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Letter to the Editor

Zebrafish, Killifish, Neither Fish, Both Fish?

To the Editor:

In their recent report (1), Herrera and Jagadeeswaran offer data supporting the use of killifish (annual fish) species over zebrafish as a genetic model for aging. We write to emphasize that, despite the limitations that killifish may circumvent, the zebrafish holds great promise as a model for aging research (2). In gerontology, as well as in other fields, a variety of model systems are used to a collective advantage. For each system, its inevitable disadvantages do not invalidate the exploitation of its advantages. For example, despite the inability to develop cancer, important cancer-related research continues to be conducted in yeast (3). Similarly, *Caenorhabditis elegans* is widely used in gerontology despite the lack of cell division potential in somatic cells of adults. No model is perfect, and based upon its numerous experimental advantages and its track record, the zebrafish should receive serious consideration in any area of biology.

A key advantage cited by Herrera and Jagadeeswaran for use of the killifish species was a short life span (<1 year). This contrasts with the ''relatively long'' life span of mice, which other authors have recently categorized as relatively short (4), and with zebrafish, which live longer than mice (5). The apparent divergence of views regarding longevity highlights an apparent contradiction in gerontology. Although a primary goal for the use of most genetic models is to identify genes that extend life span (6), a major criterion for the appropriateness of such models appears to be a short life span. We agree that the time required to obtain longevity data is a major practical consideration for gerontologists, especially given the time constraints imposed by the traditional 5-year funding period for investigator-initiated grants from the National Institutes of Health (NIH). Organisms with life spans exceeding 5 years are certainly a tougher sell than organisms whose longevity would fit within a standard grant period. As shown by Herrera and Jagadeeswaran, the life span of zebrafish may be well within this limit, depending upon whether median or maximum life span and a number of other factors (discussed below) are considered.

As indicated in the accompanying commentary by Austad (7), the life span of the zebrafish population studied by Herrera and Jagadeeswaran was much shorter than previously reported (5). A number of extrinsic variables may have contributed to this difference, such as temperature, although the primary difference may be due to the use of an inbred line, as discussed by Herrera and Jagadeeswaran. The inbred fish were also fed a more energy-rich diet than those reported previously, therefore caloric restriction may have played an important role. Another difference from previous longevity data, obtained using standing water tanks, was Herrera and Jagadeeswaran's use of a recirculating water filtration system (now conventional for zebrafish). The uniformity of water conditions plus the much larger population size may have contributed to the relatively rectangular zebrafish survival curves reported by Herrera and Jagadeeswaran.

The recirculating water filtration system seemed to have an even larger impact upon killifish life span but in the opposite direction. The killifish had an extremely short life span (~2 months) in the recirculating conditions that allow for very large zebrafish populations as mentioned by Austad. However, when housed in standing water tanks at a 5–10-fold lower population density, the killifish survival curves were longer but also much less rectangular. Neither Gompertzian-type analysis of the survival curves nor any documentation of the senescent morphology were reported, thus clues to causes of mortality were not evident. Based upon gathering experience with zebrafish (8), and previously with the guppy (9), a likely cause of premature deaths is infectious disease. Specific pathogen-free zebrafish (10) have recently become available.

Much investment has been made in invertebrate models of aging, which have life spans of weeks to months. However, as pointed out by Austad, their biological relevance to vertebrates may be distant based on evolutionary grounds. Indeed, we are in whole agreement that a vertebrate model with genetic tractability similar to invertebrates is needed in gerontology. This was one of the primary reasons that the zebrafish has risen to prominence first in developmental biology research (11), and now for disease modeling (12). A significant amount of funding has been devoted to building a research infrastructure for the zebrafish as a biological model. As shown in Table 1, the number of CRISP (Computer Retrieval of Information on Scientific Projects, http://crisp.cit.nih.gov) database grant hits as a proportion of all grants for the National Institute on Aging (NIA) versus all of NIH is about the same for mouse, *Drosophila*, and killifish, but two-fold higher for *elegans*, and many fold lower for zebrafish.

A Trans-NIH Zebrafish Initiative (13) has provided essential support for a wide variety of reagents and resources for zebrafish research, which have not yet been widely exploited for studies on aging (2,14–16). A key resource is the expected completion of the genome sequence by the end of 2005 (http://www.sanger.ac.uk/Projects/D_rerio/). This now seems to be a primary criterion for genetic models, as

Table 1. Absolute Number (% of All Grants) of CRISP Database Hits for Keywords *Killifish*, *Zebrafish*, *Elegans*, *Drosophila*, and *Mouse* Over the Past 20 Years for the NIA and All of NIH

	1984–1988	1989–1993	1994–1998	1999–2003
Killifish-NIA	0 (0)	0 (0)	0 (0)	1 (0.006)
Killifish-NIH	5 (0.002)	6 (0.002)	4 (0.001)	24 (0.006)
Zebrafish-NIA	0 (0)	0 (0)	2 (0.017)	10 (0.064)
Zebrafish-NIH	18 (0.008)	87 (0.034)	435 (0.138)	1327 (0.333)
Elegans-NIA	17 (0.310)	18 (0.222)	86 (0.717)	174 (1.135)
Elegans-NIH	245 (0.105)	465 (0.182)	889 (0.282)	2071 (0.520)
Drosophila-NIA	46 (0.84)	72 (0.888)	100 (0.833)	164 (1.070)
Drosophila-NIH	1906 (0.815)	2301 (0.898)	2908 (0.924)	4881 (1.225)
Mouse-NIA	194 (3.542)	731 (9.011)	1367 (11.390)	2988 (19.120)
Mouse-NIH	5477 (4.541)	8112 (9.754)	12002 (12.345)	15628 (19.852)

Note: CRISP = Computer Retrieval of Information on Scientific Projects; NIA = National Institute on Aging; NIH = National Institutes of Health.

evidenced by a requirement (NOT-DK-04-006) for a recent grant program on the use of model organisms for obesity-related traits (RFA-DK-03-018), cosponsored by the NIA, which requires that the model organism's sequence be completed. This emphasizes the point that, while it may eventually prove feasible to introduce mutations into the killifish genome (techniques that have yet to be implemented), and map them using reagents borrowed from zebrafish, identifying the underlying genes may be extremely difficult. Part of the rationale for the zebrafish genome sequence project was to facilitate the identification of the genes underlying the thousands of induced mutants obtained over the past 5–10 years.

Austad makes a strong case for the use of a fish species as a model for aging research, long overdue given the work of Comfort over 40 years ago (17). Herrera and Jagadeeswaran present interesting data for the killifish, which also follows and builds upon previous studies (18–20). However, the true power of the zebrafish and the killifish may lie in the combination of the two. A central approach in the comparative biology of aging is to compare two species whose life spans differ significantly and identify factors that could explain the difference (21). For example, findings in one invertebrate species are often examined in another invertebrate or in mice (22). Zebrafish and killifish may present an opportunity to investigate aging in two relatively closely related vertebrate species whose life spans differ by at least 3–4-fold. This was part of the rationale for studies on *Peromyscus leucopus* and *Mus musculus* (23), although the difference in life span between the species was approximately two-fold and the longevity of *Peromyscus* was 6–7 years. Indeed, a recently funded NIA grant to investigate the naked mole rat (R01AG022891), with a life span of well over 20 years, proposes to exploit a comparative approach with other shorter-lived species including *Mus musculus*. In comparison with these rodent models, the zebrafish and killifish may offer a far more efficient and productive pair of species on which to pursue comparative studies.

The explosion of information about zebrafish biology represents an exciting opportunity for gerontologists. The work by Herrera and Jagadeeswaran may help focus attention on the merits of zebrafish as a model for aging research.

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