the former contributing with between 75,000 – 87,000 off the west coast of Baja California, and approximately 30,000 in the Gulf of California (Jefferson, Webber & Pitman, 2015). The IUCN (2015) believes that the California sea lion population was thought to be reaching carrying capacity in the USA as earlier as 2007, however their numbers are still increasing. Nevertheless, Jefferson, Webber & Pitman (2015) noted that the populations in the Gulf of California have declined 20% – 35% over the past 15 – 20 years. Overall, the current population trend reveals an increase, and there is an estimate of 180,000 mature wild individuals (IUCN, 2015).

3.3.3. Threats

The most well-known threats to the wild populations of CSL are the occasional occurrence of El Niño events. These are large-scale atmosphere climate interactions that cause a warming in the sea surface temperatures (NOAA, 2020). During these periods there is a significant decrease in prey and a large number of pups, as well as weaker animals of all age classes, that die of starvation. However, the populations of the Gulf of California don't seem to be as influenced by these occurrences (IUCN, 2015).

Human-related causes also affect the mortality of this species. The conflicts tend to be with fisheries, by poaching, and through entanglement in marine debris. Pollutants can also be a reason of threat, through its accumulation in the food chain. For example, the large amounts of DDT (Dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyl) that have been continuously discharged, in the past, tend to accumulate in coastal marine food chains, evidenced by toxins found in their tissues and organs. Also, the waste from agriculture and urban sectors, continues to be discharged into coastal marine habitats, where they have very negative effects on the overall health, and notably on the sea lion's immune systems (Jefferson, Webber & Pitman, 2015).

Other causes include periodic outbreaks of planktonic organisms, that cause paralytic shellfish poisoning, diseases contracted from terrestrial animals, like leptospirosis, as well as brucellosis, which may cause miscarriages (IUCN, 2015).

3.3.4.   Status

The most recent IUCN (2015) assessment considers the California sea lion wild population to be in the Red List Category & Criteria of Least Concern (LC). This because, as described previously, CSLs have recovered from their historical exploitation and their population is currently large and still expanding, with the only major threats being the periodical El Niño events. The previously published Red List Assessments have dictated LC in 2008; Lower Risk/least concern (LR/lc) in 1996; and Very Rare and believed to be decreasing in numbers, in 1965. All the Red List Categories are shown in Figure 3.
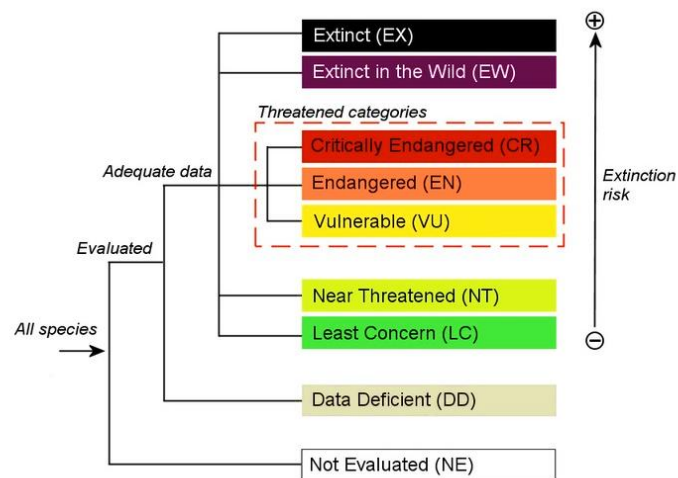
Figure 3 - IUCN Red List Categories. (Adapted from IUCN, 2012).

The main criteria for assessing the risk of a species are: Population reduction; Restricted geographic range; Small population size and decline; Very small or restricted population; Extinction probability analysis; Evaluation of the Population and Population Size; Subpopulations; Number of Mature individuals; Generation length; Reduction of population; Continuing decline; Extreme fluctuations; Severely fragmented population; Extent of occurrence; Area of occupancy; Location; Quantitative analysis (IUCN, 2012).

As this species does not belong in the "threatened" categories it was thought that a further explanation of the above criteria would be unnecessary. Further information can be found on the IUCN website.

3.4.   Species Characteristics

3.4.1.   Morphology

California sea lions are highly sexually dimorphic (Figure 4) with males achieving 3–4 times the weight of adult females, and 1.2 times their length. As the male becomes sexually mature, the sagittal crest enlarges, creating a high peaked crown, and giving them the known

appearance of a tall forehead. Adult females and juveniles do not have a sagittal crest, which makes them very difficult to distinguish from each other. (Jefferson, Webber & Pitman, 1994; 2008; 2015). This sagittal crest is unique to *Zalophus* (Perrin, Wursig, & Thewissen, 2008). Contrary to this feature, the flippers are similar for all ages and both sexes, they are naked except for a short stubble of dark fur partially covering the upper surface and overall flippers have a contrasting black colour (Jefferson, Webber & Pitman, 1994).

The coloration (Figure 4) of California sea lions is variable even within the same age and gender. However, the pups tend to be dark brown to black, until they molt to a tawny brown colour at about 4–6 months. In the females, this remains throughout adulthood, while the males' coat tends to darken as they age, with a full adult male being, normally, dark brown. Still, when wet, all individuals seem darker (Perrin, Wursig, & Thewissen, 2008).

Figure 4 - California sea lion physical characteristics. (Adapted from Jefferson, Webber & Pittman, 2008).

3.4.2.   Habitat and Ecology

California sea lions are frequent in shallow coastlines, of continental shelf and slope zones, and with sandy beaches being preferred for haul-out sites. However, as previously stated, they also occupy several man-made structures (Meijer, 2017). They tend to breed on these beaches, as well as in rocky areas on remote islands. Because the female needs to forage at sea during the lactation period, the breeding areas are restricted to regions of high marine productivity (Perrin, Wursig, & Thewissen, 2008).

3.4.3.   Social/Mating System

California sea lions have a harem system, with only the strongest and largest males able to maintain harems of many sexually mature females (Meijer, 2017), with each bull having 5 – 20 females in the wild (Grainger, 2005). All the other mature males are called bachelors and are driven away during the breeding period (Meijer, 2017), which in the Californian sea lion ranges from May to July (Dolphin Research Centre, 2020), with the number of individuals ashore rapidly increasing in May. It is also at this time that adult males start to fight for territories

along the shorelines of the rookeries, with the majority being unsuccessful and retreating to sea or to a "bachelor beach" nearby. The males that can establish territories, maintain their boundaries with ritualized displays and frequent barking. They fast throughout this period, surviving only on the fat that was accumulated during the off-season (Perrin, Wursig, & Thewissen, 2008). Males can hold this territory for up to 45 days, with some maintaining territories for multiple breeding seasons (Jefferson, Webber & Pitman, 2015).

All throughout this period, until late July, these males must keep a constant vigil over their females. Rarely, a young, strong bachelor succeeds in beating an older bull, thereby acquiring the herd (Seal Conservation Society, 2011). By the end of this period, the male has severely depleted his fat reserves, has suffered weight loss and is exhausted, with the remainder of the summer being spent by himself, resting and regaining his strength (Meijer, 2017).

3.4.4. Reproductive Characteristics

CSLs become sexually mature between the ages of 4 – 5 years, though males tend to achieve territorial status only at 8 – 9 years of age (Meijer, 2017). Estrus occurs around 27 days after the birth, which is one of the longer intervals in *Otariid* (Perrin, Wursig, & Thewissen, 2008; Jefferson, Webber & Pitman, 2015). This means, that the female is only not pregnant for about one month out of the entire year (Dolphin Research Centre, 2020).

Once fertilization occurs, the female can delay implantation of the egg to stop the embryo from growing. This delayed implantation can last up to 3 months, with the average active gestation period being 9 months long (Meijer, 2017). Like many mammals, each dam usually gives birth to a single pup, though it has been shown that not all females breed every year (Perrin, Wursig, & Thewissen, 2008).

Throughout May, June and July females give birth, usually 4 – 5 days after coming ashore (Grainger, 2005). The mother will nurse her pup, after birth, for about one week, after which she departs for the first of many foraging trips that usually last 2 – 3 days, returning then to the pup and staying for 1 – 2 days. Upon her return, she will call the pup, using a vocal and olfactory imprinting, to which the pup will answer. The mother is able to pick her own pup out of dozens of others. This pattern, of feeding herself and nursing, continues until the pup is weaned, and the cycle continues until the pup is old enough to swim and keep up with her. Most pups are weaned at around 10 months old, though some receive care as yearlings, and even as 2-year-olds. It has been stated that this length of lactation period tends to increase with the increasing age of the female (Perrin, Wursig, & Thewissen, 2008; Jefferson, Webber & Pitman, 2015; Meijer, 2017).

 After the breeding season ends, at the end of July and early August, most adult males leave the southern California rookeries and migrate north, while females and juveniles appear

to disperse to feed in the general vicinity of the breeding site. Some authors have noted that the US and Mexico populations have some notable differences in regard to the duration of the pupping season, and the interval between birth and mating. Furthermore, some reports reveal that temperature influences this species' behaviour (Perrin, Wursig, & Thewissen, 2008).

3.4.5. Longevity

Meijer (2017) and Price (2002) declare that the wild California sea lions reach an average age of about 17 years, while Perrin, Wursig, & Thewissen (2008) and SeaWorld Parks & Entertainment (2020) describe it to be between 15 and 25 years. Jefferson, Webber & Pitman (2015), suggest that males live up to 19 and females to 25 years of age. For the captive population, both SeaWorld Parks & Entertainment (2020) and Price (2002), agree on an average life expectancy of about 30 years.

4. AIMS OF THE STUDY

The main objective of this study was to characterize the entire known captive population of California sea lions, registered in the European studbook. The aim was to determine several demographic and genetic parameters that would help characterize this species, as well as to assess its progression since the initial captures. Additionally, it was intended to identify possible factors which may affect the genetic variability of the species and determine how best to minimize its loss. All the while, allowing for the comparison to its wild counterpart, when the information was available. Therefore, with the use of the CSL studbook it was possible to have access to and study this species known pedigree, and through it, assess the genetic diversity of this species, and its evolution.

This was thought to be important, because in order to improve the management of this and other wild species in captivity, the first step is to understand where it has stood until now. This would then allow to determine what needs to be changed and/or adapted, for a better and possibly easier set of tasks in the holding of this species by the institutions.

It was also meant to investigate the usefulness of different, and never applied in this taxon before, software in addition to the ones currently in use for the management of CSL, that could possibly identify new information and offer a different viewpoint. These were ENDOG (Gutiérrez, Goyache & Cervantes, 2010) and CFC (Sargolzaei, 2006).

Another potential purpose of this study would be as a foundation for additional specific genetic research, possibly with the various types of genetic markers.

5. MATERIAL AND METHODS

5.1. Studbook Description

The information contained in the California sea lion studbook was made available by the studbook manager and exported to Microsoft Excel, on the 22nd of April of 2020. All information and data gathered after this date has not, therefore, entered for analysis. The total number of individuals, at this point was of one-thousand-nine-hundred-and-ninety-eight (1998), with the oldest record from the 1st of June of 1930 (± 6 months), and the most recent, a birth from the 22nd of July of 2019.

The CSL studbook offered information on the animals' studbook ID (the number given to them in their current location); GAN (their international identification number); Their current location (if the individual was dead, this referred to the last known institution they were housed in); Sex; Birth date (with day, month and year); Their current status; Birth location; And the studbook ID of their sire and dam.

As the ease to record and share information, especially between different countries, has not always been as reliable as it is nowadays, some of the individuals on the CSL studbook, especially the ones born before the 2000's, had some incomplete information. Therefore, for the purpose of this pedigree analysis, some changes were made to facilitate the process and make possible to attain the desired results. These changes did not significantly affect the overall results and were all, previously to being made, checked with Dra. Sónia Matias for validation.

The changes were made to:
- Birth dates: on all the individuals who had a date of birth with a margin of error (for example, born on the 1st of June of 1930 (± 6 months)), it was decided that this interval would be ignored. There were a total of 380 individuals in this situation, and the intervals varied between 1 day and 2 years, with the majority being 6 months. After their removal, all the data was re-checked to make sure that the sire lines had suffered no error.
- Parents: on all the individuals with multiple possibilities for a sire and/or dam, as well as those with undetermined parents, and where it was impossible to ascertain the information, it was decided that their parents should be considered "Unknown". Which, when using the software, would be the same as them being WILD born. This was especially important in order for the software to be used properly. There were a total of 239 individuals with a sire in this situation and 180 with a dam unknown, 163 of which coincided for both parents.
- Birth location: all those animals with an undetermined institution and with a date of birth before the 1970's; as well as all the individuals with at least one "WILD" parent, were considered to have a "WILD" birth location.

- Status: Some of the animals had a "Lost to Follow Up (LTF)" status, this meant that, most likely, they had gone to a private facility or to an institution that didn't belong to EAZA, and therefore, no more data had been sent about these individuals since then. For this reason, these animals were considered dead. The number of individuals in this situation was 260.

After these adaptations, the final CSL studbook had a total registration of:
- 1050 females, 825 males and 123 with undetermined sex;
- 477 alive animals and 1521 dead ones (including the LTF);
- A total of 1561 individuals with a known birth location, distributed in 96 institutions of origin, and 437 with a "WILD" birth;
- 105 institutions that currently have at least one California sea lion;
- 1411 individuals with a known sire and 587 with a "WILD" sire;
- 1470 animals with a known dam, and 528 with a "WILD" dam;
- 17 individuals had only a known sire, and 76 only a known dam;
- 1394 individuals had both their sire and dam known.

As shown in Figure 5, only 66% of the entire population had a fully complete pedigree, meaning that only 1314 individuals had, simultaneously, a known Sex, Birth Date, Sire, Dam and Birth Location. Table 1 identifies both the number (N), and percentage of individuals, in the total population that had these missing records.
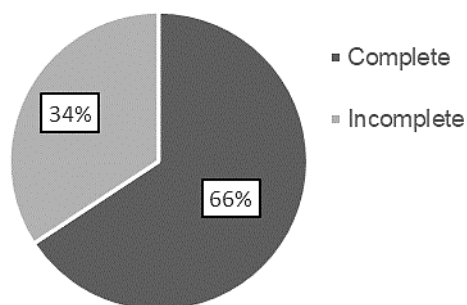
Figure 5 - Distribution of pedigree completeness.

Table 1 - Number and percentage of individuals for each type of missing record.

MISSING RECORD	N	%
Sex	123	6
Birth Date	72	4
Sire ID	587	29
Dam ID	528	26
Birth Location	434	22

It was decided that a reference population needed to be established. This was done such that an animal could not appear twice (as an individual and as a parent). Consequently, it was determined that only the animals born after 2017 were included in the reference population. The number of individuals in this population was of 85 animals. In addition to the analyses considering a reference population, a global analysis including the whole population (N=1998) was also carried out. The establishment of these two populations allowed for the

comparison of several results throughout the study. The reference population was established, mainly to understand how gene contribution has changed for this, more recent population.

5.2. Software

5.2.1. Microsoft Excel

As previously mentioned, an Excel file, with all the records of the CSL compiled up to the 22nd of April of 2020, was sent by the studbook manager. This studbook information was exported from the ZIMS software, and it was the basis for all the pedigree analysis, after application of appropriate adaptations.

Microsoft Excel was the primary tool used to help construct/design the graphs that are used in this study, allowing a more summarized visualization of the data, and making it easier to understand the results and draw the conclusions. Nevertheless, the majority of the data used for these graphs was primarily obtained from analysis carried out with ENDOG and CFC, presented next.

5.2.2. CFC

After the initial validation and analysis of base data, with Excel, the CFC (Sargolzaei et al, 2006) was used, for a preliminary assessment of the information. This software is different than other pedigree analysis software because it has been shown to be extremely efficient in analysing very large data sets (up to several hundred individuals), due to underlying algorithms (Sargolzaei et all, 2006). The CSL studbook did not have that high a number of animals, but CFC was used for preliminary analyses and for renumbering of individuals, which was a required input for other software packages such as ENDOG.

CFC software (Coancestry, inbreeding (F) and Contribution) uses an indirect method developed by Colleau (2002) to compute population statistics on relationships, such as the average coancestries between and within groups of individuals, which are necessary to monitor genetic diversity. The monitoring of this information, through probability of gene origin, can reveal the past management of populations and can be used for devising future management to minimize the loss of diversity (Sargolzaei et all, 2006).

This software was used for preliminary inbreeding calculations, coancestry between all individuals, determination of an overall brief pedigree structure (no. of sires, no. of dams, no. of individuals with and without progeny, no. of founders, no. of full siblings, etc.). It also allowed to check pedigree for possible errors, which was extremely useful since the ENDOG software used afterwards was a lot more sensitive to the presence of said errors. Therefore, this was done before any other calculations, and the use of the other software. One of its limitations was that the number of options and parameters that CFC is able to calculate, is much shorter than with ENDOG.

5.2.3.   ENDOG 4.8

After the initial assessment of the data through CFC, the ENDOG software (Gutiérrez & Goyache, 2005) was used for a more thorough analysis of the California sea lion studbook information. Most of the results reported in this study were obtained with this software, either by itself or in combination with CFC and/or Excel.

ENDOG is a population genetics computer program that conducts several demographic and genetic analyses on pedigree information. It is, therefore used to help researchers or those responsible for management of populations, to monitor the changes over time in genetic variability and population structure (Gutiérrez, Goyache & Cervantes, 2010).

For this study, ENDOG was used to obtain various pedigree analysis parameters, such as, inbreeding coefficient, no. of known generations, effective population size, founder and ancestor contributions, to determine high inbred matings, as well as other information regarding the institutions, such as genetic distances, contribution to gene diversity and genetic importance. In nearly all cases, these parameters were obtained both for the total population and for the reference population.

This software is able to calculate a larger variety of parameters, than CFC, however its limitations fall with the harder learning curve and the low flexibility of the file formats that can be used on it.

5.2.4.   SAS

This programme was used further along in the analysis of the results taken from the studbook. It was mainly applied in various statistical analysis, including linear regression analysis of some of the results that were obtained from the other software.

SAS (Statistical Analysis System) is a data analytics tool used to explore huge volumes of data quickly to identify patterns, trends, and opportunities for further analysis (SAS Institute, 2020).

SAS was used specifically to determine frequencies of birth by age of parent, and to obtain some of the regression graphs for the inbreeding and coancestry values.

5.3.   Pedigree Analysis

The degree of pedigree completeness was assessed by computing the percentage of ancestors known by generation, both for the whole population and for the reference population. These analyses were carried out with the ENDOG software, as in, Vicente, Carolino & Gama (2012) and Piccoli et al. (2014). Pedigree completeness was further assessed by computing the equivalent number of complete generations known per animal (ni) and calculated as

$$ni = \frac{ns + nd}{2} + 1,$$

where ns and nd correspond to the number of generations known for the sire (s) and dam (d), respectively, when s and d are known. When the parent was unknown, the corresponding parent was given the value of -1. For founders, 0 was the assigned number for ni.

ENDOG software (Gutiérrez, Goyache & Cervantes, 2010) was used to compute the Generation Interval (GI), also referred to as generation length. This is defined as, the average age of parents at the birth of their progeny, which are kept for reproduction (James, 1977). This is different than the average age of parents at the birth of their offspring, used for reproduction or not. The Generation Interval was computed (Table 3) for the sires and dams of all pups born, with the 4 different paths of selection (father- son, father - daughter, mother - son and mother - daughter).

An individual was considered to be a founder if it had no parents known. The genetic contribution of founders, to the total and reference populations was computed by ENDOG software by calculating the Average Relatedness of each founder with the whole or reference populations. This, average relatedness coefficient (AR), is defined as the probability that an allele randomly chosen from the whole population in the pedigree, belongs to a given animal (Gutiérrez, Goyache & Cervantes, 2010). In other words, it represents the mean relationship of each individual with all (or reference) animals in the pedigree (Vicente, Carolino & Gama, 2012).

The effective number of founders (f_e) corresponds to the number of founders which would originate the observed genetic diversity, if they all had the same genetic contribution to the population. It was computed from those contributions as:

$$f_e = \frac{1}{\sum_{k=1}^{f} q_k^2} \,,$$

where qk is the probability of a gene originating from the k ancestor. An ancestor was considered any individual in the pedigree (founder or not) with progeny recorded. The contribution of ancestors to the total and reference populations was estimated with ENDOG, by computing the marginal genetic contribution of an ancestor in addition to what is already calculated for other related individuals. The effective number of ancestors (f_a) was then calculated as:

$$f_a = \frac{1}{\sum_{j=1}^{a} q_j^2} \,,$$

where qj is the marginal contribution of an ancestor j, which is the genetic contribution made by an ancestor that is not explained by other ancestors chosen before. Furthermore, ENDOG establishes the founder genome equivalent (f_g), as described by Caballero and Toro (2000), where the parameter f_g was obtained by the inverse of twice the average coancestry between the individuals of the population (Gutiérrez, Goyache & Cervantes, 2010).

This same software also allowed for the computation of the Genetic Conservation Index (GCI), which was computed for each individual. The index corresponds to the diversity of the

contribution represented in an animal genotype, and is computed from the genetic contributions of all the identified founders as:

$$GCI = \frac{1}{\sum pi^2}$$

where pi^2 is the proportion of genes of founder i in the pedigree of a certain animal. The Alderson's (1992) GCI index is based on the assumption, that the objective of a conservation program is to retain the full range of alleles possessed by the base population (Gutiérrez, Goyache & Cervantes, 2010).

Information about the different subpopulation/institutions was firstly assessed by computing the coefficient of coancestry between subpopulation/institutions. This coefficient is the average pairwise coancestry coefficient (fij) between individuals of two subpopulations, i and j, of a given metapopulation including all $Ni \times Nj$ pairs (Gutiérrez, Goyache & Cervantes, 2010).

The parameters Fis and Fst, developed by Sewall Wright (1969, 1978), are both a measure of genetic structure, where Fis represents the mean inbreeding of a given subpopulation and Fst represents the genetic distance between pairs of subpopulations. These were computed in ENDOG as:

$$Fis = \frac{\tilde{F}-\bar{f}}{1-\bar{f}} \text{ and } Fst = \frac{\bar{f}-\tilde{f}}{1-\tilde{f}},$$

where $\tilde{f}$ and $\tilde{F}$ are respectively, the mean coancestry and the inbreeding coefficient for the entire overall population, and $\bar{f}$, the average coancestry for the subpopulation (Gutiérrez, Goyache & Cervantes, 2010).

The Inbreeding Coefficient (F) was calculated, for each individual, by means of the ENDOG software, in which the inbreeding coefficient, of an individual (Fi), is:

$$Fi = [\left(\frac{1}{2}\right)^{n+1} (1 + Fc)],$$

where n is the number of generations between the two parents of the individual (i), "through" to their common ancestor, and Fc is the inbreeding coefficient of this common ancestor (Gama, 2002).

Also, based on the individual F, the rate of inbreeding per year was estimated by the regression coefficient of F on the year of birth. The rate of inbreeding, per generation was determined in two different ways. In the first,

$$\Delta Fg = \Delta F/year \ x \ (GI),$$

where GI is the generation interval. In the second method, the rate of inbreeding per generation was obtained from the individual rate of inbreeding (ΔFi), which was computed as in Gutiérrez, Goyache & Cervantes (2010),

$$\Delta Fi = 1 - (1 - Fi)^{\frac{1}{(ni-1)}},$$

where Fi is the coefficient of inbreeding of an individual and ni is its equivalent number of equivalent complete generations. Piccoli et al. (2014) mentions that since the latter considers the evolution of inbreeding throughout the period analysed, it should be considered to be more reliable. The rate of inbreeding per generation for a population on N animals was then computed as:

$$\Delta F = \frac{\Delta F_i}{N}$$

The effective population size was assessed by different methods, besides the typical

$$Ne = \frac{1}{2\Delta F} ,$$

where ΔF is the rate of increasing in inbreeding, per generation. ENDOG gives three additional estimates of Ne, by computing the regression coefficient (b) of the individual inbreeding coefficient over: i) the number of full generations traced; ii) the maximum number of generations traced; and iii) the equivalent complete generations. These, especially when available information is scarce, can be useful to inform on the lower (i), upper (ii) and 'real' (iii) limits of Ne in the analysed population. Besides these, ENDOG computes Ne in an alternative approach, followed by Piccoli et al. (2014), described as the "realised" Ne . Where it uses the same formula but through addressing the individual rate of inbreeding (ΔFi) (Gutiérrez, Goyache & Cervantes, 2010). This last way is not dependent on the whole reference population mating policy but on the matings carried out throughout the pedigree, of each individual (Gutiérrez et al., 2008).

6. RESULTS AND DISCUSSION

6.1. Pedigree Information

Using the ENDOG software, the known pedigree completeness by generation was assessed for the whole population, as shown in Figure 6. Additionally, this was also done for the reference population (Annex 1).
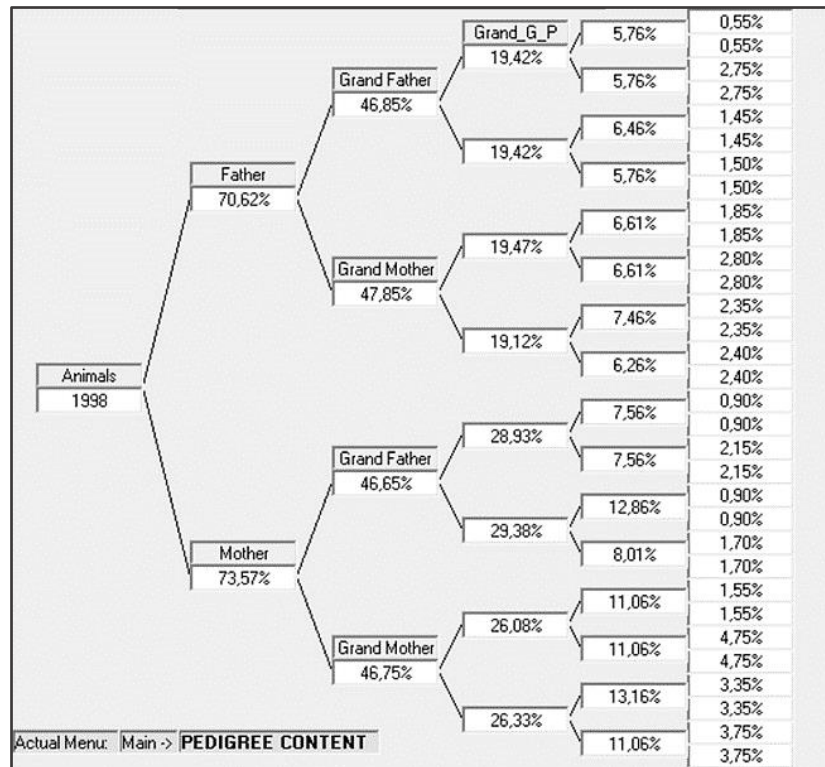
Figure 6 - Known pedigree content for the total population.

Overall, in the total population grandparents were known for slightly less than one-half of the animals. The situation improved for the reference population represented by animals born from 2017 onwards, where all animals had maternal grandparents known and nearly 85% to 90% had paternal grandparents known.

The degree of pedigree completeness can be further assessed by computing the mean number of individuals with ancestors known generation by generation in the past, as shown in Figure 7, for the total and reference populations.
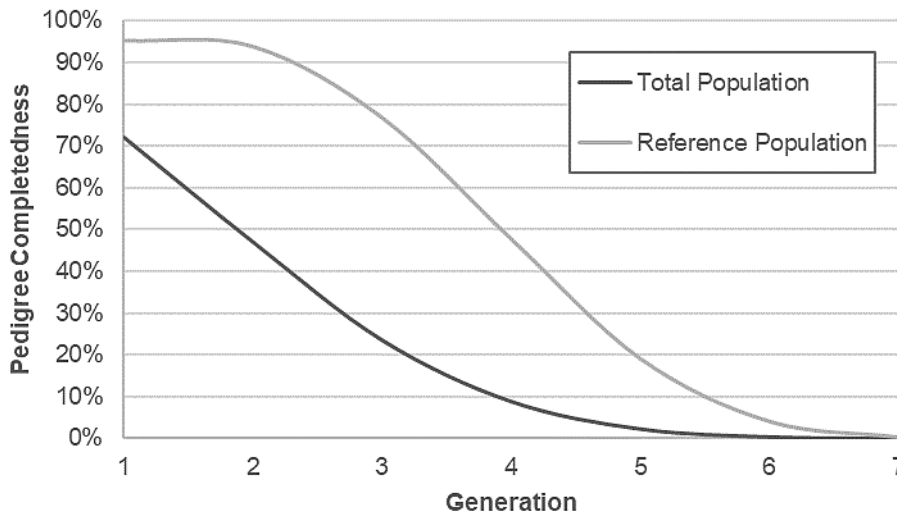
Figure 7 - Comparison of pedigree completeness per generation, in the total and reference populations.

Estimates of genetic variability depend largely on the amount of available pedigree information, which may be measured via pedigree completeness (Stephens & Splan, 2013). Within the total California sea lion population, pedigree completeness peaked at 72% in the first generation and then steadily decreased as more generations were considered. In contrast, pedigree completeness for the reference population remained above the 90's percentile for the 1st and 2nd generations, before it began to decline. Still, nearly 50% of the animals had ancestors known in the fourth generation. Overall, maximum pedigree depth extended to 7 generations.

The values of completeness, for this population, were somewhat lower than most other studies with domestic species. Although these were not performed in California sea lions, they had a pedigree completeness, at least at the 1st generation, in the 90's percentile, for Lusitano horses (Vicente, Carolino & Gama, 2012), American Shire horses (Stephens & Splan, 2013), Maremmano horses (Giontella et al., 2018) and Lusitano horses in Brazil (Da Silva Faria et al, 2018). With Giontella et al. (2018), even referring that the completeness was considered "good", because it was above 90%, for the overall population. Still, Piccoli et al. (2014), with various cattle breeds were able to conduct a pedigree analysis which had a completeness similar to that of the current study.

Nevertheless, it is important to understand that a lack of complete pedigree information will result in an underestimation of the inbreeding coefficients and an incorrect assignment of influence to founders and ancestors (Stephens & Splan, 2013), both of which will be mentioned further along.

Figure 8 shows the evolution of the level of pedigree completeness, assessed through the mean number of Equivalent Complete Generations, by year of birth for the CSL population analysed here.
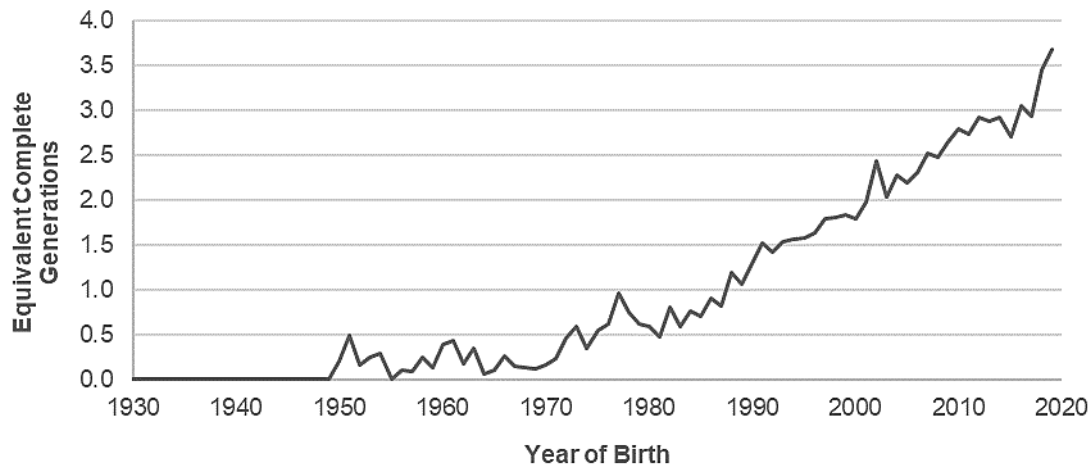
Figure 8 - Mean equivalent complete generations by year of birth.

This revealed that over time, the number of generation equivalents has increased almost steadily, meaning that there has been a gradual increase in pedigree information. Still, there was a mean of only 1.54 (±1.20) Equivalent Generations known for the entire population. However, this has risen to an average of 2.98 (±0.78) in the last 10 years, and 3.37 (±0.76) in the reference population, with 2019 having the highest average of 3.67 (±0.59) complete generation equivalents.

6.2. Demographic Analysis

As stated by Foose (1980), demography assessment can provide specific recommendations that can be used in aiding the management of wild species, in captivity. A demographic analysis can be used in comparative studies to evaluate husbandry practices or to assess vulnerability to inbreeding depression. These studies can lead to improvements in both husbandry and in promoting exchanges between regional programmes to keep inbreeding within regions at acceptable (low) levels (Princée, 2016). Sometimes, simple demographic parameters have a large impact on the evolution of the genetic variability and largely depend on the management of the population (Gutiérrez, Goyache & Cervantes, 2010).

Therefore, assessing not only these demographic parameters, but also their evolution in time can help in the management of the population in question. Moreover, the use of the means for these parameters, over time, absorbs some of the fluctuations typically observed in specific years (Foose, 1980).

6.2.1. Demographic Overview

Table 2 summarizes some relevant demographic parameters for the entire known population of the California sea lion present in the European studbook. In further chapters these will be, separately, discussed more thoroughly.

Table 2 - Demography overview.

PARAMETER	Total Population
Total number of animals	1998
Number of progeny	1458
Number of sires	177
Number of dams	363
Average number of offspring per sire	7.97 (±8.57)
Average number of offspring per dam	4.05 (±3.16)
Mean equivalent number of complete generations	1.54 (±1.20)
Mean generation interval	11.52 (±4.70)
Births per year	25.34 (±15.71)
Births per Institution	16.26 (±2.15)
Average sire age	12.21 (±5.40)
Average dam age	10.67 (±4.29)
Dam's Interbirth Interval (in days)	661.85 (±522.17)
Dam's Age at 1st Birth	7.15 (±3.22)
Dam's Age at Last Birth	12.96 (±5.09)

6.2.2. Current Population Overview

6.2.2.1. Sex and Age Distribution

It was thought that a first brief analysis of the current living population was important. There were, at the time of this study, 477 alive individuals in captivity, that had been recorded on the CSL studbook, that is managed by the Lisbon Zoo. Of these, 2 had no recorded birth date and 5 had an undetermined gender. Thus, only 276 females and 196 males were considered. These numbers are similar to the 2016 studbook analysis report, which revealed 443 individuals, with 178 males, 263 females and 2 of unknown sex (Meijer, 2017).

The most conventional visual representation of a population is an age pyramid, which provides an overview of the population status, at a glance (Ballou, et al., 2010). Princée (2016), citing Bodenheimer (1938), refers that there are three major shapes that are often used to interpret these pyramids:

- Pyramids with younger age classes that are larger than older classes are associated with population growth.
- A bell or pillar shape, with age classes similar in size, where the oldest age classes can be smaller are typically associated with a stable population.

- And an urn or beehive shape, with younger age classes that are smaller in size than older age classes are associated with a population decline.

However, these should not be immediate conclusions, but should be suggestions. This interpretation was made with a human population in mind, and therefore does not necessarily apply to other species. Additionally, the age structure can be in a phase of converging to a stable distribution. This means that the shape does not represent the dynamics but is just a phase. Therefore, they should be interpreted in context, with the census data (Princée, 2016).

Figure 9 illustrates the sex and age distribution of the current population, represented by the number of males and females by year of birth.
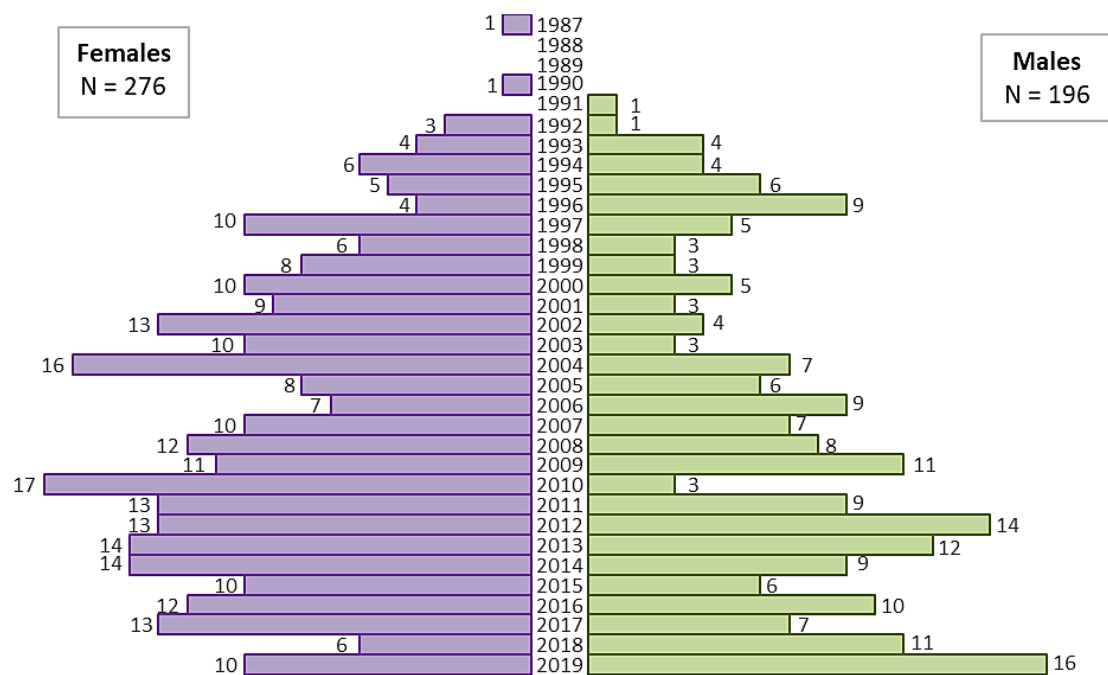
Figure 9 - Distribution of the current living population, by sex and year of birth.

The CSL population revealed a different "shape" for the female and male populations. In the former, the shape resembled a bell, with most younger classes more or less similar, and the older classes smaller, suggesting a somewhat stable population. For males, the pyramid suggested an increase in population, since the younger classes tended to be larger. For the females, the most abundant age classes were between 9 - 10 years old, and 15 - 16. In the males, the predominance of individuals were in the 0 - 1 and 7 – 8 age groups. This seems to be in agreement with the previous "shape" suggestion, which revealed a much older overall population of females than males. However, in both genders 50% of individuals were between 0 – 11 years old.

There were two females older than the oldest male, and the oldest female was born 4 years before the oldest male. This seems to suggest a higher life span for the female

population, as well as their higher average age within the population. Which seems to agree with the consensus for the wild population, addressed in the literature review, that indeed the females have a longer lifespan. The mean age of active animals is, currently, 11.66 (±7.86) years old for males, 12.86 (±7.60) years old for females, with an overall population's average of 12.32 (±7.35) years of age.

In the current population, the oldest individual is 33 years old, which also seems to be in accordance with the average of around 30 years of maximum age previously reported for captive California sea lions by SeaWorld Parks & Entertainment (2020) and Price (2002).

Meijer (2017) also suggested that the breeding age for this species, in captivity, for females, is between 3 and 23 years of age. Following this recommendation, at the time of that report, there were a total of fourteen females that were too old to breed. At present, this number has increased to twenty-four individuals. This, once again, suggests a "top-heavy" pyramid, that seems to agree with the previously stated idea that, in contrast with the males, the female population is not growing. Ballou et al. (2010) suggests that these "top-heavy" populations, may experience some difficulty, seeing as current breeders will age and be replaced by a smaller number of new breeders, which may not be sufficient to form breeding pairs to sustain the population.

6.2.2.2.   Current Institutions

Pertaining to the locations of the current population, Figure 10 was made to illustrate the frequency of the number of individuals present per their current institution. There were a total of 105 known institutions, on the studbook, with at least one California sea lion. Out of the 477 animals, 3 had an either unknown or private current location.
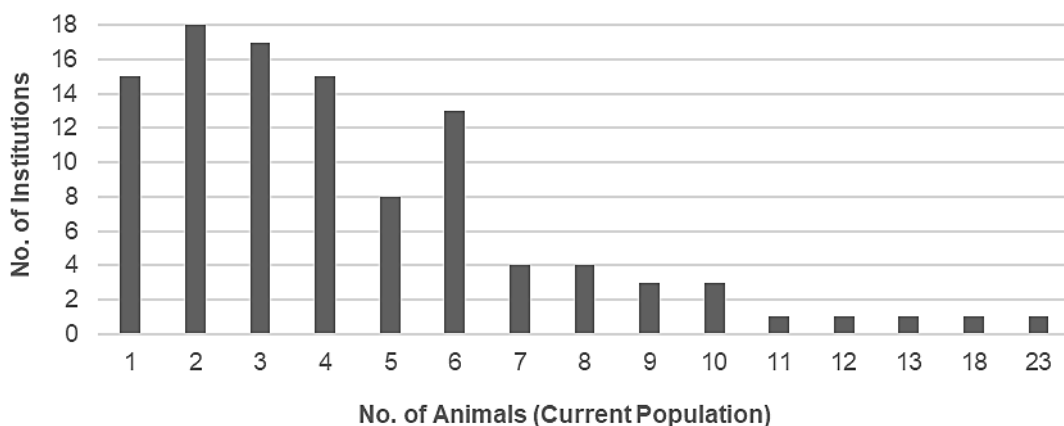
Figure 10 - Frequency of current institutions by number of individuals.

There were 15 institutions with only 1 recorded animal, and the highest number of individuals, per institution, was 23 California sea lions. These belong to an institution in

Germany, which in fact, among the countries represented in this studbook, holds the highest number of California sea lions, with a total of 112 individuals, which reflects 23% of the current population, divided between 15 different institutions.

On average, there were 4.51 (±3.51) individuals per organization, though the majority had only 2 recorded animals, and only 18% had more than 6 individuals. The fact that there were several institutions with only one animal seems to contradict this species social behaviour, however, the "Current Location" of this STD includes some of the institutions that do not belong in EAZA. Yet, they were still taken into account for this analysis. This would mean that, at least some of the institutions that are listed to have only 1 individual, very likely are from other continents, and therefore the rest of their records are with their own studbook holders.

6.2.3.   Births

Out of the 1998 recorded animals in the studbook, 72 did not have a known birth year. Therefore, there were a total of 1926 individuals with a recorded date of birth: 1020 females, 789 males and 117 with an unknown sex. The earliest record was from June 1st of 1930, and the latest was from July 22nd of 2019. These birth records are shown by year and by sex in Figure 11.
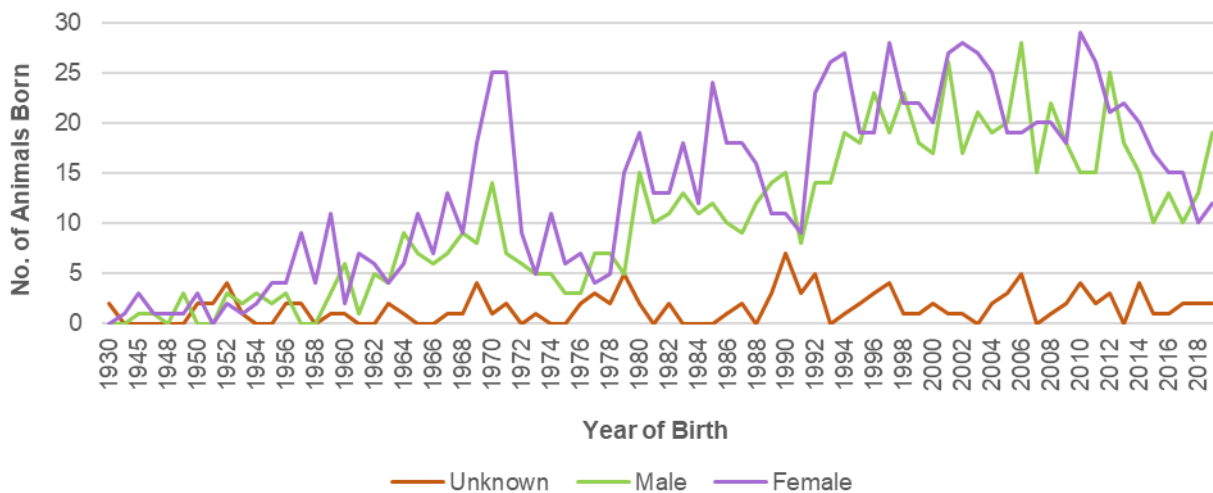
Figure 11 - Number of births by year and by sex.

The data showed an average of 25.34 (±15.71) births per year, with 2001 being the year with the most births (54 individuals). However, if only the records from 1980 onwards are considered (since it seems to be the year when the records became more consistent, which reflects the previously mentioned fact that, according to Princée (2016) the California sea lion studbook was more or less officially established in the early 1980s), this average increases to 37.43 (±9.09) individuals born per year.

The number of females born per year was, almost always, higher and had an average of 13.42 (±8.66), versus the average for the males, which was 10.38 (±7.38) births. The number of individuals born per year with an undetermined sex has been very consistent since the first record in 1930, with an average of 1.54 (±1.53) individuals each year. The number of recorded births appear to have been decreasing since 2013, however in 2019 they seem to be rising again.

Concerning sex determination systems in the animal kingdom, these are known to vary between strict genetic control to strict environmental control. In mammals, as it is in birds, this is a genotypic (heterogametic) sex determination (GSD) system, that results in sex determination at fertilisation. Therefore, the chances of being male or female in a GSD system are expected to be equal and should result in a sex–ratio at fertilisation, that is expected to be 0.5. Even though, the random nature of GSD systems explains fluctuations in annual sex–ratio in small populations, over a long period of time this is expected to be equal. However, male, or female bias have been reported in both birds and mammals (Princée, 2016).

The sex ratio at birth, in relation to females, by year of birth was calculated for the overall population of California sea lions (Figure 12).
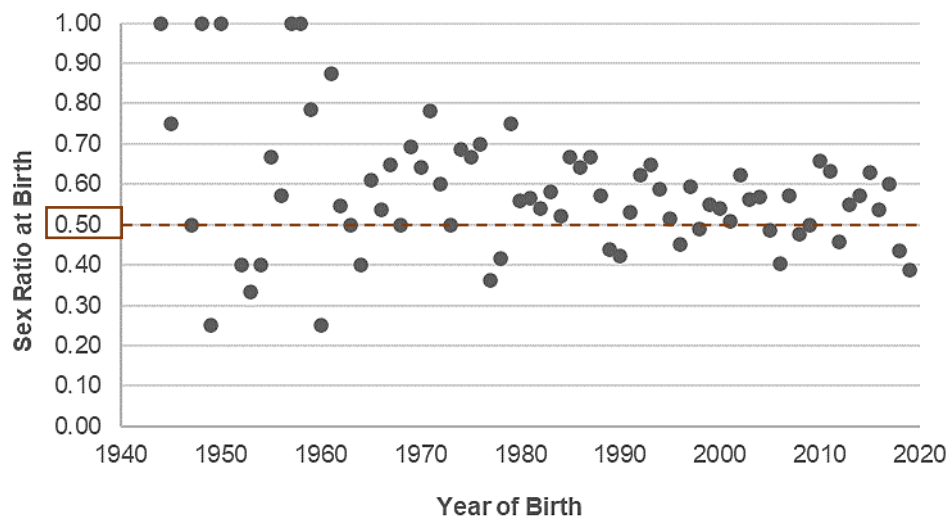
Figure 12 - Sex ratio at birth in relation to females.

This population had a mean female/male sex–ratio at birth of 0.56 (Males:789; Females:1020), which suggests a slight female bias. However, as shown in Figure 12, there have been several years where the sex-ratio at birth was male biased, though less frequently. Additionally, it is also possible to notice that since the 1980's this ratio has been much closer to being equal, as is expected of mammals. And both in 2018 and 2019, the sex-ratio at birth was 0.43 and 0.39, respectively, which means it was male biased.

As Zuckerman (2009), citing Darwin (1868) mentions, "any change in the habits of life, whatever these habits may be, if great enough, tends to affect in an inexplicable manner the powers of reproduction. The result depends more on the constitution of the species than on the nature of the change; certain whole groups are affected more than others". Even though the California sea lion is known to have the ability to breed readily in captivity (Reeves & Mead 1999), there have been studies showing that its reproductive success can be affected. Temte (1993), undertaking a study on the influence of latitude on the birth dates of CSL pups, confirmed a previous report of Schusterman et al. (1982), demonstrating the existence of marked latitudinal variation that affects, not only the timing of birth, but also the variance of the mean birth date.

Figure 13 was established to assess if changes of the seasonality of births, which are known to happen in the wild, also occur in captivity. This figure shows the number of individuals born, for each month of the year. This was done for the whole population, however in order to make sure that the results where realistic some individuals had to be counted off. These were all the ones who had an either undetermined or uncertain month of birth, as well as those that were considered to be wild born. The total, after this, was of 1544 recorded births, which are represented in Figure 13.
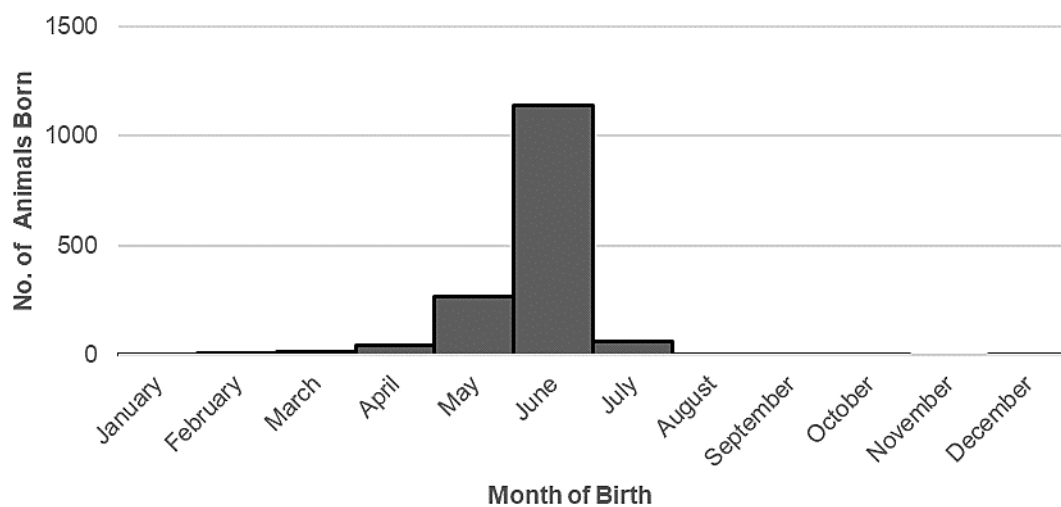
Figure 13 - Seasonality of births.

These results revealed that the majority of births occurred in June, with 74% of the individuals falling in this category, while 95% of all individuals were born between May and July, which is the typical time frame for the wild populations (Perrin, Wursig, & Thewissen, 2008 & Jefferson, Webber & Pitman, 2015). Around 3% of the population gave birth in April, which according to Meijer (2017), can also happen. The remaining 2%, born during the other months, may actually just belong to old data that was not accurate.

Regarding the population's birth locations, only animals with a captive birth, and a known location were considered for analysis (Annex 2). There were a total of 1561 individuals born in 96 institutions. However, 50% of the animals were born in only 14 institutions, and 47 locations accounted for just 5% of the overall captive population of California sea lions. On average, there were 16.26 (±21.03) births recorded per institution, with the highest number being 100 animals born in one location. This was from an institution in Germany, though not the same that currently has the largest number of individuals. In fact, Germany supplied 36% of the total captive born population of California sea lions registered in the studbook, born between 14 different institutions.

There were a total of 29 establishments (30%) with only 1 recorded birth and 59 (61%) with a maximum of 3 animal birth records. This, however, does not mean that they only house one individual, just that only one birth has been recorded.

6.2.4. Sires and Dams
6.2.4.1. Offspring per Parent

The CSL studbook contained a total of 177 known sires, with a progeny of 1411 individuals between them, and a total of 363 dams with a progeny of 1470 individuals between them. This indicates a sire/dam ratio of around 0.49, which means that for breeding purposes, on average, there is 1 male for every 2 females. However, if only the living population is considered, the sire/dam ratio lowers to 0.32, which implies that, on average during breeding, there now is 1 male for every 3 females. This is fairly different than in wild populations, where each bull has between 5 – 20 females in a harem (Grainger, 2005). However, for matings in captivity, selection is artificially made for a breeding pair. Also, of a total of 825 males, in the overall population, only around 21% had at least one offspring and of the 1050 females, only 33% were dams.

Figure 14 shows the distribution of the number of progeny per parent, for the overall population, considering that the earliest recorded captive offspring was born on April 1st of 1950, and the most recent on July 22nd, 2019.

% of Parents by No. of Offspring
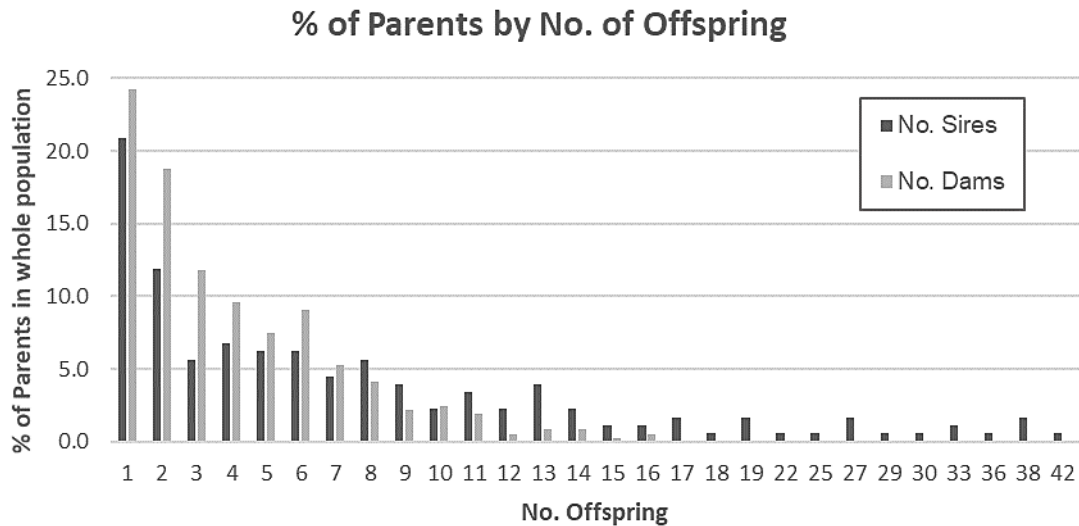
Figure 14 - Percentage of sires and dams by number of offspring.

As can be seen in Figure 14, the majority of recorded sires (21%) only had 1 offspring, with the highest number being 42 individuals from 1 sire. Additionally, only 46 out of the 177 sires (26%) had over 10 offspring. On average, in this studbook, one sire had 7.97 (±8.57) offspring.

For the dam group, 24% of the females (88 dams) only had 1 offspring, and the highest number of offspring born to the same dam was 16, observed in 2 females. Also, only 28% of the females (102 dams) had more than 5 offspring. On average, the total captive population of California sea lions, had 4.05 (±3.16) offspring per dam. This number was much lower than with the males, being almost half the progeny per dam than per sire, which is in accordance with the sire/dam ratio mentioned previously. No information on the number of offspring per parent was found, in regard to the wild population and other captive populations.

6.2.4.2.   Parents Age at Progeny's Birth

The information about the sires' and dams' age at their progeny's birth is summarized in Figure 15. For these calculations 2 sires (with a total of 4 offspring), and 3 dams (with a total of 3 offspring) were disregarded due to lack of a recorded birth date.
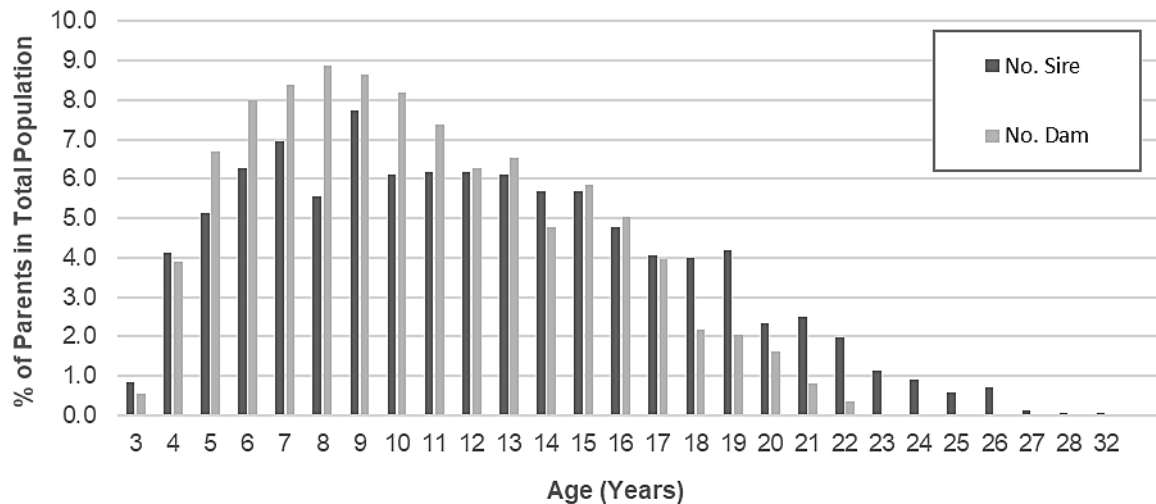
Figure 15 - Percentage of sires' and dams' age at progeny's birth.

As seen in Figure 15, the youngest age for a sire to have had an offspring was 3 years old (12 occurrences), with the oldest one being a sire at 32 years old. The average age for a male to become a sire was 12.21 (±5.40) years, and the sire age with the largest number of recorded births was 9 years. Furthermore, just over 50% of the individuals were born with a sire aged between 7 and 14 years old. This, can possibly be explained by the fact that, at least in the wild, males may not successfully reproduce until about 8 or 9 years of age, when they attain full size and are able to compete for females (SeaWorld Parks & Entertainment, 2020; Meijer, 2017), even if in captivity the mating pairs are purposely selected.

For the dams, the data revealed that the youngest age at the time of birth, was also around 3 years old (8 occurrences), with the oldest recorded dams (5 individuals) giving birth at 22 years of age. This showed a substantial difference, of 10 years, with the oldest sire to have had an offspring. However, the most common age, for the dams to have offspring was 8 years old, which was very close, to the age of the sires. The average dam age at which to have calves in this population was 10.67 (±4.29) years old, almost 2 years younger than the males. Overall, nearly 50% of the population had their dam aged between 6 and 11 years old.

Both for the sires and dams, the youngest ages to have offspring seem to be consistent with other studies on captive populations (Meijer, 2017), with SeaWorld Parks & Entertainment (2020) referring that in zoological environments, successful mating has been observed in males as young as two years, which if considered the average 1-year gestation period for CSL, is in accordance with the population under study. Additionally, the oldest age for an individual to have an offspring also seemed to be very close with previous studies on this captive population, with the females having a breeding longevity up to 23 years of age, while the males were able to continue having offspring up to ages close or higher than the average longevity

for this species in captivity (Meijer, 2017). However, according to Grainger (2005) the age of the last breeding of females and males, in this species, is unknown.

The evolution of the parents' age at birth, through the different decades, was also determined (Figure 16).
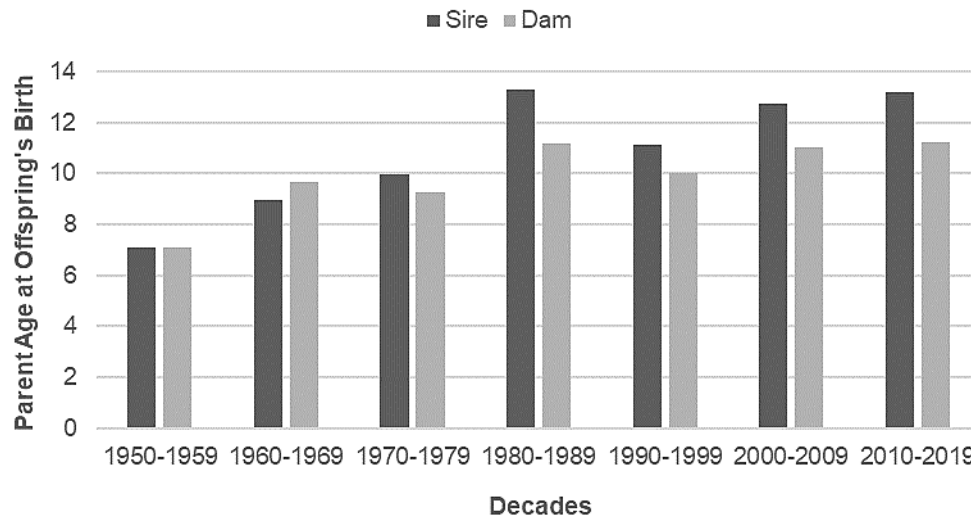
Figure 16 - Mean sire's and dam's age at progeny's birth by decade.

For the sires, the mean age for producing offspring, per year, has suffered some changes, with an overall increase, and reaching some of its highest numbers in the last decade, when the average reached 13.19 (±5.92) years of age. However, if only parents of the individuals from the reference population (animals born between 2017 and 2019) were considered, this average decreased to 11.13 (±5.06) years old.

The average of a dam's age at birth has not seen as much variation, mainly since 1960, but especially since the 1980's. However, in the last decade, the average has reached its highest, at 11.23 (±4.17) years of age, and even in the reference population, it has shown an even larger increase, with the individuals in this population having dams with a mean age of 11.68 (±4.18) years. Both these ages were higher than the average for the total population, which does not happen in the sires where, at least in the reference population, it showed a decrease. This can be due to an effort in delaying the loss of genetic variation, by delaying female breeding (Mace, 1986, Ballou & Foose, 1996; Ballou & Cooper, 1992; Ballou et al., 2010). This method will be more thoroughly discussed later on.

ENDOG software (Gutiérrez et al., 2010) computed the Generation Interval (Table 3) for the sires and dams of all pups born, with the 4 different paths of selection (father- son, father - daughter, mother - son and mother - daughter).

Table 3 - Mean generation interval for each selection path.

TYPE OF INTERVAL	N	MEAN INTERVAL
Father - Son	104	13.21
Father - Daughter	240	12.26
Mother - Son	107	10.36
Mother - Daughter	238	10.53
Total	689	11.52

The overall mean generation interval, based on the age of dams and sires used in the CSL population, was 11.52 years, and it was the highest in bulls by about 2.3 years. For the different selection paths, the Sire – Son was the longest, with the shortest being the Mother – Daughter, at 10.53 years. There was a clear variation in generation intervals especially between the sires and the dams, where in the latter the difference between son and daughter was only of around 0.3 years, while for the sires, there was a difference of nearly 1.0 year between sons and daughters.

Meijer (2017) referred a generation interval of 11.2 years in the 2016 studbook report, which shows only a slight increase of about 0.3 years, in the analysis of the current studbook. Possibly due to the strategy of decreasing the loss of genetic diversity, by increasing the duration of the GI. The Marine Mammal TAG's, Regional Collection Plan (2012) referred an average GI of about 12.7 years, for the American studbook. This shows that the individuals in that studbook have an average generation interval of around 1.2 years longer.

Regarding the generation length of a population, Stephens & Splan (2013) describe that longer generation intervals are often present in breeds with an extensive evaluation or a breeding approval process, while shorter ones tend to be seen in populations with the heavy use of artificial insemination. Also, Farquharson, Hogg & Grueber (2017) refer that species characterised by short generation lengths, have a higher risk of adaptation to captivity, over time, than species with longer ones. Furthermore, several studies discuss the advantage of a longer GI as a way of reducing the rate of inbreeding, per generation and thus delaying the loss of genetic variation (Mace, 1986, Ballou & Foose, 1996; Ballou & Cooper, 1992; Ballou et al., 2010).

However, Farquharson, Hogg & Grueber (2017) found that there may be a downside to this strategy, and that reproductive success may show a decline, as dam age at first breeding increases. This indicates that, captive breeding programs that delay female breeding, with this goal in mind, can risk reduced productivity, which can translate into negative population growth and demographic instability. And though this was a study on Red Pandas, it has also been researched for California sea lions, with Hernández-Camacho, Aurioles-Gamboa & Gerber (2008) finding that, for a wild population of *Zalophus californianus*, in the Gulf of California, Mexico, the birth rates among older females, do suffer a decrease.

6.2.4.3. Interbirth Interval

For the female population, it was thought to be important to assess other more specific parameters, including a dam's age at the first and last offspring's birth, and the interval between consecutive births, assessing its variation between different individuals, and the evolution throughout the years.

Considering the interbirth interval, in the 360 known dams with recorded birth dates it was possible to ascertain 1074 intervals. It is important to recall that, especially in the earlier records, the registered date of birth was not fully reliable, with several individuals having the 1st of January as their day and month of birth. Nevertheless, in more recent years the information on date of birth is believed to be more reliable. The overall distribution of frequency of dams for each interbirth interval is shown in Figure 17.
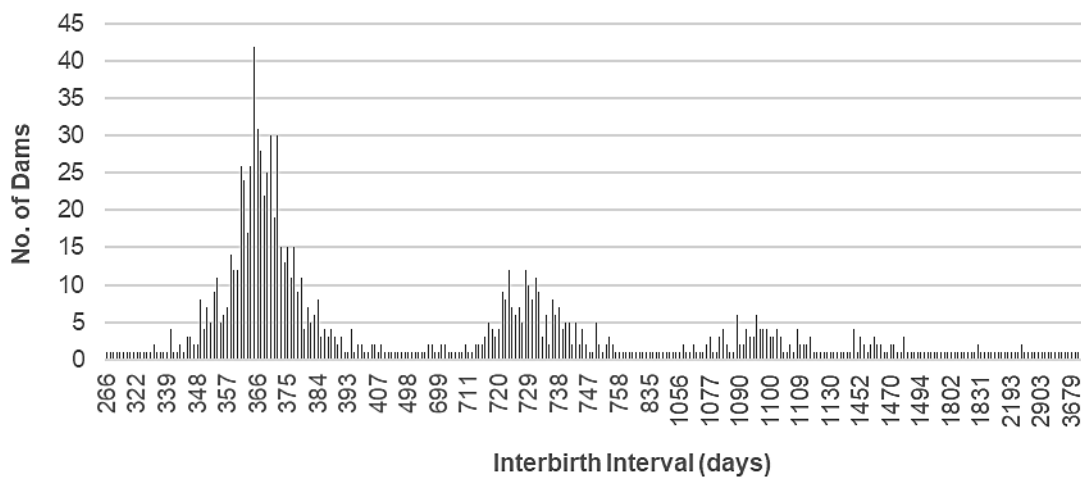
Figure 17 - Frequency of dams by interbirth interval.

The general pattern observed is a very consistent yearly cycle, with most intervals being close to one year, followed by intervals of two years, three years, etc. The data revealed 266 days (just under 9 months) to be the lowest number between two consecutive births. Which is, in fact, not long enough for this species, however the dam could have given birth early, due to a stillborn, and it was still recorded. The longest interval was 5493 days (around 15 years), probably as a result of incomplete recording. This revealed a very significant variation between births, which can possibly be explained with the transfer of this second female to a different location during that period, where no male was available.

The average number of days between two sequential births was 661.85 (±522.17) days, which is just under 2 years. Figure 17 very clearly revealed two prominent clusters, the first being the more obvious one, around the 340 to 390 days (which is around the 1-year mark), and the second between 715 to 745 days (which is around 2 years). The first, more obvious one, consisted of 54% of all intervals recorded, and the second 17% of the recordings.

It also showed another two, much less obvious, clusters in the 1080 to 1100 days (about 3 years), and the 1440 to 1470 days (roughly 4 years). Overall, 78% of dams had an interval within these four clusters. This indicates that, although the data showed a lot of discrepancy, the majority of individuals still seemed to keep their known seasonality, with mating and parturition in very specific times of the year. And, as previously stated, at least a considerable part of the other intervals were due to an uncertain recorded date of birth. Also, even in the wild, with available mature males, females can skip a year where they don't have a pup (Perrin, Wursig, & Thewissen, 2008). The fact that there was still some variation is addressed by the same authors, who refer these also happen in the wild populations, based on the different temperatures, and consequentially, their geographical location.

A study on wild populations of California sea lions, referred a mean interbirth interval of 547.5 days (Jones et al., 2009). This is only two and a half months less than in the overall captive population registered in the studbook considered in the present analysis, while the "AnAge: The Animal Ageing and Longevity Database (2017)", refer an average of 730 days for captive populations. This reflects an increase of around three and a half months, compared to the studbook's CSL population.

Throughout the years, the evolution of the average interbirth interval, has shown some variation (results not shown), reaching its lowest during the 1990's with 553.93 (±378.44), and it has only increased since then, with the mean for the last decade being 783.14 (±679.25) days, which is around 5 months longer than the average for the overall population.

The possible influence of a dam's age on the interbirth interval was assessed and the results of this analysis are in Figure 18, which shows the mean interbirth interval by the average dam's age. This was assessed because, Gagliardi et al. (2007), in a study on Rhesus Macaques (*Macaca mulatta*), found that the interval between births proved to be affected, primarily, by the dam's age.
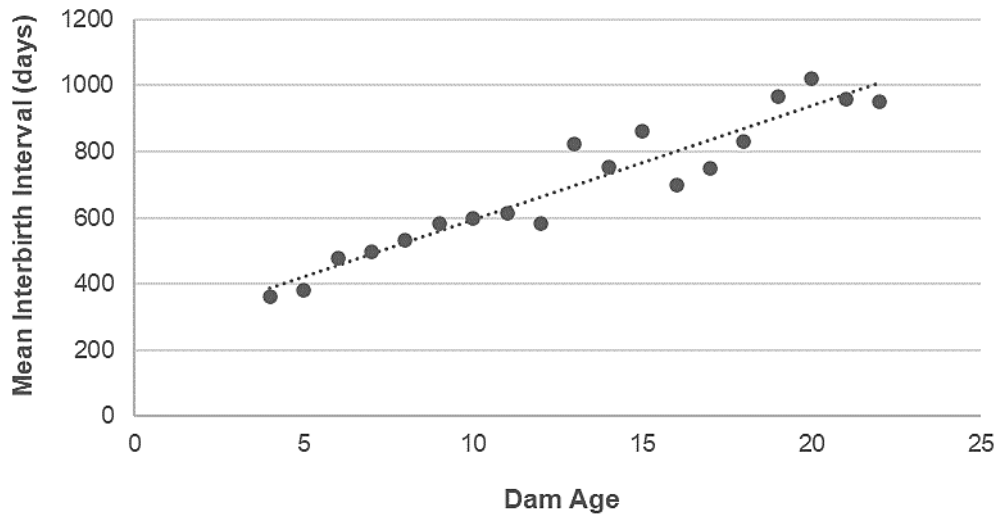
Figure 18 - Mean interbirth interval by dam age.

These results, shown in Figure 18, were in accordance with what was previously suggested by Gagliardi et al. (2007), with the mean number of days between two consecutive births, increasing with the age of the dam.

6.2.4.4.   Dam's Age at First Progeny's Birth

Regarding the dam's age at the recorded birth of her first offspring, the mean for the entire population was 7.15 (±3.22) years old. However, the most common age to have her first progeny, was 5 years old, with the oldest dam having her first pup at 21 years of age. Overall, around 55% of all the dams had their first offspring between the ages of 4 – 6 years, and only 10% had their first parturition after reaching 12 years of age.

Concerning the evolution of the mean of this parameter through time, the results are presented in Figure 19.
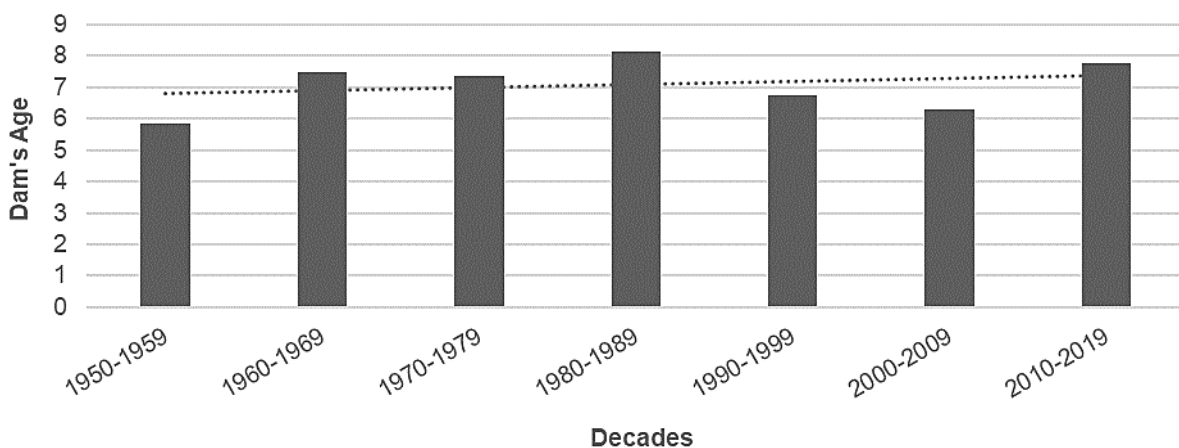
Figure 19 - Mean dam's age at first birth by decade.

These revealed some variation, especially if the mean was determined year by year. Though, if assessed decade by decade (Figure 19) this variation was less prominent, demonstrating neither an obvious increase nor decrease since the initial records.

Nevertheless, in the last decade, age at first birth suffered a small increase, especially compared with the previous 2 decades, even though when compared to the overall population, it was not as evident. In the reference population, on the other hand, a significant increase, up to 8.96 (± 4.28) years was observed, which means that in the last 3 years, on average, dams have been having their first offspring almost 2 years later, compared to the overall population. This was, possibly, due to an effort of delaying the loss of genetic variation, by increasing generation intervals (Mace, 1986, Ballou & Foose, 1996; Ballou & Cooper, 1992; Ballou et al., 2010). In comparison to the known age of sexual maturity, this showed that on average, the female population "waits" 2.5 years after reaching sexual maturity, before having a pup.

6.2.4.5. Dam's Age at Last Progeny's Birth

For the assessment of age at last progeny, only the deceased population was considered, so as to ensure more accurate results. The overall mean for the global population, was 12.96 (±5.09) years of age. However, the most common age in which to have their last offspring was 15 years of age. In the data set, 55% of dams had their last offspring between the ages of 15 – 20 years.

Concerning the evolution of this parameter in time, the results showed a more prominent variation throughout the years, in comparison to the previous parameter. Showing an evident overall increase of age at last parturition over time, as shown by the regression line with a prominent increasing tendency (Figure 20).
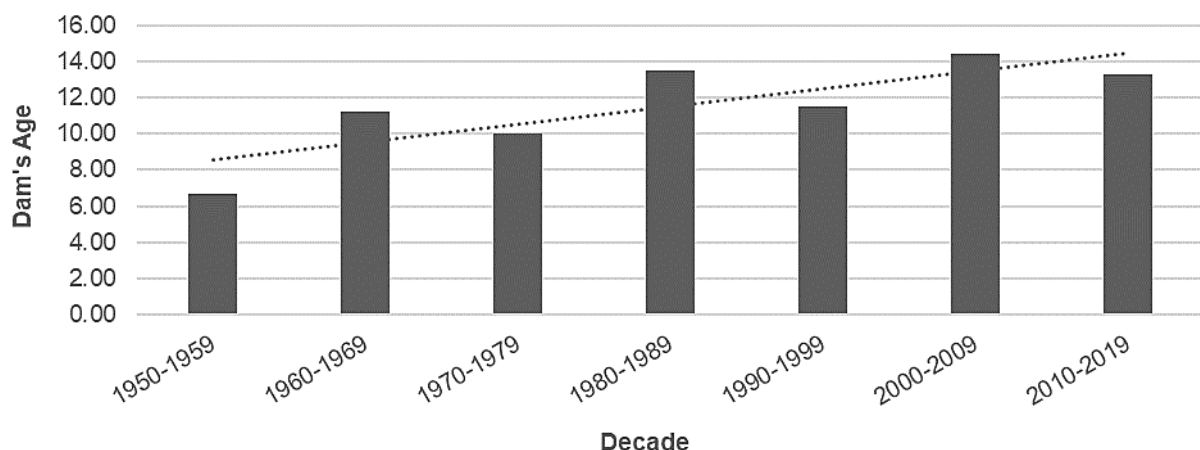
Figure 20 - Mean dam's age at last birth by decade.

This revealed that, in the last two decades, the mean age of last parturition has risen to 14.43 (±5.29) and 13.26 (±5.07), respectively, indicating that reproductive longevity has

increased, such that dams have been able to have up to, a year and half and 6 months longer to have offspring, respectively. As previously stated by Meijer (2017), the breeding age range is up to 23 years old, which means that on average, dams stop breeding 10 years before they reach this stage.

Additionally, and referring back to the age at first birth, the results showed that this population of California sea lions, has a mean interval of reproductive activity of almost 6 years.

6.3. Genetics Analysis

The maintenance of the original (wild) genetic variation is an important objective in the management of small populations of wild species (Princée, 2016). Genetic variation is considered an important requirement, for both short–term and long–term survival of a species, as it influences the individuals' ability to cope with environmental variation and therefore, lessen their extinction risk (Mace, 1986; Ballou et al., 2010; Frankham, 2010; Princée, 2016 & Ralls et al., 2017). Also, maintaining genetic diversity preserves future management options, for both wild and captive populations (Ballou et al., 2010).

Conventionally, modifications in genetic variability of a population have been assessed by evaluating the degree of inbreeding and its variations over time (Vicente, Carolino & Gama, 2012), because small, genetically isolated populations tend to lose genetic diversity, mainly by random processes, and become increasingly inbred with each generation (Ralls et al., 2017). In addition, most studies on the effects of inbreeding, in both captive and wild populations, have documented deleterious effects (Ballou et al., 2010). These negative effects, of inbreeding and the importance of genetic variation in zoo populations, were recognised in the late 1970s. This was, in fact, what caused changes in the perspective taken by studbooks, from being merely a register, into cooperative management programmes in which population genetics can be considered the "backbone" (Princée, 2016).

As stated by Gutiérrez, Goyache & Cervantes (2010) the goal in conservation programs, for endangered species, is to restrain the rate of inbreeding. Fortunately, Ralls et al. (2017), state that the negative effects of inbreeding and low genetic diversity can sometimes be reversed, by crossing at-risk populations with genetically distinct ones, which can provide genetic variation that over time can mask harmful effects and promote beneficial phenotypes.

6.3.1. Genetic Indicators Overview

Table 4 summarizes some important parameters in the genetic analysis of the California sea lion studbook population, both for the total and reference populations. These will be thoroughly addressed further on.

Table 4 - Genetic Indicators Overview for total and reference populations.

PARAMETER	Total Population	Reference Population
Total number of animals	1998	85
Average inbreeding coefficient, %	2.03 (±5.92)	3.34 (±6.08)
Inbred animals, %	16.87	52.94
Average inbreeding coefficient for inbred animals, %	12.01 (±9.39)	6.32 (±7.17)
Maximum inbreeding coefficient, %	37.5	30.27
Animals with inbreeding coefficient above 6.25%, %	11.36	22.35
Average relatedness, %	1.43 (±1.24)	2.44 (±0.90)
Rate of inbreeding/year, %	0.102	\
Rate of inbreeding/generation (%), from the ΔF/year	1.2	\
Rate of inbreeding/generation (%), from ΔFi	1.6	\
Number of founders	604	82
Effective Number of founders (f_e)	96.31	34
Number of Ancestors	182	61
Effective Number of Ancestors (f_a)	54	31
f_e / f_a	1.78	1.10
Founder Genome Equivalent (f_g)	69.94	\
Number of founders explaining 50% of the gene pool	36	12
Effective population size (Ne) from ΔFg	31.17	\
Effective population size (Ne) from ΔFi	35.83	\
Average genetic conservation index	3.12 (±2.19)	7.00 (±2.71)
Mean relationship among animals from the same herd	0.1015	\
Mean relationship among animals in different herds	0.0071	\

6.3.2.   Founders

A founder is defined as an individual with no known genetic ancestry in the pedigree. In the context of captive management, founders refer to wild–born individuals and individuals with unknown parentage (Ballou et al., 2010 & Princée, 2016). However, animals that have not reproduced are not (yet) founders since they have not contributed genetically to the captive population. Therefore, the number of founders is a rough indication of how well the source population has been sampled to provide genetic diversity to the captive population.

A large number of founders is indicative that the source population was well sampled and probably could be managed to retain much of its original genetic diversity (Ballou et al., 2010). Breeding recommendations, in the early years of population management should focus on equalising founder representation, to maintain genetic diversity in the long term (Princée, 2016).

Founders will typically have an unequal genetic contribution to the current population, because of bottlenecks occurring over time. Founder contribution can be quatified as the expected percentage of an individual or a population gene pool that have descended from each founder. Founder contributions in most captive populations are highly skewed, usually due to disproportionate breeding of a small proportion of the founders, early in the population's

history. Therefore, genetic diversity, potentially contributed by the underrepresented founders, is at a high risk of being lost due to genetic drift. Extreme cases of genetic drift are often referred to as pedigree bottlenecks, occurring when the genetic contribution of a founder passes through only one or a few individuals. The genetic drift caused by such bottlenecks can occur at any point in the pedigree, resulting in gradual erosion of the founder alleles. The more pathways a founder has in contributing its genes to the current living population, the less likely will be the loss of its alleles (Ballou et al., 2010).

For the CSL population under study, it is important to remember that just over 250 individuals had no known recorded parents. Also, even before the adjustments mentioned at the beginning of this study, the data had 348 individuals with parents classified as "wild born", for the same reasons, many of which were given this classification due to lack of records, and not because they were indeed wild caught.

Therefore, ENDOG indicated a total of 604 founders, for the whole population. However, 93 of these were considered to be "phantom" founders. This happens when one parent of a listed animal is unknown so the contribution of this unknown parent to the population is considered to correspond to a 'Phantom' founder (Gutiérrez, Goyache & Cervantes, 2010). Without these, the population had a total of 511 real founders. For the reference population, there were 82 founders, 11 of which were "phantom". This points to a much narrower contribution of founders to the reference population, indicating that genetic diversity has been lost over time. The cumulative genetic contribution of founders to the total and reference populations is represented in Figure 21.
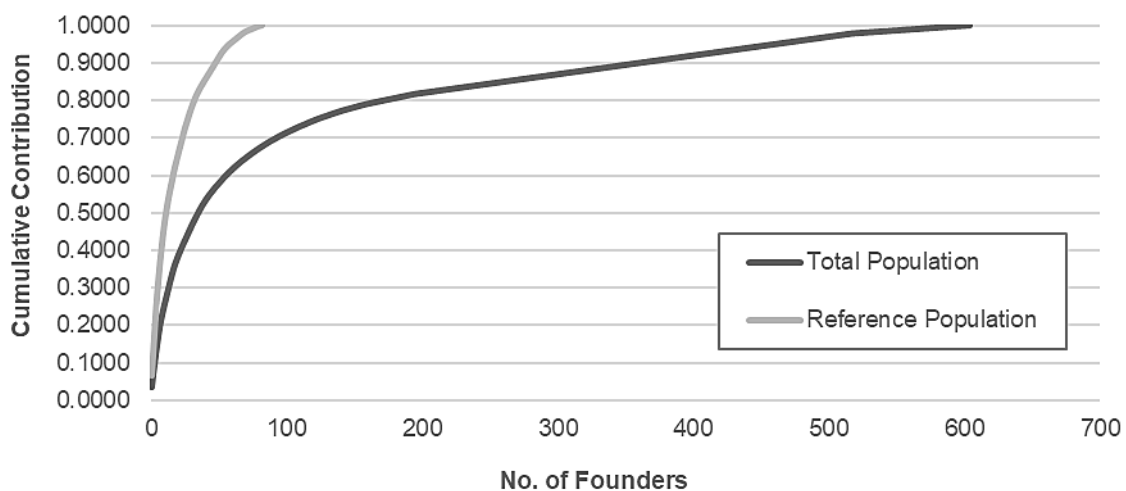
Figure 21 - Founders cumulative contribution for the total and reference populations.

From Figure 21 it is noted that for the total population, there were 36 founders explaining 50% of the gene pool, while, for the reference population, only 12 founders

explained 50% of the existing genetic diversity. These results show the unequal contribution between all the founders of the population. Additionally, in both cases, the numbers are quite low, and support the need for careful management of the existing genetic diversity.

Equalising family sizes among founders, and their descendants in subsequent generations, will not only reduce random genetic drift, but will also minimise possible selection effects through equal distribution of founder genomes in the population. However, this condition is rarely met in populations of wild animals (Princé, 2016).

The effective number of founders (f_e) was assessed and can be defined as the number of equally contributing founders that would be expected to produce the same genetic diversity as in the population under study (Gutiérrez, Goyache & Cervantes, 2010). For the total and reference populations, this was 96.31 and 34, respectively. A higher ratio of f_e relative to the total number of founders, in a population, indicates that their founders are better represented (Vicente, Carolino & Gama, 2012). This does not seem to have been the case for this studbook, where the ratio of effective to total number of founders was 0.16 and 0.41 for the total and reference populations, respectively, indicating the occurrence of bottlenecks in the pedigree. This will later be translated into increased population inbreeding. For the overall population, ENDOG also computed the expected inbreeding caused by the unbalanced founder contribution, which in this case was of 0.52%.

Regarding the number of ancestors, for this population, there were a total of 182 and 61 for the total and reference populations, respectively. As to the effective number of ancestors (f_a), this was 54 and 31, respectively. Parameter f_a is the minimum number of ancestors, not necessarily founders, explaining the complete genetic diversity of a population if they all had the same genetic contribution to the population. This parameter complements the information offered by the effective number of founders, accounting for the losses of genetic variability produced by the unbalanced use, over time, of reproductive individuals producing bottlenecks (Gutiérrez, Goyache & Cervantes, 2010).

Therefore, the f_e /f_a ratio can provide important information about the population. It should be close to 1, if important bottlenecks have not occurred in the population (Boichard et al., 1997). For this population, it was around 1.78, which seems to suggest that there have been important bottlenecks in this captive population.

Since both the skewed founder contributions and loss of alleles due to genetic drift will result in the loss of founder genetic diversity, the actual genetic contribution of the founders to the gene pool may be less than expected (Ballou et al., 2010). Lacy (1989, 1995) introduced the concept of founder genome equivalent to illustrate the combined effect that skewed founder contribution and genetic drift have on the genetic diversity of a population.

This founder genome equivalent (f_g), can be defined as the number of founders that would be expected to produce the same genetic diversity as in the population under study, if

the founders were equally represented, and no loss of alleles occurred (Gutiérrez, Goyache & Cervantes, 2010). The California sea lion population revealed an f_g of 69.94.

This parameter, compared with the f_e, would then allow to determine if there was an unequal contribution of founders and the possible random loss of alleles throughout successive generations, due to genetic drift. An unequal genetic contribution by founders decreases the founder equivalents, which leads to greater inbreeding in future generations, than would be desirable, and reflect a greater loss of the genetic diversity initially present in the founders (Lacy, 1989). For the whole population, f_g was around three quarters that of the effective number of founders, confirming the occurrence of bottlenecks in the population.

6.3.2.1.   Genetic Conservation Index

The GCI index of Alderson (1992) reflects the contribution of different founders in an individual gene pool, and is based on the assumption, that the objective of a conservation program is to retain the full range of alleles possessed by the base population (Gutiérrez, Goyache & Cervantes, 2010).

In this respect, animals with higher indexes exhibit greater conservation value, due to a greater balance in the number of founders and should, therefore, be used (preferentially in the choice of breeding animals) in genetic selection programs, to maintain the genes transmitted by founders (Da Silva Faria et al., 2018). However, there can be benefits in a GCI of 1, because it maintains the genome of a particular founder. This can be used as an advantage when specific founders have a lack of representation. They then can be used to breed, and thus increase their representation in future generations.

The Genetic Conservation Index for this species was calculated, and Figure 22 shows the frequency of animals for each GCI category.
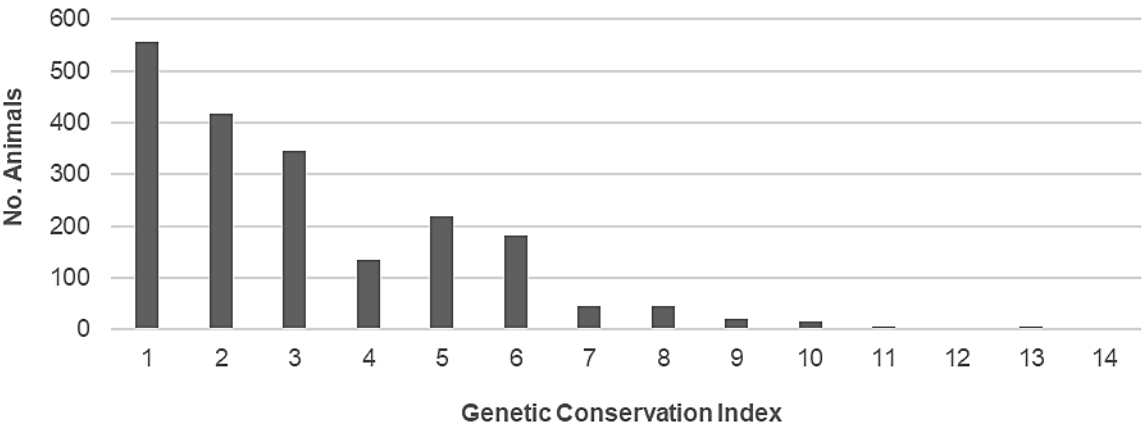
Figure 22 - Frequency of individuals by genetic conservation index.

The GCI indicated a mean number of founders per individual of 3.11 (±2.19). This is quite low, indicating a small, unbalanced contribution of the founders in the population, with the highest being approximately 14 founders. In the group analysed, 28% of individuals had a Genetic Conservation Index of 1, and almost half of the overall population had a GCI of 2 or less. Moreover, only around 2% of individuals in the studbook had a GCI of 10 or higher founders.

Throughout the years, the average GCI has been increasing, especially in the last 30 years (not shown here), with the last decade having an average GCI of 5.71 (±2.49) founders, and the reference population with an even higher GCI, at 7.00 (±2.71) founders. This reflected an increase of the mean, of around 2.5 and 4 founders per individual in the two categories above, compared to the overall population. Which seems to suggest that recently there has been some care in increasing founder representation.

The GCI parameter can, be actively used in the current captive population, when selecting breeding individuals, to maintain the founder pool or even selectively grow the contribution of a specific founder to the population.

6.3.3.   Relationship Between Institutions of Origin

In a population with different groups separated between institutions, the average inbreeding and mean kinship values of these different populations (average relatedness within and between subgroups) can be calculated. This information, then allows managers to identify which groups should send or receive migrants with other groups. Research in this area is still ongoing (Ralls et al., 2017).

For this pedigree analysis, the individual's birth location was preferred to their last known location, since it reflects the breeding decisions underlying the history of the population, which was one of the aims of this study. The population has a total of 1998 individuals, where 1561 originated from 96 known institutions and the remaining were either wild born or had an unknown birth location. Table 5 highlights important genetic parameters for the whole population, computed by the ENDOG software.

Table 5 - Mean coancestry within and between institutions, Fis and Fst.

PARAMETER	Value
Mean Coancestry Within an Institution	0.1015
Mean Coancestry Between the Different Institutions	0.0071
F_{is}	-0.0904
F_{st}	0.095

As shown in the table, within the same birth institution, the mean coancestry coefficient was 0.1015 and between the different birth locations it was much lower, at 0.0071. The coefficient of coancestry of a pair of individuals, indicates the probability that one allele, derived from the same common ancestor, is identical by descent in the two individuals (Rédei, 2008). The fact that the mean coancestry between different birth locations was low indicated that animals from different institutions were mostly unrelated, and that careful exchange of breeders between groups may be a good strategy for maintaining inbreeding at low levels.

The last two parameters in the table (Fis and Fst) are both measures of genetic structure, developed by Sewall Wright (1969, 1978). The first, Wright's inbreeding coefficient, can be defined as the proportion of the variance in the subpopulation, contained in an individual. The second, is the normalized variance in allele frequencies between subpopulations, in other words, it will assess the proportion of genetic diversity in the whole population, that is justified by the variance between subpopulations (McDonald, 2013 & Zhivotovsky, 2015).

For the population under study, Fis was negative, which suggests that there has been introduction of individuals in the subpopulation, and that breeding is carried out in a way to avoid matings between close relatives. In other words, there has been at least some rotation between the different subpopulations/institutions (McDonald, 2013).

Regarding Fst, values can range from 0 to 1, with a high value implying a considerable degree of differentiation among populations. In this analysis, the estimated Fst indicated that nearly 10% of the total variability was due to genetic differences between locations.

Also, with the ENDOG software it was possible to determine another important parameter termed "*PWithin*" which reflects mating strategies. This determines the mean proportion of males in the population, that are used as sires in their own birth locations. Where 0 indicates that an institution did not use any of its males as sires, and 1, the opposite, that they only used males born there as fathers. For this assessment, only 53 institutions had the necessary factors to be considered. Figure 23 shows the results of this analysis.

Figure 23 - Frequency of institutions by proportion of males used as sires in their own birth locations.

This demonstrated that the majority (58%) of institutions did not use their own males as sires, at all. On the other hand, only 17% of institutions used only their own males, which means that the remaining 25%, used as sires, both their own males and males born in different institutions. If associated with the information of the previous table, one would suggest that the 17% of institutions not use, at least not exclusively, their own males as sires, due to the fact that the relationship between individuals born on the same institution was much higher than the one with individuals from different locations.

As the work was done closely with the manager of the California sea lion studbook, at the Lisbon Zoo, it was thought to be of interest to build a Tree/Diagram with the genetic distances between all the institutions of origin, for the whole population (Annex 3). ENDOG calculates this distance by means of the average pairwise coancestry coefficient (Gutiérrez, Goyache & Cervantes, 2010). This diagram demonstrated four obvious clusters of institutions with a closer genetic proximity, and that therefore should limit their individuals breeding with each other. This tool can be of great use for the selection of an animal for breeding purposes in the future, in order to have the least related individuals allowed to have offspring. For this, the same diagram with only the living population, would possibly be more beneficial.

Gene diversity (GD) is the probability that two alleles from the same locus sampled at random from the population, are not identical by descent from a common ancestor, and it estimates the expected proportion of heterozygous loci, in the population (Balloue et al., 2010 & Princée, 2016). ENDOG calculated the founder genome equivalent (f_g) for each institution (Annex 4), as well as the Gene Diversity (GD) of each, their contribution to the gene diversity of the whole population and the equivalent complete generations of each institution (Annex 4). This revealed that, both the GD and equivalent generations seemed to increase with the

number of individuals per institution (not shown here), probably indicating that larger institutions tend to have a more organized data collection system.

Small populations are affected by demographic and genetic processes that can result in loss of genetic variation. Meaning, they are more likely to experience the loss of diversity over time, by random chance, in a process known as genetic drift (Wright, 2005). Concerns about genetic diversity are, therefore, especially important with large mammals due to their small population size and high levels of human-caused population effects.

A genetic bottleneck can occur when a population goes through a period of low number of individuals, resulting in a rapid decrease in genetic diversity. Populations of endangered species, whether in captivity or in the wild, are in general small in size and, therefore, subject to genetic loss. Three major causes of genetic loss can be identified: inbreeding, genetic drift and selection. Therefore, the gene diversity of the founders should be maintained as much as possible, by having an equal founder representation in the living population at all times (Princée, 2016).

The average GD for the total population was 0.9929 (±0.0062), and for the current living population it was 0.9891 (±0.0056).

6.3.4.   Inbreeding

For this population of California sea lions, using the studbook information available, an Inbreeding Coefficient (F) was calculated for each individual, where F is defined as the probability that an individual has two identical alleles, by descent (Gama, 2002).

In populations under selective pressure, the inbreeding within the progeny of reproducing individuals, can be higher than that expected under pure genetic drift with random selection and mating. Nevertheless, the goal in conservation programmes, for endangered breeds, is to restrain the rate of inbreeding (Gutiérrez, Goyache & Cervantes, 2010). The result of inbreeding is an increase in homozygosity, in offspring, because they possess alleles that originate from ancestors common to the parents. This increases the probability that deleterious alleles will be expressed in the homozygous state (Princée, 2016).

Figure 24 shows the evolution of the mean inbreeding by year of birth, in order to assess its progression, over time.
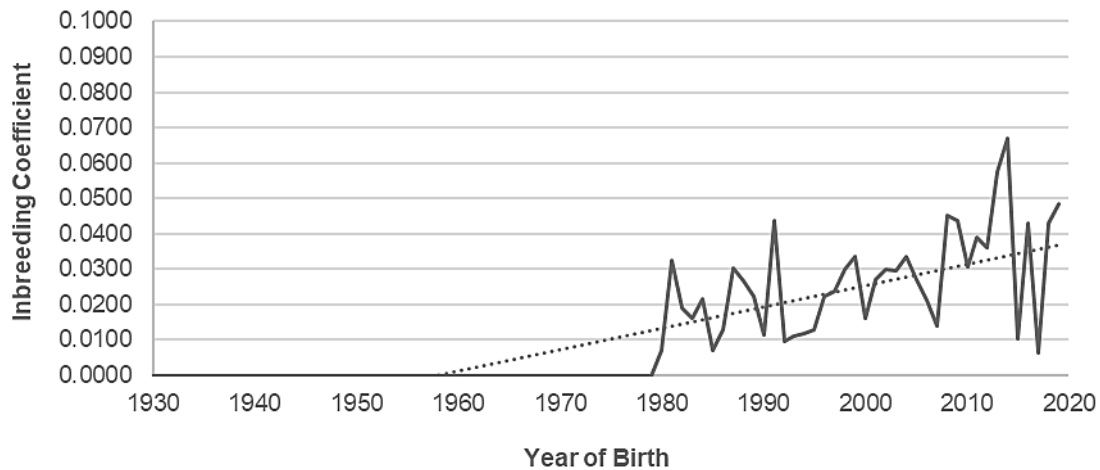
Figure 24 - Mean inbreeding coefficient by year of birth.

As shown in Figure 24, the mean inbreeding coefficient, per the individual's year of birth, has been increasing since 1980, which was when the records became more comprehensive. The overall average inbreeding for the whole population was 0.0203 (±0.0592), and the mean for the reference population (individuals born in the last three years) was 0.0334 (±0.0608). The highest mean, occurred in 2014, with an inbreeding coefficient of 0.0670. The current living individuals had a mean inbreeding coefficient of 0.0324 (±0.0106).

Beuchat (2015) suggest that the deleterious effects of inbreeding, begin to become evident at a coefficient of about 5%. And at around 10%, there is a significant loss of vitality in the offspring, as well as an increase in the expression of deleterious recessive mutations. Because of this, the latter is considered the so called "extinction vortex" - the level of inbreeding at which its consequences have a negative effect on the ability of the population to remain viable. And as these get smaller, the rate of inbreeding increases, resulting in a negative feedback loop, that eventually can drive the population to extinction. Hence, a coefficient of less than 5% was the suggested level.

For this population, there were only 2 years where mean F was higher than 5% (2013 and 2014). However, there were 228 individuals, 10 of which born in 2019, with a coefficient higher than Beuchat's (2015) recommended level.

Based on the individual F, the rate of inbreeding per year was estimated by the regression coefficient of F on the year of birth. For the CSL population, this revealed an increase of 0.102%/ year (±0.044).

The rate of inbreeding, per generation was determined in two different ways. The first estimate was obtained from the annual rate of inbreeding and the mean generation interval and resulted in an increase in inbreeding of 1.2% in every generation. The second estimate

was obtained from the individual rate of inbreeding (Gutiérrez, Goyache & Cervantes (2010), and resulted in, a rate of inbreeding of 1.6%, per generation.

Information about the percentage of inbred individuals, per year of birth, was also obtained (not shown here), and revealed a similar distribution as the previous figure, meaning an almost constant increase since 1980. Out of the 1998 animals, 337 were considered inbred, which represents around 16.8% of the entire recorded population. When only the reference population was considered, 45 out of 85 (52.9%) individuals had an inbreeding coefficient higher than zero. This means that in the last three years, over half the population born, was inbred. In fact, 2019 was the year with the highest frequency of inbred individuals, with a total of almost 70% of inbred animals being born. The current living population had a percentage of inbred individuals at around 36.7%.

However, the average value of the Inbreeding coefficient, within the inbred animals has been decreasing over time (Figure 25).
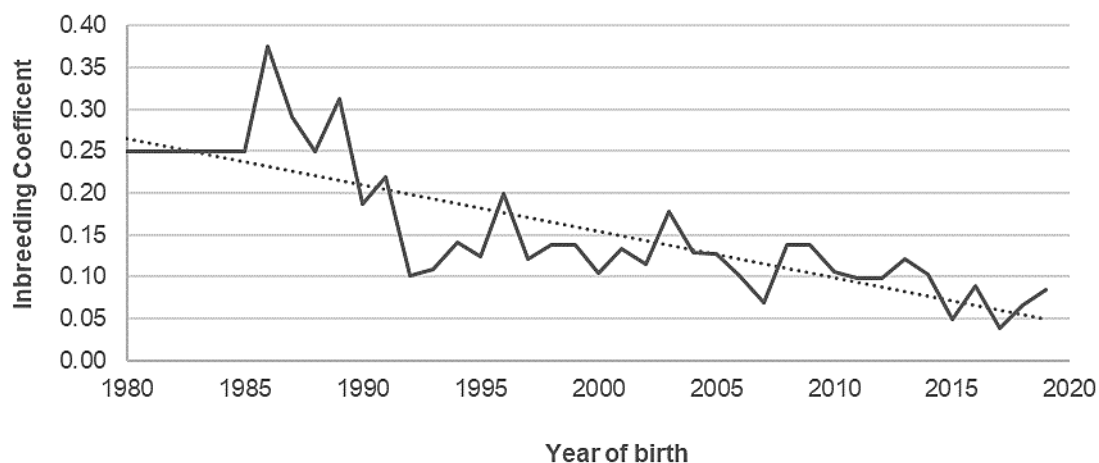
Figure 25 - Mean inbreeding coefficient within the inbred individuals by year of birth.

The average inbreeding for inbred animals was 0.12 (±0.09) for the whole population and for the reference population, it was 0.06 (±0.07).

Taken together, these results indicated that, per year, there have been more inbred animals being born, with an increasing average Inbreeding Coefficient in the whole population, even though, with an overall lower mean value within the inbred animals. This suggests that some care has been made to avoid high inbreeding coefficients.

The distribution of these coefficients within the inbred population, was summarized in Figure 26, which displays the percentages of individuals for different categories of F, in both the total and reference populations.
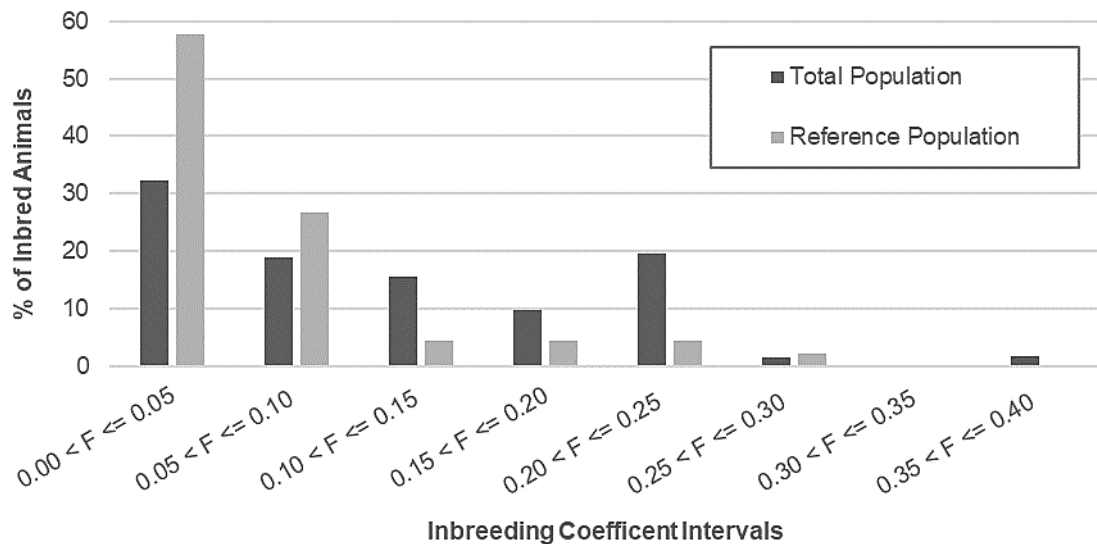
Figure 26 - Percentage of inbred individuals by inbreeding coefficient interval for the total and reference populations.

This showed that, for both populations, the highest percentage of individuals had an inbreeding coefficient between >0 – 0.05, with the total population having 32% and the reference population 58% of animals in this interval. However, the second highest category was not the same for these populations. For the first, it was between >0.20 – 0.25, with 20% of the whole inbred population in this category, and for the reference population it was 27% between >0.05 – 0.10. This has, once again, confirms the previous statement, that the actual value of the coefficient within the inbred individuals has been decreasing. Additionally, the highest coefficient for the entire population was for 6 individuals with $F = 0.38$, while in the reference population there was only one animal with the highest inbreeding coefficient of $F = 0.30$.

With the ENDOG software it was also possible to compute the highest inbred matings between individuals. Table 6 shows these results, both for the total and reference populations.

Table 6 - Percentage of high inbred matings in the total and reference populations.

MATING TYPE	Total Population	Reference Population
Matings between half-siblings	3.35%	0.25%
Matings between full siblings	0.50%	0.05%
Parent–offspring matings	3.30%	0.05%

For the entire population, there were a total of 153 high inbred matings, and only 7 of these occurred in the reference population. For all these high inbred matings, the reference population had a much lower percentage of occurrences. This suggests that, in recent years there has been some care to avoid closely related individuals, acting as breeding pairs.

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The comparison of these results with wild populations is difficult, as much uncertainty exists regarding the extent and effect of inbreeding in wild populations of mammalian carnivores. Long-term behavioural studies of lions, spotted hyenas, and African wild dogs indicate that close inbreeding (between parents and offspring or siblings) is rare. Among these carnivores, as in other mammals, dispersal by young animals is widely viewed as the principal means by which matings by close relatives are avoided (Keane, Creel & Waser,1996).

In marine mammals, a study by Brakes & Dall (2016) cites that social species (as is the case for sea lions) may benefit in regard to several factors, one of which would be an increased availability of suitable mates and reduction of inbreeding. It is also mention that there is a variability in response to inbreeding across marine mammals, which suggests that some species may be more sensitive to inbreeding depression than others.

6.3.5.   Effective Population Size

An important aspect, in any genealogical analysis, is the study of the probability of gene loss between generations and consequent decrease in genetic variability (Machado, Carolino & Ferreira, 2020).

The extent and rate of loss of genetic diversity is highly dependent, on the size of the population. However, the size of relevance is not simply the number of individuals (N), but rather the genetically effective population size (Ne). The effective size of a population is a measure of how well the population maintains its gene diversity, from one generation to the next (Ballou et al., 2010). Meaning, it is a good indicator of the risk of genetic erosion (Carolino & Gama, 2008), as genetic diversity is lost at the rate of $\frac{1}{2Ne}$, per generation (Ballou et al., 2010). In other words, it is defined as the number of breeding individuals that would lead to the current increase in the inbreeding coefficient (F) , if they contributed equally to the next generation (Gama, 2002; Gutiérrez, Goyache & Cervantes, 2010).

Knowledge of this parameter over time, allows for the assessment of changes in genetic variability, as it is a reflection of the number and choice of breeders in previous generations. However, the Ne parameter is extremely sensitive to the quality of pedigree file data and, therefore it makes more sense in long-term analyses (Machado, Carolino & Ferreira, 2020).

Populations with a small effective population size, lose genetic diversity at a faster rate than those with a larger one. The concept of Ne is based on the genetic characteristics of a theoretical or ideal population, that experiences no selection, no mutations, no migrations and in which all individuals are asexual and have an equal probability of contributing offspring to the next generation (Ballou et al, 2010). Therefore, Ne would equal the census population size (N) only when all the assumptions of an 'ideal population' are met: random mating (which

assumes an equal sex ratio among breeders); no migration; no mutation; no selection; non-overlapping generations (Mace, 1986).

In general, the effective size of a population, is based primarily on three characteristics: the number of breeders, their sex ratio, and the relative number of offspring that they produce and are kept for breeding. Overall, a large number of breeders, will pass on a larger proportion of the parental generation's genetic diversity. On the other hand, an equal sex ratio is preferable, since it assures that the gene pool will not be disproportionately determined by the sex with the smaller census, compared to a sex ratio that is highly skewed. Differences in family size also result in loss of genetic diversity, since it means that some individuals contribute few or no offspring to the gene pool of breeders (Ballou et al., 2010).

There are many methods for estimating a population's effective size (Ballou et al., 2010). However, as stated by Gutiérrez, Goyache & Cervantes (2010), in small populations, with shallow pedigrees, whatever method is used, this parameter fits poorly with real populations, giving an overestimate of the actual effective population size. Table 7 displays the Ne for all four of the, already mentioned, methods used to estimate this parameter. The percentages in this table represent the increase in inbreeding per generation, for each of the methods.

Table 7 - Effective population size.

METHOD	Ne
by Complete Generation (2,11%)	23,72
by Maximum Generation (0,84%)	59,84
by Equivalent Generation (1,60%)	31,17
'realized' Ne	35,83

Effective population sizes are also normally presented, as the ratio of the effective size to the census size (Ne/N). Interpreting this ratio is often more useful for management purposes than simply looking at Ne, as it relates the N to its capability of preserving gene diversity (GD) (Allendorf, Hohenlohe & Luikart, 2010; Ballou et al., 2010).

Ne can, theoretically, range from 0 to around twice the population's census size. The Ne/N ratio, for most species in captivity, typically ranges from 0.15 – 0.40 (average of about 0.3), with the low end being species managed as groups with unequal sex ratios and the high end being long lived and monogamously paired species. In the wild, Ne/N ratios are closer to 0.11 (Ballou et al., 2010).

For the current CSL population, the Ne was calculated using the formula by Frankel & Soulé (1981), $Ne = \frac{4NmNf}{NmNf}$, where Nm and Nf were the number of proven breeder (individuals that have had progeny) males and females, respectively. This revealed an effective population

size of 121.21, the resulting Ne/N ratio was around 0.27, which seems to agree with the previously suggested ratio seen in captive species.

Another important ratio is fe/Ne, with fe being the, previously mentioned, effective number of founders. And, according to Sorensen et al. (2005), fe should be close to $Ne/2$, in a population where genetic drift has stabilized, since it suggests a greater representation of founders (Vicente, Carolino & Gama, 2012; Da Silva Faria, 2018). This was the case for this population, with a ratio of around 2.69 and 3.09, for the "realised" Ne and the Ne by increase in inbreeding by equivalent generations, respectively.

The relationship between Ne and fe (as well as with fa) provides information on the occurrence of bottlenecks in the population, since genetic drift occurs at a higher intensity when the population undergoes a drastic reduction in its effective size (Vicente, Carolino & Gama, 2012; Da Silva Faria, 2018).

The aim of captive breeding protocols has been to reduce genetic drift, by maximizing effective population size, which is especially suitable for mammal species in zoos, considering that they tend to have a small number of individuals (Allendorf, Hohenlohe & Luikart, 2010). The procedures to maximize a population's Ne tend to focus on maximizing the number of breeding individuals, equalizing the sex ratio of breeders, and rotating breeding animals among sublines, so that each breeding group or pair produces similar numbers of offspring (Ballou et al., 2010).

6.3.6.   Average Relatedness

Choosing which individuals to breed can be very complex when founder lineages are, almost inevitably, mixed over the generations. Therefore, the possibility of identifying animals that are least related, to others in the population, is extremely advantageous (Princée, 2016). In a study on wild California sea lions, Acevedo-Whitehouse et al. (2003), reported that sick individuals had a higher-than normal parental relatedness, suggesting that mortality in natural populations may not be entirely random and that inbred individuals could act as more effective reservoirs of infectious agents.

The Average Relatedness (AR) coefficient between all individuals was computed for the entire CSL population. This coefficient, described by Gutiérrez and Goyache (2005), represents the genetic relationship of a given animal, with the whole population and was obtained based on the probability that a randomly selected allele of the population, belongs to a given animal.

The mean AR was then calculated by year of birth, to investigate its evolution in time (Figure 27).
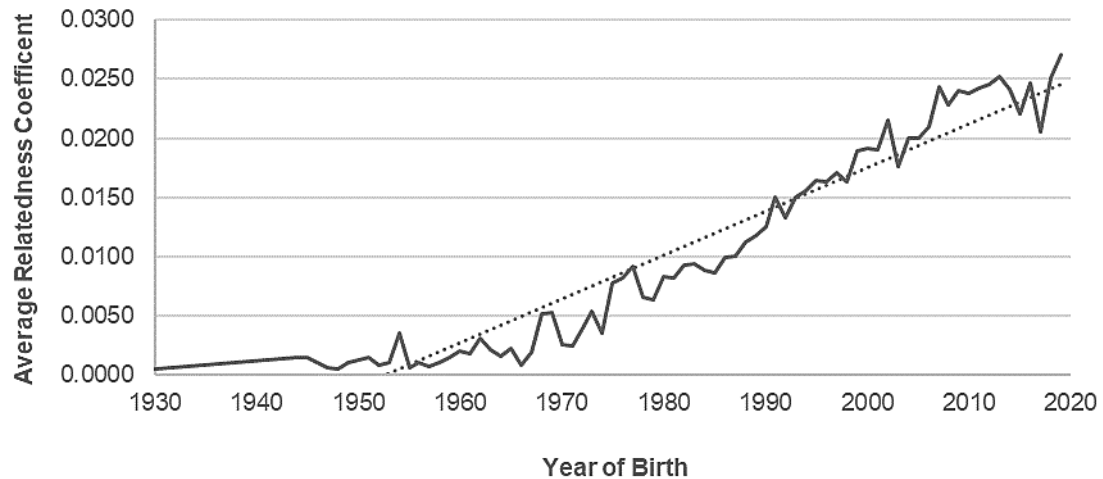
Figure 27 - Mean average relatedness by year of birth.

The mean AR has been increasing consistently, particularly in the last 30 years, such that the mean for the entire population was 0.0143 (±0.0124), while for the reference population it was 0.0244 (±0.0090). This showed an increase of almost double, for individuals born in the last three years, relative to the overall population, reaching the highest value in animals born in 2019, with a mean of 0.0270. For the current living population of captive *Zalophus californianus*, in this studbook, the mean AR coefficient was 0.0217 (±0.0106).

Gutiérrez, Goyache & Cervantes (2010) describe some of the advantages of investigating average relatedness, including the possibility of using the AR of a founder as an indication of its genetic contribution to the population; its use as a measure of inbreeding of the population, as AR takes into account both inbreeding and coancestry coefficients; also, AR can be used as an index to maintain the initial genetic stock by using as breeding animals those with the lowest AR value with the global population; and finally, AR, as an alternative or complement to F, can be used to predict the long-term inbreeding of a population, because it takes into account the percentage of the complete pedigree, originating from a founder at population level, and can be used to predict the level of inbreeding in future generations.

As suggested by Carolino & Gama (2008), the degree of non-random selection and mating, practiced in the population can be evaluated by comparing the observed inbreeding of the individuals, with the inbreeding that would be expected from the existing relationship, one generation back. For this California sea lion population, almost 88% of individuals had a lower inbreeding than expected, which reflects the concern of breeders in avoiding matings among closely related individuals. However, the fact that the mean inbreeding was higher than the mean average relatedness (Table 4), and is still increasing, for both the total and reference populations, indicates that a further effort in avoiding mating of related individuals needs to continue.

6.3.7. Inbreeding Impact

Burrow (1993) has stated that inbreeding can have a negative effect on all measurements of female fertility. Therefore, to assess the possible impact of inbreeding in the CSL population, the possible association of some variables with the dam's corresponding inbreeding coefficient were investigated. Mostly, this was carried out in order to assess if the results for inbred versus non-inbred individuals showed a significant difference. However, it should be taken into account, that several of the individuals in this studbook had unknown parents and therefore they were attributed an inbreeding coefficient of zero. This can, consequently, affect the results shown next. Therefore, particularly in this section, the results should be thoroughly re-evaluated through more precise and detailed methods, including the use of genetic markers that allow the quantification of the degree of homozygosity and, thus, the level of genomic inbreeding.

6.3.7.1. Interbirth Interval

The first parameter to be examined was a dam's interval between consecutive births. Different authors have estimated an increase in the interval between births due to the undesirable effects from inbreeding. A number of human studies have shown that inbred woman, tend to have longer interbirth intervals (Ober, Hyslop & Hauck, 1999; Robert et al, 2009; Postma, Martini & Martini, 2010 & Kenji Ikehara, 2011). Also, though in a smaller number, studies have been conducted on animals such as dairy cows but showing the same detrimental effects of inbreeding on parturition interval (McParland, Kearney & Berry, 2009; Doekes, 2019 & Gutiérrez-Reinoso, 2020).

For the population under study, the results were assessed by the average of days between births, compared to the inbreeding coefficient of the dam in question (Figure 28).
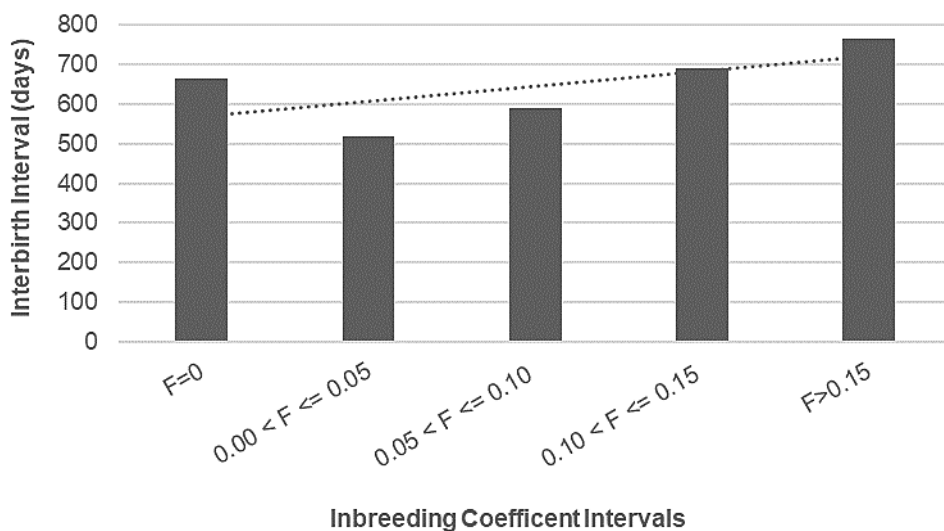
Figure 28 - Mean dam's interbirth interval by inbreeding coefficient interval.

The means between the inbred and the non-inbred individuals only showed a 7-day difference. However, when the Inbreeding Coefficient was separated into intervals, there was a more significant difference in the number of days. On average, the dams with an inbreeding coefficient higher than 0.10, seemed to have a longer interbirth interval then the rest. And comparing with the non-inbred dams, there was an increase of 1 and 3.5 months, in the mean parturition interval of females in the $0.10 < F < = 0.15$ and $F > 0.15$ categories, respectively. These results suggested that the number of days between births increased with the increase of the inbreeding coefficient. This seems to concur with the previously stated information that the deleterious effects of inbreeding become more apparent above the 10% level of inbreeding (Beuchat, 2015).

6.3.7.2.   Dam's Age at First and Last Progeny's Birth

Several authors have demonstrated that inbreeding can have a negative effect on a dam's age at first parturition. However, most of these studies have been performed in cows (Burrow, 1993; Fioretti et al., 2001 & Biffani, Samoré & Canavesi, 2002).

Concerning the CSL population in study, the age of the dam at the birth of her first offspring did not seem to suggest the influence of inbreeding. Overall, the average of this age was consistently between 6 and 7 years old in all intervals of the dam's inbreeding coefficient, and with the average for the whole population being around 7 years old.

Regarding the dam's age at the birth of her last offspring, a similar situation to that of the interbirth interval occurred, particularly, when comparing the average age between the inbred and non-inbred individuals. However, since for this particular trait only the already deceased dams were considered in the analysis the number of individuals with an inbreeding coefficient higher than zero, were relatively low. Figure 29 illustrates the results, indicating some decline in longevity in inbred animals.
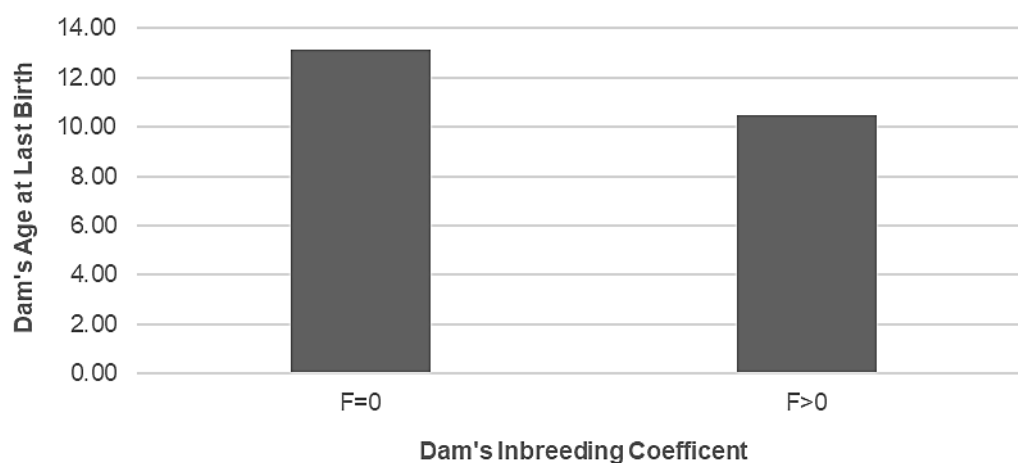
Figure 29 - Mean dam's age at last birth in inbred versus non-inbred dams.

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Globally, the average age at last parturition for the inbred animals was 10.50 years old, which is almost two and half years younger than the average age at last offspring for the overall population. Also, an even larger variation occurred, between the inbred and non-inbred dams, with an almost 3-year decrease for the former. This could, possibly suggest a decrease in longevity, for inbred individuals.

7. CONCLUSION

Our study, using pedigree information collected over the years, from the California Sea Lion population kept in captivity in Europe, clearly demonstrates the importance of maintaining a sound program aimed at its genetic management, including careful selection and mating decisions, to avoid further losses of genetic diversity. These results can be further used to assist in the continuous management of this population, as they show what has been working and what still needs a more focussed approach.

Overall, the findings of the demographic assessment seem to be in accordance with the expected results, as well as with previous studies, both in this species and in other captive wild animals. The results show that an effort has been made to keep a healthy genetic diversity in this population, however the increase of the percentage of individuals being born inbred and the limited effective population size, among other parameters, can be worrying and clearly reveals a continuous need for improvement.

The most noticeable limitation in this study was the incompleteness of some of the records, especially in the earlier years of record keeping. In particular, the limited pedigree depth, as would be expected in a population which initiated record keeping a few decades ago. On the other hand, the studbook used as the basis for pedigree analysis, as any other studbook, is dependent on the quality of information received from the holders, which can be misleading in some cases, due to common human error. Also, as only the records from the European studbook were used, the results did not take advantage of the full information on the captive population of the California sea lion species, as a whole.

Now that this initial assessment exists, the hope is to achieve a more thorough evaluation of the population. The next step should be to obtain DNA samples from all the individuals in the current population and genotype those animals to assess their genomic diversity. This would complement the results obtained in this dissertation and would provide additional knowledge, which is important to better assess the evolution of genetic diversity in the California sea lion and develop management plans based on genomic information.

Decisively, for the management of a population there needs to be a continuous and accurate record keeping and its periodical analysis, to appraise the evolution of key diversity parameters. Since an EEP programme is a process that rotates between implementation, monitoring, feedback, and adjustment, the monitoring of the population, which allows for the assessment of the performance of the program, needs to be done regularly, in order to allow the adoption of the necessary adjustments and adaptations, in accordance with the results obtained.

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9. ANNEX

Annex 1 – Pedigree Completeness for Reference Population
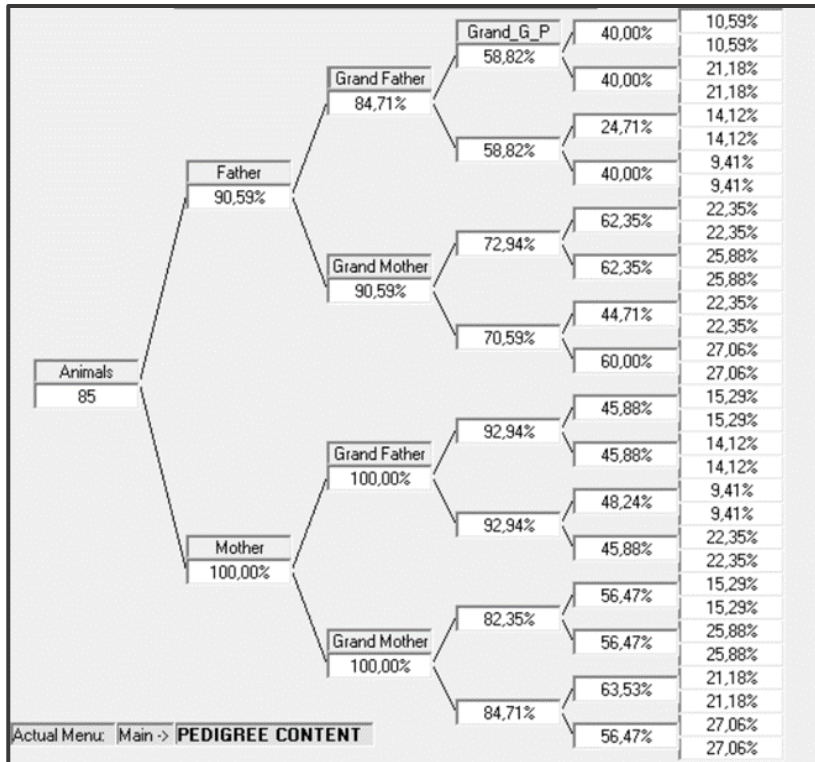
Annex 2 – Distribution of Individuals by Birth Institution with Cumulative Contribution

Institution	No. of Births	Cumulative Contribution (%)
NURN	100	6.41
BASE	97	12.62
ROTT	88	18.26
WUPP	57	21.91
GELS	50	25.11
STUT	49	28.25
BLAC	47	31.26
CHEN	46	34.21
MUNI	45	37.09
RHEN	45	39.97
LONG	44	42.79
DUIS	43	45.55
BELF	41	48.17
KREF	39	50.67
COPE	37	53.04
AMST	35	55.29
BARC	34	57.46
HANR	34	59.64
OSNA	32	61.69
BERL	30	63.61
LOND	30	65.53
DUBL	29	67.39
KARL	28	69.19
BRUG	27	70.92
KOLN	26	72.58
BEAU	24	74.12
MARD	23	75.59
MOSC	23	77.07
EDIN	22	78.48
HARD	21	79.82
TENE	20	81.10
COLW	19	82.32
WHIP	18	83.47
CHER	17	84.56
DORT	17	85.65
ANTI	16	86.68
ANTW	13	87.51
EMME	13	88.34
LA_P	13	89.17
MUNS	12	89.94
ODEN	12	90.71
ARNH	10	91.35
AMIE	9	91.93
WOBU	9	92.50
COUL	8	93.02
LISB	8	93.53
ZAGR	8	94.04
OPOL	6	94.43
KLAI	5	94.75
MYST	5	95.07
NY_B	5	95.39
LODZ	4	95.64
SEA_	4	95.90
ZOOM	4	96.16
HANB	3	96.35
HILV	3	96.54
MMC_	3	96.73
MUND	3	96.93
PUER	3	97.12
DUSS	2	97.25
FTLA	2	97.37
GANS	2	97.50
GERM	2	97.63
HALL	2	97.76
PEAU	2	97.89
SAND	2	98.01
SOLT	2	98.14
AALB	1	98.21
ATTI	1	98.27
BANH	1	98.33
BAR-	1	98.40
BUDA	1	98.46
CALG	1	98.53
CALI	1	98.59
CENT	1	98.65
DRES	1	98.72
FUER	1	98.78
HONO	1	98.85
INDI	1	98.91
LA_R	1	98.98
LEIP	1	99.04
MARA	1	99.10
MARN	1	99.17
MAR_	1	99.23
MINA	1	99.30
MIUR	1	99.36
OCEA	1	99.42
OSAK	1	99.49
PALA	1	99.55
PALV	1	99.62
PLAI	1	99.68
PRAH	1	99.74
SING	1	99.81
VERG	1	99.87
WYAR	1	99.94
ZOOA	1	100.00

Annex 3 – Diagram of Genetic Distances Between Institutions of Origin

Cluster 1:
85: Singapore
83: San Diego (USA)
66: Munster (Germany)
87: Stuttgart (Germany)
19: California (USA)

Cluster 2:
46: Klaipeda (Lithuania)
71: Odense (Denmark)
29: Duisburg (Germany)
61: Miura (Japan)
73: Osaka (Japan)
88: Tenerife (Spain)
81: Rhenen (Netherlands)
60: Minatu (Japan)

Cluster 3:
63: Moscow (Russia)
7: Attica (Greece)
34: Fuerteventura (Spain)
54: London (England)

Cluster 4:
13: Belfast (Ireland)
44: Indianapolis (USA)
74: Osnabruck (Germany)
20: Central Park (USA)

Annex 4 – Genetic Parameters of each Institutions of Origin

Institution of Origin	Gene Diversity	Contribution to Total Gene Diversity	N	Equivalent Complete Generations	*f*g
AALB	0.9929	0.0000	1	1.7	1.00
AMIE	0.9929	0.0077	9	3.0	1.00
AMST	0.9930	0.0109	35	2.7	1.00
ANTI	0.9928	-0.0056	16	1.8	2.19
ANTW	0.9929	0.0016	13	1.8	1.00
ARNH	0.9928	-0.0065	10	0.9	1.00
ATTI	0.9928	-0.0007	1	1.0	1.00
BANH	0.9928	-0.0002	1	2.9	1.00
BAR-	0.9928	-0.0007	1	1.0	3.13
BARC	0.9928	-0.0037	34	2.0	2.00
BASE	0.9929	0.0051	97	2.1	3.09
BEAU	0.9929	0.0031	24	2.2	2.00
BELF	0.9927	-0.0132	41	1.8	4.00
BERL	0.9929	0.0080	30	2.1	5.00
BLAC	0.9929	0.0010	47	1.6	3.00
BRUG	0.9927	-0.0131	27	1.5	1.00
BUDA	0.9928	-0.0007	1	1.0	1.80
CALG	0.9928	-0.0007	1	1.0	1.00
CALI	0.9928	-0.0007	1	0.0	5.17
CENT	0.9928	-0.0005	1	0.0	1.00
CHEN	0.9927	-0.0174	46	1.1	4.72
CHER	0.9928	-0.0068	17	1.7	6.50
COLW	0.9928	-0.0094	19	1.6	14.78
COPE	0.9927	-0.0173	37	1.6	3.90
COUL	0.9929	0.0044	8	3.0	1.00
DORT	0.9929	0.0009	17	1.9	6.69
DRES	0.9928	-0.0007	1	0.5	4.95
DUBL	0.9929	0.0098	29	2.7	3.00
DUIS	0.9930	0.0113	43	1.7	2.00
DUSS	0.9928	-0.0011	2	0.5	3.77
EDIN	0.9927	-0.0135	22	0.6	1.00
EMME	0.9929	0.0058	13	3.4	2.00
FTLA	0.9928	-0.0014	2	0.0	5.09
FUER	0.9928	-0.0007	1	0.0	4.89
GANS	0.9928	-0.0014	2	0.0	2.59
GELS	0.9931	0.0215	50	2.7	1.00
GERM	0.9928	-0.0014	2	0.0	5.08
HALL	0.9928	-0.0014	2	1.0	2.00
HANB	0.9928	-0.0021	3	0.0	4.35
HANR	0.9927	-0.0191	34	1.4	4.54
HARD	0.9928	-0.0096	21	1.5	4.07
HILV	0.9929	0.0010	3	3.3	5.73
HONO	0.9928	-0.0007	1	0.0	6.70
INDI	0.9928	-0.0003	1	1.0	1.88
KARL	0.9931	0.0239	28	2.4	1.00
KLAI	0.9929	0.0016	5	2.1	1.00
KOLN	0.9929	0.0001	26	1.9	2.51
KREF	0.9930	0.0134	39	1.9	1.33
LA_P	0.9928	-0.0084	13	1.1	2.56
LA_R	0.9929	0.0002	1	3.7	4.32
LEIP	0.9928	-0.0007	1	1.0	1.00
LISB	0.9929	0.0020	8	3.0	2.94
LODZ	0.9928	-0.0027	4	1.0	2.77
LOND	0.9927	-0.0143	30	1.1	3.99
LONG	0.9928	-0.0074	44	2.2	1.00
MARD	0.9927	-0.0139	23	1.2	4.19

MARA	0.9928	-0.0007	1	0.0	4.66
MARN	0.9928	-0.0006	1	0.0	4.24
MAR_	0.9928	-0.0007	1	0.0	4.09
MINA	0.9928	-0.0007	1	0.0	4.02
MIUR	0.9928	-0.0007	1	0.0	5.60
MMC_	0.9928	-0.0020	3	0.3	4.80
MOSC	0.9928	-0.0009	23	2.6	4.42
MUND	0.9928	-0.0021	3	0.0	1.00
MUNI	0.9930	0.0189	45	2.3	4.27
MUNS	0.9928	-0.0024	12	1.8	2.89
MYST	0.9928	-0.0031	5	0.0	5.84
NURN	0.9933	0.0484	100	2.5	1.00
NY_B	0.9928	-0.0031	5	0.9	2.32
OCEA	0.9928	-0.0003	1	1.8	3.99
ODEN	0.9929	0.0041	12	2.4	4.32
OPOL	0.9929	0.0036	6	3.6	1.00
OSAK	0.9928	-0.0007	1	1.0	2.42
OSNA	0.9928	-0.0005	32	1.7	1.00
PALA	0.9928	-0.0005	1	1.0	3.21
PALV	0.9928	-0.0006	1	0.0	2.38
PEAU	0.9929	0.0003	2	3.0	2.97
PLAI	0.9929	0.0003	1	4.2	2.91
PRAH	0.9928	-0.0007	1	1.0	2.02
PUER	0.9929	0.0018	3	3.0	6.72
RHEN	0.9930	0.0156	45	2.0	1.33
ROTT	0.9933	0.0479	88	2.4	1.59
SAND	0.9928	-0.0013	2	0.8	3.25
SEA_	0.9928	-0.0025	4	0.1	1.00
SING	0.9928	-0.0007	1	1.8	2.03
SOLT	0.9928	-0.0014	2	0.0	1.73
STUT	0.9931	0.0301	49	2.7	1.67
TENE	0.9927	-0.0121	20	1.4	0.89
VERG	0.9928	-0.0007	1	0.0	1.00
WHIP	0.9928	-0.0055	18	2.3	2.34
WOBU	0.9929	0.0018	9	2.4	0.99
WUPP	0.9933	0.0403	57	2.3	1.00
WYAR	0.9928	-0.0006	1	0.0	1.00
ZAGR	0.9929	0.0032	8	2.9	1.60
ZOOA	0.9928	-0.0004	1	0.0	0.89
ZOOM	0.9929	0.0000	4	2.4	1.00