**Supplemental table 1:** Rationale for using difference physiological metrics that were examined in this study.

|  |  |
| --- | --- |
| Trait | Rationale |
| Metabolic traits |  |
| Resting metabolic rate (MO2Rest) | Baseline metabolic rate can be defined in different terms including, standard metabolic rate (MO2standard) – rate of oxygen consumption when organisms exhibit minimal function activity (i.e., absence of voluntary muscle movement and digestion); and resting metabolic rate (MO2Rest) – rate of oxygen consumption when organisms display minor activity in respirometer (i.e., minor cost of activity) (Chabot et al., 2016). In this experiment MO2rest was used because *A. polyacanthus* spend most of the time using small fin movements to maintain position in the water column, making in more ecologically relevant for the study species in this experiment. |
| Maximum metabolic rate (MO2max) | Maximum metabolic rate (MO2max) aims to quantify the maximum rate of oxygen consumption an organism can perform. MO2max is typically determined by measuring oxygen consumption during exercise or immediately after (Clark et al., 2013). |
| Absolute aerobic scope (AAS) | Absolute aerobic scope (AAS) is obtained from subtracting MO2rest from MO2max. AAS provides an indication of oxygen consumption rates that can be achieved above baseline levels (Clark et al., 2013). Under the oxygen- and capacity limitations and thermal tolerance hypothesis (OCLTT), AAS is hypothesized to provide an indication of organism performance across a thermal performance curve (Pörtner et al., 2017; Pörtner and Farrell, 2008; Pörtner and Knust, 2007). However, the OCLTT does not appear to be universally applicable and remains contested within the literature (Lefevre et al., 2021). AAS (and therefore MO2rest from MO2max) have been widely used in physiological studies, including previous research on *A. polyacanthus* (Donelson et al., 2012; Donelson and Munday, 2012; Gardiner et al., 2010; Rummer et al., 2014). |
| Immunocompetence traits |  |
| Immunocompetence (Phytohemagglutinin; PHA) | The phytohemagglutinin (PHA) skin-swelling test acts as an *in vitro s*urrogate metric for immunocompetence (Martin et al., 2006). PHA triggers the proliferation of leukocytes (primarily T-lymphocytes) upon injection, however, the overall function sense of test remains unclear (e.g., does double the swelling mean double the immunocompetence?; Martin et al., 2006). The use of PHA skin swelling test has been piloted in *A. polyacanthus* previously by Donelson and Yasutake (2024). Additionally, it has been used on other coral reef fish species including rabbitfish (i.e., *Siganus doliatus* and *S. lineatus*; LaMonica et al., 2021). |
| Enzyme traits |  |
| Lactate dehydrogenase (LDH) | Lactate dehydrogenase (LDH) serves as an index for anaerobic glycolytic potential (Jayasundara et al., 2013). LDH is measured by monitoring the decrease of NADH in samples (using a spectrophotometer), as LDH is involved in converting NADH to NAD+ (Jayasundara et al., 2013). An increase in LDH activity would indicate an increased reliance on anaerobic glycolysis to meet ATP demands (Ekström et al., 2017). LDH has been used to assess the response of organisms in several aquatic species including crown-of-throne sea stars (*Acanthaster* sp.;Lang et al., 2021), rainbow trout (*Oncorhynchus* mykiss; Pichaud et al., 2017), European perch (*Perca* fluviatilis; Ekström et al., 2017), emerald rockcod (*Gillichthys* mirabilis; Jayasundara et al., 2013), as well as several coral reef species including *Amphiprion melanopus*, *Lates calcarifer, Caesio cuning*, *Cheilodipterus quinquelineatus,* and *Acanthochromis polyacanthus* (Illing et al., 2020; Johansen et al., 2021). |
| Citrate synthase (CS) | Citrate synthase (CS) serves as an index for aerobic potential and a proxy for mitochondrial volume density (Illing et al., 2020). CS is measured by monitoring the production of citrate within samples (using a spectrophotometer). CS catalyzes the condensation of acetyl-CoA and oxaloacetate to product citrate during the first step of the citric cycle (Illing et al., 2020). CS has been used to assess aerobic metabolism in several species (*see species and references listed in LDH section above*) |
| Hematological traits |  |
| Hematocrit | Hematocrit measurements refer to the percentage of the whole blood that is composed of packed red blood cells. Red blood cells are responsible for the transport of oxygen, therefore, in vertebrates the fraction of rec blood cells (found in whole blood) will partly determine the oxygen carrying capacity of blood within organisms (Gallaugher et al., 1995). Hemoglobin can also impact oxygen carrying capacity of blood within organisms (although hemoglobin was not analyzed in this study). According to the OCLTT hypothesis if oxygen capacity is limiting performance at higher temperatures, one approach organisms could use to acclimate to warmer conditions could be to increase oxygen carrying capacity of blood via increasing red blood cell concentration in the blood (Gallaugher et al., 1995). Hematocrit has been used to assess responses to warming temperatures in aquatic organisms previously including the Pacific spiny dogfish (*Squalus suckleyi*; Bouyoucos et al., 2023), *Caesio cuning*, and *Cheilodipterus quinquelineatus* (Johansen et al., 2021). |