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SHORT TAKE

Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish *Nothobranchius furzeri*

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Summary

Temperature variations are known to modulate aging and life-history traits in poikilotherms as different as worms, flies and fish. In invertebrates, temperature affects lifespan by modulating the slope of age-dependent acceleration in death rate, which is thought to reflect the rate of age-related damage accumulation. Here, we studied the effects of temperature on aging kinetics, aging-related behavioural deficits, and age-associated histological markers of senescence in the short-lived fish *Nothobranchius furzeri*. This species shows a maximum captive lifespan of only 3 months, which is tied with acceleration in growth and expression of aging biomarkers. These biological peculiarities make it a very convenient animal model for testing the effects of experimental manipulations on life-history traits in vertebrates. Here, we show that (i) lowering temperature from 25 °C to 22 °C increases both median and maximum lifespan; (ii) life extension is due to reduction in the slope of the age-dependent acceleration in death rate; (iii) lowering temperature from 25 °C to 22 °C retards the onset of age-related locomotor and learning deficits; and (iv) lowering temperature from 25 °C to 22 °C reduces the accumulation of the age-related marker lipofuscin. We conclude that lowering water temperature is a simple experimental manipulation which retards the rate of age-related damage accumulation in this short-lived species.

Key words: animal model; aging biomarker; age-dependent mortality; vertebrate aging.

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A total of 256 *Nothobranchius furzeri* were hatched and raised at the standard temperature of 25 °C as described (Genade *et al*., 2005). At age 4 weeks, when the fish are sexually mature (Genade *et al*., 2005), they were divided into two groups. One group of 145 (for a total of five independent trials) remained at 25 °C and a group of 101 fishes (for a total of two independent trials) was moved to 22 °C. The age-dependent survival of the two groups is reported in Fig. 1(A). Lowering ambient temperature significantly increased longevity by extending median lifespan from 9 to 10 weeks and maximum lifespan from 11 to 12.5 weeks (log-rank test, $P < 0.0001$). Analysis of age-specific mortality rates revealed that lowering the temperature causes a significant reduction in the slope from 1.19 to 0.50 (WinModest, log-likelihood ratio test, $\chi_1^2 = 8{,}3$; $P < 0.01$) and a significant increase of the age-independent mortality (WinModest, log-likelihood ratio test, $\chi_1^2 = 7{,}4$; $P < 0.01$) (Pletcher, 1999) (Fig. 1B). This datum confirms the effects of temperature shifts on longevity reported in the annual fish *Austrolebias bellotii* (Liu & Walford, 1975). Kinetic analysis revealed that, similarly to observations in *Drosophila*, lowering temperature reduces the slope of mortality rate acceleration. A possible interpretation is that increased longevity is tied with a reduction in the rate of age-dependent accumulation of biochemical damage. In fact, lowered temperature reduces age-dependent oxidation in *Drosophila* (Zheng *et al*., 2005).

Lowering water temperature also increases the age-independent mortality. This effect is different from that observed in *Drosophila* (Mair *et al*., 2003; Partridge *et al*., 2005), but it is well known that tropical fish become more susceptible to infectious diseases when exposed to low temperatures (Untergasser, 1989) and 22 °C is substantially lower than the average temperatures observed in the habitat of *N. furzeri* (average temperature in the Breitbrigde area during the rainy season January–March is 26.9 °C; data from www.worldweather.org).

Quantification of size at 9 weeks showed that fish moved to 22 °C were significantly smaller than the control group at 25 °C (Mann–Whitney *U*-test, $P < 0.0001$) (Fig. 1C). This result is not unexpected, as lowering temperatures is well known to reduce growth rates in fish (Brett & Groves, 1979). It is important to emphasize that, unlike worms and flies whose adult tissues contain mainly postmitotic cells, fishes continue to grow by cell mitosis after reaching sexual maturation (Charnov *et al*., 2001).

Locomotor activity was quantified by video-tracking. Control animals showed age-dependent reduction in average

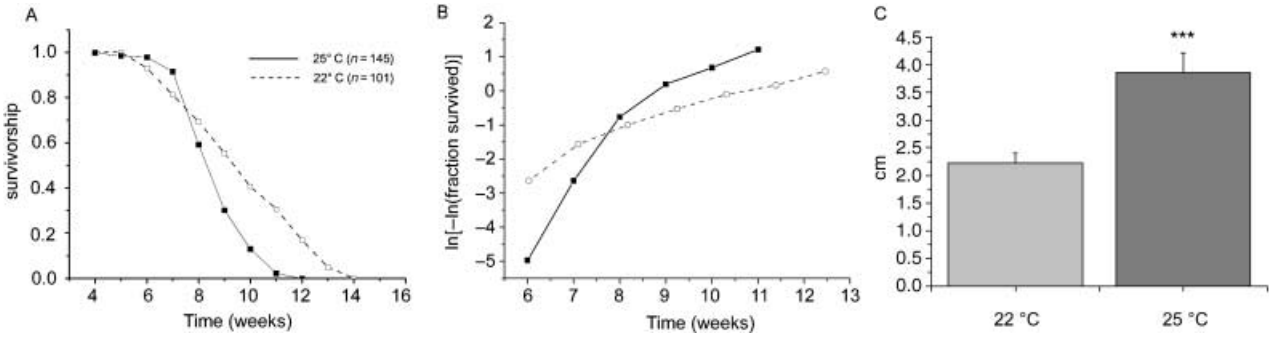

Fig. 1 Effects of temperature on survival and size in captive *Nothobranchius furzeri*. Survival curves (A) and death trajectories (B) in 256 fishes moved at age 4 weeks to tanks at 25 °C ($N = 145$) or 22 °C ($N = 101$) (log-rank test, $P < 0.0001$). The linear interpolations are performed for both groups from week 6 to maximum lifespan (week 11 for fish at 25 °C, week 12 for fish at 22 °C fishes). (C) Differences in size of 9-week fishes kept at 25 °C and 22 °C (***Mann–Whitney *U*-test, $P < 0.0001$).

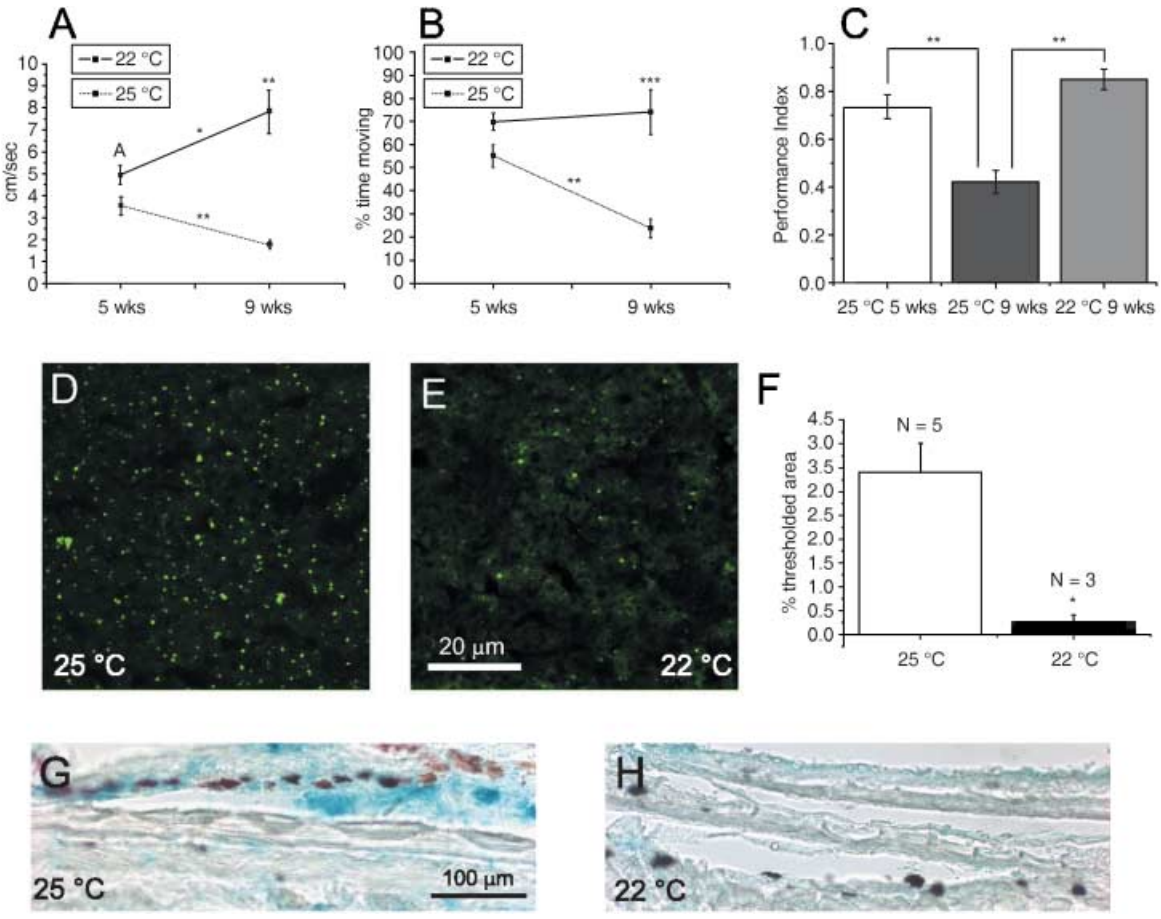

Fig. 2 Aging-related markers in fishes kept at 25 °C and 22 °C. Average velocity (A) and percentage of time spent moving (B) in open field exploration in fishes kept at 25 °C and 22 °C. (C) Learning scores in Active Avoidance task (see Supplementary materials) in fishes at 25 °C and 22 °C. The mark * above the line indicates statistical significance in score differences within the same group of animals at different ages. The marks * and ^ above points indicate statistical significance in difference among different groups of animal at the same age. $^P < 0.05$; $*P < 0.01$; $**P < 0.001$, $***P < 0.0001$. (D–E) Lipofuscin autofluorescence in livers of 9-week-old fishes at 25 °C (D) and at 22 °C (E) visualized with confocal microscopy and 488-nm laser excitation. (F) Quantification of labelling intensity. For each subject, three rectangular frames of fixed size were acquired and the images were thresholded at a fixed intensity and the number of pixels over threshold was measured using Metamorph ®. Senescence-associated beta-galactosidase activity (Kishi *et al*., 2003; Genade *et al*., 2005) in coronal sections of the fish caudal peduncle at 25 °C (G) and at 22 °C (H). Specific staining is the pale blue precipitate. Black cells are melanocytes and do not contain reaction product. The images are representative of three control and three experimental fish.

velocity (one-way ANOVA, $P < 0.001$) and time spent moving (one-way ANOVA, $P < 0.001$) from age 5 weeks to age 9 weeks. Motility deficit was totally prevented in animals moved to 22 °C (average velocity: Mann–Whitney *U*-test, $P < 0.001$; percentage of time spent moving: Mann–Whitney *U*-test, $P < 0.0001$) (Fig. 2A,B). Interestingly, fish moved to 22 °C showed higher mean velocity compared to those moved to 25 °C at 5 weeks of age (Mann–Whitney *U*-test, $P < 0.05$, Fig. 2A). In addition, at age 9 weeks, fish moved at significantly higher velocity than at age 5 weeks (one-way ANOVA, $P < 0.01$) (Fig. 2A). This indicates that lower ambient temperature induces a paradoxical increase in motility, although cold adaptation in fish up-regulates genes involved in muscular atrophy (Gracey *et al*., 2004). We cannot offer at the moment any explanation for this datum.

We quantified operant learning in *N. furzeri* by a modified shuttle-box originally developed for zebrafish and goldfish (Laudien *et al*., 1986; Pradel *et al*., 1999). The fish associates a light stimulus with a mechanical disturbance and learns to avoid the negative stimulus by crossing a hurdle upon light onset (Valenzano *et al*., 2006). Control fish were tested at 5 and 9 weeks. Young fish learned the task effectively and attained a 73% success rate after 50 consecutive trials (Fig. 2C). Nine-week-old fish kept at 25 °C showed significant learning but, at the end of the training, their success rate only reached 43%. The difference in the success rates between young and old fish was highly significant (Mann–Whitney *U*-test $P < 0.001$). Fish moved to 22 °C and tested at age 9 weeks attained an average success rate of 85%, which was significantly better than the controls kept at 25 °C (Mann–Whitney *U*-test $P < 0.001$).

We quantified lipofuscin autofluorescence, a known age-related marker of senescence (Brunk *et al*., 1992; Genade *et al*., 2005), in the liver of 9-week-old fish kept at 25 °C and 22 °C. We found a significantly lower autofluorescence in fish kept at lower temperature (Fig. 2D–E, two-tailed *t*-test, $P < 0.01$), indicating a lower amount of age-dependent lipid oxidation compared to fish kept at 25 °C. Analysis of senescence associated beta-galactosidase activity in the skin, a putative marker of cellular senescence in fish (Kishi *et al*., 2003; Genade *et al*., 2005), revealed lower expression in cold-adapted fish (Fig. 2G–H). These results confirm and extend the observation that lowered ambient temperature retards the onset of macroscopic aging symptoms in annual fish (Liu & Walford, 1966; Walford & Liu, 1965).

Recent molecular studies have revealed the pattern of genes regulated by long-term and short-term adaptation to cold in fish (Gracey *et al*., 2004; Malek *et al*., 2004; Podrabsky & Somero, 2004). Cold adaptation causes differential expression in genes involved in mitochondrial metabolism. A decrease in oxidative damage accumulation and a lower production of reactive oxygen species in the mitochondrial electron transport chain could be one of the mechanisms responsible for increased longevity and functional preservation in fish kept at lower temperature.

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The authors would like to acknowledge the following glants: FIRB RBAUOIA7BT to AC and FIRB FBAUO15YHM to LD.

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Supplementary material

The following supplementary material is available for this article online at www.blackwell-synergy.com:

Scheme S1.

(A) Scheme of the apparatus used for training. A tank (38 × 23 × 18 cm) was divided in two by a hurdle with a rectangular hole (3 × 3 cm). The two compartments are wedged-shaped to funnel the fish through the hurdle. The tank was filled with water from the home tank and the fish was left to acclimatize for 15 min before starting the test. Then the conditioned stimulus (red light) was delivered in the compartment were the fish was present followed by an aversive stimulus (a plastic stick whirling in the compartment). The fish always responded to the disturbance by moving to the other compartment. The aim of the test was to detect the acquisition of a strategy to escape the aversive stimulus by crossing the hurdle upon presentation of the conditioned stimulus.

(B) Scheme of the temporal structure of the test. The conditioned stimulus lasts for 30 s, if the fish does not move to the other compartment after 15 s, the aversive stimulus is delivered for 15 s. The fish moves to the other compartment, rests for 30 s and then the cycle is repeated. If the fish crosses the hurdle within 15 s before onset of the red light (i.e. before administration of the aversive stimulus) the trial is scored as a success, otherwise the trial.