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Short report

Lack of metabolic ageing in the long-lived flatworm *Schmidtea polychroa*

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ABSTRACT

Freshwater planarians have a large totipotent stem cell population allowing high rates of cell renewal and morphological plasticity. It is often suggested that they are able to rejuvenate during fission, regeneration and starvation. These features, together with the rapidly expanding molecular toolset, make planarians such as *Schmidtea polychroa* and *S. mediterranea* interesting for ageing research. Yet, the basic demographic and physiological data are lacking or still based on fragmentary observations of one century ago.

Here, we present the first longitudinal physiological study of the species *S. polychroa*. Survival, size and metabolic rate, measured by microcalorimetry, of a cohort of 28 individuals were followed over a period of three years. Sexual maturity was reached during the second month after which the worms continued growing up to 5 months. This initial growth phase was followed by alternating periods of synchronised growth and degrowth. Although mass-specific metabolic rates declined during the initial growth phase, no changes were found later in life. The absence of metabolic ageing may be explained by the very high rate of cell renewal during homeostasis and alternating phases of degrowth and growth during which tissues are renewed.

Surprisingly, all deaths occurred in pairs of worms that were housed in the same culture recipient, suggesting that worms did not die from ageing. Taking into account the metabolic and demographic data, we suggest that *S. polychroa* shows negligible ageing.

Detailed analyses of size and metabolic rate revealed a remarkable biphasic allometric scaling relation. During the initial growth phase (months 1–5) the allometric scaling exponent *b* was 0.86 while later in life, it increased to an unusually large value of 1.17, indicating that mass-specific metabolic rate increases with size in adult *S. polychroa*.

1. Introduction

Due to their high regenerative capacity and active stem cell population, freshwater planarians have drawn attention of biogerontologists. These worms maintain a stable population of totipotent stem cells, which are the only proliferating cells in the adult body (Baguñà and Slack, 1981; Newmark and Alvarado, 2002). These cells, called neoblasts, are responsible for the very high rate of somatic cell renewal during normal tissue homeostasis (Pellettieri and Alvarado, 2007). They also allow an impressive morphological plasticity of the planarian during fission (asexual reproduction), regeneration (ability to regenerate a complete organism from any tiny body fragment), and starvation (controlled shrinkage during prolonged starvation) (Saló, 2006).

Interestingly, these three processes are often claimed to induce rejuvenation in these worms. This is based on the observations of an everlasting clonal lifespan in asexuals, a lifespan extension after inducing regeneration or starvation and the classical metabolic experiments of Child and Hyman (Child, 1911, 1915; Egger et al., 2006; Haranghy and Balázs, 1964; Hyman, 1919a, 1919b, 1919c). Child and Hyman indirectly measured oxygen consumption by means of the susceptibility and Winkler methods and found that planarian metabolic rate appeared to decrease with age. After fission, regeneration and starvation, they noticed that metabolic rates were restored to juvenile levels, leading to the conclusion that these are rejuvenating transformations (Child, 1911, 1915; Hyman, 1919a, 1919b, 1919c). These metabolic data should be interpreted with caution. Hyman studied metabolic changes only during development and not during the ageing process (Hyman, 1919a). Child measured metabolic rates during both development and adulthood, but the adults were selected based on their size, which was used as a proxy of physiological age (Child, 1911, 1915). Because adult planarians may repeatedly grow and shrink during adulthood, this study is severely flawed and little can be derived concerning metabolic patterns in ageing planarians. In addition, the rejuvenation hypothesis became equivocal as later metabolic studies of fission, regeneration and starvation reported contradictory results (Allen, 1919; Brøndsted, 1969; Pedersen, 1956). Surprisingly, no genuine longitudinal metabolic studies of ageing flatworm cohorts

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have been performed and it is yet unknown whether these animals show a clear metabolic decline at advanced age or whether metabolic rejuvenation occurs after regeneration or starvation.

In the three-year longitudinal study presented here, we analyse the correlation among size, age and metabolic rate during both development and adulthood of the planarian *Schmidtea polychroa*. Our data provide evidence that mass-specific metabolic rate decreases during the initial growth period, but remains constant during adulthood. Our demographic data suggests that this planarian species may not age significantly after all. We further show a remarkable morphological plasticity in our flatworm cohorts and suggest that there is an age-related biphasic allometric scaling relation.

2. Material and methods

2.1. Species, culture and design of the longitudinal experiment

S. polychroa is a free-living freshwater planarian (Platyhelminthes). Because it is a cocoon-laying species, the lifespan can be simply defined as the time from hatching till death.

Standard lab cultures were maintained in square plastic containers with a surface of about 200 cm^2, filled with about 300 ml 1:1 tap water: distilled water and incubated at 20 °C in the dark. The worms were fed weekly with raw veal liver. Small pieces of liver, purchased locally every few months, were kept at −20 °C and before feeding, several of these pieces were chopped into thin slices while they thawed. After the worms stopped showing feeding behaviour (usually about 4 h) cocoons were collected, containers were cleaned and the medium was renewed. The collected cocoons were kept in a Petri dish with fresh medium.

For the longitudinal study, individuals of the same age were obtained by collecting juveniles that hatched within a 24-hour time frame from several cocoons produced by a batch of about 30 parthenogenic adults. The experiment started with 14 replicates. Each replicate contained 2 animals as sufficient biomass was necessary for accurate microcalorimetry. The experimental animals were maintained, per replicate, in a Petri dish under the same conditions as the standard cultures. Survival was scored weekly, after feeding. If one of the two individuals died, the replicate was excluded from further metabolic measurements. Metabolic rate was measured monthly, three days after feeding to control for the influence of food intake and digestion on metabolic rate. One day after microcalorimetry, pictures were taken for automated analysis of body surface. As accurate life tables of *S. polychroa* do not exist, we ran the study until the median lifespan of the cohort was reached.

2.2. Measuring metabolic rate and normalisation to body surface

During the longitudinal study, metabolic rate and a normalisation parameter were measured in a non-destructive manner. Metabolic rate was determined by means of microcalorimetry, a very accurate technique that directly measures metabolic heat which reflects total catabolism, including fermentation (Braeckman et al., 2002a, 2002b). The metabolic heat produced by each replicate was quantified with the thermal activity monitor from Thermometric (Järfälla, Sweden). Before measurement, the two worms of a replicate were transferred to a 20-ml glass ampoule containing 2 ml culture medium. After a 1–2 h equilibration period, stable power (μW) recordings were obtained for at least 3 h at 24 °C.

Mass-specific metabolic rate was obtained by dividing the total heat production rate of a replicate by the summed surface area of the two worms. Worm surface area was measured by transferring the slow moving animals to a moist rolling paper. At each time point, five replicate photographs of each individual were taken with an Olympus C-5050 Zoom camera mounted on an Olympus SZX12 stereo microscope. Body surfaces were quantified by semi-automated image analysis (KS400 software, ZEISS).

To verify whether body surface area is an appropriate normalisation parameter, body surface and the commonly used biomass proxies protein content and wet weight were measured in 48 individuals of different sizes. We found a highly significant correlation between body surface and both protein content ($R^2 = 0.97$; $P<0.0001$) and wet weight ($R^2 = 0.97$; $P<0.0001$) (Fig. 1).

2.3. Statistical analyses

The accuracy of body surface as normalisation parameter was verified by performing Pearson's product-momentum correlation tests between body surface, wet weight and protein content.

Because of the limited population size, mortality rates were calculated over 5-month intervals. To test if the log mortality rate significantly increases with age, a linear regression analysis was performed.

To investigate whether age and/or size had a significant effect on mass-specific metabolic rate, a linear mixed model was fitted to the data (Proc mixed SAS 9.2). Here, we related the effect of 'age', 'body surface' and their interaction 'age*body surface' to the mass-specific metabolic rate. Both independent variables were treated as continuous variables. As the same replicates were repeatedly measured over time, replicate and its interaction with age were included as random effects. Detailed analyses were performed on the following data subsets: development (months 1–3), the initial growth phase (months 1–5) and adulthood (≥3 months).

For the determination of the scaling exponent *b* during the complete lifespan, we log-transformed all body surface and metabolic rate data. Data from worm replicates undergoing the typical severe degrowth (<35mm^2) which is preceding death were censored. Next, linear regression was performed and the parameter values were determined. This function can be written as the log form of the allometric scaling law,

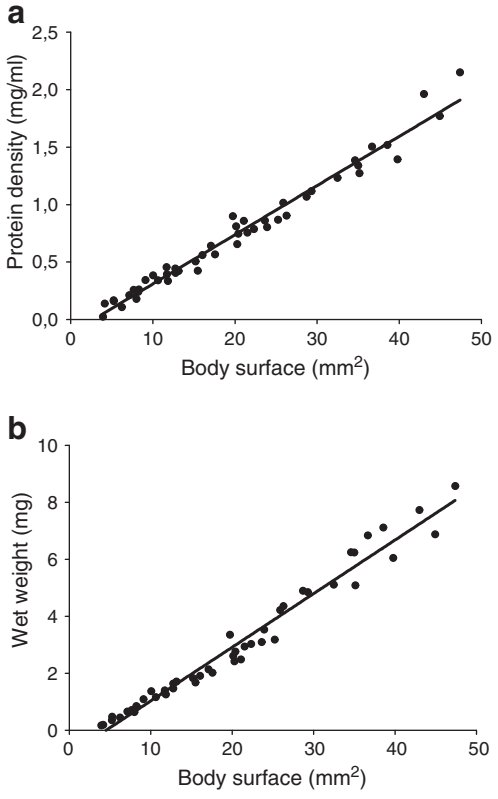

Fig. 1. (a) Protein density as a function of body surface. (b) Wet weight as a function of body surface.