Stress responses of reproduction and behavior in the marine rotifer *Brachionus* sibling species: Adaptation to temperate and tropical habitats

（海産ワムシ姉妹種の生殖及び行動のストレス応答と温帯・熱帯域への適応）

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Rotifers are significant second consumers in hydrosphere food webs, which can transfer both the energy and pollutant to higher trophic levels. Therefore, they have widespread applications in aquaculture and ecotoxicology studies. Rotifer mass production is important for improving aquaculture productivity. However, they may encounter different kinds of stressors during cultivation, such as high levels of unionized ammonia (NH_3-N), increased as the rotifer population expands, and overdosed trace metals. In aquaculture operations, iron (Fe) and zinc (Zn), as essential trace nutrients, are added to the culture medium of marine microalgae. The algae suspension without filtration, known as a “green water” technique, was commonly supplied in the culture tanks of rotifers or fish larvae. Whilst this procedure may put rotifers at a risk of trace metal overexposure or overload, which could induce deleterious effects. Furthermore, some rotifer sibling species from different habitats, such as temperate water species *Brachionus plicatilis* and tropical species *Brachionus rotundiformis*, exhibit distinct tolerance to ecological factors and show different growth characteristics even under similar culture conditions. It is expected that the two species may represent varied reproductive strategies regarding stressful situations because of their habitat-evolutionary experience, nevertheless, the underlying mechanisms have not been uncovered. Based on these considerations, this study aims to (1) evaluate the stress tolerance and reproductive flexibility of the two rotifer sibling species, *B. plicatilis* and *B. rotundiformis*, and (2) identify the underlying mechanisms that regulate their stress-reproductive adaptations following exposure to different levels of Fe (ferrous sulfate) (Chapter II), Zn (zinc chloride) (Chapter III), and ammonia (ammonium chloride) (Chapter IV), respectively.

First, the median lethal concentrations (LC_{50}) of $FeSO_4 \cdot 7H_2O$, $ZnCl_2$, and NH_4Cl (expressed as NH_3-N form) to the employed two rotifer species were determined after 24 h acute exposure. Because the tolerance of toxicants was significantly affected by the animal size, thus, to compare the toxic tolerance in the two species, I transformed the LC_{50} values by dividing the dry weights (DW) of rotifers. In this transformation, the Fe and NH_3 tolerance in *B. rotundiformis* were greater than in *B. plicatilis*, with 1.9- and 1.5-fold higher LC_{50}/ng DW values, respectively. While in response to Zn exposure, the two rotifers showed similar transformed values.

Second, rotifer reproductive patterns and swimming speed, a sensitive behavior biomarker in rotifer toxicology studies, were investigated following chronic exposure. Two species were

exposed to $FeSO_4 \cdot 7H_2O$ (0–96 mg/L), $ZnCl_2$ (0–2 mg/L), and NH_3-N (0–29.3 mg/L) individually or in batches. The results revealed that the two rotifer species displayed species-specific reproductive sensitivity associated with chemical concentrations, especially in sexual reproduction. The mixis induction and resting egg production in *B. plicatilis* were more susceptible to either the trace elements or NH_3-N exposure. Conversely, *B. rotundiformis* showed higher resilience in response to the same treatments, with enhanced sexual reproductivity. In behavior observations, both species demonstrated increased swimming speed following Zn and NH_3-N exposure. Whereas exposure to Fe affected differently in females and males in both species. Fe concentrations of 20–45 mg/L resulted in a decrease in male swimming speed but an increase in female swimming speed.

Third, to uncover the underlying mechanisms involved in the different reproductive flexibility of the two species, the biological activities that regulate rotifer reproductive fitness, such as lipid (an energy source) accumulation in female reproductive organs and male sperm viability were estimated. Additionally, stress response-associated metabolisms, such as intracellular reactive oxygen species (ROS) production and antioxidant activities were evaluated. Consequently, the two rotifers demonstrated differential modulations in lipid accumulation, sperm viability, and ROS scavenging responses, with overall inhibition in *B. plicatilis* but stimulation in *B. rotundiformis*. The reduced neutral lipid accumulation and downregulated lipid-synthesis-related biomarkers (e.g., *acetyl-CoA carboxylase* and *mitochondrial cytochrome*) were observed in *B. plicatilis*. The suppressed antioxidant activities in *B. plicatilis*, evidenced by inhibited SOD, CAT, and CYP, suggested that stress-induced ROS was not neutralized by its defense systems, thereby negatively affecting rotifer reproduction. Furthermore, Fe and Zn exposure sensitively inhibited the sperm viability of *B. plicatilis*. In contrast, *B. rotundiformis* exhibited active defensive responses, with enhanced antioxidant activity and lipid synthesis, implying the higher resilience of *B. rotundiformis* in response to stressful situations.

In conclusion, this study determined species-specific stress response patterns in tolerance and reproductive flexibility between the temperate and tropical marine rotifer sibling species. The potential mechanisms involved were established by lipid and ROS scavenging metabolisms. Different sexual reproductive sensitivity associated with inhibited/stimulated lipid metabolic in two rotifers, implied the significance of lipids in regulating rotifer sexual reproduction. Furthermore, stress stimulating effects on rotifer swimming speed suggested an indirect impact of rotifer reproduction via behavior regulations. Additionally, in aquaculture practice, the greater tolerance or resilience of *B. rotundiformis* in response to stress exposure, supported its high-density endurance during mass cultivation. Finally, the different modes of stress response in the two rotifers might be related to their habitat evolutionary adaptations, which should be investigated further.