

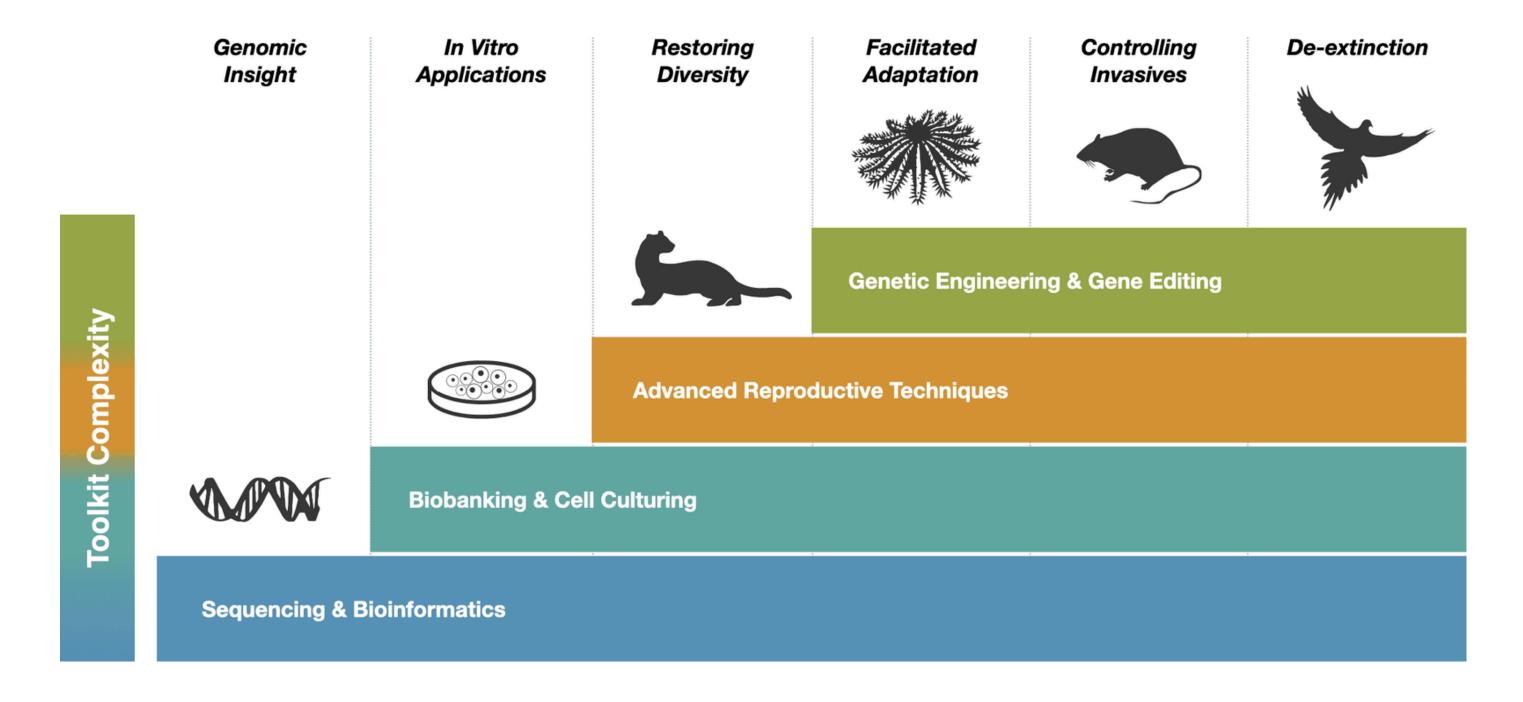
Annual Report 2024

Table of Contents

Revive & Restore works around the world to expand conservation's scientific toolkit. Here are five ways we're transforming the field today:

Halting the Harvest	Page 2
Debunking Myths About Cloning	Page 4
Advancing Stem Cell Technologies	Page 6
Bridging Science and Practice	Page 8
Fortifying Ocean Ecosystems	Page 10
Amplifying our Impact	Page 11

These achievements are made possible by drawing upon the Genetic Rescue Toolkit. We use this term to describe our suite of biotechnology tools with direct conservation applications. From bottom to top, these biotechnologies form the building blocks of genetic rescue. Sequencing and biobanking, for example, are foundational tools that enable more advanced technologies, like cloning and genetic engineering.



Thank you to the donors and partners who made all this possible in 2024. Each milestone we reach is a direct result of your steadfast support.

The American Horseshoe Crab: Halting the Harvest

Our goal is to accelerate the adoption of synthetic alternatives to protect horseshoe crabs and the ecosystems that rely on these living fossils.

American horseshoe crabs are vital to coastal ecosystems, yet approximately 1.1 million crabs are harvested annually for their blood, which pharmaceutical companies use to test for endotoxins (contaminants) in drugs and vaccines. This practice is not only ecologically unsustainable but also lethal for up to 30% of bled crabs. There are animal-free synthetic alternatives for endotoxin testing created through genetic engineering, and some pharmaceutical companies have begun to make the transition away from horseshoe crab blood.

2024 Accomplishments

In collaboration with conservation, advocacy, and pharmaceutical industry partners, this effort has achieved several significant milestones:

- Announcing the Sustainability Scorecard for Endotoxin Testing, which tracks and measures pharmaceutical companies' progress as they transition to synthetic alternatives
- The US Pharmacopeia's final approval of Chapter 86 in November 2024 offers guidance for using synthetic alternatives for endotoxin testing
- The continued development of the Horseshoe Crab Recovery Coalition, engaging over 30 organizations

Next Steps

The Sustainability Scorecard will accelerate the pharmaceutical industry's transition to using synthetic alternatives for drug and vaccine testing. This initiative will help ensure accountability and transparency to the public as companies adopt sustainable, animal-free alternatives to horseshoe crab blood.

We will continue to advocate for the full adoption of synthetic alternatives globally, leveraging the Sustainability Scorecard to monitor progress in the industry. Our focus will remain on raising public awareness, accelerating the transition to synthetic alternatives, and ensuring the long-term survival of horseshoe crabs and the ecosystems that depend on them.



American Horseshoe Crabs in a bleeding facility. Photo credit: Timothy Fadek



"There is no excuse to continue using horseshoe crab blood when readily available alternatives are recognized as equivalent. This decision by USP presents a tremendous opportunity for pharmaceutical companies to rise to the occasion and do the right thing."

-Ryan Phelan, Executive Director & Co-founder

Ryan Phelan examines a horseshoe crab during spawning season. Since 2018, Revive & Restore has collaborated with scientific, legal, and industry partners to promote the adoption of rFC and stop the bleeding of this threatened keystone species Photo credit for cover and above: Jason Sussberg, Structure Films.

The Black-footed Ferret: Debunking Myths About Cloning

Our goal is to move black-footed ferrets from their current state of conservation reliance to a future in which they can be independent of human help.

While other partners restore and secure habitat, we are working to accomplish two critical goals: 1) to increase genetic diversity for long-term viability and 2) to provide genetic resilience to sylvatic plague, which threatens their full recovery.

2024 Accomplishments

Our program made history this year when a black-footed ferret clone named Antonia became a mother, giving birth to Sibert and Red Cloud at the Smithsonian National Zoo. Antonia is a clone of Willa, who died in 1988 with no descendants. Before our cloning efforts, all black-footed ferrets were descended from just 7 wild founders saved from extinction in the 1980s. Antonia's kits officially make Willa an eigth founder of the species—dramatically increasing available genetic diversity.

This is the first time a clone of an endangered species has reproduced successfully to help save its species, and the first time pre-bottleneck genetic diversity has been restored to an endangered species. The latter is a capability only possible with biotechnology.

Next Steps

Proving cloning as a viable tool for the breeding program was necessary for our next step: delivering a long-term solution to sylvatic plague. We are currently funding research into multiple forms of inheritable resilience, including a genetic vaccine and allele replacement. Either approach could allow the species to thrive in the face of disease outbreaks. In the future, these technologies could save other wild species from extinction.

As for Antonia, Sibert, and Red Cloud, we hope their family grows in years to come!



Newborns Sibert and Red Cloud. Photo credit: Adrienne Crosier, NZCBI



Sibert and Red Cloud, six weeks old. Photo credit: NZCBI



We are grateful for our partners in this critical work: U.S. Fish & Wildlife Service, Smithsonian's National Zoo and Conservation Biology Institute, ViaGen Pets & Equine, the San Diego Zoo Wildlife Alliance, and our incredible donors. This work is made possible by your belief in our shared mission. **Thank you.**



"Nearly 30 years since Dolly the sheep, our program has achieved an incredible moment for the field—the first time a clone of an endangered species has given birth. These babies are proof to the world that cloning can contribute to saving species."

-Ben Novak, Lead Scientist

A black-footed ferret emerges from a prairie dog burrow. Revive & Restore and its partners have worked for over a decade to restore genetic diversity in black-footed ferrets through strategic conservation cloning. Photo credit: Kerry Hargrove

Stem Cells: Advancing Technologies for Conservation

Our goal is to catalyze this field, engaging the global stem cell community to advance crucial research in this area.

Induced pluripotent stem cells (iPSCs) have the capacity to transform conservation efforts. They can become any cell type in the body, including sperm, eggs, and even embryos. While tractable methods exist for model species, like the lab mouse, translating this technology to wildlife encounters considerable hurdles. We aim to explore opportunities in AI to predict better ways to create stem cells for more species, and fund breakthrough uses of stem cell technology for conservation. In human medicine, the ability to create stem cells from any cell in the body has revolutionized therapies, disease research, and reproductive technologies. For wildlife, iPSCs hold the promise of transforming limited samples into an infinite resource.

2024 Accomplishments

Through our recently launched "Poo Zoo" project, we are funding cutting-edge research into the derivation of cell cultures from scat samples. This non-invasive method to obtain and bank cell lines from species will be a huge step towards safeguarding genetic diversity, enabling the derivation of biomaterials for rare species that are challenging to sample. If successful, this high-risk, high-reward project will enable standardized optimization and production of stem cell lines from a wide range of species for deployment in conservation. Our funded team includes researchers from Oxford University and collaborators at Chester Zoo. The initial phase focuses on three extraordinary species sampled in the zoo environment: the okapi, sun bear, and black rhino.

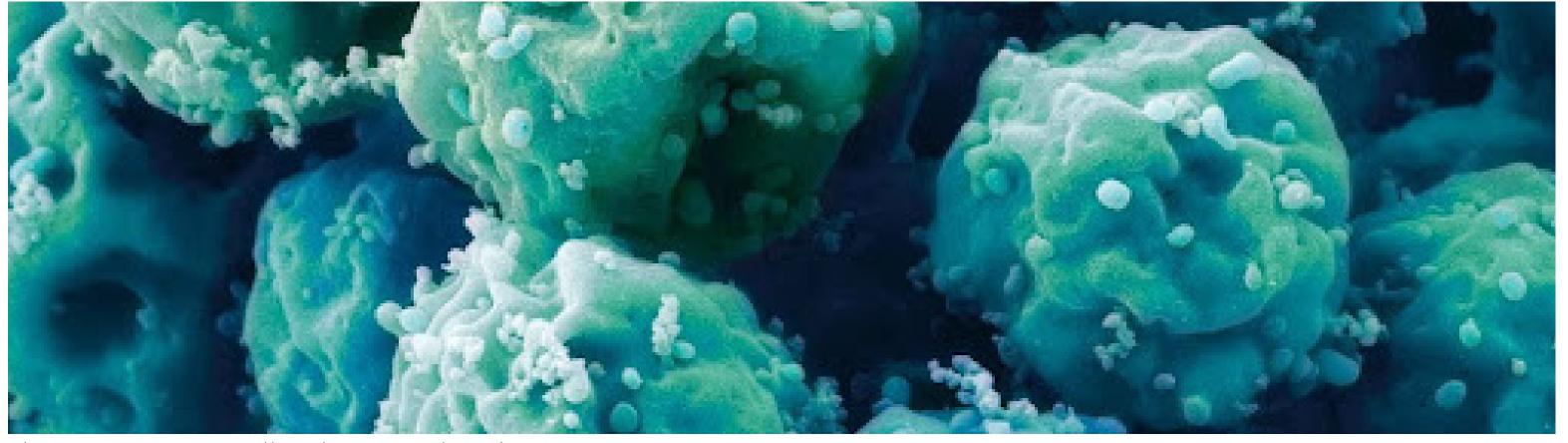
Our 2024 highlights include:

- The Poo Zoo finishes the year as our first funded project resulting from our inaugural Stem Cell Technology For Genetic Rescue Workshop in 2023
- Our stem cell community now includes 50 exceptional global leaders from 12 countries across 5 continents
- Workshop participants contributed to a joint paper, "Advancing Stem Cell Technologies For Conservation of Wildlife Biodiversity," which was published in the journal Development in 2024
- We scoped and developed an outline for how AI can accelerate advances in this area and presented our vision at Reinvent Futures' event, How Can AI Accelerate Progress In The Bio-World

Next Steps

Through a generous donor, we are creating a research fund for innovations in applied stem cell science for conservation. Revive & Restore will announce our first open request for proposals, aiming to accelerate the integration of stem cell technology into conservation efforts worldwide.

We are committed to mobilizing the stem cell community and increasing the visibility of this research. Through continued publications in scientific journals and outreach efforts, we aim to demonstrate the value of these tiny tools and shape a more resilient future for wildlife.



Pluripotent stem cells. Photo credit: The New Economy



"Stem cells are the enabling technology for every level of the Genetic Rescue Toolkit, providing a way to produce offspring from a skin sample, transforming biobanked material into an infinite resource. For endangered species, like the okapi, this technology cannot come soon enough."

-Ashlee Hutchinson, Program Manager

The okapi, a forest-dwelling relative of the giraffe, plays a key role in the health of its Central African habitat. By preserving its cell lines, Revive & Restore aims to ensure the species' long-term survival and genetic diversity. Photo credit: hipproductions via Getty

Biotech for Birds: Bridging Science and Practice

Our goal is to overcome the scientific challenges facing bird biotechnology and revolutionize conservation.

Every revolution needs an army—ours is the International Avian Genetic Rescue Consortium. We've been gathering scientists and conservationists to grow this community, building a shared vision to save birds with biotechnology.

2024 Accomplishments

Our 11 funded teams are working to make breakthroughs never achieved before—from culturing primordial germ cells of diverse species, stem cells for reproduction, rewriting bird genomes, to hatching doves from quail eggs! With foundational science in place, teams are on their way to exciting experiments in 2025. Biotechnologies to help endangered birds will soon move from hypothetical science to plausible applications. The Consortium is pivotal to this transition. In 2024, we gathered Consortium members from eight nations in person for the first time in Toronto for an intensive 3-day workshop.

Our funded scientists and others presented their work developing a range of cellular, reproductive, and gene editing tools. Conservationists showcased their incredible efforts to save birds, including heroic stories of rescuing South African vultures, establishing new Pacific seabird breeding colonies, and breeding back American grouse and quail from near extinction.

Next steps

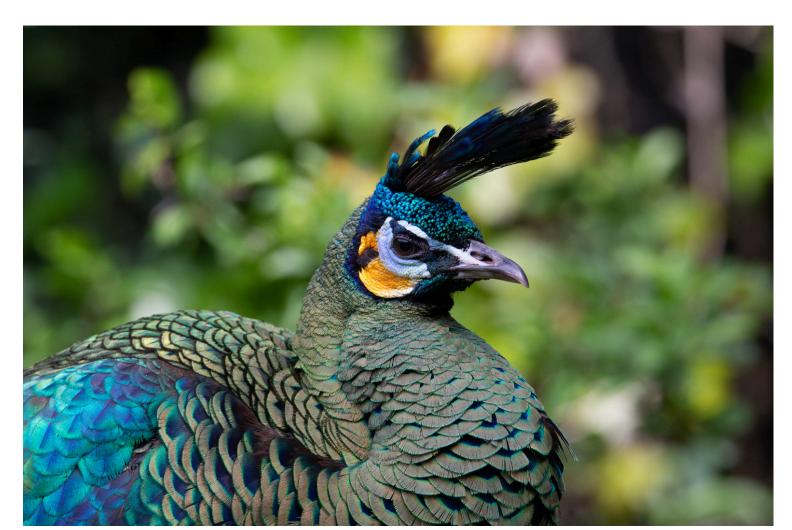
Combining their expertise, we asked participants to brainstorm the next "big ideas" to push biotechnology concepts further. The three big, final ideas were:

- Discovering candidate genes to fill the immense knowledge gaps for climate adaptation
- Developing chickens to lay eggs of dozens of wild bird species, to use as an ingenious disease or pathology array to get ahead of outbreaks and test treatments
- Perhaps the most ambitious: envisioning the tools necessary to recover a viable species from just a single, sole surviving member or a frozen cell line!

These ideas are thrilling, and one day may come to fruition, but conservationists need tools in the present to safeguard avian diversity: enter biobanking. Conservationists new to the Consortium were shown how to collect key biosamples from birds at different life stages. Using these new skills, and dissection kits gifted upon departure, they will be able to biobank critical genetic diversity of the species they are working to save—enabling future genetic rescue options as yet to be imagined.



A masked bobwhite quail. Photo credit: Gerald Corsi



A green peafowl. Photo credit: Ian Peter Morton



"The science we are developing over the next 5 years will create new opportunities for bird conservation so that biotechnologists and conservationists working together can make human-caused extinctions a thing of the past."

-Ben Novak, Lead Scientist

An Australian Zebra Finch, one of many bird species Revive & Restore is funding work to better understand. In 2022, Revive and Restore began funding a project to generate germ cells from zebra finch embryonic stem cells. Photo credit: Neil Bowman

Advanced Coral Toolkit: Fortifying Ocean Ecosystems

Our goal is to integrate novel biotechnologies with existing coral conservation approaches to advance the scale, efficiency, and success of reef restoration.

Coral reefs are the literal building blocks of ecosystems, supporting a quarter of the ocean's species. They also protect coastline communities and support livelihoods for over a billion people. However, they face unprecedented threats from climate change, pollution, disease, and overfishing. Our Advanced Coral Toolkit provides urgently needed genetic rescue solutions to help restore resilient coral reefs that can survive in the 21st century.

2024 Accomplishments

Now entering its fifth year, the Advanced Coral Toolkit encompasses a variety of innovative capabilities with enormous potential to transform coral restoration efforts. To date, we have awarded more than \$8 million across 13 projects. Awardees have pioneered first-of-their-kind efforts that push the boundaries of coral conservation practice. These include cryopreserving coral fragments to safeguard genetic diversity, transplanting stem cells between corals to transfer resilience, creating portable devices that diagnose coral stress in near-real-time, and developing probiotics to treat deadly disease outbreaks.

Advanced Coral Toolkit highlights:

- One People One Reef: In Ulithi, Micronesia, we fund work to integrate traditional knowledge and practices with scientific tools (including genomic sequencing, metabolomics, and eDNA). This project has been so successful that the communities on Ulithi have created an atoll-wide management plan informed by this toolkit, demonstrating how to develop sustainable, community-driven conservation approaches.
- **Coral stem cells:** A new study demonstrates, for the first time, the isolation and transplantation of stem cells in Hexacorallia. This subclass of cnidarians includes beloved creatures like stony corals and sea anemones. The study marks a pivotal step towards genetic therapies to advance coral restoration and resilience. It is also an important step forward for marine species for whom the derivation of cell cultures has been challenging.
- **Coral Probiotics:** Awardees at UNCW completed the development of a high-throughput platform for isolating, culturing, and testing hundreds of bacterial strains as potential probiotics to prevent and treat the devastating stony coral tissue loss disease. They are currently testing different strains and delivery methods in Florida, Montserrat, and Turks and Caicos, with the goal of scaling to save diseased corals and increase restoration success.

Next Steps

In 2025, we will transition the most promising technologies to scale or to field applications and optimize them by integrating feedback from restoration practitioners. In addition to awarding new grants to address the most daunting threats that corals face, we also plan to explore applications of the technologies our awardees have proven in corals to other marine species in need of novel conservation solutions.



Program Manager Liv Williamson examines wild coral. Photo credit: Trip Jennings



"Coral reefs are among the planet's most important—and most imperiled—ecosystems. Compounding threats are outpacing corals' natural capacity to evolve and adapt, jeopardizing their very persistence. The coral crisis demands innovation."

-Liv Williamson, Program Manager

Fish swim over a thicket of restored staghorn coral (Acropora cervicornis) off the coast of Miami, FL. The Advanced Coral Toolkit supports the development and field testing of new biotechnologies that have the potential to greatly benefit coral resilience and restoration efforts. Photo credit: Liv Williamson.

Our Impact in Numbers

Revive & Restore brings together a global network of conservationists, geneticists, technology developers, and field scientists to identify opportunities for conservation innovation. The breadth of our work includes:

- \$25M granted to innovative solutions to wildlife conservation challenges since our founding
- **\$8.7M** awarded to new research grants in 2024
- 54 ongoing research projects funded and overseen
- 33 nations with active research projects across the globe
- 23 projects successfully completed

Amplifying our Impact

Thank you to the journalists and media outlets that have helped our researchers increase their impact by reaching so many people in 2024.

<u>Visit our News & Media page to see the latest coverage</u>, as reported by:









The New York Times

Alta

The Washington Post





Thank You From Our Core Team

Our bold, science-driven mission allows us to address even the most intractable of conservation challenges. Yet this progress is only possible because of the extraordinary network of scientists, conservationists, and advocates who share their expertise, collaborate across disciplines, and remain steadfast in their commitment to the planet's biodiversity.

We extend our deepest gratitude to this community and our incredible donors. Your belief in the power of innovation allows us to push boundaries and transform conservation science. Together, we are not just preserving life; we are shaping a future where ecosystems can thrive in the face of unprecedented challenges.

Thank you.

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