UNIVERSITÀ DEGLI STUDI DI PADOVA
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How reproduction in captivity can save an endangered species

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1. Summary

The jaguar is the largest feline native to the Americas. It is a key component of ecosystems as it helps to maintain biodiversity and ecological processes via multiple food web pathways. Similar to most wild felids, the jaguar is classified as 'near-threatened' under the International Union for Conservation of Nature (IUCN 2009) in all the Americas. By the end of the 20th century, hunting for the fur trade, persecution for livestock depredation, and habitat loss caused an estimated 54% reduction in the historic range of the jaguar, with high levels of habitat fragmentation.

Jaguar breeding has been documented year-round in the tropics and captivity, but in northerly and southerly parts of the range (i.e. temperate zones), breeding tends to occur seasonally. Usually it happens in the course of the rainy season (spring) when native prey animals are more abundant.

During my traineeship activity at the "Los Jaguares" rescue centre in Ecuador, I had the opportunity to evaluate the reproduction history of the jaguars' mating pair hosted at the centre and I'm going to deal with it thereafter in my thesis, providing an example of successful captive breeding.

Although natural breeding is the first reproductive management of choice for sustaining the endangered wildlife populations in captivity, it is often unsuccessful. This is due to the difficulty of replicating the natural environment, including nutritional, social, and habitat needs. Consequently, intensive captive breeding management using assisted reproductive technologies (ARTs), such as artificial insemination (AI) or *in vitro* fertilization have been explored.

As a result, captive breeding of ex-situ populations in zoos, ecological parks, NGOs, and other conservation organizations offers a promising alternative for species preservation and potential reintroduction into natural habitats, providing a safeguard against extinction.

2. Introduction

2.1 Description of jaguar species

The jaguar (Panthera onca) is the largest felid in the Americas, being the only one of the five members of genus Panthera in the continent. At the beginning of the 20th century, the jaguar's range extended from the states of Arizona, Texas and Louisiana, in the United States, through the Amazon basin to the Rio Negro in the pampas' region of Argentina. Today, the jaguar's distribution has contracted by approximately 54% and is expanding from northern Mexico to northern Argentina (Figure 1), and they are found in all countries except for El Salvador and Uruguay. Currently the world's jaguar population is estimated at 173,000 individuals (2018), with Brazil being the most important country for this felid, as the holder of half of the world's population followed by Peru *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012; Johnson & Van Pelt, 2011a; Payán Garrido et al., n.d.-a)(Ríos et al., 2014)*.

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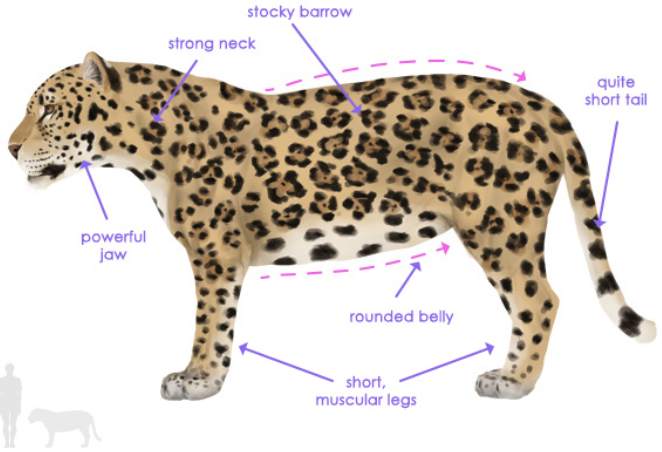
The jaguar inhabits a variety of ecosystems, primarily below an altitude of 2000 m, as an example the arid shrub systems in northern Mexico, the floodplain ecosystems such as the extensive savannah and gallery forest mosaics that make up the Llanos of Colombia and Venezuela, or the tropical rainforests that dominate the Amazon region *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012; Johnson & Van Pelt, 2011a)*.

Like most large carnivores, jaguars have relatively large home ranges (can vary from 40 to 300 km2) that are highly variable and depend on habitat quality including topography, availability of prey and population dynamics. Home range sizes vary between seasons (wet vs. dry) and between sexes.

Generally, males have larger living areas than females, which may use smaller areas within the territory of males. There may also be overlapping home ranges between individuals of the same sex, (within females is more common) so that for example we can find multiple females with the same male territories *(Action Plan for Jaguar's Conservation 2022-2031, 2021) (Ríos et al., 2014)*.

However, jaguars are solitary animals and use different signals, e.g. excretions, urine, scratching on trunks or vocalisations to demarcate areas of exclusivity, or territories and to avoid encounters with other individuals *(Payán Garrido et al., n.d.-b)*.

Jaguars are characterized by a relatively big and robust head, a compact but muscular body with short limbs and tail (Figure 2).



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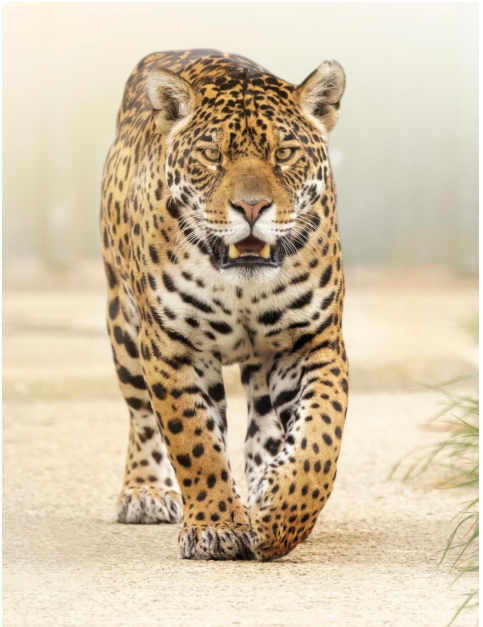
Their short, muscular limbs are well suited to climbing, swimming and crawling. They have the strongest teeth and jaws of any New World cat, being able to exert a pressure that can perforate tissues as hard as the carapaces of the turtles *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012)*.

A jaguar's coat is typically pale yellow, tan or reddish yellow above and generally whitish on the throat, belly, insides of the limbs and underside of the tail, with prominent dark spots, called rosettes, present along the whole body and whose pattern is defined at birth and will not change throughout its life. Every jaguar has a unique coloration and rosette pattern that may differ even from one side of a jaguar to the other, enabling identification of specific individuals (Figure 3).

However, melanistic individuals can occur naturally and are totally dark due to excess pigmentation. These are the ones that people often call erroneously "panthers" *(Action Plan for Jaguar's Conservation 2022-2031, 2021)(Payán Garrido et al., n.d.-b; Ríos et al., 2014),* (Figure 4).



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Jaguars are sexually dimorphic, with males being 20-30% larger than females. The average size of the species varies significantly between regions, with the largest individuals being found in the Pantanal region of Brazil and in the Venezuelan Llanos. Here the average weight of males is 99,5 and 104,5 kg, and females 76.7 kg and 66.9 kg. In contrast, in Central America the average weight of males is 56.1 kg and that of females is 41.4 kg *(Johnson & Van Pelt, 2011a; National Action Plan for the Jaguar in Brazil, 2012)*.

Jaguars are considered opportunistic feeders, this means that they can hunt the first thing they find, and their diet varies according to prey density and ease of prey capture. Their prey list includes more than 85 species of native wildlife. More commonly they eat large mammals such as collared peccaries, tapirs, capybaras as well as medium-sized, terrestrial and arboreal mammals such as armadillos, sloths, and agoutis. In addition, jaguars also feed on reptiles such as caimans, crocodiles, sea turtles and terrestrial turtles, very rarely on birds also *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012)*.

In the areas where the jaguars' natural prey have decreased due to the negative impact of human activities, jaguars also prey on domestic animals, including cattle, although they generally prey on calves *(Action Plan for Jaguar's Conservation 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012; Johnson & Van Pelt, 2011b)(Ríos et al., 2014)*. Their killing technique varies with the prey; they may hunt at any time of day or night, taking live prey by ambush or by stalking and then making a short rush-attack.

The jaguar's activity varies according to the site; it is mainly nocturnal, but may be diurnal or crepuscular.

Although this feline is primarily terrestrial, it is a very good swimmer and exploits resources available in aquatic or floodplains systems.

In the wild, jaguars live about 10–12 years, but there are some cases that lived up to 15–16 years. In captivity, however, they can exceed 20 years of age *(Johnson & Van Pelt, 2011b)*.

2.2 Biology of jaguars reproduction

Adult jaguars are solitary but join together temporarily for a couple of weeks for courtship and copulation. Female jaguars are polyestrous; however, mating is not limited to a single season. This depends on several factors including geographic space, photoperiod, temperature, food availability and social-sexual environment. Considering geographic location, jaguar mating in Mexico (northern hemisphere) occurs mainly in winter (short days) and in South America (southern hemisphere) in autumn and spring. In tropical areas where there is not such marked seasonality, it seems that reproduction occurs at any time, since light and humidity remain constant. Therefore, it is believed that

the jaguar's reproductive season is determined by day length, so that it is inhibited when day length is longer (more daylight hours) and females have seasonal anestrus and estrus in the short-day season *(fewer daylight hours) (Reproductive Biology and Assisted Reproductive Technologies in Wild Felids, 2021; Viau et al., 2020)*.
Females enter peri-pubertal phase approximately from 18 to 20 months of age and reach sexual maturity from 24 to 30 months, while for males it is later, between 36 and 48 months of age *(López-Pérez et al., 2021)*.

The reproductive cycle consists of 4 stages: proestrus, estrus, diestrus, and anestrus *(Brown, 2011)*.
Proestrus usually lasts less than a day, and is associated with the presence of ovarian follicles and increasing circulating estrogens. There's no sexual interest but there may be copulation with the males *(López-Pérez et al., 2021; Ortiz et al., 2022; Viau et al., 2020).*

Estrus accompanies advanced follicular development and peak concentrations of estradiol. The duration of estrous phase is up to 12 days. This phase is characterised by the females' expression of sexual receptivity towards males with the display of some estrous behaviours. The observable signs are vocalisations in the form of grunts to call the male, rubbing against inanimate objects, lordosis, rolling on the ground and the female allows the male to sniff the vulva and accommodates it in order to perform the mating. On the other hand, males can identify if the female is in estrus, depending on the pheromones present in the urine and faeces. During the estrous, fertilization is likely to occur, corpora lutea will develop and there will be progesterone secretion to maintain gestation *(Barnes et al., 2015; López-Pérez et al., 2021; Ortiz et al., 2022; Viau et al., 2020)*.

After ovulation, females enter **diestrus**, characterized by elevated progesterone concentrations lasting throughout pregnancy or nonpregnant luteal phase *(Barnes et al., 2015; Ortiz et al., 2022)*.

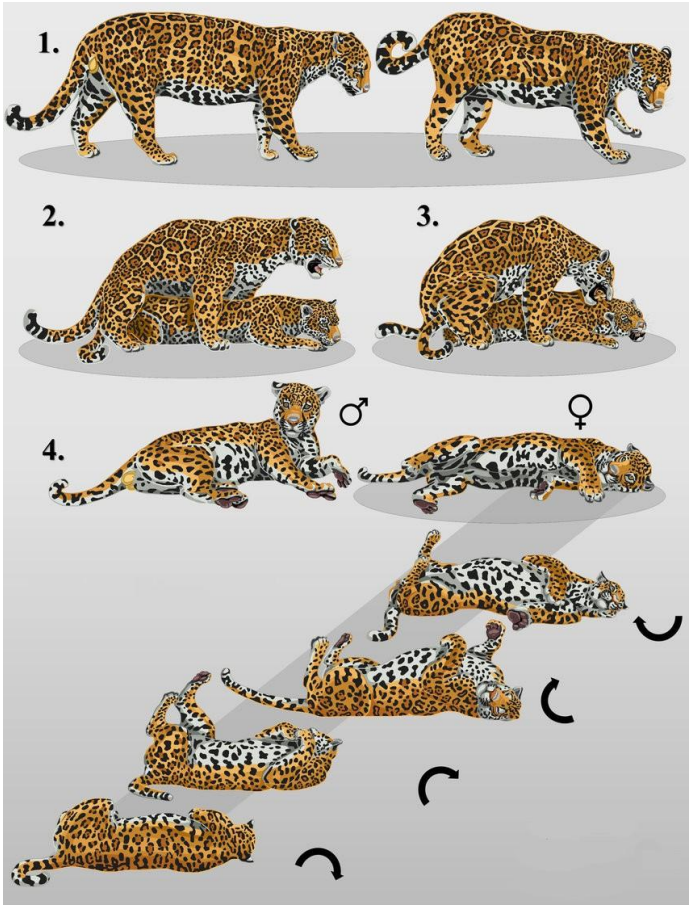
At the end of diestrus the females enter **interestrus** phase, which is the interval of ovarian quiescence when circulating estrogens remain at basal concentrations and females do not attract males. After this period the female resumes sexual activity or goes into anestrus if the reproductive season ends *(López-Pérez et al., 2021; Ortiz et al., 2022)*.

Anestrus is designated by the absence of ovarian activity and male acceptance.
In case of resumption of estrous cycles, the estrus-to-estrus period varies from 30 to 40 days *(López-Pérez et al., 2021; Ortiz et al., 2022)*.

The mating behaviour can be identified by some most characteristic activities such as vocalizations, mating attempts, naso-genital contact (the male approaches the female, performing an exploration and

sniffs the vulva) or naso-anal contact, genital preening and urine sniffing. When the female is receptive, she lies down and accommodates herself adequately for penetration (Figure 5.1). The male arrives and mounts from the back and on top of her, keeping her between his front paws (Figure 5.2). He then approximates his genital region to the female's one, squatting with the pelvic limbs, in the meanwhile the female deviates the tail, so the male initiates pelvic impulse, then bites the nape of the female's neck and penetrates her *(MORATO et al., 1999; Reproductive Biology and Assisted Reproductive Technologies in Wild Felids, 2021; Thongphakdee et al., 2020)*, (Figure 5.3).

After copulation the female usually starts rocking and rolling in lateral-dorsal decubitus and the male leaves her *(Jorge-Neto et al., 2018),* (Figure 5.4).



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Not all mating attempts are with penile penetration that's why usually there are multiple copulations during female's estrous, also during the same day (can be up to 100 times per day). This behaviour may be necessary to promote multiple ovulations and to ensure a sufficient number of successful penile penetrations with ejaculations, thereby ensuring proper numbers of normal fertilising sperm are deposited in the vagina *(Jorge-Neto et al., 2018; MORATO et al., 1999)*.

The jaguars are **induced ovulators**, this means that ovulation follows the stimulation of the vaginal floor during copulation. This mechanical stimulation triggers a neurological reflex that is responsible for

the release of GnRH by the stimulation of the mid-basal hypothalamus and subsequent releases of LH by the anterior pituitary, inducing ovulation between 24 and 48 h after the *copulation *(López-Pérez et al., 2021; Ortiz et al., 2022)*.

After insemination, the fertilised oocytes become implanted in the uterine horns and gestation is established with a duration of 91 to 111 days. Female jaguars give birth to up to four cubs, although births of one to two cubs are more common. Offspring are born in sheltered sites, such as caves, under fallen trees and among rocks. As females separate from males immediately after mating, they have to provide all parenting to the newborns *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Johnson & Van Pelt, 2011a; Modena et al., 2023)*.
The cubs open their eyes only after one week and are fully dependent on their mother's milk. During all the dependent stage of the cubs (approximately 5 weeks), it is critical to ensure their protection and continual care, which includes maintaining body temperature, cutting the umbilical cord, and encouraging breastfeeding, urination, and defecation *(Johnson & Van Pelt, 2011a; Modena et al., 2023)*.
From the sixth week, jaguar cubs are able to follow their mothers to start exploring and learn hunting, and are weaned at approximately three months. The calves remain with their mother for about 18 to 24 months, after which they separate in search of their own territory.
Although young and other jaguars succumb to a variety of natural causes, rangewide the major cause of adult mortality is killing by humans *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012; Johnson & Van Pelt, 2011a)*.
For the mother the interbirth period is approximately 2 years *(Brown, 2011; López-Pérez et al., 2021)*.

2.3 Modalities of reproduction in captivity and difficulties

Jaguar breeding has been documented year-round in captivity, but even in relatively naturalistic enclosures, reproduction in captive felids remains poor. Many of them cannot reproduce well due to various reasons, for example, behavioural incompatibility and infertility. Consequently, intensive captive breeding management using assisted reproductive technologies (ARTs), i.e. artificial insemination (AI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT) have been explored to help expanding the captive population *(Alexandre R. Silva & Alexsandra F. Pereira, 2016; Jorge Neto, 2019; Lee & Evans, 1994; Thongphakdee et al., 2020)*. However, a prerequisite to the development of effective breeding programs is a basic knowledge of species biology, including fundamental reproductive traits and status *(Ortiz et al., 2022)*.

Natural breeding in captivity is dependent on the reproductive health of the captive jaguars that is influenced by its genetic make-up, husbandry practices, good veterinary care and proper nutrition *(Brousset, n.d.; Morris, 2018a; Spindler Bush Heritage Australia, n.d.)*.

It is known that inbreeding depression (mating between relatives) influences reproductive fitness resulting in loss of heterogeneity due to unmasking and expression of recessive traits, which are often lethal, and lead to the appearance of congenital abnormalities. Loss of genetic diversity initially affects the reproductive system, newborns, and the immune system. In females may cause irregular estrous and abortions, while in males might give rise to decreased spermatozoa viability and motility. The newborns can suffer from increased prevalence of diseases or delayed puberty, resulting in an increment of juvenile mortality *(Brousset, n.d.; MORATO et al., 1999; Morris, 2018b; Spindler Bush Heritage Australia, n.d.)*.

Another factor affecting reproductive success in captivity is the stress that can be defined as an imbalance in homeostasis brought about by environmental stimuli. Stress may result from suboptimal housing and poor management mostly due to the fact that animals like the jaguar require comparatively large territories in nature that simply can't be mimicked in captive conditions *(Garcia et al., 2021; Spindler Bush Heritage Australia, n.d.)*.

Non-infectious disease, infectious and parasitic disease are known to cause morbidity and mortality in jaguars (as in all the other animals) and have direct effects on their breeding success. Additionally, health and nutrition are intimately linked to many disorders *(Spindler Bush Heritage Australia, n.d.)*.

The diet plays a fundamental role in the overall animal's body, including in the reproductive fitness influencing the sperm production, egg quality, hormone production and the ability to maintain a pregnancy *(Spindler Bush Heritage Australia, n.d.)*.

Muscle meat only diets, for example, are deficient in micronutrients like vitamins, magnesium or calcium and these deficiencies can be detrimental to the general health and reproductive fitness.

However, obesity is more often of concern in the captive felids with respect to nutrient deficiency, due to lack of exercise and overeating.

All these elements have an impact on the breeding success of jaguars in captivity, and furthermore the effect of inbreeding depression on the reproductive traits highlights the need to establish genetic management strategies in both captive and wild population to minimise the loss of valuable diversity.

Assisted reproduction techniques can play a significant role in these strategies by extending generation length and preserving genetic variation without the spatial and availability limitations facing most captive populations *(Alexandre R. Silva & Alexsandra F. Pereira, 2016; Lee & Evans, 1994; Spindler Bush Heritage Australia, n.d.; Thongphakdee et al., 2020)*.

Artificial insemination (AI) is the process of collecting sperm cells from a donor male and manually depositing them into the reproductive tract of an ovulating female to achieve pregnancy *(Lee & Evans, 1994; Thongphakdee et al., 2020)*.

It increases the efficient use of valuable male genetics, ensures reproduction between two valuable but behaviorally incompatible animals, eliminates the risks of animal transport, and can infuse new genetics into a stagnant population from semen collected in the wild.

Surgical and non-surgical AI methods are available *(Alexandre R. Silva & Alexsandra F. Pereira, 2016; Lee & Evans, 1994; Thongphakdee et al., 2020)*.

Surgical AI involves general anaesthesia and surgery. An incision is made into the abdomen, the uterus is then identified and exteriorized. The semen is then injected directly into the vagina (intravaginal) or uterus (intrauterine). This is then followed by replacement of the uterus into the abdomen, and closure of the incision site. This procedure can be done also laparoscopically and, in this case, the cervix is bypassed and the spermatozoa is placed in close proximity to the site of fertilization. Surgical AI is usually done with frozen or poor-quality semen.

Non surgical AI does not generally involve a surgical operation and the use of anaesthesia (in domestic animals) and consist in the use of an insemination pipette to deposit semen into the vagina just in front of the cervix (transvaginal). This technique should only be used with good quality semen (fresh or fresh-chilled). If the semen is of poor-quality it should be deposited directly into the uterus bypassing the cervix (transcervical). In this technique the use of palpation or an endoscope is required to better visualise the cervix *(Alexandre R. Silva & Alexsandra F. Pereira, 2016; Lee & Evans, 1994; Thongphakdee et al., 2020)*.

Successful pregnancies after AI with fresh or frozen semen in wild felids have been achieved by transcervical, intrauterine (either laparotomy or laparoscopy) or laparoscopic oviductal insemination in both small felids, like ocelot and Palla's cat and larger felids such as clouded leopard and tigers *(Lee & Evans, 1994; Thongphakdee et al., 2020)*.

Other AI methods weren't effective due to the poor semen quality associated with loss of genetic variability, as semen is usually collected from captive individuals. Specifically, species lacking heterozygosity tend to produce more malformed sperm than genetically diverse counterparts, so the percentage of available sperm to use for AI methods is reduced. Because of the danger posed by the conscious animal to personnel, semen is acquired from captive jaguars almost exclusively using electroejaculation or postmortem collection *(Jorge-Neto et al., 2023; Silva et al., 2020)*.

The most problematic aspect of AI programmes is potentially the detection of the fertile period in the female oestrous cycle. The stage of estrus needs to be known, Therefore, ovarian activity is often induced with exogenous gonadotropins to allow assisted reproduction techniques to proceed with the best chance for success. Failure is also associated with improper site of insemination, alteration of endocrine scheme with use of exogenous gonadotropins or inhibition of ovulation and uterine motility due to anaesthesia *(Barnes et al., 2015; Lee & Evans, 1994; Thongphakdee et al., 2020)*.

With jaguars the use of AI techniques is even more difficult due to the fact that they are induced ovulators, so usually, there has to be a vaginal stimulation to trigger ovulation. However, most recent

studies suggested that other forms of physical or social interactions with conspecifics during estrus, may also occasionally result in ovulation without copulation, but not entirely stimulus-free as in spontaneous ovulatory species. Therefore, physical contact through mating may not be the only mechanism that stimulates ovulation, but it can be induced via visual, olfactory and/or auditory cues by the males. They are capable of spontaneous ovulation if females are kept in proximity but without physical contact with males *(Alexandre R. Silva & Alexsandra F. Pereira, 2016; Jorge-Neto et al., 2020; Lee & Evans, 1994)*. This information is very useful for increasing the knowledge about reproductive physiology of jaguars as well as for the design of future assisted reproduction strategies.

In vitro embryo production is a technique that consists briefly of oocyte aspiration, in vitro maturation, oocyte selection, in vitro fertilization (IVF), embryo development and transfer.
The oocyte collection is often performed via transabdominal laparoscopic aspiration from pre-ovulatory follicles to maximise the available number. Once aspiration has occurred the oocytes are placed in cell culture medium for evaluation and assessment. The mature ones are prepared for in vitro fertilization. The spermatozoa and the oocytes are co-cultured together till the fertilisation has occurred and then are placed in the incubator for embryo development. The embryos are then examined and the excellent ones are collected in cell culture medium at the two to four cell stage for transfer into the oviducts of the recipient. The recipient animals may be the same individual or another female of the same species *(Alexandre R. Silva & Alexsandra F. Pereira, 2016; Jorge Neto, 2019; Lee & Evans, 1994)*.
This technique allows for more efficient use of the genetics of a valuable female that may be physically incapable of reproducing due to injury, disease, or age. Furthermore, the need to detect overt estrus is eliminated as the gametes are cultured and brought together in vitro under optimal conditions.
Although live offspring have been produced in a number of wild felids (for example, the birth of a Siberian tiger cub was accomplished at Omaha's Henry Doorly ZOO), in vitro embryo production has not been widely used in captive breeding programs for several reasons. First, there is limited information on species-specific reproductive endocrinology, gamete biology and embryogenesis. Second, the complexity of the procedure and the need for specialised equipment and facility to recover oocytes, perform IVF and culture resulting embryos. Third reason is the limited availability of developmentally competent oocytes, especially in ageing females or those with poor health *(Jorge Neto, 2019; Lee & Evans, 1994; Thongphakdee et al., 2020)*.

Lack of basic research due to lack of money, lack of interest, and the difficulty of working with the rare, non-domestic felids like the jaguar has been the primary obstacle to the widespread successful utilisation of assisted reproduction technologies in this species. Preliminary successes have been

achieved, however, further studies and implementation is needed for the successful development of captive breeding programmes.

3. Critical revision of traineeship activity with focus on the reproduction of the jaguars at the centre

3.1 Traineeship centre organization and structure with an emphasis on jaguar's enclosure

My traineeship lasted 2 months and I've done it at "Los Jaguares" rescue centre in Ecuador. I arrived in Quito (the capital city) by the end of March 2023 with another Italian volunteer of my same university course, Gaia Montini. The rescue centre is located in the province of Morona Santiago, more specifically in the city of Macas which is situated in the Ecuadorian part of the amazonian rainforest and is up to 6 h by bus from Quito.

"Los Jaguares" rescue centre is a non-profit organisation born in 2017 with the aim of creating a refuge where the wild animals rescued from maltreatment, being pets and abandoned, illegal traffic or affected by deforestation can live.

The general objective of the centre is to develop tools that allow the conservation of species in situ as well as the care, rehabilitation and protection of wildlife ex-situ, within the Province of Morona Santiago. This is done by means of the promotion of educational, socio-cultural, eco-tourism and research processes that enable the integral care of the ecosystems.

The centre is an NGO against domestication and illegal traffic of animals and it is self-sustained without governmental funds. It works through a volunteering program and earns direct profit from visitors' tours and donations, fundraising and projects of environmental education.

Currently the centre is directed by Agustin Paredes, who is the manager and the person in charge of bureaucracy, accounting and volunteer programs. Then there's the designated vet, Dr. Enrique Inga, who lives at the centre and is responsible for the animals' diet, including going to the local market to buy the missing food for the daily feeding session.

Another important person at the centre is the animal caretaker, Dr. Matteo Caccin, who is also the volunteers coordinator. He is in control of the animals' handling and care as well as overseeing the volunteers' work . Last person working at the centre is Anita Lora, the social media manager who manages instagram and facebook accounts to advertise the centre sharing contents on volunteers daily activities and missions of the refugee.

The centre is structured as follows:

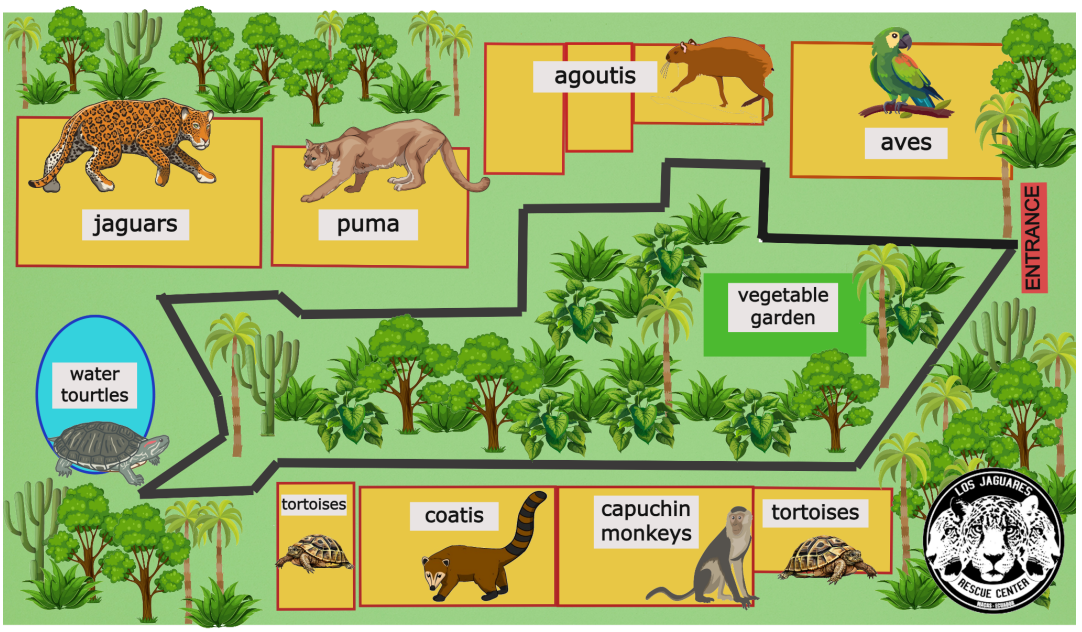
- Parking area: located in front of the centre entrance.

- Entrance: for visitors and new volunteers welcoming. Here information about timetables, rules to behave in the centre and social accounts can be found.
- Volunteers area: big space with wooden stools, amahs and tv where volunteers have daily meetings and can chill out. Is also the place where environmental educational activities are carried out as well as recreational or management activities when the weather is rainy.
- Kitchen: where breakfast, lunch and dinner are prepared with the help of the volunteers.
- Volunteers dorms: there are 4 rooms with at least 3 beds per room and individual bathroom and shower.
- Laundry: where volunteers can wash their clothes and sheets of the beds.
- Outside toilets: for the visitors.
- Guayusa's house: guayusa is the national drink of Ecuador. Its leaves are traditionally used to infuse tea and contain a level of caffeine very similar to that of coffee. In this space the leaves of the guayusa's trees are dried and kept in a cool and dry place to then be sold.
- Animals' kitchen: where the feeding for the animals is prepared everyday. Is located close to the animals enclosure and is equipped with its own kitchen tools, freezer and containers for food storage. Here there is also the necessary material for cage cleaning.
- Vegetable garden: here different types of vegetables are growing for both people and animals consumption. This to make the centre more self-sustainable.
- Quarantine area: where the animals are kept once arrived, for physical evaluation and assessment of the health status. This is for prevention of the possible spread of diseases, if present. In this structure are present cages of different sizes to suit all animals.

The animals' area is composed of 10 enclosures which can be roughly divided into 2 zones according to their geographical position (Figures 6) :

1. In the first part we find at the beginning the birds' aviary with 3 different species of amazonian parrots, then we have 3 different cages containing the agoutis. Lastly we have the felines part with a cage for the puma and one for the 3 jaguars.
2. In the second area there are the tortoises divided in 2 cages, then we have an enclosure for the 3 coatis and next to it the enclosure for the 2 capuchin monkeys.

Between these 2 main areas there is also a pond where we can find water turtles and fishes.



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All the cages are built in the same way with only one access and exit, for this reason we couldn't enter safely in all of them. The only exceptions are the enclosure of the felines where there's the presence of a management cage where we could lock up the animals. In the case of the jaguars, their enclosure is the biggest and is divided in two parts connected only in the middle by a small doorway that was opened and closed by a top-to-bottom sliding gate. Corresponding to it there is the entrance from where one can manage the confinement of the jaguars from one side or the other. This architecture allowed us to go into the free part of the cage for the feeding session or to isolate jaguars from one another when needed.

All the enclosures are then furnished with natural vegetation, hiding spots from visitors, different feeding and watering areas and some artificial objects as ambiental enrichment that was different for each cage. For example, ropes for the monkeys to climb on or platforms for the jaguars to have some rest.

3.2 Activities carried out during the traineeship

During my traineeship at "Los Jaguares" rescue centre the main daily activities that I carried out consisted in preparing the food for the animals and providing it adequately to them. Successively there was the cleaning routine of all the enclosures, this included also the provision of fresh water to the animals replacing the old one.

The working day started with the alarm at 8 a.m. and at 8:30 I met in the kitchen with the other volunteers to have breakfast. We used to prepare breakfast all together with both salt and sweet food to meet any taste. After this we usually had a meeting with all the volunteers at 9:30 in the volunteers area to decide what to do in the morning. There were activities that were to be done everyday like the

feeding of the animals, while others once during the week such as general cleaning of the common areas. Me or the other Italian volunteer Gaia Montini were always assigned to the feeding and watering of the animals with other 2 volunteers.

The feeding routine started with the checking of all the diet requirements of each animal and then we prepared the feed dividing it for the different enclosures. There are specific diets for the needs of all the animals and most of them eat 2 times during the day, morning and afternoon with the exception of the felines. Once we prepared the food, we started with the feeding giving it to the animals according to their different feed habits. We tried to minimise as far as possible hand-feeding, favouring feeding enrichment instead, for example hiding, hanging or throwing the food in the enclosure so that the animals have to actively search for it and also entertain themselves for more time. To make sure that all the animals in the same enclosure were eating we usually put the feed in different feeding spots within it. This was also to avoid crowding of all the animals at the same feeders and possible competition.

The feeding of the carnivores was performed differently compared to all the other animals because we adopted in most of the feeding sessions the scatter feeding method. As I previously described, the cages of both jaguars and the puma are divided in 2 sections, so we could close up the jaguars/puma in one of the 2 parts and safely enter in the other. This was done initially with a piece of meat to attract them in the desidered part, lately we started a successful training protocol with a whistle to make the animals associate its sound with the feeding time. The quantity of meat given to each animal was carefully weighed before and and then once entered in the cage we hid it in the vegetation of the enclosure, hang it on the trees or we also used to wrap some pieces of meat in 1-2 big leaves to make a sort of “burrito” and hide in this way to make it more difficult for them to find. Some blood left from the meat was also spread in the cage to confuse the animals a bit while looking for the meat.

The meat given was usually cow head or chicken heads and legs, this because these types of meat are rich in bones that are important for the teeth cleaning and maintenance but even more as mineral source, such as calcium and magnesium that are critical for the prevention of bone diseases development, like arthritis or arthrosis.

After the feeding, there was the cleaning of food leftovers and faeces and the watering. We went through each enclosure with brooms, a dustpan and brushes. Clean water was used both for cleaning and provision to the animals. For the majority of the enclosure we used to give water manually transporting it with a watering can and change the water in the drinking trough, but for the felines there was a watering system that could be opened and closed when needed, (as it used to rain a lot, it wasn’t necessary every day and also because felines prefer drinking rain water).

Cleaning of the felines was done at the same time of the feeding to limit the access in the cage, minimising the stress on the animals. In the morning, during the feeding session, was also performed a daily monitoring of the general conditions of each resident animal, checking firstly if all were present,

then a rapid physical evaluation, to assess the possible presence of any injury or parasites and finally if they were eating, drinking or performing any abnormal behaviour.
Important was that at the end of the feeding routine the kitchen of the animals was cleaned and kept in order and if something for the feeding was missing to communicate it so that the day after it would be bought. All the food for the animals was bought in the early morning at the local markets with the vet of the centre .

Morning work usually finished around 12:30/13 p.m. and then we had lunch time. We had a break to chill out till 3 p.m. and then we had the second meeting with all volunteers to split out the work. In the afternoon we had the second feeding session of the day that proceeded as the first. While watering and cleaning was done in the afternoon only if it hadn't been done in the morning.
The afternoon was dedicated to perform other activities like maintenance of enclosures and environmental enrichment for different animals. For example as environmental enrichment we build a ladder for the monkeys, peanuts feeders for the amazonian parrots and a small wooden house for the agoutis. The rescue centre is rich in material, like bamboo and wood and to build all this we didn’t have to buy almost anything else.
The biggest project of environmental enrichment we made while I was in the centre, was the building of a swimming pool for the jaguars enclosure, obviously we were helped by external workers too. The entire work took about 2 months, due to weather conditions not always favourable and disponibility of the workers. The pool is placed in only one of the 2 sections of the jaguars enclosure and is as big as one third of the entire cage and deep enough to swim in it freely. It was constructed with concrete and embedded with stones to make the pool banks less slippery and give it a more "natural" appearance (Figure 7).
It is complete with a filling system that utilises rainwater by collecting it and directing it towards the pool and a drainage system at the bottom also useful for collecting any debris. The project was huge and a lot of volunteers took part in its realisation and the final result was amazing and satisfying to see the jaguars swimming soon after it was ready (Figure 8 - 9).

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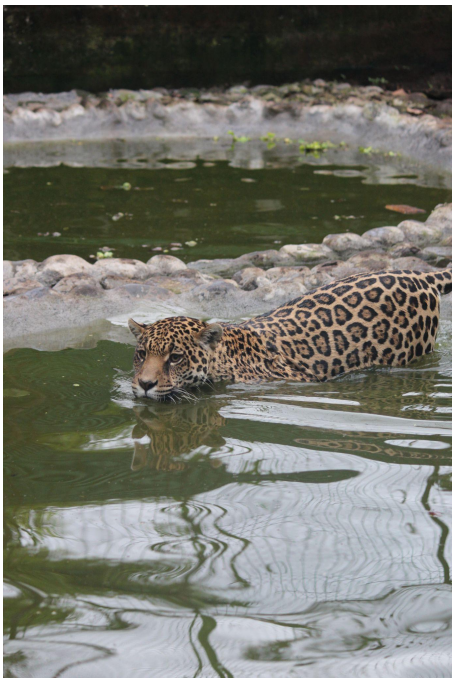

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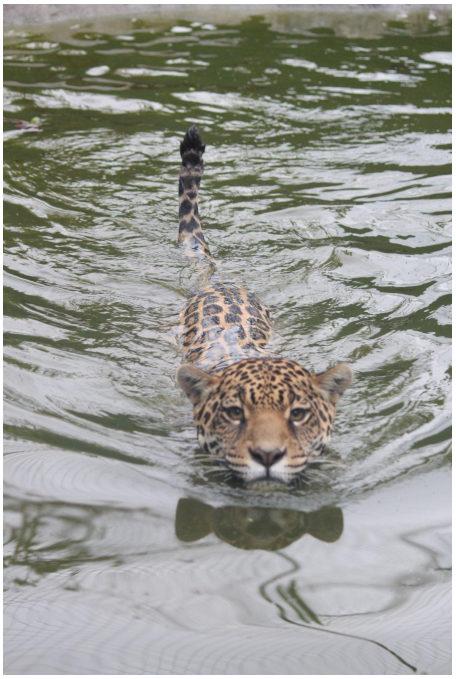

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Activities were done also for the maintenance of the rescue centre, like general cleaning and, as the centre is surrounded by nature, we also had to cut some plants or grass to preserve its appearance. In the centre there is also a vegetable garden with the aim of being more self- sufficient, producing some food by ourselves. Crop management of it was one of our weekly duties.

When the weather was rainy, recreational activities like painting and decorating the common areas were done as well as some research or just chilling a little more. Of course, the feeding routine was always done.

The working day finished around 5/5:30 p.m. and then we had time for taking a shower and relaxing till dinner time.

We worked 5 days a week and usually we had the weekend free. This time was used to make some trips and ecotourism around the province to get to know different places and the culture of Ecuador.
At weekends, naturally, the animals have to eat, the vet helped us with the feeding when we were away or we did it ourselves if we stayed at the centre.
These days were also the busiest ones for visitors. Us, as volunteers, have also the duty to carry out guided tours through the rescue centre. We elaborated and revised a speech (in different languages) to introduce the centre and its mission and the stories of all the animals hosted. The important purpose was to make the people aware of the role of the different animals within an ecosystem, how every single animal is essential for the maintenance of the equilibrium and biodiversity in a given habitat. This to make tourists realise that more effort has to be put in the conservation of nature and everyone can do a little.
Great results were achieved when we saw the visitors understanding our mission and all the work and efforts we are doing to preserve Ecuador's wildlife. There were people willing to do their part cooperating with donations or simply sharing contents on social media to give the centre more visibility and all this was very gratifying.
During the week it happened that sometimes we had schools visiting the centre and we did some environmental education with them. This was done through making guided tours through the centre, speaking about each animal rescued and underlying their roles for the maintenance of the biodiversity in Ecuador. As with the visitors the aim of the environmental education was to make them understand what biodiversity is and how important it is to preserve it. In addition there were also different kinds of educational activities figured out for scholars of any age. For the youngest scholars, for example, we did some interactive games to get them to know the different animals in the rescue centre, what they eat and some curious facts about their biology.

The main purpose of my traineeship activity was focused on the management, research and reproduction of jaguars. This involved the behavioural observation of the jaguars hosted in the centre, paying attention to how they interact with each other and the surrounding environment, the display of normal behaviour (eat, drink, sleep, etc. …) or stereotypic behaviours. Making research about how to improve their quality of life, finding out possible different forms of enrichment (food based, environmental, etc. …) to keep them stimulated, active and observe their reactions. Looking also for information about proper husbandry and management to minimise stress on them, given also by the presence of tourists.
We put more effort in their handling rather than the other animals, because the jaguars hosted at the centre are candidates for the reintroduction into the wild, so we were always doing research looking for what we could do to meet this objective. That’s why we firstly decided to build the pool for them, because in nature they are used to swim and prey along the rivers, (they are the feline species which

interact the most with water) and by staying at the centre they didn't have the opportunity to develop this behaviour before.
Of course, during the swimming pool building time, the jaguars couldn't always access the interested part and this put a lot of stress on them. First, because they were confined in just half of their enclosure and, secondly, we weren't able to perform the scatter feeding session so they were lacking any stimuli. They seemed bored mostly, but the final result was worth it because the jaguars were very curious and didn't hesitate a moment to enter the pool. We also encouraged them by putting some floating plants as embellishment and to oxygenate the water and some fishes inside for the jaguars to hunt.
Behavioural observations on jaguars weren't always possible due to lack of time or bad weather. Sometimes it happened that at the centre we were just few people and there was much to do so that we barely had time to stop. Another disadvantage was that in Ecuador we only had daylight till 6 p.m. so once we had finished working we didn't have that much time left. Also the rain wasn't that helpful because the jaguars stayed in their refuge inside the enclosure.

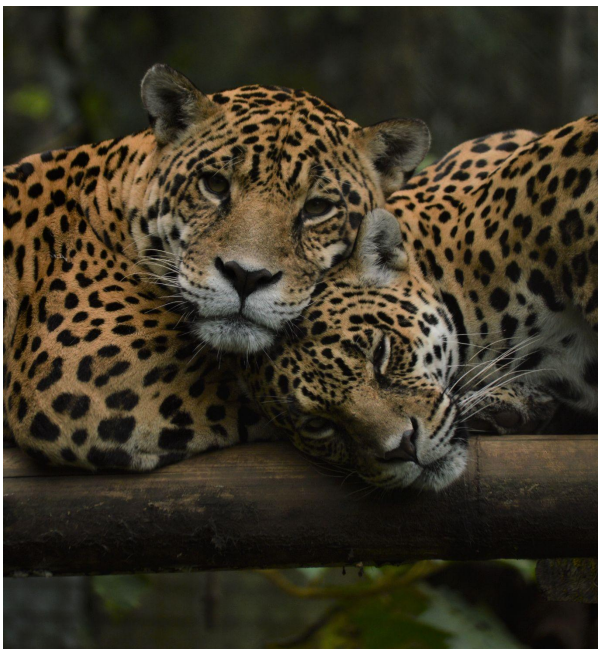
As my main daily activities were the feeding and cleaning routine of the animals I focused on how I could improve their diets or the way the food was prepared and given to the animals. I started from an organisational approach with writing a daily feeding routine that any new volunteers coming could see and understand what is to be done. Regarding the diets improving I turned my attention particularly to the capuchin monkeys that were a little under stimulated by having the same food almost every day that consisted mainly in fruits and sometimes seemed to be tired and not willing to eat it. I just simply enriched their diets by adding some vegetables and varying the types of fruits given each day during the feeding, trying not to give the same food in the afternoon with respect to the morning. As they are omnivorous animals, to implement their diet we also thought about giving them insects as a source of protein and Gaia elaborated a successful protocol for crickets breeding. Before, the monkeys were given a piece of cow or chicken lungs that are too rich in calories and fats for the monkeys' body. On the contrary, the crickets are almost calories-free and very rich in protein so they turned out to be a successful compromise. As the capuchin monkeys are very intelligent animals I also tried to entertain them with some environmental enrichment, hanging ropes and building a ladder for them to use. The result achieved with all of this was satisfying because we have seen the monos respond positively to both the new food given and the objects put in their enclosure. For organisational reasons I didn't have the time to concentrate enough on what I could improve for all the other animals hosted in the centre.

During the traineeship I also started a protocol for the construction and maintenance of hummingbird feeders. Ecuador is the second country with the biggest number of hummingbird species after Columbia and at the rescue centre there were many. To construct the feeders we recycled glass bottles and attached them to a small container, that could be a jar or plastic little box, where we practised some small holes for the hummingbird beak to pass. The container was filled with a nectar made of

just sugar and water that must not be too sugary because it can be dangerous for the hummingbirds (can't process it). To attract the hummingbirds we decorated them with small colourful plastic flowers and finally hung it on a tree. Essential is then its correct maintenance with regular cleaning and refilling.

3.3 History of jaguars' breeding which occurred at the traineeship centre

The jaguars' breeding couple at "Los Jaguares" rescue centre is composed of the female **Nantar** and the male **Amaru**. They arrived at the centre in 2009 when they were approximately 4 months old.
They were found tied to a tree in an indigenous community and rescued by the vet of the centre who brought them to "Los Jaguares".
Once they arrived at the centre, a small cage (5x5 m) was built for each of them. They stayed there until they were 6 months old, when they were too big to fit in.
At this point a larger cage (18x12x5 m) was built for each of them. The 2 cages were side by side, separated by a net, but connected by a small gated opening. Each cage was equipped with natural vegetation, small trees and wooden platforms. This allowed the animals to rest and get some sunlight. The enclosures were also provided with a shelter for sleeping and repairing in case of bad weather. To make the handling of the 2 jaguars easier, management cages were positioned next to the feeding area, allowing some clinical assessment to take place as well.
The 2 jaguars were kept separated during development for a period of 6 months, one in its own cage, able to see and smell each other but unable to touch. It was then decided to try and bring them together by simply opening the gate that connected them. The first reaction was an aggression by the female towards the male, which ended up in a fight. As a result, they stayed away from each other for a while, even though they were in the same enclosure. Gradually, they began to come closer to each other in a distrustful way, growling a bit until they got to know one another. From that moment on, they began to trust each other and they became a loving pair. They were always playing together, especially at feeding time and cuddling (Figure 10 - Amaru on the left and Nantar on the right).
After a year of being together, the female showed her willingness to mate. The heat was reported to last approximately 5 days per oestrus cycle and during this period there were multiple copulations with vocalisations or growls (especially during mounting) at a frequency of 1 or 2 per hour, preferably early in the morning or at sunset.



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After a few months it became clear that the female was pregnant, as her belly was enlarged, and she refused further attempts by the male to mate with her. However, their relationship remained lovely throughout this time, with the 2 still playing or cuddling. About a month before the birth, the male was separated from the female to allow her to relax and avoid a possible abortion. The shelter inside the female enclosure was cleaned, disinfected and provided with fresh dry hay to prepare it for the impending birth.

On 13th of March 2012 the first cub was born. The mother stayed with it all day until the next day, when she went for feeding. During this time, the vet was able to get close to the newborn to do a quick vet check and saw that the temperature in the cage was too low and the humidity was too high, which could affect the survival of the cubs. This was because the enclosure was not equipped with a warming system. To prevent its death, the cub was separated from the mother and taken to an incubator at a local vet clinic and fed pet milk and some vitamins to ensure its chances of survival. After ten days the newborn returned to the rescue centre in good health. One month old the cub was able to eat some meat provided by the mother and after three months it was eating only that.

The first cub was a female and her name is **Kiara** that is now at a zoo in Quito.

A year later, on 26th of May 2013, the second cub was born. Affiliative behaviour was observed between January and February, and after several mating attempts in April, pregnancy was evident. The female's belly became progressively larger and she refused male company. As with the first pregnancy, the 2 were separated for the month prior to the pregnancy and this time a thermostat was placed in the shelter where the mother would have given birth. This made it possible to control the temperature inside and prevent any risk of cooling. Once again, the mother stayed all day after giving birth, only

leaving to feed. Thanks to the thermostat, the newborn stayed with its mother without risk. At the end of one month, the condition of the cub was still healthy and it was growing without any problems.
The newborn this time was a male and his name is **Ikiam** and is now at the biopark "Amaru" in Cuenca, Ecuador.
On February 7th, 2015, there was another birth. This time two felìmale puppies, named **Gaya** y **Goya,** were born. The birth went as smoothly as the previous one and both newborns were healthy and strong.
Now Goya is located at the same biopark where her brother Ikiam is staying. They were transferred together when Ikiam was 4 years old and Goya was 2 years old, on 10 September 2017.
Instead, Gaya was killed in an attack by her mother that was motivated by her sexual competence on the 20th of July 2019.
In August 2019, the Ecuadorian law on reproduction in captivity changed. This law states that the breeding of animals in captivity for purposes other than conservation would have been a crime.
So the centre had to change its management to stop Nantar and Amaru breeding. This was done by physically separating the animals when the female was in heat, to prevent any possible mating attempts. This caused some stress to the animals as they had been used to being together for many years and as a result they started to eat and move less. However, due to restructuring work in one part of the enclosure, the pair stayed together for 3 days, during which time a possible pregnancy occurred. This was noticed about a month before the birth when the mother isolated herself in her den and stopped eating. The male was therefore allowed to enter the female's part of the enclosure to look after her and he began to bring her food. This continued until the morning of 7 January 2020, when the male also stayed in the cave of the female and did not come out to feed her. For three days the mother stayed in the den and only the male came out and fed her too until she was able to walk out and to feed herself. This made it possible to see that she was in good condition and it was also a chance to check on the health of the cubs. This birth resulted in two more newborns, two females named **Wayus** and **Yaona**, who are now 3 years old and are still living at the centre with their mother. Naturally, this birth was reported to the Ecuadorian authorities, who were merciful enough not to give them a penalty.
The father, Amaru, tragically died in an accident in his management cage on 9 March 2020, which is why there have been no more newborns at the centre *(Informe Jaguares Bebe Zoo, n.d.)*.

This breeding pair has given birth to a total of 6 cubs, representing approximately 1/3 of the entire captive jaguar population in Ecuador. All of these breedings have occurred naturally and have been successful. As previously described in my thesis, this is not an easy event to occur due to many possible causes (i.e. incompatibility of couples, death of newborns, etc.) but this breeding pair have met the necessary conditions at "Los Jaguares" rescue centre to mate. There's no way to explain why, but the fact that the jaguars have grown up in captivity and have not possibly felt as much pressure may have played a role. Also, because the centre is quite small and has developed gradually over the

years, the jaguars have not experienced the stress of having to interact with too many people. Food has always been plentiful and space sufficient. All these conditions potentially favoured successful mating. All of these newborns at the rescue centre would have been suitable candidates for release in the wild as they have good genes, but the Ecuadorian government has not focused on this before. After years in captivity, this goal is now more difficult to achieve.

4. Discussion

4.1 Conservation status of the jaguar in Ecuador and threats to it

In Ecuador, the jaguar has a disjunct distribution, on both sides of the Andes: in the Amazon and on the coast, mainly below an altitude of 2,000 m. These two populations are isolated from each other and represent different evolutionary units.

The largest population of jaguar in the country is found in the Amazon region, where there is the largest amount of available habitat, approximately 80,000 km^2, which corresponds to 80% of its original extent. Jaguar populations are reported to exist in the lower foothills of the Andes, in pre-montane forests and in the large remnants of lowland rainforest along the border with Colombia and Peru *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Ríos et al., 2014)*.

The main jaguar populations in the Ecuadorian Amazon are found within the large protected areas (e.g. Yasuní National Park) and indigenous territories that maintain forest cover. In the Amazon, it is estimated that the range of the jaguar has been reduced by approximately 30%.

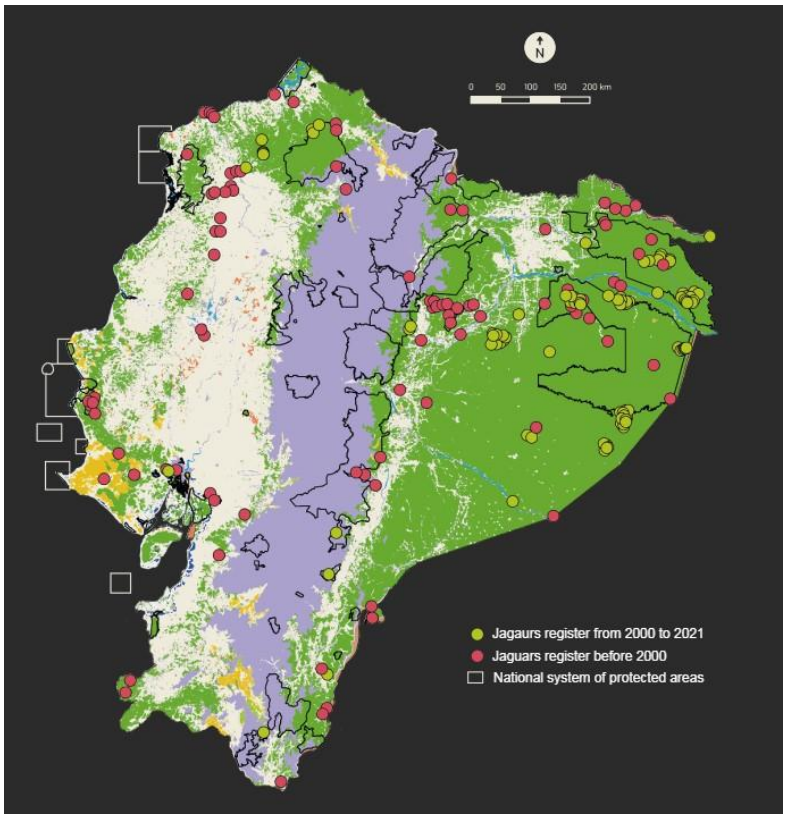
However, the jaguar may also use areas with a high degree of agricultural influence, such as in the provinces of Sucumbíos, Orellana, Pastaza and Morona Santiago *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*.

It is supposed that the jaguar population in the Ecuadorian Amazon is approximately 1,200-2,000 individuals (2016). Additionally, this population is part of a larger one that includes the neighbouring countries, Peru and Colombia of about 2,000 individuals *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*.

On the other side, the distribution of the jaguar on the Ecuadorian coast is very fragmented, with the jaguars species considered extirpated from most of this region. The degree of habitat fragmentation on the coast is very high and there are only 26,000 km2 of forest remnants.

In the last 20 years, the coastal jaguar distribution has been recorded only in the north-western region, in the area corresponding to the Cotacachi-Cayapas National Park and the Pambilar Wildlife Refuge (2013); and in the south-west, in the Cerro Blanco Protected Forest (2017). It is estimated that on the coast of Ecuador the jaguar population does not exceed a few dozen individuals (2016) (Figure 11).

Connectivity among Jaguar populations is being lost at local and regional scales, the isolated populations have fewer individuals and are more prone to local extinctions *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*.



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The International Union for Conservation of Nature (IUCN), in its Red Data Book, classifies the jaguar as a globally Near Threatened species (2017) due to a suspected 20-25% decline over the past three generations (21 years) in area of occupancy and due to the extent of occurrence *(THE IUCN RED LIST OF THREATENED SPECIES, 2018)*.

In Ecuador, due to the differences in the conservation status of the species on both sides of the Andes, the jaguar species has been evaluated in the Red Book of Ecuadorian Mammals (2011) independently, dividing the Coastal and Amazonian populations into subspecies. The Coastal populations are considered to be a Critically Endangered species, while those of the Amazon are considered just Endangered.

Moreover, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has included the jaguar in Appendix I since 1975. The species listed in this appendix are critically endangered and CITES prohibits international trade in specimens, body parts or products of these species, except when the import is for non-commercial purposes *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Ríos et al., 2014)*.

Currently, the main threats to jaguar conservation in Ecuador include loss of habitat and reduced levels of connectivity, direct hunting caused mainly by conflicts with people for livestock, and a decline of its prey due to the increasing demand for wild meat (both for subsistence and commercial hunting). The intensity, and relative importance of these threats are not uniform, and vary from locality to locality. In many cases, the effects of these threats form a synergy, exacerbating the negative effects on jaguar populations *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012; Boron, 2012; Johnson & Van Pelt, 2011b; Paviolo et al., 2016; Payán Garrido et al., n.d.-b; Povilitis, 2015; Ríos et al., 2014)*.

Habitat destruction and fragmentation are probably the most critical threats to jaguar conservation.

Deforestation in Ecuador continues to be significant; for example, the Ecuadorian Amazon, where the country's largest jaguar population is concentrated, has an annual net deforestation rate of over 60,000 ha per year (2019). Deforestation rates in Latin America are the highest in the world together with tropical Africa (2015).

The main drivers of deforestation have been attributed to unplanned land use over forested areas, expansion of road infrastructure, unsustainable land and water-use practices (due to agriculture, cattle ranching, extractive activities, etc.). The agricultural sector is, at the moment, the main source of deforestation, primarily through the cultivation of livestock pastures, like soy. The produce is mainly used for export as raw products for developing countries, not to feed local populations *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Boron, 2012; Paviolo et al., 2016)*.

On the other hand, the over-exploitation of wildlife through subsistence and commercial hunting have a direct influence, with a negative impact on the abundance and distribution of jaguars *(Boron, 2012; Ceballos et al., 2021; Paviolo et al., 2016; Ríos et al., 2014)*.

The use of prey species, such as white-lipped peccaries, white-fronted peccaries, collared peccaries, deer, among others, by indigenous communities has been increasing to satisfy the commercial wild game meat demand, using also the hunting for subsistence as a pretext.

As an example, in the Yasuní Biosphere Reserve, the increase in access to the landscape, facilitated by the construction of roads built for the oil exploitation, is associated with the intensive use of wildlife by the indigenous communities that live there. Indeed, it has been recently noted that jaguar density in Yasuní is lower in the areas that are more accessible to hunters, i.e. next to roads or navigable rivers *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*.

The procurement of their parts for trade is a constant menace. In a problem analysis workshops, it was confirmed that there is an incipient market of jaguar parts (skin, bones and teeth) aimed at commercial purposes for ornamental use and possibly for export, despite the fact that it is a protected species in the country. As an example, based on a research conducted in Suriname between September 2017 to July 2018, it has been recorded that different jaguar parts (e.g., bones, teeth and a product known as jaguar paste) are being shipped to China as substitutes for

traditional medicine *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; National Action Plan for the Jaguar in Brazil, 2012; Payán Garrido et al., n.d.-b)*.

The conflicts between people and jaguars for predation of livestock, have increased in recent years in areas where jaguars and human populations coexist and share limited resources.
In the absence of management, particularly small and poorly resourced farms, livestock are more vulnerable to feline attacks. Small cattle keepers are more heavily impacted by predation and are less tolerant to losses, which results in active persecution of jaguars *(Bredin et al., 2018; Kelly, 2019; Steinberg, 2016)*.
As the density of people increases, the demand for resources and access to land for agriculture, industry and urbanisation necessary for sustaining the incremental number of people will continue to rise, making the wildlife easier accessible for hunting. The greater need for food means increased commercialization and growth of the prizes for wildlife game species, which are all jaguars' prey species. The jaguar-people conflicts will not be solved without the engagement of the human communities in the development of strategies to ensure the conservation of wildlife species that are part of the jaguar's diet. The negative effects of human activities have caused the local extinction of jaguar populations in large areas of its historical distribution, the decrease in population and reduced levels of relatedness, increasing isolation and reducing gene flow between populations *(Kelly, 2019; Paviolo et al., 2016; Steinberg, 2016)*.
Many jaguar's populations require connectivity between core sites to survive in the long term, and these linking corridors are most of the time outside protected areas, and therefore vulnerable to human impacts *(Gutiérrez-González et al., 2015; Jaguar Conservation in Brazil: The Role of Protected Areas, 2008; Morato et al., 2014)*.
The vulnerability of the Jaguar to persecution is demonstrated by its disappearance by the mid-1800 from Uruguay and El Salvador and by the mid-1900's from the southwestern US. These countries were the first areas to show rapid population increase with large scale land conversion. Retaliatory, killing of the remaining exposed Jaguars led to their extinction. These processes are now taking place on a continental scale and therefore there are few areas within the jaguar range that can be considered safe *(Paviolo et al., 2016)*.

Large carnivores such as the jaguar are keystone species that maintain the structure of an ecological community in diversely forested habitats and protecting biodiversity.
Being a top and a generalist predator, it helps maintain healthy prey populations by eliminating old and sick animals. In addition, predators such as the jaguar contribute to maintaining herbivore populations at sizes that allow for adequate regeneration of the vegetation they use for food.
It has been observed that the removal of large predators from the landscape can lead to drastic changes in the composition and structure of the landscape, even lead to alterations in its hydrological dynamics

due to the lack of demographic regulation of prey species. For example, in areas where jaguars and pumas have been extirpated, the density of large rodents, primates, iguanas and leafcutter ants is 10 to 100 times greater than in areas where they are present.

At the same time, the density of seeds and seedling of canopy tree species in the predator-free areas show levels of 80% reduction compared to areas where jaguars still exist and fulfil their ecological function.

Jaguars are, however, relevant not only ecologically but also culturally as they have served as a cultural icon for many indigenous American people. The jaguar has been an emblem of hierarchy, being a symbol of kings and warriors with an intense intrusion in creation myths, cosmogony and mythological symbolism.

The jaguars have a high cultural value, and this makes them ideal as a flagship species, and can therefore play a fundamental role in increasing the conservation efforts and for triggering awareness processes *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Paviolo et al., 2016; Ríos et al., 2014)*.

4.2 How reproduction in captivity can help jaguars' conservation

Over the last decade, the jaguar in Ecuador has shifted from being a nearly disregarded species to being the focus of conservation activities, mainly by different international non-governmental organisations (NGOs) and researchers.

For example, WWF has embraced the jaguar as one of its priority species and is currently developing a communication and awareness-raising strategy at national level in order to promote the jaguar as one of the symbolic species of the country's natural heritage and to obtain public and political support for the conservation of the species *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Luczinski et al., 2023)*.

There is ongoing research, mainly within the Cuyabeno Fauna Production Reserve and the Yasuní National Park, with the support of the academic sector, that seeks information that can be used for the conservation of the species.

On the other hand, the MAATE (Ministry of Environment, Water and Ecological Transaction) has worked to improve the management of the protected areas in which the jaguar lives, through activities such as the strengthening of the control and surveillance systems, with the implementation of camera-trap monitoring devices in jaguar habitats *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*.

The Organic Code of the Environment (CODA), which came into force in 2018, constitutes the country's most important environmental regulation. It establishes, implements, and encourages mechanisms and instruments for the conservation, sustainable use, and restoration of ecosystems, and the biodiversity including its components, genetic patrimony, national forest heritage, national forest

patrimony services, coastal marine zone and natural resources *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*.

The main objective of conservation actions is to maintain and restore viable jaguar populations, in coexistence with human populations, as an integral part of the ecosystems and landscapes in Ecuador. In this context, it is also necessary to create a network of actors interested in jaguar conservation (local communities, central governments, universities, etc.) *(De Angelo et al., 2013; Povilitis, 2015)*.

The lines of action, in a context of adaptive management, consider the conservation of jaguar populations and subpopulations throughout their geographic range on both sides of the Andes.

Reintroduction, restoration, and rewilding are all becoming more important conservation approaches in the 21st century, since they are valuable tools aimed at reestablishing or supplementing an extinct or declining population. In the case of the jaguar species, it would be useful to increase the population in the core sites and try to connect the fragmented populations. Individuals for reintroduction can be from captive bred programs or from translocation from existing wild populations *(Gasparini-Morato et al., 2021; Luczinski et al., 2023)*.

Nevertheless, for a successful reintroduction plan, the joint action between **in situ** and **ex situ** management of wildlife populations species is a critical key component for jaguars' conservation.

With regard to the **ex situ** management of the species, there is an updated inventory of the jaguar population in captivity, which to date includes 18 specimens (eight males and ten females) distributed in seven holding centres *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021)*. The existence of jaguars in captivity is not only related to the development of environmental education activities for the general public. Of course, this plays an essential role in raising awareness about the importance of the jaguars and other species in an ecosystem and their issues for conservation.

Once a jaguar is captured, its role for the conservation of the species is not lost. Most individuals kept in captivity are wild-born and are genetically valuable founders and play a significant role in species survival, serving as hedge populations and as educational and research resources *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Johnson & Van Pelt, 2011a; Payán Garrido et al., n.d.-b)*.

It's obvious that captivity changes the relationship with jaguars and humans by being fed by them. In this way the animal will always remember that humans are associated with food and after being reintroduced, may forage in villages and houses, thus creating risky situations for both the jaguar and humans.

However, this is not necessarily true for the cubs that a captive jaguar might have, because they still could have a chance to be raised without human care and be reintroduced in the wild.

In fact, the main objective of ex situ management for conservation purposes, is to learn and develop jaguar husbandry techniques to raise animals that can live in the wild. This means that the animals will be able to form part of an ex situ population of jaguars in Ecuador, or be integrated into in situ

conservation initiatives that are approved and supported by the competent authorities *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Ignacio, n.d.; Luczinski et al., 2023; Sanderson et al., 2021)*.

In captivity, the jaguars can live and reproduce until they are approximately 20 years old, which would allow the development of different captive management programs in Ecuador.

The ex situ population of jaguars in Ecuador is managed with standard parameters and protocols, in close coordination between the Environmental Authority and the wildlife holding centres. They require very specific actions and must be separated in its management from populations in the wild. The animals chosen for ex situ programs should be jaguars belonging to the same genetic group as the jaguars in Ecuador (i.e. the core group located in the amazonia), and directly related animals have not to be crossed, to avoid possible loss of heterogeneity in the newborns or any abnormalities *(Ignacio, n.d.)*.

Due to the possible deleterious effects the captivity can have on the reproducing capacity of the animals, the individuals that are confined for less time should be preferred *(Ignacio, n.d.; Spindler Bush Heritage Australia, n.d.)*.

Considering the cheapest option for the development of a captive breeding program, the occurrence of natural reproduction will be the one. The goal is that the captive breeding pairs reproduce as many pups as possible that can learn to survive in the wild. Using breeding pairs already established should be favoured as it reduces the risk of incompatibility and crossbreeding should be selected to minimise the level of relatedness of the offspring born. The newborns will never have any contact with the humans from the first moment. As the jaguar is the top predator in its natural habitat, the learning of behaviours that guarantee predator recognition in cubs should be aimed at establishing a neutral relationship with humans. For this purpose, the use of indirect feeding systems may be the best way to prevent pups from developing an association between caretaker and food that could lead to excessive habituation to human presence. On the other hand, there shouldn't be any interest in encouraging negative associations between the two, as this could lead to the development of certain aggressiveness towards people on the part of the puppies. Feeding will be a combination of live and dead prey encouraging the feed on wild prey especially abundant in the wild and not chicken or other livestock that are not their natural prey. Monitoring of the cubs will be evaluated through camera tramps.

Dead prey should be fed initially and then small live prey should be used to encourage play and chase behaviour in the puppies. Progressively, and if the mother's hunting skills and physical condition allow it, live prey of larger calibre shall be provided. To encourage predatory behaviours in the cubs, the complexity and diversity of the live prey shall be gradually increased.

Establishing routines should be avoided to prevent habituation and prediction by newborns of the time and place of prey appearance. Fasting periods and the sporadic use of starvation should be used to stimulate predation behaviours and resemble a bit how life in the wild would be.

The final development phase begins when the pups start to become independent from the mother. During this period, of variable duration depending on each individual, the management to be carried out shall be the minimum and indispensable and shall basically consist of the replenishment of wild prey through the automatic feeding systems and the elimination of carcasses and biological remains.
Objective evaluation systems must be followed to assess the minimum criteria for animals to be considered as suitable for release. Jaguars tend to move in areas with which they are familiar, (evidence of cognitive capacity and spatial memory) and therefore building enclosures in the release area and keeping individuals there prior to release may facilitate acclimation.
Prior to liberation into the wild, a health check and marking of the animals shall be carried out.
The animals can be tagged with microchips and GPS/VHF (very high frequency) collars to allow their monitoring in the wild. The reintroduction is considered successful when the jaguars are reported to have home ranges, and movement and activity patterns similar to those reported for free living individuals, including the exhibition of social interactions and reproduction *(Gasparini-Morato et al., 2021; Ignacio, n.d.; Sanderson et al., 2021)*.

As a pre-condition for reintroduction, the highest priority must be to address the threats that are causing jaguar population declines. Once jaguars are reintroduced, concerted work will be required to monitor the health and welfare of jaguar and prey populations, minimise livestock losses, and share information with local communities, tourists, and the public *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; De Angelo et al., 2013; Sanderson et al., 2002; Seddon et al., 2014)*.
In situ management proposes activities to strengthen protected area management and incorporate other forms of conservation to maintain and connect jaguar habitat in Ecuador *(Sanderson et al., 2002)*. This is especially necessary for those jaguar subpopulations that are proximal to areas where people are active, potentially resulting in negative interactions. In such areas, law enforcement to prevent killing of jaguars, and alleviation of negative interactions with ranchers, are required and should play a central role in planning any reintroduction. Activities for the prevention, mitigation and compensation of jaguar-people conflict are discussed through the development of response protocols and implementation of measures to avoid conflict by improving livestock husbandry practices, as well as a series of incentives to stimulate paradigm changes in the means of livestock production *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Povilitis, 2015; Seddon et al., 2014)*.
Environmental education is also a key aspect to support jaguar conservation. This line of action should include activities aimed at disseminating knowledge, raising awareness about the importance and current status of the species, and to generate changes in the way people relate to the species in favour of preservation of the jaguar in Ecuador. The activities should focus on the implementation of campaigns, workshops and communication and awareness-raising tours for different groups of actors, including students, educators, decision-makers, communicators, the private sector and the and the

general public *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; De Angelo et al., 2013)*.

On the other hand, in areas that have undergone deforestation or defaunation, it is important to evaluate habitat quality and prey availability before establishing a reintroduction programme. Activities should be promoted for the management of the jaguar's prey and the exploration of alternative sources of protein for communities in the surrounding of protected areas. In addition, the need has been identified to evaluate the social interaction before the reintroduction, because it is useful to get to know which sex is better to release in that area. As an example, the absence of intersexual territoriality suggests that reintroducing female jaguars is likely to increase the success of reintroductions in areas where male jaguars are present *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Sanderson et al., 2002)*.

Habitat fragmentation is also a big threat to conservation that needs to be addressed. For this reason, wildlife corridors are considered a valuable conservation tool to promote the ability of individuals to move among habitat patches and provide, in this way, an opportunity to mitigate the negative effects of demographic and environmental stochasticity and to sustain the population's genetic diversity and maintain the evolutionary processes associated *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; De Angelo et al., 2013; Rabinowitz & Zeller, 2010)*. However, designing corridors can be challenging due to the lack of methodological examples found in the literature and few available practical examples of field assessment of wildlife corridors, thus further research is needed.

Essential is also to reinforce the judicial systems so that they can address and respond to complaints of wildlife trafficking and other environmental crimes that may affect jaguar conservation *(Action Plan for Jaguar's Conservation in Ecuador 2022-2031, 2021; Sanderson et al., 2002)*.

Last point to consider in a reintroduction plan through a captive breeding programme is the cost (usually conspicuous) and it needs to be compared to alternative strategies before being approved *(Gasparini-Morato et al., 2021; Luczinski et al., 2023)*.

5. Conclusion

5.1 Personal review

I would like to start reviewing my experience during the traineeship. My time working at "Los Jaguares" Rescue Centre was simply amazing! Personally, I think it was not just a work opportunity, but a whole life experience. I have learned how to properly manage feeding and cleaning of wild animals, particularly how to handle wild felines safely. I challenged myself with different enrichments and overcame difficulties in realising them. At the rescue centre, I was not just a volunteer working

there, but I felt a part of it, my ideas were considered and put into practice, my opinions were asked and accepted.
Any volunteer had the possibility to express himself and do whatever he was good at, propose ideas and realize something. That is why I initially decided to write my thesis in collaboration with them. When I expressed this interest, they immediately made themselves available to discuss with me how concrete my idea could be developed and then they provided me with the material in their possession. I have to say that sometimes the organization was lacking because there was not always a referent person and people did not know what to do exactly, but things always worked out well.
I have learned how to work with others and brainstorming solutions to problems but I've also experienced being an authoritarian person and giving instructions and orders to others. I have met a lot of new people, coming from different parts of the world, having different backgrounds and living all together at the centre was such an experience. Sometimes it could have been a little overwhelming because I did not really have any time to myself, and on some days there was a lot of work to be done even on the day off. The only way to chill out was to go out of the rescue centre and go for some excursions. Life in South America was also not that easy because of the rainy weather, a lot of mosquitos, humidity, cold water and a lot of noise, so you have to get used to it a bit. Nonetheless, people and culture are amazing and it is not that easy to get in love with it.

Revising my thesis, I must say that it was sometimes difficult to find the information I needed, partly because research on this subject was scarce or not too detailed. It could have been easier if the information at the centre was clearer and better documented, but some information was lost and I did not have all the data I needed. Being the first to write about it meant that I had to work harder, but I am pretty happy with the result.

Last point I would like to discuss is the conservation of a species, which is always a sensitive subject. There may be many projects, but they are not always feasible. This is because there are many parties involved in these plans, ranging from individual conservation organisations to political authorities and even individual people. However, those who have the power to get the engine moving sometimes wait until the situation becomes critical, either because of the costs of implementing or because of a lack of interest, and stop conservation efforts.
Those who fail to see the importance of these projects are very often private individuals who do not feel part of it and become disinterested. In this world where climate emergencies, habitat destruction and species extinction are commonplace, being aware of what everyone can do can make a difference.

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