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# Respiration by the Opportunistic Spionid Polychaete *Streblospio gynobranchiata* during Adjustment to and Recovery from Moderate Hypoxia

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**Abstract:** Understanding the capacity of estuarine organisms to acclimate to stressful conditions provides insights into how communities cope within fluctuating environments. The opportunistic spionid polychaete, *Streblospio gynobranchiata* Rice and Levin, 1998, regularly experiences intermittent moderate hypoxia within shallow sedimentary habitats. To better understand fine-scale adjustments by this opportunistic species to short-term moderate hypoxia, the aerobic respiration response of three size classes was examined over a 12 h period and after 24 h of exposure to moderate hypoxia (i.e., 20% air saturation) at 25 °C. In addition, the capacity to resume standard respiration was examined over a 12 h period following a 24 h period of exposure to moderate hypoxia. Mass-specific respiration varied with body size during both exposure and recovery from hypoxia. Small worms switched from an oxyregulating to an oxyconforming strategy within 6 h of exposure to moderate hypoxia at 25 °C. After 24 h of hypoxia exposure, small worms hypo-regulated at 81% of the preceding 24 h normoxic reference level. By contrast, medium and large worms hyper-regulated during the first 12 h exposure period, but hypo-regulated at 70% and 79% of the preceding 24 h normoxic reference levels after 24 h of hypoxia exposure. Fluctuations in respiration levels during the recovery period revealed a temporal recovery pattern implying cycling energetic processes. The recovery pattern also indicated some respiration overshoot to compensate for oxygen debt. The timing of the cycling recovery pattern also differed with body size. The ability of *S. gynobranchiata* to dynamically adjust its metabolic response to low oxygen stress underscores the ecologically important role of tolerant organisms within estuarine benthic habitats subject to recurrent diel or intermittent hypoxia.

**Keywords:** respiration; hypoxia; tolerant polychaete; oxyregulation; oxygen debt

## 1. Introduction

Interpreting physiological effects of hypoxia in ecological terms is challenging, considering variation in the magnitude, duration, and frequency of hypoxic exposure regimes. Moreover, because coastal zones typify productive habitats in which hypoxia often occurs naturally, many opportunistic estuarine organisms are adapted to hypoxic regimes [1]. Understanding the capacity of marine organisms to acclimate to stressful conditions provides insights into how they cope with fluctuating environments. As hypoxia is rapidly exacerbating globally [2], understanding the capacity of marine organisms to adjust to hypoxic conditions is also of special concern.

Adaptations of aquatic organisms vary relative to the severity and duration of low oxygen stress [3,4]. Oxyregulating adaptations include ventilation behavior, circulation of internal fluids, the use of blood pigments, and internal branching networks for enhancing transport and diffusion of oxygen between the organism and its surrounding environment [5]. The expression of oxyregulating

adaptations varies with duration of exposure to hypoxia and by life stage. Initial organismal responses help maintain aerobic respiration during short hypoxia episodes [4], such as during diurnal tidal cycles [1]. Thus, during early exposure to hypoxia, organisms may oxyregulate. However, these same organisms may oxyconform when the aerobic demands of resting metabolism cannot be maintained [6,7]. Metabolic depression associated with oxyconforming entails different molecular mechanisms [4], reduced feeding, and the onset of anaerobic metabolism [1,4,8].

Changes in aerobic respiratory rates during exposure to and recovery from hypoxia should vary with adaptations used by aquatic organisms to cope with hypoxic conditions [9]. Responses to hypoxia may occur over distinct phases during early exposure, full adjustment, and recovery periods, in accordance with changing metabolic costs and compensation mechanisms [10]. Although initial responses to hypoxia vary among species, they typically involve mechanisms for maintaining oxygen delivery, including increases in respiration rates, changes in oxygen binding affinities, or other molecular adaptations [4]. Together, such mechanisms constitute a dynamic response to hypoxia, and may involve the need to conserve energy through metabolic depression involving the down-regulation of metabolic processes. These responses can lead to reductions in growth, feeding, reproduction, and ultimately, overall fitness [4,11]. Consequently, the severity and duration of exposure directly affect the risk of mortality [12], especially for immobile organisms.

Constraints on the ability to obtain energy and maintain normal metabolic functioning under hypoxia vary among and within species, and the ability to return to normal metabolic activity following exposure to hypoxia should vary accordingly. Recovery of aerobic respiration refers to the return to unrestricted metabolic function following a hypoxic event [9]. Several types of aerobic responses expressed during recovery from hypoxia can be characterized by the direction and magnitude of compensatory adjustment in oxygen consumption [10]. Early adjustments to hypoxia may incur metabolic costs that must be repaid later when oxygen is more available. Oxygen debt is defined as the repayment of metabolic costs of hypoxia, as marked by a period of supernormal oxygen consumption following the resumption of normoxia [9]. The expression of oxygen debt may provide insights into physiological and biochemical responses to hypoxic conditions [13].

Macrobenthic organisms cope with hypoxia more by virtue of their physiological tolerance than by their opportunistic traits [14]. The widely distributed opportunistic benthic polychaete, *Streblospio benedicti*, is abundant within shallow estuarine habitats that intermittently become hypoxic [15]. Like its cosmopolitan ecological analog *S. benedicti*, *S. gynobranchiata* is a small, common, tolerant, early colonizer within shallow intertidal and subtidal sediments [16,17]. Thus, it can be considered as an indicator of hypoxic conditions when dominant [18]. Recently, *S. gynobranchiata* has been found to be an invasive indicator of polluted conditions in the eastern Mediterranean Sea, where it is having noticeable impacts on the ecosystem by replacing some native opportunistic species [19]. Although tolerance and behavioral responses to low dissolved oxygen (DO) and hydrogen sulfide ($H_2S$) have been examined for *S. benedicti* [15], respiration under hypoxia has not been examined for this species or for *S. gynobranchiata.* Because *S. benedicti* is apparently well adapted to cope with intermittent periods of hypoxia [15], *S. gynobranchiata* should be an ideal species for investigating dynamic aerobic respiration responses to hypoxia.

Here we examine dynamic responses in aerobic respiration by *S. gynobranchiata* at predefined time intervals during and immediately following exposure to moderate hypoxia (i.e., 20% air saturation at 25 °C), which this species would experience naturally. Two experiments were conducted to consider dynamic responses in aerobic respiration during exposure to hypoxia and during metabolic recovery from hypoxia. The objective of the first experiment was to examine the capacity of three size classes of *S. gynobranchiata* to oxyregulate during a 12 h period of exposure to hypoxia. The objective of the second experiment was to examine the capacity of three size classes of *S. gynobranchiata* to resume standard rates of respiration during a 12 h period of normoxia following 24 h of exposure to hypoxia. In addition, we compared aerobic respiration following 24 h of hypoxia exposure to the preceding 24 h of normoxia within the recovery experiment. We expected that mass-specific respiration would vary

with exposure time and body size, as a function of different adaptive capabilities. Deeper knowledge of respiratory responses to and recovery from exposure to hypoxia will contribute to the eco-physiological characterization of opportunistic estuarine species.

## 2. Materials and Methods

### *2.1. Specimen Collection and Culture*

Adult *S. gynobranchiata* were collected during low tide from the top 5 cm of sediment within sparsely vegetated mud-sand bottom tidal creek habitat in Weeks and Simmons Bayous, Mississippi in April 2014. Polychaetes were recovered by gently washing single cups of sediment over a 0.5 mm nitex mesh sieve, and gently picking and retaining specimens for laboratory culture. Methods for the culture of *S. gynobranchiata* mostly followed procedures developed and used for the culture of *Capitella* spp. in the Grassle laboratory (J. Grassle, pers comm). Briefly, groups of 50 or fewer adult polychaetes were maintained in 4.5" diameter culture dishes along with approximately 1 tablespoon of sediment enriched with TetraMin® fish flakes and 2 cm of standing artificial saltwater (ASW) at 23 ppt. Larval culture methods were developed based on the culture of other spionid polychaetes [20,21], and modified as described in [22]. Planktonic larvae were kept under natural light at room temperature (~20 °C) and 23 ppt at densities of about 1 larva $mL^{-1}$, and fed a regular supply of unicellular algae, including *T-isochrysis*, *Rhodonomas*, and *Chaetoceros*. Newly settled juveniles were allowed to grow within adult culture conditions for 4 weeks prior to handling.

### *2.2. Experimental Conditions*

Two experiments each using 20 different individual worms of various sizes were conducted separately to consider dynamic responses in aerobic respiration during exposure to hypoxia (exposure experiment) and during metabolic recovery from exposure (recovery experiment).

For the exposure experiment, conducted from 11 July to 26 August 2016, the capacity of three size classes (small, medium, and large) (0.42–2.22 mg) of *S. gynobranchiata* to oxyregulate during a 12 h period of exposure to hypoxia was tested. For this experiment, oxygen consumption measurements (0 h) were made following established protocols [13,23,24] for 20 individual worms, after 24 h of acclimation to normoxia (100% air saturation) at a salinity of 23 (i.e., same salinity as culture conditions) and 25 °C. Next, repeated measurements of oxygen consumption rates for the 20 subjects were made individually at 3 h intervals after 3, 6, 9, and 12 h during the 12 h period of exposure to hypoxia (20% air saturation) at 25 °C in ASW at 23 ppt.

For the recovery experiment conducted from 27 July 2016 to 19 August 2016, the capacity of three size classes (small, medium, and large) (0.31–2.01 mg) of *S. gynobranchiata* to resume standard rates of respiration was examined. For this experiment, normoxic respiration rates were measured for 20 individual worms following exposure to 100% air saturation at 25 °C for 24 h. The subjects were different than those used for the exposure experiment. Next, a hypoxic respiration measurement was made after 24 h of hypoxia exposure. This hypoxia respiration measurement also provided a longer-term period of adjustment to hypoxia (24 h) for comparison with the exposure experiment. Finally, repeated respiration measurements were made every 2 h (i.e., after 2, 4, 6, 8, 10, and 12 h) during a 12 h period of normoxia. The 2 h interval in the recovery experiment allowed for a finer scale of detection of differences in aerobic respiration during recovery.

To allow for physiological adjustment to normoxia (i.e., 100% air saturation) at 25°C, subjects were placed into individually labeled 5 cm diameter glass petri dishes with sediment within an incubator (Precision™ Low Temperature Incubator Model 815, Marietta, OH, USA). Exposure to hypoxia (20% air saturation) was accomplished within sealed air chambers (BioSpherix ©, Parish, NY, USA) into which nitrogen gas was pumped and regulated at the designated level of DO (Proox© Model 110 O2 regulator, Parish, NY, USA). Sealed chambers were housed within a Precision© Low Temperature Incubator (Model 815) to maintain the appropriate temperature. Prior to the addition of subjects,

the chamber was held for 24 h at the desired DO and temperature to ensure that the air-water interface had reached equilibrium and DO had stabilized. DO and temperature of the treatment water were checked daily using a handheld optical dissolved oxygen meter (YSI ProODO® Digital Professional Series, Yellow Springs, OH, USA).

Respiration measurements followed a fixed protocol developed in our lab. Respiration was measured for 1 h periods using a FireStingO2™ (2 channel) (Aachen, Germany) oxygen sensing meter. All respiration observations were made in complete darkness, following other studies [25–28]. Corresponding background DO measurements accounted for any bacterial respiration or other sources of drift [22]. Repeated observations on individuals were made using the same channel of the oxygen sensing meter. Worms were transferred into respirometry syringes containing 2 mL of treatment water within pyramid shaped mesh pouches to minimize the movement of subjects. The bare fiber-optic DO probe was secured to the respirometry syringe by an adapter and attached to the oxygen meter. The decline in oxygen concentration was recorded continuously by means of Oxygen Logger™ software (Aachen, Germany). The treatment temperature was maintained using a Boekel Grant Optima™ Model GD100 (Feasterville, PA, USA) circulating water bath. Syringes were secured in the water bath and allowed to float within the gentle surface turbulence to help circulate water within the syringe and avoid local depletion of DO near the subject. After each successive interval, subjects were returned to petri dishes within the sealed air chamber and repeatedly exposed to experimental conditions prior to taking respiration measurements. After recording oxygen consumption for the final time interval, the blotted wet weight for each subject was measured to the nearest $10^{-5}$ g on an Ohaus model AP250D™ microbalance (Melrose, MA, USA).

Decline in oxygen concentration was used to estimate mass-specific respiration rate ($MO_2$) for each individual subject. To avoid measurement noise due to sensor equilibration, the decline over the last 10 min of the measurement period provided the definitive $MO_2$. Oxygen consumption rates were obtained as the slopes from regressions of oxygen concentration versus time [29–31]. Each respiration estimate was defined as the difference in $O_2$ consumption in the presence of the subject minus any equipment drift in the absence of the subject. The mass-specific respiration rate, $MO_2$ (mg $O_2$ $g^{-1}$ $h^{-1}$) was calculated as:

$$\dot{M}O_2 = \frac{dDO}{dt} \times \left( \frac{V_r - V_a}{m} \right)$$

where $MO_2$ = the instantaneous mass-specific respiration rate (mg $O_2$ $g^{-1}$ $h^{-1}$); dDO/dt = the rate of decrease of DO (mg $O_2$ $L^{-1}$ $h^{-1}$); Vr = respirometer volume (L) (with volumetric correction for mesh pouch); Va = volume of experimental subject (L); and m = subject mass (g).

### *2.3. Data Analysis*

To examine mass-specific respiration rates over 12 h of exposure to hypoxia, individual subjects were first grouped within three size classes to treat body size as an explicit categorical factor. Accordingly, individual subjects were grouped into small ($n$ = 3) (≤ 0.75 mg), medium ($n$ = 14) (> 0.75 and ≤ 1.5 mg), or large ($n$ = 3) (> 1.5 mg) size classes. Unequal group sizes resulted from, (1) the necessary selection of subjects by eye (i.e., subjects were not pre-weighed to avoid harming them); and (2) limited availability of some size classes within cultures. Each subject was represented by five serial respiration measurements. A repeated measures Linear Mixed Model ANOVA using a diagonal covariance structure (DIAG) was conducted using the SPSS linear mixed model (LMM) procedure (SPSS version 18). The diagonal covariance structure was more parsimonious than the heterogeneous first order autoregressive covariance structure (ARH1). $MO_2$ data were $\log_{10}$ transformed to fulfill the LMM normality assumption. Time, Size, and the Time × Size interaction served as fixed factors, and Time also served as a repeated factor. The Time factor comprised the initial normoxic (0 h) in addition to four 3-h hypoxia intervals (3, 6, 9, and 12 h). An intercept was included in the model.

Profile plots of mean $MO_2$ were examined across the time intervals for the three size classes to formulate six custom or interaction contrasts of differences prior to and during exposure (Table 1):

(1) the normoxic reference (0 h) versus the first hypoxic period (3 h) for all three size classes; (2) the mean for the 6 h and 9 h versus the 12 h hypoxic period for all three size classes; (3) the 9 h versus the 12 h hypoxic period for all three size classes; (4) the 3 h versus the mean of the 6 h, 9 h, and 12 h hypoxic periods for small versus medium and large size classes; (5) the normoxic (0 h) versus the mean of the 6 h, 9 h, and 12 h hypoxic periods for small versus medium and large size classes; and (6) the mean of the normoxic (0 h) and 3 h hypoxic periods versus the mean of the 6 h, 9 h, and 12 h hypoxic periods for small versus large size classes. The latter three interaction contrasts addressed whether mass-specific respiration differed qualitatively with respect to size class during exposure to hypoxia.

To examine mass-specific respiration rates over the 12 h recovery period, individual subjects were first grouped within three size classes to treat body size as an explicit categorical factor. Thus, individual subjects were grouped into small ($n$ = 9) (≤ 0.75 mg), medium ($n$= 8) (> 0.75 and ≤ 1.5 mg), or large ($n$ = 3) (> 1.5 mg) size classes. Each subject was represented by eight serial respiration measurements. A repeated measures Linear Mixed Model ANOVA using a first order autoregressive covariance structure (ARH1) was conducted using the SPSS linear mixed model (LMM) procedure (SPSS version 18). $MO_2$ data were $\log_{10}$ transformed to fulfill the LMM normality assumption. Time, Size, and the Time × Size interaction served as fixed factors, and Time also served as a repeated factor. The Time factor comprised the normoxic reference (−24 h) in addition to a hypoxic measurement (0 h), followed by six normoxic 2 h recovery intervals (2, 4, 6, 8, 10, and 12 h). An intercept was included in the model.

Profile plots of mean $MO_2$ were examined across the time intervals for the three size classes to formulate six custom or interaction contrasts of differences prior to and during recovery: (1) the normoxic reference (−24 h) versus the 24 h hypoxic period (0 h) for all three size classes; (2) the normoxic reference (−24 h) versus the mean of the six normoxic recovery periods (2, 4, 6, 8, 10 and 12 h) for all three size classes; (3) the 24 h hypoxic period (0 h) versus the mean of the six normoxic recovery periods (2, 4, 6, 8, 10 and 12 h) for all three size classes; (4) the early recovery period (2 and 4 h) versus the late recovery period (10 and 12 h) for small versus large size classes; (5) the early recovery period (2 and 4 h) versus the mid recovery period (6 and 8 h) for small versus large size classes; and (6) the mid recovery period (6 and 8 h) versus the late recovery period (10 and 12 h) for small versus large size classes. The latter three interaction contrasts addressed whether recovery differed qualitatively with respect to size class.

## 3. Results

### *3.1. Exposure*

Mass-specific respiration varied with size class and time interval during 12 h of exposure to hypoxia (Figure 1). Mass-specific respiration was inversely related to body size under normoxia, averaging 19.6 ± 1.4, 2.0 ± 0.6, and 1.0 ± 0.3 mg $O_2$ $g^{-1}$ $h^{-1}$ (mean ± 1 se), for small, medium, and large subjects, respectively. Initially, all three size classes oxyregulated under hypoxia. Mass-specific respiration by small worms was particularly high under normoxia (19.6 ± 1.4 mg $O_2$ $g^{-1}$ $h^{-1}$), as well as being high and variable after the 3 h hypoxia period (19.5 ± 8.6 mg $O_2$ $g^{-1}$ $h^{-1}$). By the time 6 h of hypoxia had ensued, mass-specific respiration by small worms had fallen off to levels comparable with medium and large worms, signifying oxyconforming by small worms. Conversely, mild hyper-regulation by medium and large size classes was indicated by noticeably elevated mass-specific respiration during hypoxia than under normoxia, especially at 12 h for large worms (5.0 ± 2.8 mg $O_2$ $g^{-1}$ $h^{-1}$).

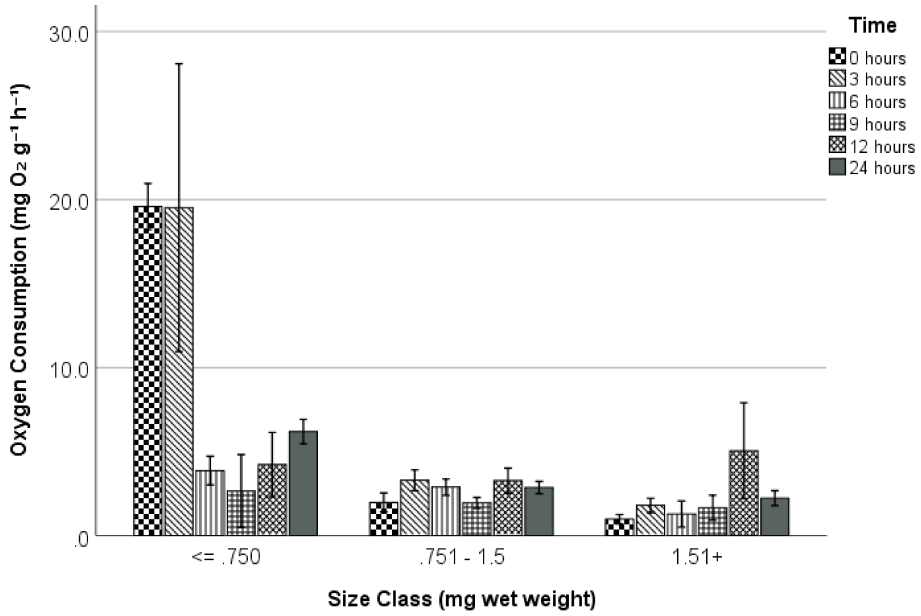


**Figure 1.** Mass-specific respiration rates ($MO_2$ (mg $O_2$ $g^{-1}$ $h^{-1}$)) (mean ± 1 one se) across the three body- size classes during 12 h of moderate exposure to hypoxia (20% air saturation). Respiration was measured for all subjects at a normoxic reference (0 h) and at four 3-h hypoxia exposure intervals (3, 6, 9, and 12 h). Solid grey bar represents measurements after 24 h of hypoxia exposure for 20 different individuals from the recovery experiment.

**Table 1.** Custom and interaction contrasts for the exposure experiment (see text for explicit descriptions of time intervals) (bold $p < 0.05$).

| Exposure-Custom Contrasts | *t*-Value | df | *p* |
|---|---|---|---|
| (1) 0 vs 3 h for all 3 sizes | 1.054 | 33.7 | 0.299 |
| (2) 6 and 9 h vs 12 h for all 3 sizes | 0.324 | 41.0 | 0.748 |
| (3) 9 h vs 12 h for all 3 sizes | 2.313 | 33.7 | **0.027** |
| (4) 3 h vs 6, 9, and 12 h for small vs med and large | 2.835 | 32.1 | **0.008** |
| (5) 0 h vs 6, 9, and 12 h for small vs med and large | 4.113 | 29.5 | **<0.001** |
| (6) 0 and 3 h vs 6, 9, and 12 h for small vs large | 3.594 | 74.4 | **0.001** |

The Linear Mixed Model ANOVA (LMM) for the exposure experiment showed respiration differed overall in terms of the Time factor (F = 3.26; $p = 0.024$), Size Class (F = 11.9, $p < 0.001$), as well as for the interaction between Time and Size Class (F = 3.5; $p = 0.006$). Heterogeneous variance was accommodated by discrete covariance estimates across the repeated time intervals.

Contrasts highlighted differences in mass-specific respiration during the hypoxia exposure period (Table 1). For all three size classes combined, mass-specific respiration did not differ between the reference normoxic period (0 h) and the 3 h hypoxic period ($p = 0.299$), nor for the combined 6 and 9 h periods versus the 12 h period ($p = 0.748$). However, mass-specific respiration was notably higher in the 12 h period than in the previous 9 h period for all three size classes combined ($p = 0.027$). There were also meaningful interactions between size class and time. Mass-specific respiration was relatively high for small worms in the first hypoxic period (3 h) than in the latter three hypoxic periods (6, 9, and 12 h), as opposed to being relatively lower for medium and large worms during the first hypoxic period (3 h) ($p = 0.008$). Similarly, mass-specific respiration was relatively high for small worms in the reference normoxic period (0 h) as opposed to being relatively high for medium and large worms during the latter three hypoxic periods (6, 9, and 12 h) ($p < 0.001$), confirming hyper-regulation by medium and large size classes. Finally, mass-specific respiration was higher for small worms in both the reference normoxic and first hypoxic periods (0 h and 3 h) combined as opposed to being relatively higher for

large worms during the latter three hypoxic periods (6, 9, and 12 h) ($p = 0.001$), underscoring that mass-specific respiration for small worms was relatively low during late exposure (oxyconforming), whereas for large worms it was relatively high during late exposure (hyper-regulating).

A longer-term period of adjustment to hypoxia by *S. gynobranchiata* was observed in the recovery experiment for which a different set of subjects was exposed to 20% DO saturation for 24 h at 25 °C (Figure 1). Compared to the latter half of the exposure experiment (i.e., mean for 6, 9, and 12 h), mass-specific respiration was markedly higher after 24 h of hypoxia exposure for small worms (6.19 ± 0.92 vs. 3.58 ± 0.90; t = 4.81, $p = 0.001$), and comparable for medium worms (3.00 ± 0.35 vs. 2.70 ± 0.32; t = 0.96, $p = 0.37$) and large worms (2.23 ± 0.44 vs. 2.67 ± 1.06; t = −0.98, $p = 0.43$). In addition, compared to the preceding normoxic period in the recovery experiment, worms hypo-regulated at somewhat lower than normoxic levels following the 24 h hypoxic period (i.e., 81%, 70%, and 79% for small, medium, and large, respectively) ($p = 0.01$ for all three size classes combined) (Figure 2). By contrast, small worms oxyconformed while medium and large worms hyper-regulated somewhat during the latter half of the 12 h exposure experiment.

### 3.2. Recovery

Mass-specific respiration rates also varied with body size during 12 h of hypoxia recovery (Figure 2). Mass-specific respiration was generally inversely related to body size across the three size classes during the recovery experiment, averaging 7.3 ± 0.5, 4.8 ± 0.3, and 2.7 ± 0.2 mg $O_2$ $g^{-1}$ $h^{-1}$ (mean ± 1 se), for small, medium, and large subjects across all treatment levels, respectively. As mentioned, mass-specific respiration was consistently lower for all three size classes after the 24 h hypoxic period (0 h) compared to the preceding normoxic period (−24 h). Mass-specific respiration rates were generally higher during recovery than for the reference normoxic and hypoxic periods. Moreover, a temporal cycling pattern that varied across size classes was revealed by fluctuating levels during the recovery period (Figure 2). When considered as three recovery phases, each comprising two 2 h observation periods, relatively low rates of mass-specific respiration occurred during the mid-recovery phase for small and medium worms and during the early recovery phase for large worms. Conversely, mass-specific respiration was relatively higher during early and late phases for small and medium body sizes, and relatively lower during the early phase for large body sizes.

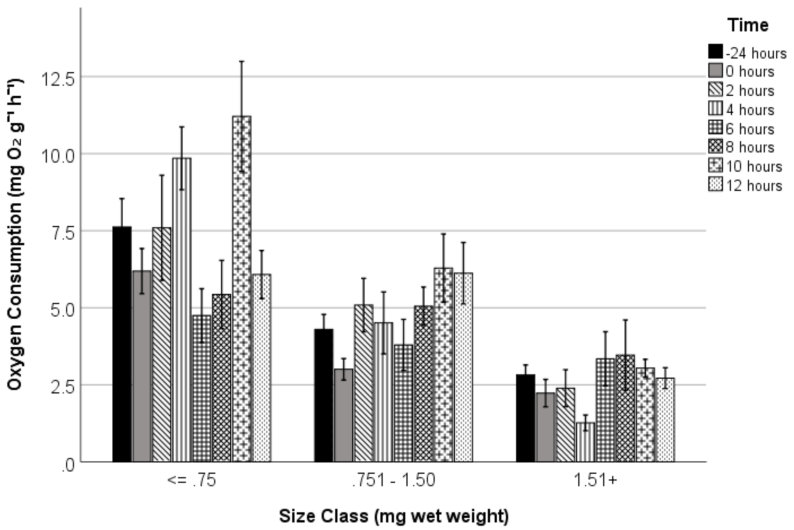


**Figure 2.** Mean mass-specific respiration rates ($MO_2$ (mg $O_2$ $g^{-1}$ $h^{-1}$)) (mean ± 1 one se) over the 12 h period of recovery from 24 h of moderate hypoxia (20% air saturation). Respiration was measured for all subjects at a normoxic reference (−24 h), after 24 h of hypoxia (0 h), and at six 2h normoxic recovery intervals (2, 4, 6, 8, 10, and 12 h).

The Linear Mixed Model ANOVA (LMM) for the hypoxia recovery experiment revealed respiration differed overall in terms of Time (F = 2.58; $p = 0.033$), Size Class (F = 19.8, $p < 0.001$), and for the interaction between the Time and Size Class factors (F = 2.2; $p = 0.034$). Heterogeneous variance as well as serial autocorrelation was accommodated by separate covariance estimates and the ARH1 rho term across the repeated time intervals.

Contrasts highlighted differences in mass-specific respiration during the hypoxia recovery period (Table 2). Mass-specific respiration was lower for the 24 h hypoxic period (0 h) than for the preceding reference normoxic period (-24 h) for all three size classes combined ($p = 0.01$). However, mass-specific respiration did not differ between the reference normoxic period and the mean of the six recovery periods for all three size classes combined ($p = 0.569$). On the other hand, mass-specific respiration was lower for the 24 h hypoxic period than for the mean of the six recovery periods for all three size classes combined ($p = 0.043$). Three interaction contrasts compared the three recovery phases between small and large worms. Mass-specific respiration did not differ between early and late recovery phases ($p = 0.138$) or between mid and late recovery phases ($p = 0.107$) for small versus large worms. However, mass-specific respiration differed between early and mid-recovery phases for small versus large worms ($p = 0.006$). Mass-specific respiration was relatively higher for small worms and was relatively lower for large worms in the early recovery phase than in the mid recovery phase (Figure 2).

**Table 2.** Custom and interaction contrasts for the recovery experiment (see text for explicit descriptions of time intervals) (bold $p < 0.05$).

| Recovery-Custom Contrasts | *t*-Value | df | *p* |
|---|---|---|---|
| (1) −24 h vs 0 h for all 3 sizes | −2.784 | 25.9 | **0.01** |
| (2) −24 h vs 2, 4, 6, 8, 10, and 12 h for all 3 sizes | −0.574 | 45.8 | 0.569 |
| (3) 0 h vs 2, 4, 6, 8, 10, and 12 h for all 3 sizes | 2.074 | 53.6 | **0.043** |
| (4) 2 and 4 h vs 10 and 12 h for small vs large | 1.509 | 45.4 | 0.138 |
| (5) 2 and 4 h vs 6 and 8 h for small vs large | 2.865 | 51.1 | **0.006** |
| (6) 6 and 8 h vs 10 and 12 h for small vs large | −1.640 | 48.2 | 0.107 |

## 4. Discussion

### *4.1. Exposure*

The respiration response to moderate hypoxia exposure varied with body size for *S. gynobranchiata*; initially, small worms oxyregulated before oxyconforming, whereas medium and large worms hyper-regulated somewhat during the entire 12 h exposure period. Small *S. gynobranchiata* adjusted to moderate hypoxia by adopting an oxyconforming strategy within 6 h of exposure to hypoxia. A dichotomy in respiration strategy based on body-size has been documented for the tolerant polychaete, *Capitela telata* [7]. Likewise, mass-specific respiration was markedly higher for small *Arenicola* than for large worms during an early phase of hypoxia adjustment [32]. *Arenicola* retained the ability to oxyregulate after as long as 2 h of anaerobic conditions. A size-related dichotomy in respiration in our study was evidenced by the lack of major differences in mass-specific respiration between small and larger worms after 6 h of exposure. In addition, elevated respiration for all three size classes at 12 h of hypoxia suggests higher levels of energy were required to maintain aerobic respiration during the latter part of the 12 h exposure period. The polychaete, *Hydriodes elegans*, also showed a dynamic respiratory response involving an initial decrease followed by a gradual increase in respiration when exposed to low oxygen at 2 or 4 mg $O_2$ $L^{-1}$ for 4 days [33]. Indeed, oxyconformation and oxyregulation represent extreme end points at opposite poles of a plastic respiration strategy gradient, the expression of which can vary with respect to body-size, species, oxygen availability, duration of exposure, and temperature [7]. The dynamic biological response over the course of exposure to hypoxia likely involves the expression of different underlying respiratory adaptations over time [34].

*Streblospio gynobranchiata* possesses various adaptations for maintaining aerobic respiration, including high surface area:volume, ventilation behavior, enhanced internal circulation, and increased concentrations of blood pigments. Surface area seems to play a large role in oxygen exchange for this small, elongated organism. This species has a pair of external banded branchiae, which increases the surface area and efficiency of oxygen absorption. Like *Streblospio benedicti*, *S. gynobranchiata* may spread its branchiae onto the sediment surface outside of its tube or into the water column to obtain more oxygen [15]. *S. gynobranchiata* also may possess efficient red blood cells with high oxygen binding affinities or alternative blood pigments. Red pigment through their palps and branchiae suggests the presence of hemoglobin in *S. gynobranchiata* (S. Rice, pers. comm.). The idea that opportunistic species often oxyregulate under low oxygen is also supported by a study of aerobic respiration of the estuarine polychaete, *Alitta succinea* [6]. In any case, successful oxyregulation under low oxygen suggests tolerant organisms maintain aerobiosis for as long as possible in order to delay switching to less efficient or more energetically costly methods of meeting metabolic demands. From our study, it appears that the capacity of *S. gynobranchiata* to maintain aerobic respiration under moderate hypoxia and temperature varies with body size and time of exposure.

Depressed respiration by small worms observed during much of the first 12 h of hypoxia in the present study likely reflected certain underlying physiological mechanisms for coping with metabolic demands. Small organisms with higher surface/area volumes and less prolific branching networks underlying the 2/3 and 3/4 scaling rules, reach much higher mass-specific respiration rates than larger organisms. Thus, they are also more susceptible to early effects of stress when conditions change, or to delayed induction of feedback systems in response to low oxygen. The observed depressed respiratory response for small worms during early hypoxia exposure may also be required for reducing energetic costs in preparation for potentially longer periods of hypoxia. Early down-regulation in metabolism in response to hypoxia might mitigate oxygen supply deficits and ATP demands [35]. A common early response to hypoxia on the cellular level is the release of reactive oxygen species like the Hypoxia Inducing Factor (HIF) within mitochondria in order to induce a suite of adaptive mechanisms to counteract effects of low oxygen. Metabolic depression also accompanies anaerobic metabolism. Thus, oxyconformity during hypoxia adjustment signifies a stress response to a quickly changing oxygen regime.

A longer-term hypoxia adjustment period for *S. gynobranchiata* inferred from our recovery experiment implied that all three size classes oxyregulated at somewhat lower than normoxic levels, within 24 h of hypoxia. By contrast, small worms oxyconformed while medium and large worms hyper-regulated somewhat during the latter half of the first 12 h of exposure to hypoxia. Even longer periods of exposure have been used to examine tolerance to low DO and $H_2S$ for the closely related *Streblospio benedicti* [15]. This species can withstand fairly long periods of hypoxia at 26 °C. After 14 d, 89.5% survived the 14.5% DO saturation treatment, whereas 70.0% survived the 7.0% DO saturation treatment, and 94.7% survived control air saturated conditions. Long-term tolerance to hypoxia was apparently facilitated by the resumption of feeding after 3.5 d in the 14.5%, and after 4.5 d in the 7.0%, treatment. However, although *Streblospio* is fairly tolerant of low DO and $H_2S$, it does not survive well under anoxia. The LT50 under anoxia was 43 h. Tolerant species vary in their resilience to stressors.

### *4.2. Recovery*

Mass-specific respiration during recovery from exposure to hypoxia also varied inversely with body size for *S. gynobranchiata*. Respiration varied during the recovery period and was often higher than in the normoxic reference period. Fluctuations in respiration levels during the recovery period revealed a temporal recovery pattern implying cycling energetic processes. The recovery pattern also indicated some respiration overshoot to compensate for oxygen debt. The timing of the cycling recovery pattern also differed with body size. Mass-specific respiration for small and medium size classes was higher during early and late recovery periods, whereas, mass-specific respiration was relatively low during the early recovery period for the large size class. A return to normal cycling

in the synthesis and use of ATP could explain such a temporal pattern [9,36]. Following exposure to hypoxia, a major part of the metabolic recovery process is thought to require recharging the ATP pools. The recharging process apparently occurs very quickly in invertebrates, with ATP potentially returning to normal levels within 15 min to 1 h [9]. Metabolism is also subject to feedback induced production of ATP. However, further study involving direct assays of metabolites is required to understand the role of ATP cycling during hypoxia recovery in *S. gynobranchiata*.

Respiratory responses expressed during recovery from hypoxia are characterized by compensatory adjustment in oxygen consumption [10]. Aquatic species can show different recovery responses indicating subnormal oxygen debt, normal oxygen debt, supernormal oxygen debt, or no oxygen debt. Apparent respiratory overshoot during our study suggested some supernormal oxygen consumption associated with oxygen debt. Such overshoot presumably reflects heightened energy needs related to anaerobic end product disposal and re-saturation of body tissues with oxygen [26]. Oxygen debt is generally associated with the accumulation of anaerobic end products in multiple tissues [13], potentially leading to variable hypoxia recovery responses. In crustaceans, oxygen debt is often marked by respiratory overshoot following exposure to hypoxia. Vismann and Hagerman [29] showed that lactate oxidation accounted for almost half of the oxygen debt incurred during an 8 h recovery period for the isopod *Saduria entomon*. In most cases, the extent of respiratory overshoot is commensurate with the duration and severity of hypoxia [26]. Accordingly, only minor respiratory overshoot was observed in our study of recovery from 24 h of moderate hypoxia.

Many polychaetes appear to be particularly well adapted to hypoxia, and often show the ability to quickly return to normal respiration [34]. Furthermore, polychaetes can avoid accumulation of anaerobic end products by excreting them in real time during exposure [9]. For example, the polychaetes, *Arenicola marina* and *Euzonus mucronata*, and the leech, *Hirudo medicinalis*, excrete large amounts of succinate and proprionate directly into the medium. Dales [32] found that *A. marina* was able to store enough oxygen in its blood to sustain it for some period of time when exposed to anoxia. In most cases, repayment of the oxygen debt is needed to reoxidize lactic acid; however, many polychaete species do not use the lactate pathway during anaerobic respiration. In the case of *A. marina*, an anaerobic pathway involving products other than lactic acid precluded accumulations of lactate or pyruvate. The minor oxygen debt observed for *S. gynobranchiata* in our study might reflect only slight induction of anaerobic metabolism under moderate hypoxia. It is also possible that *S. gynobranchiata* uses anaerobic pathways involving less toxic by-products that can be quickly excreted. Activities of various dehydrogenases involved in anaerobic metabolism have also been documented for *S. gynobranchiata* exposed to moderate hypoxia (pers. obsv.).

The response to moderate hypoxia by *S. gynobranchiata* likely reflects its ecological status as a common opportunistic resident within intertidal and shallow subtidal habitats experiencing frequent periodic moderate hypoxia. In tidal habitats, organisms must endure periods of low to no oxygen during low tide [5]. Accordingly, *Streblospio gynobranchiata* appears to be well adapted to periodic hypoxia in such habitats (pers. obsv.). Its metabolic resilience under low DO enables *S. gynobranchiata* to respond quickly to diurnal exposure to hypoxia within organic-rich sediments of shallow benthic habitats of marshes and tidal creeks. The duration and severity of hypoxia at the temperature (25 °C) experienced by *S. gynobranchiata* in our study falls well within the normal range of conditions this species experiences within its natural shallow subtidal and intertidal soft-sediment environment on a diel cycle or intermittently in summer. Benthic function is buffered and vital ecosystem services maintained by such tolerant species [6]. Mortality due to hypoxia detrimentally changes macrobenthic populations and ecosystems [37–40], leading to altered food webs and a loss of fisheries production [41–43], as well as altered sedimentary biogeochemical processes [44]. Resilience in benthic function provided by tolerant species promotes the recovery of hypoxic habitats by facilitating the succession of benthic organisms once conditions recover [45]. Tolerant opportunistic species like *S. gynobranchiata*, also help maintain critical ecosystem functions in support of benthic-pelagic coupling, including bioturbation and nutrient regeneration, while sustaining secondary production [6,15]. The ability of *S. gynobranchiata*

to dynamically adjust its metabolic response to low oxygen stress underscores the ecologically important role of tolerant organisms within estuarine benthic habitats subject to recurrent diel or intermittent hypoxia.

## 5. Conclusions

Opportunistic estuarine species presumably evolved to tolerate frequent exposure to mild hypoxia. Depending on physical demands and body size, respiration strategies can range from oxyregulating to oxyconforming within the same species. Accordingly, the metabolic response by *S. gynobranchiata* was dynamic relative to time and plastic relative to body size during exposure to and recovery from moderate hypoxia. Small worms switched from an oxyregulating to an oxyconforming strategy within 6 h of exposure to moderate hypoxia at 25 °C. Moreover, hyper-regulation during the first 12 h of hypoxia exposure implied elevated costs of maintenance for medium and large size classes. Hypo-regulation by all three size-classes after 24 h of hypoxia exposure implied further metabolic adjustment. Fluctuations in respiration levels during the recovery period revealed a temporal respiration pattern implying cycling energetic processes and some respiration overshoot to compensate for oxygen debt. Metabolic resilience under low DO likely enables *S. gynobranchiata* to respond quickly to diurnal exposure to hypoxia within organic-rich sediments of shallow benthic habitats of marshes and tidal creeks. The ability of *S. gynobranchiata* to dynamically adjust its metabolic response to low oxygen stress underscores the ecologically important role of tolerant organisms within estuarine benthic habitats subject to recurrent diel or intermittent hypoxia

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