Journal of
Zoological and Botanical Gardens

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Article

By Bits and Pieces: The Contributions of Zoos and Aquariums to Science and Society via Biomaterials

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Abstract: Scientific research has long been recognized as one of the four pillars of the zoo or aquarium mission, alongside recreation, conservation, and education. This study sought to quantify a sample of zoos' participation in scientific research via the provision of biomaterials from animals to outside scientists and the associated training of undergraduate and graduate students that resulted from these projects. A convenience sample of zoos provided data on their participation in biomaterials-related projects and a focused analysis of biomaterials-related research facilitated by the Saint Louis Zoo was conducted. In addition, the Association of Zoos & Aquariums' conservation & science database was queried to uncover what research projects AZA members engaged in over the last five years that likely involved biomaterials research and training of students. Results demonstrate that zoos are making significant contributions to science by agreeing to participate in large numbers of biomaterials-related projects across different areas of focus involving a variety of animal species annually, with results applicable to human and non-human animals. Support of such research often involves student thesis projects, thus contributing to the education of future scientists.

Keywords: education; genetics; health; microbiome; physiology; research; students

Citation: Powell, D.M.; Meyer, T.G.; Duncan, M. By Bits and Pieces: The Contributions of Zoos and Aquariums to Science and Society via Biomaterials. *J. Zool. Bot. Gard.* **2023**, *4*, 277–287. https://doi.org/10.3390/jzbg4010023

Academic Editor: Courtney Collins

Received: 30 January 2023
Revised: 7 March 2023
Accepted: 8 March 2023
Published: 13 March 2023

1. Introduction

Zoos and aquariums, hereafter zoos, have long been venues for scientific research. For example, the Zoological Society of London's original charter in 1826 had as its goal to assemble a collection of living wild animals for the purpose of scientific research [1]. Similarly, the original charter of the Wildlife Conservation Society, then known as the New York Zoological Society in 1895, included the study of zoology as one of its goals [2], alongside the creation of a zoological park and the preservation of wildlife. A research emphasis in zoos grew throughout the 20th century and by the 1990s many zoos included research in their mission statements and created on-site research programs [3]. Facilities accredited by the Association of Zoos & Aquariums (AZA) published 5,175 peer-reviewed papers in the period from 1993 to 2003 [4]. The contributions of Japanese [5], German [6], and zoos and aquaria that are members of the European Association of Zoos and Aquaria (EAZA) have also been quantified [7]. This history of zoo research worldwide has been reviewed previously [8,9]. The scope of this research spans the fields of anatomy, physiology, reproductive biology, veterinary medicine, nutrition, animal behavior, welfare, and genetics among others [10–13].

While many zoos have conducted in-house research through the activities of curators, veterinarians, nutritionists, and other in-house scientists, they have also contributed to research by the provision of biomaterials (e.g., hair, skin, blood, carcasses, gametes, tissue and saliva samples) from animals in the zoo's collection to outside researchers for studies in the disciplines mentioned above as well as for studies of epidemiology & human health, forensics, evolution, and ecology. These samples may be collected opportunistically (e.g.,

during physical exams), collected at necropsy, or may be obtained through positive reinforcement training. Banked samples stored frozen or with tissue preservation methods may also be available at individual facilities. Additionally, some regions are establishing regional biobanks of samples for use by researchers, for example the EAZA Biobank [14] or the Australian Frozen Zoo [15]. Zoo animal collections provide access to biomaterials that could be costly, invasive, or logistically difficult to obtain from wild animals. Certain kinds of biomaterials from zoo and aquarium animals may also be easier to obtain from a regulatory perspective. Many animals in zoos may be trained to provide biological samples (e.g., blood or urine [16], allowing collection of biomaterials while minimizing the effect on animal welfare.

The goal of this paper is to describe the contribution of zoos to society via the provision of biomaterials for scientific research and the associated benefit of supporting the training of graduate and undergraduate students. We do this by presenting data from a convenience sample of zoo participation in biomaterials-contingent research conducted by non-zoo personnel and the Saint Louis Zoo's history of providing biomaterials to outside researchers to support research. Records of external research project approvals were used to build this dataset rather than a review of the literature as zoo staff are not always co-authors on resulting manuscripts, acknowledgement sections of published papers are not readily searchable, and in some cases, zoos may not be individually identified in publications as having provided biomaterial samples. The AZA Conservation & Science database was also used to get a sense of biomaterials project participation across AZA, as well as the number of students obtaining advanced degrees based on research done at AZA facilities.

2. Materials and Methods

2.1. Convenience Sample of Zoos' Participation in Externally-Driven Biomaterials Research

In 2018, we contacted 42 individuals charged with receiving, reviewing, and administering biomaterials requests for a number of AZA-accredited zoos. We obtained a list of contacts from the chair of AZA's Biobanking Scientific Advisory Group. These individuals had identified themselves to the chair as fulfilling these roles at their facility. We explained that we were gathering data on the role of zoos and aquariums in supporting external research by providing biomaterials and asked if they would be willing to complete a form detailing their facility's participation in biomaterials research. Facilities were asked to provide data for as many years as they had reliable records. The form asked for the number of biomaterials projects approved for participation each year, the number of unique principal investigators (PIs) on projects approved that year, the number of different institutions with which the principal investigators were affiliated, the participating institution type (i.e., zoo/aquarium, university, non-government organization, government agency, museum), the number of approved biomaterials projects that had a zoo staff member as a principal investigator, the number of samples provided that year, the number of individual animals and different species sampled, the sample types, and whether the facility maintained a repository of stored biomaterials available to researchers. Descriptive statistics were calculated for these variables when data quality allowed statistical analyses were run in Sigmastat 4.0 (Inpixon, Inc., Palo Alto, CA, USA).

2.2. Saint Louis Zoo Participation in Biomaterials Research 2016–2022

As Director of Research at the Saint Louis Zoo, the first author receives and reviews all research proposals from internal and external principal investigators seeking to conduct research on the Zoo's animals, biomaterials produced by them, and/or associated animal records. Proposals are reviewed by a committee of zoo staff including scientists, curators, and veterinarians, and if the proposal requests biomaterials, it is further reviewed by the Zoo's biomaterials committee which includes the Zoo's pathologist (third author), registrar and representatives from each of the managers of animal care and veterinary technical staff.

For each proposal approved by the research and biomaterials committees, we extracted the following: number of principal investigators, number of institutions aside from the

Saint Louis Zoo that were involved in the project, a list of the types of institutions involved (i.e., zoo/aquarium, university, non-government organization, government agency, museum), species involved in the research, keyword descriptions of the project (e.g., genetics, physiology), number of individual animals to be sampled, sample type (e.g., hair, feces, blood, whole carcass), and whether the project was part of an undergraduate or graduate thesis. Descriptive statistics were calculated for variables when data quality allowed.

2.3. Saint Louis Zoo Participation in Biomaterials Research Prior to 2016

Full research proposals received prior to 2016 were not archived, but we were able to extract data from past, approved biomaterials forms for the period of 2009–2015. The data we were able to extract from these forms included: requesting facility type (e.g., zoo, university, museum, etc.), species for which biomaterials were requested, general area of research focus (e.g., physiology, genetics, health), number of individuals to be sampled, and sample type (e.g., feces, blood, hair). We calculated descriptive statistics for these parameters where data quality allowed.

2.4. AZA Conservation and Science Database Query

The AZA Conservation and Science database of research projects conducted by its member facilities is updated annually by its members. Reporting to the database is encouraged but was not required until 2021. In 2022, we asked the AZA staff to query the database looking for projects with the following non-exhaustive list of keywords: non-invasive hormone, gut microbiome, blood values/characteristics, and genetic. These keywords were chosen as these were the most common types of projects requesting biomaterials from the Saint Louis Zoo in recent years and because these types of projects almost certainly would require biomaterials. Projects containing any of these keywords were extracted and the project titles further reviewed. Any projects whose title reflected work on free-ranging animals only were eliminated from the calculations. The resulting descriptive statistics should be considered minimum estimates. The database captures the number of graduate (Ph.D. and Master's) degrees and professional degrees (D.V.M.) based on research done at the submitting facility, samples and/or data obtained from the facility or where staff serve as the primary mentor or advisor for a research-based advanced degree. These figures are reported for the last five years as they represent contributions of AZA-accredited zoos and aquariums to society via the education of students, even though it is probable that not all of these projects were based on biomaterials, and it was not possible to determine which degrees involved biomaterials research.

3. Results

3.1. Convenience Sample of Zoo's Participation in Externally-Driven Biomaterials Research

We obtained data from 10 AZA-accredited (24% response rate) zoos in eight states, mostly in the central-western United States. These zoos varied in size from 2–235 hectares. Zoos provided on average 4.6 years of data (range: 1–8 years). Zoos agreed to participate in an average of 7.6 ($\pm$0.85 SE) projects per year (range: 1–28). On average about half the projects approved by these zoos in a given year had an internal staff member or staff member from another zoo listed as a principal investigator (mean # of projects approved per year that involved a zoo-based PI: 3.2 $\pm$ 0.49, range: 0–11). Projects generally had large numbers of unique principal investigators (mean: 8.0 $\pm$ 1.04, range: 1–30) from multiple institutions (mean: 6.8 $\pm$ 0.91, range: 1–28). Institutions included other zoos, universities, research facilities, hospitals, sanctuaries, natural history museums, high schools and middle schools, local parks/preserves, non-government and government agencies. Universities and other zoos were the most common facility types requesting biomaterials. Across the ten zoos and the years of data they provided, the average approval rate of proposals was 88.5% (range: 33–100%).

The total number of samples provided by a zoo each year was not always available but was available for enough years (31 institution-years) to calculate robust descriptive

statistics. Numbers of samples provided per year were high (mean: 138.5 ± 36.46, range: 1–749). These samples were derived from 16.7 (±2.87) individuals on average (range: 1–98) from 6.7 (±0.70) species (range: 1–17). Feces (27.1%) or blood/serum (22.9%) were the most common sample types requested (Figure 1), but a variety of sample types were requested.

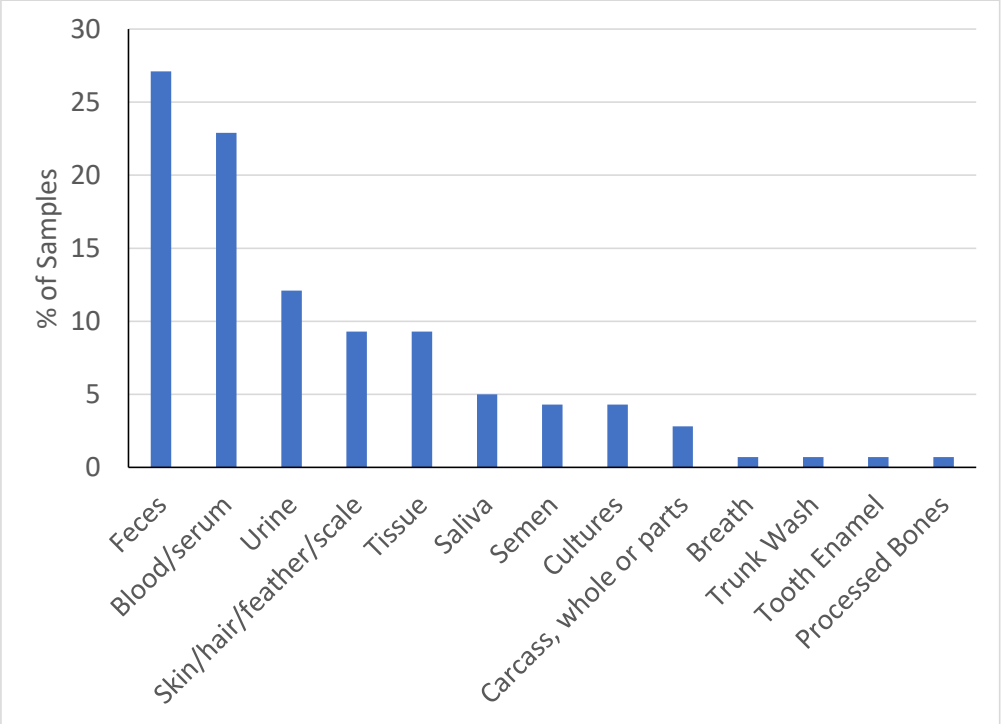

Figure 1. Percentages of different biomaterial types zoos agreed to provide to researchers in the convenience sample of zoos in this study.

A little more than half of responding facilities (56.5%) indicated that they had a repository of stored biomaterials that could be made available to researchers. Having a repository or not had no impact on the number of biomaterials proposals reviewed (t = 0.19, 6 d.f., $p = 0.86$) or approved (t = 0.47, 8 d.f., $p = 0.65$).

3.2. Saint Louis Zoo Participation in Biomaterials Research 2016–2022

In the period of 2016–2022, the Saint Louis Zoo agreed to participate in 45 biomaterials-related projects (mean 6.4 projects/year, range: 3–11 projects). The projects included an average of 2.6 different principal investigators or co-principal investigators (range: 1–9) who came from an average of 2.0 facilities (range: 1–10) external to the Saint Louis Zoo. Again, most proposals came from universities, followed by zoos, and then non-government and government agencies. A Saint Louis Zoo staff member was a principal or co-investigator on only 11.5% of the proposals, indicating that most projects were fully external to the Zoo. Of the 45 proposals approved, 37.8% were part of a graduate or undergraduate thesis project.

On average, proposals requested biomaterials from 2.1 species (range: 1–20). Primates and elephants were the most common focus of biomaterials requests. Requests were also approved for canids, felids, ursids, ailurids, macropods, rhinoceros, bovids, camelids, suids, crocodilians, snakes, chelonids, beetles, and five species or species groups (e.g., Psittacidae) of birds. A total of 65 species plus three species groups (e.g., "vipers", "macaws") were targets of biomaterials requests. We could not reliably extract the desired number of individuals to be sampled, as investigators commonly stated requests in the form of "any available from XX taxa" or something similar. As in the convenience sample of other zoos, fecal and blood/serum samples were the most commonly requested samples the Saint Louis Zoo agreed to provide (Figure 2).

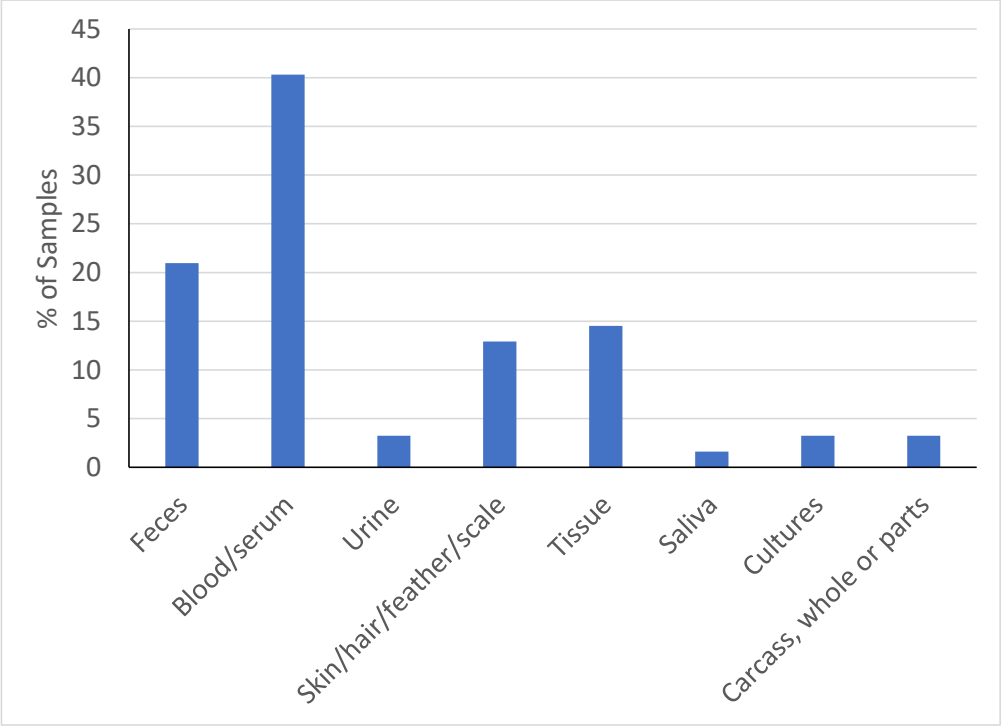

Figure 2. Percentages of different biomaterial types the Saint Louis Zoo agreed to provide to researchers in the period of 2016–2022.

Biomaterials research approved by the Zoo in this period predominately focused on genetics (40%), animal health (22.2%), microbiomes (8.9%), nutrition and reproduction (each 6.7%), physiology, forensics and anatomy (each 4.4%) and welfare (2.2%).

3.3. Saint Louis Zoo Participation in Biomaterials Research Prior to 2016

The Zoo agreed to participate in 40 biomaterials projects between 2008 and 2015. Again, zoos and universities were the vast majority of PI facilities involved in these collaborative projects with some representation from government and non-government agencies. Biomaterials were requested from most major vertebrate groups (mammals, birds, reptiles, amphibians, but no fish) and invertebrates. In total, biomaterials were requested from 59 species. Again, primates and elephants were the most commonly requested species. Requests were also approved for canids, felids, ailurids, ursids, macropods, rhinoceros, equids, snakes, turtles & tortoises, lizards, amphibians, beetles, and birds. As was true in the other datasets, fecal and blood/serum samples were the most commonly requested samples the Saint Louis Zoo agreed to provide (Figure 3).

Biomaterials research approved in this period mostly focused on genetics (34.1%), health (26.8%), and physiology (17.1%). Reproduction accounted for 4.9% of studies. Studies of anatomy, microbiomes, welfare, and forensics were also represented (each 2.4% of projects). Other areas of research focus in this period included ecology (4.9%) and behavior (2.4%).

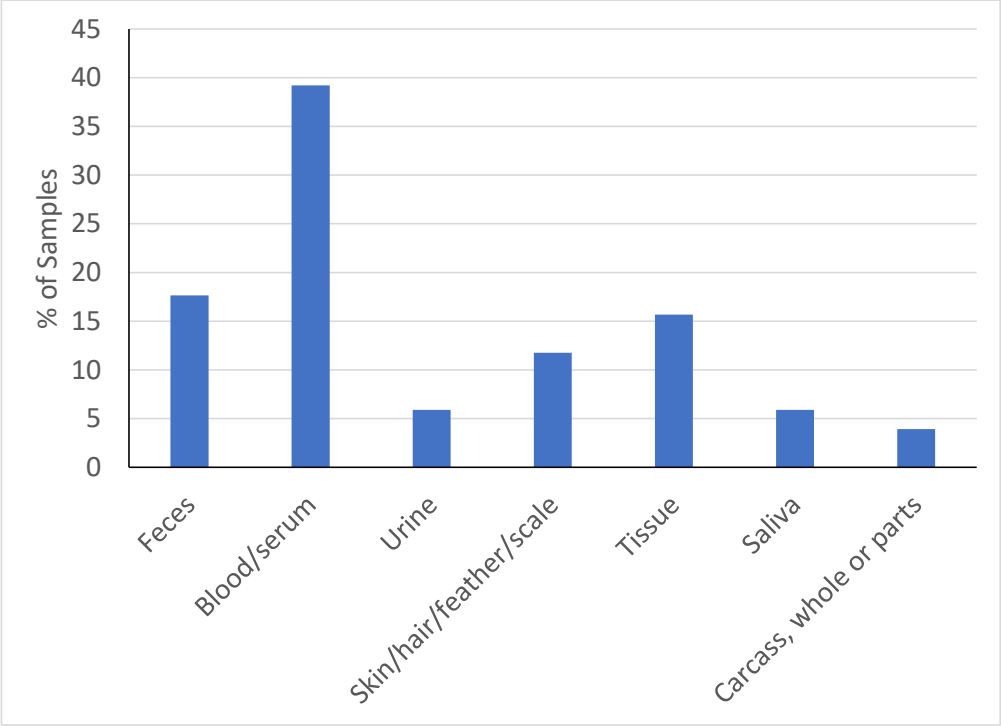

Figure 3. Percentages of different biomaterial types the Saint Louis Zoo agreed to provide to researchers in the period of 2008–2015.

3.4. AZA Annual Report on Conservation and Science Database Query

The AZA database extract revealed that at least 67 unique AZA zoos and aquariums, or currently 28% of the current membership, participated in 66 unique biomaterials-related projects between 2000 and 2021. Many of these projects were multi-institutional studies that spanned several years. Mammals, birds, reptiles, amphibians, and invertebrates were represented in the projects, and topics covered were predominantly molecular genetic analyses of populations and projects related to veterinary health and epidemiology of disease in animal populations.

On average, 104.6 Ph.D., Master's or Doctor of Veterinary Medicine degrees are conferred to students doing research associated with AZA-accredited zoos annually. In the last five years (2017–2021), 523 advanced degrees were completed involving zoo and aquarium collections, biomaterials, or staff mentors.

4. Discussion

In this study, we sought to quantify the contribution that zoos and aquariums make to society through provision of biomaterials from zoo and aquarium animals for scientific research and the sometimes-associated training of students. We find that zoos are significant contributors to the research efforts of external scientists. Extrapolating across our various datasets, we estimate that, across the AZA membership, zoos are making contributions to hundreds of projects annually, in the form of over one hundred biomaterial samples, impacting the research of hundreds of scientists at many different institutions in a variety of basic and applied science disciplines. Though our most detailed datasets (convenience sample and Saint Louis Zoo records) are based on only 11 zoos, these zoos are vastly different in size, which could be a proxy for facility budget. In addition, we found that having an established biomaterials repository did not affect how many biomaterials requests these zoos received or approved. All zoos can provide at least some kind of biomaterial (e.g., feces), so there is no reason to believe that facility size would necessarily impact biomaterials participation. Proximity to a university or having an on-site research department should also be irrelevant because samples can easily be shipped anywhere,

and because in many cases, it is a member of the veterinary staff that handles biomaterials requests in our experience. Since all AZA-accredited zoos have at least a basic veterinary staff, not having a research department does not preclude participation in externally-lead biomaterials research. Our less detailed dataset, from the AZA conservation & science database still reflects the efforts of a large number of zoos & aquariums and shows that they are contributing to many projects.

Other zoos and universities are the most common requestors of biomaterials, which greatly affects how much zoos participate in externally-lead biomaterials research. Most projects are focused on health, genetics/genomics, or physiology but also cross over into applied sciences like animal welfare and forensics. A broad array of taxonomic groups from invertebrates to mammals are represented in the biomaterials research supported by zoos. Mammals tend to predominate the list, but reptiles are also a common focus of biomaterials research in our data. Bird, amphibian, and invertebrate samples appear to be requested less frequently but this likely depends on what is in zoos' collections when they receive requests. Our convenience sample included only one zoo with a significant aquarium and no stand-alone aquariums, so we don't have reliable estimates of how often aquatic species are the targets of biomaterials research. Blood and blood components (e.g., serum) and feces are the most commonly requested sample types in all of the datasets we analyzed, but zoos are providing a wide range of sample types. Proposals approved by the Saint Louis Zoo reflected many of the same categories of biomaterial type requests seen in the sample of other zoos. Different zoos may receive different kinds of requests from researchers based on the zoo's animal collection, the number of individuals it houses, and proximity to the scientist, which can affect shipping costs, among other factors. It was nice to see in our convenience sample that over half of reporting zoos indicated they had a biomaterials repository of some kind from which samples are available to scientists, though we found that having an existing repository of biomaterials was unrelated to how many biomaterials proposals a zoo received or approved.

Most often, the results of zoo-facilitated, biomaterials-related research appear to be of direct relevance to non-human animals, in the wild or in human care. Examples include genomic analysis [17] of an avian retrovirus identified in endangered Attwater's prairie chickens (*Tympanuchus cupido attwateri*) that threatens the viability of the wild and captive population. Blood protein analysis in a large sample of Grant's zebra (*Equus burchelli*) was used to generate normal reference ranges for the species to improve health monitoring [18]. Genetic analyses of zoo animal samples have been used to identify inbreeding depression in assurance populations of endangered species [19] and to improve captive population management [20]. Studies of the micro-organisms living in or on animals (the "microbiome") have increased recently, these may have implications for animal health ([21] and references therein) and possibly conservation [22,23], as studies of captive animals have revealed that they may not share the full extent or distribution of microbiome diversity that their wild counterparts do. Analyses of the parasites of zoo animals have implications for animal health as well as parasite ecology and management [24]. Blood samples from zoo polar bears (*Ursus maritimus*) were used to validate assays for plasma cytokines and contaminant concentrations in wild polar bears to measure impacts of climate change [25]. Finally, tissue from captive wood turtles (*Glyptemys insculpta*) was used to develop a technique law enforcement could use in correctly classifying confiscated turtles as wild- or captive-born [26].

In addition, there are notable examples of research studies supported by zoos via the provision of biomaterials that have relevance to human health and well-being. For example, American alligator (*Alligator mississippiensis*) and Komodo dragon (*Varanus komodoensis*) blood plasma were used in bio-prospecting studies to look for anti-microbial proteins that might have therapeutic relevance for treating disease or overcoming antibiotic resistance [27,28]. In both studies, candidate proteins were identified. Great ape, Afro-Eurasian monkey, and New World primate samples provided by zoos were used to help understand the evolution of the ABO blood group system in humans and to understand

the selective processes that maintain this genetic polymorphism [29]. Scientists studied the brains of zoo gorillas (*Gorilla gorilla*) to help understand the origins of and pathology associated with Alzheimer's disease [30]. Samples from elephants and other Afrotherians in zoos have been studied to understand tumor suppression in these species with an eye to better understand and treat cancer in humans [31,32]. Finally, samples from zoo animals were used early in the prediction of the possible range of hosts for COVID-19 [33].

Biomaterials provided by zoos also support the undergraduate, graduate, and professional education of students. Over one third of the biomaterials projects approved by the Saint Louis Zoo in the last seven years were part of a student thesis project. Although our queries of the AZA Conservation and Science database were likely not able to capture all possible biomaterials projects, we used keywords to find projects that in all likelihood would have required biomaterials (e.g., endocrine studies) and keywords that covered the types of proposals we typically see that request biomaterials. Due to this and the possibility that some zoos under-reported their data, our estimates of projects from this database are likely conservative. Thee database does not capture whether individual projects involve a student thesis but rather captures a total number of degrees facilitated by the zoo or aquarium in a given year. Thus, some student projects found in our query possibly did not involve biomaterials, and our estimate of students trained is possibly an over-estimate. Still, the contributions that zoos make to student projects that lead to advanced or professional degrees should not be ignored. Hundreds of students annually are completing advanced education in collaboration with zoos, and their research frequently involves biomaterials. A publication analysis based on zoo-derived biomaterials was not possible because zoos are not always represented among the authors on publications and text in other parts of manuscripts (i.e., acknowledgments) is not searchable by databases.

Clearly zoos have much to offer to science, and thus society, by providing access to living (or deceased) animals from a vast array of species. Increasing zoos' contributions to science via biomaterials is possible, but challenges exist. Maintaining biomaterials repositories requires space and often significant financial resources to properly store samples. Staff time and record keeping systems are also required to review, document, and fulfill biomaterials requests as well as curate and maintain inventories. The role that natural history museums may have in alleviating some of the storage issues zoos may face and the mutual benefits, challenges and opportunities of stronger zoo-museum partnerships were recently highlighted [34]. Those authors also point out that there may be institutional cultural differences that can present challenges.

External researchers may be frustrated with paperwork or approval processes zoos require for biomaterials transfer and the associated fact that zoos don't all have a universal research approval form or process that provides the researcher with access to multiple zoo animal collections simultaneously. Years ago, the AZA's Research and Technology Committee, with input from AZA's Biobanking Scientific Advisory Group, created an AZA standardized research proposal form (see: https://www.aza.org/research_and_technology_committee?locale=en to obtain this form, accessed on 1 February 2023), which includes biomaterials requests, and this has helped to streamline approval-seeking in many cases. However, use of the form is voluntary, and for biomaterials in particular, there may be legal idiosyncrasies for a given zoo or a given species that do not apply to others (i.e., if zoo animals are considered city property for municipally-funded zoos), limiting the applicability of a universal form. Ideally zoos should try to use the standardized research form whenever possible. The EAZA biobank mentioned earlier has also created standardized biomaterials request forms to hopefully streamline these processes to the extent possible.

When a repository of appropriate samples does not exist that addresses a researcher's needs, they may also be frustrated that zoos may not immediately obtain samples from living animals. Outside researchers may not understand the complexities or risks involved in obtaining certain kinds of biomaterials. Researchers should be aware that zoos receive many requests for biomaterials that commit the use of staff time and bud-

getary resources to review and fulfill these requests—in addition to the stress that may be placed on the animals. In our experience, zoos consider the ease of obtaining the sample(s), whether or not the samples can be obtained opportunistically rather than on a timeline prescribed by an outside researcher, and the relevance of the proposed research to the zoo's mission, among other factors. Because there is, in our experience, a preference among zoos for providing biomaterials samples that are opportunistically collected (e.g., coincident with a regularly scheduled physical exam), outside researchers should plan well in advance if they are seeking biomaterials from zoos to meet a research deadline. The AZA's Behavior Advisory Group produced a resource document with the goal of facilitating behavioral research collaborations between zoos and outside scientists (see: https://assets.speakcdn.com/assets/2332/(aza_formatted)_behavior_sag_tips_for_conducting_research_guide_(aza_formatted).pdf, accessed on 1 February 2023), and many of the same considerations, for example discussing logistical challenges in obtaining samples with zoo staff and requesting samples well in advance of laboratory analysis, in that document apply to facilitating biomaterials research as well.

In conclusion, zoos have a strong history of contributing to science and the training of future scientists through provision of biomaterials for scientific research. These contributions increase society's knowledge of the natural world and how to conserve it as well as ways to enrich human health and well-being. Providing biomaterials for scientific research is one way that zoos increase the societal impact of the animals in their care and allows zoo animals to contribute to sustaining all of the planet's inhabitants, even after death. Zoos and outside researchers should continue to address challenges to collaboration in order to reap the mutual benefits of biomaterials research.

Author Contributions: Conceptualization, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing, project administration, D.M.P. Data collection, curation, manuscript review and editing, T.G.M. Data collection, curation, manuscript review and editing, M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data collected for this study may be available upon reasonable request from the author.

Acknowledgments: Gillian Cannataro conducted the AZA conservation & science database queries. We thank the ten zoos that provided data on their biomaterials research participation.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Olney, P.J.S. London Zoo. In *Great Zoos of the World: Their Origins and Significance*; Zukerman, S., Ed.; Weidenfeld and Nicholson: London, UK, 1980; pp. 35–48. [CrossRef]
2. Our History. Available online: https://www.wcs.org/about-us/historical-timeline (accessed on 20 January 2023).
3. Hutchins, M.; Wiese, R.J.; Smith, B. Introduction: Research in zoos and aquariums: Purpose, justification, utility, and welfare. In *Scientific Foundations of Zoos and Aquariums: Their Role in Conservation and Research*; Kaufman, A.B., Bashaw, M.J., Maple, T.L., Eds.; Cambridge University Press: Cambridge, UK, 2019; pp. 1–42. [CrossRef]
4. Loh, T.L.; Larson, E.R.; David, S.R.; de Souza, L.S.; Gericke, R.; Gryzbek, M.; Kough, A.S.; Willink, P.W.; Knapp, C.R. Quantifying the contribution of zoos and aquariums to peer-reviewed scientific research. *Facets* **2018**, *3*, 287–299. [CrossRef]
5. Anzai, W.; Ban, K.; Hagiwara, S.; Kako, T.; Kashiwagi, N.; Kawase, K.; Yamanashi, Y.; Murata, K. Quantifying the 60-year contribution of Japanese zoos and aquariums to peer-reviewed scientific research. *Animals* **2022**, *12*, 598. [CrossRef] [PubMed]
6. Kögler, J.; Pacheco, I.B.; Dierkes, P.W. Evaluating the quantitative and qualitative contribution of zoos and aquaria to peer-reviewed science. *J. Zoo Aquar. Res.* **2020**, *8*, 124–132. [CrossRef]
7. Hvilsom, C.; Welden, H.L.Å.; Stelvig, M.; Nielsen, C.K.; Purcell, C.; Eckley, L.; Bertelsen, M.F. The contributions of EAZA zoos and aquariums to peer-reviewed scientific research. *J. Zoo Aquar. Res.* **2020**, *8*, 133–138. [CrossRef]

8. Wemmer, C.; Thompson, S.D. A short history of scientific research in zoological gardens. In *The Ark Evolving: Zoos and Aquariums in Transition*; Wemmer, C.W., Ed.; Smithsonian Conservation and Research Center: Front Royal, VA, USA, 1995; pp. 70–90.
9. Wharton, D. Research by zoos. In *Zoos in the 21st Century: Catalysts for Conservation*; Zimmerman, A., Hatchwell, M., Dickie, L., West, C., Eds.; Cambridge University Press: Cambridge, UK, 2007; pp. 178–191.
10. Anderson, U.S.; Keiling, A.S.; Maple, T.L. Twenty-five years of zoo biology: A publication analysis. *Zoo Biol.* **2008**, *27*, 444–457. [CrossRef] [PubMed]
11. Maple, T.L.; Bashaw, M.J. Research trends in Zoos. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*, 2nd ed.; Kleiman, D.G., Thompson, K.V., Kirk Baer, C., Eds.; The University of Chicago Press: Chicago, IL, USA, 2010; pp. 288–300. [CrossRef]
12. Melfi, V.A. Diversity of zoo primate research: A sampler. *Int. J. Primatol.* **2007**, *28*, 1413–1415. [CrossRef]
13. Kleiman, D.G. Behavioral research in zoos: Past, present, and future. *Zoo Biol.* **1992**, *11*, 301–312. [CrossRef]
14. EAZA Biobank. Available online: https://www.eaza.net/conservation/research/eaza-biobank (accessed on 8 February 2023).
15. Australian Frozen Zoo. Available online: https://australianfrozenzoo.org.au (accessed on 8 February 2023).
16. Mellen, J.; MacPhee, M. Animal learning and husbandry training for management. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*, 2nd ed.; Kleiman, D.G., Thompson, K.V., Kirk Baer, C., Eds.; The University of Chicago Press: Chicago, IL, USA, 2010; pp. 314–328. [CrossRef]
17. Barbosa, T.; Zavala, G.; Cheng, S.; Villegas, P. Full genome sequence and some biological properties of reticuloendotheliosis virus strain APC-566 isolated from endangered Attwater's prairie chickens. *Virus Res.* **2006**, *124*, 68–77. [CrossRef]
18. Cray, C.; Hammond, E.; Haefele, H. Acute phase protein and protein electrophoresis values for captive Grant's zebra (*Equus burchelli*). *J. Zoo Wildl. Med.* **2013**, *44*, 1107–1110. [CrossRef]
19. Hammerly, S.C.; Morrow, M.E.; Johnson, J.A. A comparison of pedigree- and DNA-based measures for identifying inbreeding depression in the critically endangered Attwater's prairie-chicken. *Mol. Ecol.* **2013**, *22*, 5313–5328. [CrossRef]
20. Norman, A.J.; Putnam, A.J.; Ivy, J. Use of molecular data in zoo and aquarium management: Benefits, challenges, and best practices. *Zoo Biol.* **2019**, *38*, 106–118. [CrossRef]
21. Hyde, E.R.; Navas-Molina, J.A.; Song, S.J.; Kueneman, J.G.; Ackermann, G.; Cardona, C.; Humphrey, G.; Boyer, D.; Weaver, T.; Mendelson, J.R.; et al. The oral and skin microbiomes of captive Komodo dragons are significantly shared with their habitat. *Msystems* **2016**, *1*, e00046-16. [CrossRef] [PubMed]
22. Redford, K.H.; Segre, J.A.; Salafsky, N.; del Rio, C.M.; McAloose, D. Conservation and the microbiome. *Conserv. Biol.* **2012**, *26*, 195–197. [CrossRef]
23. Zhu, L.; Wang, J.; Barhndorff, S. Editorial: The wildlife gut microbiome and its implication for conservation biology. *Front. Microbiol.* **2021**, *12*, 697499. [CrossRef]
24. Amin, O.M.; Chaudhary, A.; Heckmann, R.A.; Swenson, J.; Singh, H.S. Redescription and molecular characterization of *Pachysentis canicola* Meyer, 1931 (Acanthocephala: Oligacanthorhynchidae) from the maned wolf *Chrysocyon brachyurus* (Illiger, 1815) in Texas. *Acta Parasitol.* **2021**, *67*, 275–287. [CrossRef]
25. Bourque, J.; Desforges, J.P.; Levin, M.; Atwood, T.C.; Sonne, C.; Dietz, R.; Jensen, T.H.; Curry, E.; McKinney, M.A. Climate-associated drivers of plasma cytokinds and contaminant concentrations in Beaufort Sea polar bears (*Ursus maritimus*). *Sci. Total Environ.* **2020**, *745*, 140978. [CrossRef] [PubMed]
26. Hopkins, J.B., III; Frederick, C.A.; Yorks, D.; Pollock, E.; Chatfield, M.W.H. Forensic application of stable isotopes to distinguish between wild and captive turtles. *Biology* **2022**, *11*, 1728. [CrossRef]
27. Bishop, B.M.; Juba, M.L.; Devine, M.; Barksdale, S.M.; Rodriguez, C.A.; Chung, M.C.; Russo, P.S.; Vliet, K.A.; Schnur, J.M.; van Hoek, M.L. Bioprospecting the American alligator (*Alligator mississippiensis*) host defense peptidome. *PLoS ONE* **2015**, *10*, e0117394. [CrossRef]
28. Bishop, B.M.; Juba, M.L.; Russo, P.S.; Devine, M.; Barksdale, S.M.; Scott, S.; Settlage, R.; Michalak, P.; Gupta, K.; Vliet, K.; et al. Discovery of novel antimicrobial peptides from *Varanus komodoensis* (Komodo Dragon) by large-scale analyses and de-novo-assisted sequencing using electron-transfer dissociation mass spectrometry. *J. Proteome Res.* **2017**, *16*, 1470–1482. [CrossRef] [PubMed]
29. Ségurel, L.; Thompson, E.E.; Flutre, T.; Lovstad, J.; Venkat, A.; Margulis, S.W.; Moyse, J.; Ross, S.; Gamble, K.; Sella, G.; et al. The ABO blood group is a trans-species polymorphism in primates. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18493–18498. [CrossRef]
30. Perez, S.E.; Raghanti, M.A.; Hof, P.R.; Kramer, L.; Ikonomovic, M.D.; Lacor, P.N.; Erwin, J.M.; Sherwood, C.C.; Mufson, E.J. Alzheimer's disease pathology in the neocortex and hippocampus of the western lowland gorilla (*Gorilla gorilla gorilla*). *J. Comp. Neurol.* **2013**, *521*, 4318–4338. [CrossRef] [PubMed]
31. Abegglen, L.M.; Caulin, A.F.; Chan, A.; Lee, K.; Robinson, R.; Campbell, M.S.; Kiso, W.K.; Schmitt, D.L.; Waddell, P.J.; Bhaskara, S.; et al. Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. *JAMA* **2015**, *314*, 1850–1860. [CrossRef] [PubMed]

32. Vazquez, J.M.; Lynch, V.J. Pervsasive duplication of tumor suppressors in Afrotherians during the evolution of large bodies and reduced cancer risk. *eLife* **2021**, *10*, e65041. [CrossRef] [PubMed]
33. Damas, J.; Hughes, G.M.; Keough, K.C.; Painter, C.A.; Persky, N.S.; Corbo, M.; Hiller, M.; Koepfli, K.; Pfenning, A.R.; Zhao, H.; et al. Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 22311–22322. [CrossRef] [PubMed]
34. Poo, S.; Whitfield, S.M.; Shepack, A.; Watkins-Colwell, G.J.; Nelson, G.; Goodwin, J.; Chakrabarty, P. Bridging the Research Gap between Live Collections in Zoos and Preserved Collections in Natural History Museums. *BioScience* **2022**, *72*, 449–460. [CrossRef] [PubMed]