

Colder rotifers grow larger but only in oxygenated waters

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Abstract. Why do colder ectotherms grow more slowly but mature at a larger size? Some researchers have argued that oxygen supply and demand play a crucial role in these processes, but many studies conflated the effects of oxygen and temperature. We studied the body sizes of rotifers (*Keratella cochlearis*) at different depths in 20 European lakes, taking advantage of gradients in oxygen and temperature during summer, when dense, cool waters sink to low depths and become hypoxic. Rotifers were larger in colder waters, but only in the presence of abundant oxygen. In hypoxic waters, rotifers remained small regardless of temperature. We propose that oxygen supply generates a ceiling for maximal possible body size, especially in environments that elevate metabolic demands. Under this condition, any of several processes—developmental plasticity, genetic divergence, size-dependent mortality, or size-dependent selection of microhabitats—could cause a wider range of body sizes in more oxygenated waters, where the maximal possible size exceeds the adaptive size at any temperature.

Key words: Bergmann's rule; body size; cell size; *Keratella cochlearis*; lakes; oxygen limitation; Poland; rotifers; temperature-size rule; thermal gradient.

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INTRODUCTION

Whether they be protists, plants or animals, warmer ectotherms usually grow faster but mature at a smaller size (Atkinson 1994). This unintuitive pattern of thermal plasticity, called the temperature-size rule, has fuelled much debate about its adaptive significance (Sibly and Atkinson 1994, Angilletta and Dunham 2003, Kozłowski et al. 2004). Following principles of life-history evolution, an organism's size at maturity reflects a compromise between the benefit of being large and the cost of delaying reproduction. Environmental temperature presumably modulates this benefit and cost (Angilletta et al. 2004, Kozłowski et al. 2004). The same principles explain the evolutionary divergence of

body size among populations. For example, body sizes of *Drosophila melanogaster* diverged among populations along latitudinal clines (Zwaan et al. 2000) and in laboratory experiments (Partridge and French 1996) such that larger size evolved in colder environments—a pattern consistent with Bergmann's rule (Partridge and Coyne 1997).

Body size depends on the number and size of cells, as well as the volume of extracellular matter (Arendt 2007, Hessen et al. 2013). In drosophilids, simultaneous changes in cell size and number underlay thermal plasticity and geographic clines of body size (Zwaan et al. 2000). Changes in the number and size of cells also occur when body size evolves between species (Kozłowski et al. 2010). These changes have consequences for physiological functions (Szarski

1983, Woods 1999, Kozłowski et al. 2003, Atkinson et al. 2006, Starostova et al. 2009, Czarnoleski et al. 2013, 2015). Collectively, small cells have a large surface area relative to volume, which requires more ATP to turnover phospholipids and generate electrochemical gradients. Nevertheless, organs with small cells should metabolize faster because of greater surface area for transport, shorter distances for diffusion, and more nuclei for transcription. A thermal dependence of cell size should emerge from this tradeoff between meeting metabolic demands and conserving energy.

Although oxygen supply and demand must influence body size and cell size, many researchers measuring the thermal dependence of body size and cell size have conflated the effects of oxygen and temperature (but see Chapelle and Peck 1999, Atkinson et al. 2006). Here, we use samples from different depths of lakes to study the body sizes of rotifers (*Keratella cochlearis* Gosse 1851) in natural gradients of temperature and oxygen. We took advantage of the thermal stratification within lakes during summer, when dense, cool waters sink deeply and become hypoxic. Because all individuals of *K. cochlearis* develop the same number of somatic cells (Wallace 2002), a difference in body size between two rotifers reflects a difference in cell size. This property enabled us to infer how environmental conditions influenced cell size as well as body size. We predicted that hypoxic waters, whether warm or cold, favor small cells and small bodies, whose large surface relative to volume facilitates the uptake of oxygen. On the other hand, rotifers in well oxygenated waters, which experience relaxed limits on body size and cell size, should exhibit a thermal dependence that follows the temperature-size rule and Bergmann's rule.

METHODS

Water temperature, oxygen concentration, and body size of *K. cochlearis* were measured in twenty lakes, located in three districts of Northern Poland. Lakes from Suwałki district (Hańcza, Jaczno, Jegłówek, Kamendul, Kojle, Perty, Szelment Mały, Szelment Wielki, Szurpity) were studied in 1983–1985. Lakes from Mazury district (Beldany, Kisajno, Mikołajskie, Ryńskie, Święcajty, Tałtowisko, Tałty, Tyrkło) were studied in

1996. Lakes from Konin district (Licheńskie, Skulsk, Ślesieńskie) were studied in 2010. The biotic and abiotic conditions of these lakes were described elsewhere (Hillbricht-Ilkowska and Zdanowski 1988, Zdanowski et al. 2002).

During a period of stagnation (July/August), water was collected with a 5-L Bernatowicz's sampler at depth intervals of 1 m in the deepest part of each lake. Water temperature and oxygen concentration were measured to the nearest 0.1°C and 0.1 mg/L, respectively, with an electronic meter (Hillbricht-Ilkowska and Wiśniewski 1994). Thermal profiles were used to assign samples to epilimnion, metalimnion, or hypolimnion. Water from the same layer was pooled within each lake, filtered through a plankton net (mesh = 30 µm) and lengths of at least 30 adult rotifers were measured with a calibrated ocular micrometer (Nikon Eclipse-E600, Nikon, Tokyo, Japan) to the nearest µm, excluding appendages (Fig. 1). Consequently, each layer of each lake was characterized by a mean water temperature and oxygen concentration and a mean body size of rotifers.

We used the nlme library (Pinheiro et al. 2011) of the R Statistical Package (R Development Core Team 2011) to model effects of water temperature and oxygen concentration on body size. Geographic district and lake (nested within district) were included as random factors. We started with a model that included the interaction between temperature and oxygen and used AIC to decide whether to keep this interaction.

RESULTS AND DISCUSSION

Although colder water holds more oxygen (Verberk et al. 2011), the cold water deep within lakes becomes depleted of oxygen, which reverses the typically negative relationship between water temperature and oxygen concentration. Experiments on various species have shown that either lower temperature or more oxygen causes ectotherms to grow larger bodies (Atkinson 1994, Stelzer 2002, Harrison et al. 2010) and larger cells (Heinrich et al. 2011, Czarnoleski et al. 2013, Walczyńska et al. 2015). Indeed, we observed larger rotifers of *K. cochlearis* in cold ($F_{1,47} = 3.91$, $P = 0.05$) or oxygenated waters ($F_{1,47} = 14.93$, $P < 0.001$), whereas cold but hypoxic waters produced rotifers of intermediate size (Fig. 1).

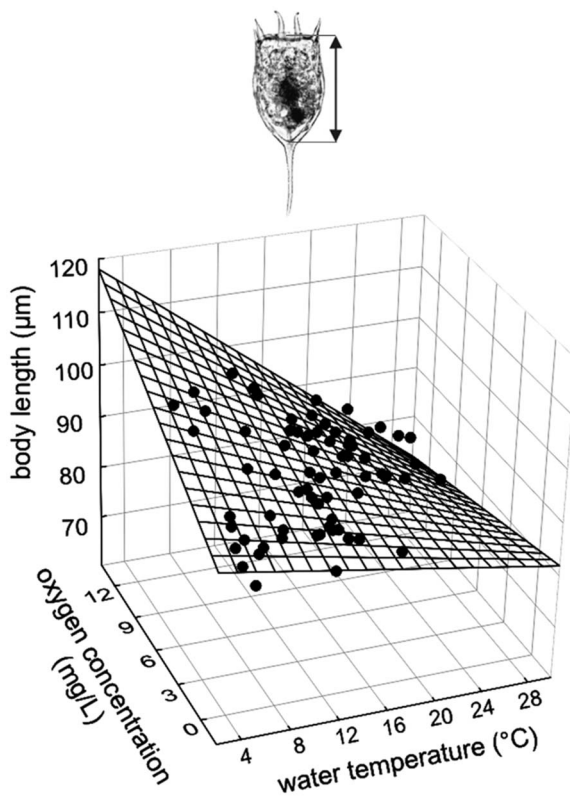


Fig. 1. Body size of rotifers increased with decreasing temperature, but this relationship was steeper in waters with more oxygen. Symbols represent data from different layers in twenty lakes. The plane displays the fit of the most likely statistical model.

Importantly, oxygen supply determined the relationship between temperature and body size (interaction: $F_{1,47} = 7.45$, $P < 0.01$). Where oxygen was abundant, rotifers followed the temperature-size rule and Bergmann's rule, such that colder waters contained larger rotifers (Fig. 1). In hypoxic waters, rotifers remained small regardless of temperature. This interaction between oxygen supply and environmental temperature could have resulted from metabolic constraints. As animals become larger, oxygen must travel longer distances through more pathways to reach cells. At a certain size, the circulatory system cannot cope with the demand for oxygen during activity, creating a ceiling for metabolic performance (Chapelle and Peck 1999, Peck et al. 2009, Glazier 2010, Pörtner 2010, Verberk et al. 2011). This concept was used to understand evolutionary patterns of size among terrestrial insects

(Harrison et al. 2010) or ecological patterns of size among marine amphipods (Chapelle and Peck 2004). Following this theory, hypoxia should lower the ceiling on maximal possible body size such that rotifers become smaller regardless of their temperature. This response should speed metabolism because the reduction of body size reduces diffusion distances. Moreover, the concomitant reduction of cell size should speed diffusion within tissues (Woods 1999, Atkinson et al. 2006, Czarnoleski et al. 2013, Czarnoleski et al. 2015), because oxygen diffuses more readily through membranes than through cytoplasm, especially under warm conditions (Subczynski et al. 1989). By contrast, an abundant supply of oxygen raises the maximal body size, such that adaptive sizes in warm and cold waters fall within the range of possible sizes. Under this condition, any of several processes—developmental plasticity, genetic divergence, size-dependent mortality or size-dependent selection of microhabitats—can cause the relationship between water conditions and body size or cell size that we observed.

An abundance of oxygen enables an organism to grow larger but does not dictate this outcome. Although larger individuals typically reproduce faster and avoid predators better, growth drains resources from other functions and prolonged growth increases the risk of death before reproduction. Consequently organisms should rarely develop their physiologically maximal size, because risky environments favor earlier maturation at a smaller size (Stearns 1992). Moreover, reaching the maximal size would deprive an organism of surplus energy for reproduction, which would be maladaptive (Czarnoleski and Kozłowski 1998, Kozłowski et al. 2004). In other words, the temperature-size rule emerges when natural selection favors genotypes that mature earlier at a smaller size in warmer environments (Kozłowski et al. 2004). Accordingly, Walczyńska et al. (2015) demonstrated a fecundity advantage of large rotifers (*Lecane inermis*) in cold water, but small rotifers in warm and hypoxic water. For eutelic species, such as *K. cochlearis*, growth and reproduction involve a tradeoff between cell size and body size. Developing small cells to enhance oxygen delivery (Czarnoleski et al. 2013, 2015) would force a rotifer to remain small. Fusing cells into multinucleated syncytia, a common phe-

nomenon in rotifers (Wallace 2002), creates larger cells relative to body size, but no mechanism exists for creating smaller cells relative to body size. This constraint poses an unusual problem, because most other metazoans can change cell size independently of body size (Czarnoleski et al. 2013, Maciak et al. 2014).

If our findings generalize to other species, temperature should exert the weakest effect on body size in the most hypoxic environment, where the fewest sizes are possible. Because water holds less oxygen than air does (all else being equal), aquatic environments should impose a lower ceiling for the body mass of ectotherms compared to terrestrial environments. Yet, comparisons of aquatic and terrestrial species revealed that aquatic species exhibit either equal (Klok and Harrison 2013) or greater (Forster et al. 2012) thermal plasticity of body size. According to Verberk and Atkinson (2013), greater thermal plasticity among aquatic species results from unique physical challenges of respiring in water coupled with a greater supply of oxygen in cold water. Yet, our study included samples from cold, hypoxic waters, an environmental scenario ignored by synthetic analyses at the interspecific level. Moreover, interspecific comparisons cannot control for adaptations of body plan, cardiovascular system, or life history that influence body size independently of temperature and oxygen. Thus, our intraspecific comparison revealed an interaction between temperature and oxygen by eliminating the confounding factors that plague interspecific comparisons. Such studies will help to forecast impacts of climate change on species that experience hypoxia in the depths of oceans or on the tops of mountains.

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