

Allometric scaling improves characterizing complex community transcriptomes

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Allometric scaling, i.e. scaling patterns in physiological processes relative to body size, can be used to overcome the current limitations in metatranscriptomics. Metatranscriptomics refers to the use of RNA transcripts to characterize a complex community. In contrast to metagenomics, metatranscriptomics allows you to simultaneously address community composition and functionality through the characterization of the community transcriptome. Hence, insights into metabolic processes and molecular pathways can also be obtained. Despite its increasing use in community ecology, a major limitation and source of error is the variation in RNA transcript abundance across organisms varying in body size. Hence, this may lead to incorrect estimations of the community structure and functioning as larger RNA quantities from larger individuals may overestimate their community contribution.

The recent approach put forward by Lopez et al. (2022) focusses on the extension of two major ecological concepts: (1) the growth rate hypothesis (GRH) and (2) the metabolic theory of ecology (MTE) to validate allometric scaling in RNA transcript abundance using metatranscriptomic analysis. The GRH hypothesis predicts an increase in RNA and phosphorus content with an increasing growth rate, both within as among taxa (Elser et al., 2008). In essence, it links growth rate to the stoichiometry of element use by an individual organism. The latter hypothesis predicts how the metabolic rate controls all ecological processes at all levels of biological organization (Brown et al., 2004). The integration of these two concepts predicts that whole body RNA and P content are tightly linked to energy fluxes in organisms that should influence growth rate. It also assumes that RNA transcript production per mass of tissue is expected to vary with both body size and temperature (Gillooly et al., 2004). Lopez et al. (2022) now aimed to provide empirical genomic and gene-level evidence using next generation sequencing of a mock community.

Using five model organisms, for which reference genomic sequences are available, in a mock community, Lopez et al. (2022) were able to investigate transcriptome wide analyses of the relationship between RNA transcript abundance and body size. Given that RNA abundance is expected to vary with body size and temperature, they studied these communities at three different temperature conditions. Their empirical evidence supports the MTE-GRH theory that faster growing, smaller species harbour more RNA per tissue mass compared to slow-growing, larger species, on the genomic level, as well as on the gene level (Figure 1). Ultimately, Lopez et al. (2022) validated their gene-specific allometric exponents obtained from the model organism mock community in a field study. There, they observed that allometrically scaled biomass is better suited to explain proportions of RNA transcript abundance within the metatranscriptome of a zooplankton community. In addition to this, the study also contributes to more wider implications of the MTE-GRH theory. First, the RNA abundance in function of body size is an inherent consequence of the effect of body size on mitochondrial density and ATP production. As such, mitochondrial encoded transcripts can be used as

proxy measurements for energy fluxes within a community. Therefore, metatranscriptomics becomes a relevant tool for community structure and functioning, but can also serve as an estimate of the energy flows within the ecological system.

However, Lopez et al. (2022) noticed a less pronounced body size dependence on transcript abundance at the gene level, among the studied mitochondrial and nuclear-encoded genes, than observed at the genomic level. Their findings contradict the universality of the MTE assumption that mass-specific metabolic processes scale with biomass to the $-1/4$ power. Therefore, Lopez et al. (2022) suggest the 'metabolic level boundaries' (MLB) hypothesis as an alternative explanation for the mechanistic relationship between the organisms' ecology and the scaling of metabolic processes with body size. This MLB, as postulated by Glazier (2005), concludes that the scaling of metabolism is not the simple result of a physical law, but rather is the more complex result of diverse adaptations that have evolved in the context of both physico-chemical and ecological constraints. Hence, the MLB suggests that several ecological factors drive metabolic rate and, thus, explain variations in the allometric exponent. Lopez et al. (2022) also propose the dynamic energy budget (DEB) hypothesis as an alternative explanation, which describes simple rules for metabolic organization, i.e., how organisms acquire and use resources for growth, development and reproduction (Kooijman, 2000, 2001). In contrast to the MTE assumption, DEB assumes that allometric scaling of the weight specific metabolic rate should be $-1/3$, considering that energy/resource acquisition is proportional to surface area, while energy demand is proportional to volume, thus, essentially originating from the surface to volume relationship (Kooijman, 2000, 2001). In order to arrive at a sound theoretical underpinning of RNA metabolic scaling, future studies should fully investigate all these different views.

Additionally, the assumed temperature dependence of RNA transcript abundance was not observed in the study of Lopez et al. (2022). They found that the correlation between temperature and mass-corrected transcript abundance regarding temperature dependence contradicts the MTE prediction, as different genes respond differently to fluctuations in the temperature.

In conclusion, their approach puts forward a first step to reduce and/or remove biases caused by wide size variation in meta-community samples. A further extension and validation with more species with wider size structures is required to lead to more improvements in the application of metatranscriptomics. This is especially true for zooplankton communities (e.g. Figure 2), which can be very diverse and are known to contain organisms in the microscale up to organisms that can be a couple of meters long (megaplankton). Understanding the consistency of the effect of allometric processes on RNA transcript abundance, as well as conditions under which allometric patterns might emerge, is crucial for evaluating the extent to which integrating allometry can improve RNA abundance/biomass correlations and, ultimately, help operationalize RNA to monitor abundance, biodiversity and ecophysiological processes in natural ecosystems. Nevertheless, these findings do provide a foundation for extending the utilization of transcript abundance data, derived from metatranscriptomics, as a unit to quantify several ecological processes. Ultimately, the field of metatranscriptomics will be able to improve our understanding on the dynamics of ecosystem functions within complex communities.

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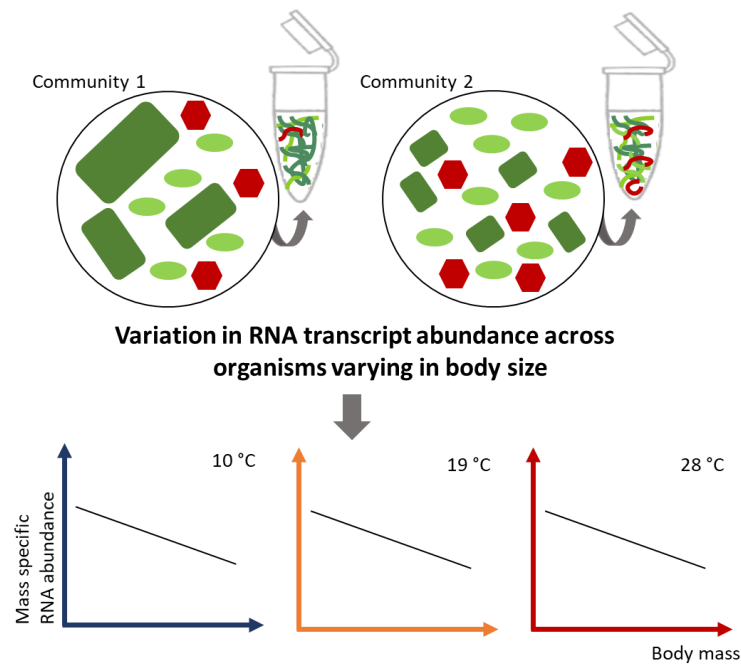


FIGURE 1 Conceptual figure illustrating the variation in the number of RNA transcripts originating from organisms with different body sizes. This variation might introduce bias/errors in metatranscriptomic analyses. Lopez et al. (2022) found a negative correlation between total transcript abundance and individual body mass and tried to align these results with assumptions of several metabolic theories. They found that faster-growing smaller species harbour more mRNA and rRNA transcripts per tissue mass compared to slow-growing larger species. Testing at three different temperatures, the authors did not find the assumed temperature dependence of this relationship.



FIGURE 2 A zooplankton sample from the Belgian part of the North Sea, including copepods, amphipods, tunicates and fish larvae. The article by Lopez et al. (2022) uses zooplankton samples to validate whether allometrically scaled biomass is suitable to explain RNA transcript abundance. Image courtesy of Ilias Semmouri.