

Effects of Immune Activation Through Bacterial Lipopolysaccharides and Food Deprivation on the Behaviour and Standard Metabolic Rate of Western Pygmy Perch (Nannoperca vittata)

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Western pygmy perch (*Nannoperca vittata*; an endemic freshwater fish of south-western Australia) infected with *Photobacterium damsela* subsp. *damsela* (Pdd) exhibit increased standard metabolic rate (SMR), activity, and boldness in a previous study. However, it is unclear whether these changes in metabolic rate and behavior were caused by direct manipulation by the parasite, the host immune response, a side effect of infection (e.g., nutritional stress), or a combination of all of these variables. To investigate the causal relationships between infection, metabolic rate, and fish behaviour, uninfected fish were exposed to a one-week immunological challenge (to provoke an immune response) with bacterial lipopolysaccharides (LPS) and food deprivation using a fully factorial experimental design. SMR, activity trait track length (TL), boldness trait emergence time (ET), and time spent in the risky zone (RZ) of the same individual fish were measured weekly for three weeks before and after immune challenge/food deprivation using intermittent flow respirometer and standard behavioural tests (open field test, emergence test). The impact of immunological challenge and food deprivation on metabolic and behavioral aspects of *N. vittata* were assessed using generalized linear mixed models. Food deprivation led to an increase in activity trait, TL, and in SMR, but had no effect on the boldness traits RZ and ET. Injection with LPS had no effect on any traits, with no evidence for any additive or synergistic effects when in combination with food deprivation. Food-deprived fish displayed an increase in activity immediately following treatment, while SMR rose three weeks later. These findings indicate that nutritional stress, rather than parasite manipulation or host immunological response, is a significant driver of the alterations in metabolic rate and behavior observed when *N. vittata* is challenged with Pdd. In food-deprived fish, an increase in activity may be linked to hyperphagia, which is associated with compensatory growth after a period of weight loss. This increase in activity is followed by an increase in SMR.

Keywords: Behavior, Food deprivation, Immune activation, *Photobacterium damsela* subsp. *damsela*, Standard metabolic rate