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Metabolic Rate, Critical Oxygen Partial Pressure, and Oxygen Supply Capacity of *Farfantepenaeus duorarum* at their Lower Thermal Limit

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by

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ABSTRACT

Temperature and environmental oxygen availability affect oxygen supply and demand in ectotherms, which are hypothesized to control the geographic limits of many marine species. The oxygen supply capacity (α) is calculated from commonly measured metabolic traits, including the standard metabolic rate (SMR) and critical oxygen partial pressure at SMR (P_{crit}). It may be used to estimate the metabolic capacity and aerobic scope across changes in temperature and oxygen partial pressures as α reflects adaptations of the cardiorespiratory system to meet maximum energy demands at a given oxygen partial pressure (PO_2). In this study, α was measured for the Tampa Bay region's Pink Shrimp (*Farfantepenaeus duorarum*) via respirometry and compared at two temperatures, 20°C and 23°C, near the apparent lower thermal limit of Pink Shrimp. Loss of equilibrium was observed between 18°C and 20°C. The α, and the parameters used to calculate it, were not significantly different between these measurement temperatures and provided an aerobic scope sufficiently large to support activity beyond basal maintenance. This suggests that physiological oxygen limitations do not define the lower temperature limit of Pink Shrimp analyzed in this study (20°C). Further, reported metabolic trait values were within the range reported for closely related penaeid shrimp. A significant difference in α was found when calculated with different time intervals between oxygen measurements, and with time intervals between 10- and 20-minutes resulting in similar mean values with variability that decreased as the time interval increased. The 15-minute time interval is reported here as a representative data set.

INTRODUCTION

Anthropogenic inputs of carbon dioxide have contributed to global climate change and ocean warming (IPCC, 2019; Dansgaard et al., 1993; Charlson et al., 1992; Rayner et al., 2003). Global temperatures have warmed an average of about 1^{o}C above pre-industrial levels (IPCC, 2019). Regionally, in the Central Gulf of Mexico, sea-surface temperatures have been warming at a rate between +0.17 to +0.3°C per decade since the 1980s (Muller-Karger et al., 2015). Increasing seawater temperatures reduce gas solubility and accelerate respiration leading to oxygen removal while increased water column stratification constrains reoxygenation from the atmosphere (IPCC, 2019; Gattuso et al., 2015; Breitburg et al., 2018). It is increasingly important to gain a mechanistic understanding of the impacts of these continuing environmental changes on marine organisms, particularly at the changing edges of thermal envelopes, to predict the future movements, distributions, and responses of the marine ecosystem. In particular, the lowest observable sea temperatures of many regions are increasing, often co-occurring with the above listed environmental changes, and baseline information of organismal responses at the lowest temperatures is needed to help predict future biological responses.

While temperature affects environmental oxygen supply, it also impacts oxygen demands. Reported physiological effects include changes to cardiorespiratory system function, gill structure, hemoglobin and other respiratory protein oxygen affinity, heart and ventilation rates, and hemolymph oxygen partial pressures (Wu et al., 2013; Chen et al., 2019; Sollid and Nilsson, 2006; Jacobs et al., 1981; Pan et al., 2017; Jayasundara et al., 2013; Frederich and

Pörtner, 2000). The resulting mismatch between oxygen supply and demand is hypothesized to set biogeographical limits in many marine species (Deutsch et al., 2015; Ern et al., 2016; Pörtner et al., 2017; Seibel and Deutsch, 2020). Additionally, these changes restrict the aerobic scope for growth, reproduction, activity, and survival (Smith et al., 2014; Li et al., 2006; Neilan and Rose, 2014; Breitburg et al., 2018). Changes in rates and the total amount of oxygen taken up across a population can in turn further change environmental oxygen conditions (Altieri and Gedan, 2014). However, other studies suggest that oxygen supply evolves to match maximum demand across a species' native temperature range, with biogeography more heavily influencing physiology (Seibel and Deutsch, 2020).

The factorial aerobic scope (FAS), the ratio of the maximum metabolic rate (MMR) to the standard, or resting, metabolic rate (SMR), represents the metabolic capacity to support activities beyond basal maintenance, including growth, reproduction, locomotion, etc. (Clark et al., 2013; Deutsch et al., 2015; 2020; Seibel and Deutsch, 2020). These metabolic traits are important because marine organisms adjust their habitat distributions in response to oxygen availability changes (Craig and Crowder, 2005; Craig, 2012; Deutsch et al., 2015; Jakob et al., 2016; Pörtner and Farrell, 2008; Wishner et al., 2018). The FAS will decline with reduced environmental oxygen and increasing temperature because it is a ratio of metabolic rates that reflects changes in oxygen-related physiological functions. A FAS = 1 indicates a lower limit where oxygen supply equals demand and oxygen supply may only sustain resting metabolism. FAS typically ranges between about 2-6, with a mean FAS = 3.3 that coincides with the warm edge of many marine species habitat ranges (Deutsch et al., 2015; 2020). This suggests that the capacity to supply oxygen for metabolism needed to support a population is three times the resting rate of an individual. As a result of this response, documenting the relationship of

temperature and metabolism in marine organisms has become increasingly important for predicting changes to species habitats. Some studies suggest a similar mechanism operates in setting the biogeographic limits at the cold end of a species' range as well (Pörtner et al., 2017). However, most studies focus on a species' upper thermal limits. Here oxygen supply capacity was measured to test the role of setting lower thermal limits in the shrimp, *Farfantepenaeus duorarum*.

METABOLIC TRAIT DEFINITIONS

Studies of the physiology of oxygen uptake in ectotherms have been a research topic for many decades (e.g., Fry and Hart 1948; Steffensen, 1989; Clark, 2013; Svendsen et al., 2016; Frederich and Pörtner, 2000; Seibel, 2011). This research has been critical to understanding species' physical limitations and habitat distributions as outlined above. As a result of these studies, an extensive set of terminology and commonly used acronyms are found in the literature (Table 1). Among these terms are directly measurable parameters relating to metabolism in organisms.

The oxygen supply capacity (α) is the maximum rate of oxygen supplied for cellular respiration per unit available oxygen pressure and mass of the individual (μmol O_2 $g^{-1}h^{-1}hPa^{-1}$, Table 1; Seibel and Deutsch, 2020; Seibel et al., 2021). Changes in α represent adaptations of the cardiorespiratory system that permit sufficient oxygen delivery to meet maximum energy demands (Seibel et al., 2021). Because α is a measure of the maximum functionality of the cardiorespiratory system, α varies with temperature in ectotherms in proportion to the MMR (Ern et al., 2016; Seibel and Deutsch, 2020; Seibel et al., 2021). Likewise, the critical oxygen partial pressure (P_{crit}), the minimal partial pressure of oxygen at which the organism can maintain the

SMR, is temperature-dependent (Table 1, Chabot et al., 2016; Claireaux and Chabot et al., 2016; Rogers et al., 2016; Priede, 1985; Seibel and Deutsch, 2020; Wood, 2018). The level of hypoxic tolerance of a species has often been characterized by P_{crit} (Pörtner and Knust, 2007; Seibel, 2011; Rogers et al., 2016), but this characterization has been questioned by Wood (2018) and Seibel et al. (2021), in part because of the lack of definitive and consistent responses across species at P_{crit}. However, $P_{critmax}$, the critical oxygen partial pressure below which the MMR is oxygen-limited, is a more direct measure of hypoxic tolerance as it also represents the metabolic response to the oxygen partial pressure (PO_2) but at what would be considered a more inhabited level of environmental oxygen values (Seibel and Deutsch 2020; Seibel et al., 2021). For many coastal normoxic species, $P_{critmax}$ is typically near air-saturation, and any reduction in environmental oxygen concentrations will linearly reduce MMR and FAS, and with a slope proportional to α (Seibel and Deutsch, 2020). Thus, α can be determined at P_{crit} for any metabolic rate, and can then be used to estimate MMR and FAS for any PO_2 up to $P_{critmax}$.

SUMMARY OF OXYGEN SUPPLY CAPACITY CALCULATION

Measuring oxygen-related physiological parameters, as outlined above, at multiple temperatures can be used to identify the temperature dependence of oxygen supply and the cardiorespiratory system (Seibel and Deutsch, 2020; Seibel et al., 2021). Recent new methods to calculate P_{crit} relative to α have been proposed by Seibel et al. (2021; Seibel and Deutsch, 2020) which provide insight into the relationship between these metabolic parameters, making them particularly useful to analyze oxygen constraints and compare species. To date, P_{crit} has been estimated by various methods that determine the PO_2 at which a critical breakpoint in measured metabolic rate values (MO_2) occurs (Chabot et al., 2016; Reemeyer and Rees, 2019; Harianto et

al., 2019; Yaeger and Ultsch, 1989; Farrell and Richards, 2009; Pörtner and Grieshaber, 1993; Richards, 2011; Ultsch and Regan, 2019). The α is equivalent to a measured metabolic rate divided by its corresponding P_{crit}, as shown in Eq. 1 (Seibel and Deutsch, 2020).

$$\text{Eq. 1} \qquad \alpha = \frac{\text{SMR}}{P_{crit}} = \frac{\text{MMR}}{P_{critmax}}$$

The relationship in Eq. 1 can be rearranged to yield Eq. 2, which indicates that the ratio of the critical environmental oxygen partial pressures is equal to the FAS, which reflects adaptations in aerobic scope for given environmental oxygen concentrations.

$$\text{Eq. 2} \qquad \text{FAS} = \frac{\text{MMR}}{\text{SMR}} = \frac{P_{critmax}}{P_{crit}}$$

When further rearranged to Eq. 3, the α can be used to estimate the MMR for the corresponding partial pressure, which is constant at $P_{critmax}$ and above (Seibel et al., 2020; 2021).

$$\text{Eq. 3} \qquad \text{MMR} = \alpha \times P_{critmax}$$

However, the maximum possible MMR is dependent on environmental oxygen availability which is represented by the oxygen partial pressure (PO_2). Therefore, Eq. 3 can also be written as Eq. 4.

$$\text{Eq. 4} \qquad \text{MMR} = \alpha \times \text{PO}_2$$

The relationships between Eqs. 1-4 are illustrated in Figure 1. These relationships suggest that P_{crit}, rather than being a standalone point with biological information on its own, is a rate-specific measure of the α.

$$\text{Eq. 5} \qquad \text{SMR} = \alpha \times P_{crit}$$

To obtain MMR as in Eq. 4 for a given temperature, α must first be calculated for that temperature. During a respirometry trial, the oxygen supply (α_0), which is the measured oxygen consumption rate at a given partial pressure of oxygen, is calculated across all partial pressures (Eq. 6). Oxygen supply increases towards a maximum value as the partial pressure of oxygen

declines or as the metabolic rate increases. The α is reached at the maximum possible metabolic rate for a partial pressure that is observed (Seibel et al., 2021). Equivalently, α is reached at the critical PO_2 for a given metabolic rate.

$$\text{Eq. 6} \qquad \alpha_0 = \frac{\mathrm{MO_2}}{\mathrm{PO_2}}$$

This calculation method for P_{crit} and metabolic rate estimation is more precise and less ambiguous in interpretation than other calculation methods for P_{crit} (Seibel et al., 2021). Further, using α for a species as outlined above, the MMR for any given environmental PO_2 can be extrapolated for a given temperature.

TIME INTERVALS IN CALCULATION OUTPUTS

Oxygen supply depends on metabolic rates, which can be obtained via respirometry between oxygen measurements spanning any length of time. Due to decreasing amounts of change and increasing noise over short measurement durations, metabolic rate and oxygen supply depend upon duration (i.e., time intervals) employed. Previous studies on respirometry methods have used measurement intervals anywhere between 5- to 30-minutes, depending on total trial duration (Clark et al., 2013 and references therein; Chabot et al., 2016; Priede, 1985). To increase the accuracy of α measurements, analysis of the effect of time intervals was conducted, following the Seibel and Deutsch (2020) α calculation methods, to understand the scale at which measurement errors influence outcomes.

STUDY SPECIES

Penaeid shrimp are found across the planet, and many are widely cultured for food, in addition to being wild-caught for bait and/or human consumption (Rothlisberg, 1998; Benzie,

2009; Hart et al., 2009; Abarca-Arenas et al., 2007). Further, these species reside at low trophic levels near the base of the food chains in their respective regions (Stoner and Zimmerman, 1988; Bhathal and Pauly, 2008; Abarca-Arenas et al., 2007). As a result, they are economically important across the Gulf of Mexico and around Florida (Bielsa et al., 1983; Farfante, 1970; 1988; Criales et al., 2003; 2011). Due to this economic and ecologic importance, impacts of environmental changes on the physiology of these species have been investigated, including measurements of metabolic rates and oxygen-related parameters (Table 2).

However, metabolic traits, as in Table 2, have yet to be analyzed for *Farfantepenaeus duorarum*, known locally as Pink Shrimp. Pink Shrimp are a species of omnivorous penaeid shrimp found across the Gulf of Mexico and waters near Florida, Bermuda, and along the US East coast northward to the lower Chesapeake Bay (Abele, 1986; Bielsa et al., 1983). They live between depths of 2-70 m, most frequently between 9-44 m (Costello and Allen, 1964; 1967; Farfante, 1970; Bielsa et al., 1983). Their life cycle, which includes dynamic habitat changes, has been well documented in southern Florida waters, a main nursery for the species (Criales et al., 2003; 2011; Costello and Allen, 1964). This dynamic life cycle includes settlement to estuarine nurseries after an offshore development period, followed by postlarval and juvenile diurnal burial in seagrass beds in nursery areas, and then returning to deeper waters offshore to spawn as adults (Bielsa et al., 1983; Criales et al., 2011; Browder, 1985; Costello and Allen, 1964; Bishop and Herrnkind, 1976). Such drastic habitat adjustments and movement throughout their life cycle have been associated with changes in salinity and temperature (Browder et al., 2002; Criales et al., 2011; Perez-Castaneda and Defeo, 2005; Zink et al., 2013; 2017).

Penaeid shrimp gills function by diffusion of gasses across the gill epithelia via cross-current exchange aided by channels across the surface structure (Foster and Howse, 1978). As in

all species, PO_2 is the driving factor in how much gas is taken up across the gill lamina. Thus, Pink Shrimp's burial within the sandy substrate of seagrass beds may impair oxygen diffusion, requiring physiological adjustments to adjust oxygen uptake or metabolism (Bielsa et al., 1983; Costello and Allen, 1967; Reynolds and Casterlin, 1979; Bishop and Hernkind, 1976). To date, no studies have been performed to show whether burial produces significant changes to oxygen uptake and metabolism. Further, the relatively modest hydrostatic pressures experienced by Pink Shrimp as a result of depth are also unlikely to have a substantial impact on oxygen consumption (Oliphant et al., 2011).

GOALS OF STUDY

The physiological and population responses of Pink Shrimp to oxygen availability and temperature in tandem have not been examined previously. Because changes in ocean temperatures can affect metabolic performance and therefore alter suitable habitat of these organisms, this study examines how the α of Pink Shrimp responds to temperature at the apparent lower thermal limit (18°C, 20°C, and 23°C). These temperatures represent the average winter (December-February) sea surface temperatures of Tampa Bay (19.8°C ± 1.4°C, ten-year average, 2011-2020, National Data Buoy Center, Station SAPF1, accessed April 2021). Application of the calculation methods in Seibel et al. (2021; Seibel and Deutsch, 2020) for α are used to compare metabolic traits of Pink Shrimp to those of other penaeid shrimp species (Table 2). Additionally, this study examines the effect of altering the time interval over which the rate of oxygen consumption is calculated on the consistency of the calculated α.

METHODS

HUSBANDRY

Pink Shrimp (Figure 2) were collected from local bait shops in St. Petersburg, Florida, and transferred to holding tanks at the University of South Florida's College of Marine Science within 15-minutes. Shrimp were fed to satiation on a diet of cucumbers for short-term retention prior to experiments. Seawater was circulated through the main tank system and routed through a cycle of sand filter, biological media filters, and UV sterilizer. Tanks were monitored and tested several times weekly to ensure chemical and nutrient levels remained stable. A list of equipment brands, models, and relevant tool details for all experiments and tank monitoring is found in Table 3. The holding tank salinity was maintained at 32, within an observed range of 30-34. Holding tank water temperature was observed to vary with outside ambient temperatures, ranging from 20°C-28°C. Tanks were maintained at a pH of 8.1, with ammonia < 0.02 mg/L, alkalinity < 2 mEq/L, and nitrate and nitrite levels below detection limits.

In preparation for experiments, shrimp were fasted for 36-48 hours to reach a post-absorptive state and simultaneously acclimated to temperatures of 18°C, 20°C, or 23°C (Clark et al., 2013; Svendsen et al., 2016). Each shrimp was isolated and maintained in a separate chamber during the acclimation period to experimental temperatures. Shrimp were moved to experimental chambers about 30-45 min before the start of the experiments, and chambers were promptly sealed to begin measurement recording. The duration of each trial was between 10-12 hours to allow enough time for individuals to become unstressed from the initial sealing within the

chamber. Keeping trials within this time duration also ensured that comparable rates of change in oxygen partial pressure were observed and that stress due to the rate of oxygen removal was maintained at the same level.

Trials were concluded when PO_2 fell below 50 hPa and Pink Shrimp exhibited behaviors consistent with hypoxic exposure, including sudden activity with repeated swimming to the top of the chamber and loss of equilibrium (LOE). This level of hypoxia was considered sufficient to reach environmental hypoxia (equivalent to 2 mg/L) and surpass P_{crit} of Pink Shrimp (Breitburg et al., 2018). Wet weight was immediately measured following trials and shrimp were frozen at -80°C for preservation and identification of sexual maturity of individuals under a dissecting microscope as outlined by Farfante (1970; 1988) and Abele (1986). Eighteen shrimp in total, nine at each temperature, were analyzed.

RESPIROMETRY SETUP

A closed respirometry system was used in this study (Figure 3); standard practices and considerations for general respirometry techniques were adapted as described in Clark et al. (2013), Svendsen et al. (2016), and Steffensen (1989). Custom-built chambers were used with a netted barrier to prevent disruption or injury to test specimens from the stir bar that was used to continuously mix water in the chambers. Two chamber sizes (645mL and 2240mL) were available for experiments and selected depending on the mass of the test specimen to maintain relative consistency in the rate of oxygen partial pressure decline and trial duration (Clark et al., 2013; Svendsen et al., 2016). Water temperature and oxygen partial pressure were measured and recorded using a Pyroscience FireStingO2, Oxygen Meter fiber optic probes, and Pyro Oxygen Logger Software (V. 3.313, 2015). Oxygen was measured as a unit of partial pressure by the

FireSting system. Chambers were covered with black plastic material during trials to reduce disturbances from light or movement within the vicinity. Filtered seawater with 50 mg/L ampicillin sodium salt was used during trials to reduce bacterial respiration. A single, additional trial was run at each temperature to estimate background bacterial respiration in a shrimp-less, sealed chamber filled with ampicillin seawater solution at each temperature (Steffensen, 1989; Svendsen et al., 2016).

DATA ANALYSIS

Oxygen partial pressure data were taken directly from the Pyro Oxygen Logger .txt output file and analyzed in MATLAB (2020). Code was written to locate time endpoints corresponding with the 1-, 2-, 5-, 10-, 15-, and 20-minute time intervals across the data. The oxygen consumption rate was calculated from measured oxygen across the time intervals and converted to units of micromoles O_2 per liter seawater using conversions available from Loligo® Systems (Available unit converter, Table 3). Background bacterial respiration rates, obtained from the corresponding shrimp-less trial, were subtracted from their corresponding MO_2 values (Steffensen, 1989; Svendsen et al., 2016). Rates of oxygen consumption were then adjusted by mass and are expressed per unit wet body mass. This study did not explore the effects of variable body sizes because an order of magnitude of mass is required to properly analyze the effects of body size on metabolic rates, and here the range of body masses was not an order of magnitude different (3.00-6.57 g; Seibel and Drazen, 2007; Kleiber, 1932; White and Seymour, 2003).

The α and MMR equation were derived as outlined in Seibel and Deutsch (2020) and Seibel et al. (2021; Eqs. 1-6) where α_0 was calculated across each trial using Eq. 6, and the maximum value, representing α, was used to produce an estimate for the maximum possible

MMR (Eq. 4; Figure 4). As outlined in Timpe et al. (in prep.), a small error in oxygen calibration can result in a large error when calculating α values, due to division by near-zero PO_2 values. For datasets exhibiting this error of increasing α_0 values, often toward infinity at lower PO_2 or a y-intercept in Eq. 4 at high PO_2, a correction was applied following the method outlined in Timpe et al. (in prep.). These corrected values are reported and used here for calculating SMR, MMR, and P_{crit}.

SMR was calculated according to the code from Chabot et al. (2016, supplementary material). Chabot et al. (2016) found that the mean lowest normal distribution (MLND) or quantiles assigning either 20% or 25% ($q_{0.2}$ and $q_{0.25}$) of the MO_2 data to be below SMR were advantageous to use for determining SMR. To decide between these reportable SMR values, the coefficient of variance of the mean of the lowest normal distribution (C.V.MLND) is calculated and used to select between them. At or below a breakpoint of C.V.MLND = 5.4, SMR should be reported as the MLND of MO_2 values, and above this breakpoint the SMR should be reported as the MO_2 value below which 20% or 25% of the input MO_2 values lie. Here, only MO_2 measurements made within a range 50–75% air saturation (between 100–160 hPa) were used to calculate SMR, because organisms under these conditions are not likely to be oxygen-limited (Clark et al., 2013; Svendsen et al., 2016). In the case of the C.V.MLND > 5.4, the quantiles assigning 20% of the MO_2 data to be below SMR ($q_{0.2}$) will be reported for all trials.

Using α and SMR, all other variables were calculated, and P_{crit} values were estimated as in Seibel et al. (2020; Eq. 5). Since respirometry trials for MMR were not performed, two assumptions were made to calculate MMR, $P_{critmax}$, and FAS from listed equations (Eqs. 2-4). The first estimation for MMR was with the assumption that it would occur at a $P_{critmax}$ = 210 hPa, which corresponds to 100% air-saturation (Eq. 3). This is a reasonable assumption for organisms

that would be adapted to regions with consistently air-saturated water. The second MMR estimation was made based on the assumption that FAS should not exceed 6 (Seibel and Deutsch, 2020; Eq. 2). This assumption represents a maximum possible value that could be expected based on observations for other organisms, particularly at either end of their thermal limits (Clark et al., 2013; Deutsch et al., 2015; 2020; Seibel and Deutsch, 2020). A FAS was estimated using the MMR from the 100% oxygen saturation and compared with the assumed FAS = 6 (Seibel and Deutsch, 2020). $P_{critmax}$ was calculated from Eq. 3 using the measured α and the MMR estimated with the assumption of FAS = 6. Estimated values were used to compare these metabolic traits of Pink Shrimp to other penaeid shrimp. The mean, median, and standard deviation of all metabolic traits for Pink Shrimp were reported. The values reported for other shrimp are found in Table 2.

STATISTICAL ANALYSIS

A two-sample *t*-test, assuming unequal variance, was used to analyze differences in α, SMR, and P_{crit} values between 20°C and 23°C. Metabolic trait values are reported as determined for 15-minute time intervals, following results and discussion below, with a stated significance level at 0.05. For each individual shrimp, MO_2 and α were calculated for each of the six time intervals (1-, 2-, 5-, 10-, 15-, and 20-minutes) using data collected at 23°C. Only shrimp tested at 23°C were used to analyze the effect of time intervals on MO_2 calculations to eliminate any noise effects or variability that could be present as a result of temperature differences. The variability due to the time intervals was visualized by creating a box plot to show the medians and variation about α values.

To better understand variability, dispersion analyses were performed to quantify the variability and verify the ANOVA assumption of homogeneity of the variance across groups. These analyses also help identify the relationship between time intervals and determine the differences in α values between them. The methods from Anderson (2006) were first applied to calculate variability, as the mean residual, about each time interval group's mean α value. Differences in mean variability across time intervals were then tested. This test evaluated the size and significance of differences between groups' residuals to determine if the chosen time interval violates the assumption of equal variability.

A pairwise PERMANOVA method, as developed by Anderson (2001), was further performed upon the residuals to determine which time intervals have different variance from each of the other time intervals. This test gives a clearer understanding of the distinguishing differences in variability for each time interval, if any, and provides greater context to the results of the Anderson (2006) methods. Holm's adjusted *p*-values were used for determining significance to reduce the possibility of Type-I error (Holm, 1979).

Where needed, the outputs of a Canonical Analysis of Principal Coordinates (CAP), as outlined in Anderson and Willis (2003), were paired with a Leave-One-Out Cross-Validation (LOO-CV) to further quantify and visualize results of differences in mean variability about α for each time interval. The LOO-CV takes each observation, removes it, recalculates a CAP solution, and reclassifies the removed observation into which group it best matches based on the new CAP model's results. Results indicate the frequency at which all observations for each time interval were correctly reclassified back into the original groups they were removed from, or incorrectly into another time interval, and they also serve as a relative visualization of similarity between groups.

A two-way PERMANOVA of α values, not their variability, was performed to check for differences in mean values across grouping factors. The two factors chosen in this test were time intervals and the effects of individual shrimp trials resampled across each time interval. An additive model is reported with no interaction variable as the individual shrimp factor was not truly replicated within time intervals, and the effects of one individual would theoretically be equal across the time intervals factor. Further, time intervals were chosen and applied independently of testing conditions and were not a factor that could have interacted with or upon the individual shrimp factor, and likewise in the opposite direction, though each may separately affect oxygen outputs. A one-way PERMANOVA of α values against each of the time intervals was performed to determine whether differences in mean α due to the time intervals could occur by chance. Subsequently, another pairwise PERMANOVA procedure (Anderson, 2001), as performed for the residuals, was performed on the α values to determine which time intervals have different mean α from each of the other time intervals. A second CAP and associated LOO-CV, this time for α values, was used to visualize differences in the time intervals. For α, the analysis indicates those time intervals with similar α results and those that are distinct from the others. The results of these analyses were considered in conjunction with the variability analyses.

RESULTS

ACCLIMATION TEMPERATURES

Attempts to acclimate four shrimp to 18°C led to loss of equilibrium (LOE), whereby the shrimp were lying on their side with no other apparent signs of stress or mortality. Three were observed and documented with LOE, with one mortality shortly after the observation. The fourth shrimp was a documented mortality without observation of LOE. Two shrimp completely recovered after being placed in the main tank (20.4°C). One of the recovered shrimp was reintroduced to acclimation conditions at 18°C and again showed LOE. This temperature was abandoned for comparisons after these four shrimp failed to acclimate because LOE was considered an untestable condition. LOE was not observed in shrimp at the other tested temperatures (20°C and 23°C).

CALCULATED PARAMETERS AND TEMPERATURE *t*-TEST

Correction methods from Timpe et al. (in prep.) were used to analyze all reported data series across time intervals for error. Correction factors were added only when conditions were met indicating systematic error in PO_2 determination. Before applying correction factors, when needed, α for some time intervals were overestimated as indicated by a poor fit to the data (Figure 5, red). Following the application of the correction methods, the newly estimated line for MO_2 (green) more closely aligned with the observed data (blue) (Figure 5). Where there were no visible red lines, corrections made with the Timpe et al. (in prep.) method were minimal.

A summary of the results for α, SMR, P_{crit}, and estimated values for MMR, $P_{critmax}$, and FAS for Pink Shrimp at 20°C and 23°C, all calculated for the 15-minute time interval, is shown in Table 4. An example plot of α vs. PO_2, used to determine maximum α values and created using the collected data, is shown in Figure 4. Mean oxygen supply capacities were 0.0031±0.0009 and 0.0033±0.0014 µmol O_2/g/min/hPa at 20°C and 23°C, respectively. These values were within the range for other shallow-living benthic aquatic animals reported by Seibel et al. (2021). Additionally, these values were within the range of other closely related shrimp (Table 2; Maggioni et al., 2001).

The C.V.MLND values were calculated across three time intervals (10-, 15-, 20-minutes), around the reported time interval of 15-minutes, to determine how to report SMR, and, of the fifty-four values calculated, all but three resulted in a C.V.MLND > 5.4 (Chabot et al., 2016). Due to the consistency of the C.V.MLND results, the results from the $q_{0.20}$ are reported for all SMR values. Mean standard metabolic rates were 0.09±0.03 and 0.10±0.05 µmol O_2/g/min at 20°C and 23°C, respectively, for the 15-minute time interval (Table 4).

The P_{crit} values were calculated to be 31.7±14.7 hPa at 20°C and 33.0±17.6 hPa at 23°C (Table 4). Assumptions were made to estimate MMR, $P_{critmax}$, and FAS. Minor differences in the two estimations for MMR can be seen in Table 4. The MMR estimates assuming a $P_{critmax}$ of 210 hPa (100% air-saturation) were higher at both temperatures than MMR estimates made assuming a FAS = 6. Mean estimated MMR values for 100% air-saturation were 0.65±0.19 and 0.62±0.35 µmol O_2/g/min at 20°C and 23°C, respectively, and mean MMR values assuming FAS = 6 were 0.55±0.20 and 0.59±0.28 µmol O_2/g/min at 20°C and 23°C, respectively (Table 4). Mean FAS, calculated under the assumption of 100% air-saturation, was greater than 6 (7.9±3.2 at 20°C, 8.5±5.1 at 23°C; Table 4). These FAS values are larger than reported for other penaeid shrimp at

similar temperatures (Table 2). Further, these FAS values are just outside the expected range of about 2 to 6 for most aquatic animals (Seibel and Deutsch, 2020; Killen et al., 2016; Peterson et al., 1990). FAS values outside of the expected FAS range suggest that $P_{critmax}$ should be below air-saturation, potentially indicating adaptation to persistently hypoxic estuarine environments. Mean $P_{critmax}$, as estimated using the MMR assuming a maximum FAS = 6, was indeed below total air saturation (210 hPa) at 190.3±88.1 hPa at 20°C and 197.7±105.5 hPa at 23°C (Table 4). The two-sample *t*-test results for α, SMR, and P_{crit} for the 15-minute time interval can be found in Table 5. For all of the listed parameters, $p > 0.05$ resulted, and thus I fail to reject the null hypothesis for each that there is no difference in the mean values between the two temperatures.

TIME INTERVAL VARIABILITY

A table of mean, median, and standard deviations of α values for each time interval at 23°C are found in Table 6, with corresponding box plots shown in Figure 6. Variability about mean α values appears to be unequal across time intervals, where the nonparametric dispersion calculation of variability showed that smaller time intervals had the larger residuals (Table 7). A *p*-value = 0.040 was obtained from the PERMANOVA about each time interval's residual α values (Table 7), and, thus, I rejected the null hypothesis that there is no difference in the mean residual α across time intervals.

Table 8 reports the results of the pairwise PERMANOVA procedure, indicating which time intervals had significantly different variability. Time intervals of 15- and 20-minutes did not have significantly different residuals from each other. However, time intervals from 1- through 10-minutes were significantly different from 20-minute time intervals. Several time intervals produced α values with significantly similar variability, which could be further grouped (Table

8). The similar groups were the 1-, 2-, 5-, and 10-minute time intervals; the 5-, 10-, and 15-minute time intervals; and the 15- and 20-minute time intervals. The corresponding CAP and associated LOO-CV further visualized differences in the variability of α, with respect to the time interval factor (Table 9). Statistically, the 1- and 20-minute intervals were the most different from one another based upon having the largest *t*-statistic of all pairs tested ($t = 5.3$; Table 8). The 20-minute interval largely reclassified back into itself and was only reclassified into the 15-minute interval (Table 9). The 1-minute time interval largely reclassified back into itself, though variability was also similar enough to 5- and 20-minutes leading to a singular reclassification into both of these time intervals (Table 9).

For time intervals other than 1- and 20-mintues, a gradation of reclassification occurred (Table 9). The removed observations for α corresponding to time intervals between 2- and 15-minutes were, as a whole, more often reclassified into other intervals than those they were initially drawn from (Table 9). Additionally, observations were predominantly reclassified into the next greater experimental time interval (i.e., 2-minutes to 5-minutes). In the case of 15-minutes, reclassification into the 15-minute time interval and the 20-minute interval was the same. The 2-minute time interval included reclassification into all but the 20-minute interval and no other time intervals were reclassified into the 2-minute interval. The 5-minute and 10-minute time intervals were reclassified into the 1-minute interval and into each of the longer time intervals. The largest reclassification into a time interval other than the observed experimental interval occurred for the 10-minute time interval, which was reclassified into the 15-minute interval.

TIME INTERVAL EFFECTS ON MEAN OXYGEN SUPPLY CAPACITY

Results of the two-way PERMANOVA applied directly to the α values are outlined in Table 10. The result for the time interval factor was a p-value = 0.0001 and F = 13.5 and the result for the individual shrimp factor was a p-value = 0.0050 and F = 3.5. As such, I rejected both null hypotheses that there is no difference in mean α as a result of the time interval or each individual shrimp tested. Examining the F-statistics revealed that time intervals had a greater effect than repeat sampling of the same individual shrimp. A one-way PERMANOVA test was performed to determine how much of a difference in mean α from time intervals could occur by chance, and results are found in Table 11. The analysis resulted in a p-value = 0.0001 and F = 9.4. Therefore, I once again rejected the null hypothesis that there is no difference between α based on time intervals. However, the results of this analysis should be considered in conjunction with the above results related to the residual variability differences across time intervals.

An additional pairwise PERMANOVA comparing mean α for each time interval to each other time interval (n = 9 per time interval) is found in Table 12. Mean α for the 1-minute time interval was significantly different from the 10-, 15-, and 20-minute intervals. The 2-minute time interval was significantly different from the 15- and 20-minute intervals. Two groups of time intervals with mean α values that were not significantly different were observed: the 1-, 2-, and 5-minute intervals; and the 5-, 10-, 15-, and 20-minute intervals (Table 12).

The results of the CAP and associated LOO-CV for the α values (Table 13) share some similarity to the results of the CAP analysis of the residual variability (Table 9), with a gradation of reclassifications across time intervals. For example, both tests exhibit 1- and 20-minute time interval values that were the most distinguishable from each of the other time intervals. The 20-minute interval was again only observed to be reclassified into the 15-minute time interval for α

values. Time intervals between the 2- and 15-minute intervals were, as a whole, more often reclassified into time intervals other than the observed experimental interval for both tests, and the 2-minute time interval was reclassified into all but the 20- interval.

In contrast to the CAP analysis and associated LOO-CV of residual variability about mean α, analysis for the means of α values showed the 1-minute time interval was reclassified into itself and 2-minute time interval. Beginning with the 5-minute time interval and greater, more than half of the reclassifications were into the 15- and 20-minute intervals. Additionally, the 5-minute time interval was reclassified into all but the 2-minute interval. The 15-minute time interval was reclassified into the 5- through the 20-minute intervals, and with the dominant time interval being 20-minutes. All but the 1-minute time interval were reclassified into the 15-minute interval. The 5- through 20-minute time intervals were reclassified into the 20-minute interval, which contained the largest number of reclassifications.

DISCUSSION

TEMPERATURE AT THE LOWER THERMAL LIMIT

The Pink Shrimp studied here did not exhibit differences in their oxygen consumption, nor α, between 20°C and 23°C (Tables 4, 5). This may indicate that there is a level of cardiorespiratory robustness in Pink Shrimp at low temperatures, or other physiological adjustments not observable via respirometry, that mitigate any changes that would otherwise occur in the metabolic rate. Changes at higher or lower temperatures may still be likely and should be investigated in future studies.

Other studies report that Florida Pink Shrimp are found in temperatures from 10-36°C (Zink et al., 2018; Bielsa et al., 1983). The review by Bielsa et al. (1983) also reports Florida shrimp will become narcotized at 13.3°C, though stating Pink Shrimp tolerances vary by latitude. In contrast, 20°C was the lowest tolerable temperature for shrimp in lab conditions during this study. Attempts to acclimate shrimp to 18°C led to LOE, with recovery at 20°C. Shrimp were collected during February and March, and thus, should be well adjusted to the low temperatures tested in this experiment (February average = 20.3 ± 1.2°C; March average = 23.3 ± 1.5°C; 2011-2020, National Data Buoy Center, Station SAPF1, accessed April 2021). However, Florida Gulf waters' surface temperatures are not representative nor encompass all temperatures experienced across the entire habitat range, up the United States East coast, and the lowest range of temperatures listed in these studies may not apply to local Pink Shrimp tested here. Further, genetic drift between subpopulations may contribute to differences in temperature tolerances.

While observations in this study correspond with the lowest end of the thermal range of Pink Shrimp for the Tampa Bay region, temperatures over time are expected to increase. Since the 1980s, Muller-Karger et al. (2015) observed a trend of increasing sea surface temperatures ranging between +0.17 to +0.3°C per decade in the Gulf of Mexico. This suggests that within the next century, Tampa Bay water temperatures may increase up to 3°C. The lowest temperature measured in this study is coincident with the average winter temperature for Tampa Bay (19.8°C ± 1.4°C, ten-year average, 2011-2020, National Data Buoy Center, Station SAPF1, accessed April 2021) and the higher temperature measured in this study corresponds to a 3°C increase, as forecasted from Muller-Karger et al. (2015). Since the metabolic traits at these two temperatures in this study were not significantly dissimilar, it can be assumed that metabolically available habitat will not be reduced for Pink Shrimp during the winter season in the Tampa Bay region in the next century. Changes in metabolic traits at higher temperatures within the Pink Shrimp thermal range, that may correspond to other seasons within Tampa Bay, were outside the scope of this study.

The Oxygen Capacity Limitation of Thermal Tolerance hypothesis (OCLTT) proposes that an organism's upper and lower thermal limits are determined by the difference between oxygen demands and environmental oxygen supply (Pörtner et al., 2001; 2009). In short, the OCLTT theory suggests that oxygen supply, as a result of physiological effects, limits aerobic scope at both temperature limits of a species and is responsible for providing a thermal habitat barrier. Although widely accepted, other studies have challenged the OCLTT hypothesis (Clark et al., 2013; Jutfelt et al., 2014; 2018). These studies contend that many species appear to reach thermal limits with no decline in aerobic scope, a contention that this study also supports.

Several observations in this study provide evidence for this contention against the OCLTT hypothesis. The lower temperature of this study, 20°C, appears very near the observed thermal limit for Pink Shrimp (LOE reached at 18°C). Further, this lower thermal limit at 20°C had a negligible effect on SMR and P_{crit} when compared to 23°C, showing no signs of significant reduction in aerobic capabilities suggested to occur at thermal limits (Table 5). Finally, α was sufficient to support a substantial aerobic scope at test temperatures (20°C) very near temperatures at which unrelated system failures occurred (18°C; Table 4). Even assuming that MMR is reached at an unusually low 50% air-saturation ($P_{critmax}$ at ~100 hPa), the measured α could still support a FAS = 3.3, which is the mean value defining metabolically available habitat in marine species (Deutsch et al., 2020). Therefore, contrary to the OCLTT hypothesis, it appears that the oxygen supply may not be involved in or be the driving force behind setting the lower thermal limit for this species. There may be more mitigation of physiological aerobic changes that affect metabolic rates between temperatures than is assumed by the OCLTT. Measurements over a larger range of temperatures need to be collected in Pink Shrimp before a statement can be made on the upper thermal limit for Pink Shrimp concerning this trend opposing the OCLTT.

PENAEID SHRIMP COMPARISONS

Shrimp comparisons in Table 2 are considered in the context of many differences in ecology and physiology between listed penaeid shrimp species. Temperatures reported in Table 2 vary in relation to position within the habitable temperature range and/or absolute heat content. Additionally, body sizes and life stages also vary between species. For example, *Penaeus monodon* are rather large shrimp compared to *F. duorarum*, and *P. esculentus* were not sexually mature as were the Pink Shrimp tested here. Postlarvae of *Litopenaeus setiferus* were reported

with metabolic rates corrected by dry weight versus the wet weight used for all other studies, and thus are not directly comparable to other reported values, but are included for reference purposes (Table 2; Brito et al., 2000; Rosas et al., 1999).

For the few species that data are available, α for *F. duorarum* was within the range reported, with *P. monodon* and *P. esculentus* having the lowest oxygen supply capacities. The α in *F. duorarum* was most similar to that in *L. schmitti*, which are closely related (Maggioni et al., 2001). Comparatively, SMR was lowest for *P. esculentus* at 25°C, followed by *P. monodon* at 30°C *then F. duorarum* (Table 2). These species have the closest SMR values to *F. duorarum*, closer than even the species within its own genus, *F. aztecus*, measured at the same temperature. P_{crit} was also lowest for *F. duorarum* in this study, followed closely by *L. vannamei*. The low P_{crit} measured in Pink Shrimp may suggest that their aerobic scope may be better maintained at, and thus more resilient to, lower oxygen levels. This could be an adaptation to living in an environment where toxic algal blooms are frequent and lead to hypoxic conditions (Milbrandt et al., 2021). Additionally, Pink Shrimp burial in sandy substrate could contribute (Bielsa et al., 1983; Criales et al., 2011; Browder, 1985; Costello and Allen, 1964; Bishop and Herrnkind, 1976). Differences in metabolic traits may also be explained, in part, by differences in measurement temperature, body size, and life stage reported between species. The high α, low SMR, and low P_{crit} values are likely representative of adaptations to an estuarine habitat that experiences large fluctuations in oxygen content (Seibel and Deutsch, 2020).

The MMR from this study that is reported in Table 2 was the estimate using an assumption of a maximum metabolic rate at 100% air-saturation (where $P_{critmax}$ = 21 kPa). The second MMR, estimated assuming a maximum possible MMR occurring at FAS = 6, is reported in Table 4. With either estimation, MMR for Pink Shrimp was higher than for all other penaeid

shrimp in Table 2. When calculated using the air-saturated $P_{critmax}$, FAS is at the upper end of the range of 2-6 reported for the vast majority of species, including the penaeid shrimp outlined here (Tables 2, 4; Seibel and Deutsch, 2020; Killen et al., 2016; Peterson et al., 1990). The highest FAS is typically found at the colder end of a species temperature range, and my observations suggest that this colder temperature for Tampa Bay Pink Shrimp is near 20°C. Resting and maximum metabolic rates or critical partial pressures could vary at different temperatures to accommodate and support a higher aerobic scope in this species. However, a $P_{critmax}$ near air saturation is typical for many species. $P_{critmax}$ estimated assuming a maximum possible FAS = 6 is near what would be observed for water at air-saturation. However, fluctuations in oxygen levels are common in estuarine habitats and it would not be unexpected for Pink Shrimp to be adapted to lower oxygen levels, with a lower $P_{critmax}$, that would be more consistent with the lower FAS reported for other penaeid shrimp. The higher P_{crit} values in other species suggest their aerobic scope is very limited. In studies reporting on postlarval stages, it may be that oxygen transport systems are not yet fully developed (Bouaricha et al., 1994). Further, *L. schmitti* appear to be oxyconformers, which suggests they lack an aerobic scope, and, as such, MMR and aerobic scope values for this species were not reported in Table 2 (Rosas et al., 1997; Seibel et al., 2021).

Because MMR was not measured in this study, further research should be done on this measure to confirm any assumptions made here, as MMR may be achieved at lower or higher air saturation levels indicative of long-term oxygen adaptations (Seibel and Deutsch, 2020). Estimations for MMR, $P_{critmax}$, and FAS reported here should be interpreted with caution as the reported MMR and FAS appear to be so much different compared to other penaeid shrimp, despite $P_{critmax}$ aligning with expectations.

Based upon the parameters α, SMR, and P_{crit} measured in this study, it appears that the cardiorespiratory system of *F. duorarum* may be more ancestral in characteristics, sharing much in common with individuals from the genus *Penaeus*, and may be a distinguishing point of evolutionary difference from other species in more genetically related genera (Maggioni et al., 2001). A closer look into such distinguishing characteristics of the cardiorespiratory system of *F. duorarum* may be beneficial to understand this distinction better.

EFFECT OF TIME INTERVALS

The analysis shows that time intervals impact the α values obtained using the calculations in Seibel and Deutsch (2020). Figure 6 visually suggests that mean α is similar for a time-intervals of 5-minutes and greater. The analysis of mean α values in Table 12 for the time intervals resulted in two groups having similar mean values. Table 12 and Table 13 confirm that the mean α values converge above 5-minutes because the mean α at this time interval was not significantly different from any other interval, and reclassifications were into time intervals of 5-minutes and greater more than half of the time. Of these intervals in the group of 5-minutes and longer, the largest total number of reclassifications into a time interval was into the 20-minute interval (Table 13). Therefore, it appears that of this group of 5-minutes and longer, the α values produced using a time interval of 20-minutes is most representative.

While mean α values may converge at 5-minutes and above, with high representation by the 20-minute time interval, differences in variability about α need to be considered to ensure that differences in the data can be captured. Referring to Figure 6, it is visually apparent that the 1-minute time interval has large variability. This large variability is supported and quantified by the average distance to the centroid reported in Table 7. The 1-minute time interval is likely not

representative of any other time interval besides itself because of this large variability, which suggests inclusion of too much noise, and a mean α value that is consistently higher than the other time intervals, even when not significantly different (Tables 5, 6, 12). The analysis of residual variability about the mean α for the time intervals resulted in three groups having similar variability (Tables 8, 9). Table 8 shows variability for the 15-minute time interval was not significantly different from either 10- or 20-minute intervals, but the 20-minute interval was significantly different from the 10-minute. As a result of these observations, it can be inferred that a difference in variability is occurring between the 10- and 15-minute time intervals.

From above, starting with the most representative α values, which was for the 20-minute time interval, the variability about the mean α can be accommodated. When looking at the variability, the 20-minute time interval was similar to the 15-minute interval, but significantly different from the 10-minute. From Table 8 and Table 12, it is clear that there is no significant difference in mean α or associated residual variability between the 15- and 20-minute time intervals. However, variability does significantly differ between the 20-minute interval and any other interval aside from the 15-minute time interval. The 15-minute interval, with respect to variability, was similar to the 5-, 10-, and the 20-minute time intervals. Thus, it appears that the 15-minute time interval is a reasonable compromise to accommodate the best representation of α, and encompass enough variability that may be found in the other time intervals with converging means. For this reason, data in Table 4 were reported using the 15-minute time interval. However, reporting with the 10- or 20-minute intervals would likely result in comparable α values, the main difference being variability about mean α.

CONCLUSION

This study shows that oxygen capacity and critical oxygen consumption measurements of Pink Shrimp (*Farfantepenaeus duorarum*) are not significantly different between 20°C and 23°C. Oxygen supply capacity at this low end of the temperature range for Pink Shrimp is sufficient to support an aerobic scope of 3.3 even at an unlikely assumption of MMR at 50% air saturation. Because of this substantial scope at a near the lower thermal limit of this species, the observed loss of equilibrium at 18°C for Pink Shrimp is unlikely to result from oxygen consumption limitations. Results further suggest, based on high α and low P_{crit}, with an estimated $P_{critmax}$ below air-saturation, that Pink Shrimp are likely adapted to persistent hypoxia for a portion of their life cycle.

In review of α calculation methods from Seibel and Deutsch (2020), time intervals did impact mean α values and the variability about them. Reporting values for the α and related metabolic variables using time intervals of 10-, 15-, or 20-minutes are likely to adequately address measurement uncertainties and variabilities from different studies and should provide comparable values between studies.

Future studies should measure MMR in Pink Shrimp to better understand their true aerobic scope. Additional studies should also expand upon the temperatures evaluated for Pink Shrimp to address upper and lower thermal limits and their relationship to the OCLTT hypothesis. Additional focus should be looked at for Pink Shrimp responses and the mechanism(s) behind LOE between 18°C and 20°C. Observation of a greater body mass range of

different life stages of Pink Shrimp may be of interest due to changes from different stages of the dynamic life cycle of the species. Additionally, the behavior of Pink Shrimp burying themselves within seagrass beds is a potential topic for future studies in aerobic respiration to help understand any related physiological mechanisms that may affect oxygen uptake, consumption, and metabolism that was beyond the range of this study.

Table 1: Key terms and definitions along with key sources for each.

Term/shorthand	Working Definition	Pertinent Citations
Maximum Metabolic Rate (MMR)	The maximal rate at which an organism utilizes oxygen. It is typically measured with no oxygen limitations (at 100% air-saturated water) during maximum exertion.	Fry and Hart, 1948 Clark et al., 2013
Standard Metabolic Rate (SMR)	The lowest rate of oxygen consumption required to maintain a rested, fasting organism at a given temperature.	Fry and Hart, 1948 Chabot et al., 2016 and sources therein
MO_2	The rate of oxygen consumption in moles (MO_2) of oxygen per unit time and mass. $\mathrm{MO_2} = \alpha_0 \times \mathrm{PO_2}$	Chabot et al., 2016 Clark et al., 2013 Equation relationship from Seibel and Deutsch, 2020
Oxygen Partial Pressure (PO_2)	The portion of the gas pressure exerted by oxygen gas in a mixture, expressed relative to seawater's capacity at equilibrium with air. At saturation, PO_2 is 21% of the total, or 210 hPa.	----
Critical Oxygen Partial Pressure (P_{crit})	Critical oxygen partial pressure (P_{crit}) or the minimal partial pressure of oxygen at which the given metabolic rate may be maintained by the organism. In this study representing the SMR.	Seibel et al., 2021
Critical Oxygen Partial Pressure at MMR ($P_{critmax}$)	The environmental oxygen partial pressure below which MMR is oxygen-limited.	Seibel and Deutsch, 2020
Absolute Aerobic Scope (AAS)	Measure of the amount of oxygen available for activities beyond basal maintenance. Including growth, reproduction, swimming, etc. $\mathrm{AAS} = \mathrm{MMR} - \mathrm{SMR}$	Fry and Hart, 1948 Clark et al., 2013

Table 1 (Continued)

Term/shorthand	Working Definition	Pertinent Citations
Factorial Aerobic Scope (FAS)	The factorial difference between resting and active metabolic rate. $$\mathrm{FAS} = \frac{\mathrm{MMR}}{\mathrm{SMR}}$$	Clark et al., 2013 Seibel and Deutsch, 2020
Oxygen Supply (α_0)	The rate of supply of oxygen to the rest of the body at a given point in a respirometry trial. $$\alpha_0 = \frac{\mathrm{MO_2}}{\mathrm{PO_2}}$$	Seibel and Deutsch, 2020 Seibel et al., 2021
Oxygen Supply Capacity (α)	The maximum possible oxygen supply. Reached at P_{crit} or MMR, describes the rate dependence of P_{crit} or the PO_2 dependence of MMR. $$\alpha = \mathrm{SMR} \div P_{crit}$$ $$\alpha = \mathrm{MMR} \div P_{critmax}$$	Seibel and Deutsch, 2020 Seibel et al., 2021

Table 2: Key oxygen parameters measured in related penaeid shrimp species. *P.* = *Penaeus*, *L.* = *Litopenaeus*, *F.* = *Farfantepenaeus*. Values that are from figures in referenced papers were obtained using apps.automeris.io/wpd/. Organized based on genus and genetic diversity as reported in Maggioni et al. (2001). Values reported in studies (green highlight) include SMR, P_{crit}, and MMR. All other metrics (red highlight) are calculated from those measurements assuming a $P_{critmax}$ at 21 kPa, as is MMR for the current study, unless otherwise denoted (* = AAS or FAS values reported by study). Oxygen supply capacity (α) was preferentially calculated from Eq. 5 where possible. Otherwise, α was calculated with Eq. 3 from the assumed $P_{critmax}$ = 21 kPa and reported MMR, and then P_{crit} was estimated from that α (** = P_{crit} estimated with Eq. 3). FAS was estimated as from Eq. 2. Absolute Aerobic Scope (AAS) is calculated as MMR − SMR. *L. schmitti* appear to be oxyconformers and therefore MMR, AAS, and FAS are not reported.

Species/ Temperature	α (µmol g^{-1} min^{-1} kPa^{-1})	SMR (µmol g^{-1} min^{-1})	MMR (µmol g^{-1} min^{-1})	P_{crit} (kPa)	$P_{critmax}$ (kPa)	AAS (µmol g^{-1} min^{-1})	FAS --	Source
F. duorarum								
20°C	0.031±0.009	0.09±0.03	0.65±0.19	3.17±1.47	21	0.56	7.9±3.2	*Current study*
23°C	0.033±0.014	0.10±0.05	0.62±0.35	3.30±1.76	21	0.52	8.5±5.1	
F. aztecus (juvenile)								
20°C	--	0.17±0.04	--	--	--	--	--	Latournerié et al., 2011
24°C	--	0.40±0.05	--	--	--	--	--	
26°C	--	0.20±0.04	--	--	--	--	--	
30°C	--	0.536±0.068	--	--	--	--	--	
32°C	--	0.31±0.04	--	--	--	--	--	
L. vannamei (subadult)								
24°C	0.030-0.051	0.18	--	3.5-6.1	--	--	--	Song, 2015
28°C	0.008-0.015	0.1135-0.1383	0.1605-0.3056	7.6-17.3**	21	--	2.0-3.3*	Ponce-Palafox et al., 2017
L. stylirostris (postlarvae)								
27°C	--	2.89±0.60	--	--	--	--	--	Gaudy and Sloane, 1981

Table 2 (Continued)

L. setiferus								
24°C	0.021-0.023	0.11	--	4.8-5.2	21	--	4.0-4.2	Song, 2015
28°C (postlarvae)	--	80.0–298.3 (dry mass)	--	--	--	--	--	Brito et al., 2000
28°C (postlarvae)	--	18.5-151.0 (dry mass)	--	--	--	--	--	Rosas et al., 1999
29°C (postlarvae)	--	--	--	13.0-15.2	21	--	1.4-1.6, 1.75-2.6	Rosas et al. 1997
L. schmitti								
25°C (oxyconformers)	0.040-0.087	0.620±0.031-1.125±0.052	—	15.5-12.9	--	—	—	Rosas et al., 1997
P. monodon								
20°C	--	--	--	10.9	21	--	1.9	Liao and Murai, 1986
25°C	--	--	--	11.8	21	--	1.8	
27°C (postlarvae)	--	2.63±0.68	--	--	--	--	--	Gaudy and Sloane, 1981
30°C	0.016	0.072	0.342	4.5**	21	0.267*	4.5*	Ern et al., 2015
30°C	0.006-0.007 (Using SMR above from Ern et al., 2015)	--	--	10.4-12.0	21	--	1.75-2.0	Liao and Murai, 1986
34°C	0.019	0.128	0.395	6.7**	21	0.263*	3.1*	Ern et al., 2015
38°C	0.020	0.202	0.415	10.1**	21	0.206*	2.0*	
P. esculentus								
25°C	0.005-0.009	0.047	0.170	5.3-8.7	--	0.123	3.6	Dall, 1986

Table 3: List of equipment used for experiments. Relevance of their purpose and parameters are also listed.

Equipment type	Brand	Uses
Chemical testing	Marine Basic MultiTest®	Tank and seawater chemical condition monitoring, including pH, nitrate, nitrite, free and total ammonia, and alkalinity.
Salinity monitoring	American Marine Inc. Pinpoint® Salinity Monitor	Tank water maintenance/monitoring
Temperature and environmental control system	Harris Environmental Systems Inc. environmental room	Isolation and acclimation of shrimp prior to experiments
Pump	AquaClear®30, PH301, AC 120V, 60 Hz, 8W)	Pumping temperature controlled water through chamber water jacket to maintain experiment temperature
Temperature bath	LAUDA Ecoline Staredition RE120	Controlling experimental temperature
Temperature probe	Firesting ADVIAL20 temperature cable	Temperature monitoring of water bath and water jacket temperature
Oxygen and temperature monitoring system	Pyroscience FireStingO2, Fiber-Optic Oxygen Meter system	Measuring and monitoring of oxygen and temperature during experiments
Oxygen monitoring sensors	FireSting fiberglass optode cables (SPFIB-CL2 Optical Fiber (2ST-plugs) plugged into SPADLNS Lens Spot Adapters (for 2-6mm thickness) and sensor spots (SC7-539-203)	Monitoring oxygen in experimental chambers
Monitoring and recording program	Pyro Oxygen Logger Software V. 3.313 (2015)	Operation of and recording of measurements made with probes
Coding and/or analysis program	MATLAB R2020a; Excel 2018; R Studio	Analysis of data
Available unit converter	Loligo® Systems (https://www.loligosystems.com/convert-oxygen-units) Date of access: 2021	Conversion of units

Table 4: Summary of results for the means, medians, and standard deviations of α, SMR, MMR, FAS, and P_{crit} values estimated from each tested *Farfantepenaeus duorarum* at 20°C (n = 9) and 23°C (n = 9) using 15-minute time intervals. SMR was calculated using Chabot (2016). P_{crit} values were extrapolated from Eq. 3 ($MO_2 = \alpha \times PO_2$; from Seibel et al., 2020) with SMR input to MO_2 to solve for the PO_2. MMR is calculated with the assumption of FAS equivalent to 6 ($MMR = 6 \times SMR$). $P_{critmax}$ is calculated as from MMR and α (Eq. 3). MMR.210 and FAS.210 are calculated based on assumption that MMR occurs at 210 hPa ($MMR.210 \times \alpha$. FAS.210 is calculated as $MMR.210 \div SMR$.

15-min	α	SMR	MMR	P_{crit}	$P_{critmax}$	MMR.210	FAS.210
20°C	µmol O_2/g/min/hPa	µmol O_2/g/min	µmol O_2/g/min	hPa	hPa	µmol O_2/g/min	--
Mean	0.0031	0.09	0.55	31.7	190.3	0.65	7.9
Median	0.0030	0.08	0.47	27.8	166.7	0.63	7.6
Std. Dev.	0.0009	0.03	0.20	14.7	88.1	0.19	3.2
23°C							
Mean	0.0033	0.10	0.59	33.0	197.7	0.62	8.5
Median	0.0028	0.07	0.43	28.0	168.3	0.57	7.5
Std. Dev.	0.0014	0.05	0.28	17.6	105.5	0.35	5.1

Table 5: Results of two-sample *t*-tests assuming unequal variance comparing the (A) α (B) SMR and (C) P_{crit} between the two reported temperatures at the 15-minute time interval (n = 9; n = 9). Stated level of significance = 0.05. Both a one-tail and two-tail test were performed. Values are reported for the one-tail tests compare Variable A (values at 20°C) to Variable B (values at 23°C), where positive values indicate Variable B is larger. Code from Excel v2105.

A)

α	*Variable A 20°C*	*Variable B 23°C*
Mean	0.0031	0.0033
Variance	8.36E-07	1.84E-06
Observations	9	9
Hypothesized Mean Difference	0	
Degrees of Freedom	14	
t-Statistic	-0.329	
p-value one-tail	0.373	
t-Critical one-tail	1.76	
p-value two-tail	0.747	
t-Critical two-tail	2.145	

Table 5 (Continued)

B)

SMR	*Variable A 20°C*	*Variable B 23°C*
Mean	0.092	0.099
Variance	0.0011	0.0023
Observations	9	9
Hypothesized Mean Difference	0	
Degrees of Freedom	14	
t-Statistic	-0.333	
p-value one-tail	0.372	
t Critical one-tail	1.761	
p-value two-tail	0.744	
t Critical two-tail	2.145	

C)

P_{crit}	*Variable A 20°C*	*Variable B 23°C*
Mean	31.7	33.0
Variance	215.8	309.5
Observations	9	9
Hypothesized Mean Difference	0	
Degrees of Freedom	16	
t-Statistic	-0.161	
p-value one-tail	0.437	
t Critical one-tail	1.746	
p-value two-tail	0.874	
t Critical two-tail	2.120	

Table 6: Mean, median, and standard deviation of α values (µmol/ g min hPa) at 23°C for time intervals of 1-, 2-, 5-, 10-, 15-, and 20-minutes (n = 9).

	1-minute	**2-minutes**	**5-minutes**	**10-minutes**	**15-minutes**	**20-minutes**
Mean	0.0188	0.0094	0.0060	0.0042	0.0033	0.0026
Median	0.0193	0.0067	0.0036	0.0029	0.0028	0.0026
Std Dev.	0.0093	0.0078	0.0070	0.0038	0.0014	0.0004

Table 7: Multivariate dispersion applied in a univariate context, used to calculate the variability as a mean residual about the mean α for each time interval tested (n = 9 per time interval). I reject the null that there is no difference in the dispersion about mean α between time intervals (stated significance level of 0.05).

==========
NP-DISP: Homogeneity of Multivariate Dispersion

F = 5.22 p = 0.040 (iter=10000)

Pos Eigenvalues = 1
Neg Eigenvalues = 0

Average distance to centroid:
1-minute = 0.0078
2-minute = 0.0053
5-minute = 0.0042
10-minute = 0.0023
15-minute = 0.0009
20-minute = 0.0003

Table 8: Pairwise nonparametric multivariate ANOVA (PERMANOVA) comparing the mean variance about α for each time interval to each other (n = 9 per time interval). Highlighted rows indicate time intervals that were significantly different (stated significance level of 0.05).

Pairwise comparisons between time intervals				
Compared time intervals	t-statistic	Unadjusted p-value	Holms adjusted p-value	Significant difference?
1 vs. 2	1.102	0.296	0.887	
1 vs. 5	1.597	0.136	0.817	
1 vs. 10	3.253	0.006	0.052	
1 vs. 15	4.791	0.0007	0.008	YES
1 vs. 20	5.334	0.0004	0.005	YES
2 vs. 5	0.450	0.521	0.914	
2 vs. 10	1.506	0.142	0.817	
2 vs. 15	2.439	0.0008	0.008	YES
2 vs. 20	2.812	0.0002	0.003	YES
5 vs. 10	0.940	0.457	0.914	
5 vs. 15	1.800	0.007	0.058	
5 vs. 20	2.162	0.0003	0.004	YES
10 vs. 15	1.338	0.169	0.817	
10 vs. 20	2.023	0.0005	0.006	YES
15 vs. 20	1.786	0.025	0.174	

Table 9: Canonical Analysis of Principal Coordinates (CAP) and Leave-One-Out Cross-Validation (LOO-CV) of the residual variability about α. In the confusion matrix, rows represent the group that was input for reclassification while columns represent which group was given as the suggested output (n = 9 per time interval).

==========
CAP - Canonical Discriminant Analysis:

Trace Stat = 0.3520 p = 0.00120
Greatest Root = 0.3520 p = 0.00120
No. of permutations = 10000

No. of axes of Q used (m) = 1
Variability of yDis explained = 100.00 %
Canonical Correlations:
0.5933
Squared Canonical Correlations (= delta^2):
0.3520
==========

LOO CROSS-VALIDATION
Classification Success:

Group Correct
1 77.8 %
2 22.2 %
3 22.2 %
4 22.2 %
5 44.4 %
6 77.8 %
Total Correct = 44.44 %
Total Error = 55.56 %

Confusion Matrix (%)						
Time interval:	1-min	2-min	5-min	10-min	15-min	20-min
1-min	77.8	0	11.1	0	0	11.1
2-min	11.1	22.2	33.3	22.2	11.1	0
5-min	11.1	0	22.2	44.4	11.1	11.1
10-min	11.1	0	0	22.2	55.6	11.1
15-min	0	0	11.1	0	44.4	44.4
20-min	0	0	0	0	22.2	77.8

Table 10: Two-way PERMANOVA of the effects of interval size and individual upon mean α.

PERMANOVA					
Source	Degrees of Freedom	Sum of Squares	Mean Square	*F* ratio	*p*-value
Factor 1: time interval	5	0.0017	3.33e-4	13.49	1.00e-4
Factor 2: individual shrimp	8	7.07e-4	8.84e-5	3.58	0.0050
Residual	40	9.88e-4	2.47e-5	--	--
Total	53	0.0034	--	--	--

Table 11: PERMANOVA test of α values against time intervals (stated significance level = 0.05).

Nonparametric (Permutation based) MANOVA					
Source	Degrees of Freedom	Sum-of-Squares	Mean Square	*F* ratio	*p*-value
Factor 1: time interval	5	0.0017	3.33e-4	9.43	1.00e-4
Residual	48	0.0017	3.53e-5	--	--
Total	53	0.0034	--	--	--

Table 12: Pairwise PERMANOVA comparing the mean α for each time interval to each other (n = 9 per time interval). Highlighted rows indicate time intervals that were significantly different (stated significance level = 0.05).

Pairwise comparisons between time intervals				
Compared time intervals	*t*-statistic	Unadjusted *p*-value	Holms adjusted *p*-value	Significant difference?
1 vs. 2	2.303	0.036	0.287	
1 vs. 5	3.282	0.007	0.067	
1 vs. 10	4.348	0.0001	0.0015	YES
1 vs. 15	4.931	0.0001	0.0015	YES
1 vs. 20	5.199	0.0001	0.0015	YES
2 vs. 5	0.981	0.387	1.000	
2 vs. 10	1.822	0.061	0.424	
2 vs. 15	2.339	0.0025	0.0275	YES
2 vs. 20	2.636	0.0001	0.0015	YES
5 vs. 10	0.693	0.527	1.000	
5 vs. 15	1.157	0.248	0.992	
5 vs. 20	1.471	0.007	0.067	
10 vs. 15	0.682	0.680	1.000	
10 vs. 20	1.264	0.130	0.764	
15 vs. 20	1.457	0.127	0.764	

Table 13: CAP and LOO-CV of the α values. In the confusion matrix, rows represent the group that was input for reclassification while columns represent which group was given as the suggested output (n = 9 per time interval).

==========
CAP - Canonical Discriminant Analysis:

Trace Stat = 0.4956 p = 0.00010
Greatest Root = 0.4956 p = 0.00010
No. of permutations = 10000

No. of axes of Q used (m) = 1
Variability of yDis explained = 100.00 %
Canonical Correlations:
0.7040
Squared Canonical Correlations (= delta^2):
0.4956
==========
==========
LOO CROSS-VALIDATION
Classification Success:

Group Correct
1 55.6 %
2 22.2 %
3 11.1 %
4 11.1 %
5 11.1 %
6 88.9 %
Total Correct = 33.33 %
Total Error = 66.67 %

Confusion Matrix (%)						
Time interval:	1-min	2-min	5-min	10-min	15-min	20-min
1-min	55.6	44.4	0	0	0	0
2-min	11.1	22.2	33.3	22.2	11.1	0
5-min	11.1	0	11.1	11.1	33.3	33.3
10-min	0	11.1	0	11.1	22.2	55.6
15-min	0	0	11.1	11.1	11.1	66.7
20-min	0	0	0	0	11.1	88.9

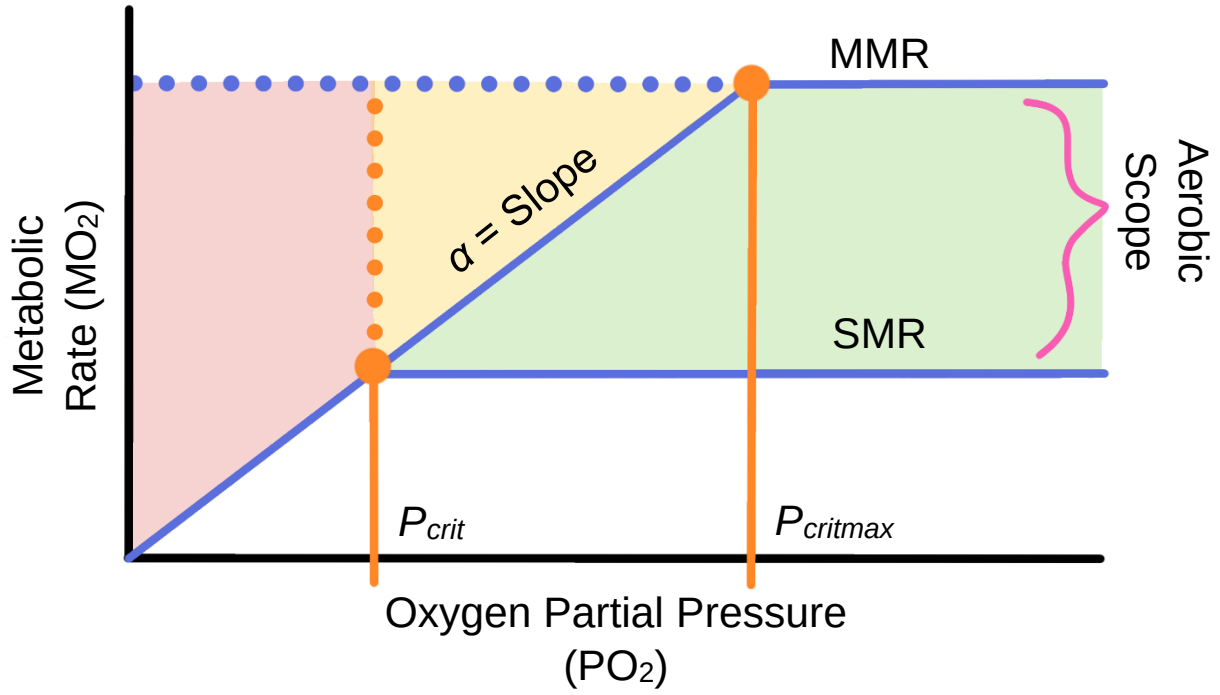


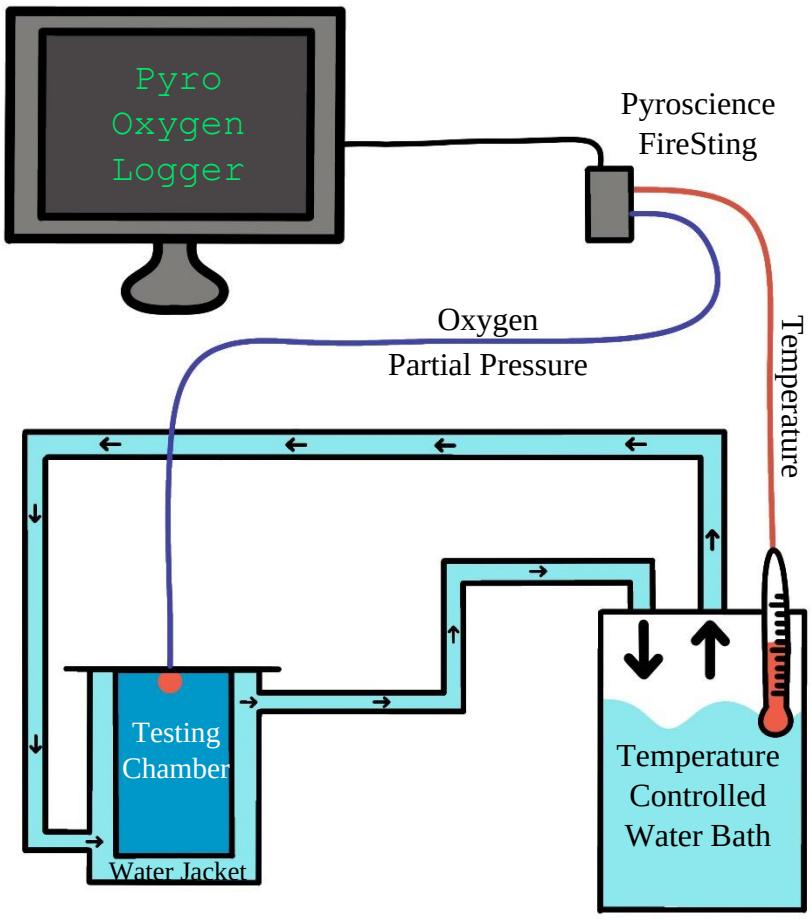
Figure 1: Illustration of the relationship between the metabolic parameters as outlined in Seibel and Deutsch (2020). The oxygen supply capacity, α, can be seen as the relationship between the metabolic rate, or rate of oxygen uptake, and the partial pressure of oxygen, or oxygen availability ($MO_2 = \alpha \times PO_2$). At PO_2 values less than the P_{crit} long term organism survival is not possible and is denoted by the red space. At PO_2 values greater than P_{crit} but less than $P_{critmax}$ it is theoretically possible for an organism to continue activity above SMR so long as they drop metabolic needs along the diagonal blue line, between the green and yellow regions; the region that indicates a required drop in metabolic needs is shown in yellow. The region of the Aerobic Scope, highlighted in green, is where all activities beyond basic physiological maintenance of an organism can occur.

A) 

B) 

Figure 2: Photos of (A) Pink Shrimp (*Farfantepenaeus duorarum*) frozen under a dissecting microscope for identification and (B) in separated acclimation chambers. Central behind the rostrum in both images, indicated by an arrow, an opaque, off-white coloration can be identified as the stomach and other organs of pink shrimp.

A)



B)

Figure 3: (A) Photo of closed respirometry system attached to water bath for temperature control and (B) diagram of the closed respirometry setup. A water jacket encompasses, but is not connected to, the testing chamber. This water jacket contains temperature-controlled water pumped from a water bath. Chambers are sealed at the top and then separately covered to run experiments in the dark. The tubing connecting the pump and water jackets surrounding

Figure 3 (Continued)

experimental inner chamber is insulated to reduce any heat exchanges. A stir bar within the chamber is used to ensure adequate mixing.

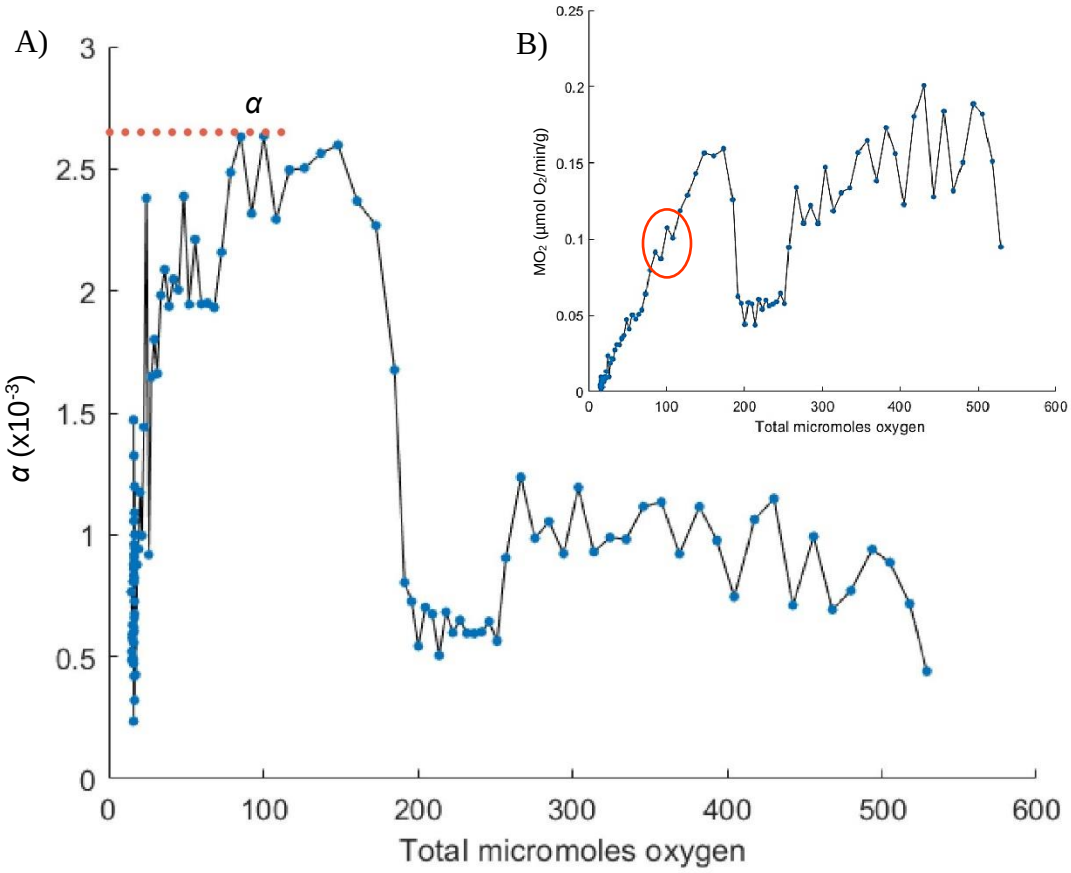


Figure 4: (A) Example plot of oxygen supply (α) versus total amount of oxygen within a chamber. (B) Comparison of the corresponding MO_2 versus total oxygen. The highest α observed during a trial was used to estimate MMR across oxygen partial pressures (as seen in Fig. 5), and is denoted by the red dotted line in (A) and corresponding location circled in (B).

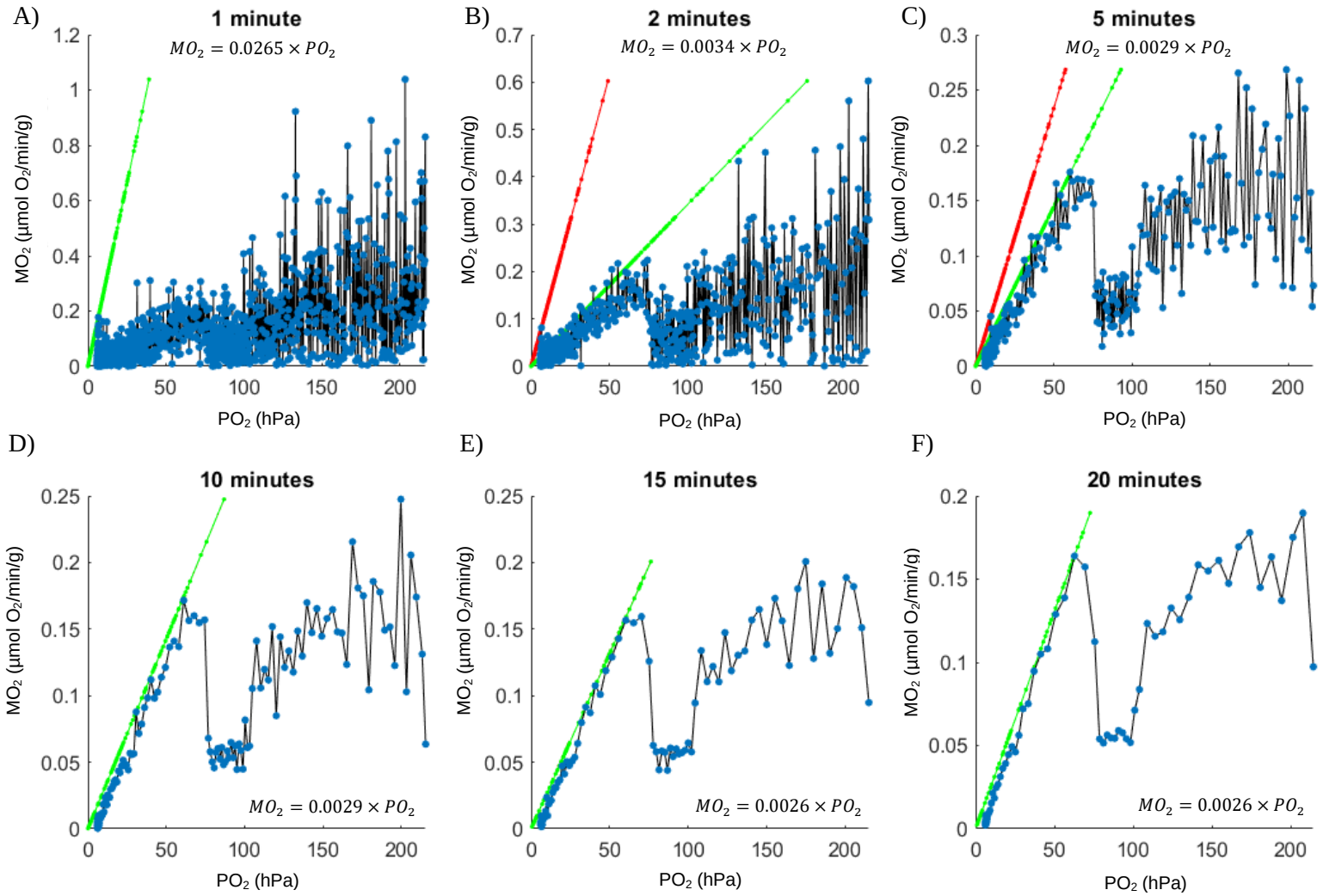


Figure 5: Plots of estimated metabolic rate versus partial pressure of oxygen within chamber for an individual trial at 23°C using (A) 1-min (B) 2-min (C) 5-min (D) 10-min (E) 15-min (F) 20-min time intervals to calculate the metabolic rates. Red lines denote $MO_2 = \alpha \times PO_2$ before application of corrections via the methods outlined by Timpe et al. (in progress), green lines denote $MO_2 = \alpha \times PO_2$ after correction (Seibel et al., 2020). MO_2 equations for the corrected values (green line) are found at the top center in (A),

Figure 5 (Continued)

(B), and (C) and in the bottom right along the x-axis in (D), (E), and (F). Blue circles indicate measured metabolic rates (MO_2) across the experiment. Where no red line is seen indicates that the Timpe et al. (in prep.) correction found little to no error, so the green directly overlies the red. Data shown from a single individual shrimp.

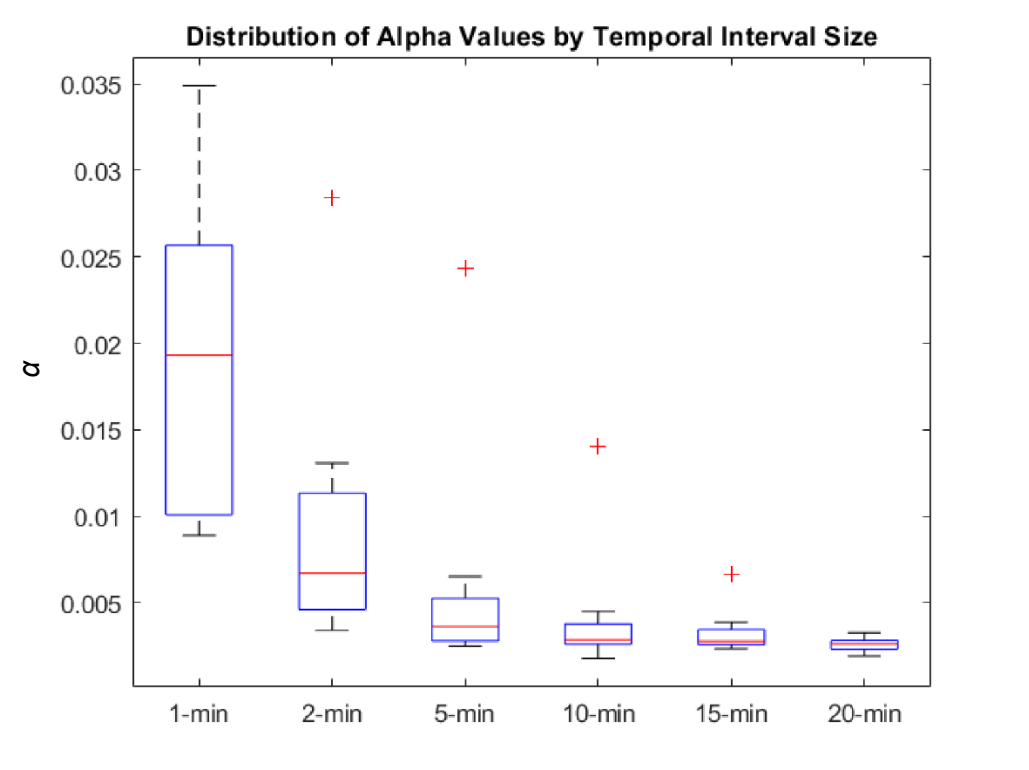


Figure 6: Box plot of α values, as determined for 23°C, distributed by the time interval size (n = 9 per time interval). Red central lines denote the median, the blue edges of the box denote the 25th and 75th percentiles, the black whiskers are the most extreme data points that are not considered outliers, and red (+) indicate outliers.

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APPENDICES

Appendix A: Supplemental Tables

Table A1: Summary of results for the means, medians, and standard deviations of α, SMR, MMR, FAS, and P_{crit} values from tested *Farfantepenaeus duorarum* at 23°C (n = 9) for each time interval. SMR was calculated using Chabot (2016) for time intervals 10-minutes and larger, and thus values, other for α, are only reported for these time intervals. P_{crit} values were extrapolated from Eq. 3 ($MO_2 = \alpha \times PO_2$; from Seibel et al., 2020) with SMR input to MO_2 to solve for the PO_2. MMR is calculated with the assumption of FAS equivalent to 6 ($MMR = 6 \times SMR$). $P_{critmax}$ is calculated as from MMR and α (Eq. 3). MMR.210 and FAS.210 are calculated based on assumption that MMR occurs at 210 hPa ($MMR.210 \times \alpha$). FAS.210 is calculated as $MMR.210 \div SMR$.

23°C	α	SMR	MMR	P_{crit}	$P_{critmax}$	MMR.210	FAS.210
1-min	µmol O_2/g/min/hPa	µmol O_2/g/min	µmol O_2/g/min	hPa	hPa	µmol O_2/g/min	--
Mean	0.0188	--	--	--	--	--	--
Median	0.0193	--	--	--	--	--	--
Std. Dev.	0.0093	--	--	--	--	--	--
2-min							
Mean	0.0033	--	--	--	--	--	--
Median	0.0028	--	--	--	--	--	--
Std. Dev.	0.0014	--	--	--	--	--	--
5-min							
Mean	0.0033	--	--	--	--	--	--
Median	0.0028	--	--	--	--	--	--
Std. Dev.	0.0014	--	--	--	--	--	--
10-min							
Mean	0.0042	0.09	0.55	29.6	177.6	0.79	12.1
Median	0.0029	0.07	0.40	26.3	158.0	0.60	7.8
Std. Dev.	0.0038	0.05	0.27	18.8	113.0	0.80	12.5
15-min							
Mean	0.0033	0.10	0.59	33.0	197.7	0.62	8.5
Median	0.0028	0.07	0.43	28.0	168.3	0.57	7.5
Std. Dev.	0.0014	0.05	0.28	17.6	105.5	0.35	5.1
20-min							
Mean	0.0026	0.10	0.60	38.5	230.9	0.49	6.6
Median	0.0026	0.07	0.44	20.5	183.1	0.55	6.9
Std. Dev.	0.0004	0.05	0.28	17.6	105.5	0.19	2.8

Table A2: Summary of results for the means, medians, and standard deviations of α, SMR, MMR, FAS, and P_{crit} values from tested *F. duorarum* at 20°C (n = 9) for time intervals of 10- and 15-minutes. SMR was calculated using Chabot (2016) for time intervals 10-minutes and larger, and thus values, other for α, are only reported for these time intervals. P_{crit} values were extrapolated from Eq. 3 ($MO_2 = \alpha \times PO_2$; from Seibel et al., 2020) with SMR input to MO_2 to solve for the PO_2. MMR is calculated with the assumption of FAS equivalent to 6 ($MMR = 6 \times SMR$). $P_{critmax}$ is calculated as from MMR and α (Eq. 3). MMR.210 and FAS.210 are calculated based on assumption that MMR occurs at 210 hPa ($MMR.210 \times \alpha$). FAS.210 is calculated as $MMR.210 \div SMR$.

20°C	α	SMR	MMR	P_{crit}	$P_{critmax}$	MMR.210	FAS.210
10-min	µmol O_2/g/min/hPa	µmol O_2/g/min	µmol O_2/g/min	hPa	hPa	µmol O_2/g/min	--
Mean	0.0034	0.08	0.50	26.7	160.0	0.66	9.0
Median	0.0029	0.07	0.42	25.7	155.2	0.61	8.1
Std. Dev.	0.0011	0.03	0.19	13.1	78.8	0.32	5.5
15-min							
Mean	0.0031	0.09	0.55	31.7	190.3	0.65	7.9
Median	0.0030	0.08	0.47	27.8	166.7	0.63	7.6
Std. Dev.	0.0009	0.03	0.20	14.7	88.1	0.19	3.2

Appendix B: Supplemental Figures

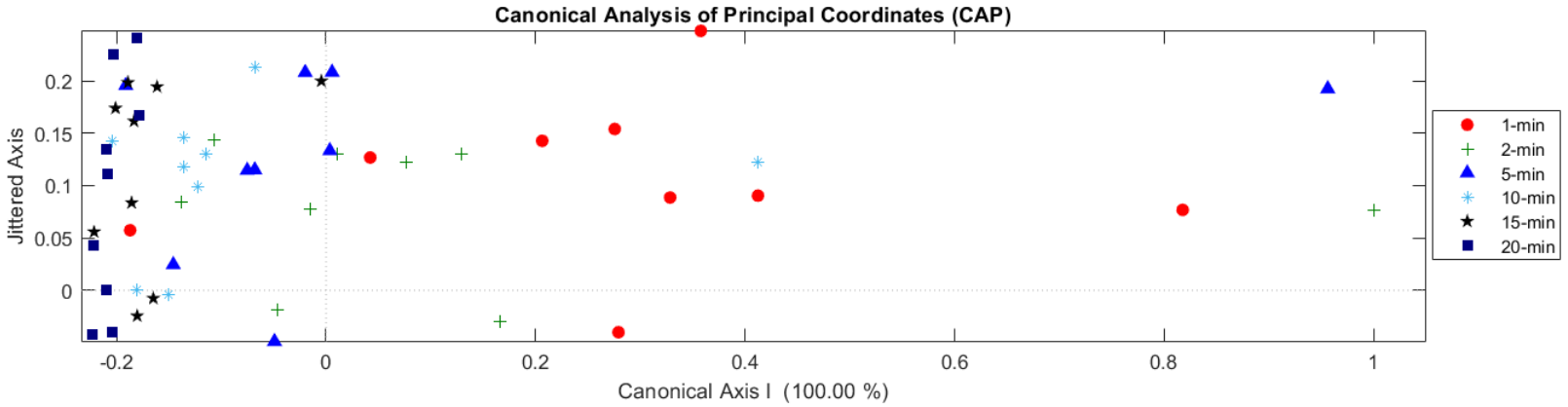

Figure B1: Canonical Analysis of Principal Coordinates (CAP) applied to residuals by time interval as outlined in Anderson and Willis (2003).

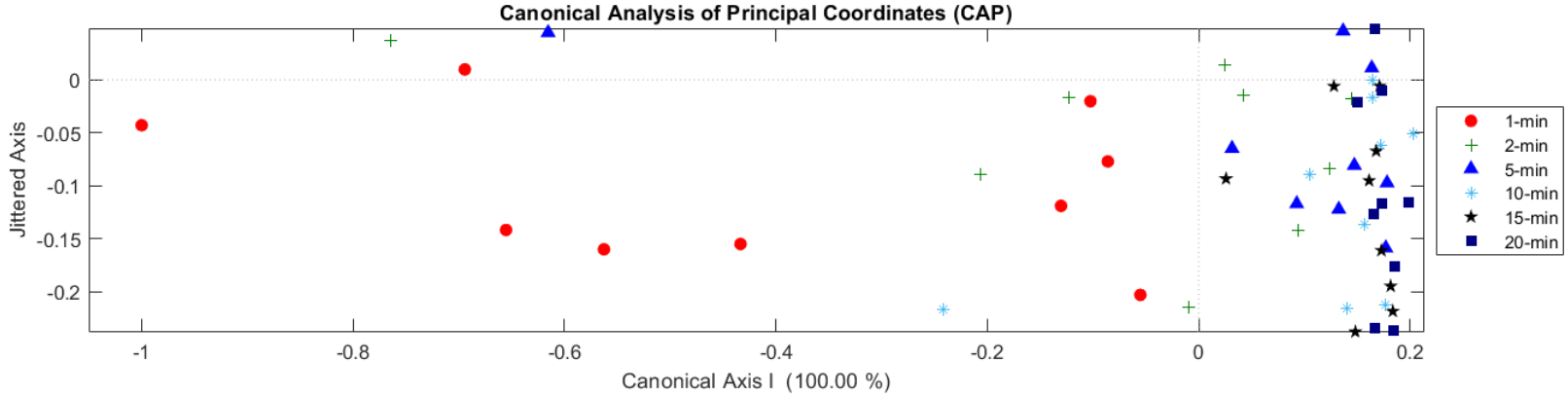

Figure B2: CAP applied to α by time interval as outlined in Anderson and Willis (2003).