

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/322950687>

Cardiac responses of the bay scallop *Argopecten irradians* to diel-cycling hypoxia

Article in *Journal of Experimental Marine Biology and Ecology* · February 2018

DOI: 10.1016/j.jembe.2017.12.011

CITATION

1

READS

113

6 authors, including:



Samuel J Gurr

University of Rhode Island

2 PUBLICATIONS 1 CITATION

[SEE PROFILE](#)



Jennifer A. Goleski

Stony Brook University

29 PUBLICATIONS 294 CITATIONS

[SEE PROFILE](#)



Fernando P Lima

University of Porto

109 PUBLICATIONS 1,647 CITATIONS

[SEE PROFILE](#)



Rui Seabra

University of Porto

29 PUBLICATIONS 324 CITATIONS

[SEE PROFILE](#)

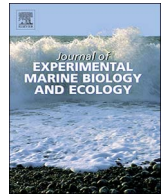
Some of the authors of this publication are also working on these related projects:



MicrobeModel: Modelling the water and fish microbiomes to monitor and predict pathogen outbreaks [View project](#)



Harmful Cyanobacterial Blooms [View project](#)



Cardiac responses of the bay scallop *Argopecten irradians* to diel-cycling hypoxia

Samuel J. Gurr^a, Jennifer Goleski^a, Fernando P. Lima^b, Rui Seabra^b, Christopher J. Gobler^a, Nils Volkenborn^{a,*}

^a School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY, USA

^b CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

ARTICLE INFO

Keywords:

Hypoxia
Cardiac activity
Ecophysiology
Environmental stress
Scallops

ABSTRACT

Bottom water oxygen concentrations in coastal environments can oscillate between fully oxygenated and hypoxic conditions on a daily basis. How benthic organisms respond to such drastic changes in oxygen availability is not well understood. Specifically, we do not know the magnitude, duration, and frequency at which diel-cycling hypoxic conditions become stressful. Here we have used non-invasive, infrared sensors to measure the cardiac activity of the Atlantic bay scallop, *Argopecten irradians*, in response to diel-cycling hypoxia *in-situ* over one-month periods as well as in controlled laboratory incubations using animals conditioned to contrasting field conditions. In the field, heartbeat rates at a well-oxygenated site were 23.0 ± 1.8 beats minute^{-1} with $12.7 \pm 2.1\%$ variance while heartbeat rates at sites with pronounced diel-cycling hypoxia were higher and more variable (site 1: 34.5 ± 3.1 beats minute^{-1} with $20.8 \pm 3.2\%$ variance; site 2: 48.4 and 45.8 beats minute^{-1} with 16% variance). Maximal heartbeat rates were commonly recorded around dawn when oxygen concentrations fell below $5 \text{ mg O}_2 \text{ L}^{-1}$ suggesting this was a threshold concentration or critical P_{O_2} (P_c) that induced a switch to oxyconformity and onset of anaerobic metabolic pathways. *In-situ* cardiac activity at locations with diel-cycling hypoxia indicate that *A. irradians* spent nearly 40% of each day in sub-optimal conditions during which metabolic activity was reduced and/or at least partially sustained by anaerobic metabolism. During laboratory experiments, an increase in heartbeat rate in response to initial declines below $5 \text{ mg O}_2 \text{ L}^{-1}$ from fully oxygenated conditions suggests a regulatory response in which cardiac activity was enhanced to maintain oxygen supply. At DO below $2 \text{ mg O}_2 \text{ L}^{-1}$, however, heartbeat rates declined reaching a state of bradycardia and acardia during anoxia, suggesting a conformer response to severe hypoxia. Heartbeat frequency was a suitable proxy for respiration under normoxia, but heartbeat and respiration rates decoupled during severe hypoxia ($< 2.0 \text{ mg O}_2 \text{ L}^{-1}$). *A. irradians* were able to survive anoxic periods between 12 and 14 h and cardiac activity rapidly returned to basal rates once full oxygen saturation was re-established. Mortality occurred after 23–32 h in anoxia regardless of prior conditioning. We speculate that repetitive exposure to periods of DO oscillations with exposure below $5 \text{ mg O}_2 \text{ L}^{-1}$ in the field can cause sub-lethal effects to *A. irradians* affecting fitness, growth, and reproductive success.

1. Introduction

In coastal ecosystems, hypoxia is a common environmental stressor with an increasing global occurrence in frequency and severity due to nutrient loading and eutrophic conditions (Diaz and Rosenberg, 2008). Periods of hypoxia or low dissolved oxygen (DO) develop when oxygen demand outweighs supply. Oxygen is consumed when algae die, sink, and decompose resulting in hypoxia in deeper waters that can last from weeks to months. Hypoxia can intensify seasonally from the joined effects of elevated temperature on microbial respiration, oxygen

solubility, and stratification (Diaz, 2001; Howarth et al., 2011; Wallace et al., 2014). Ecological effects associated with hypoxia can include mass mortalities, metabolic alterations, and escape responses of benthic and pelagic fauna (Howell and Simpson, 1994; Diaz and Rosenberg, 1995; Burnett and Stickle, 2001; Brokordt et al., 2013).

In shallow water environments, photosynthetic activity during the day and respiration during the night can lead to extremely variable oxygen availability (D'Avanzo and Kremer, 1994). This diel-cycling hypoxia can intensify in summer at coastal environments of economic and ecological importance (Tyler et al., 2009; Baumann et al., 2014).

* Corresponding author.

E-mail addresses: samjgurr@gmail.com (S.J. Gurr), nils.volkenborn@stonybrook.edu (N. Volkenborn).

Such conditions may constitute a significant burden for benthic organisms affecting their physiology, growth, and reproductive success (Baker and Mann, 1992; Thomas et al., 2007; Cheung et al., 2008; Wu, 2009; Gobler et al., 2014; Steckbauer et al., 2015; Keppel et al., 2015; Gobler et al., 2017). Sub-lethal effects of diel-cycling hypoxia on benthic organisms, however, are not well-understood. Importantly, many biological response variables (such as growth rates or larval development) integrate over relatively long temporal scales, which limits our ability to understand how dynamic environmental stressors affect organisms and to identify thresholds beyond which conditions become harmful.

Cardiac activity is widely used as a proxy of whole animal metabolism (Helm and Trueman, 1967) and can be measured at high temporal resolution to reveal immediate metabolic responses to changes in the environment (Burnett et al., 2013; Chapperon et al., 2016; Seabra et al., 2016). Heartbeat rates of invertebrates exposed to environmental stress have been comprehensively studied (DeFur and Magnum, 1979; Gainey and Shumway, 1988; Braby and Somero, 2006), although past efforts using minimally invasive techniques primarily targeted temperature stress in laboratory conditions (Aagaard et al., 1991; Xing et al., 2016; Seabra et al., 2016). Parallel measurements of heartbeat and respiration rates as a function of temperature indicate a close relationship between both physiological parameters (DeFur and Magnum, 1979). However, few studies have addressed the cardiac response of marine invertebrates to dynamically changing oxygen availability (Pörtner and Grieshaber, 1993; Grieshaber et al., 1994; Aguirre-Velarde et al., 2016), despite the commonality of diel-cycling DO concentrations in coastal waters.

Here we have used the Atlantic bay scallop, *Argopecten irradians*, as a model organism to measure cardiac and respiratory responses to diel-cycling hypoxia. Larval stage scallops have been found to be sensitive to sustained periods and diurnal oscillations of hypoxia and acidification which resulted in reduced rates of growth and survival (Gobler et al., 2017). To study the link between fluctuating oxygen availability and cardiac activity in adult scallops, heartbeat rates were recorded *in-situ* every 15 min over 4 week periods in 2015 and 2016 at three sites around Long Island, NY. During the time of measurements, the three sites strongly differed with respect to the frequency and severity of diel-cycling hypoxia. At two sites, DO concentrations fell regularly from fully oxygenated conditions to $< 2 \text{ mg O}_2 \text{ L}^{-1}$ during nighttime, while DO concentrations at the third site remained above $4.8 \text{ mg O}_2 \text{ L}^{-1}$ throughout the *in-situ* deployments. We hypothesized that in order to fulfill their respiratory oxygen demand, heartbeat rates of *A. irradians* would increase in response to declining oxygen concentrations. Bay scallops exposed to those contrasting field conditions were then used in laboratory experiments to determine if acclimation to diel-cycling hypoxia would affect the animals' ability to cope with periods of anoxia. Combined heartbeat and respiration rate measurements at various temperatures and DO concentrations were used to determine to what extent heartbeat rate can be used as a proxy for animal respiration.

2. Methods

2.1. Site selection and monitoring of *in-situ* conditions

Water quality monitoring and field experiments were performed in 2015 and 2016 in three semi-enclosed bays around Long Island, NY, USA. The three sites (Fire Island (40.626334 N, 73.25923 W), Nicoll Bay (40.727055 N, 73.139145 W), Seatuck Cove (40.81403 N 72.72598 W)) were selected because of contrasting diel-cycling DO dynamics recorded in the weeks preceding this study. Bottom water at Fire Island was continuously fully oxygenated while Nicoll Bay and Seatuck Cove bottom water was characterized by regularly occurring moderate hypoxia ($< 4.8 \text{ mg O}_2 \text{ L}^{-1}$) and occasional severe hypoxia ($< 2.0 \text{ mg O}_2 \text{ L}^{-1}$). DO was measured throughout the summer and over the duration of *in-situ* heartbeat rate monitoring to determine

frequency, magnitude, and duration of moderate and severe hypoxia at the three sites. DO and temperature were continuously recorded at each site with HOBO Dissolved Oxygen Loggers (U26-001) tied to fixed pilings and suspended $\sim 0.5 \text{ m}$ above the sea floor. Sensors recorded temperature and DO every 15 min from June to October 2015 (Fire Island and Nicoll Bay) and 2016 (Seatuck Cove); all sensors were cleaned and calibrated weekly according to manufacturer's recommendations.

2.2. Infrared heartbeat sensing

Reflective optical sensors (Vishay Intertechnology CNY70) were used for non-invasive heartbeat detection. These sensors can be glued on the exoskeleton or shell surface above the heart of the study animal and emit infrared light through the exoskeleton. Alterations in the shape or volume of the circulatory structures during heart contractions result in variations in the amount of reflected IR light which is detected by the sensor and can then be used to determine heartbeat rates (Depledge and Andersen, 1990). Signals were recorded using a custom-build amplifier and logging system which consists of a custom-build amplifier board, a microcontroller board (Arduino Uno), and SD shield (Adafruit) (Burnett et al., 2013). For *in-situ* measurements, two 8-channel amplifier and logging systems were incorporated into a waterproof housing made of PVC piping. Power was supplied by 15 V custom-build battery packs consisting of 20 1.5 V D-cell batteries, providing enough energy for one month of continuous recording. Data were analyzed with an interactive R script that allows visual inspection of the raw data and semi-automatically computes heartbeat rates for periods with regular signal oscillations.

2.3. Animal collection and lab preparation

Wild *A. irradians* (55–65 mm shell height) were collected in 2015 by hand in Orient Point Harbor, Long Island, New York, and maintained in unfiltered re-circulating seawater at 28 ppt salinity and 23–25 °C, representative of the field conditions at time of collection. Infrared heartbeat sensors were applied with super glue (Loctite) to the external upper valve on the posterior side of the valve hinge to capture data from the pericardium. Sixteen scallops for which heartbeat was detected reliably over a 12-h period were randomly divided into two groups of eight for field deployments at Fire Island and Nicoll Bay. In 2016, adult *A. irradians* (45–50 mm shell height) were collected from spawn sanctuaries in Orient Point Harbor, Long Island, New York, and eight individuals were prepared for *in-situ* deployments at Seatuck Cove as described above.

2.4. *In-situ* heartbeat monitoring

Field deployment cages were built with PVC pipe (2 cm diameter) and plastic hex netting (mesh size 1.9 cm). To avoid entanglement of sensor cables, each cage had nine cube divisions ($15 \text{ cm} \times 15 \text{ cm} \times 15 \text{ cm}$) in a 3×3 grid. Each of the eight outer cubes housed an individual bay scallop while the central cube was used to affix a Thermochron iButton® temperature logger (DS1921G). Temperature was recorded every 15 min to compare internal cage data at the same temporal resolution as the dockside water quality sensors. Each heartbeat logger was programmed to sequentially record 60 s of heartbeat data for eight *A. irradians* and repeat the cycle every 10 min throughout the deployment duration. *A. irradians* cages and heartbeat loggers were deployed over a four-week period from early September to early October of 2015 (Fire Island and Nicoll Bay) and for 18 days in August of 2016 (Seatuck Cove). Cages were staked 0.5 m above the sediment surface $< 2 \text{ m}$ from the oxygen and temperature sensors.

2.5. Laboratory set-up for post *in-situ* experiments

Following the Fire Island and Nicoll Bay *in-situ* deployments in 2015, scallops were brought back to the laboratory and put into a seawater table at 17–18 °C, which represented the *in-situ* temperature at the time of retrieval. Biofouling organisms that had grown on the shells were carefully removed with steel brushes to reduce their impact on respiration measurements. All infrared sensors were removed and reglued to ensure optimal heartbeat signals during post *in-situ* experiments. One day after the scallops were brought back from the field, experiments with animals conditioned to contrasting conditions in the field were conducted to i) simultaneously determine heartbeat and respiration rates at four different temperatures representative of the range in temperatures that the animals experienced in the field, ii) characterize cardiac and respiratory responses to short-term anoxia (12h) and recovery during water re-oxygenation, and iii) determine the duration that animals can survive without oxygen. Effects of temperature and short-term hypoxia were tested consecutively using the same individuals ($n = 3$ per site). Mortality trials were done with additional pre-conditioned individuals ($n = 3$ per site). Leak-proof wide-mouth PET bottles (Kautex) were used as respiration chambers. Heartbeat sensor cables, temperature probes, and optical oxygen probes (Pyro Science, Germany) were fed through the lids of the respiration chambers and sealed with thermoplastic adhesive. Respiration chambers were positioned in a temperature controlled water bath with magnetic stir bars that rotated at low speed to ensure homogenous mixing of water without disturbing the incubated animals. A 2 mm mesh was used to separate animals from the magnetic stir bar below. Each chamber had an inflow and outflow valve connected to a recirculating seawater system. Water in the water bath and in the respiration chambers were fed from the same head tank. During respiration measurements, both valves were closed and oxygen decline was recorded at one second intervals with a four channel oxygen meter with temperature compensation (FireStingO2 with TeX4 temperature extension module, Pyro Science, Germany). Respiration measurements were terminated by opening of inflow and outflow valves, which resulted in replacement of the water in the respiration chambers by gravity flow and re-establishment of fully oxygenated conditions within a few minutes. Estimates of respiration rates were adjusted to the water volume in each respiration chamber (approximately 1 L) and normalized to the ash free dry weight (AFDW) of the individuals and are reported as $\text{mg O}_2 \text{ min}^{-1} \text{ gAFDW}^{-1}$. AFDW of each scallop was estimated as loss on ignition after 4 h at 450 °C. Respiration rates of three individuals were measured simultaneously. Since seawater was un-filtered, the rate of oxygen depletion was measured in a fourth chamber without an animal to determine oxygen consumption not due to animal respiration. This rate of oxygen consumption was subtracted from our estimates of respiration rates and was typically 5–10% of the animal-driven oxygen consumption.

2.5.1. Simultaneous heartbeat and respiration rate measurements

Scallops from the 2015 Fire Island and Nicoll Bay *in-situ* deployments were exposed to a stepwise temperature ramp during which heartbeat and respiration rates were measured simultaneously. The short-term temperature manipulations were initiated by raising the temperature from the October *in-situ* temperature (18 °C) to the maximal summer temperature that the animals experienced in the field (28 °C). Animals were allowed to acclimate at 28 °C for 30 min. Initial tests indicated that heartbeat frequencies followed changes in temperature almost instantaneously and that heartbeat rates stabilized within 15–20 min after a new temperature was established. For respiration measurements, inflow and outflow valves were closed and DO decline was measured over a period of approximately 30 min. Respiration chambers were flushed with oxygenated water by opening the inflow and outflow valves before DO concentration fell below $3 \text{ mg O}_2 \text{ L}^{-1}$. Once fully oxygenated conditions were re-established the

recirculating seawater system was set to the next (lower) temperature, which was typically reached within 15–20 min. These combined respiration and heartbeat measurements were done at 28 °C, 23 °C, 21 °C, and 18 °C. The temperature ramp experiment runs were completed in < 3 h. The short-term exposure to various temperatures was not to mimic *in-situ* temperature dynamics, but rather to determine the relationship between heartbeat and respiration rates under fully oxygenated conditions over the temperature range that the animals experienced in the field. We had chosen to run the temperature ramp from high to low temperatures because the same individuals were used in a following sub-lethal anoxic challenge experiment, which was run at 18 °C. The short duration of temperature experiments was chosen to minimize any re-acclimation to laboratory conditions that may have masked site-specific acclimation effects in the sub-lethal anoxic challenge experiments.

2.5.2. Anoxic challenge experiments

The three animals from Fire Island and Nicoll Bay *in-situ* deployments that were used in the temperature ramp experiments were allowed to recover at 18 °C for 15–20 min before they were exposed to a sub-lethal anoxic challenge experiment. The experimental procedure was identical to the combined heartbeat and respiration rate measurements at various temperatures, but respiration chamber inflow and outflow valves remained closed. After 12 h of anoxia, inflow and outflow valves were opened and heartbeat rates were recorded for another 2 h.

Basal heartbeat rates before sub-lethal anoxic exposure, basal heartbeat rates after recovery from short-term anoxia, DO concentration at maximal heartbeat rate during oxygen decline, heartbeat rate during anoxia, and qualitative information on heartbeat rate during anoxia and during recovery were comparatively analyzed between sites for these challenges. At the end of all post *in-situ* experiments, length, width, thickness, biovolume, wet weight, dry weight, and AFDW of each individual were recorded.

Lethal anoxic exposure challenges were conducted with three individuals from Fire Island and Nicoll Bay *in-situ* deployments and followed the same experimental procedure as described above. Trials ended when all individuals expressed characteristics associated with scallop mortality, such as wide valve gaping and lack of reactive valve movements during agitation. The exact time of mortality was defined as the timepoint when heartbeat data failed to yield a minimum of three viable measurements over a period of one hour.

2.6. Data analysis

In-situ heartbeat rates, DO, and temperatures were averaged hourly across the deployment durations to compare responses in the field. Average rates of DO incline and decline at Nicoll Bay and Seatuck Cove were estimated in $\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ from the slopes of 17 and 18 diel-cycling hypoxia oscillations during heartbeat sensor deployments, respectively. Maximum and minimum heartbeat rates and coinciding DO concentrations were identified for each day of *in-situ* deployments. Means and standard deviations were calculated for each individual at each site. Respiration rates were measured from the slope of DO decline in $1.0 \text{ mg O}_2 \text{ L}^{-1}$ increments from full oxygen saturated conditions to anoxia. Individual heartbeat and respiration rates were averaged at each $1.0 \text{ mg O}_2 \text{ L}^{-1}$ increment during a continuous DO decline to calculate the mean heartbeat and respiration rates of scallops pre-conditioned at each site ($n = 3$ per site). Individual mean heartbeat and respiration rates were used to estimate heartbeat efficiency, defined as oxygen consumed per heartbeat and normalized to AFDW, in units of $\text{mg O}_2 \text{ g AFDW}^{-1} \text{ heartbeat}^{-1}$. An ANOVA followed by a Tukey Honest Significant Difference (HSD) test for multiple comparisons tested for significant difference in heartbeat efficiency between $1.0 \text{ mg O}_2 \text{ L}^{-1}$ increments of DO during oxygen decline from full saturation to anoxia.

In-situ oxygen dynamics were used to determine the durations of

Table 1

Characterization of hypoxic periods at Nicoll Bay, Fire Island, and Seatuck Cove during *in-situ* heartbeat rate monitoring. Percentage of time with hypoxic conditions (%) and the frequency (count of hypoxic periods = *f*) and duration (mean duration of hypoxic periods in hours = *d*) of hypoxia were calculated using two DO concentration thresholds. One hypoxic event below 4.8 mg O₂ L⁻¹ persisted for 43.1 h at Nicoll Bay and was not included into this site characterization.

		Fire Island	Nicoll Bay	Seatuck
Hypoxia threshold mg L ⁻¹	Lat. Long.	40.626334 N 73.25923 W	40.727055 N 73.139145 W	40.81403 N 72.72598 W
< 2.0	%	0%	2.4%	5.3%
	<i>f</i>	–	5	6
	<i>d</i>	–	3.1 ± 1.9	3.8 ± 4.1
< 4.8	%	0%	28%	32.5%
	<i>f</i>	–	24	22
	<i>d</i>	–	6.7 ± 4.2	6.8 ± 3.7

intervals with “moderate” (below 4.8 mg O₂ L⁻¹) or “severe” (below 2.0 mg O₂ L⁻¹) hypoxia, in accordance with water quality criteria established by US Environmental Protection Agency (US EPA, 2000) and implemented in associated monitoring efforts (CT DEEP, 2016). “Hypoxic periods” were defined as intervals below threshold concentrations for > 1 h. “Hypoxic events” were defined as intervals below threshold concentrations for > 24 h, i.e., hypoxic conditions persisted throughout daytime.

3. Results

3.1. Diel Cycling DO

The three sites used for *in-situ* heartbeat monitoring exhibited strong contrasts in the frequency, duration, and magnitude of moderate and severe hypoxic intervals during *A. irradians* deployments (Table 1, Fig. 1). At Fire Island DO never fell below 5.1 mg O₂ L⁻¹ (Table 1, Fig. 1A). Nicoll Bay exhibited strong diel-cycling DO fluctuations with hypoxic conditions during morning hours (Fig. 1B). An average minimum DO of 2.6 ± 1.1 mg O₂ L⁻¹ (mean of 17 days) occurred during early mornings followed by an increase at a rate of 1.00 ± 0.35 mg O₂ L⁻¹ h⁻¹ from late morning to evening or nightfall. Maximum DO of 10.4 ± 2.2 mg O₂ L⁻¹ was followed by a 0.63 ± 0.15 mg O₂ L⁻¹ h⁻¹ rate of decline throughout the night until morning. Nicoll Bay underwent 24 moderate and 5 severe hypoxic periods with an average duration of 6.7 ± 4.2 and 3.1 ± 1.9 h, respectively. A moderate hypoxic event persisted for 43.1 h while the longest period of severe hypoxia persisted for 4.9 h (Table 1). Moderate and severe hypoxia at Nicoll Bay occurred during 28% and 2.4% of the time during heartbeat sensor deployments. Bottom water at Seatuck Cove exhibited 22 moderate hypoxic periods with an average duration of 6.8 ± 3.7 h and six severe hypoxic periods with an average duration of 3.8 ± 4.1 h. The longest duration of severe hypoxia (including short-term anoxia) persisted for 11.8 h (Fig. 1C). Moderate and severe hypoxia occurred during 32.5% and 5.3% of the heartbeat sensor deployment. Average minimum DO of 1.8 ± 1.0 mg O₂ L⁻¹ (mean of 18 days) occurred in early to late mornings followed by a DO concentration increase at a rate of 0.93 ± 0.53 mg O₂ L⁻¹ h⁻¹ from afternoon to nightfall. A maximum DO of 10.0 ± 1.3 mg O₂ L⁻¹ was followed by a 0.64 ± 0.24 mg O₂ L⁻¹ h⁻¹ rate of decline throughout the night until morning.

During *in-situ* heartbeat rate monitoring in September 2015 (Nicoll Bay and Fire Island) and August 2016 (Seatuck Cove) average temperatures were 23.4 ± 1.8 °C at Nicoll Bay, 21.6 ± 1.4 °C at Fire Island, and 27.6 ± 1.4 °C at Seatuck Cove. Temperature data recorded within each cage directly matched temperatures recorded by external loggers. Diurnal temperatures occurred with a daily maximum after nightfall and minimum temperatures around noon; daily temperature

variations were always < 3 °C with temperatures changing at rates near 0.2–0.5 °C hour⁻¹ (Figs. 1A–C). Average daily temperatures decreased over the duration of the heartbeat sensor deployment period in September 2015 from 26.8 ± 0.2 °C to 20.5 ± 0.4 °C at Nicoll Bay and from 23.6 ± 0.4 °C to 19.5 ± 0.3 °C at Fire Island while temperatures peaked in the middle of the August 2016 deployment at Seatuck Cove.

3.2. In-situ heartbeat rates

Heartbeat rates of *A. irradians* recorded in September 2015 at Fire Island and Nicoll Bay ranged between 13.2 and 57.6 beats per minute (bpm). Cardiac activity differed between the two sites with average heartbeat rates of 34.5 ± 3.1 bpm (*n* = 6) at Nicoll Bay and 23.0 ± 1.8 bpm (*n* = 7) at Fire Island (Fig. 2). Variance around mean heartbeat rate also differed between the two sites with average percent variance of 20.8 ± 3.2% at Nicoll Bay and 12.7 ± 2.1% at Fire Island. Heartbeat rate data during *in-situ* deployments at Seatuck Cove in 2016 were only recorded from two individuals. Average heartbeat rates of these two individuals were 48.4 and 45.8 bpm with a variance of 16% around the individual means. Heartbeat rates at Fire Island with fully oxygenated conditions were relatively stable (Figs. 1A, D and 2) while scallops at Nicoll Bay in 2015 and Seatuck Cove in 2016 showed daily oscillations of cardiac activity with a dynamic range of nearly 20 bpm (Figs. 1B, C, F, and 2). These diurnal excursions in heartbeat rates were closely associated with changes in DO concentrations (Fig. 1). Daily cardiac fluctuations showed a lagged trend of peaks and troughs in synchrony with DO. Maximal heartbeat rates of 42.0 ± 3.1 bpm at Nicoll Bay and 58.4 ± 2.9 bpm at Seatuck Cove occurred during early mornings when DO declined below 5 mg O₂ L⁻¹. Minimum DO always occurred approximately 4 h after peak heartbeat rate. Cardiac activity decreased 10 bpm below maximum rates when DO reached the daily minimum. Cardiac responses decreased to a rate 20 bpm below peak cardiac activity during midday when oxygen availability increased to 7.1 ± 2.3 mg O₂ L⁻¹ at Nicoll Bay and 5.2 ± 2.5 mg O₂ L⁻¹ at Seatuck Cove. Daily peaks in oxygen availability consistently occurred after minimum heartbeat rates when rates increased 10 bpm above minimum cardiac activity. Heartbeat rates from different individuals at the same site were almost identical with average rates of 30–38 bpm at Nicoll Bay and 44–50 bpm at Seatuck Cove. The overall higher cardiac activity at Seatuck Cove was likely due to the warmer average temperatures (27.6 ± 1.4 °C) relative to the Nicoll Bay (23.4 ± 1.8 °C). Persistent elevated cardiac activity (tachycardia) during exposure to reduced oxygen concentration was evident throughout most of a 43.1 h long hypoxic event at Nicoll Bay (Fig. 1E). Average heartbeat rates towards the end of this long hypoxic event declined by 42% from 47.5 ± 4.0 to 27.5 ± 2.9 bpm while DO remained constant at 4.2 ± 0.5 mg O₂ L⁻¹ (Fig. 1E).

3.3. Cardiac activity and respiration rates: response to temperature

Cardiac activity proved to be a good proxy for respiration at different temperatures; heartbeat and respiration rates of *A. irradians* declined linearly as temperatures were reduced stepwise from 28 °C to 23 °C to 21 °C, and to 18 °C (Figs. 3 and 4). Cardiac activity and respiration rate reached a maximum of 60.3 ± 7.3 bpm and 0.046 ± 0.015 mg O₂ minute⁻¹ gAFDW⁻¹ at 26–28 °C and minimum of 25.9 ± 3.8 bpm and 0.020 ± 0.01 mg O₂ minute⁻¹ gAFDW⁻¹ at 18 °C (Fig. 4). Responses to temperature were not affected by site pre-conditioning. Scallops from Nicoll Bay exhibited similar heartbeat rates *in-situ* and when exposed to a range of temperatures comparable to field conditions. Fire Island scallops yielded higher heartbeat rates in the lab than *in-situ* under comparable field temperatures (Figs. 2 and 4). Compared to animals pre-conditioned to Nicoll Bay, respiration rates from animals pre-conditioned at Fire Island were greater and more variable (Fig. 4), but this result was largely driven by higher rates by one of the three individuals.

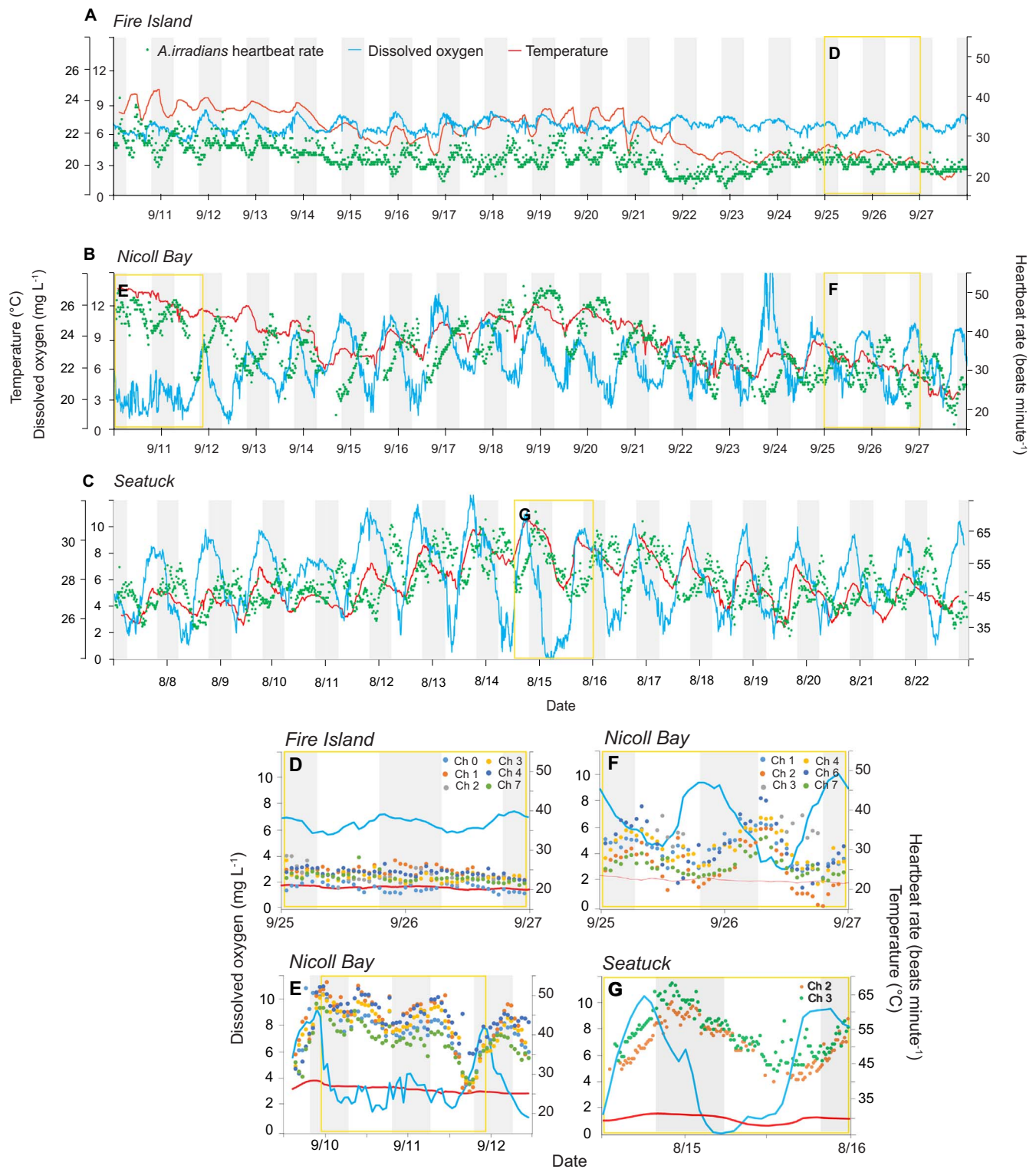


Fig. 1. Continuous *in-situ* data of dissolved oxygen and temperature in ambient bottom water (blue and red lines, respectively), and *A. irradians* heartbeat rates (colored dots) recorded at Fire Island (A), Nicoll Bay (B), and Seatuck Cove (C). A – C show cardiac activity of a single *A. irradians* at each site. Boxed regions in yellow represent time periods enlarged in panels D – G. Panels D – F show all channels with viable heartbeat signals throughout the time periods ($n = 6$ per site). Panel E displays tachycardia at Nicoll Bay during an extended moderate hypoxia event. Panel G shows heartbeat data from two *A. irradians* at Seatuck Cove (G) during an anoxic period. Grey and white regions indicate night and day, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Cardiac activity and respiration rates: DO dependence

Oxygen consumption in respiration chambers induced a $3.7 \pm 0.9 \text{ mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ rate of decline from full oxygen saturation to severe hypoxia. It took 2–3 h for *A. irradians* to deplete the oxygen in

sealed chambers. Heartbeat rates peaked at 29.7 ± 2.9 (range 24.6–32.7) bpm when DO fell below $5 \text{ mg O}_2 \text{ L}^{-1}$. Heartbeat rates declined to a minimum of 19.4 ± 4.2 (range 14.4–24.9) bpm as DO fell below $1 \text{ mg O}_2 \text{ L}^{-1}$ and approached anoxia (Fig. 5). Respiration rates at $0.034\text{--}0.028 \text{ mg O}_2 \text{ minute}^{-1} \text{ gAFDW}^{-1}$ after the initial 1 mg

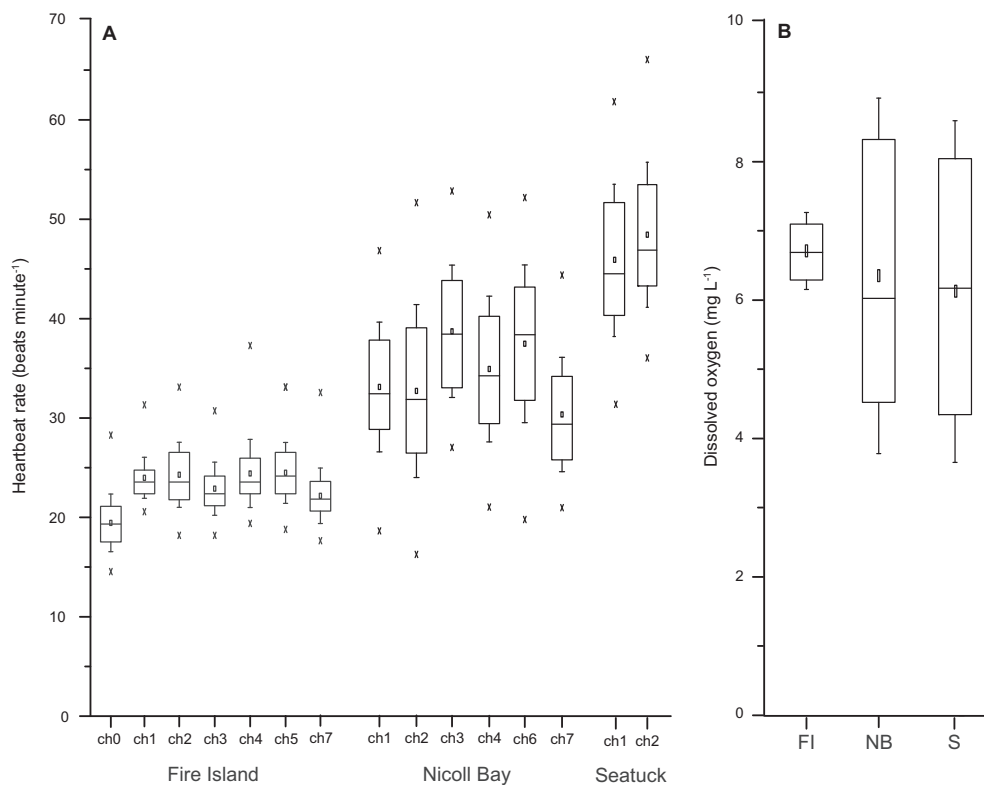


Fig. 2. Box whiskers plots of continuously recorded *A. irradians* heartbeat rates (A) and DO concentrations (B) at Fire Island (FI), Nicoll Bay (NB), and Seatuck Cove (S) where average temperatures were 21.6 ± 1.4 °C, 23.4 ± 1.8 °C, and 27.6 ± 1.4 °C, respectively. All sensors yielded > 1000 viable heartbeat rate measurements. Displayed are the mean (small squares), standard deviation (whiskers), 25–75% (large box), and 1–99% range (x).

$\text{O}_2 \text{ L}^{-1}$ decline of DO decreased $81.56 \pm 5.26\%$ to an average minimum respiration rate of $0.005 \text{ mg O}_2 \text{ minute}^{-1} \text{ gAFDW}^{-1}$ when DO fell below $1 \text{ mg O}_2 \text{ L}^{-1}$ (Fig. 5). Heartbeat rates proceeded to decline to bradycardia (abnormal decrease in heartbeat rate) and acardia (complete absence of cardiac activity) under sustained anoxic conditions (Figs. 6 and 7). *A. irradians* pre-conditioned at Nicoll Bay and Fire Island had significantly higher heartbeat efficiency ($p = 0.0186$ and $p = 0.007$, respectively) at $6\text{--}5 \text{ mg O}_2 \text{ L}^{-1}$ than at DO concentrations below $1 \text{ mg O}_2 \text{ L}^{-1}$. Heartbeat efficiency at $6\text{--}5 \text{ mg O}_2 \text{ L}^{-1}$ was also significantly higher than at $2\text{--}1 \text{ mg O}_2 \text{ L}^{-1}$ for individuals pre-conditioned at Fire Island ($p = 0.049$) (Fig. 5).

3.5. Cardiac activity of preconditioned animals in response to oxygen decline and anoxic stress

In laboratory experiments scallops increased cardiac activity from a basal rate of 25–30 bpm when DO initially declined from full oxygen saturation, irrespective of pre-conditioning in the field. Scallops from Nicoll Bay yielded heartbeat rates throughout the 12 h of anoxic exposure without one instance of acardia; two scallops exhibited bradycardia with rates < 5 bpm and one individual maintained relatively high activity of 20 bpm. Scallops pre-conditioned at Fire Island exhibited both bradycardia and acardia throughout the anoxic period (Fig. 7B). All *A. irradians* survived 12–14 h of anoxic conditions. Scallops exhibited a gradual elevation (Fig. 7A) or slight upswing (Fig. 7B) of heartbeat rates upon reestablishment of normoxia conditions; all

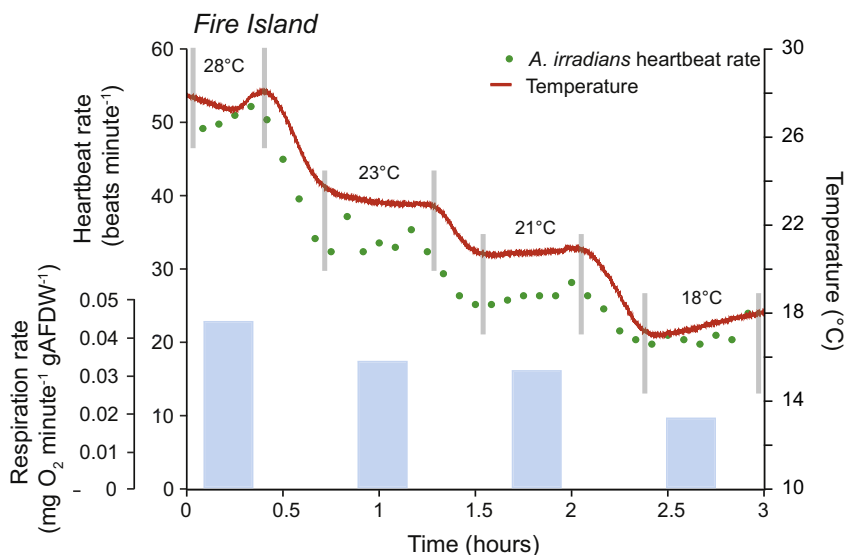


Fig. 3. Simultaneous cardiac activity and respiration rate measurements during a short temperature ramp experiment on an *A. irradians* individual pre-conditioned to Fire Island. Respiration rates (in blue) and mean heartbeat rates were calculated at four temperatures (between grey lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

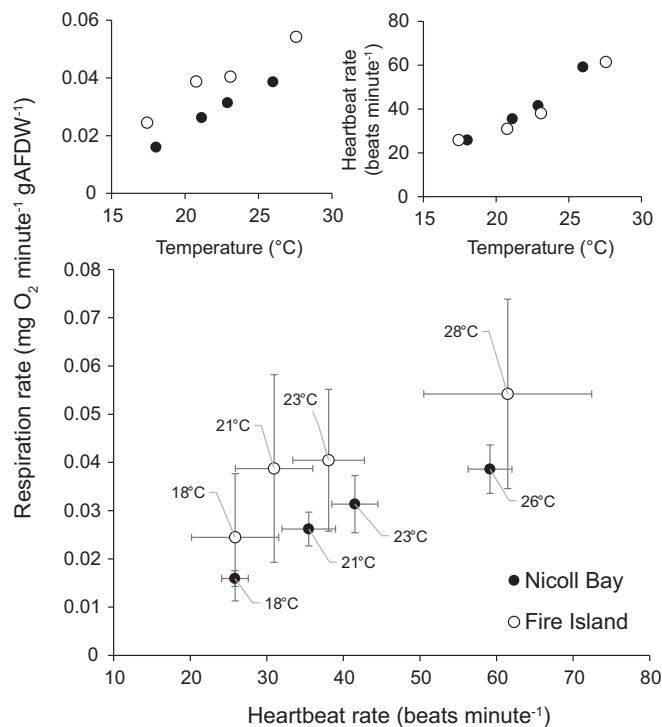


Fig. 4. Respiration and cardiac responses (mean \pm standard deviation; $n = 3$) to temperature by *A. irradians* pre-conditioned to diel-cycling hypoxia (Nicoll Bay) and continuously oxic conditions (Fire Island).

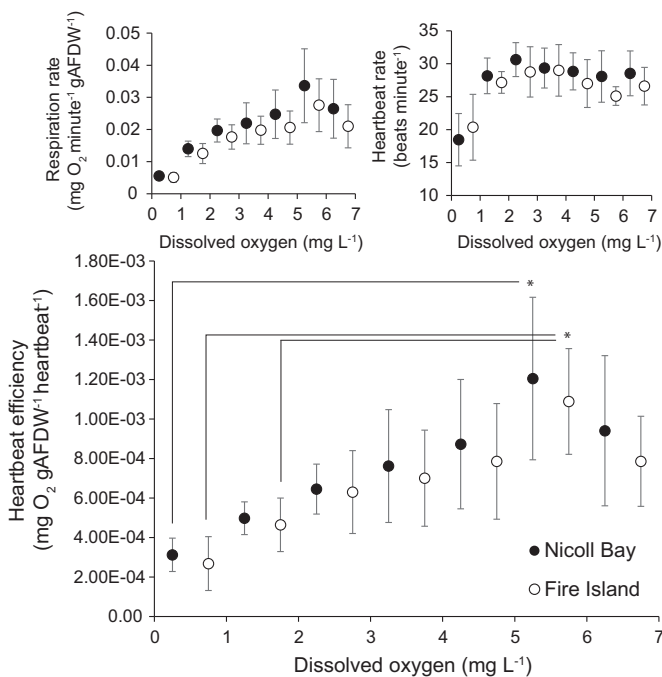


Fig. 5. Effect of DO decline on heartbeat and respiration rates (mean \pm standard deviation; $n = 3$) of *A. irradians* pre-conditioned to diel-cycling hypoxia (Nicoll Bay) and continuously oxic conditions (Fire Island). Heartbeat and respiration rates were averaged during $1.0 \text{ mg O}_2 \text{ L}^{-1}$ increments throughout a continuous DO decline. Heartbeat efficiency combines both cardiac and respiratory activity; asterisks display significant differences ($p < 0.05$) from a Tukey HSD test.

scallops reached basal rates after 1 h.

Initial cardiac responses in anoxic mortality experiments were similar as those observed in non-lethal anoxic stress challenges with elevated heartbeat rates when DO initially declined, maximum

heartbeat rates at $1\text{--}3 \text{ mg O}_2 \text{ L}^{-1}$, and bradycardia and acardia as DO depleted below $1 \text{ mg O}_2 \text{ L}^{-1}$ and during anoxia (Figs. 6 and 7). Heartbeat rates were consistently measurable during full oxygen saturation and the initial DO decline, but data became inconsistent during prolonged periods of anoxia (Figs. 6 and 7B). Detectable cardiac responses under anoxia reached bradycardia with minimal activity of $< 5 \text{ bpm}$, leading to mortality (Fig. 6). *A. irradians* reached mortality after $27.9 \pm 3.5 \text{ h}$ of exposure to anoxia; Nicoll Bay and Fire Island scallops endured 28 ± 3.2 and $27.8 \pm 4.5 \text{ h}$ of anoxia before mortality, respectively (Fig. 6).

4. Discussion

4.1. Heartbeats under diel-cycling of DO

This study is the first continuous *in-situ* non-invasive measurement of cardiac activity in a marine invertebrate under diel-cycling hypoxia. Daily DO oscillations had a strong effect on the heartbeat rate of *A. irradians*. Our data suggest that *in-situ* heartbeat measurements can be used as a proxy for respiration under fully oxygenated conditions, but that this relationship falls apart at low DO concentrations. Heartbeat rates elevated and declined in co-occurrence with diel-cycling hypoxia while cardiac activity was less variable under continuously oxygenated conditions (Figs. 1 and 2). Heartbeat rate as a response variable allowed us to characterize the immediate response of the organism to dynamic changes in DO concentration and identify thresholds that caused a switch from an oxyregulatory response to oxyconformity.

4.2. Cardiac activity helps define oxyconformity

The *in-situ* cardiac activity decline in response to falling DO concentrations during diel-cycling hypoxia documented here are indicative of the onset of oxyconformity. Oxy-conformers and oxy-regulators are organisms with linear dependence or independence of ambient oxygen availability for their respiratory function (Prosser, 1973). This categorization has since been criticized and developed to recognize species' variation and altered responses to multiple stressors (Herreid, 1980; Grieshaber et al., 1994; Pörtner, 2010). During the decline of ambient DO, regulatory respiration rates reach a transitional breakpoint at a DO concentration threshold that stimulates oxyconformity; this is referred to as the critical P_{O_2} or P_c (Prosser, 1973). The P_c is identified as the start of anaerobic metabolism because ambient DO concentrations below P_c are insufficient for regulation of aerobic functions (Pörtner et al., 1985; Van den Thillart et al., 1992; Pörtner and Grieshaber, 1993). It has been suggested that oxygen depletion below P_c should be defined as “severe” hypoxia. (Grieshaber et al., 1994). Maximum heartbeat rates recorded *in-situ* under exposure to periods of diel-cycling hypoxia suggest that the P_c for *A. irradians* is around $5.0 \text{ mg O}_2 \text{ L}^{-1}$. Decreases in cardiac activity at DO concentrations below P_c resemble the metabolic activity decline at upper thermal limits (Frederich and Pörtner, 2000) which has been related to the transition to anaerobic metabolism (DeFur and Magnum, 1979; Frederich and Pörtner, 2000; Pörtner, 2010).

Respiration rates of *A. irradians* were consistent with the response of a “poor” regulator because of the prompt transition to oxyconformity at relatively high DO concentrations and linear decrease in respiration rates as DO was declining below $5 \text{ mg O}_2 \text{ L}^{-1}$. (Herreid, 1980). The Great Scallop *Pecten maximus* also exhibits oxyconformity under similar DO conditions (Artigaud et al., 2014; Brand and Roberts, 1973). Under severe hypoxia and anoxia, *A. irradians* decreased oxygen consumption to a minimum rate that was 25% of basal respiration rates at full oxygen saturation (Fig. 5). Aguirre-Velarde et al. (2016) found that *Argopecten purpuratus* exhibited a similar proportional difference in respiratory activity above and below its P_c . Common respiration responses among different scallop species could indicate an evolutionary response to cope with low oxygen and hypoxic stress.

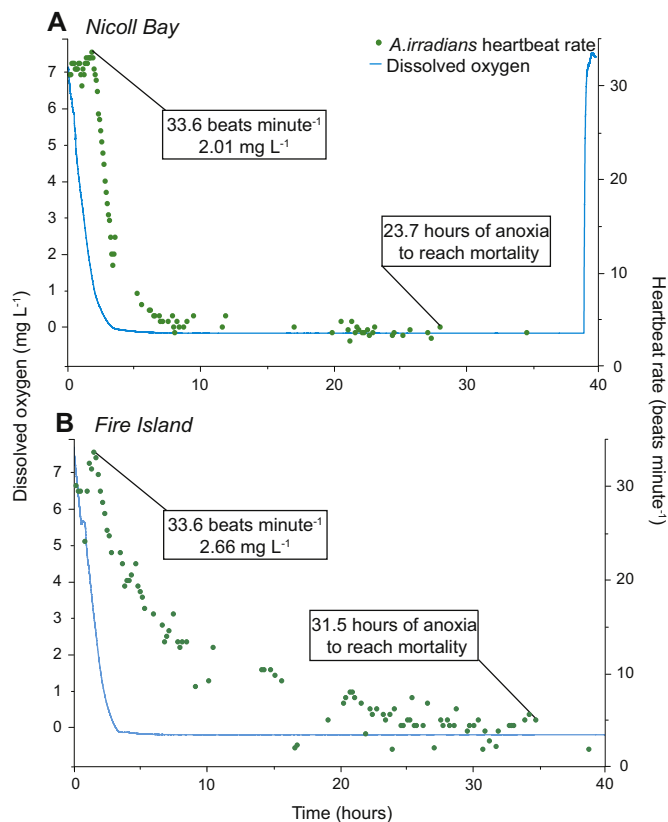


Fig. 6. Cardiac response of an *A. irradians* pre-conditioned to diel-cycling hypoxia (Nicoll Bay) and continuously oxic conditions (Fire Island) to an extended period of anoxia until mortality. Heartbeat rates and ambient water DO concentrations reveal a cardiac up-swing, bradycardia, and duration of anoxia until mortality.

Contrary to oxygen consumption rates, cardiac responses presented in this study were consistent with the response of a “good” regulator during DO decline (Herreid, 1980). *A. irradians*’ heartbeat rates in laboratory experiments were up-regulated when DO concentrations started to decline and reached maxima at DO concentrations between 1 and 2.5 mg O₂ L⁻¹ (Figs. 6 and 7). Field measurements indicated extended periods with elevated heartbeat rates during which DO concentrations remained low (Fig. 1E). Only when DO concentration fell below 2–3 mg O₂ L⁻¹ cardiac activity declined, consistent with the oxyconformer response (Figs. 6 and 7). Unlike the linearity between heartbeat and respiration rates observed during acute temperature stress (Figs. 3 and 4), heartbeat rates became decoupled from respiratory responses during DO decline (Fig. 5). While respiration rates decreased consistently with ambient DO decline, cardiac activity elevated to a maximum before rates declined at lower ambient DO concentrations (Figs. 6 and 7). Brand and Roberts (1973) found that *P. maximus* exhibited a cardiac increase and decline under similar ambient oxygen conditions and proposed this as an attempt to maintain aerobic functions by increasing oxygen uptake until conditions were unfavorable. *A. irradians* regulated oxygen uptake until DO declined to severe hypoxia as heartbeat efficiency decreased significantly when DO declined below 2.0 mg O₂ L⁻¹ (Fig. 5). The decoupling of heartbeat and respiration rates during DO decline suggests that cardiac activity can be used to define P_c and as an indicator of the onset of anaerobic stress.

4.3. Sub-lethal physiological responses and hypoxia thresholds

Metabolic transitions, as interpreted from simultaneous cardiac and respiratory activity measurements obtained in this study, provide a new perspective on the definition of hypoxic stress thresholds. When heartbeat and respiration rates under oxygen limitation are visually

overlaid (Fig. 5) they illustrate conformer and regulator responses that define thresholds of “moderate” and “severe” hypoxia in the scheme suggested by Grieshaber et al. (1994), in which “moderate” hypoxia has been suggested as the start of oxyconformity by a “poor” regulator such as *A. irradians* (Herreid, 1980; Grieshaber et al., 1994). DO concentrations at maximum *in-situ* cardiac activity (Nicoll Bay: 5.0 ± 1.2 mg O₂ L⁻¹; Seatuck Cove: 4.9 ± 1.7 mg O₂ L⁻¹) closely match the DO concentration of 4.8 mg O₂ L⁻¹ that has been set as target for the protection of aquatic life in US East Coast waters (USEPA, 2000; CT DEEP, 2016). As DO continues to decline, the threshold of “severe” hypoxia is most apparent when a regulator transitions to oxyconformity (Grieshaber et al., 1994). Under oxygen limitation (< 5 mg O₂ L⁻¹), *A. irradians*’ heartbeat rates responded as a regulator, independent of oxygen availability until DO concentration dropped below 2 mg O₂ L⁻¹ (Fig. 5). This is also in agreement with the “severe” hypoxia threshold in coastal systems native to *A. irradians* (US EPA, 2000; CT DEEP, 2016). With the cardiac activity of *A. irradians* remarkably responsive to DO levels that matched US EPA thresholds, further experimental effort is needed to confirm these patterns under comparable dynamics associated with diel-cycling DO.

4.4. Response to stress duration

Cardiac activity of *A. irradians* in a diel-cycling hypoxic environment is widely dependent upon the frequency and magnitude of stress periods. However, *A. irradians* displayed a strong dependence on stress duration as indicated by sustained tachycardia (elevated heartbeat rate) for 30 h of a long-term (43.1 h) moderate hypoxic event before heartbeat rates declined independent of ambient DO concentration (Fig. 1E). Findings during long-term hypoxia suggest that stress duration is an important component of the P_c. Metabolic transitions under long-term hypoxia has been studied on other bivalves. Van den Thillart et al. (1992) found that the ark clam *Scapharca inaequivalvis* increased anaerobic metabolism under long-term severe hypoxia, but continued to uptake oxygen and relied greatly upon aerobic pathways. Sudden decline of heartbeat rates demonstrates that *A. irradians* diminishes aerobic capacity to cope with extended durations of hypoxia and likely undergoes a transition to anaerobic pathways. If anaerobic transitions affect growth, survival, and reproduction (Wang and Widdows, 1991, 1993; Pörtner, 2010), *A. irradians* may be particularly vulnerable to extended periods of hypoxia relative to other bivalve species. Continuous *in-situ* tachycardia demonstrates sensitivity to both magnitude and duration of DO depleted conditions for regulation of aerobic function (Fig. 1E). Cardiac responses recorded in this study call for integration of stress duration in species-specific P_c to account for internal oxygen reserves and diverse aerobic capacity over extended hypoxic periods (Pörtner et al., 1985; Van den Thillart et al., 1992).

4.5. Adaptation to anoxia

Cardiac responses by *A. irradians* in laboratory anoxia incubations resembled activity measured *in-situ* during diel-cycling hypoxia at Nicoll Bay and Seatuck Cove (Fig. 1B and C); heartbeat rates peaked at DO concentrations around 2.0 mg O₂ L⁻¹ but decreased as DO concentrations declined further (Figs. 6 and 7). However, patterns evident in laboratory challenges were not observed *in-situ* such as bradycardia and evidence of acardia during absence of oxygen. It should be noted that the animals used in the sub-lethal anoxia trials had been exposed to a short-term heat stress event prior to the hypoxic challenge. In a strict sense, we therefore looked at the response of individuals to anoxia following a heat stress event. However, respiration and heartbeat rate measurements in sub-lethal and lethal trials, the latter using animals that were not exposed to short-term heat stress, did not suggest that the short-term exposure to elevated temperatures affected the ability of animals to cope with anoxia (Figs. 6 and 7).

Bradycardia is a common response of marine invertebrates to

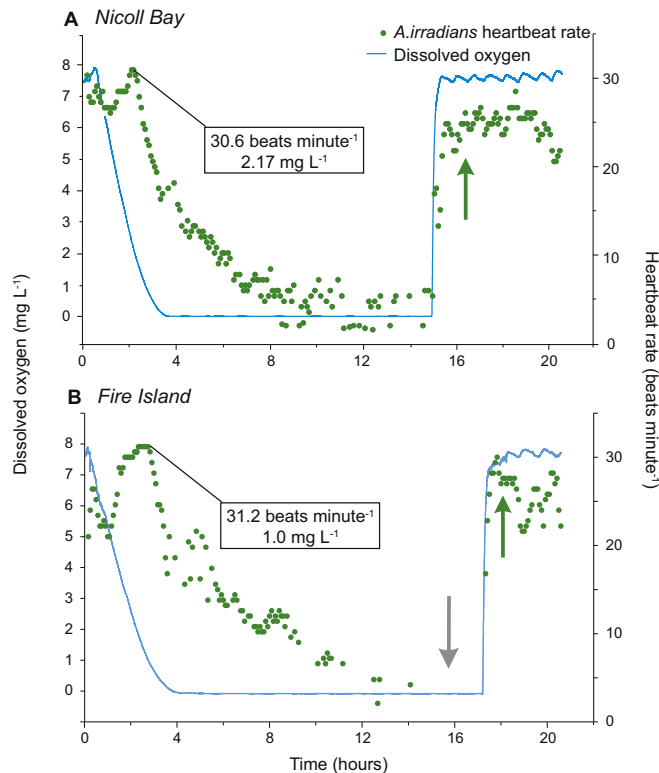


Fig. 7. Cardiac responses to short-term anoxia in respiration chambers. Ambient water DO and heartbeat rates of two *A. irradians* preconditioned to diel-cycling hypoxia (Nicoll Bay) and continuously oxic conditions (Fire Island) show a cardiac upswing, bradycardia, acardia (grey arrow), and recovery (green arrows) during sub-lethal anoxic challenges. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hypoxia and it is presumed to exemplify a high capacity for anaerobic metabolism (Bayne, 1971; Taylor et al., 1973; Taylor and Brand, 1975). It has also been proposed that abnormally low heartbeat rates are indicative of indefinite capability to tolerate low DO concentrations (DeFur and Magnum, 1979). A 40–50% decline of heartbeat rates during severe hypoxia (including short-term anoxia) at Seatuck Cove suggests that cardiac activity would reach bradycardia if anoxia persisted (Fig. 1G). Acardia was recorded in few cases by scallops exposed to sub-lethal and lethal anoxic challenges. Bradycardia was a precursor to mortality in long-term anoxic challenges (Fig. 6). Regardless of acclimation to contrasting site conditions at Fire Island and Nicoll Bay, all *A. irradians* survived and recovered cardiac activity after short periods (12–14 h) of anoxia (Fig. 7). Brand and Roberts (1973) found that *P. maximus* readily responded with an immediate return to elevated cardiac activity upon reintroduction of full oxygen saturation after exposure to anoxia. *A. irradians* responded similarly in this study, with restoration of vital aerobic function even after hours in a severely low metabolic state of acardia (Fig. 7). Mortality after extended durations of anoxia (> 24 h) demonstrated the physiological limits in stress tolerance and maintenance of vital functions (Fig. 6).

Survival, time of mortality, recovery, and cardiac responses under anoxia were not affected by site-specific environmental pre-conditioning. However, only scallops from Fire Island, which were never exposed to hypoxia, displayed acardia during short-term anoxic challenges (Fig. 7). Assuming that bradycardia is a physiological response leading to better resilience than a complete absence of cardiac activity, our data suggest that pre-exposure to hypoxia may strengthen *A. irradians*' ability to cope with diel-cycling hypoxia.

4.6. In-situ versus laboratory cardiac response to hypoxia

Under the assumption that full oxygen saturation in the laboratory inflicted minimal stress, basal heartbeat rates for *A. irradians* exposed to temperatures comparable to field deployments (20–25 °C) were 30–45 bpm (Fig. 4). Deviations in cardiac activity from this range during fully oxygenated conditions at Fire Island and Nicoll Bay may indicate physiological responses to other environmental factors such as temperature, salinity, or food availability (Ansell, 1973; Widdows, 1973; Braby and Somero, 2006). However, the relatively stable *in-situ* temperatures over the duration of heartbeat sensor deployments and the consistent co-occurrence of maximal heartbeat rates and minimal DO suggests that diel-cycling DO was the main driver of *A. irradians*' daily heartbeat rate excursions. Heartbeat rates consistently peaked at DO concentrations around 5 mg O₂ L⁻¹ in the field, while maximal heartbeat rates in the lab were recorded around 2 mg O₂ L⁻¹. This mismatch suggests that the cardiac response in *A. irradians* is not a simple function of oxygen concentration, but also depends on the rate of oxygen decline. Oxygen decline induced by animal respiration in our laboratory experiments was faster than the typical DO decline during diel-cycling hypoxia in the field (3–5 mg O₂ L⁻¹ h⁻¹ versus 0.3–0.8 mg O₂ L⁻¹ h⁻¹). Maximal rates of DO decline in the field were 1.5–2.5 mg O₂ L⁻¹ h⁻¹. The discrepancy between the *in-situ* and laboratory DO concentrations with peaks in heartbeat rates may therefore relate to a longer exposure to declining DO conditions, which triggered an earlier establishment of maximal heartbeat rates. Aguirre-Velarde et al. (2016) simulated a gradual decline in O₂ at a realistic rate and found that the Peruvian scallop *Argopecten purpuratus* was able to regulate oxygen uptake down to DO concentration of 24% air saturation, suggesting that the Peruvian scallop is better adapted to diel-cycling hypoxic conditions, but potentially also that the rate of O₂ decline may significantly affect the regulatory response. In future studies, it will be important to identify to what extent the rate of oxygen decline affects the ability of organisms to respond and adjust their metabolic activity.

4.7. Conceptual model: Cardiac responses to diel-cycling of DO

A prominent feature found in both field and laboratory data was the offset of DO concentration peaks and troughs and heartbeat rates (Figs. 1, 6, and 7). This phenomenon can be put into perspective with a simple conceptual model relative to regulation of aerobic functions and transition to oxyconformity. Our data suggest that cardiac activity during diel-cycling DO (CRD_{DO}) includes four phases of physiological responses (Fig. 8). Phase 1 begins after the initial DO decline from daily maximum accompanied by an increase in cardiac activity and is the longest of the four phases at our study sites with a mean duration of 8–10 h (Fig. 9). Elevated cardiac activity during phase 1 can be assumed to be an oxyregulatory response to maintain oxygen uptake and aerobic metabolism as DO availability declines (Bayne, 1971; Brand and Roberts, 1973; Grieshaber et al., 1994). *A. irradians* reached a maximum heartbeat rate when DO declined to 5 mg O₂ L⁻¹ (Fig. 9) suggesting a metabolic threshold dependent upon oxygen availability at a concentration higher than previously identified to cause harm to marine invertebrates. If cardiac activity is assumed to be indicative of respiratory rates, 5 mg O₂ L⁻¹ is the P_c during diel-cycling hypoxia at which breakpoint respiration rate symbolizes a switch to oxyconformity and onset of anaerobic pathways in Phase 2.

Phase 2 represents the transition to oxyconformity and occurs from peak heartbeat rate until DO declines to oxygen minimum (Fig. 8). This period had a mean duration of 4–4.5 h over which cardiac activity declined by 10 bpm regardless of different temporal, thermal, and environmental conditions at Nicoll Bay and Seatuck Cove (Fig. 9). It can be speculated that the cardiac activity and oxygen uptake decline during phase 2 was due to the increasing cost to maintain aerobic metabolism when ambient oxygen availability is inadequate (DeFur and Magnum, 1979). This phase may indicate an elevated dependence or

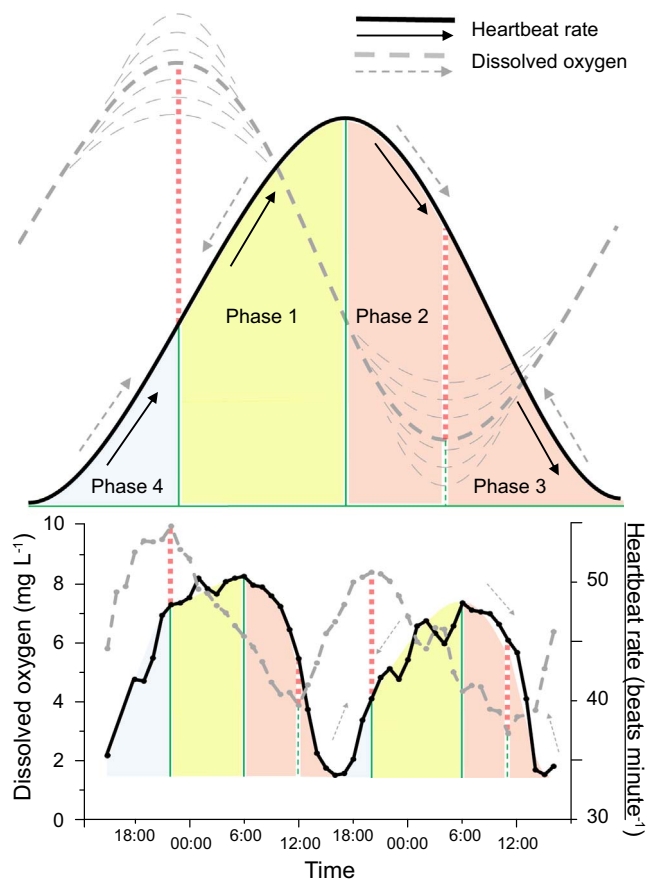


Fig. 8. The upper panel depicts a conceptual model of cardiac response to diel-cycling DO concentrations (CRD_{DO}). CRD_{DO} synthesizes the consistent pattern between *in-situ* DO concentrations and *A. irradians* heartbeat rate oscillations at Nicoll Bay and Seatuck Cove, displaying respiratory and metabolic implications and transitions during diel-cycling hypoxia. The lower panel is an example of the model criteria overlaid on two days of hourly mean DO concentrations and *A. irradians* heartbeat rates from the Nicoll Bay deployment. Colors represent the regulator response (yellow), conformer response (red), and cardiac recovery (blue) during diel-cycling hypoxia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

onset of anaerobic pathways when DO concentrations fall below the P_c of 5 mg O₂ L⁻¹ (Pörtner et al., 1985; Pörtner and Grieshaber, 1993).

Oxyconformity continues in Phase 3 as a period of “stress and rest”. Cardiac activity continues to decline to a minimum rate although DO increases (Fig. 8). *A. irradians* decreased heartbeat rates an additional 10 bpm during both Nicoll Bay and Seatuck Cove deployments during this phase (Fig. 9). *A. irradians* were not initially responsive to oxygen incline *in-situ* at a rate of 1 mg O₂ L⁻¹ h⁻¹. In the laboratory, however, *A. irradians* immediately responded with a return to basal cardiac activity upon reintroduction to full oxygen saturation (Fig. 9). Prompt cardiac recovery was also evident in the Great Scallop (*Pecten maximus*) after exposure to anoxia (Brand and Roberts, 1973). However, investigations of cardiac responses have not included more realistic (slower) DO increase relative to diel-cycling hypoxia measured *in-situ* (Fig. 9). Phase 3 in our field data lasted 5–6 h. Combined with phase two, scallops at Nicoll Bay and Seatuck Cove exhibited a 20 bpm range between their mean maximum and mean minimum cardiac activity during diel-cycling hypoxia (Fig. 9). DO concentrations at minimum heartbeat rates were 5–7 mg O₂ L⁻¹ (Fig. 9) perhaps representing a favorable DO threshold that stimulates a return to aerobic metabolism. Interestingly, DO concentrations were nearly identical at minimum heartbeat rate (aerobic recovery threshold) and maximum heartbeat rate (P_c and onset of anaerobiosis) suggesting that the rates of DO

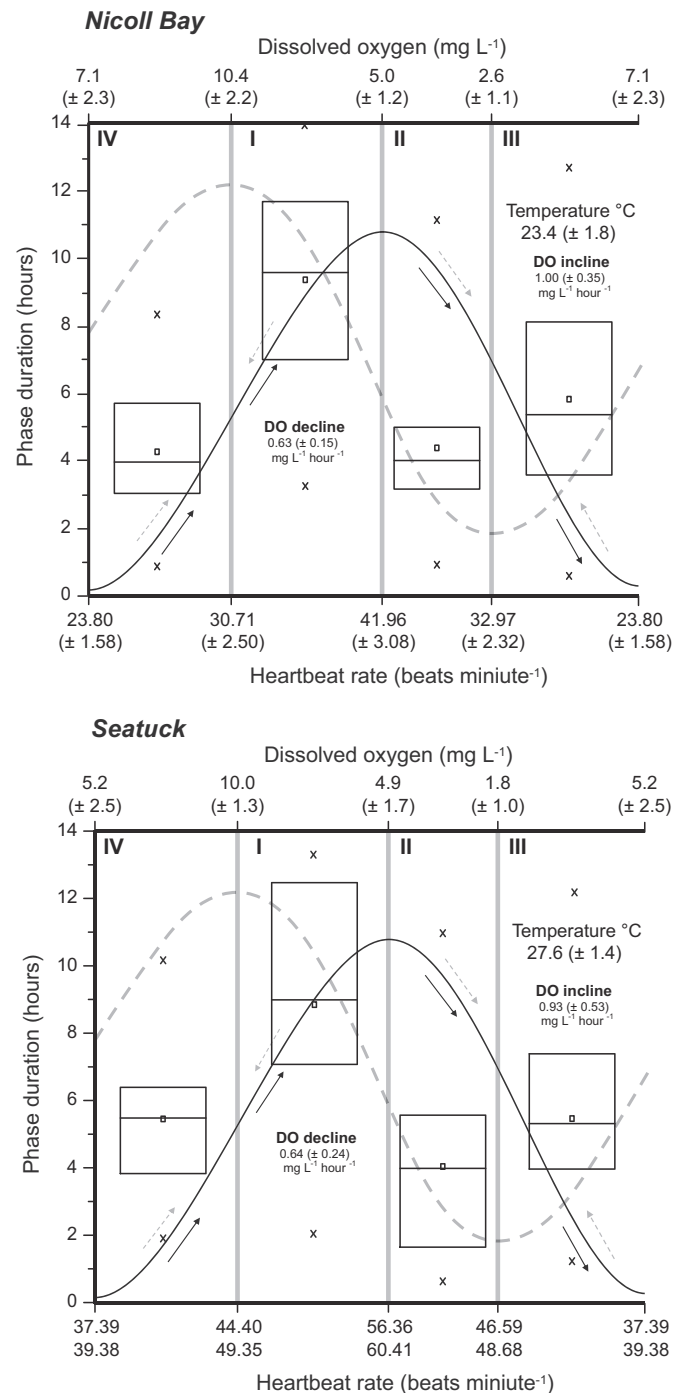


Fig. 9. Heartbeat rates, DO concentrations, and phase durations (mean ± standard deviation) that define the CRD_{DO} criteria. Phase durations are displayed with the mean (small squares), 25–75% (large box), and 1–99% range (x). Nicoll Bay displays individual variance among the mean heartbeat rates of six scallops over 17 diel-cycling oscillations. Mean heartbeat rates are listed from two scallops at Seatuck Cove over 18 diel-cycling oscillations.

decline and rise during diel-cycling hypoxia and not absolute instantaneous values control physiological responses in this species. Phase 3 ends at minimum heartbeat rate when an incline of DO concentrations stimulate recovery of aerobic functions and elevation of heartbeat rates in Phase 4 (Figs. 8 and 9).

Phase 4 of CRD_{DO} is the “recovery” period and is the only interval when heartbeat rate and DO increase simultaneously (Fig. 8). Rates increased 10 bpm above the daily minimum in phase one. This suggests

an initial effort to restore aerobic functions to basal rates. Phase four ends at maximum daily DO and had a mean duration of 4–6 h. Visual observation of combined responses in phases 1 and 4 and 2 and 3 show a dynamic cardiac range of 20 bpm from mean daily minimum to maximum under exposure to diel-cycling DO (Fig. 9). Cardiac activity of *A. irradians* in diel-cycling hypoxic environments is widely dependent upon the frequency and magnitude of stress periods as well as duration of exposure as indicated by sustained tachycardia during a long-term moderately hypoxic event (Fig. 1E).

At the sites used in the present study, days of diel-cycling hypoxia were dominated (approximately 10 h) by phase 1, an oxy-regulatory response during which DO declines and heartbeat rates increase. This suggests adaptive capability to maintain aerobic metabolism in response to a decrease in oxygen availability. However, we speculate that the frequency and overall duration of oxy-conformity in phase 2 and 3 is particularly critical for the performance of *A. irradians* because declining heartbeat rates at declining DO concentrations suggest that aerobic metabolism cannot be maintained. From our data, we conclude that *A. irradians* spent almost 40% of its days within Nicoll Bay and Seatuck Cove under suboptimal conditions (phase 2 and phase 3) during which metabolic activity was reduced and/or at least partially sustained by anaerobic metabolism.

5. Conclusion

Application of heartbeat rate sensors in the field and in laboratory experiments revealed immediate cardiac responses to diel-cycling hypoxia. Daily alterations in cardiac activity demonstrated that bay scallops are sensitive to natural fluctuations in oxygen availability. Scallops showed repetitive oxy-regulatory and oxy-conformer responses over diurnal DO fluctuations. Maximal heartbeat rates were measured when DO fell to 5 mg O₂ L⁻¹. This response occurred at oxygen concentrations higher than what is typically considered to be hypoxic. Further decline in oxygen concentrations and the parallel decline in heartbeat rates suggest an onset of anaerobic metabolism, a potentially sub-lethal implication of periodic exposure to diel-cycling hypoxia. Short-term acclimation to diel-cycling hypoxia did not affect *A. irradians*' ability to cope with severe hypoxic or anoxic events. Our data suggest that at fully oxygenated conditions cardiac activity is a good proxy for respiration over a wide temperature range, but that variations in DO cause dynamic cardiac responses in which heartbeat frequencies and respiration rates are decoupled.

Acknowledgements

This study was supported by New York Sea Grant (Project number 1124181). FPL was supported by Fundação para a Ciência e a Tecnologia (FCT) under the contract IF/00043/2012 and RS was supported by MARINFO - NORTE- 01-0145-FEDER-000031, funded by Norte Portugal Regional Operational Program (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). We thank all members of the Long Island Water Quality Index program that provided the temperature and oxygen *in-situ* data for this study. We also thank Steven Tettelbach (LIU Post), Joe Hinton (Marine Field Technician), the Cornell Cooperative Extension Marine Program, and Andrew Griffith (SBU) for providing specimen for this study. We also thank Tom Wilson and Alex Sneddon from the Instrument Laboratory (eShop) at the Stony Brook School of Marine and Atmospheric Science for development of waterproof housings used in this study. This research would not have been possible without assistance from Ian Dwyer and Molly Graffam (Volkenborn lab) in addition to the combined effort, generosity, and innovations from fellow scientists and friends.

References

- Aagaard, A., Andersen, B.B., Depledge, M.H., 1991. Simultaneous monitoring of physiological and behavioral activity in marine organisms using noninvasive, computer-aided techniques. *Mar. Ecol. Prog. Ser.* 73, 277–282.
- Aguirre-Velarde, A., Jean, F., Thouzeau, G., Flye-Sainte-Marie, J., 2016. Effects of progressive hypoxia on oxygen uptake in juveniles of the Peruvian scallop, *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture* 451, 385–389.
- Ansell, A.D., 1973. Changes in oxygen consumption, heart rate and ventilation accompanying starvation in the decapod crustacean cancer pagurus. *Neth. J. Sea Res.* 7, 455–475.
- Artigaud, S., Lacroix, C., Pichereau, V., Flye-Sainte-Marie, J., 2014. Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus spp.* *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 175, 135–140.
- Baker, S.M., Mann, R., 1992. Effects of hypoxia and anoxia on larval settlement, juvenile growth, and juvenile survival of the oyster *Crassostrea virginica*. *Biol. Bull.* 182, 265–269.
- Baumann, H., Wallace, R.B., Tagliaferri, T., Gobler, C.J., 2014. Large natural pH, CO₂ and O₂ fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. *Estuar. Coasts* 38, 220–231.
- Bayne, B.L., 1971. Ventilation, the heartbeat and oxygen uptake by *Mytilus edulis* L. In: *Declining Oxygen Tensions*. *Comp. Biochem. Physiol.* Vol. 40A, pp. 1065–1085.
- Braby, C.E., Somero, G.N., 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *J. Exp. Biol.* 209, 2554–2566.
- Brand, A.R., Roberts, D., 1973. The cardiac responses of the scallop *Pecten maximus* (L.) to respiratory stress. *J. Exp. Mar. Biol. Ecol.* 13, 29–43.
- Brokordt, K., Pérez, H., Campos, F., 2013. Environmental hypoxia reduces the escape response capacity of juvenile adult scallops *Argopecten purpuratus*. *J. Shellfish Res.* 32, 369–376.
- Burnett, L.E., Stickle, W.B., 2001. Physiological responses to hypoxia. In: Rabalais, N.N., Turner, R.E. (Eds.), *Coastal Hypoxia: Consequences for Living Resources and Ecosystems*. American Geophysical Union, pp. 101–114.
- Burnett, N.P., Seabra, R., de Pirro, M., Wetthey, D.S., Woodin, S.A., Helmuth, B., Zippay, M.L., Sara, G., Monaco, C., Lima, F.P., 2013. An improved noninvasive method for measuring heartbeat of intertidal animals. *Limnol. Oceanogr.* 11, 91–100.
- Chappon, C., Volkenborn, N., Clavier, J., Séité, S., Seabra, R., Lima, F.P., 2016. Exposure to solar radiation drives organismal vulnerability to climate: evidence from an intertidal limpet. *J. Therm. Biol.* 57, 92–100.
- Cheung, S.G., Chan, H.Y., Liu, C.C., Shin, P.K.S., 2008. Effect of prolonged hypoxia on food consumption, respiration, growth and reproduction in marine scavenging gastropod *Nassarius festivus*. *Mar. Pollut. Bull.* 57, 280–286.
- Connecticut Department of Energy and Environmental Protection (CT DEEP), 2016. Long Island Sound Hypoxia Season Review.
- D'Avanzo, C., Kremer, J.N., 1994. Diel oxygen dynamics and anoxic events in an eutrophic estuary of Waquoit Bay, Massachusetts. *Estuar. Coasts* 17, 131–139.
- DeFur, P.L., Magnum, C.P., 1979. The effect of environmental variables on the heart rates of invertebrates. *Comp. Biochem. Physiol. A* 62, 283–294.
- Depledge, M.H., Andersen, B.B., 1990. A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. *Comp. Biochem. Physiol. A* 96, 473–477.
- Diaz, R.J., 2001. Overview of hypoxia around the world. *J. Environ. Qual.* 30, 275–281.
- Diaz, R.J., Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanogr. Mar. Biol.* 33, 245–303.
- Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. *Science* 321, 926–929.
- Frederich, M., Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R1531–R1538.
- Gainey, L.F., Shumway, S.E., 1988. Physiological effects of *Protonotaulax tamarensis* on cardiac activity in bivalve molluscs. *Comp. Biochem. Physiol. C Comp. Pharmacol.* 91, 159–164.
- Gobler, C.J., DePasquale, E.L., Griffith, A.W., Baumann, H., 2014. Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. *PLoS One* 9, e83648.
- Gobler, C.J., Clark, H.R., Griffith, A.W., Lusty, M.W., 2017. Diurnal fluctuations in acidification and hypoxia reduce growth and survival of larval and Juvenile Bay scallops (*Argopecten irradians*) and hard clams (*Mercenaria mercenaria*). *Front. Mar. Sci.* 3, 282.
- Grieshaber, M.K., Hardewig, I., Kreutz, U., Pörtner, H.O., 1994. Physiological and metabolic responses to hypoxia in invertebrates. *Rev. Physiol. Biochem. Pharmacol.* 125, 43–147.
- Helm, M.M., Trueman, E.R., 1967. The effect of exposure on the heart rate of the mussel, *Mytilus edulis* L. *Comp. Biochem. Physiol.* 21, 171–177.
- Herreid, C.F., 1980. Hypoxia in invertebrates. *Comp. Biochem. Physiol. A Physiol.* 67, 311–320.
- Howarth, R., Chan, F., Conley, D.J., Garnier, J., Doney, S.C., Marino, R., Billen, G., 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front. Ecol. Environ.* 9, 18–26.
- Howell, P., Simpson, D., 1994. Abundance of marine resources in relation to dissolved oxygen in Long Island Sound. *Estuar. Coasts* 17, 394–402.
- Keppel, A.G., Breitburg, D.L., Wikfors, G.H., Burrell, R.B., Clark, V.M., 2015. Effects of co-varying diel-cycling hypoxia and pH on disease susceptibility in the eastern oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 538, 169–183.

- Pörtner, H.O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881–893.
- Pörtner, H.O., Grieshaber, M.K., 1993. Critical PO_2 in oxyconforming and oxyregulating animals gas exchange, metabolic rate and the mode of energy production. In: Bicudo, E. (Ed.), *The Vertebrate Gas Transport Cascade. Adaptations to Environment and Mode of Life*. CRC Press, Boca Raton FL, pp. 330–357.
- Pörtner, H.O., Heisler, N., Grieshaber, M.K., 1985. Oxygen consumption and mode of energy production in the intertidal worm *Sipunculus nudus* L.: definition and characterization of the critical PO_2 for an oxyconformer. *Respir. Physiol.* 59, 361–377.
- Prosser, C.L., 1973. *Comparative Animal Physiology*. W.B. Saunders Company, Philadelphia.
- Seabra, R., Wetthey, D.S., Santos, A.M., Gomes, F., Lima, F.P., 2016. Equatorial range limits of an intertidal ectotherm are more linked to water than air temperature. *Glob. Chang. Biol.* 22, 3320–3331.
- Steckbauer, A., Ramajo, L., Hendriks, I.E., Fernandez, M., Lagos, N., Prado, L., Duarte, C., 2015. Synergistic effects of hypoxia and increasing CO_2 on benthic invertebrates of the central Chilean coast. *Front. Mar. Sci.* 2, 49.
- Taylor, A.C., Brand, A.R., 1975. A comparative study of the respiratory responses of the bivalves *Arctica islandica* (L.) and *Mytilus edulis* L. to declining oxygen tension. *Proc. R. Soc. B* 190, 443–456.
- Taylor, E.W., Butler, P.J., Sherlock, P.J., 1973. The respiratory and cardiovascular changes associated with the emersion response of *Carcinus maenas* (L.) during environmental hypoxia, at three different temperatures. *J. Comp. Physiol.* 86, 95–115.
- Thomas, P., Rahman, M.S., Khan, I.A., Kummer, J.A., 2007. Widespread endocrine disruption and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. *Proc. R. Soc. B* 274, 2693–2702.
- Tyler, R.M., Brady, D.C., Targett, T.E., 2009. Temporal and spatial dynamics of diel-cycling hypoxia in estuarine tributaries. *Estuar. Coasts* 32, 123–145.
- US Environmental Protection Agency (EPA), 2000. Ambient aquatic life water quality criteria for dissolved oxygen (saltwater): Cape Cod to Cape Hatteras. In: EPA-822-R-00-012. Office of Water, Washington, DC.
- Van den Thillart, G., Van Lieshout, G., Storey, K., Cortesi, P., De Zwaan, A., 1992. Influence of long-term hypoxia on the energy metabolism of the haemoglobin-containing bivalve *Scapharca inaequivalvis*: critical O_2 levels for metabolic depression. *J. Comp. Physiol. B* 162, 297–304.
- Wallace, R.B., Baumann, H., Gear, J.S., Aller, R.C., Gobler, C.J., 2014. Coastal ocean acidification: the other eutrophication problem. *Estuarine coastal and shelf*. *Science* 148, 1–13.
- Wang, W.X., Widdows, J., 1991. Physiological responses of mussel larvae “*Mytilus edulis*” to environmental hypoxia and anoxia. *Mar. Ecol. Prog. Ser.* 223–236.
- Wang, W.X., Widdows, J., 1993. Metabolic responses of the common mussel *Mytilus edulis* to hypoxia and anoxia. *Mar. Ecol. Prog. Ser.* 205–214.
- Widdows, J., 1973. Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis*. *Mar. Biol.* 20, 269–276.
- Wu, R.S., 2009. Effects of hypoxia on fish reproduction and development. *Fish Physiol. Biochem.* 27, 79–141.
- Xing, Q., Li, Y., Guo, H., Yu, Q., Huang, X., Wang, S., Hu, X., Zhang, L., Bao, Z., 2016. Cardiac performance: a thermal tolerance indicator in scallops. *Mar. Biol.* 163, 1–9.