

Preface

We are excited to present this collection of protocols for the African turquoise killifish *Nothobranchius furzeri*—a promising vertebrate model system for the study of a wide range of traits and processes, including aging, age-related diseases, ecology and evolution, development, and “suspended animation.”

Killifish species populate the globe and often live in very harsh habitats—for example, transient ponds that completely dry up for most of the year. Many killifish species have short life spans, an adaptation to life in a harsh environment, and have been recognized since the 1960s for their potential use to study aging. About 20 years ago, Stefano Valdesalici from the Italian Killifish Association and Alessandro Cellerino from the Scuola Normale Superiore in Italy performed the first life span studies of the African turquoise killifish *Nothobranchius furzeri*, and the Cellerino laboratory proposed this species as a model for aging research. Dario Valenzano, then a graduate student in the Cellerino laboratory, conducted the first experimental aging studies on *N. furzeri*—examining behavior and histology during aging and testing the effect of external interventions on aging. As a postdoctoral fellow in the Brunet laboratory at Stanford University in the United States, Dario Valenzano developed the African turquoise killifish as a genetic model and sparked the interest of trainees in the Brunet laboratory. In parallel, the Cellerino laboratory set up collaborations with several groups at the Leibniz Institute on Aging in Germany to study the African turquoise killifish. In the past decade, there has been an explosion in the number of research groups pioneering crucial genomic tools for the study of the African turquoise killifish, including genome sequencing and genome-editing techniques, and using this model species to address multiple biological questions. We anticipate that this species will not only facilitate novel discoveries in vertebrate aging and other areas of research but may also help uncover new phenomena because of its “extremophile” properties: dispersed cell stage in development and long-term diapause, a state of “suspended animation.”

This protocol collection is designed both for research groups that already have an established African turquoise killifish colony in their laboratories and for groups who would like to start their own colony. It includes an introduction to the species, as well as standardized husbandry protocols for raising and breeding this fish efficiently, for maintaining a healthy colony, and for preserving lines via sperm freezing and in vitro fertilization. It also contains protocols for genetic manipulation—generation of knockout and transgenic lines—and their genotyping and sexing. Furthermore, a series of protocols present methods for the generation and analysis of genomics data sets. As the African turquoise killifish is frequently used for studies on aging, several protocols focus on standardized life span studies and assaying aging hallmarks. This collection additionally encompasses both general and specific methods to study different organs (notably in young and old individuals), with a particular focus on the brain. Finally, this collection also contains protocols for other interesting areas that can be probed using the African turquoise killifish, including injury repair, development, and diapause.

We would like to thank all the scientists who have contributed to this protocol collection. We are incredibly grateful for their excitement, careful work, and thoughtful contributions in preparing the different protocols. We would also like to thank Maria Smit, Inez Sialiano, Christin Munkittrick, Kathleen Bubbeo, Alejandro Montenegro-Montero, and Richard Sever at Cold Spring Harbor Laboratory Press for their invaluable help and enthusiasm about this book.

We hope that this protocol collection will be helpful for a wide range of researchers who work on the African turquoise killifish and other species of killifish such as the South American killifish, and

that it will be enhanced by additional online protocols as the community continues to grow. We also hope that this protocol book will help gather the growing killifish community and allow exciting studies in many different areas in the future.

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