

**EFFECTS OF DIEL-CYCLING HYPOXIA ON THE CARDIAC
ACTIVITY AND GROWTH OF *ARGOPECTEN IRRADIANS***

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by

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Abstract of the Thesis

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ABSTRACT

Bottom water oxygen concentrations in coastal environments can oscillate between fully oxygenated and hypoxic conditions on a daily basis. How benthic organisms deal with such drastic changes in oxygen availability is not well understood. Specifically, we do not know the magnitude, duration, and frequency at which diel-cycling hypoxia conditions become harmful. Here we have used non-invasive infrared sensors to measure the cardiac activity of the Atlantic bay scallops, *Argopecten irradians*, in response to diel-cycling hypoxia *in-situ* over one-month periods as well as in the laboratory in controlled incubations using animals conditioned to contrasting field conditions. In the field, heartbeat rates at a well-oxygenated site were relatively stable while scallops deployed at two sites with pronounced diel-cycling hypoxia were elevated and more variable. Maximal heartbeat rates during diel-cycling hypoxia were commonly recorded around dawn when oxygen concentrations fell to 5 mg L⁻¹ indicating a sub-lethal response to dissolved oxygen (DO) concentrations higher than what is typically defined as hypoxia. Laboratory incubations confirmed the tight link between DO and cardiac activity. An increase of heartbeat rate in response to an initial decline from fully oxygenated conditions was indicative of a regulatory response in which cardiac activity was enhanced presumably to maintain oxygen supply. At DO below 3 mg L⁻¹ heartbeat rates declined reaching a state of ascardia during anoxia, suggesting a conformer response to severe hypoxia. *In-situ* and laboratory data was integrated into a novel conceptual model to characterize four phases that interpret cardiac and respiratory activity in diel-

cycling hypoxia. Heartbeat frequency was a suitable proxy for respiration under normoxia, but scallops were unable to compensate for reduced oxygen availability by increasing heartbeat rates below hypoxic thresholds. Pre-conditioning to diel-cycling hypoxia did not affect survival or cardiac activity in anoxic and severe hypoxic treatments. However, *A. irradians* pre-conditioned to diel-cycling hypoxia were less responsive to normoxia with heightened effort to maintain vital aerobic functions after long-term exposure to severe hypoxia. Survival after 12-14 hours of anoxia and mortality after 23-32 hours of anoxia convey physiological limitations unaffected by acclimation. We speculate that repetitive exposure to periods of DO oscillations with exposure below 5.0 mg L⁻¹ in the field can cause sub-lethal effects to *A. irradians* affecting fitness, growth, and reproductive success.

DEDICATION PAGE

I dedicate this Master's thesis to my mother and father. Their encouragement to pursue my interests and explore the Long Island Sound shoreline throughout my growing years has driven me to become the scientist I am today.

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1. INTRODUCTION

1.1 Hypoxia in coastal environments

In coastal ecosystems hypoxia is a common physicochemical 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 (DO) develop when oxygen demand outweighs supply. Hypoxia intensifies seasonally from the effects of elevated temperature, light intensity, stratification and eutrophication (Diaz 2001; Howarth et al. 2011; Wallace et al. 2014). Large quantities of oxygen are consumed by whole community nighttime respiration resulting in hypoxic conditions within bottom waters that can last weeks to months. In shallow water environments, photosynthetic activity during the day and community respiration during night can lead to extremely variable oxygen availability (D'Avanzo and Kremer 1994). Diel-cycling hypoxia seasonally intensifies in summer and co-occurs with acidification among coastal environments (Wallace et al. 2014). Coupled stressors pose dynamic stress oscillations on seasonal as well as on daily time scales to organisms of commercial and ecological importance (Tyler et al. 2009; Baumann et al. 2014; Gobler and Baumann 2016; Gobler et al. 2017).

1.2 Effects of hypoxia on benthic invertebrates

Ecological issues and alterations of community structure associated with hypoxia are undisputed and result in mass mortalities, metabolic alterations, and escape responses of benthic and pelagic fauna (Howell and Simpson 1994; Diaz and Rosenberg 1995; Burnett and Stickle 2001). Such conditions may constitute a significant burden for benthic organisms affecting 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. 2016; Gobler et al. 2017), but sub-lethal effects of diel-cycling hypoxia on benthic organisms are not well understood. Importantly, many biological response variables (such as growth rates, development, mortality, etc.) integrate over relatively long time scales that limit the ability to understand and identify effects of dynamic environmental stressors and thresholds at which conditions become harmful.

1.3 Cardiac activity as response variable

Cardiac activity is a widely used proxy of whole animal metabolism (Helm and Trueman 1967) and can be measured with high temporal resolution to reveal immediate metabolic responses to changes in the environment (Burnett et al. 2013; Chapperon et al. 2016). Heartbeat rates of invertebrates exposed to environmental stress have been extensively studied (DeFur and Mangum 1979; Gainey and Shumway 1988; Braby and Somero 2006), but past efforts with minimally invasive techniques were mostly constrained to laboratory use (Aagaard et al. 1991; Rovero et al. 1999; Xing et al. 2016; Seabra et al. 2016). Maximum heartbeat rate at upper critical temperature (T_{cII}) and Arrhenius breakpoint temperature (ABT) identify thermal windows of optimal aerobic performance and acute physiological limitations for marine invertebrates (Fredrich and Pörtner 2000; Pörtner 2012; Xing et al. 2016; Chapperon et al. 2016; Tagliarolo and McQuaid 2016). For example, the European spider crab *Maja squinado* reaches maximum cardiac activity at 31.5°C (T_{cII}) followed by an increase in products of anaerobic metabolism at 33°C when heartbeat rates decline (Fredrich and Pörtner 2000). This provides evidence that heartbeat rate is closely linked with scope of aerobic performance and metabolic transitions. Parallel measurements of heartbeat and respiration rates as a function of temperature (Defur and Magnum 1979) indicate a strong relationship between both physiological parameters, but 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). Some marine invertebrates exhibit a short cardiac upswing under the initial DO decline from full oxygen saturation and drastically decrease heartbeat rate under hypoxia and anoxia exposure (Brand and Roberts 1973; Nicholson 2002). An abnormal decline, elevation, and cease of heartbeat rate are referred to as bradycardia, tachycardia, and acardia, respectively. Slight tachycardia during DO decline followed by bradycardia under severe oxygen limited conditions is evidence of an attempt to maintain aerobic function and transition to anaerobic metabolism (Bayne 1971; DeFur and Pease 1988). Here we have used non-invasive heartbeat sensors in an ecophysiological approach to measure cardiac activity *in-situ* during diel-cycling hypoxia and in the laboratory under hypoxia and anoxic simulations to increase an understanding of immediate physiological responses to stress conditions.

1.4 Model organism: *Argopecten irradians*

We chose *Argopecten irradians* as a model organism to measure stress response of a commercially and ecologically important species to diel-cycling hypoxia. Benthic invertebrates such as *A. irradians* with limited locomotive capability to avoid unfavorable conditions must alter physiological responses under stress (Burnett and Stickle 2001). The bay scallop fishery around Long Island suffered a severe decline as a result of brown tide blooms in 1985 (Bricelj et al. 1987), motivating efforts to restore populations (Tettelbach et al. 2015) and discover potential stressors that prevent natural population recovery (Shumway 1990; Gainey and Shumway 1991; Rheault and Rice 1996; Shriver et al. 2002; Hégaret and Wikfors 2005; Talmage and Gobler 2009). Environmental stressors such as harmful algae, hypoxia, and acidification have been simulated in laboratory studies and found to impact the histology, behavior, growth, and survivorship of scallop larvae and early-life stages (Chun-de and Fu-sui 1995; Wikfors 2005; Brokordt et al. 2013; Gobler et al. 2014; Gobler 2017). However, hypoxic-induced effects on adult *A. irradians* are poorly understood.

1.5 Working hypotheses and approaches

In-situ monitoring of heartbeat frequencies, DO, and temperature at locations that contrasted with respect to the severity of diel-cycling hypoxia as well as laboratory measurements of heartbeat rates under temperature and DO concentrations were used to study the link between fluctuating DO concentrations and cardiac activity. The effects of diel-cycling hypoxia on animal performance was analyzed with respect to the frequency, severity and duration of low oxygen conditions. Our working hypothesis were:

- 1) *A. irradians* alters cardiac activity under exposure to *in-situ* diel-cycling dissolved oxygen (DO)

To test this hypothesis, *A. irradians* with heartbeat sensors were deployed *in-situ* during summer months to continuously monitor cardiac responses to daily DO and temperature oscillations.

- 2) Cardiac activity is a suitable proxy for respiration at different temperatures and DO concentrations.

To test this hypothesis, heartbeat and respiration rates were recorded simultaneously in respiration chambers at several temperatures and DO concentrations.

- 3) Acclimation to diel-cycling hypoxia affects the ability for *A. irradians* to cope with, recover from, and survive periods of anoxia.

To test this hypothesis, *A. irradians* were deployed at two sites with contrasting diel-cycling DO dynamics to determine if short-term field acclimation affects the ability to cope with anoxic stress in laboratory simulations. Heartbeat sensors were glued to pre-conditioned scallops to monitor cardiac responses during short-term anoxia and aerobic recovery and determine the lethal duration of anoxic exposure.

4) *A. irradians* acclimated to pronounced diel-cycling hypoxia are best suited to cope with a severe hypoxic event.

To test this hypothesis, *A. irradians* pre-conditioned to five contrasting hypoxic sites were returned to the lab for a post-acclimation mesocosm experiment. Cardiac activity was continuously monitored for 24-hours of normoxia and severe hypoxia to investigate whether multi-week acclimation has an effect on the cardiac response to severe hypoxia and recovery upon return to normoxia.

5) *Environments with greater frequency, severity, and duration of hypoxic periods impede shell growth and reduce condition index of A. irradians.*

To test this hypothesis, scallop shell dimensions were measured for 180 tagged adult *A. irradians* prior to cage deployments at nine contrasting sites. Scallop shell size was measured upon return to the lab for estimation of shell growth and four individuals from each site were sacrificed for condition index determination.

2. METHODS

2.1. Characterization of diel-cycling hypoxia

Nine sites on the south shore of Long Island, NY, at which water quality is monitored as part of the Long Island Water Quality Index Program (LIWQI) were used in this study (Table 1). As part of the LIWQI program, DO and temperature were recorded at 15 minute intervals from June to October at each site with HOB0 Dissolved Oxygen Loggers (U26-001) fixed to floating docks and suspended 0.5 meters above the sea floor. Loggers were calibrated and cleaned weekly. Water sampling occurred weekly with measurements of chlorophyll, secchi depth, and salinity where the sensors were deployed. Field readings were taken for DO and salinity with a YSI Pro2030 which was calibrated monthly.

2.2. Heartbeat measurements with infrared sensors

Eight-channel heartbeat amplifier and logging systems (Burnet et al. 2013) incorporated into waterproof housings were designed to record heartbeat rates. The system uses minimally invasive infrared sensors that are glued onto the exoskeleton or shell surface of marine invertebrates. For *A. irradians*, sensors were adhered to the external upper valve on the posterior side of the valve hinge to capture data from the pericardium (photo below). Sensors emit infrared light through the hard substrate and detect the infrared light reflected from subtle alterations in shape or volume of the heart and cardiovascular vessels (Burnett et al. 2013). Data were analyzed with an interactive R script (first written and implemented by Fernando Lima) to allow visual inspection of the raw data and deduct heartbeat data for time periods with regular signal oscillations (Burnett et al. 2013; Chapperon et al. 2016; Seabra et al. 2016).

Each heartbeat logger was programmed to sequentially record 60 seconds of heartbeat data for each of eight *A. irradians* and repeat the cycle every 10 minutes throughout the deployment duration. Loggers were deployed in waterproof housings (photo below) with custom built 15 V battery packages consisting of 20 alkaline D cells providing sufficient power for one month of continuous recording. Sensor wires were spliced and fed through the lid to avoid water intrusion through sensors cables.

A. irradians cages and heartbeat loggers were deployed in 3 coastal bays of Long Island, NY. Cages were staked 0.5 meters above the sea floor near (2 – 3 m) the oxygen and temperature sensors. 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 cm x 15 cm x 15 cm) in a 3 x 3 grid (photo below). 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 fifteen minutes to compare internal cage data at the same temporal resolution as the dockside LIWQI temperature sensors.

In-situ heart rate was monitored at Fire Island and Nicoll Bay from 9 Sept – 28 Oct in 2015. This was in part of a site acclimation deployment to test hypothesis in Exp. 1 (2.3). Cardiac responses in a second *in-situ* heart rate deployment in Seatuck during August 2016 sought to supplement previous findings with responses to severe mid-summer hypoxia and anoxia. Heartbeat rate data was recorded during *A. irradians* site acclimation deployments to test hypothesis in Exp 2. (2.4).



Photos: *A. irradians* with an infrared heartbeat rate sensor (left), waterproof housing for *in-situ* deployment of heartbeat monitoring equipment (center), and a cage with cube divisions for each scallop during heartbeat sensor deployments. Photos taken by Sam Gurr and Nils Volkenborn.

2.3. Exp 1: Conditioning to diel-cycling hypoxia and effects on the ability to cope with anoxia

A. irradians were collected by hand in Orient Point Harbor in Long Island, New York and maintained in the laboratory in unfiltered re-circulating seawater (replaced weekly). Laboratory conditions were maintained at constant 28 ppt salinity and 23 – 25°C representative of the field conditions. Sixteen scallops were acclimated for one day before infrared heartbeat sensors were applied. Scallops were deployed to two sites (Fire Island and Nicoll Bay) from 9 Sept and 12 Oct.

After a 4 week conditioning period at Fire Island and Nicoll Bay scallop cages were brought back to the lab. Infrared sensors were removed and re-glued on scallops to ensure strong signals during post *in-situ* experiments. Biofouling organisms that had grown on the shells were carefully removed with steel brushes to reduce their impact on respiration measurements. Experiments were designed to expose the conditioned scallops to anoxia to 1) determine the time period of anoxia to reach mortality and 2) measure cardiac and respiratory responses to a short-term hypoxic period. Wide mouth PET bottles (Kautex) were used as respiration chambers. Each chamber had in inflow and outflow valve which were connected to the recirculating seawater system. Respiration chambers were positioned in a temperature controlled water bath above magnetic stir plates. Each chamber contained a magnetic stir bar that was rotated at low speed to ensure homogenous mixing of water within the respiration chamber without disturbing the incubated animals. A 2 mm mesh was used to separate the animals from the magnetic stir bar below. Heartbeat sensor cables, temperature probes and optical oxygen probes (Pyro Science, Germany) were fed through the lids of the respiration chambers and sealed with hot glue.

Oxygen concentrations were recorded with a 4 channel FireStingO2® oxygen meter (Pyro Science, Germany) taking oxygen and temperature readings at 1 second intervals.

Lethal anoxic exposure experiments were completed on three *A. irradians* from the Nicoll Bay and Fire Island deployments. Trials were initiated after *A. irradians* were acclimated to a temperature of 17.5°C. Trials ended when all individuals expressed characteristics associated with scallop mortality such as wide valve gaping and lack of reactive valve movements during agitation. Time of mortality was determined when heartbeat data failed to yield a minimum of three viable signals over a period of one hour.

Sub-lethal anoxic exposure challenges were simulated in a second experiment in which animals were exposed to 12 – 14 hours of anoxia after the initial oxygen decline period. The linear decline in oxygen concentrations within each respiration chamber was used to derive respiration rates which were normalized to the ash-free dry weight of each individual. Basal heartbeat rates before sub-lethal anoxic exposure trials, basal heart rates after recovery from short-term anoxia, DO concentration at maximal heartbeat rate during oxygen decline, heartbeat rate at anoxia, and qualitative information on heart rate during anoxia and during recovery were analyzed for animals from both sites.

2.4. Exp 2: Conditioning to diel-cycling hypoxia and effects on the ability to cope with severe hypoxic events

Adult *A. irradians* (200 specimen) with a length of 47.11 ± 3.66 mm were provided by Cornell Marine Peconic Bay Scallop Restoration Program and collected in Orient Point Harbor, NY. Shell size, biovolume, and wet weight were measured for all scallops on return to the laboratory. Scallops were maintained in unfiltered seawater on a temperature controlled recirculating table at 28 ppt and 25° C for one day. A total of 180 scallops were individually labeled with plastic embossing labels and randomly divided to nine cages (20 *A. irradians* per cage). Field deployment cages were built identical to *in-situ* heartbeat monitoring cages (2.2), but without cube divisions to separate individuals. Cages were deployed from mid-July to early September (50 days) at nine coastal locations with contrasting environmental parameters monitored continuously as part of the Long Island Water Quality Index Program (Table 1.). To determine diel-cycling patterns of DO and its influence on the growth and condition index of *A. irradians*, all alive animals were measured and weighed at the end of the field deployments and four individuals per site were sacrificed to estimate condition index (2.6).

After pre-conditioned for nearly two months, the cardiac response to severe hypoxia was investigated using 40 scallops from five of the nine sites (Fire Island, Nicoll Bay, Quantuck, Sag Harbor, Moneybogue). Each scallop was affixed with infrared heartbeat sensors immediately on arrival to the laboratory and divided into eight 20-liter mesocosms each with one individual representative of each site. Mesocosms were $\frac{3}{4}$ filled with 28 ppt unfiltered seawater and positioned in a temperature controlled water bath (25°C) above magnetic stir plates. Each mesocosm contained a magnetic stir bar that was rotated at low speed to ensure homogenous mixing of water without disturbing the incubated animals. A 2 cm mesh was used to separate the animals from the magnetic stir bar below.

Experimental design contained eight mesocosms. Four mesocosms were controlled at severe hypoxia (1.5 – 2.0 mg L⁻¹) for a 24 hour period by bubbling with air (air pump) and N₂ premixed with 400ppm CO₂ gas. Four mesocosms were maintained at full oxygen saturation throughout the experiment by bubbling air. Heartbeat rates were monitored during 60 second intervals every ten minutes and initiated under full oxygen saturation before the start of severe hypoxia. The experiment was terminated after severe hypoxic mesocosms were reoxygenated to normoxia for one hour. DO was measured continuously in severe hypoxia mesocosms with a 4 channel FireStingO2® oxygen meter (Pyro Science, Germany) taking oxygen and temperature readings at 1 second intervals. Sensors were occasionally positioned in adjacent control mesocosms for one hour to record DO concentrations in normoxic replicates.

2.5. Respiration measurements

Animals in respiration chambers were exposed to full oxygen saturation with open inflow and outflow valves for one hour before starting experiments. Heartbeat and respiration rate measurements were recorded simultaneously during DO decline in anoxic exposure experiments (2.3) and a temperature ramp. Heartbeat and respiration rates were monitored under acute stress in a short-term temperature ramp to uncover a link in these physiological responses. Once respiration chamber valves were closed, the decline in DO was used to calculate respiration rates. Respiration rates were estimated upon arrival to new temperatures (28, 23, 21, and 18°C, respectively) to correspond with the acute changes in heartbeat rate. The linear decline of oxygen concentrations was used to determine oxygen consumption rates. Oxygen was resupplied before concentration fell below 3 – 4 mg L⁻¹. Fully

oxygenated conditions in the chambers rapidly reestablished (within 2 – 3 minutes) when valves were opened.

Estimates of respiration rates were adjusted to the water volume in each respiration chamber, taking into account the biovolume of the incubated scallop, and normalized to the ash free dry weight (AFDW) of the individuals ($\text{mg L}^{-1} \text{ minute}^{-1} \text{ gram}^{-1}$). Respiration rates were measured for three individuals 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. Cardiac and respiratory responses to the decline of DO were assembled in “heartbeat efficiency” calculated as the quotient of heartbeat rate and respiration rate resulting in units of $\text{mg L}^{-1} \text{ gram}^{-1} \text{ beat}^{-1}$.

2.6. Scallop shell growth, biovolume, tissue biomass, and condition index measurements

Shell size dimensions length (l), width (w), and thickness (t) in mm were recorded with calipers in addition to biovolume (ml), wet weight (g), dry weight (g), and AFDW (g) for each *A. irradians* in Exp. 1 and 2. Biovolume was estimated by the displacement of seawater with live organisms. AFDW was estimated as loss on ignition after 4 hours at 450°C. Exp 1. included the shell during initiation, while tissue was separated in Exp 2

Condition index and pre and post-deployment shell size in Exp. 2 was used to determine biomass and shell growth of *A. irradians* deployed at the nine contrasting sites. Post-deployment AFDW was estimated with shucked scallops to avoid inclusion of the shell during ignition. Condition index was calculated as $\text{AFDW} \div \text{Vcap}_{\text{scallop}}$. $\text{Vcap}_{\text{scallop}}$ encompasses use of l , w , and t to standardize condition index with scallop shell volume converted from the equation for the volume of a spherical cap (Harris and Stocker 1998);

$$\text{Vcap}_{\text{scallop}} = 1/3\pi \cdot (t \div 2) \cdot (3 \cdot ((l \div 2 + w \div 2) \div 2)^2 + (t \div 2)^2)$$

2.7 Data Analysis

In-situ heartbeat rates, DO, and temperature were averaged hourly across the deployment durations to compare responses in the field. Average daily rates of the elevation and decline in DO at

Nicoll Bay and Seatuck were estimated in $\text{mg L}^{-1} \text{ hour}^{-1}$ from the slopes of 17 and 18 diel-cycling hypoxia oscillations during heartbeat rate deployments, respectively. Maximum and minimum heartbeat rates and the DO concentrations when they occurred were identified for each day during *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 mg L^{-1} increments from full oxygen saturated conditions to anoxia in respiration chambers. Mean heartbeat and respiration rates at each 1.0 mg L^{-1} DO increment were averaged to estimate individual variance between individuals pre-conditioned at Fire Island and Nicoll Bay. Mean respiration and heartbeat rates were used to estimate heartbeat efficiency in units of $\text{mg L}^{-1} \text{ gram}^{-1} \text{ beat}^{-1}$ (2.5). Heartbeat efficiency followed a normal distribution from a shapiro-wilk normality test (Fire Island $p = 0.582$; Nicoll Bay $p = 0.095$). An ANOVA followed by a Tukey Honest Significant Difference (HSD) test for multiple was used to determine whether there was a significant difference in heartbeat efficiency between 1.0 mg L^{-1} DO increments of during oxygen decline from full saturation to anoxia. Shell growth and condition index from all sites were correlated with weekly site sampling characteristics (chlorophyll, salinity, and secchi depth) and hypoxic descriptors such as percentage of time as well as the frequency, mean duration, and mean oxygen concentration during hypoxic periods. A chi-square test determined mean shell growth from Sag Harbor as an outlier; shell growth from this site was excluded in regression analysis. Analysis of *in-situ* DO data was completed at five hypoxia thresholds ($4.8, 3.5, 3.0, 2.0$, and 1.0 mg L^{-1}) to test Exp. 2 (Table 1). Shell growth was right skewed with one negative value; shell growth was not normally distributed and was transformed as $\sqrt{(\text{shell growth} + 1)}$ (shapiro-wilk normality test, $p = 0.3216$). Condition index and $\sqrt{(\text{shell growth} + 1)}$ were used to determine differences among sites with an ANOVA followed by a Tukey HSD test for multiple comparisons among sites. All statistics were completed with use of OriginPro™ (v6.1) and R (v3.2.0).

To quantitatively characterize the frequency, duration, and magnitude of hypoxic conditions at each site, intervals during which oxygen concentrations remained below defined thresholds for at least one hour in duration were defined as hypoxic “periods”. “Events” were intervals during which hypoxia persisted for more than 24 hours. The criteria was first used to investigate the count (frequency), mean duration, and mean DO concentration of periods and events defined as ‘moderate’ (4.8 mg L^{-1}) and ‘severe’ (2.0 mg L^{-1}) hypoxia during Fire Island and Nicoll Bay deployments in 2015 before an R (v3.2.0) script was written for rapid computation of hypoxia for nine sites at several DO thresholds in 2016. The script was designed to calculate hypoxic periods and events below $4.8, 3.5, 3.0, 2.0$, and 1.0

mg L⁻¹. All DO thresholds are defined in water quality criterion stated by US Environmental Protection Agency (US EPA, 2000) and implemented in associated monitoring efforts (CT DEEP, 2016).

3. RESULTS

3.1. Patterns of diel-cycling hypoxia in bays and harbors around Long Island

3.1.1 Exp.1: Late-summer deployments at two sites

Nicoll Bay and Fire Island exhibited strong contrast in frequency, duration, and magnitude of moderate and severe hypoxic periods and events with normoxic conditions at Fire Island and frequency diel-cycling hypoxia at Nicoll Bay (Fig. 1). The lowest DO measurement at Fire Island was 5.15 mg L⁻¹; DO at this site never fell below 4.8 mg L⁻¹ during deployments (Fig. 1A). Nicoll Bay underwent 24 moderate hypoxia periods with an average duration of 6.7 ± 4.2 hours and one moderate hypoxia event that persisted for 43.1 hours. Five cases of severe hypoxia occurred at Nicoll Bay with a mean duration of 3.4 ± 1.9 hours. The longest period of severe hypoxia persisted for 4.9 hours (Figs. 1B and 1E). Overall, moderate and severe hypoxia at Nicoll Bay occurred for 28% and 2.4% of the *A. irradians* deployment duration in late summer.

Nicoll Bay exhibited strong diel-cycling DO with hypoxic conditions during morning hours (Fig 1B). An average minimum DO of 2.6 ± 1.1 mg L⁻¹ occurred during early mornings followed by an increase at a rate of 1.00 ± 0.35 mg L⁻¹ hour⁻¹ from late morning to evening or nightfall. Maximum DO of 10.4 ± 2.2 mg L⁻¹ was followed by a 0.63 ± 0.15 mg L⁻¹ hour⁻¹ rate of decline throughout the night until morning. Although the magnitude of diel-cycling oxygen availability differed between Nicoll Bay and Fire Island, both sites demonstrated temporal consistencies in their daily maximal and minimal oxygen conditions (Figs. 1A and 1B).

Temperatures remained within 20 – 27 °C at Nicoll Bay (23.4 ± 1.8 °C) and Fire Island (21.6 ± 1.4 °C) (Figs. 1A and 1B). Diel-cycling temperatures occurred inconsistently with 1 – 2 °C oscillated that reached daily maximum temperatures after nightfall followed by 0.1 °C hour⁻¹ decline until minimum temperatures at noon the following day. Average daily temperatures decreased slowly over the duration of the heartbeat sensor deployment period from 26.8 ± 0.2 to 20.5 ± 0.4 at Nicoll Bay and 23.6 ± 0.4 to 19.5 ± 0.3 at Fire Island.

3.1.2 Exp. 2: Early-summer deployments at nine sites

Sites strongly differed with respect to the frequency, duration, and severity of diel-cycling hypoxia below five DO thresholds (Table 1). Moneybogue exhibited the greatest percent time and frequency of hypoxic periods at all DO thresholds (Fig. 2). As an example, moderate ($< 4.8 \text{ mg L}^{-1}$) and severe ($< 2.0 \text{ mg L}^{-1}$) hypoxia occurred for 41.87% and 14.13% of the 50-day deployment period. Moneybogue, Nicoll Bay, Quantuck, and Seatuck had the greatest frequency and severity of diel-cycling hypoxia with conditions below 4.8 mg L^{-1} nearly every day. These four sites exhibited DO oscillations below 4.8 and 2.0 mg L^{-1} for more than 30% and 5% of the *A. irradians* deployment period and the greatest percent time below 1.0 mg L^{-1} (2.71 – 8.06%) (Table 1, Fig. 2). Bellport, Tiana Beach, and Penniman Creek were intermediately hypoxic sites with a lower percent time below 4.8 (18.6 – 24.25%) and 2.0 mg L^{-1} (0.18 – 1.12%), but comparable frequency of moderate hypoxia periods to sites with the most severe conditions. Fire Island and Sag Harbor yielded the least number of hypoxic periods at all DO thresholds and conditions rarely fell below 4.8 ($< 10\%$) or 2.0 mg L^{-1} ($< 0.27\%$). DO at Fire Island never fell below 3.5 mg L^{-1} (Table 1, Fig. 2).

Frequency, mean duration, mean concentration of hypoxic periods and overall percent time of hypoxia were strongly associated with environmental parameters recorded weekly throughout the *A. irradians* pre-acclimation period. There was a strong positive correlation between mean chlorophyll and salinity ($r = 0.9767$, $p < 0.001$). Percent time, count, and duration of hypoxic periods increased with chlorophyll and salinity and decreased at sites with higher secchi depth. Sites with higher chlorophyll and salinity yielded a lower mean DO concentration during hypoxic periods at all thresholds (Table 1). Mean chlorophyll was positively correlated with the percentage of time below 3.5 mg L^{-1} ($r = 0.7910$, $p = 0.011$), count of hypoxic periods below 3.5 mg L^{-1} ($r = 0.7648$, $p = 0.016$), and duration of hypoxic periods below 2.0 mg L^{-1} ($r = 0.8863$, $p = 0.001$). Chlorophyll had a strong negative association with mean concentration of hypoxic periods below 4.8 mg L^{-1} ($r = -0.8218$, $p = 0.007$).

Seatuck was investigated further to supplement *in-situ* heartbeat sensor deployments from 5 – 23 August 2016 (3.2; Fig. 1C) and compare responses to Nicoll Bay (3.1.1) (Fig. 1B). Average temperature was $27.6 \pm 1.4^\circ\text{C}$ and diel-cycling temperature occurred throughout the heartbeat deployment with $2 - 3^\circ\text{C}$ oscillations (Figs. 1C). During 18 days of heartbeat measurements, Seatuck exhibited 22 moderate hypoxic periods with an average duration of 6.8 ± 3.7 hours and six severe hypoxic periods with an average duration of 3.8 ± 4.1 hours. The longest duration of severe hypoxia persisted for 11.8 hours on

15 August (Fig. 1C). Moderate and severe hypoxia occurred for 32.5% and 5.3% of the heartbeat sensor deployment at Seatuck. Average minimum DO of $1.8 \pm 1.0 \text{ mg L}^{-1}$ occurred in early to late mornings followed by an increase at a rate of $0.93 \pm 0.53 \text{ mg L}^{-1} \text{ hour}^{-1}$ from afternoon to nightfall. A maximum DO of $10.0 \pm 1.3 \text{ mg L}^{-1}$ was followed by a $0.64 \pm 0.24 \text{ mg L}^{-1} \text{ hour}^{-1}$ rate of decline throughout the night until morning. Diel-cycling hypoxia at Nicoll Bay and Seatuck behaved with identical temporal dynamics during heartbeat sensor deployments (Figs. 1B and 1C).

3.2. *In-situ* heartbeat monitoring

A. irradians heartbeat rates recorded in late-summer 2015 at Fire Island and Nicoll Bay ranged between 13.2 and 57.6 beats minute⁻¹ (bpm). Cardiac activity differed between the two sites with average rates $34.3 \pm 7.8 \text{ bpm}$ from the Nicoll Bay deployment and $23.0 \pm 3.4 \text{ bpm}$ from the Fire Island deployment (Fig. 3). Relative to the stable heart rates expressed by scallops exposed to oxygenated conditions at Fire Island (Figs. 1A, 1D and 3), intermittent hypoxia at Nicoll Bay was associated with daily oscillations of cardiac activity at a scale of nearly 20 bpm (Figs. 1B, 1F, and 2). A deployment at Seatuck in early-summer 2016 yielded two *A. irradians* with viable heartbeat signals. Cardiac activity at Seatuck resembled the Nicoll Bay 2015 data with identical daily cardiac activity excursions but at higher temperatures and more severe diel-cycling hypoxia (Figs. 1C and 1G).

A. irradians increased cardiac activity under reduced oxygen conditions at Nicoll Bay and Seatuck. Daily cardiac fluctuations reveal a lagged trend of peaks and troughs in sync with DO. Heartbeat rate elevations to a maximum of $42.0 \pm 3.1 \text{ bpm}$ at Nicoll Bay and 55 – 60 bpm at Seatuck occurred during early mornings when DO declined to 5.0 mg L^{-1} . Minimum DO always occurred approximately four hours 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 $7.1 \pm 2.3 \text{ mg L}^{-1}$ at Nicoll Bay and $5.2 \pm 2.5 \text{ mg L}^{-1}$ at Seatuck. Daily peaks in oxygen availability consistently occurred after minimum heartbeat rates when rates elevated 10 bpm above minimum cardiac activity. Daily DO maximum and minimum stimulated near identical heartbeat rates among scallops from both sites with average rates of 30 – 33 bpm at Nicoll Bay and 44 – 50 bpm at Seatuck. Higher activity at Seatuck is likely due to the warmer average temperatures (27.6 ± 1.4) relative to the Nicoll Bay (23.4 ± 1.8).

Persistent elevated cardiac activity (tachycardia) during exposure to reduced oxygen availability was evident throughout most of a 43.1 hour long hypoxic event at Nicoll Bay (Fig. 1E). However average heartbeat rates towards the end of this long hypoxic event declined 42% from 47.5 ± 4.0 to 27.5 ± 2.9 bpm while DO remained constant at 4.2 ± 0.5 mg L⁻¹ (Fig. 1E). Bradycardia did not occur in field deployments, but heartbeat rates declined 50% during severe hypoxia (including short term anoxia) at Seatuck (Fig. 1G).

3.3. Cardiac activity and animal respiration

3.3.1 Response to temperature

Heartbeat and respiration rates of *A. irradians* increased linearly with a short-term temperature ramp (Figs. 4 and 5). Cardiac activity and respiration rate reached a maximum of 60.3 ± 7.3 bpm and 0.046 ± 0.015 mg L⁻¹ minute⁻¹ gram⁻¹ at 26 – 28°C and minimum of 25.9 ± 3.8 bpm and 0.020 ± 0.01 mg L⁻¹ minute⁻¹ gram⁻¹ at 18°C (Fig. 5). Cardiac responses to temperature were not affected by *in-situ* pre-conditioning. Compared to animals pre-conditioned at Nicoll Bay, mean respiration rates from scallops pre-conditioned at Fire Island were higher and had more individual variability (Fig. 5). Scallops from Nicoll Bay exhibited similar heartbeat rates in the laboratory when exposed to a range of temperatures comparable to field conditions (Figs. 1, 3, and 5). Fire Island scallops yielded higher heartbeat rates in the lab than *in-situ* under comparable field temperatures (Figs. 3 and 5).

3.3.2 DO dependence

Oxygen consumption in closed respiration chambers induced a 3.7 ± 0.9 mg L⁻¹ hour⁻¹ decline from full oxygen saturation to severe hypoxia. It took 2 – 3 hours for *A. irradians* to consume all available oxygen in sealed chambers until anoxic conditions (Figs. 6, 7, and 8). Heartbeat rates peaked to 24.6 – 32.7 (29.7 ± 2.9) bpm when DO fell below moderate hypoxia before rates declined to a minimum heartbeat rate of 14.4 – 24.9 (19.4 ± 4.2) bpm as DO approached anoxia (below 1.0 mg L⁻¹) (Fig. 6). Scallops respiration rate at $0.034 - 0.028$ mg L⁻¹ minute⁻¹ gram⁻¹ after the initial 1 mg L⁻¹ decline of DO declined 81.56 ± 5.26 % to an average minimum respiration rate of 0.005 mg L⁻¹ minute⁻¹ gram⁻¹ under anoxia (Fig. 6). Heartbeat rates proceeded to bradycardia and ascardia under sustained anoxic conditions (Figs. 7 and 8). *A. irradians* pre-conditioned at Nicoll Bay and Fire Island had

significantly greater heartbeat efficiency at $6 - 5 \text{ mg L}^{-1}$ than when DO concentrations fell between $2 - 1 \text{ mg L}^{-1}$ ($p = 0.0492$, Fire Island only) and below 1 mg L^{-1} ($p = 0.0186$ and $p = 0.007$, respectively) (Fig. 6). Respiration rate, cardiac activity, and heartbeat efficiency were independent of site pre-conditioning.

3.4. Cardiac activity of acclimated scallops in response to oxygen decline and anoxia

Cardiac responses of *A. irradians* in laboratory incubations resembled cardiac responses measured *in-situ* during diel-cycling hypoxia at Nicoll Bay and Seatuck (Figs 1B, 1C, 7, and 8); heartbeat rates peaked during DO decline but decreased as DO concentration declined further (Figs. 7 and 8). However, in laboratory challenges some response patterns were evident that were not observed in field such as bradycardia (abnormal decrease in heartbeat rate) and evidence of ascardia (complete absence of cardiac activity) which occurred during extended exposure to DO concentrations below 2.0 mg L^{-1} (Figs. 7 and 8).

Scallops acclimated to 17.5°C in lethal anoxic stress challenges reached maximum heartbeat rates of $25 - 34 \text{ bpm}$ ($30.5 \pm 3.1 \text{ bpm}$) when DO declined to hypoxic conditions with DO concentrations of $1.3 - 3.2 \text{ mg L}^{-1}$ ($2.23 \pm 0.66 \text{ mg L}^{-1}$) (Fig. 7). Even though heartbeats were consistently measureable during full oxygen saturation and the initial DO decline, clean signals were discontinuous during prolonged periods of anoxia (Fig. 7). Detectable cardiac responses under these conditions reached bradycardia with minimal activity of less than 5 bpm , leading to mortality (Fig. 7). Since heartbeat sensors recorded consistent signals at full oxygen saturated conditions, it is plausible that the absence of heartbeat signals during anoxia was due to ascardia. *A. irradians* reached mortality after $23 - 32$ (27.9 ± 3.5) hours of exposure to anoxia; Nicoll Bay and Fire Island scallops endured 28 ± 3.2 and 27.8 ± 4.5 hours of anoxia exposure before mortality occurred (Fig. 7).

Comparable to the responses during field deployments and lethal anoxia exposure experiments (Figs. 1B, 1C, and 7), all *A. irradians* used for sub-lethal anoxic challenges elevated heartbeat rates when DO initially declined and decreased activity to bradycardia as DO depleted to severe hypoxia and anoxia (Fig. 8). Nicoll Bay scallops yielded heartbeat rates throughout the 12 hours of anoxic exposure without an instance of ascardia; two scallops exhibited bradycardia with rates less than 5 bpm and one individual maintained relatively high activity of 20 bpm . Scallops pre-conditioned at Fire Island exhibited both bradycardia and ascardia throughout the non-lethal anoxic period (Fig. 8B). All *A. irradians* survived the 12 – 14 hour period of anoxic conditions. Scallops exhibited both a gradual

elevation (Fig. 8A) and slight upswing (Fig. 8B) of heartbeat rates upon reestablishment of normoxia conditions.

3.5. Cardiac activity of acclimated scallops in response to severe hypoxia

Nicoll Bay, Moneybogue, Quantuck, Fire Island, and Sag Harbor exhibited differential exposure to hypoxic stress (Table 1 and Fig. 2), but preconditioning did not have an effect on the cardiac activity during 24-hours of severe hypoxia (Fig. 9). All scallops survived 24 hours of exposure to severe hypoxia. Cardiac responses were unaffected by site acclimation. *A. irradians* exhibited a mean heartbeat rate of 30 bpm under normoxia and 40 bpm under severe hypoxia; a 10 bpm elevation persisted throughout oxygen depleted conditions (Fig. 9). *A. irradians* pre-conditioned to Fire Island and Sag Harbor exhibited an immediate decline of cardiac activity during the final hour of the experiment when oxygen in severe hypoxic mesocosms was returned to normoxia (Fig. 10). Scallops from Moneybogue and Quantuck did not alter cardiac activity and individuals from Nicoll Bay exhibited an upswing of heartbeat rate upon return to normoxic conditions (Fig. 10).

3.6. Effects of diel-cycling hypoxia on scallop growth

A. irradians shell growth from Sag Harbor was significantly different than shell growth from Fire Island, Bellport, and Penniman Creek ($p < 0.05$, $p < 0.001$, and $p < 0.05$, respectively). Scallops deployed at Bellport grew an average length of 5.81 ± 3.15 mm after 50 days *in-situ* with the greatest mean shell growth of 116 ± 63 $\mu\text{m day}^{-1}$ whereas scallops deployed at Sag Harbor on average grew 1.95 ± 1.52 mm with the least shell growth of 39 ± 30 $\mu\text{m day}^{-1}$ (Table 1). Mean shell growth was strongly associated with all hypoxia descriptors addressed in this study (Figure 11, Appx. 1 and 2). Strongest regressions were with percent time and count of hypoxic periods below 3.0 mg L^{-1} ($r^2 = 0.8484$; $r^2 = 0.8682$) and mean duration and concentration of hypoxic periods below 4.8 mg L^{-1} ($r^2 = 0.83$; $r^2 = 0.8547$) (Appx. 1 and 2). Condition index demonstrated a weak positive relationship with hypoxic descriptors (Fig. 11), mean chlorophyll, and mean salinity ($r^2 = 0.2581$; $r^2 = 0.2862$).

4. DISCUSSION

4.1. Cardiac activity during diel-cycling hypoxia

This study is the first attempt to measure continuous *in-situ* non-invasive cardiac activity of a marine invertebrate under diel-cycling hypoxia. Daily DO oscillations had a strong effect on the heartbeat rate of *A. irradians*. Daily heartbeat rate oscillations with a dynamic daily range of 20 bpm appear to be stimulated by diel-cycling hypoxia since cardiac activity was relatively stable under well-oxygenated conditions (Figs. 1 and 3). It is evident that exposure to realistic short-term hypoxic periods has ramifications to the growth and development of *A. irradians* (Gobler et al. 2017). Interpretation of post-*in-situ* experiments and related findings support *in-situ* heartbeat measurements as a metabolic indicator of sub-lethal stress.

An *in-situ* cardiac response to diel-cycling hypoxia provides a new perspective on the onset of oxyconformity. Conformers (oxyconformity) and regulators (oxyregulatory) 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 (Herried 1980; Grieshaber et al. 1994; Pörtner 2010). During the decline of ambient DO, regulatory oxygen consumption rates independent of ambient conditions reach a transitional breakpoint at a DO 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 below P_c is insufficient for regulation of aerobic functions (Pörtner et al. 1985; Van den Thillart et al. 1992; Pörtner and Grieshaber 1993). It is also suggested that DO depletion below P_c should be defined as “severe” hypoxia. (Grieshaber et al. 1994). The linear relationship of *A. irradians* heartbeat and respiration rates with temperature (Fig. 4 and 5) reflects dependence of cardiac activity, ventilation rate, and oxygen uptake at optimal thermal windows until a breakpoint at T_{cII} (Fredrich and Pörtner 2000) or ABT (Prusina et al. 2014; Chapperon et al. 2016; Tagliarolo and McQuaid 2016; Xing et al. 2016). *A. irradians* were not exposed to their ABT in this study (32.2°C; Xing et al 2016), but the drop in cardiac activity after T_{cII} and ABT found in previous studies resembles the decline in oxygen consumption rates after P_c ; this suggests that breakpoints in cardiac activity during DO decline should translate to insufficient aerobic function, a transition to anaerobic metabolism, and decreased respiration rate during diel-cycling hypoxia (DeFur and Magnum 1979; Frederich and Pörtner 2000; Pörtner 2010).

A prominent feature found in field data was the offset of DO peaks and troughs before heartbeat rates (Fig. 1). At first glance, this appears to demonstrate a staggered response reflective of a low metabolic or respiratory sensitivity to changes in oxygen. However, this phenomenon can be put into

perspective with a simple conceptual model with implications on the regulation of aerobic functions and transition to oxyconformity during diel-cycling hypoxia. *In-situ* DO and all *A. irradians* heartbeat rate data from Nicoll Bay and Seatuck show ubiquitous sequential occurrences of daily heartbeat rate and DO peaks and troughs that can be designated into four phases. Cardiac response to diel-cycling DO (CRD_{DO}) includes these four phases in a model to characterize cardiac responses of *A. irradians* to diel-cycling hypoxia (Fig. 12).

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 hours (Fig. 13). Elevated cardiac activity during phase 1 can be assumed to be an oxyregulatory response to maintain oxygen uptake and aerobic metabolism as DO becomes less available (Bayne 1971; Brand and Roberts 1973; Grieshaber et al. 1994). *A. irradians* reached a maximum heartbeat rate when DO declined to 5 mg L^{-1} (Fig. 13); this suggests 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 anaerobic transitions, 5 mg L^{-1} is the P_c during diel-cycling hypoxia at which a breakpoint in heartbeat rate symbolizes a switch to oxyconformity and onset or elevation 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. 12). This period had a mean duration of 4 – 4.5 hours over which cardiac activity declined by 10 bpm regardless of different temporal, thermal, and environmental conditions at Nicoll Bay and Seatuck (Fig. 13). 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 onset or elevated allocation to anaerobic pathways when DO falls below the P_c (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. *A. irradians* decreased heartbeat rates an additional 10 bpm during both Nicoll Bay and Seatuck deployments (Fig. 13). *A. irradians* were not initially responsive to oxygen incline *in-situ* at a rate of $1 \text{ mg L}^{-1} \text{ hour}^{-1}$. In the laboratory, *A. irradians* immediately responded after anoxia with a return to basal cardiac activity upon reintroduction to full oxygen saturation (Fig. 8). Prompt cardiac recovery was also shown by the great scallop after exposure to anoxia (Brand and Roberts 1973). However, investigation of cardiac responses in this study did not

simulated a more realistic (slower) DO increase relative to diel-cycling hypoxia measured *in-situ* (Fig. 13). Phase 3 in our field data lasted 5 – 6 hours. Combined with phase two, scallops at Nicoll Bay and Seatuck exhibited a 20 bpm range between their mean maximum and mean minimum cardiac activity during diel-cycling hypoxia (Fig. 13). DO at minimum heartbeat rates was 5 – 7 mg L⁻¹ (Fig. 13); this may represent a favorable DO threshold that stimulates a return to optimal aerobic performance. Interestingly, DO concentrations were nearly identical at minimum heartbeat rate (aerobic recovery threshold) and maximum heartbeat rate (P_c and onset of anaerobiosis). Phase 3 ends at minimum heartbeat rate when DO incline stimulates recovery of aerobic functions and elevation of heartbeat rates in Phase 4 (Figs. 12 and 13).

Phase 4 of CRD_{DO} is the “recovery” period and is the only interval when heartbeat rate and DO increase simultaneously (Fig. 12). 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 hours. 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. 13).

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* also show a strong dependence on stress duration as indicated by sustained tachycardia during a long-term moderately hypoxic event (Fig. 1E) and a 24-hour severe hypoxia simulation (Fig. 9). CRD_{DO} addresses diel-cycling DO as the prominent explanatory factor responsible for changes in heartbeat rates but cannot be applied to cardiac responses over extended stress durations.

Findings during an unexpected long-term hypoxic event (Fig. 1E) shed light on stress duration as an important component of the P_c . *A. irradians*’ aerobic capacity under persistent moderate hypoxia appeared to be dependent on time of exposure and independent of ambient DO concentration. 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 rely greatly upon aerobic pathways. *A. irradians* maintained elevated heartbeat rates indicative of an attempt to sustain oxygen uptake for over 30 hours in moderately hypoxic conditions before heartbeat rates declined. Continuous *in-situ* tachycardia demonstrates sensitivity to both magnitude and duration of DO depleted conditions in regulation of aerobic functions (Fig. 1E). Stress duration must be integrated with P_c to acknowledge use

of internal oxygen reserves and the diverse aerobic capacity over extended hypoxic periods (Pörtner et al. 1985; Van den Thillart et al. 1992).

4.2. Multi-stress environments and effects on scallop performance

Stress from exposure to DO depleted conditions is not optimal for shell growth nor condition of adult *A. irradians* although hypoxia coincided with greater food availability (Fig. 11). Strong associations between mean chlorophyll and hypoxia descriptors (Fig. 11) identified two opposing types of coastal systems in this study: 1) high food and diel-cycling hypoxia 2) low food and minimal diel-cycling hypoxia. High nutrient concentrations and food supply in eutrophic systems can increase tolerance of some marine invertebrates to hypoxic and high $p\text{CO}_2$ conditions (Thomsen et al. 2013; Ramajo et al. 2014), but this is not persistently the case (Cheung et al. 2008; Hettinger et al. 2013). Food supply did not seem to increase physiological tolerance to adverse conditions for *A. irradians*.

Undisputed coupled dynamics of hypoxia with acidification (Melzner et al. 2013; Wallace et al. 2014; Baumann et al. 2015; Gobler and Baumann 2016) pose decreased calcification rates for shellfish exposed to systems frequented by periods of severe hypoxia. *A. irradians* deployed at sites with greater percentage time, frequency, duration, and severity of hypoxic conditions exhibited a decrease in mean shell growth relative to growth among sites with infrequent hypoxia (Fig. 11). Wallace et al. (2014) investigated the seasonal co-occurrence of DO with pH among locations nearby to the sites addressed in this study; their results suggest strong linear associations between DO and pH at three coastal systems (r^2 0.70 – 0.94). Based upon Wallace et al. (2014), thresholds such as 4.8 and 2.0 mg L⁻¹ translate to a pH of 7.5 and 7.3, respectively. Hypoxia and acidification in isolation impede shell growth, development, and survival of *A. irradians* and other bivalves (Gazaeu 2007; Cheung et al. 2008; Talmage and Gobler 2009; White et al. 2013); deleterious effects can be intensified when both hypoxia and acidification co-occur in naturalistic diel-cycling simulations (Pörtner 2010; Goyer et al. 2014; Wallace et al. 2014; Gobler et al. 2017). While most studies that investigate this phenomenon had been focused on the development of bivalve early life stages, this study demonstrated a considerable hypoxia-induced effect on shell growth also in adult *A. irradians* after only 50 days in the field.

Condition of *A. irradians* did not differ among deployment sites; biomass accretion appears to be influenced by a cost versus benefit trade-off between hypoxia and food supply (Fig. 11). Scallops within systems characterized by low food supply and minimal diel-cycling hypoxia may not allocate energy to

respiratory or cardiac stress responses, but low food availability may reduce biomass accretion to rates equivalent to individuals within stressful systems and high food supply. Interplay of hypoxia-induced stress effects in nutrient rich eutrophic systems can consume energy reserves of marine bivalves although food supply is ample (Huhn et al. 2016). Cardiac oscillations and respiratory inquiry proposed in CRD_{DO} show potential for a significant energy sink in maintenance of vital functions likely responsible for identical biomass accretion rates among sites with differed food supply.

Metabolic transitions, as interpreted from simultaneous cardiac and respiratory measurements obtained in this study, suggest a new perspective on the definition of hypoxia thresholds. Cardiac activity proved to be a good proxy for respiration at different temperatures, but responses to oxygen conditions were complex. When visually overlaid, heartbeat and respiration rates under oxygen decline (Fig. 6) resemble the conformer and regulator responses that define thresholds of “moderate” and “severe” hypoxia in the scheme by Grieshaber et al. (1994). DO at maximum *in-situ* cardiac activity (5 mg L⁻¹) and the threshold for growth and protection of aquatic life, DO below 4.8 mg L⁻¹ (US EPA, 2000), closely align in agreement to the establishment of “moderate” hypoxia as the start of oxyconformity by a “poor” conformer (Herreid 1980; Grieshaber et al. 1994). As DO continues to decline, the threshold that signifies “severe” hypoxia is most apparent when a regulator transitions to oxyconformity (Grieshaber et al. 1994). *A. irradians* heartbeat rates under oxygen decline responded as a regulator until concentrations depleted below 2 mg L⁻¹ (Fig. 6). This is also in agreement to the “severe” hypoxia threshold in coastal systems native to *A. irradians* (US EPA, 2000). Further experimental effort is needed to confirm the sensitivity of cardiac activity to thresholds under DO oscillations comparable to *in-situ* rates.

A. irradians under severe hypoxic conditions decreased oxygen consumption rates to a minimum that was a fourth of basal respiration rates under full oxygen saturation (Fig. 6). Aguirre-Velarde et al. (2016) found that Peruvian scallop *Argopecten purpuratus* exhibits the same proportional difference in respiratory activity above and below its P_c . A prompt transition to oxyconformity during DO decline demonstrated *A. irradians* as a “poor” conformer (Herreid 1980). The great scallop *Pecten maximus* also exhibits oxyconformity under the same ambient DO conditions (Brand and Roberts 1973; Artigaud et al. 2014). Compiled findings suggest a common adaptive response to hypoxic conditions by scallops. Respiratory activity supplemented with additional physiological factors of aerobic and anaerobic processes can expand upon associations with oxygen consumption (Pörtner and Grieshaber 1993).

Contrary to oxygen consumption rate, cardiac responses show activity of a “good” regulator during ambient oxygen decline (Herreid 1980). *A. irradians*’ heartbeat rates in laboratory experiments were regulated during the early onset of oxyconformity defined by respiration rates (Fig. 6). Unlike the linearity between heartbeat and respiration rates during acute temperature stress (Fig. 4), heartbeat rates become decoupled from respiratory responses during DO decline (Fig. 6). While respiration rates decrease consistently with ambient DO decline, cardiac activity elevated to a maximum before heartbeat rates declined at a much lower ambient DO concentration (Figs. 7 and 8). Brand and Roberts (1973) found that *P. maximus* exhibits a cardiac upswing and decline under the same ambient oxygen conditions and proposed this as an attempt to maintain aerobic functions by increasing oxygen uptake until conditions are unfavorable. *A. irradians* regulated oxygen uptake until DO declined to below 3.0 mg L⁻¹. Relative to heartbeat efficiency during the decline of DO, rates decreased significantly when oxygen was severely depleted below 2.0 mg L⁻¹ (Fig. 6). The decoupling of heartbeat and respiration rates, highlights cardiac activity as a potential indicator of ambient DO conditions that stimulate ineffective aerobic function and define the onset of anaerobic stress at P_c.

4.3. Acclimation to diel-cycling hypoxia and effects on resilience to hypoxia

Pre-conditioning did not affect cardiac responses under exposure to anoxia and severe hypoxia. *A. irradians* either reduced heartbeat rates to bradycardia or ceased cardiac activity (acardia) during anoxia. Bradycardia is a common response of marine invertebrates to hypoxia and it is presumed to exemplify a high capacity for anaerobic metabolism (Bayne 1971; Taylor et al. 1973; Taylor and Brand 1975; Nicholson 2002). It is also proposed that abnormally low heartbeat rates are indicative of indefinite capability to tolerate low oxygen concentrations (DeFur and Magnum 1979). However, bradycardia was a precursor to mortality in long-term anoxic challenges (Fig. 7). Regardless of acclimation to dissimilar site conditions at Fire Island and Nicoll Bay (3.1.1), all *A. irradians* survived and recovered cardiac activity after short periods (12 – 14 hours) of anoxia (Fig. 8). Mortality after extended durations (>24 hours) demonstrated physiological limitations in stress tolerance and maintenance of vital functions depended on stress duration (Fig. 7). Only one scallop from Fire Island showed acardia throughout 12 hours of anoxia in sub-lethal challenges, but other individuals revealed short periods of acardia before cardiac recovery upon reestablishment of normoxic conditions (Fig. 8B). If assumed that bradycardia is a more resilient physiological response than a complete absence of cardiac activity, heartbeat measurements may reveal a spectrum of susceptibility to low oxygen

conditions. Similar to the cardiac recovery of the great scallop *P. maximus* (Brand and Roberts 1973), *A. irradians* immediately restored basal cardiac activity after hours in a severely low metabolic state or acardia (Fig. 8). Survival, time of mortality, and cardiac recovery under anoxia were not strengthened nor dampened by site-specific environmental conditioning.

Peaks in cardiac activity during oxygen decline to anoxia (Figs. 7 and 8) and sustained tachycardia throughout a moderate hypoxia event in Nicoll Bay (Fig. 1E) suggested that elevated heartbeat rates persist when ambient DO is maintained at or near P_c . *A. irradians* in Exp 2 showed a physiological consistency of slight tachycardia 10 bpm above basal rates (at 25 °C) when exposed to constant severe hypoxia between 1.5 – 2.0 mg L⁻¹ (Fig. 9). Our data supports elevated aerobic activity during severe hypoxia as an attempt to regulate metabolism that is unaltered by acclimation and likely an adaptive response for *A. irradians*.

Similar to the immediate increase of heartbeat rate after a period of anoxia (Fig. 8), slight tachycardia during severe hypoxia should readily decline to basal rates when normoxia is reestablished. Scallops acclimated to infrequent hypoxic periods at Fire Island and Sag Harbor promptly returned to basal heartbeat rates after severe hypoxia, but individuals acclimated to severe diel-cycling hypoxia at Moneybogue, Quantuck, and Nicoll Bay lacked this immediate response (Fig. 10). Diel-cycling hypoxia prolongs exertion of aerobic stress responses in adult *A. irradians* even when conditions become favorable. The fact that *A. irradians* pre-conditioned to pronounced diel-cycling hypoxia were less responsive to the reestablishment of normoxia indicates a potentially negative impact of hypoxia that extends beyond actual exposure to low DO conditions.

4.4 Conclusions and Future Directions

Application of heartbeat rate sensors in the field and laboratory revealed immediate cardiac responses to changes in DO concentrations. Daily alterations in cardiac activity demonstrated that bay scallops are metabolically sensitive to natural fluctuations in oxygen availability. *A. irradians* reached maximal heartbeat rates during diel-cycling hypoxia when DO fell to 5.0 mg L⁻¹; this sub lethal response occurred at oxygen concentrations higher than what is typically considered to be hypoxic. It is important to further analyze the metabolic inferences of cardiac activity especially considering that diel-cycling hypoxia decreases growth and development of *A. irradians* (Gobler et al. 2017).

Monitoring *in-situ* cardiac activity is a useful approach to study the ecophysiology of benthic invertebrates. Obtaining real-time and high resolution sub-lethal responses to changes in physicochemical factors is especially needed in coastal and estuarine systems with temporally coupled stressors. CRD_{DO} is a malleable model in its preliminary stage of development and should be modified to encompass responses to multiple stressors.

Respiration is a key perimeter used to study the performance of organisms, but it is not possible to measure respiration *in-situ*. In this thesis I have shown that cardiac activity can close this gap in our knowledge. Simultaneous heartbeat and respiration rates should be measured under realistic diel-cycling hypoxia (and $p\text{CO}_2$) simulations at various temperatures to model respiratory activity from *in-situ* heartbeat rates. Heartbeat and respiratory measurements will integrate oxygen consumption rates into CRD_{DO}. A cohesive model on the sub-lethal physiological responses to environmental stressors has application for scope for growth and dynamic energy budget models that can facilitate selection of optimal sites for restoring threatened shellfish species. Refinement of this concept will incorporate shellfish heartbeat rate biosensors as a live water quality standardization technique for monitoring coastal and estuarine systems that undergo periodic physicochemical stress events.

Studies on gene expression supplemented with biochemical analysis of organism tissues can shed further light into anoxic stress responses (David et al. 2005) and anaerobic inferences with abnormal cardiac and respiratory activity in diel-cycling hypoxic environments (Frederich and Pörtner 2000; Pörtner 2010). Heartbeat and respiratory measurements supplemented with extraction of tissues samples in temporal increments throughout an extended hypoxia event and during normoxia recovery can investigate linkages between real-time activity and biochemical production. This directly links hypoxia-induced stress effects on the dynamic energy budget of commercially and ecologically important shellfish species among coastal systems threatened by physicochemical stressors.

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Table 1. Characterization of environmental conditions (hypoxia, chlorophyll, salinity, and secchi depth) among nine sites between 17 July and 1 September 2016 to supplement the pre-acclimation deployments and measurements of shell growth and condition of *A. irradians* in Exp 2 (2.1 and 2.4). Percentage of time with hypoxia conditions (%), frequency (count of hypoxic periods = f), duration (mean duration of hypoxic periods in hours = d), and magnitude (mean concentration of hypoxic periods in mg L^{-1} = m) were calculated using five DO thresholds. Percent mortality is also included for each deployment site. EQ compiles f , d , and m to rank hypoxic stress among sites: $EQ = f \cdot d \div m$. Sites are ordered by EQ calculated at 4.8 mg L^{-1} .

Hypoxia threshold		Sag Harbor 41.000203 N -72.307287 W	Fire Island 40.626334 N -73.25923 W	Penniman Creek 40.81847 N -72.58652 W	Bellport 40.75341 N -72.93243 W	Tiana Beach 40.8285 N -72.53179 W	Nicoll Bay 40.727055 N -73.139145 W	Seattuck 40.81403 N -72.72598 W	Quantuck Bay 40.8197 N -72.62354 W	Moneybogue 40.80501 N -72.64044 W
< 1.0	%	0.17	0	0	0	0.37	3.33	2.71	2.81	8.06
	f	1	0	0	0	1	11	8	10	16
	d	1.8	-	-	-	4.2	3.4 ± 2.5	3.4 ± 2.5	3.2 ± 1.7	5.6 ± 4.7
	m	0.81	-	-	-	0.73	0.78 ± 0.4	0.55 ± 0.31	0.89 ± 0.46	0.85 ± 0.28
	EQ	2.26	-	-	-	5.71	47.81	49.75	35.39	105.52
< 2.0	%	0.27	0	0.30	0.18	1.12	7.18	7.73	6.48	14.13
	f	1	0	1	1	5	15	18	18	25
	d	3	-	3.3	2	2.5 ± 1.6	5.4 ± 3.2	4.3 ± 3.1	4.1 ± 3.2	6.3 ± 4.8
	m	1.34	-	1.79	1.52	1.68 ± 0.49	1.42 ± 0.45	1.48 ± 0.45	1.69 ± 0.53	1.69 ± 0.69
	EQ	2.24	-	1.85	1.31	7.44	56.73	52.66	43.46	93.7
< 3.0	%	0.65	0	1.56	1.47	3.75	14.31	16.95	16.41	21.66
	f	4	0	7	5	12	30	34	35	39
	d	1.8 ± 1.5	-	2.5 ± 1.2	3.3 ± 2.1	3.5 ± 2.9	5.3 ± 3.8	5 ± 3.5	5.2 ± 4	6.2 ± 4.9
	m	2.73 ± 0.8	-	2.77 ± 0.44	2.72 ± 0.53	2.58 ± 0.37	2.23 ± 0.56	2.31 ± 0.55	2.45 ± 0.60	2.26 ± 0.77
	EQ	2.62	-	6.32	6.06	16.28	71.84	74.03	75	107.14
< 3.5	%	6.19	0	5.36	3.80	6.11	19.38	23.45	20.74	28.39
	f	6	0	24	12	21	34	39	38	43
	d	1.9 ± 1.8	-	2.5 ± 1.6	3.6 ± 2.2	3.3 ± 2.6	6.4 ± 4.5	6.1 ± 3.7	6.3 ± 4.5	7.6 ± 4.9
	m	3.18 ± 0.52	-	3.22 ± 0.43	3.19 ± 0.39	3.04 ± 0.48	2.63 ± 0.64	2.64 ± 0.62	2.78 ± 0.6	2.63 ± 0.87
	EQ	3.66	-	18.63	13.39	22.52	82.61	89.52	85.71	123.8
< 4.8	%	6.36	8.81	18.60	20.05	24.25	35.10	38.60	37.37	41.87
	f	23	31	38	47	48	47	47	52	53
	d	3 ± 1.8	3.2 ± 2.3	5.6 ± 3.9	4.8 ± 3.6	5.8 ± 3.9	8.4 ± 5.6	8.3 ± 4.4	8.2 ± 5.8	9 ± 6.8
	m	4.39 ± 0.51	4.67 ± 0.16	4.18 ± 0.43	4.38 ± 0.42	4.18 ± 0.48	3.65 ± 0.87	3.49 ± 0.88	3.76 ± 0.91	3.51 ± 1.15
	EQ	15.93	21.11	51.18	51.34	66.37	107.78	111.51	113.4	136.06
Weekly field measurements										
chl a ($\mu\text{g L}^{-1}$)		15.96 ± 10.92	6.65 ± 5.46	17.39 ± 6.71	14.59 ± 3.59	8.41 ± 4.22	23.77 ± 8.09	20.83 ± 10.95	18.68 ± 8.15	20.91 ± 8.00
salinity (ppt)		26.47 ± 0.90	23.67 ± 0.80	26.65 ± 1.22	25.88 ± 0.97	25.03 ± 0.96	27.8 ± 0.85	27.32 ± 1.46	27.17 ± 1.07	27.53 ± 1.03
secchi depth (m)		1.51 ± 0.32	2.04 ± 0.45	0.64 ± 0.2	0.93 ± 0.31	0.89 ± 0.30	0.71 ± 0.17	0.76 ± 0.23	0.56 ± 0.15	0.61 ± 0.15
Mortality										
<i>A. irradians</i>		15%	25%	35%	10%	15%	40%	20%	30%	15%
Shell growth ($\mu\text{m day}^{-1}$, length)										
<i>A. irradians</i>		39 ± 30	105 ± 57	101 ± 59	116 ± 63	90 ± 55	58 ± 42	58 ± 39	73 ± 53	61 ± 41
Condition index ($\text{AFDW} / \text{Vcap}_{\text{Post}}$) $\times 100$										
<i>A. irradians</i>		2.02 ± 0.44	1.80 ± 0.40	2.35 ± 0.26	1.85 ± 0.36	2.20 ± 0.41	2.91	2.15 ± 0.61	1.92 ± 0.18	2.43 ± 0.50

Figure 1. Continuous *in-situ* data of DO, temperature, and *A. irradians* heartbeat rates recorded between 10 September and 28 September at both Fire Island (A) and Nicoll Bay (B) and between 7 August and 23 August at Seatuck (C). Boxed regions in yellow represent data enlarged in panels D – G. Hourly mean temperature, DO, and heartbeat rates from Fire Island (D), and Nicoll Bay (E and F) show all channels with viable heartbeat signals throughout the deployments. Panel E displays prevalent tachycardia at Nicoll Bay during an extended moderate hypoxia event (Table 1). Panel G shows all heartbeat data from two *A. irradians* in Seatuck (G) during an anoxic period. Grey and white regions indicate night and day, respectively.

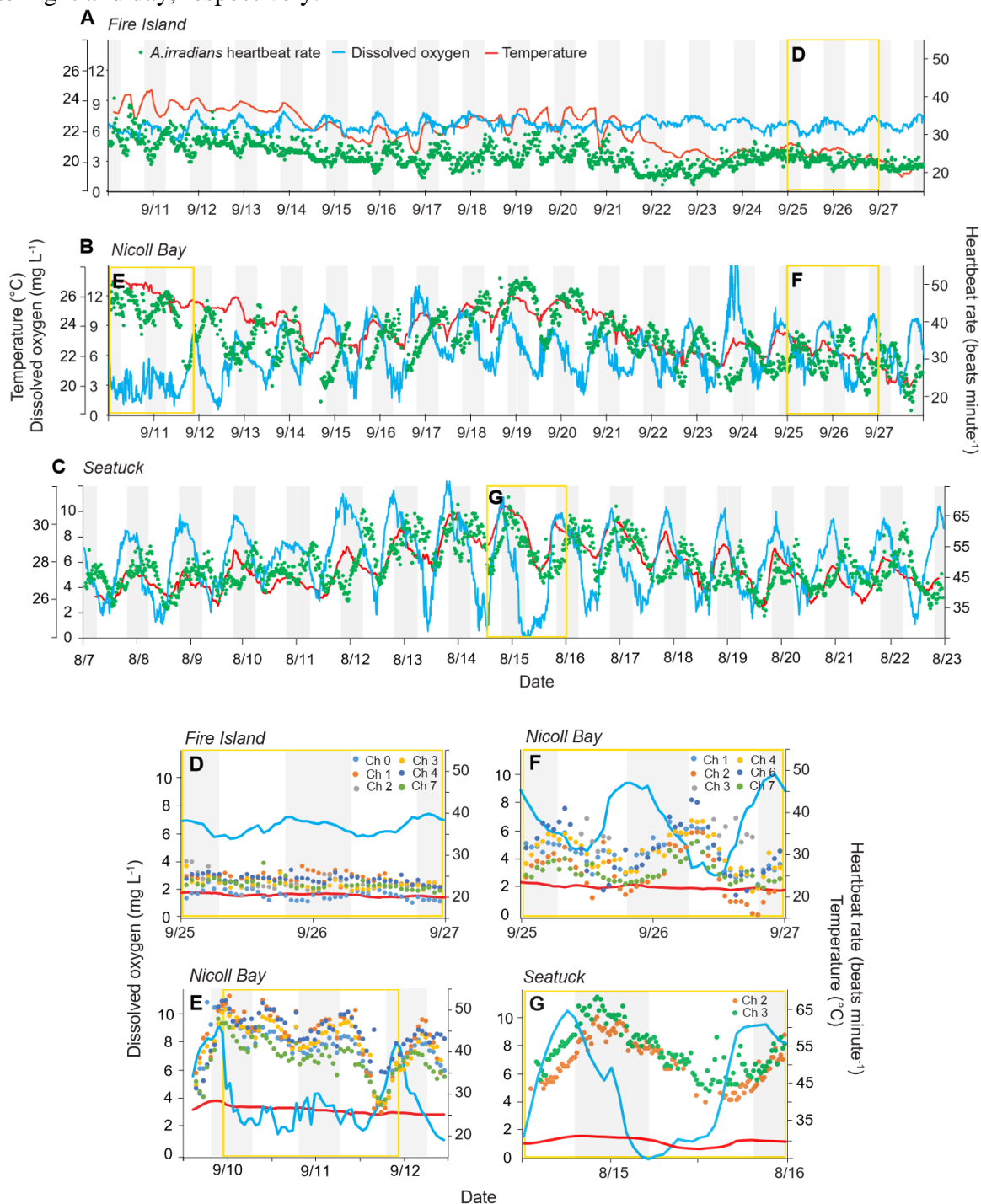


Figure 2. Cumulative distribution of percent time of hypoxia at each threshold for the nine *A. irradians* deployment sites in Exp. 2. Site are depicted in the color scheme in Table 1, but in reference to percent time of hypoxic periods.

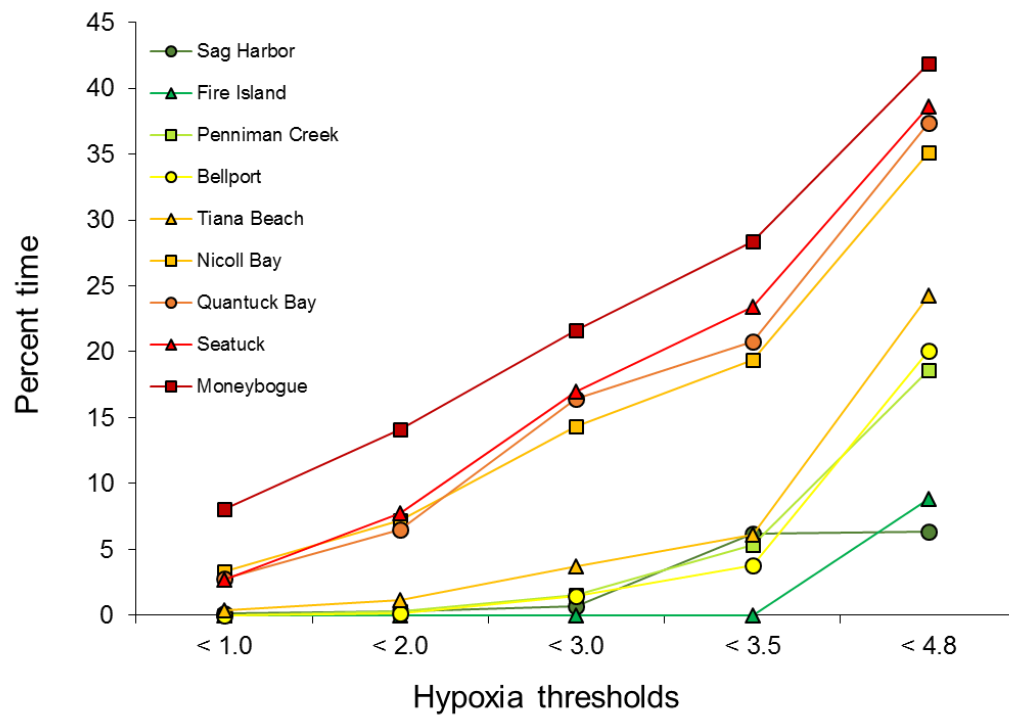


Figure 3. Box whiskers plots of continuously recorded *A. irradians* heartbeat rates (A) and DO concentrations at Nicoll Bay (B) and Fire Island (C) recorded between 10 September and 28 September. All sensors yielded > 1,000 viable heart rate measurements. Displayed are the mean (small squares), standard deviation (whiskers), 25-75% (large box), and 1-99% range (x).

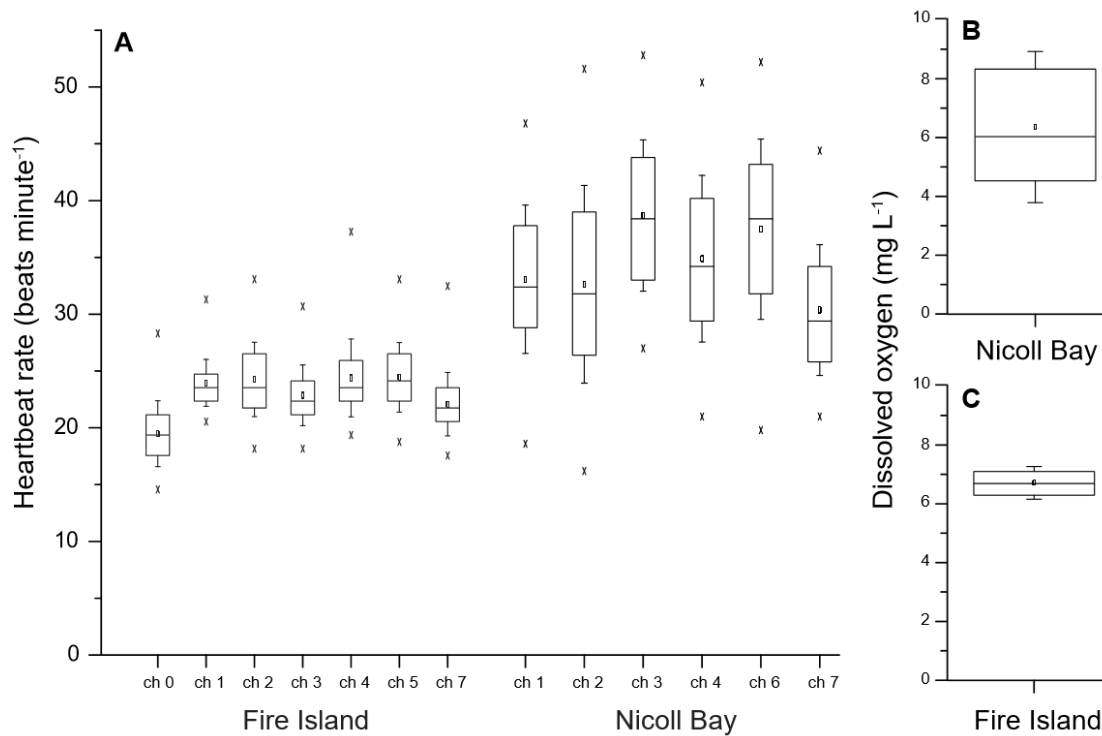


Figure 4. Simultaneous cardiac activity and respiration rate ($\text{mg L minute}^{-1} \text{ gram}^{-1}$) measurements during a short temperature ramp experiment on an *A. irradians* individual pre-conditioned to Fire Island. Mean heartbeat rate is displayed above the coinciding rate of respiration at each of the four temperatures (between grey lines).

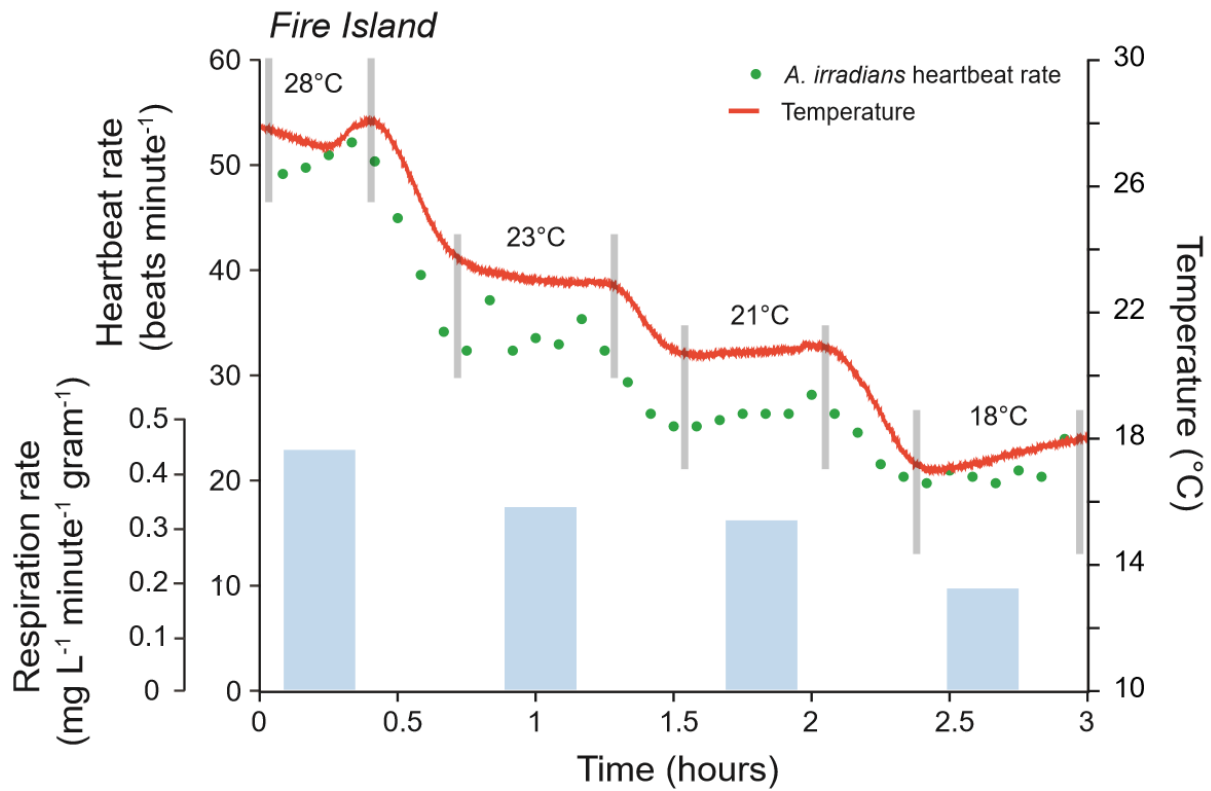


Figure 5. Respiration and cardiac responses (mean \pm standard deviation) to temperature by *A. irradians* pre-conditioned to Nicoll Bay and Fire Island (n=3).

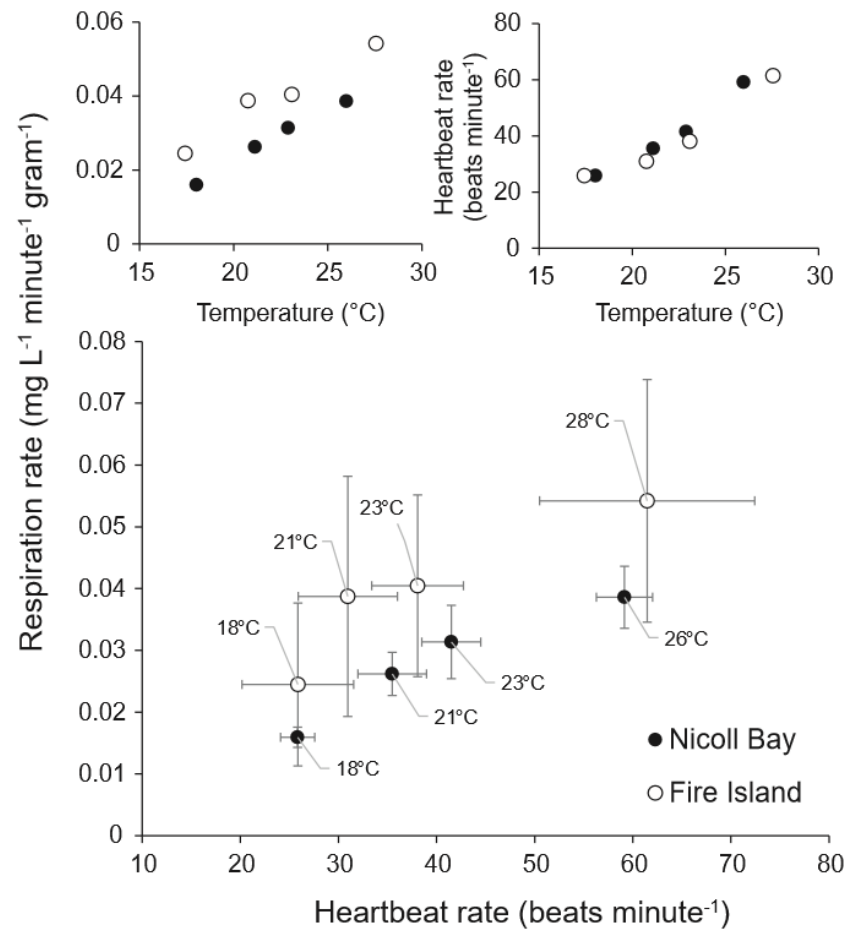


Figure 6. Effect of DO decline on heartbeat and respiration rates of pre-conditioned *A. irradians*. Shown are mean and standard deviations of respiration and heartbeat rates for 3 individuals averaged over 1.0 mg L⁻¹ increments throughout a continuous DO decline. Heart beat efficiency combines both cardiac and respiratory activity; asterisks display significant differences ($p < 0.05$) from a Tukey HSD test.

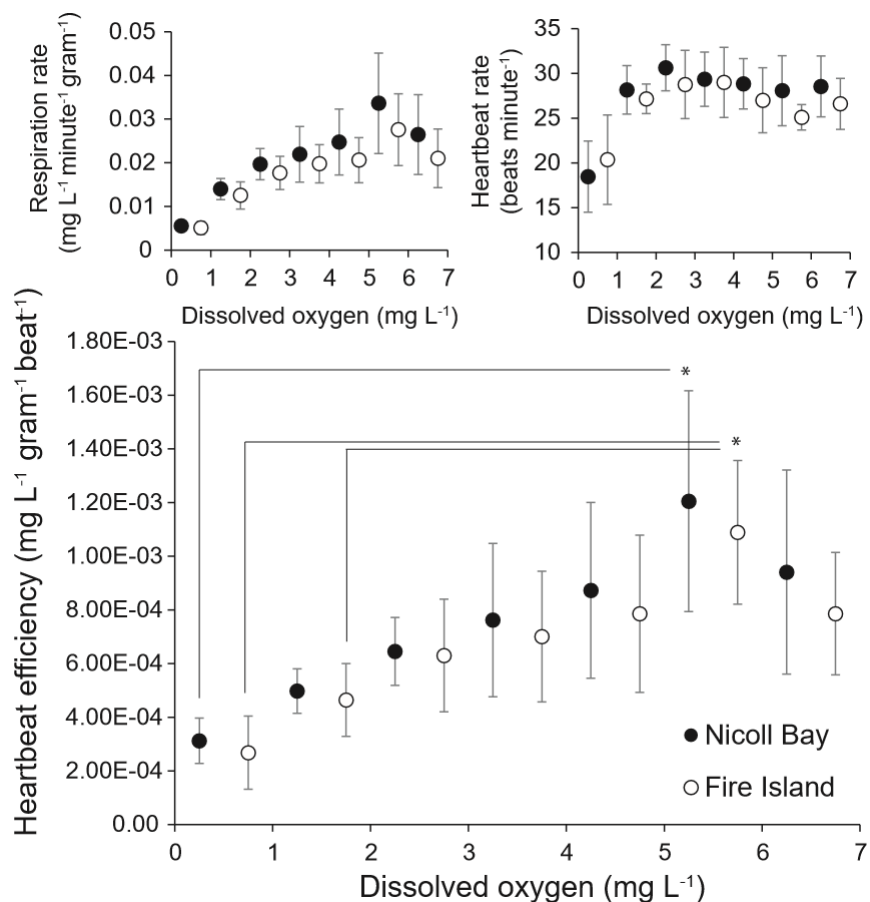


Figure 7. Cardiac response of *A. irradians* pre-conditioned to Nicoll Bay (A) and Fire Island (B) exposed to an extended period of anoxia until mortality. Heartbeat rates reveal a cardiac upswing, bradycardia, and duration of anoxia until mortality.

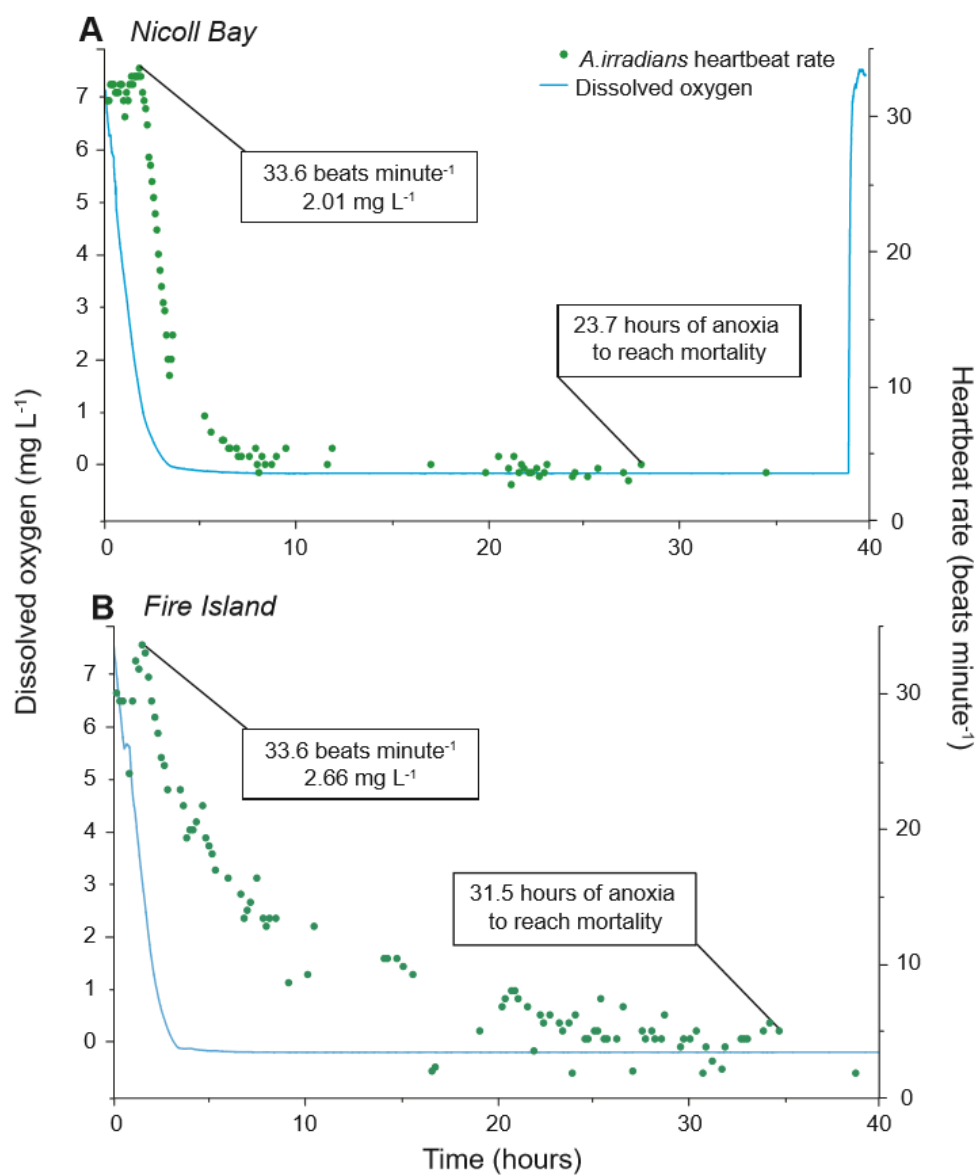


Figure 8. Cardiac responses to short-term anoxia in respiration chambers. Displayed are two *A. irradians* preconditioned to Nicoll Bay (A) and Fire Island (B) to show cardiac upswing, bradycardia, acardia (grey arrow), and recovery (green arrows) during sub-lethal anoxic challenges.

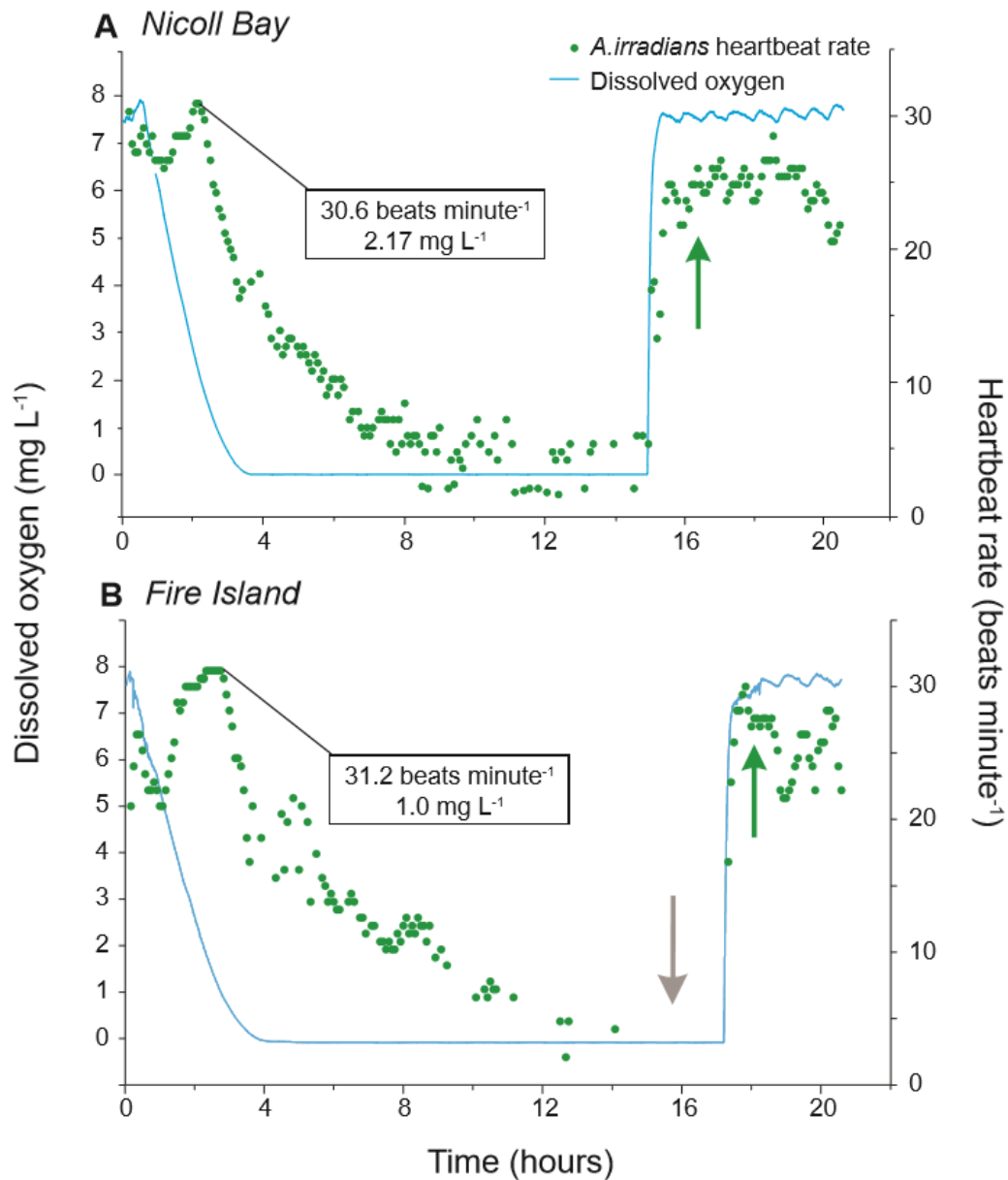


Figure 9. Heartbeat rates (mean \pm standard deviation of 4 individuals) recorded every 10 minutes in normoxia and 24-hours of severe hypoxia (1.5 - 2.0 mg L⁻¹). *A. irradians* were pre-conditioned to five sites that differed with respect to diel-cycling hypoxic descriptors. Mean heartbeat rates show basal heartbeat rate during normoxia and an extended severe hypoxia event. Grey lines border the 24-hours of severe hypoxia and show heartbeat rates during the initial DO decline (left) and reestablishment of normoxia (right).

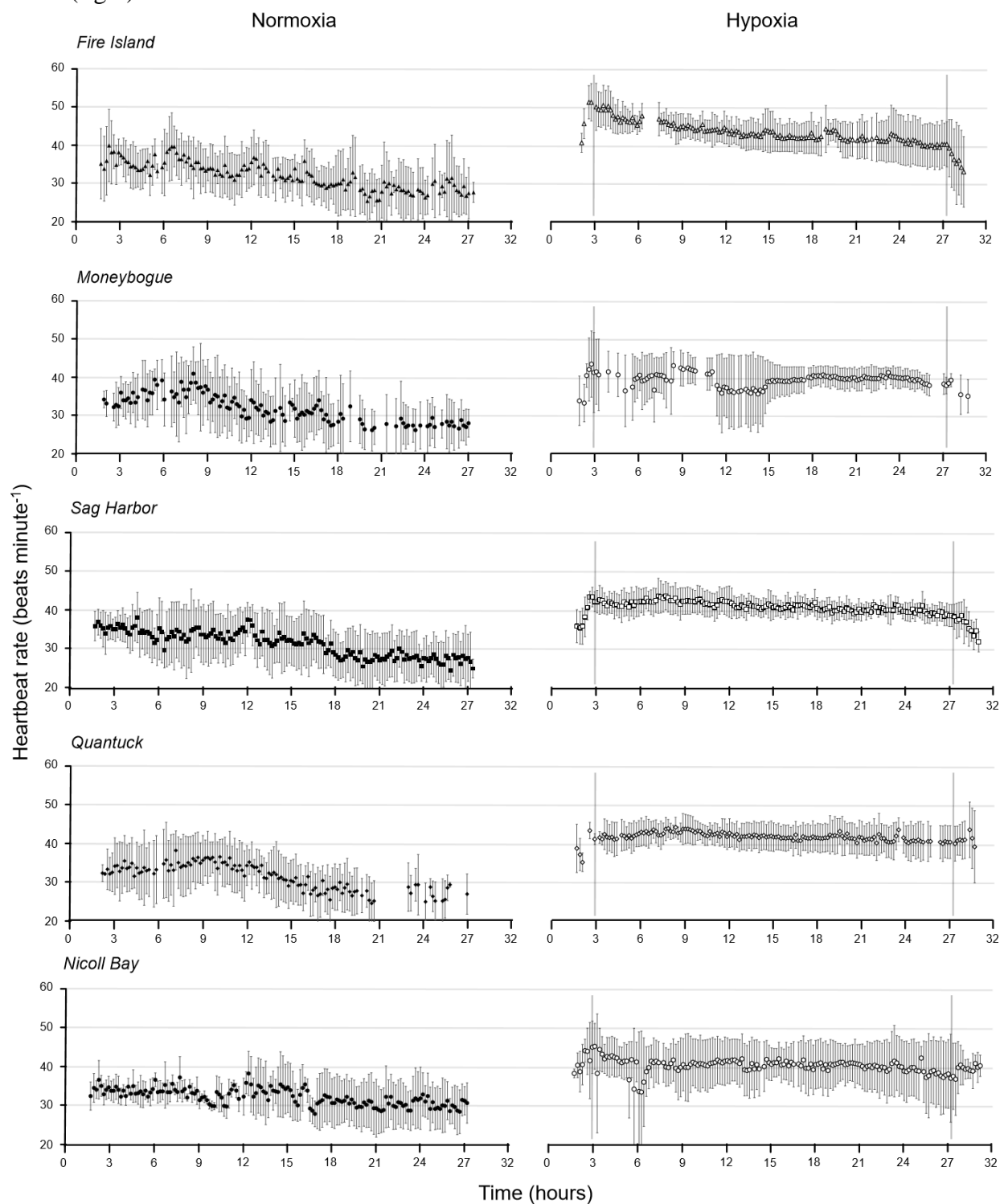


Figure 10. Cardiac recovery of *A. irradians* after 24-hours of exposure to severe hypoxia in the post-deployment mesocosm experiment. Displayed are mean heartbeat one hour prior (negative x-axis) and after reestablishment of normoxic conditions (positive x-axis). Cardiac responses at time zero occurred nearest to when DO increased above 2.0 mg L⁻¹ during reoxygenation of mesocosms. Sites are depicted in the color scheme for hypoxic descriptors in Table 1.

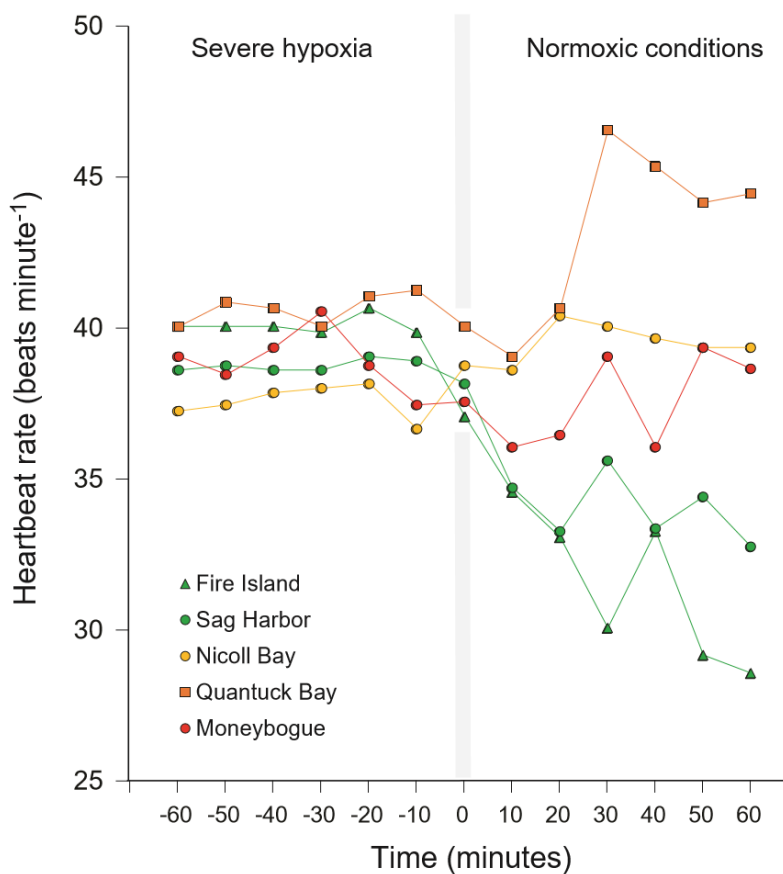


Figure 11. Mean shell growth (A) and condition index (B) of *A. irradians* after deployed for 50 days at nine sites that contrasted with respect to diel-cycling hypoxia descriptors. Food supply (C) is displayed as mean chlorophyll from weekly measurements. Each point is representative of a site, however shell growth at Sag Harbor was omitted as an outlier. All figures are regressed with percent time of hypoxic periods below 4.8 mg L⁻¹. Percent time below 3.5 mg L⁻¹ had the strongest association with mean chlorophyll between sites and is also shown in C.

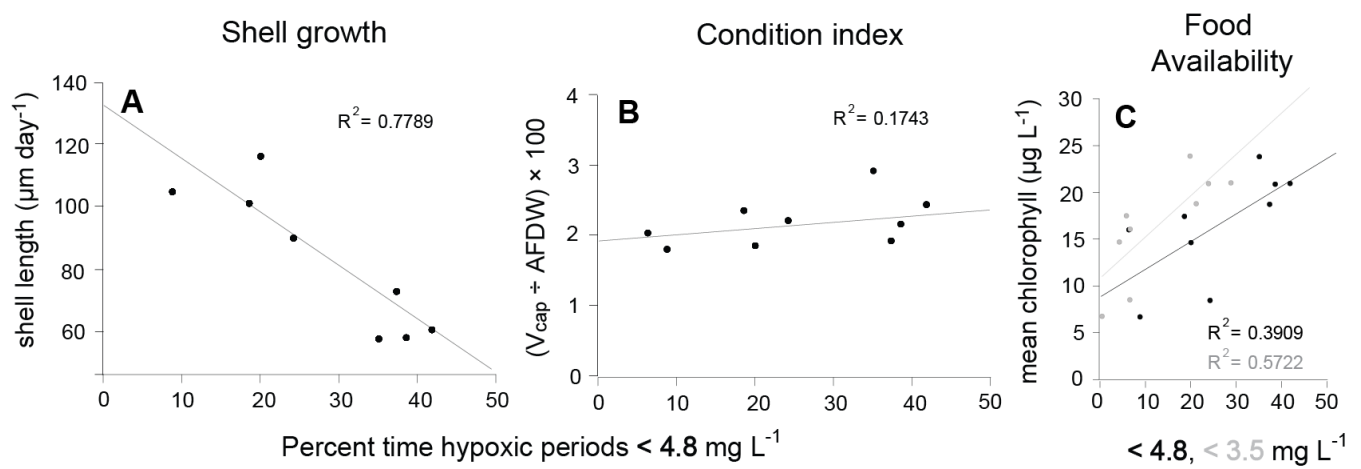


Figure 12. Conceptual model on the cardiac response to diel-cycling dissolved oxygen (CRD_{DO}). CRD_{DO} synthesizes the consistent pattern between *in-situ* DO and *A. irradians* heartbeat rate oscillations at Nicoll Bay and Seatuck to suggest respiratory and metabolic implications and transitions among diel-cycling environments. Below is an example of the model criteria overlaid on two days of hourly mean DO and *A. irradians* heartbeat rates from the Nicoll Bay deployment.

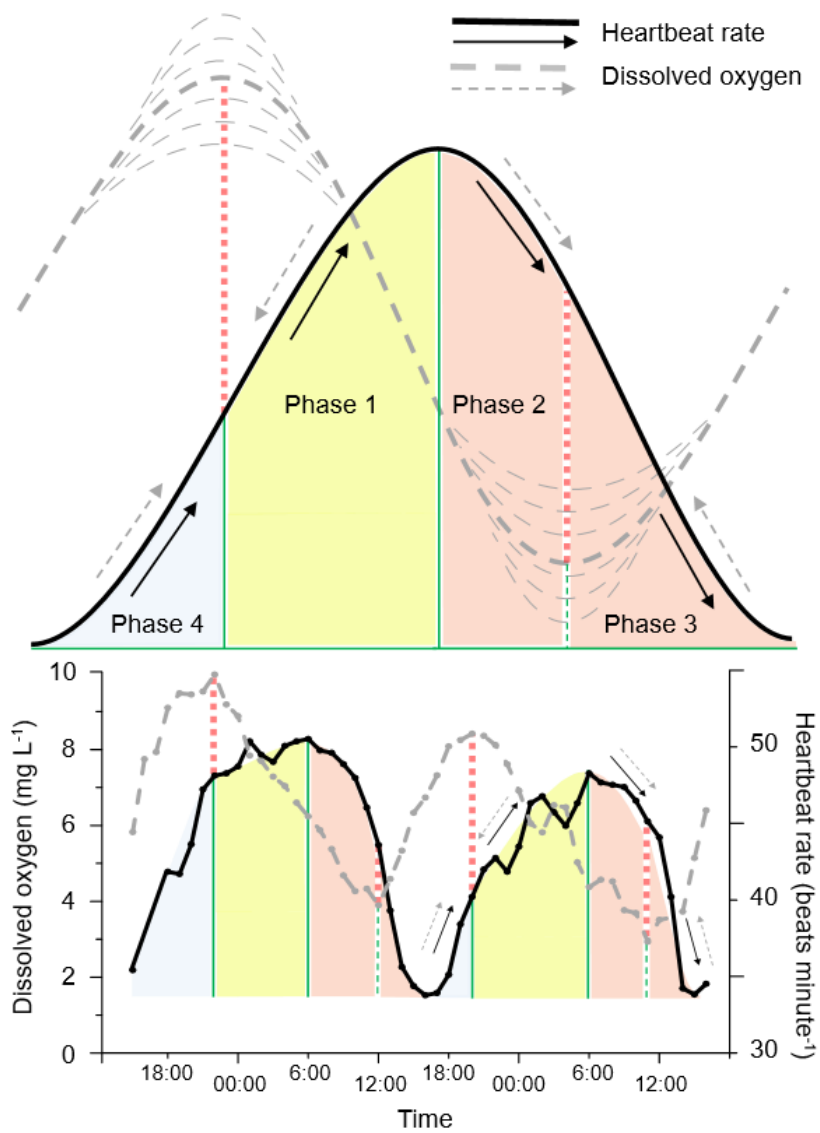
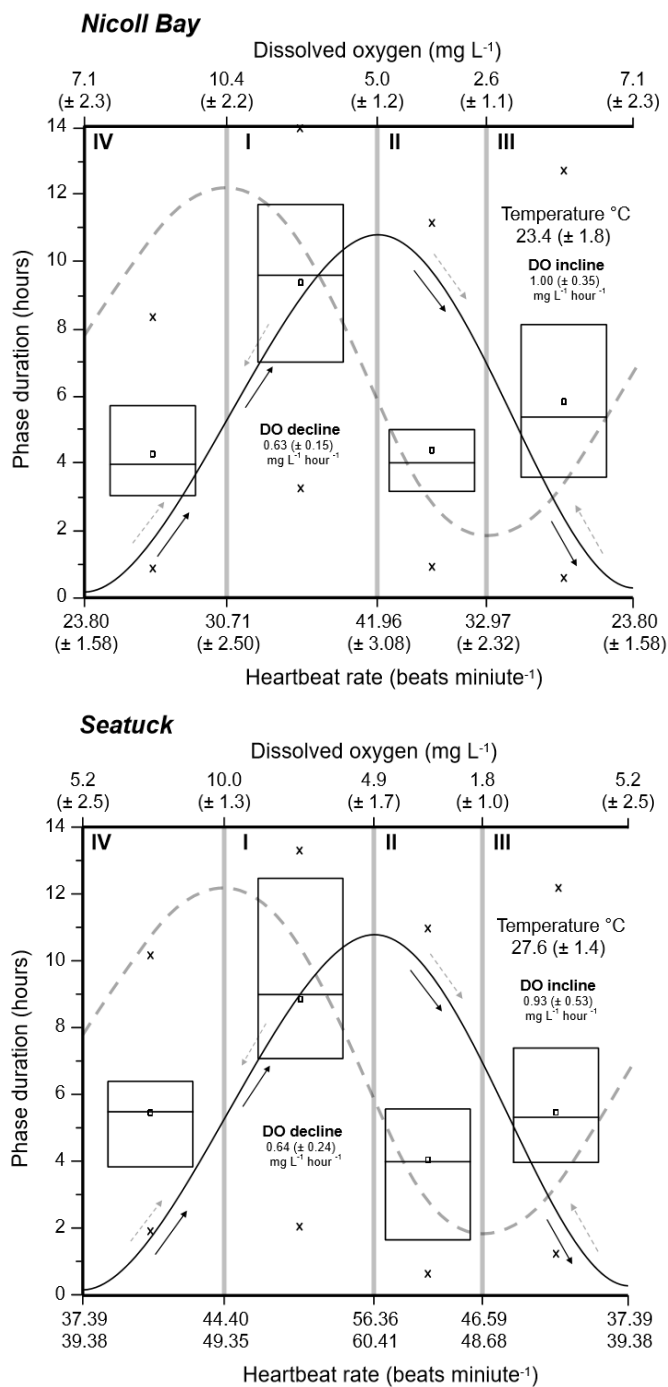
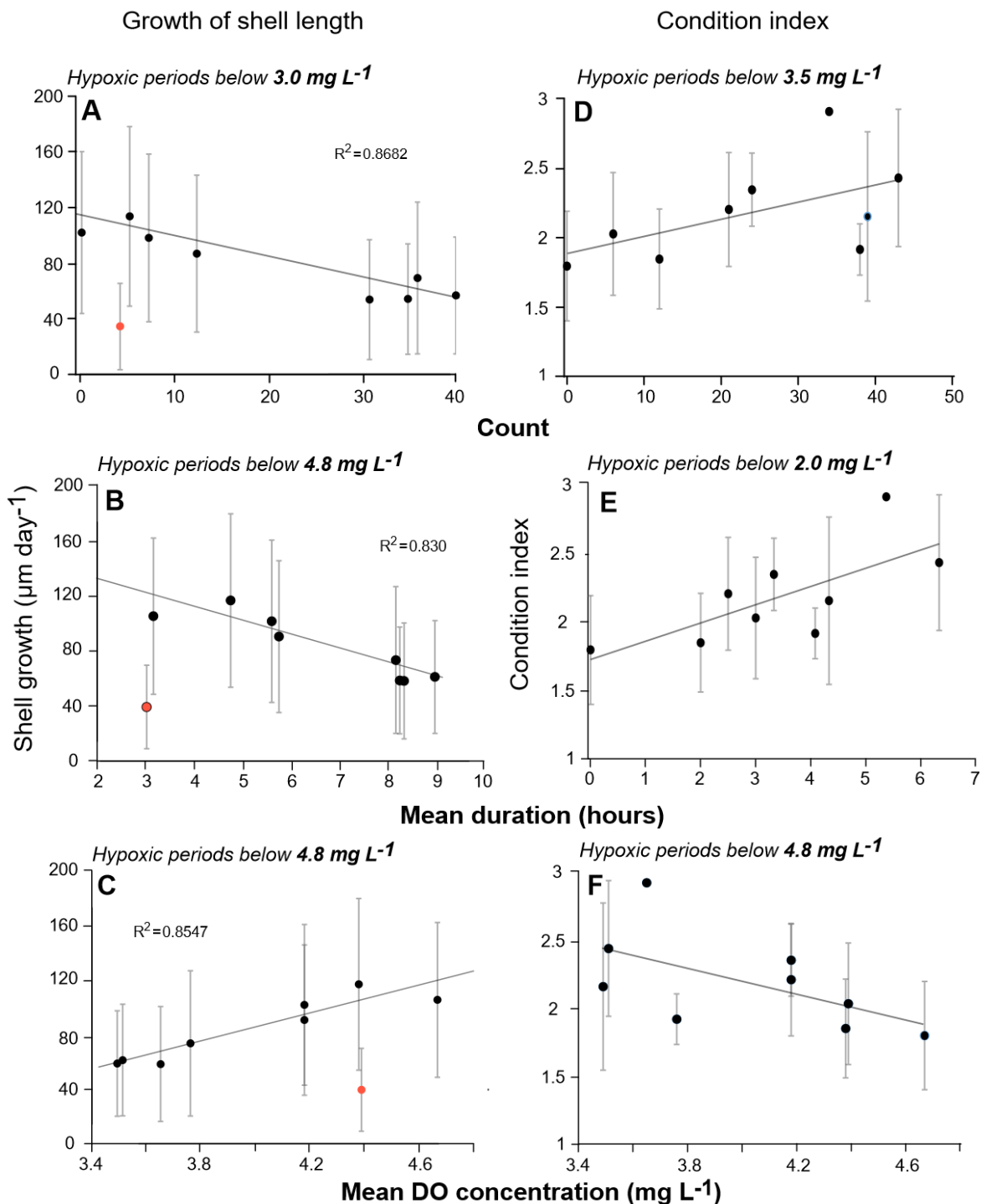


Figure 13. Heartbeat rates, DO concentrations, and phase durations (mean \pm standard deviation) that define the CRD_{DO} criteria. Phase durations are displayed with the mean (small squares), 25-75% (large box), and 1-99% range (x). Nicoll Bay displays individual variance among the mean heartbeat rates of six scallops over 17 diel-cycling oscillations. Mean heartbeat rates are listed from two scallops at Seatuck over 18 diel-cycling oscillations.

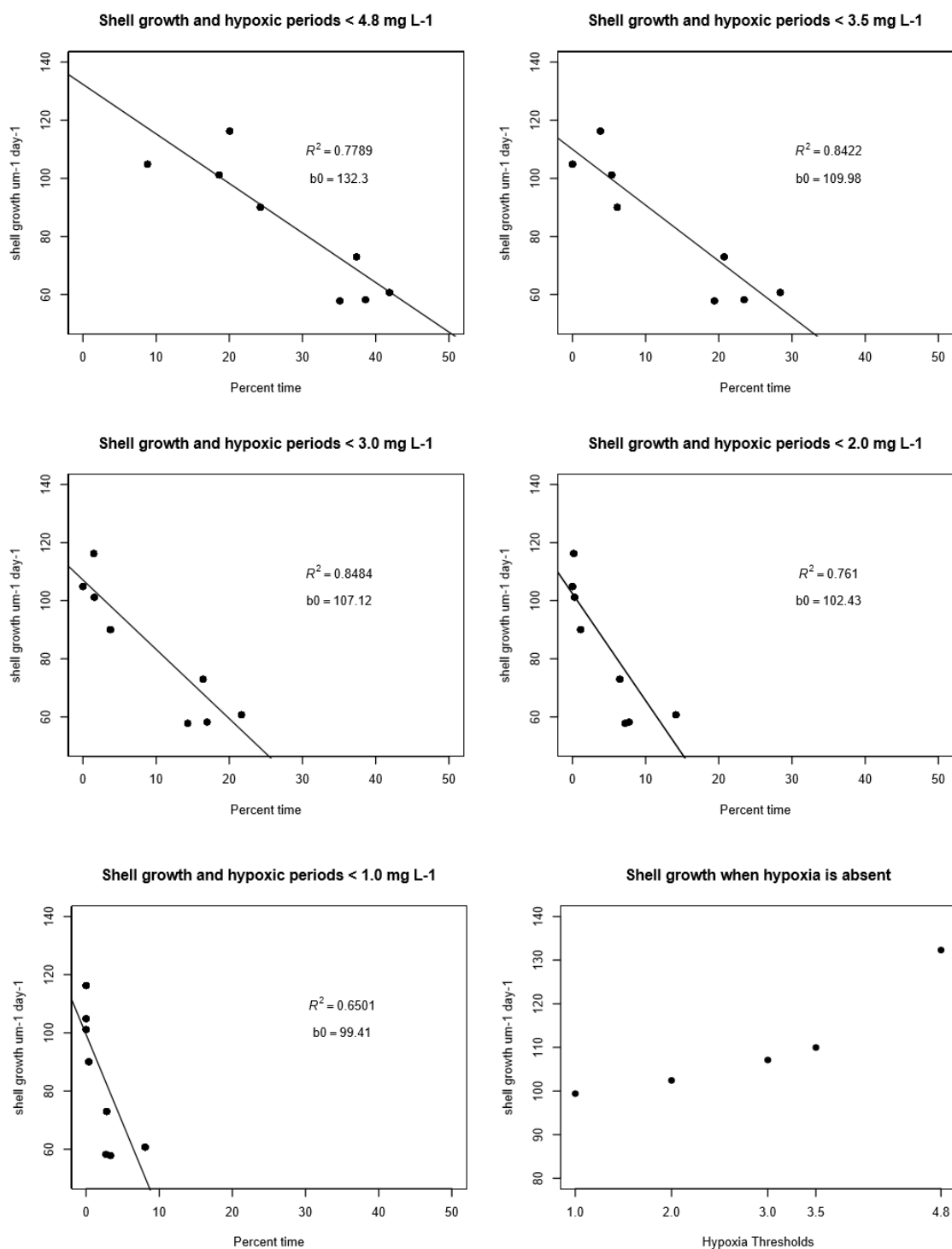


APPENDIX

1. Strongest correlations of *A. irradians* growth (A – C) and condition index (D – E) with descriptors of diel-cycling hypoxic periods – count of periods below specified threshold (A and D), mean duration (B and E), and mean DO concentration (C and F). Mean shell growth in Sag Harbor is shown as an outlier (red) and was not included in regressions of shell growth.



2. Regressions of *A. irradians* shell growth with percent hypoxia at all thresholds addressed in this study. Intercepts give a visual of shell growth rates in absence of hypoxia under each DO threshold (bottom right).



3. Regressions of *A. irradians* condition index with percent hypoxia at all thresholds addressed in this study. Intercepts give a visual of condition index in absence of hypoxia under each DO threshold (bottom right).

