Introduction to the Laboratory Axolotl and Ambystoma Genetic Stock Center

S. Randal Voss & Chris Muzinic

Department of Neuroscience, Spinal Cord and Brain Injury Research Center, and Ambystoma Genetic Stock Center, University of Kentucky, Lexington, KY 40536

*For correspondence: S. Randal Voss – srvoss@uky.edu

Abstract

Laboratory populations of the Mexican axolotl have been sustained for over 150 years in support of biological research. With recent advances in genetic and genome resource development, the axolotl is attracting considerable attention from new researchers, especially in the area of tissue regeneration. To reduce the learning curve and generally facilitate broader use of the axolotl, we introduce the Ambystoma Genetic Stock Center (AGSC), a research resource center that sustains and makes axolotl stocks available to researchers nationally and internationally. Also, to assist researchers that are unfamiliar with axolotl biology, we describe AGSC methods of husbandry, highlighting water quality and extrinsic environmental variables that are controlled and monitored to ensure axolotl health and well-being.

1. Introduction

In this chapter we introduce the primary salamander model in laboratory research, the Mexican axolotl (*Ambystoma mexicanum*). We also introduce the Ambystoma Genetic Stock Center (AGSC), which is funded by the National Institutes of Health to provide axolotls in support of biomedical research. Our objective is to provide useful information to those that use, or are planning to use, axolotls in research and educational activities.

2. The axolotl model organism

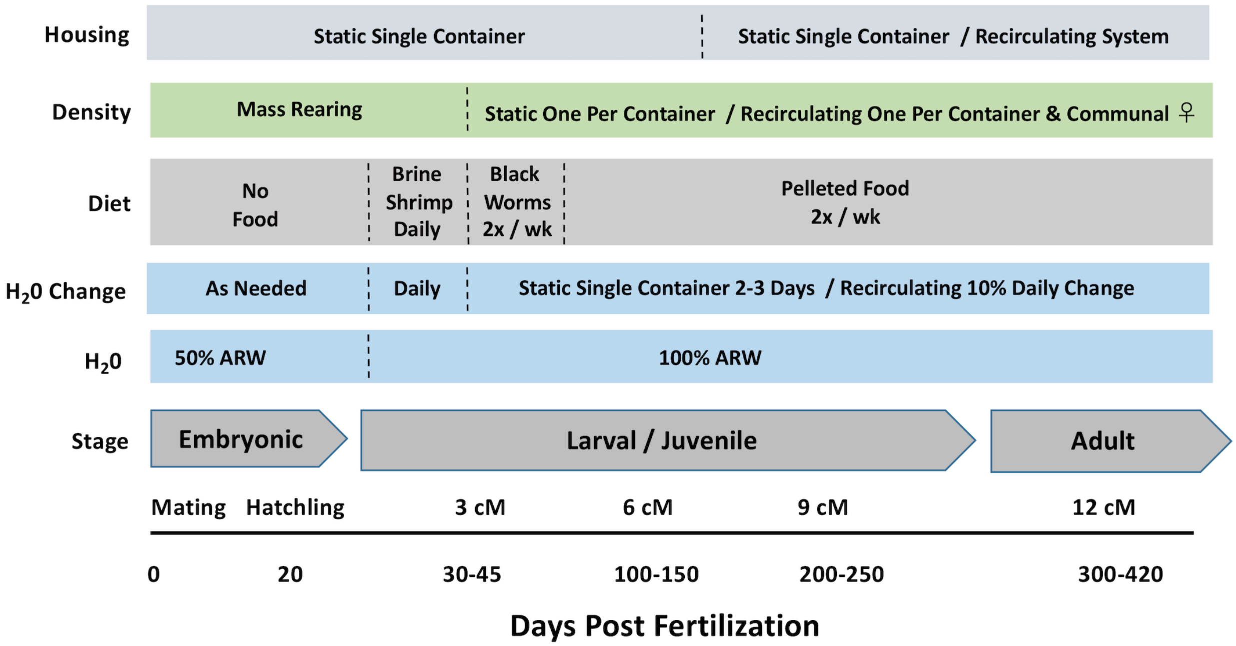
The axolotl has a deep and rich history as a laboratory model organism (Fig. 1). Present day laboratory populations trace their ancestry back to an original collection of 34 axolotls that were shipped to Paris in 1863 from aquatic habitats near present day Mexico City (Reiß et al. 2015). Over the next few decades, axolotls were propagated in laboratories across Europe and used to study questions in development and evolution, and these studies helped to originate the field of experiment zoology. During the 20th century, axolotls factored prominently in studies of embryonic and post-embryonic development, sex determination, cloning, and tissue regeneration (Smith and Smith 1971, Voss et al. 2009). Today, axolotls are attracting considerable interest among biomedical researchers because of their unrivaled ability to regenerate entire organs. They share the body plan of tetrapod vertebrates and are unique in their ability to regenerate a broad spectrum of damaged organs throughout life, including limbs, spinal cord, brain, lens, skin, ovary, and heart (Tazaki et al. 2017, Haas and Whited 2017, Erler et al. 2017, Amamoto et al. 2016, Cano-Martínez et al. 2010, Suetsugu-Maki et al. 2012, Ponomareva et al. 2015, Yokoyama et al. 2018). Understanding the cellular and genetic mechanisms by which axolotls regenerate tissues could have clinical significance for treating human trauma, disease, and aging.

In several ways, the axolotl was pre-adapted to become a laboratory model. Axolotls are members of the Tiger salamander complex, a group of ambystomatids that exhibit considerable variation in life history and modes of development (Shaffer and Voss 1996, Voss et al. 2015). Some species undergo a metamorphosis after an aquatic larval phase and gain traits for terrestrial life. Other species like the axolotl are paedomorphic and remain in the aquatic habitat throughout their life cycle. Paedomorphosis evolved so recently in axolotls that the ancestral metamorphic mode of development can be induced by adding thyroid hormone to an axolotl's rearing water (Page and Voss 2009). While it is possible to rear metamorphic forms in the lab, the axolotl's totally aquatic life history greatly simplifies laboratory culture. Moreover, axolotls are capable of breeding more than one time a year and produce considerably more offspring per spawn than metamorphic forms, which are seasonal breeders. Axolotls are ideal laboratory models because they can be propagated as captive managed populations that are self-sustaining and capable of providing living stocks (embryos, larvae, juveniles, and adults) to meet the needs of a research community. In contrast, other salamanders that are used in biomedical research are annual breeders that are harvested from natural populations. Because amphibians in general are declining around the world, it is difficult to justify the collection of salamanders from natural populations for laboratory studies when axolotl stocks are available from a sustainable, captive-bred population (Baddar et al. 2015).

3. Ambystoma Genetic Stock Center

Almost all of the domesticated axolotls in the world trace their ancestry to the Ambystoma Genetic Stock Center (AGSC) at the University of Kentucky, which is funded by the National Institutes of Health (P40-OD019794) to provide axolotls in support of research and educational efforts. This collection is irreplaceable because decades of inbreeding have yielded homogeneous, genetic stocks that thrive in the lab (Voss and Kump 2016). The collection provides standard living stocks (embryos, larvae, and adults) and supplies to culture axolotls in investigator labs. By supplying living material from a single facility, the AGSC obviates the need for smaller satellite collections. This effectively reduces the cost of animal use for investigators; yearly expenses for the entire research community are greatly reduced because axolotl material is obtainable on demand. Many AGSC users are located at institutions that do not have the equipment, expertise, or dedicated space to rear axolotls. For investigators that maintain relatively large numbers of axolotls, the AGSC serves as a back-up facility to replenish stocks and a source of genetic variation to maintain vigor of strains. For investigators that need early stage embryos, the AGSC provides breeders and advice about performing crosses and maintaining axolotls. Thus, the AGSC serves the needs of investigators in a variety of ways and it is the historical and contemporary source of axolotl stocks in the world.

The AGSC has approximately 3000 sq ft for maintaining approximately 1000 juvenile and adult axolotls, and thousands of embryos and larvae. The AGSC primarily functions as a custom order business as it is too costly to maintain all axolotl stocks in sufficient numbers to meet immediate user needs. Thus, it is important that users plan their experiments well ahead of time as it can take several months to produce some stocks and a year to produce adults (Fig. 2). Users initiate the purchasing process by first completing an online registration form that is available from the AGSC webpage (http://www.ambystoma.org/genetic-stock-center). After registering, users are put into contact with AGSC staff to discuss their order and the timeframe for receiving purchased stocks.



In addition to supplying axolotl stocks and associated husbandry supplies, the AGSC serves as an informatics hub where investigators obtain information about the collection, technical procedures, potential collaborators, and research findings. Several times each week AGSC staff respond to user questions and every effort is made to provide answers to queries within 24 hours. An independent website was developed for the AGSC to ensure flexibility and control of web and database design, networking, and file storage. Information on the AGSC website provides researchers with a list of available stocks, pricing, and instructions on how to order axolotls and related supplies. Also, the AGSC website provides information concerning axolotl husbandry, development, and genetics. A newsletter (*Axolotl*) is distributed annually to keep the community informed about axolotl services, new advances in the field, NIH policies and opportunities, and techniques.

3.1 Axolotl stocks

The AGSC maintains a variety of axolotl stocks of all life stages, as well as mutants and transgenics. Each stock is associated with a unique research resource identifier (RRID) number, as recommended by the Resource Identification Initiative at SciCrunch (SciCrunch.org). The Resource Identification Initiative is a database-driven initiative that seeks to "barcode" all of the critical reagents and tools that are used in the course of scientific research with RRIDs. In addition to wildtype, there are four pigment mutants (*melanoid, white, albino, copper*), two lethal mutants (*cardiac, short toes*), one sterile mutant (*eyeless*), and multiple transgenic and knock-out lines. Five of the mutants were recently cloned (Woodcock et al. 2017, Smith et al. 2019; Kabangu et al 2023; Cecil et al 2024). For example, the albino mutant traces to an interspecific hybridization in 1962 that brought in a mutated, *A. tigrinum tyrosinase* allele into the AGSC population, as well as additional *A. tigrinum* genomic DNA. The number of stocks in the AGSC is expected to increase over the next few years as a result of community efforts in making transgenics and mutants.

4. Extrinsic environmental variables – Ensuring reproducible axolotl research

There is growing appreciation for the need to monitor and report extrinsic environmental variables that can affect the reproducibility of scientific experiments (Federation of American Societies for Experimental Biology 2016). This includes all of the variables that are standardized in the rearing of animals in stock centers like the AGSC. Here, we review the most important extrinsic environmental variables for ensuring reproducibility of scientific research using the axolotl. This information will be useful to those that obtain axolotl stocks from the AGSC or want to establish satellite axolotl facilities with parallel standard operating procedures. These variables include water quality, temperature, housing, diet, and light.

4.1 Water quality

Axolotl health and well-being is critically tied to water quality. Axolotls are freshwater organisms that thrive in a dilute saline solution. The AGSC uses reverse osmosis (RO) water to make Axolotl Rearing Water (ARW) that contains (1.38 g NaCl, 80 mg $MgSO_4$, 40 mg $CaCl_2$, and 20 mg KCl per liter). The water is buffered by manual or automatic-dosing of NaHCO3 to achieve a pH in the range of 7.1 -7.6. Larvae, juveniles, and adults are reared in 100% ARW while 50% ARW is used to rear embryos.

Recognizing that many investigators might not have access to RO water, it is possible to use other water sources and filtration systems to prepare axolotl rearing water. In the past we used distilled water and charcoal filtered municipal water to make ARW, and some labs have reported success in rearing axolotls in minimally conditioned well-water and municipal water. Water chemistry including pH can vary dramatically when using municipal water and it is important to mitigate municipal water additives (e.g. chorine, chloramine, and ammonia) that are toxic to axolotls. Chlorine and chloramine can be removed by charcoal filtering or by aging water with an air bubbler, and ammonia can be removed by adding conditioners like *Amquel Plus* (Kordon). When using municipal water, pH, ammonia, and chlorine/chloramine should be carefully and frequently monitored before axolotl culture to ensure health. Ammonia is less of a concern when rearing axolotls statically in bowls because the frequency of water changes can be tuned to mitigate ammonia buildup. However, it is important to carefully and frequently measure ammonia when rearing axolotls in recirculating systems or large tanks with filters. When measured alongside nitrites and nitrates, ammonia provides an indicator of water quality. When ammonia levels exceed 2.0 ppm within a recirculating system, the system is not in equilibrium in regards to the nitrogen cycle. This can be a serious problem if pH is also high because high pH increases ammonia toxicity. To mitigate high ammonia and pH (> 8.0) in recirculating systems, 20-30% of the water is replaced daily with fresh, 100% ARW. Additional water changes may be needed to bring non-equilibrium systems below pH 8.00, at which time an ammonia detoxifier (e.g. Amquel Plus) can be added to alleviate ammonia buildup. However, a second reason for high ammonia levels in a recirculating system is inefficient biological filtration. To mitigate this problem, nitrifying bacteria can be added to a system to increase ammonia processing through the nitrogen cycle. The AGSC uses ProLine Nitrifying Bacteria (Pentair Aquatic Eco-Systems) following manufacturer dosing instruction for both initial set up and maintenance of recirculating systems.

4.2 Housing and Diet

Several methods are used to house axolotls in research and educational settings. The three primary methods are static housing (still water that is changed frequently), filtered aquaria, and recirculating systems. The AGSC uses static housing and recirculating systems to house all stocks (Fig. 3). An advantage of static housing is that a larger number of animals can be maintained within a small foot print. For example, 66 adult animals can be maintained on a single 36” x 21” x 60” mobile rack. However, static housing requires frequent water changes and bowl cleaning, and thus significantly more labor. Recirculating systems require less hands on cleaning because water changes are automatic and continuous, and tanks only need to be cleaned every two weeks. However, recirculating systems require a larger footprint. Each double-sided recirculating system in the AGSC supports 60 animals, but occupies the same space as three mobile racks (198 animals). Also, recirculating systems require a significant upfront financial investment and greater technical expertise to maintain. These trade-offs should be considered well in advance of establishing a facility to rear axolotls.

As a general rule, the size of a housing container should scale with animal number and body size. In the AGSC, husbandry begins with embryos that are housed in circular 1.5 l glass bowls, no more than 75 individuals per bowl. The water is manually stirred once daily to ensure oxygenation and dead embryos are removed. When necessary to slow down or speed up development, embryos are reared for short periods of time at 6 C^{o} and 24 C^{o} respectively. After larvae hatch, they are transferred to clean 1.5 L glass bowls with 100% ARW and reared in mass. When larvae make air bubbles at the surface of the water to indicate the onset of feeding behavior, they are fed newly hatched brine shrimp. Setting up a brine shrimp hatchery is fairly simple with a kit available from the AGSC (PLACE TABLE 2) and instructions provided on the AGSC website. Regardless of what method is used to rear brine shrimp, unhatched eggs and egg shells should be removed before feeding because brine shrimp shells and unhatched eggs can block the intestines of larvae. Larvae are maintained at moderate-to-low densities to prevent bite injuries to limbs and tails, however such injuries are inevitable unless individuals are housed separately. The vast majority of larvae in bowls incur bite injuries that are repaired by regeneration (Thompson et al. 2014). The AGSC offers a pay-for-fee service to rear individuals separately for investigators that require non-bite injured axolotls.

When larvae reach 3 cm, individuals are moved into 2 L plastic bowls with approximately 1 liter of water. At this time, individuals are fed California blackworms to increase growth rate and better facilitate the transition from live food to pelleted food, although axolotls can be reared throughout the adult phase on blackworms. The pelleted food is obtained from Rangen (Wilbur-Ellis Nutrition) in two sizes (4 mm for 4-8 cM animals; 5 mm for larger animals) and aged for 6 months prior to use. Aging the food is necessary because the pellets contain an ingredient (most likely thyroid hormone from bovine blood) that can induce spontaneous metamorphosis. Individuals are fed 2-3 pellets twice a week. When individuals reach 8-9 cM they are moved into larger 4 l bowls with 2.5 liters of water. The AGSC sells pelleted food and salts to make ARW to better ensure repeatability of axolotl husbandry (Table 2).

The method of husbandry (static vs recirculating system) determines the frequency of cleaning axolotl housing containers. During the embryonic period, water is changed as needed. Embryos do not produce a lot of waste but dead embryos and residual feces from the mating parents can foul water. During the larval period, all of the individuals in a bowl are strained into a net and the net is placed into a temporary holding container while the bowl is cleaned with a dilute bleach solution (5%) or baking soda, and then thoroughly rinsed. After the addition of fresh 100% ARW, larvae are returned to their original bowl. This procedure ensures that larvae from different spawns are not mixed up during the cleaning process. When larvae/juveniles/adults are reared individually in single bowls, water is changed every 2-3 days using the same cleaning method described above. In general, the frequency of cleaning is optimized for animal and container size, and as was mentioned earlier, water quality. Animals that mess-up their bowls before their scheduled cleaning are attended to immediately, as would be the case for a human patient in a hospital bed.

Both Aquarius (Aquatic Enterprises) & IWAKI recirculating systems are used within the Stock Center to house most of the adult breeding population, as well as excess juveniles for shipments. Each system is constructed of powder coated steel or aluminum racks with molded polycarbonate boxes. Aquarius life support systems include an 80-gallon sump per rack (8 per system) with screen filters, self-cleaning rotating drum filter, high efficiency pumps, 100-W UV sterilizer, and an automatic water change system that draws RO water from a 100-gal reservoir tank. Each system is automated using a NEMA protected ProFilux touchscreen monitoring system that includes sensors for pH, conductivity, temperature, and water level. Dosing tanks maintain pH at 7.5 and conductivity at 4,200 μS (based on 100% ARW). Males and females are housed individually in 8 L containers, while some larger females are housed three per 23 l container. Approximately 20% of the water in each system is changed daily. The IWAKI life support system includes a 50-gallon sump per rack (2 per system), 200 μm pre-filter bag, carbon block filter, 50 μm pleated cartridge filter, 100-W UV sterilizer, high efficiency pumps, automatic water changes drawing RO water directly from building supply, and a 1/10 HP chiller. The system is automated using the patented Walchem W600 water treatment controller, that can be operated via touchscreen and by remote access from anywhere. The system includes sensors for pH, conductivity, temperature, water flow, and water level. Onboard dosing systems maintain pH at 7.5, conductivity at 4,200 μS (based on 100% ARW). Male and female adults are housed individually in 4L containers (40 per rack on two racks). Approximately 30% of the water is changed daily.

For both Aquarius and IWAKI systems, lids are removed and cleaned weekly; containers are cleaned in place every two weeks. Quarterly, containers are removed and sanitized in Techniplast Calypso dishwasher units. The washers have programmed run cycles that descale, wash, rinse, and sanitize. Each unit uses building RO water and preset levels of aquatic safe detergent and water pressures.

Although the AGSC utilizes static and recirculating housing methods, it is possible to house axolotls in filtered aquaria. Ideally, no more than two adults should be housed together in a 10-gallon tank. The use of substrate is discouraged as axolotls will ingest small rocks and gravel during feeding. Low current, power filters with biological filtration can efficiently mitigate ammonia and nitrates, however it is still necessary to perform weekly 10% water changes and monitor water chemistry.

4.3 Temperature and Light

To maintain a relatively constant temperature of 15-17 C^{o} the AGSC relies upon both the building HVAC system and 2, 6-ton auxillary air-cooling units. A 12 hr light / 12 hr dark photoperiod is maintained throughout the year and the facility is lit by cool-white fluorescent lighting that is typical of research and educational buildings.

5.0 Pathogen Monitoring

Pathogen monitoring is essential to ensuring the health and well-being of animals housed in research facilities (Luchins and Langan 2018). The AGSC currently performs quarterly qPCR assays to monitor for five pathogens: *B. dendrobatidis*, *B. salamandrivorans*, *Ranavirus spp.*, *Dermocystidium spp.*, and *Amphibiocystidum spp*. To date, only the *B.* dendrobatidis assay has yielded a positive test result. *B. dendrobatidis* (Bd) is a fungal species that causes a skin disease called chytridiomycosis. This disease is thought to be the primary cause of global anuran declines (Scheele et al 2019). In AGSC axolotls, *Bd* is detected from skin swabs of ~ 1 year old animals, at very low abundances (Kabangu et al 2020). *Bd* positive axolotls are asymptomatic for chytridiomycosis. Although the AGSC *Bd* strain is benign and perhaps a commensal, the threat of an emerging virulent strain warrants continued testing for *Bd*. The AGSC tests for *B. salamandrivorans* and *Ranavirus spp*. because these species are known to be lethal to some salamander species, and tests were developed for *Dermocystidium spp.*, and *Amphibiocystidum spp*. in attempt to identify a pathogen during a larval mortality event in 2023.

To perform qPCR assays, DNA is isolated from axolotl skin swab samples. Samples are obtained from individuals housed statically in bowls and from individuals that are housed in recirculating systems. AGSC staff collect longitudinal data for adults, sampling the same individuals across sampling periods. Individuals are replaced in this design if they are sold, culled, or die. Ventral skin surfaces are swabbed with a MW100 swab:

1. Fore and hind limbs including feet (5 sweeps per limb)
2. Abdomen (10 sweeps)

Swabs are air dried for 1-1.5 hrs and then the cotton swab tips are removed with scissors and each placed into 2.0 ml screw cap plastic tubes for DNA isolation, using the method described by Boyle et al (2004).

6.0 Conclusions

In this chapter we introduced the Mexican axolotl and the primary stock center that provides axolotls to researchers and educators. The axolotl has the deepest laboratory pedigree of all animal models and with the recent development of a genome assembly its use will likely expand in coming years, especially in the area of tissue regeneration. This will present new opportunities and challenges for the AGSC. To meet the needs of an expanding research community it will be important to prioritize production of the most useful stocks and develop methods to cryopreserve an increasing number of transgenic and mutant lines that are being produced within the community (Coxe et al 2024). It will also be important to sustain the central role that the AGSC plays in facilitating axolotl research and education by providing homogeneous stocks, supplies, services, and useful information about axolotl husbandry.

References

Amamoto, R., V.G. Huerta, E. Takahashi, G. Dai, A.K. Grant, Z. Fu and P. Arlotta. 2016. Adult axolotls can regenerate original neuronal diversity in response to brain injury. *eLife* 2016;5:e13998. doi:10.7554/eLife.13998.

Baddar, N.W., M.R. Woodcock, S. Khatri, D.K. Kump and S.R. Voss. 2015. Sal-site: Research resources for the Mexican axolotl. *Methods in Molecular Biology* 1290:321-36.

Boyle DG, Boyle BD, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Org. 60:141–148.

Cano-Martínez, A., A. Vargas-González, V. Guarner-Lans, E. Prado-Zayago, M. León-Oleda and B. Nieto-Lima B. 2010. Functional and structural regeneration in the axolotl heart (*Ambystoma mexicanum*) after partial ventricular amputation. *Archives de Cardiologia de Mexico* 80:79-86.

Coxe N, Liu Y, Arregui L, Upton R, Bodenstein S, Voss SR, Gutierrez-Wing MT, Tiersch TR. 2024. Establishment of a Practical Sperm Cryopreservation Pathway for the Axolotl (*Ambystoma mexicanum*): A Community-Level Approach to Germplasm Repository Development. *Animals* (Basel) 14:206.

Erler, P., A. Sweeney and J.R. Monaghan. 2017. Regulation of injury-induced ovarian regeneration by activation of oogonial stem cells. *Stem Cells* 35:236-247.

Federation of American Societies for Experimental Biology. 2016. (http://www.faseb.org/Portals/2/PDFs/opa/2016/FASEB Response to NOT-OD-17-011_FINAL_Letterhead.pdf).

Fei, J.F., M. Schuez, D. Knapp, Y. Taniguchi, D.N. Drechsel and E.M. Tanaka. 2017. Efficient gene knockin in axolotl and its use to test the role of satellite cells in limb regeneration. *Proceedings of the National Academy of Science* (USA) 114:12501-12506.

Flowers, G.P., A.T. Timberlake, K.C. McLean, J.R. Monaghan and C.M. Crews. 2014. Highly efficient targeted mutagenesis in axolotl using Cas9 RNA-guided nuclease. *Development* 141:2165-71.

Gould, S.J. 1977. *Ontogeny and Phylogeny*. Cambridge, Massachusetts: Belknap Press.

Haas, B.J. and J.L. Whited. 2017. Advances in decoding axolotl limb regeneration. *Trends in Genetics* 33:553-565.

Johnson, C.K. and S.R. Voss. 2013. Salamander Paedomorphosis: Linking thyroid hormone to salamander life history and life cycle evolution. *Current Topics in Developmental Biology* 103:229-258.

Cecil R, Strohl L 2nd, Thomas ML, Schwartz JL, Timoshevskaya N, Smith JJ, Voss SR. 2024. *Tyrp1* is the mendelian determinant of the axolotl copper mutant. *Sci Rep* 14:22399.

Kabangu M, Cecil R, Strohl L 2nd, Timoshevskaya N, Smith JJ, Voss SR. 2023. *Leukocyte tyrosine tinase* (*Ltk*) is the mendelian determinant of the axolotl melanoid color variant. *Genes* (Basel) 14:904.

Keinath, M.C., N.Y. Timoshevskaya, D.L. Hardy, L. Muzinic, S.R. Voss and J.J. Smith. 2018. A PCR based assay to efficiently determine the sex of axolotls. *Axolotl* 2:5-7. (http://www.ambystoma.org).

Khattak, S., M. Schuez, T. Richter, D. Knapp, S.L. Haigo, T. Sandoval-Guzmán, K. Hradlikova, A. Duemmler, R. Kerney and E.M. Tanaka. 2014. Germline transgenic methods for tracking cells and testing gene function during regeneration in the axolotl. *Stem Cell Reports* 2:243. doi.org/10.1016/j.stemcr.2013.03.002.

Khattak, S. and E.M. Tanaka. 2015. Transgenesis in axolotl (*Ambystoma mexicanum*). *Methods in Molecular Biology* 1290:269-277.

Luchans K, Langan G. 2018. Managing animal colony health. In Management of Animal Care and Use Programs in Research, Education, and Testing, 2nd edition. Weichbrod RH, Thompson GAH, Norton JN, Eds. CRC Press/Taylor & Francis, Boca Raton, FL.

Page, R.B. and S.R. Voss. 2009. Induction of metamorphosis in axolotls (*Ambystoma mexicanum*). *Cold Spring Harbor Protocols*. doi:10.1101/pdb.prot5268.

Ponomareva, L.V., A.T. Athippozhy, J.S. Thorson and S.R. Voss. 2015. Using *Ambystoma mexicanum* (Mexican axolotl) embryos, chemical genetics, and microarray analysis to identify signaling pathways associated with tissue regeneration. *Comparative Biochemistry and Physiology, Part C*. 178:128-35.

Reiß, C., L. Olsson and U. Hoßfeld. 2015. The history of the oldest self-sustaining laboratory animal: 150 years of axolotl research. *Journal of Experimental Zoology, Part B.* 324:393-404.

SciCrunch. https://scicrunch.org.

Shaffer, H.B. and S.R. Voss. 1996. Phylogenetic and mechanistic analysis of a developmentally integrated character complex: alternate life history modes in ambystomatid salamanders. *American Zoologist* 36:24-35.

Smith, H.M. and R.B. Smith. 1971. Synopsis of the herpetofauna of Mexico. Vol. 1: Analysis of literature on the Mexican axolotl. Augusta: Lundberg.

Smith, J.J., N. Timoshevskaya, V.A. Timoshevskiy, M.C. Keinath, D. Hardy and S.R. Voss. A chromosome-scale assembly of the enormous (32 Gb) axolotl genome. *Genome Research* (In press).

Suetsugu-Maki, R., N. Maki, K. Nakamura, S. Sumanas, J. Zhu, K. Del Rio-Tsonis and P.A. Tsonis. 2012. Lens regeneration in axolotl: new evidence of developmental plasticity. *BMC Biology* 10:103. doi:10.1186/1741-7007-10-103.

Tazaki, A., E.M. Tanaka and J.F. Fei. 2017. Salamander spinal cord regeneration: The ultimate positive control in vertebrate spinal cord regeneration. *Developmental Biology* 432:63-71.

Thompson, S., L. Muzinic, C. Muzinic, M.L. Niemiller and S.R. Voss. 2014. Probability of regenerating a normal limb after bite injury in the mexican axolotl (*Ambystoma mexicanum*). *Regeneration* 1:27-32.

Voss, S.R., H.H. Epperlein and E.M. Tanaka. 2009. *Ambystoma mexicanum*, the axolotl: a versatile amphibian model for regeneration, development, and evolution studies. *Cold Spring Harbor Protocols 2009*. doi:10.1101/pdb.emo128.

Voss, S.R., M.R. Woodcock and L. Zambrano. 2015. Tale of two axolotls. *Bioscience* 65:1134-1140.

Voss, S.R. and K. Kump. 2016. A genotyping assay to identify carriers of *albino. Axolotl* 1:5-7. (http://www.ambystoma.org).

Woodcock, M.R., J. Vaughn-Wolf, A. Elias, D.K. Kump, K.D. Kendall, N. Timoshevskaya, V. Timoshevskiy, D.W. Perry, J.J. Smith, J.E. Spiewak, D.M. Parichy and S.R. Voss. 2017. Identification of mutant genes and introgressed tiger salamander DNA in the laboratory axolotl, *Ambystoma mexicanum. Scientific Reports* 7:6. doi:10.1038/s41598-017-00059-1.

Yokoyama, H., N. Kudo, M. Todate, Y. Shimada, M. Suzuki and K. Tamura. 2018. Skin regeneration of amphibians: A novel model for skin regeneration as adults. *Development, Growth and Differentiation* 60:316-325.