VERSION 1.2

FEB 2021

EAZA RMG

EAZA Reproductive Management Group

THE EAZA REPRODUCTIVE MANAGEMENT GROUP RESEARCH PRIORITIES

CONTENTS

Citation: Cowl, VB. (2020) *The EAZA Reproductive Management Group: Research Priorities*. The EAZA Reproductive Management Group, Chester Zoo, UK.

The EAZA RMG is supported in this work by the European Union LIFE NGO funding programme. The European Union is not responsible for the views displayed in publications and/or in conjunction with the activities for which the grant is used.

THE EAZA RMG RESEARCH PRIORITIES

WHO ARE THE EAZA RMG?

Established in 2008, the EAZA Reproductive Management Group (RMG), previously the EAZA Group on Zoo Animal Contraception (EGZAC), is a working group under the European Association of Zoos and Aquaria's (EAZA's) Veterinary Committee. The EAZA RMG consists of *in* and *ex situ* researchers, veterinarians and animal managers with an interest in the reproductive management of exotic wildlife. The work of the EAZA RMG compliments that of the Association of Zoos and Aquariums (AZA) Reproductive Management Center (RMC). Our vision, mission, and strategic aims are outlined below:

VISION

To support the work carried out by breeding programmes and animal managers within the European community by providing specialist advice in reproductive management.

MISSION

Our mission is to support the work carried out by EAZA *Ex-situ* Programmes (EEPs), Taxon Advisory Groups (TAGs), and animal managers at Member institutions by providing specialist advice in reproductive management. This includes ensuring the safe and informed use of contraception in exotic wildlife in human care (EGZAC Strategic plan, 2016). The EAZA RMG aims to promote a holistic approach to individual and population reproductive management in *ex situ* conservation. We identify gaps in the current knowledge on the effects of reproductive decisions (breeding/non-breeding, contraception), and advocate for the collection of data to inform current practices. More information regarding the RMG's goals can be found in our strategic plan (2016-2021).

STRATEGIC AIMS

SO 1. To ensure an excellent reputation for reproductive advice based on sound scientific data

SO 2**.** To identify and conduct applied reproductive research with measurable impact

SO 3. To ensure long term viability of the EAZA RMG by securing funding for a programme coordinator

SO 4. To become an integrated tool used for population management within EAZA institutions

SO 5. To form effective partnerships with programme coordinators and animal managers

To effectively meet SO 2. we have developed this document[1] to highlight the research priorities of the EAZA RMG as well as the work of our working group members and advisors. We aim to incorporate elements of these priorities in all EAZA RMG endorsed projects.

[1] This is a living document outlining research priorities as identified by the EAZA RMG. It will be updated every 3 years. Member and research appendices will be updated annually.

EX SITU CONSERVATION BREEDING

Ex situ conservation efforts aim to maintain species outside of their natural environment (WAZA, 2013), primarily by conserving genetic resources in gene banks, or through captive conservation breeding programmes (CBPs; EAZA, 2013). As an estimated one in seven threatened species are housed in zoos and aquaria, the maintenance of healthy captive genetic reservoirs is becoming increasingly important (Fa et al., 2011). These programmes, of which there are over 400 within Europe (called EEPs; van Lint and de Man, 2016), have played an important role in the conservation of threatened species. Indeed, in a 2011 assessment, 68 vertebrate species have recently had their IUCN threat level reduced, 25% of which have been assisted by CBPs (Conde et al., 2011), including flagship conservation species such as the golden lion tamarin (*Leontopithecus rosalia*), and the Prezwalksi's horse (*Equus ferus prezwalski*)(Hoffmann et al., 2010).

POPULATION SUSTAINABILITY

Regardless of the role of the captive breeding programme, populations should be healthy and breeding to allow for the long-term success and sustainability of the programme. Ideally, populations are genetically diverse, with low levels of inbreeding, and are minimally adapted to living in captivity. To achieve the goals of the population, coordinators analyse the demographic and genetic status of their population, examine institutional constraints, and consider husbandry requirements before creating specific breeding and transfer recommendations for the individuals in their population (EAZA, 2015; Faust et al., 2019). However, not all captive populations are sustainable. In fact, a recent review of over 110,000 AZA breeding and transfer plans found that as few as 20% of recommended pairs successfully bred before a new recommendation was issued (Faust et al., 2019)[2]. While the exact cause of the low rate of reproductive success is unknown, failure to produce offspring may arise through several processes, including institutional inactivity, the death or illness of an individual, mate incompatibility, senescence, medical issues, or subfertility (Asa et al., 2011; Faust et al., 2019; Martin-Wintle et al., 2018).

An additional challenge facing breeding programmes is limiting reproduction, for example to ensure that non-recommended individuals don't breed, to reduce the number of individuals who are surplus to the population, or to maintain family groups. In such situations, identifying safe,

[2] No similar figures have been published for EAZA.

effective, and reversible methods to limit reproduction while ensuring that population growth remains sustainable is integral to population management. Together, these two components form complimentary aspects of reproductive management.

THE EAZA RMG AND REPRODUCTIVE MANAGEMENT

With this in mind, the EAZA RMG's remit expanded in 2019 from a focus on wildlife contraception (as stipulated in our 2016-2021 Strategic Plan) to a wider overview of reproductive management.

In terms of reproductive management, our role in EAZA is:

1. To support EEPs and animal managers with specialist knowledge on reproductive management. Examples include evidence-based contraceptive guidelines that allow for the safe and effective use of contraceptive products, and knowledge on assisted reproductive techniques in exotic species.
2. To identify gaps in the current knowledge on the impacts of breeding decisions based on our experiences working in zoological institutions and through discussions with breeding programme coordinators.
3. To conduct research with relevant *in* and *ex situ* partners.
4. To collect data to inform best practice.

To achieve this, we are committed to using an interdisciplinary, holistic, and evidence based approach to support the EAZA community. We aim to foster collaborations internationally, between zoos, research institutions, and *in situ* partners. Outlined below are our key research focus areas.

RESEARCH PRIORITIES:

BASIC BREEDING BIOLOGY

To begin to understand the implications of breeding decisions on managed animals, an understanding of the species' basic breeding and reproductive biology is essential and should be interwoven into all aspects of reproductive management. While some exotic species can benefit from the generalization of existing research on domestic equivalents, for many species, even this base knowledge is unavailable (Melfi, 2009). As such, it is essential that zoological institutions conduct research into the basic biology of the species they manage.

Characterizing the basic reproductive biology of managed species is of paramount importance to ensure that zoological institutions are providing their animals with the appropriate husbandry required to support reproduction and positive welfare. Zoos must thoroughly evaluate factors that contribute to reproductive success, including the overall health of the animal and its group, its reproductive history and health, its age and reproductive state, management practices, and the behavioural, social and welfare impacts of breeding recommendations.

Moreover, as species held in zoos will not respond uniformly to breeding decisions, an understanding of species physiology and reproductive biology is key to ensure that husbandry practices do not hinder long term fertility. For example, for some species, including African wild dogs (*Lycaon pictus*), Seba's bats (*Carollia perspicillata*), and stingrays, common management techniques such as separation of the sexes for prolonged periods of time may negatively impact fertility and can, in some cases be associated with severe health consequences (Asa et al., 2014; Napier et al., 2009; Penfold et al., 2014). However, without knowledge on species behaviour and physiology, it is difficult to refine management practices to the needs of the individual species.

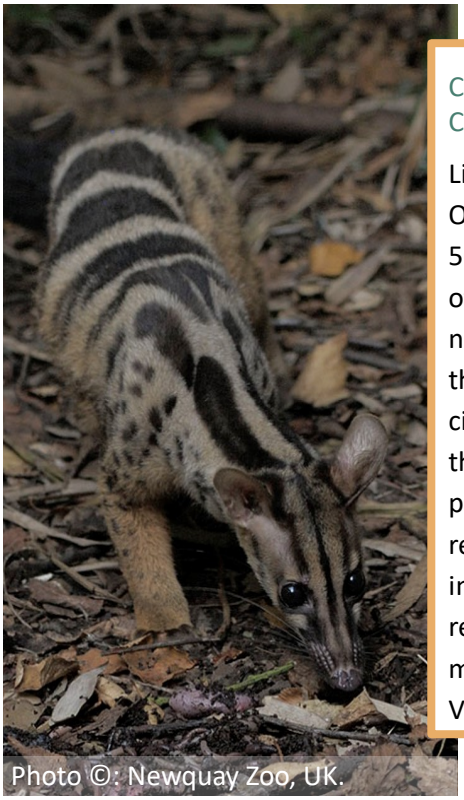

Photo ©: Newquay Zoo, UK.

CHARACTERISING THE REPRODUCTIVE BIOLOGY OF CAPTIVE OWSTON'S CIVETS (*CHROTOGALE OWSTONI*)

Little is known about the life history or biology of the Owston's civet, which has faced *in-situ* declines of close to 50% within the last 15 years, as well as historic low rates of reproductive success *ex-situ*. Using a combination of non-invasive endocrine and behavioural data collection, the EAZA RMG, in partnership with the EAZA Owston's civet EEP, aims to characterize the reproductive biology of this species, using data collected to inform management practices by identifying individuals who are most likely to reproduce, and by monitoring the effects of management interventions such as pair transfers and diet changes on reproduction. The EAZA RMG aims to replicate these methods in the *in situ* captive population held at Save Vietnam's Wildlife.

The required data can generally be collected non-invasively, for example through behavioural observations or faecal hormone monitoring, ensuring that data can easily be collected repeatedly,

and that data quality is not compromised by collection protocols (Möstl and Palme, 2002; Touma and Palme, 2005). Ideally, animals are monitored routinely to ensure that baseline profiles are established, and that institutions can monitor how changes in management or breeding decisions influence the reproductive biology of their species. These data are also essential for more advanced research, for example for the development of Assisted Reproductive Technologies (ARTs) (Jewgenow et al., 2017).

The EAZA RMG supports EAZA breeding programme coordinators with the development of practical data collection protocols to be used across collections, with the aim of understanding the basic breeding biology of exotic wildlife. EAZA RMG working group members **Dr. Franz Schwarzenberger**, Veterinary University of Vienna, Austria, and **Dr. Sue Walker**, Chester Zoo, United Kingdom (UK), are experts in the use of wildlife endocrinology to assist conservation efforts of endangered wildlife, with extensive research efforts in species such as the Eastern black rhinoceros (*Diceros bicornis michaeli*), white rhinoceros (*Cerathotherium simum*), Asian elephants (*Elephas maximus*), and Przewalski's horses (*Equus ferus przewalskii*). The EAZA RMG also collaborates with the Leibniz Institute for Zoo and Wild Animal Research (IZW) Endocrinology Laboratory, Germany who specialise in felid reproductive biology.

LIMITING REPRODUCTION

As animals living *ex situ* are generally well-provisioned and have access to veterinary care, some species have been found to have higher rates of reproductive success as a result of increased fecundity, litter size, infant survival, as well as shorter inter-birth intervals when compared to their *in situ* counterparts (Anderson and Simpson, 1979; Fairbanks and McGuire, 1984; Garcia et al., 2006; Schwitzer and Kaumanns, 2009). Increased reproductive success however, can be challenging for animal managers as space and resources may be limited (Asa, 1993), and may bear additional health costs to the individual due to the physical and energetic requirements of reproduction (Comizzoli and Holt, 2019; Edes et al., 2018; Harvey et al., 1987).

In zoos, limiting the reproductive potential of animals primarily involves the use of reversible contraception, surgical sterilization, or separation of the sexes, although in some cases alternative

methods such as breeding and culling are used. In any scenario, however, the impacts of the chosen techniques need to be carefully considered, and long-term effects must be holistically evaluated. For example, while separating individuals who should not breed may offer a 'simple' solution to limiting reproduction, isolating individuals may present a welfare concern, especially if they belong to a social species, and space constraints further limit the ease of separation. Moreover, prolonged non-reproductive periods are associated with the development of reproductive pathologies and declining fertility in a variety of species including red wolves (*Canis rufus*), African wild dogs, stingrays, Asian elephants (*Elephas maximus*), Seba's bats (*Carollia perspicillata*), wildebeest (*Connochaetes* sp.), and white rhinos (*Ceratotherium simum simium* and *C. s. cottoni*) (Asa et al., 2014; Hermes et al., 2006; Penfold et al., 2014), limiting the future reproductive potential of potentially genetically valuable individuals.

CONTRACEPTION

Hormonal contraception is increasingly being used in zoos to manage reproduction as it provides a theoretically reversible alternative to surgical sterilization procedures; useful for animals that may be required to breed in the future (Asa and Porton, 2005). However, contraception is not a 'one size fits all' solution as finding the right product and dose combination for species with varying physiologies can prove challenging, particularly as most products have been designed for use in domestic species or humans, rather than exotic wildlife (Asa and Porton, 2005). As such, the effects of contraceptives on exotic species are understudied, particularly with regards to fertility following long-term use (Fagerstone, 2002).

Wildlife contraception is still an emerging field, and knowledge on contraceptive use is extensive, but patchy in distribution within the global zoo community. In an effort to centralise contraceptive information, the AZA RMC and the EAZA RMG share a database (the "Contraception Database") detailing the efficacy of different contraceptive methods. The Contraception Database now contains over 45,000 contraceptive records, and is used to produce evidence-based contraceptive guidelines for a variety of species, with the aim of ensuring the safe and informed use of contraception in exotic wildlife. The database is used to identify gaps in current knowledge on the use and efficacy of various contraceptive methods, encouraging and focusing research in key areas of need. Notably, the Contraception Database has contributed to research by the **AZA RMC** that examined factors that are associated with the development of reproductive pathologies in wild canids (Asa et al., 2014), and as such progestin-based contraceptives are no longer recommended for long-term use in most carnivores.

Photo ©: Chester Zoo, UK.

UNDERSTANDING THE IMPACTS OF CONTRACEPTIVE USE IN FEMALE TIGERS, WITH A FOCUS ON AMUR (*PANTHERA TIGRIS TIGRIS*) AND SUMATRAN TIGERS (*P.T.SUMATRAE*)

The historic use of progestin-based hormonal contraception in captive exotic felids has been associated with the development of uterine pathologies. In more recent years, it has been recommended that GnRH agonists such as deslorelin acetate implants (Suprelorin®) are used, preceded by oral megestrol acetate to suppress the initial stimulation period. However, studies in domestic cats, African lions, and tigers demonstrate that deslorelin is long lasting and may take 30 months or more to reverse in some animals. As reproductive rates in the Amur and Sumatran tiger EEP have decreased in both male and female tigers, the EAZA RMG, in collaboration with the EAZA Amur and Sumatran tiger EEP, seek to understand the historical and current use of contraceptives in tigers. We hope that this information will help guide management decisions and promote sustainability of captive tiger populations. This project will initially use historic data from the joint AZA RMC and EAZA RMG Contraception Database as well as additional surveys to EAZA holders.

In addition to research involving the Contraception Database, several working group members and advisors are researching the use of contraception in sanctuary and *in situ* populations. **Dr. Henk Bertschinger**, University of Pretoria, South Africa is researching the effects of contraception in *in-situ* African mammals, and **Dr. Imke Lueders,** GEOlifes, Germany, is researching the impacts of Improvac use in *in-* and *ex-situ* elephants. Evidence provided by this research is used to inform and refine contraceptive protocols that are used within the EAZA zoo community. Moreover, several EAZA RMG advisors are involved in the development of contraceptive products, and the use of contraceptive products in novel contexts: **Kim Frank**, Science and Conservation Center Zoo

Montana, United States of America (USA) is leading on research on the use of the Porcine Zona Pellucida vaccine in captive and free ranging wildlife, and **Dr. Giovanna Massei**, Animal and Plant Health Agency, UK, is investigating the use of GonaCon in free living wildlife populations.

ENHANCING REPRODUCTION

A general assumption is that animals that receive breeding recommendations are fertile; however, trends towards decreasing reproductive success have been reported in several species *ex-situ* (Clubb et al., 2009; Farquharson et al., 2018; Lockyear et al., 2009). This may stem from a multitude of factors including a lack of basic knowledge of species reproduction, inappropriate husbandry and social housing, stress, inbreeding, or a genetic adaptation to captivity (Comizzoli and Holt, 2019; Farquharson et al., 2017; Holt et al., 2014; Levallois and Desvaux de Marigny, 2015; Nuss and Warneke, 2010; Wildt et al., 2010). As inappropriate management can contribute to poor welfare (Chang et al., 1999; Clarke et al., 1982; Ross et al., 2011; WAZA, 2015), reproductive success (Comizzoli and Holt, 2019; Farquharson et al., 2017; Holt et al., 2014; Wildt, 1989; Wildt et al., 2010), and health (Plowman, 2014; Videan et al., 2009), it is imperative that evidence-based practices and breeding recommendations are implemented within the zoological community to ensure the effective conservation of endangered species (Clay et al., 2011; Melfi, 2009).

The EAZA RMG promotes a holistic approach when investigating factors that may contribute to decreased reproductive success. We assess reproductive failure at both the population level as well as at an individual level, using practical methods that can be used across institutions. Working closely with EEP coordinators, we help identify trends between management and reproduction with the aim of fine-tuning best practice in captive animal care.

REPRODUCTIVE PATHOLOGIES IN KOMODO DRAGONS (*VARANUS KOMODOENSIS*)

Little is known about the reproduction of captive Komodo dragons and, in spite of the advances in the husbandry of the species there remains a significantly high rate of mortality among reproductive females. Many of these deaths have been attributable to reproductive pathologies, which have not been reported in wild individuals. In collaboration with the EAZA Komodo dragon EEP, the EAZA RMG is trying to identify whether there are any associations between captive husbandry techniques and female reproductive pathologies.

Photo ©: Chester Zoo, UK.

REPRODUCTIVE VIABILITY ANALYSIS

Reproductive Viability Analysis (RVA), developed by the **AZA RMC**, identifies how biological and reproductive factors correlate with reproductive success (Bauman et al., 2019). Using historic breeding recommendations, studbook data, data from the Contraception Database, and additional information provided by programme coordinators, RVA provides objective insight into variables that influence reproductive performance in their population and provides evidence for breeding recommendations. These analyses have so far been carried out on fennec foxes (*Vulpes zerda*) and Mexican wolves (*Canis lupus baileyi*) by the AZA RMC and the EAZA RMG has plans to trial these analyses with EAZA EEP programmes in 2020. For programme coordinators implementing RVA, it will also be essential to evaluate how changes to breeding recommendations resulting from these analyses influence reproductive success.

MATE CHOICE

Breeding recommendations *ex-situ* are primarily carried out in order to achieve the genetic and demographic goals of the population (Chargé et al., 2014). As such, opportunities for mate choice are limited *ex situ*, which may alter natural breeding strategies and ultimately reproductive success (Asa et al., 2011; Junge et al., 2009; Martin-Wintle et al., 2018; Wedekind, 2002). As mate choice

can significantly affect mating success, offspring production, and fitness (Martin and Shepherdson, 2012; Wedekind, 2002), understanding the ecological drivers for mate choice in species managed *ex-situ* may have significant positive effects on reproductive success among recommended pairs (Martin and Shepherdson, 2012). Currently however, there are few successful protocols for evaluating and providing mate choice in *ex situ* conservation breeding programmes (Martin-Wintle et al., 2018). The EAZA RMG aims to support programme coordinators with identifying the role of mate choice in their species and in integrating mate choice in their breeding recommendations, where appropriate.

USE IT OR LOSE IT?

For certain species, fertility must be established by a breeding event upon reaching sexual maturity (Yordy and Mossotti, 2016), and then must be maintained by regular pregnancies throughout the individual's lifetime. For females specifically, extended periods of non-breeding have been associated with increased risk of infertility in certain species, and pregnancies can also carry a protective function for uterine health, decreasing the risk of developing reproductive pathologies (Penfold et al., 2014). Termed 'use it or lose it', this phenomenon has been identified in various species (Penfold et al., 2014). Determining which species are susceptible to 'use it or lose it' and how husbandry and management contribute to a loss of reproductive potential requires the evaluation of breeding biology, historic studbook records, and management techniques. The EAZA RMG aims to support programme coordinators with identifying how breeding history and breeding recommendations influence fertility.

Colleagues at the **AZA RMC**, in partnership with the AZA PMC, are developing statistical models to assess the genetic and demographic impacts of different breeding recommendations. Termed Lifetime Reproductive Planning (LRP), these models will enable programme coordinators to map an individual's reproductive life, ensuring that fertility is established and maintained in individuals who should be contributing to the population, while ensuring that the demographic and genetic goals of the population are maintained, and that the production of surplus offspring is limited.

ASSISTED REPRODUCTIVE TECHNOLOGIES

Assisted reproductive technologies (ARTs) encompass a suite of techniques used by humans to enhance reproduction in animals (Jewgenow et al., 2017). These technologies include, and are not limited to, gamete recovery and cryopreservation, artificial insemination, embryo transfer, and semen sexing (Jewgenow et al., 2017). By using these techniques, deceased, post-reproductive, or individuals incapable of breeding are able to contribute to the population and females can be fertilised without the transfer of males (Jewgenow et al., 2017).

Methods underpinning ARTs have predominantly been developed for use in domestic species, such as dogs or cats, therefore extensive research is required to refine methods for more exotic species. EAZA RMG working group members **Dr. Katarina Jewgenow** and **Dr. Robert Hermes,** Leibniz IZW, Germany, and **Dr. Imke Lueders,** GEOlifes, Germany, are global leaders in developing ARTs for use in exotic wildlife who have carried out pioneering work on ARTs in species including the Iberian lynx (*Lynx pardinus*), Persian leopards (*Panthera pardus tulliana*), African elephants (*Loxodonta africana*), and Northern white rhino (*Ceratotherium simum cottoni*).

Leibniz-Institut für Zoo- und Wildtierforschung
IM FORSCHUNGSVERBUND BERLIN E.V.

GEOlifes
Animal Fertility and Reproductive Research

Furthermore, together with the **EAZA Population Management Centre (PMC)** and the **EAZA Biobank**, the EAZA RMG aims to support EAZA breeding programme coordinators with guidance for establishing ARTs in their species.

EAZA

EAZA BIOBANK

ACCESSIBLE AND PRACTICAL EVALUATIONS

The EAZA RMG aims to promote a holistic approach to reproductive management. To ensure the welfare of our animals and to remain at the forefront of best husbandry practices, a proactive approach evaluating the behavioural, social, physiological, health, and pathological effects of breeding decisions is integral. We work with programme coordinators to develop projects with

multiple levels of involvement, depending on the capacity of the involved institutions. Moreover, we aim to use data that are already available, for example studbook data, for preliminary retrospective analyses that may identify initial trends, as well as the knowledge acquired by programme coordinators and animal care staff. We also provide bespoke support to EAZA breeding programme coordinators with identifying the cause of reproductive failure using individual- and pair-based assessments using the EAZA RMG Reproductive Checklist (Appendix 2) to provide a basis for further evaluations.

The EAZA RMG benefits from having several veterinarians as members and advisors to the working group including **Dr. Tai Strike**, Zoological Society of London, UK, **Dr. Yedra Feltrer-Rambaud**, EAZA RMG, UK, **Dr. Hester van Bolhuis**, Stichting AAP, the Netherlands, **Dr. Tobias Knauf-Witzens**, Wilhelma The Zoological and Botanical Gardens, Stuttgart, Germany, **Dr. Cyriel Ververs**, Sharjah Equine Hospital, United Arab Emirates, and **Dr. Johanna Painer**, Veterinary University of Vienna, Austria who support the EAZA RMG with their extensive knowledge on wildlife contraception and animal reproduction and health. Moreover, the EAZA RMG are supported by two veterinary pathologists, **Dr. Maja Rütten**, PathoVet, Switzerland, and **Dr. Mark Stidworthy**, International Zoo Veterinary Group, UK. We also have links to the EAZA Welfare Working Group through **Dr. Holly Farmer**, Paignton Zoo, UK, and to EAZA (including the EAZA Population Management Centre and the EAZA Biobank) through the EAZA Reproductive Biology Coordinator, **Dr. Veronica Cowl**, Chester Zoo, UK, and EAZA, the Netherlands.

BALANCING POPULATION MANAGEMENT WITH REPRODUCTIVE MANAGEMENT

The effects on fertility of non-breeding and repeated breeding need to be carefully balanced with the genetic and demographic requirements for sustainability. The EAZA RMG collaborates with the **EAZA PMC** to support programme coordinators with future planning for their populations, while taking into account the contraceptive options available to them. Furthermore, EAZA RMG working group members and advisors **Dr. Holly Farmer**, Paignton Zoo, UK, **Dr. Maria-Teresa Abello**, Barcelona Zoo, Spain, and **Sarah Forsyth**, Colchester Zoo, UK bring experience in population management to the RMG through their experience managing EEPs and through their work in EAZA Taxon Advisory Groups (TAGs).

The extensive knowledge within the EAZA community, provides us with a unique opportunity for collaboration across institutions and regions for the continued improvement of best practice in the

management of exotic species. Tools such as the Zoological Information Management System (ZIMS) and the Contraception Database allow for the systematic collection of vast amounts of data across zoological collections, enabling the analysis of trends across populations and the continued advancement of best practice. However, it is essential that data entry is standardized across these platforms to ensure that data are comparable across institutions. The EAZA RMG aims to improve the standardization of data entry relating to reproduction through workshops targeted towards key stakeholders, for example, the BIAZA and EAZA Records Working Groups, and delegates at the EAZA Annual Conferences.

REFERENCES

Anderson, D.M., Simpson, M.J., 1979. Breeding performance of a captive colony of rhesus macaques (Macaca mulatta). Lab. Anim. 13, 275–281.

Antor, R.J., Antoni, M., Hans, F., Heredia, R., Lorente, L., Sese, J.A., 2007. First breeding age in captive and wild Bearded Vultures Gypaetus barbatus 114–118. https://doi.org/10.3161/000164507781646979

Asa, C.S., 1993. The Development of Contraceptive Methods for Captive Wildlife. USDA Natl. Wildl. Res. Cent. Symp. Contracept. Wildl. Manag. 235–240.

Asa, C.S., Bauman, K.., Devery, S., Zordan, M., Camilo, G.R., Boutelle, S., Moresco, A., 2014. Factors Associated with Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility. Zoo Biol. 33, 8–19.

Asa, C.S., Porton, I.J., 2005. Wildlife Contraception: Issues, Methods, and Applications. The Johns Hopkins University Press, Baltimore, Maryland.

Asa, C.S., Traylor-Holzer, K., Lacy, R.C., 2011. Can conservation-breeding programmes be improved by incorporating mate choice? Int. Zoo Yearb. 45, 203–212. https://doi.org/10.1111/j.1748-1090.2010.00123.x

Bauman, K., Sahrmann, J., Franklin, A., Asa, C., Agnew, M., Traylor-Holzer, K., Powell, D., 2019. Reproductive Viability Analysis (RVA) as a new tool for ex situ population management. Zoo Biol. 38, 55–66. https://doi.org/10.1002/zoo.21477

Chang, T.R., Forthman, D.L., Maple, T.L., 1999. Comparison of confined mandrill (Mandrillus sphinx) behavior in traditional and ?ecologically representative? exhibits. Zoo Biol. 18, 163–176. https://doi.org/10.1002/(SICI)1098-2361(1999)18:3<163::AID-ZOO1>3.0.CO;2-T

Chargé, R., Teplitsky, C., Sorci, G., Low, M., 2014. Can sexual selection theory inform genetic management of captive populations? A review. Evol. Appl. 7, 1120–1133. https://doi.org/10.1111/eva.12229

Clarke, A.S., Juno, C.J., Maple, T.L., 1982. Behavioral effects of a change in the physical environment: A pilot study of captive chimpanzees. Zoo Biol. 1, 371–380. https://doi.org/10.1002/zoo.1430010411

Clay, A.W., Perdue, B.M., Gaalema, D.E., Dolins, F.L., Bloomsmith, M.A., 2011. The use of technology to enhance zoological parks. Zoo Biol. 30, 487–497. https://doi.org/10.1002/zoo.20353

Clubb, R., Rowcliffe, M., Lee, P., Moss, C., Mason, G.J., 2009. Fecundity and population viability in female zoo elephants: problems and possible solutions.

Comizzoli, P., Holt, W.V., 2019. Breakthroughs and new horizons in reproductive biology of rare and endangered animal species. Biol. Reprod. https://doi.org/10.1093/biolre/ioz031

Conde, D.A., Flesness, N., Colchero, F., Jones, O.R., Scheuerlein, A., 2011. An emerging role of zoos to conserve biodiversity. Science (80-.). 331, 1390–1391. https://doi.org/10.1002/zoo.20369/abstract

EAZA, 2015. EAZA Population Management Manual.

EAZA, 2013. Conservation, Education & Research, in: 2 (Ed.), The Modern Zoo: Foundations for Management and Development. EAZA Executive Office, Amsterdam, pp. 77–88.

Edes, A.N., Wolfe, B.A., Crews, D.E., 2018. The first multi-zoo application of an allostatic load index to western lowland gorillas (Gorilla gorilla gorilla). Gen. Comp. Endocrinol. 266, 135–149.

https://doi.org/10.1016/J.YGCEN.2018.05.006

Fa, J.E., Funk, S.M., Connell, D.O., 2011. Zoo Conservation Biology 250–272. https://doi.org/10.1017/CBO9780511993435

Fagerstone, K.A., 2002. Wildlife Fertility Control.

Fairbanks, L.A., McGuire, M.T., 1984. Determinants of fecundity and reproductive success in captive vervet monkeys. Am. J. Primatol. 7, 27–38. https://doi.org/10.1002/ajp.1350070106

Farquharson, K.A., Hogg, C.J., Grueber, C.E., 2018. A meta-analysis of birth-origin effects on reproduction in diverse captive environments. Nat. Commun. 9, 1055. https://doi.org/10.1038/s41467-018-03500-9

Farquharson, K.A., Hogg, C.J., Grueber, C.E., 2017. Pedigree analysis reveals a generational decline in reproductive success of captive Tasmanian devil (Sarcophilus harrisii): implications for captive management of threatened species. J. Hered. 108, 488–495. https://doi.org/doi:10.1093/jhered/esx030

Faust, L.J., Long, S.T., Peri, K., Simonis, J.L., 2019. Uncovering challenges to sustainability of AZA Animal Programs by evaluating the outcomes of breeding and transfer recommendations with PMCTrack 1–12. https://doi.org/10.1002/zoo.21470

Garcia, C., Lee, P.C., Rosetta, L., 2006. Dominance and reproductive rates in captive female olive baboons, Papio anubis. Am. J. Phys. Anthropol. 131, 64–72. https://doi.org/10.1002/ajpa.20405

Harvey, P.H., Martin, R.D., Clutton-Brock, T.H., 1987. Life histories in comparative perspective, in: Smuts, B.B., Cheney, D.L., Seyfarth, R.M., Wrangham, R.W., Struthsaker, T.T. (Eds.), Primate Societies. University of California Press, Chicago, pp. 181–196.

Hermes, R., Hildebrandt, T.B., Walzer, C., Göritz, F., Patton, M.L., Silinski, S., Anderson, M.J., Reid, C.E., Wibbelt, G., Tomasova, K., Schwarzenberger, F., 2006. The effect of long non-reproductive periods on the genital health in captive female white rhinoceroses (Ceratotherium simum simum, C.s. cottoni). Theriogenology 65, 1492–1515. https://doi.org/10.1016/j.theriogenology.2005.09.002

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T.M., … Stuart, S.N., 2010. The impact of conservation on the status of the world's vertebrates. Science (80-.). 330, 1503–9. https://doi.org/10.1126/science.1194442

Holt, W., Brown, J., Comizzoli, P., 2014. Reproductive Sciences in Animal Conservation: Progress and Prospects, Advances in Experimental Medicine and Biology. https://doi.org/10.1007/978-1-4939-0820-2

Jewgenow, K., Braun, B.C., Dehnhard, M., Zahmel, J., Goeritz, F., 2017. Research on reproduction is essential for captive breeding of endangered carnivore species. Reprod. Domest. Anim. 52, 18–23. https://doi.org/10.1111/rda.12836

Junge, R.E., Williams, C.V., Campbell, J., 2009. Nutrition and behavior of lemurs. Vet. Clin. North Am. Exot. Anim. Pract. 12, 339–348.

Levallois, L., Desvaux de Marigny, S., 2015. Reproductive success of wild-caught and captive-bred cynomolgus macaques at a breeding facility. Lab Anim. (NY). 44, 387–393. https://doi.org/10.1038/laban.733

Lockyear, K.M., Waddell, W.T., Goodrowe, K.L., MacDonald, S.E., 2009. Retrospective investigation of captive red wolf reproductive success in relation to age and inbreeding. Zoo Biol. 28, 214–229. https://doi.org/10.1002/zoo.20224

Martin-Wintle, M.S., Wintle, N.J.P., Díez-León, M., Swaisgood, R.R., Asa, C.S., 2018. Improving the

sustainability of ex situ populations with mate choice. Zoo Biol. 1–14. https://doi.org/10.1002/zoo.21450

Martin, M.S., Shepherdson, D.J., 2012. Role of Familiarity and Preference in Reproductive Success in Ex Situ Breeding Programs. Conserv. Biol. 26, 649–656. https://doi.org/10.1111/j.1523-1739.2012.01880.x

Melfi, V., 2009. There Are Big Gaps in Our Knowledge and Thus Approach, to Zoo Animal Welfare: A Case for Evidence-Based Zoo Animal Management. Zoo Biol. 28, 574–588. https://doi.org/https://doi.org/10.1002/zoo.20288

Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67–74. https://doi.org/10.1016/S0739-7240(02)00146-7

Napier, J.E., Caron, S., Reavill, D.R., Murphy, H., Garner, M.M., 2009. Proliferative Endometrial Lesions in a Group of Seba's Short-Tailed Bats (Carollia perspicillata). J. Zoo Wildl. Med. https://doi.org/10.2307/27751717

Nuss, K., Warneke, M., 2010. Life span, reproductive output, and reproductive opportunity in captive Goeldi's monkeys (Callimico goeldii). Zoo Biol. 29, 1–15. https://doi.org/10.1002/zoo.20239

Penfold, L.M., Powell, D., Traylor-Holzer, K., Asa, C.S., 2014. "Use it or lose it": Characterization, implications, and mitigation of female infertility in captive wildlife. Zoo Biol. 33, 20–28. https://doi.org/10.1002/zoo.21104

Plowman, A., 2014. Diet review and change for monkeys at Paignton Zoo Environmental Park. Diet review and change for monkeys at Paignton Zoo Environmental Park, Journal of Zoo and Aquarium Research.

Ross, S.R., Calcutt, S., Schapiro, S.J., Hau, J., 2011. Space use selectivity by chimpanzees and gorillas in an indoor-outdoor enclosure. Am. J. Primatol. 73, 197–208. https://doi.org/10.1002/ajp.20891

Schwitzer, C., Kaumanns, W., 2009. Litter size, infant mortality and female body weight in captive black-and-white ruffed lemurs Varecia variegata. Endanger. Species Res. 8, 201–2019. https://doi.org/10.3354/esr00210

Touma, C., Palme, R., 2005. Measuring Fecal Glucocorticoid Metabolites in Mammals and Birds: The Importance of Validation. Ann. N. Y. Acad. Sci. 1046, 54–74. https://doi.org/doi.org/10.1196/annals.1343.006

van Lint, W., de Man, D., 2016. Anatomy of the EEPs. ZooQuaria 14–15.

Videan, E.N., Heward, C.B., Chowdhury, K., Plummer, J., Su, Y., Cutler, R., 2009. Comparison of Biomarkers of Oxidative Stress and Cardiovascular Disease in Humans and Chimpanzees (Pan troglodytes). Am. Assoc. Lab. Sci. 59, 287–296.

WAZA, 2015. Caring for Wildlife: The World Association of Zoo and Aquarium Animal Welfare Strategy. WAZA Executive Office, Gland.

WAZA, 2013. Towards Integrated Species Conservation, Towards Integrated Species Conservation. WAZA, Gland.

Wedekind, C., 2002. Sexual Selection and Life-History Decisions: Implications for Supportive Breeding and the Management of Captive Populations. Conserv. Biol. 16, 1204–1211. https://doi.org/10.1046/j.1523-1739.2002.01217.x

Wildt, D.E., 1989. Strategies for the Practical Application of Reproductive Technologies to Endangered Species, Zoo Biology Supplement.

Wildt, D.E., Comizzoli, P., Pukazhenthi, B., Songsasen, N., 2010. Lessons from biodiversity-the value

of nontraditional species to advance reproductive science, conservation, and human health. Mol. Reprod. Dev. 77, 397–409. https://doi.org/10.1002/mrd.21137

Yordy, J., Mossotti, R.H., 2016. Kinship, maternal effects, and management: Juvenile mortality and survival in captive African painted dogs, Lycaon pictus. Zoo Biol. 35, 367–377. https://doi.org/10.1002/zoo.21306

APPENDIX 1: INDIVIDUALS AND INSTITUTIONS SUPPORTING THE EAZA RMG

EAZA RMG WORKING GROUP MEMBERS

Chair: Sue Walker [1]

Vice-chair: Yedra Feltrer [2]

EAZA Executive Office Liaison: Veronica Cowl [1,3]

Programme Coordinator: Veronica Cowl [1,3]

Henk Bertschinger [4]

Hester van Bolhuis [5]

Sarah Forsyth [6]

Tobias Knauf-Witzens [7]

Katarina Jewgenow [8]

Tai Strike [9]

ADVISORS TO THE WORKING GROUP

Maria Teresa Abello [10]

Mary Agnew [11]

Cheryl Asa [11]

Linda Douglas [1]

Holly Farmer [12]

Dominik Fischer[13]

Kimberley Frank [14]

Gidona Goodman [15]

Robert Hermes [8]

Imke Lüders [16]

Giovanna Massei [17]

Tullis Matson[18]

Johanna Painer [19]

Kristen Pullen [12]

Maja Rütten [20]

Franz Schwarzenberger [19]

Mark Stidworthy [21]

Cyriel Ververs [22]

INSTITUTIONS SUPPORTING THE EAZA RMG

[1] Chester Zoo
[2] EAZA RMG
[3] EAZA
[4] University of Pretoria
[5] AAP Rescue Centre for Exotic Animals
[6] Colchester Zoo
[7] Wilhelma The Zoological and Botanical Gardens, Stuttgart
[8] IZW
[9] Zoological Society of London
[10] Barcelona Zoo
[11] AZA RMC, Saint Louis Zoo
[12] Paignton Zoo
[13] University of Gießen
[14] Science and Conservation Centre, Zoo Montana
[15] University of Edinburgh
[16] GEOlifes
[17] AHVLA
[18] Stallion AI
[19] University of Veterinary Medicine, Vienna
[20] PathoVet
[21] International Zoo Veterinary Group
[22] Sharjah Equine Hospital

APPENDIX 2: REPRODUCTIVE CHECKLIST FOR IDENTIFYING THE POINT OF REPRODUCTIVE FAILURE

Reproductive Checklist for Diagnosing the Point of Reproductive Failure

EAZA RMG
EAZA Reproductive Management Group

General

1) Natural history

- ❑ Is this a seasonal species?
- ❑ What is the normal age of puberty for this species?
- ❑ What is the normal age of sexual maturity for the species?
- ❑ What is the age of senescence for the species?
- ❑ What is the species' mating system/reproductive strategy?

2) Individual history

- ❑ Age
- ❑ Rearing
- ❑ Transfers
- ❑ Experience of parental care?
- ❑ What is the individual's demeanour?
- ❑ What is the general health of the individual?
  - ❑ Do they have any chronic conditions?
  - ❑ Have they received any hormone treatments?
  - ❑ Do they have any skeletal abnormalities?
  - ❑ Has an infectious disease screening been carried out?
  - ❑ What is their weight and body condition score?
- ❑ Imprinting/species appropriate behaviours?
- ❑ Is there a history of aggression towards conspecifics or mates?

3) Individual reproductive history

- ❑ Has a reproductive health workup been done recently?
  - ❑ What were the findings?
  - ❑ What have historic workups found?
  - ❑ Is there a history of reproductive disease or pathology?
  - ❑ Is the genital/reproductive morphology sound?
- ❑ Has this animal received any contraceptive treatments?

4) Social considerations

- ❑ Does the social structure resemble natural history?
  - ❑ Solitary, paired, grouped, harem, multi-male, multi-female?
- ❑ Which conspecifics are housed with the individual?
  - ❑ If relevant, where is the individual in the hierarchy?
- ❑ Have new individuals been introduced recently?
- ❑ When have the individuals been allowed mate access?
- ❑ Is your pair compatible?

5) Husbandry

- ❑ Is their diet species specific and nutritionally sound?
  - ❑ Are they fed on a natural feeding cycle e.g. carcass fed?
  - ❑ Are they fed supplements? Concentrates?
- ❑ Are they given any enrichment?
- ❑ What are potential stressors?
  - ❑ Presence of predators?
  - ❑ Social stress?
  - ❑ Stress from the public?
- ❑ Are they in a single or mixed species exhibit?
- ❑ Do they have access to an indoor/outdoor enclosure?
- ❑ Is there UVB provision?
- ❑ Is a light cycle being simulated?
- ❑ Do they have access to a nest box?
- ❑ Are hiding/escape areas provided?
- ❑ What substrate do they have?
- ❑ What heat provision is available?
- ❑ Does the enclosure have the appropriate humidity conditions?
- ❑ Are the right environmental cues to induce mating/courtship provided?

Reproductive Checklist for Diagnosing the Point of Reproductive Failure

EAZA RMG
EAZA Reproductive Management Group

Female

1) Natural history

- ❑ What are the signs of proestrus and oestrus in this species?
- ❑ Do we know what a normal cycle looks like in this species?
  - ❑ Are induced or spontaneous ovulators? Both?
  - ❑ What is the normal inter-oestrus interval?
- ❑ What are the signs of receptivity in this species?

2) Individual reproductive history

- ❑ Has endocrine monitoring been carried out?
  - ❑ What were the results?
- ❑ Does the female have a record of previous conception?
  - ❑ What was the age at first reproduction?
  - ❑ Does she have a history of abortions?
- ❑ Does the female have a record of previous parturition?
  - ❑ Does she have a history of dystocia?
  - ❑ Does she have a history of stillbirths?
  - ❑ What are historic litter sizes?
  - ❑ Is there a record of neonatal death?
  - ❑ Are there any incidences of congenital abnormalities?
  - ❑ Does she have a history of infanticide?
- ❑ Has the female ever had pseudopregnancies?
- ❑ What is the length of her inter-birth interval?
- ❑ Has she ever suffered from mastitis?
- ❑ Does she have a history of successful rearing?

3) Current reproduction

- ❑ Are the appropriate courtship and mating behaviours observed?
- ❑ Describe breeding events
  - ❑ Is there evidence of intromission?
- ❑ Has the male previously bred with a different female?

Male

1) Natural history

- ❑ Do we know what average testosterone concentrations are in breeding males of this species?
- ❑ Do we know what normal sperm morphology looks like in this species?

2) Individual reproductive history

- ❑ Has endocrine monitoring been carried out?
  - ❑ What were the results?
- ❑ Sperm quality?
- ❑ Does the male have a record of previously siring offspring?
  - ❑ What was the age at first reproduction?
  - ❑ What were the fate of the offspring?
  - ❑ Are there any incidences of congenital abnormalities?
  - ❑ Does he have a history of infanticide?
- ❑ Does he have a history of successful rearing?

3) Current reproduction

- ❑ Are the appropriate courtship and mating behaviours observed?
- ❑ Describe breeding events
  - ❑ Is there evidence of intromission?
- ❑ Has the female previously bred with a different male?

START → What is the problem? → What diagnostics can be used? → What treatments can be used? → What are the action items? → Who are the point people?

REFERENCES

Christensen, B. (2019) Female Infertility in Zoo Animals in *Fowler's Zoo and Wild Animal Medicine*: *Current Therapy*. 9th Ed. St. Louis:Elsevier, pp124-129.

APPENDIX 3: RESEARCH LOG

The following list details current and historic EAZA RMG endorsed research projects. The list does not include projects that are solely carried out by our working group members and advisors.

Current projects:

- Investigating the efficacy of Regumate when milled into feed as a contraceptive in giraffes.
- Investigating the efficacy of contraception in captive Rodrigues fruit bats (*Pteropus rodricensis*).
- Characterizing the reproductive biology of captive Owston's civets (*Chrotogale owstoni*).
- Evaluating the effects on reproductive health and welfare of long-term contraception in chimpanzees and other great apes.
- Characterizing the reproductive tract of chimpanzees.
- Investigating the use of contraception as a behavioural management tool in spider monkeys (*Ateles fusciceps*) (EEP project).
- Investigating factors contributing to reproductive failure in bush dogs (*Speothos venaticus*) (EEP project).
- A review of the use of GnRH vaccines in exotic wildlife: what do we know, and where are the gaps?
- Investigating the use of contraceptives for therapeutic management.

Historic projects:

- Assessing the efficacy of deslorelin implants when placed in alternative placement sites.
- A review of contraception used in EAZA ungulates, with a focus on bovids.
- A review of etonogestrel implants in Old World primates.
- Understanding the impacts of contraceptive use in European and North American female tigers using data from the Contraception Database.
- Identifying factors that contribute to the development of reproductive pathologies in captive Komodo dragons (*Varanus komodoensis*).

APPENDIX 4: RESEARCH WISHLIST

The following projects comprise a non-exhaustive list of research projects that the EAZA RMG has identified. We aim to begin research on these projects in the coming years.

Future projects:

- Monitoring and evaluating the behavioural, pathological, and endocrine effects of progestin-based contraceptives in Old World monkeys.
- Identifying the role of social factors in reproductive suppression of cooperatively breeding animals.
- Investigating causes of egg infertility in rockhopper penguins (*Eudyotes chrysocome*) (EEP project).
- Investigating causes of reproductive failure among some pairs of striped hyena (*Hyaena hyaena*) (EEP project).
- Investigating causes of reproductive failure among some pairs of cotton-top tamarins (*Saguinus oedipus*) (EEP project).
- Investigating the role of life history variables, housing, and husbandry on reproductive success in okapi (*Okapia johnstoni*) (EEP project).
- Using Reproductive Viability Analysis in EAZA populations.
- Understanding the welfare implications of breeding and non-breeding recommendations.
- Investigating how reproductive success varies between wild-caught and captive-born animals using data in the ZIMS database.
- Evaluating the role of contraception on social and learnt behaviours involved in reproduction i.e. maternal behaviours, with a focus on primates.

CONTACT:

EAZA RMG

The North of England Zoological Society, Chester Zoo

Caughall Road

Upton-by-Chester

CH2 1LH

contraception@chesterzoo.org

WEBSITE

www.egzac.org