The effectiveness of overwintering Eastern oysters (*Crassostrea virginica*) in cold-dry storage

Jannine D. Chamorro [a], Kaila J. Frazer [a], Josephine C. DeMerit [a], Andrew R. Villeneuve [a], Selina L. Cheng [a], Joseph A. Rankin [b], Brittany M. Jellison [a], & Easton R. White [a]

Affiliations:

[a] Department of Biological Sciences, University of New Hampshire, Durham, New Hampshire, United States

[b] Hidden Coast Shellfish LLC, Durham, New Hampshire, United States

Corresponding Author:
Jannine D. Chamorro
Jannine.Chamorro@unh.edu

Present Address:

38 Academic Way, Durham, NH, 03824

ABSTRACT

In the northernmost part of the Eastern oyster (*Crassostrea virginica*) range, oyster farmers face challenges of maintaining stock through freezing winters. To avoid exposing oysters to variable field conditions, many farmers overwinter oysters out of the water in cold-dry storage (CDS). Using two iterative experiments conducted over consecutive winters, we sought to add to the limited but growing empirical research on the effectiveness of CDS and factors contributing to oyster survival (i.e., size, storage orientation, and storage duration). Oysters were sourced from a local farm in the Great Bay Estuary (New Hampshire, United States) and overwintered in a temperature- and humidity-controlled chamber held at ~4.8 ºC and 82% humidity. In Experiment 1, we evaluated how oyster size and storage orientation influenced survival. To gain insight into oyster physiology during CDS and the transitions in and out of storage, we also monitored oyster cardiac activity over the course of the experiment. Mortality was not observed until oysters were re-immersed in seawater at the end of the experiment, despite monitoring during storage. We found high short-term survival (86.5%), with no significant impact of size or storage orientation. Oyster heart rate decreased with time spent in CDS, with a periodic heartbeat becoming undetectable five weeks into CDS in live oysters. Building on findings from the first year's study, our objective for Experiment 2 was to determine if the amount of time spent in CDS impacted long-term survival in the field. We did so by examining the survival of oysters redeployed into the field at staggered dates. Here, we found that oyster mortality increased with time held in CDS. Together, these findings advance our empirical understanding of oyster survival in CDS, offering farmers practical guidance for overwintering strategies while raising informed questions about the physiological processes that govern survival and determine optimal CDS in the Northeast.

KEYWORDS

Eastern oyster, *Crassostrea virginica*, overwintering, cold-dry storage, heart rate

1. INTRODUCTION

Eastern oysters (*Crassostrea virginica*) occupy an extensive geographic range along the eastern coast of North America. The species can be found from the Gulf of St. Lawrence, Canada (48ºN), down to the Gulf of Mexico (27ºN), and parts of Central and South America (Kennedy et al., 1996). Across this range, the aquaculture of Eastern oysters is a rapidly growing industry, contributing to local economies and providing numerous ecosystem services (Coen et al., 2007; USDA, 2023). As a result of covering such a broad latitudinal range, oysters experience diverse environmental conditions that can impact physiological performance (Lord and Whitlatch, 2014), and in turn, stock production. For example, near their southern range limit, elevated temperatures and storm-induced low salinity during summer months have led to mass mortality events (Du et al., 2021; Rybovich et al., 2016). In contrast, near their northern limit, one of the greatest challenges that farmers face is maintaining oysters during the winter months, when ice forms at the water surface and water temperatures drop to near freezing (Comeau et al., 2012; Masanja et al., 2023).

Limited but growing empirical evidence has improved our understanding of oyster physiology during the winter season in the Northeast. When water temperatures are below 5 ºC, oysters have been documented to cease filtering; this has been recognized as the lower thermal threshold for feeding (Comeau et al., 2017, 2012). During this period of inactivity, or quiescence, oysters minimize valve gaping by completely closing themselves off to the surrounding environment or remaining slightly open (Comeau et al., 2017, 2012). Oysters require energy to maintain this quiescent state, as they must contract their adductor muscle for three to five consecutive months, depending on the location. During this time, oysters rely on stored nutrients to fuel aerobic and anaerobic pathways for basic cellular functions (Bridier et al., 2026; Mayrand et al., 2017). As energy reserves are exhausted through the winter season, vulnerability to potential disturbance increases (Comeau, 2014; Comeau et al., 2017). For example, as temperatures rise in the spring and awaken oysters from their quiescent state, sedimentation in the natural environment can bury and harm oyster stock (Comeau, 2014; Comeau et al., 2017). Infestations of the mud-blister worm (*Polydora websteri*) peak in severity during winter months, adding additional stress by forcing awakening oysters to allocate more energy to shell construction in the spring (Davinack and Sheddy, 2025). Due to the uncontrolled variability of

field conditions, farmers and researchers have maintained oysters outside of water during winter (Hidu et al., 1988). Research focused on optimizing oyster survival and decreasing pest abundance can help farmers make decisions about best practices that fit their needs.

Several different overwintering practices are employed in the Northeast, each with its own costs and benefits (Stewart-Clark, 2013). The most common and simplest approach is dropping oyster bags or cages into deeper waters to avoid ice formation while maintaining them above the benthos to prevent sedimentation (Comeau et al., 2017; Grizzle et al., 2020). Alternatively, quiescent oysters can be maintained outside of the water in a practice termed cold-dry storage (CDS), which has proven to be effective for maintaining survival rates (Hidu et al., 1988; Morse, 2006; Wang and Amiro, 1977) and decreasing pest abundance (Hood et al., 2024). A CDS approach termed "pitting" involves storing oysters in underground pits (e.g., vegetable cellars), layered in burlap (Morse, 2006). Since pitting is the longest-standing CDS technique, the term is sometimes colloquially used to refer to all CDS. Oysters can also be kept in temperature and humidity-controlled chambers, such as a refrigerator between 0 and 6 ºC over the winter (Hidu et al., 1988; Wang and Amiro, 1977).

While CDS is a commonly used practice, its effectiveness and impact on oyster physiology remains understudied. Only a small number of early studies have investigated this topic in Eastern oysters (Hidu et al., 1988; Wang and Amiro, 1977). Wang and Amiro saw a ~50% mortality rate following three to five months in CDS at 2-5 ºC, where mortality rates were dependent on the specific storage treatment (Wang and Amiro, 1977). In contrast, Hidu and colleagues found higher survival rates, ~80%. Here, oyster size and storage duration, but not temperature (0-6 ºC), seemed to impact survival in CDS. Over two consecutive years, they found larger oysters had higher survival. Further, three to five months seemed to be the optimal storage time (Hidu et al., 1988). Another factor to consider is the oysters' storage orientation (Morse, 2006). A common practice in short-term storage is to maintain oysters cupped side down to preserve the oyster's liquor (i.e., a combination of saltwater and internal fluid); however, the effects of oyster orientation in long-term CDS have yet to be explored.

The goal of this study was to investigate CDS and its efficacy as an overwintering strategy for Northeastern *C. virginica*. Oysters were sourced from a local farm in the Great Bay Estuary (New Hampshire, United States (US)), where ice often forms at the surface from

December to March (Short, 1992). To examine oyster survival and physiology in CDS, two experiments were conducted across consecutive years, with the second designed to build on insights from the first. For Experiment 1, the overarching objective was to determine if storage orientation and size impacted survival during and shortly after CDS. Based on past studies and common storage practices, we hypothesized that larger oysters and those stored cupped side down would have higher survival rates. Additionally, in Experiment 1, we sought to gain insight into physiology during CDS and the transitions in and out of storage. In support of this aim, we fitted a subset of oysters with cardiac activity monitors throughout the experiment. As cardiac monitors become increasingly prevalent for long-term monitoring (Miller, 2022), their use in this study offers novel insights into physiology under CDS conditions, as well as approaches and limitations for processing the complex data generated. Building on the first study's findings, our objective for Experiment 2 was to determine if the amount of time spent in CDS impacted long-term survival in the field. As oysters can exhaust energy storage during dormancy, we hypothesized that mortality would increase with time held in CDS.

2. METHODS

2.1 Oyster Collection

All oysters, *C. virginica*, were sourced from a local farm located in the Little Bay region of the larger Great Bay Estuary (New Hampshire, US). Oyster seed was originally purchased from Rhody Oyster Seed Co. (Matunuck, Rhode Island, US).

2.2 Experiment 1

2.2.1 CDS Conditions and Survival Monitoring

Experiment 1 was conducted during the winter of 2024 to 2025. On November 26, 2024, 3,000 two-year-old oysters were transported to the University of New Hampshire and placed in a Darwin Walk-In Stability Chamber (Model: LAB-G2HD-12X12) held at 4.6 ± 0.2 ºC (mean ± standard deviation) and 81.2 ± 2.5% humidity. Upon initial transfer to CDS, oysters were held in 12 3/8" mesh bags at an approximate concentration of 1,000 oysters per bag. During week three (W3), monitoring for survival commenced. Here, eight oysters from each bag were taken as representative samples to monitor survival over the CDS period. Oysters (*N*=96, length ~ 35-65

mm) were weighed, and this initial weight measurement was used to examine the influence of size on survival. Initial weight for experimental oysters ranged from 2.4-32.5 g. To determine if storage orientation influenced survival, four oysters from each bag were stored cupped shell up (*N*=48, half of the total sample size), and the other four were stored cupped shell down (*N*=48). Oysters were stored on top of the bag they were collected from.

The survival of experimental oysters was checked approximately every two weeks from December 16, 2024, to March 17, 2025 (Figure 1A). Oysters were held in CDS for approximately 16 weeks. This timeframe was informed by the farmer's experience with dewinterizing (reversing winter protective measures) their farm and was in line with previous studies (Hidu et al., 1988; Wang and Amiro, 1977). Oysters were scored as alive if they were tightly shut, and dead if gaping or open when tapped. To confirm the survival status at the end of the storage period, oysters were removed from CDS and placed in a ~ 75 L tank of seawater at 4 ºC. Survival was then determined after 24 hours. Because physiological stress associated with quiescence can result in delayed mortality, oyster survival was further examined 11 days after being transferred to a seawater tank at 13 ºC. These data were reported descriptively and were not included in formal statistical analyses. However, they were intended to inform future targeted studies.

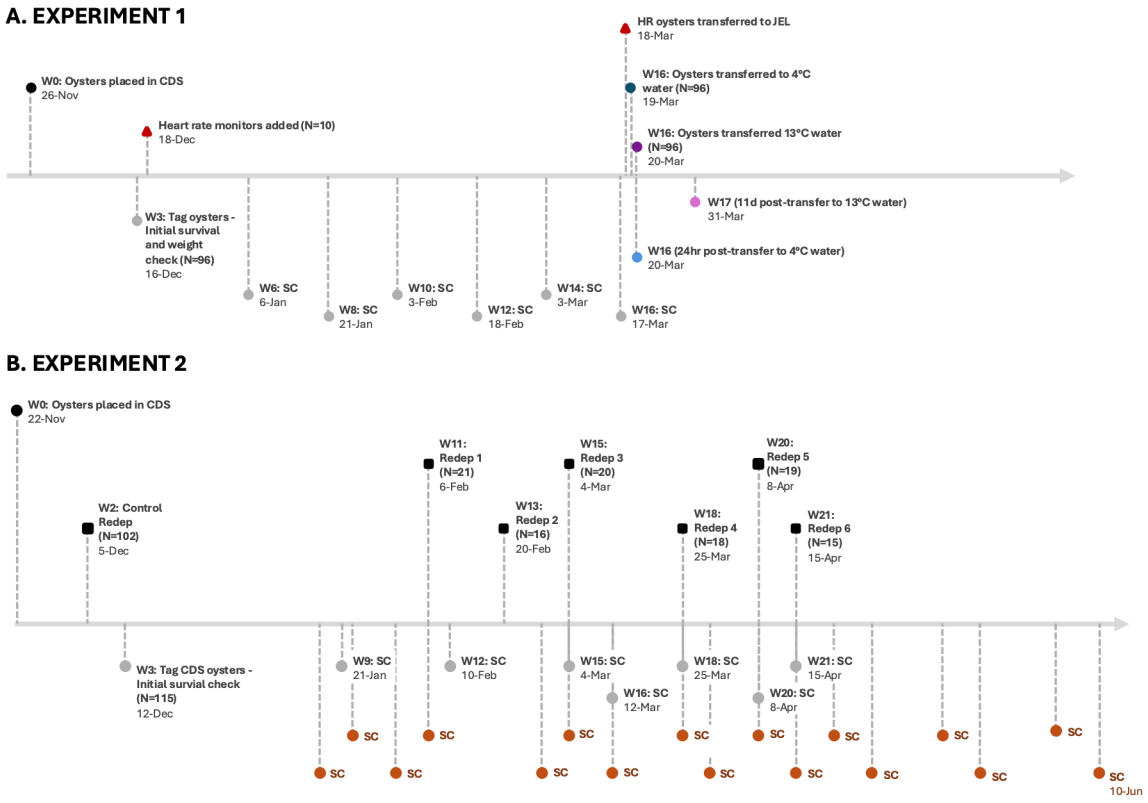


Figure 1. Experimental Timelines. (A) Experiment 1: Oysters were transferred to cold-dry storage (CDS) on November 26, 2024 (Week 0 (W0)); survival monitoring commenced on W3. Oyster survival checks (SC) were conducted approximately every two weeks, depicted by the grey circles on the timeline (e.g., W6:SC). After W16, oysters used to monitor survival were transferred to 4 °C seawater (dark blue circle) for 24 hours, then assessed for mortality (light blue circle). Following 24 hours, surviving oysters were transferred to seawater held at 13 °C (purple points). The red triangles depict when a separate subset of oysters was outfitted with cardiac monitors and transferred to the Jackson Estuarine Laboratory for spring awakening. (B) Experiment 2: Oysters were transferred to CDS on November 22, 2025; survival monitoring commenced on W3. Black squares represent each redeployment date. Grey circles represent survival checks in CDS, while orange circles represent survival checks in the field.

2.2.2 Survival Data Analysis

All analyses were conducted in R (2025.05.1+513). Oyster mortality was not observed during mid-CDS mortality checks; therefore, the effects of size (i.e., weight) and storage orientation on survival 24 hours post-transfer to 4 ºC seawater were assessed. We used generalized linear models (GLMs) (*lmer* package (Bates et al., 2015)) to determine the influence of these factors on survival. Here, survival data (alive/dead) were fit using a binomial error distribution with a logit link function. The primary model included size (continuous) and storage orientation (two factors) as fixed effects. A model containing the interaction between fixed

effects was also considered. Model residuals were examined using the *DHARMa* package (Hartig, 2024).

2.2.3 Monitoring Cardiac Activity

To further investigate oyster physiology during CDS, cardiac activity was monitored in a separate subset of individuals. On December 18, 2024, 10 oysters (mean wet mass ± standard deviation = 35.7 ± 4.8) were removed from CDS, cleaned, and weighed. Then, using a rotary tool (Dremel), a small opening in the cupped shell was made above the mantle membrane. An infrared (IR) heart frequency logger (PULSE V2, ElectricBlue, Porto, Portugal) was used to record oyster cardiac activity. This logger uses an IR detector to measure changes in the amount of light reflected from the animal's internal circulatory structures during heart contraction (Lima et al., 2025). Infrared sensors (Vishay CNY70) were attached to oyster shells with super glue so that the IR detectors were aimed into the drilled hole. Two-part aquarium epoxy was used to further secure loggers to each oyster. Cardiac activity was recorded at 20 Hz (50 ms intervals). All oysters with adhered sensors were oriented cupped shell up.

Once all sensors were attached, the oysters were placed into 13 ºC recirculating seawater to allow for short-term acclimation and ensure post-drilling survival. Following 26 hours in seawater, all oysters were placed back in CDS at 4.7 ºC on December 19, 2024. After approximately 16 weeks in CDS, on March 18, 2025, the oysters with heart monitors were transported to Jackson Estuarine Laboratory (JEL) to commence the spring awakening trial. Oysters were placed in a mesh bag inside an 11.7 L tank with a flow rate of approximately 87.6 L/hour. The tank was in a flow-through system pulling water from the Great Bay Estuary. Oyster survival, cardiac activity, and water conditions were then monitored for 14 days. The temperature and salinity during this time were 6.7 ± 0.8 ºC and 16.3 ± 1.5, respectively.

2.2.4 Cardiac Signal Processing

We investigated changes in cardiac activity during four distinct periods of the experiment: 1) in 13 ºC seawater prior to entering CDS, 2) the transition from seawater to CDS (i.e., the first 48 hours after being placed in CDS), 3) long-term CDS, and 4) spring awakening. We confirmed spring awakening from quiescence via sensor observation of increased valve gaping behavior in other CDS oysters which were different from those with heart rate sensors attached (Nagai et al., 2006). Awakening behavior is synchronous among oysters exposed to the

same conditions, so we assumed those with heart rate sensors attached also emerged from quiescence (Comeau, 2014; Comeau et al., 2012). Heart rate signals recorded over long periods (i.e., more than a few hours) tend to be highly variable among oysters and across time. This is expected, as signal detection of IR sensors can be impacted by a variety of factors, including external light sources, sensor positioning, and changes in animal behavior (Hellicar et al., 2015). Thus, the variable and long-term nature of our data required initial processing prior to estimating physiological metrics such as heart rate.

Signal processing followed the methods described in Hellicar et al. 2015 with some modifications. Here, we used an algorithm to classify raw sequences into three categories: a saturated sequence, an aperiodic sequence, or a periodic sequence (Hellicar et al., 2015). Saturated sequences were those where a significant proportion of the recorded values reached a maximum threshold, often the sensor maximum, usually caused by external factors such as light interference (Figure 2A). Aperiodic sequences were defined as having a lack of periodicity, which may have been due to the absence of a strong signal reflected off the heart surface or the absence of a heartbeat (Figure 2B). Lastly, periodic sequences were a result of the heartbeat modifying the intensity signal at regular intervals. Heart rate can be estimated from periodic sequences by converting period estimates to beats per minute (bpm) (Figure 2C) (Hellicar et al., 2015).

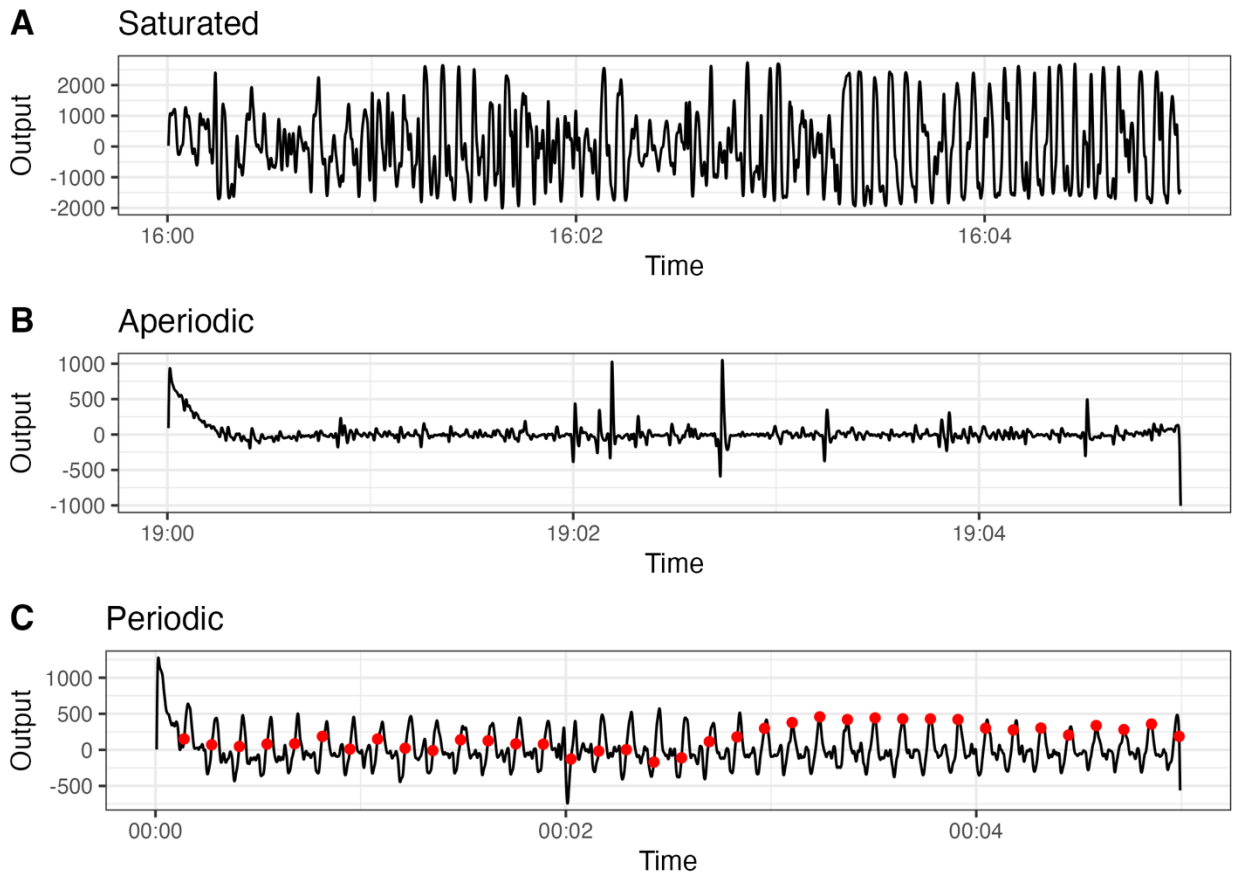


Figure 2. Classification of Cardiac Data. Examples of data sequences classified as A) saturated, B) aperiodic, and C) periodic. The black line represents the filtered data. The x-axis displays an interval of five minutes. The output on the y-axis is a unitless value representing the relative intensity of the reflected IR light. Red points on C) represent the start of each new period. Here, the estimated period length was 8.1 sec.

We subsampled the data to examine the first five minutes of each hour. Raw sequences were determined to be saturated if 5% of the recorded values reached the sensor's maximum threshold (Figure 2A). Unsaturated sequences were filtered using a bandpass filter (0.02-1 Hz) to remove high-frequency noise and low-frequency drift. The autocorrelation function (ACF) was computed using a fast Fourier transform-based approach to differentiate periodic from aperiodic sequences. The dominant periodic component was identified by searching for the maximum autocorrelation value within a biologically constrained lag range corresponding to predefined period bounds. For this study, periods between 2 and 35 seconds were considered physiologically possible. This period range equates to 1.7 – 30 beats per minute, which is observed in bivalves held under similar conditions in terms of temperature or air exposure (Bakhmet, 2017; de Carvalho et al., 2022). Sequences were classified as periodic when the

maximum autocorrelation peak exceeded a detection threshold of 0.12 and were then visually confirmed. If sequences were confirmed to be periodic, period estimates were used to estimate heart rate (Figure 2C). To characterize cardiac activity during each distinct experimental period, we described the proportion of time when a steady heartbeat was detected (i.e., visually confirmed periodic sequences) and the estimated heart rate during these times.

2.3 Experiment 2

2.3.1 CDS Conditions and Oyster Tagging

Experiment 2 was conducted during the winter of 2025 to 2026, focusing on examining the influence of storage duration on long-term survival in the field. As in the previous year, bagged oysters were placed in CDS the week of November 22, 2025 (Figure 1B); storage conditions were held at 4.9 ± 0.3 °C and 83.0 ± 1.4% humidity throughout the experiment. During W3, a subset of 115 live year-one oysters (length ~ 17- 57.3 mm) was tagged to monitor survival during CDS and after field deployment. Unlike Experiment 1, tagged oysters were stored at low density in plastic trays, without considering the storage orientation. Mortality during CDS did not differ between oysters stored at low density in plastic trays and those stored at high density in oyster bags ($p = 0.774$), validating the former as suitable representatives for survival measurements. After oysters were tagged, the next assessment of survival in CDS was conducted following W9 (Figure 1B). Oysters were assessed visually and by tapping on the exterior of the shell as in Experiment 1.

2.3.2 Redeployment and Survival of Oysters in the Field

To examine how time in CDS affected long-term survival in the field, a subset of tagged oysters (N~20) was sampled from those held in CDS and redeployed at staggered time intervals, beginning on W11 (Redep 1), with subsequent redeployments on W13 (Redep 2), W15 (Redep 3), W18 (Redep 4), W20 (Redep 5), and W21 (Redep 6). For each redeployment, oysters were first visually assessed for mortality. If dead oysters were observed, they were counted and removed from the subset. Surviving oysters were then transferred to the field. Since access to the farm was restricted due to ice conditions, oysters were deployed in a flow-through tank at JEL. Hereafter, “in the field” is in reference to JEL. Field temperature conditions can be found in Figure 3. Any oysters that died on the day of transfer were also documented and removed. The redeployment cohort for each date therefore consisted of the subset of initial ~20 oysters that

survived more than one day in the field. As a control, a separate cohort of oysters (*N*=102) was held in CDS for only two weeks after initial placement. They were then transferred to the field for the entire winter season, with survival monitored weekly (Figure 1B).

Survival of oysters in CDS and in the field was assessed at each deployment date, then weekly following redeployment of all oysters in the field. To determine the impact of time spent in CDS on long-term survival in the field, we conducted a survival analysis using the *survival* package in R (Therneau, 2026). Survival analysis models the time until a specific event, in this case death, while appropriately accounting for censored individuals, or those who did not experience the event by the last sampling date. Time was measured as days following redeployment into the field. A Kaplan-Meier survival curve was created for the control and each redeployment treatment. A between-group significance test was also conducted using a log-rank test. Pairwise differences between treatments were assessed using log-rank tests with a Benjamini-Hochberg correction for multiple comparisons.

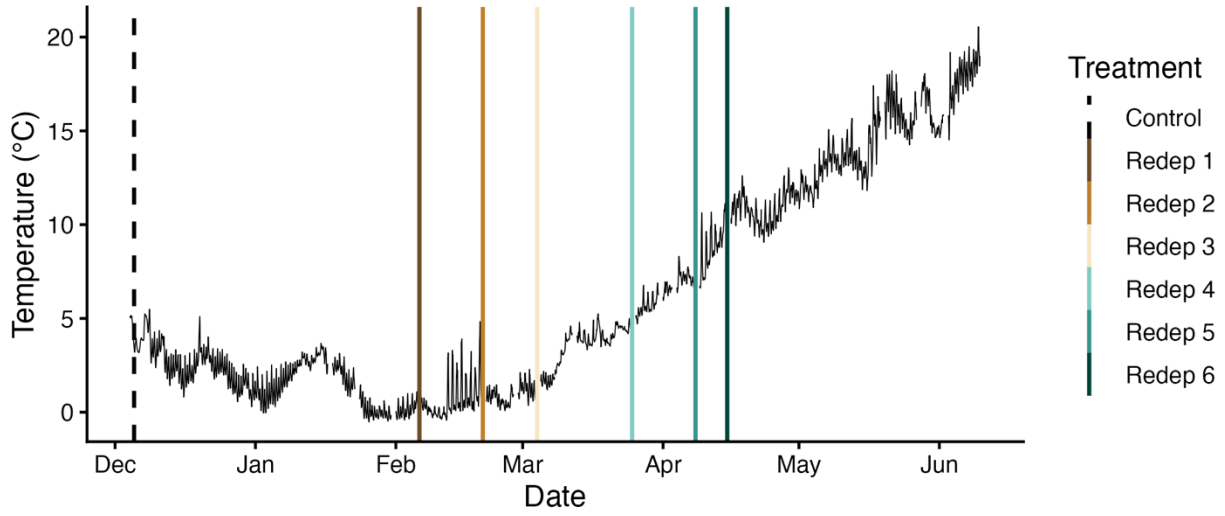


Figure 3. Field Temperature Profile. Water temperature conditions in the flow-through tank where oysters were re-deployed for Experiment 2. Dashed and solid lines indicate dates of each redeployment treatment.

2.3.3 Farm Overwintering

To compare survival of oysters overwintering in CDS with those maintained subtidally at the farm, survival was measured for farm-stored oysters once farm access was feasible (during W21 and W23). Farm sampling dates were spaced out by several weeks with the intention of capturing spring mortality that was unrelated to CDS. On each farm sampling date, four bags of year-one oysters that had been stored overwinter subtidally on the farm were haphazardly selected to

sample. On average, 77 oysters were sampled from each bag (σ = 15). Single oyster shells were recorded as 0.5 dead oysters, as dead oyster valves may be separated due to jostling or predation post-mortality. We reported descriptive statistics of these results.

3. RESULTS

3.1 Experiment 1

3.1.1 Oyster Survival

Mortality was not observed during the CDS period but was documented 24-hours after being transferred to seawater, with overall survival of 86.5% (*N*=96). Oysters stored cupped side down had a survival rate of 87.5%, while oysters stored cupped side up had a survival rate of 85.4%. A logistic regression was used to examine the effect of size and orientation on survival probability. Model selection based on the Akaike information criterion supported the exclusion of a size and orientation interaction term. While there were minor differences in survival based on size and storage orientation, their influence was not a significant predictor of survival probability (size: z = 1.09, p = 0.276; orientation: z = -0.44, p = 0.662) (Figure 4). The predicted survival probability ranged from ~77% (95% CI: 45-93%) for the smallest oysters (2.38 g) and ~97% (95% CI: 59-99%) for the largest oysters (32.48 g). The large confidence intervals at these size extremes likely reflects the small sample sizes for those values. Survival probability estimates were similar across orientation levels (down: 79-97%; up: 74-96%). While not included in formal statistical analysis, following 11 days in 13 ºC seawater, survival decreased to 77.1% of the original stock of experimental oysters.

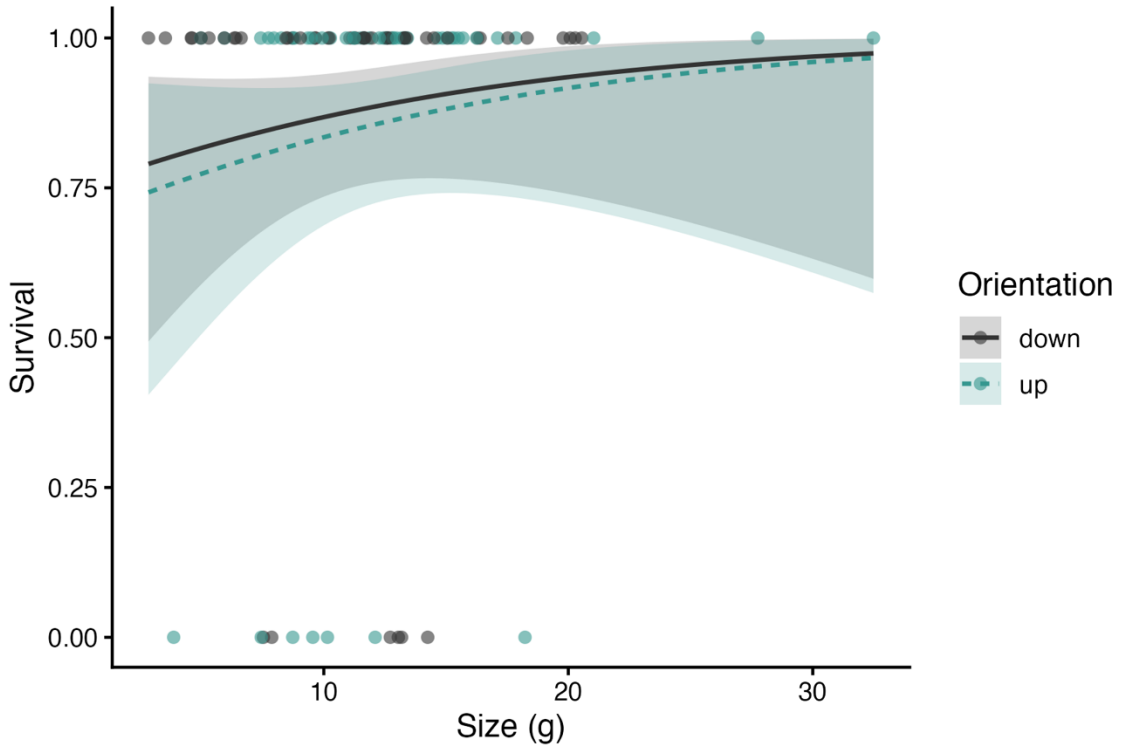


Figure 4. Cold-Dry Storage Survival (CDS). Survival of oysters held in CDS (*N*=96) following transfer to 4 ºC seawater. Size is represented on the x-axis, and the probability of survival is displayed on the y-axis, with points representing survival status (alive = 1, dead = 0). Storage orientation is represented by color and line type (black-solid: cupped side down; teal-dashed: cupped side up). The curves represent survival probabilities from a logistic regression model as a function of size and storage orientation. Shaded regions indicate 95% confidence intervals.

3.1.2 Cardiac Analysis

We examined cardiac activity during four distinct periods in our experiment: 1) in 13 ºC seawater prior to entering CDS, 2) the transition from seawater to CDS (i.e., first 48 hours after being placed in CDS), 3) long-term CDS, and 4) spring awakening. Four of the 10 oysters (Oyster ID: 2, 4, 9, 10) outfitted with HR monitors were identified as dead following transfer to seawater conditions at JEL during the spring awakening.

Seawater (13 ºC): The percentages of sequences classified as saturated, aperiodic, and periodic were 1%, 69%, and 30%, respectively. Of the periodic sequences, 50% were visually confirmed and used to estimate heart rate, and a periodic heartbeat was detected in seven oysters. Heart rates were variable across individuals and time (Supplemental Figure 1A). However, averaged over the entire acclimation period, heart rates were relatively similar, ranging from 8.2 to 10.4 bpm (Table 1).

First 48 hours in CDS (4.7 ºC): The percentages of sequences classified as saturated, aperiodic, and periodic were <1%, 72%, and 28%, respectively. Of the periodic sequences, 47% were visually confirmed and used to estimate heart rate, and a periodic heartbeat was detected in five oysters. Heart rate was generally slower during the first 48 hours in CDS than in seawater, and less variable across time (Supplemental Figure 1B). Excluding oyster 1, which only had one measurement before the signal was lost, heart rate averaged over this period ranged from 2.1 to 4.6 bpm (Table 1).

Long-term CDS (4.6 ± 0.2 ºC): There were no sequences classified as saturated. The percentages of sequences classified as aperiodic and periodic were 94% and 6%, respectively. Of the periodic sequences, 41% were visually confirmed and used to estimate heart rate, and a periodic sequence was detected in seven oysters. Estimated heart rates were relatively similar to those recorded during the first 48 hours. Heart rate averaged during this period ranged from 2.1 to 6.3 bpm (Table 1). While variability appeared to be greater, this may be an artifact of the longer dataset. All validated periodic sequences were recorded during the first five weeks of CDS, after which a periodic heartbeat became undetectable (Supplemental Figure 1C).

Spring Awakening (6.7 ± 0.8 ºC): While a significant number of sequences were classified as periodic, when visually assessed, periodicity was not evident or exceeded upper thresholds. Therefore, we could not confidently estimate oysters' heart rate during spring awakening.

Period	Oyster	Mean	SD	Max	Min	*N*	Overall Period Mean
Seawater	Oyster 1	8.2	3.2	11.9	2.4	10	
	Oyster 2	9.1	4.8	17.9	3.1	6	
	Oyster 3	9.3	5.7	13.3	5.2	2	
	Oyster 5	10.4	1.8	14.3	7.5	11	8.97
	Oyster 8	8.6	3.1	12.2	3.9	9	
	Oyster 9	8.2	1.3	9.7	7.0	5	
	Oyster 10	9.0	0.5	9.6	8.5	4	
CDS-48 hours	Oyster 1	8.2		8.2	8.2	1	
	Oyster 3	2.1	0.5	3.1	1.7	8	
	Oyster 5	4.6	0.5	5.2	3.6	34	4.06
	Oyster 9	2.2	0.2	2.5	1.9	8	
	Oyster 10	3.2	0.1	3.3	3.0	13	
CDS-Long term	Oyster 1	2.1	0.5	3.2	1.7	21	

Oyster 2	5.1	6.4	28.6	1.7	17	
Oyster 3	2.4	1.0	8.3	1.7	68	
Oyster 5	4.7	0.4	5.3	2.1	311	3.91
Oyster 8	4.1	0.5	4.5	1.7	50	
Oyster 9	6.3	7.5	25.0	1.7	91	
Oyster 10	2.7	0.8	3.3	1.7	8	

Table 1. Summary statistics of oyster heart rates (bpm) at three distinct periods in the experiment: 1) in seawater prior to entering CDS, 2) the transition from seawater to CDS (i.e., first 48 hours after being placed in CDS), and 3) long-term CDS. *N* represents the number of visually confirmed sequences classified as periodic used to calculate period estimates.

3.2 Experiment 2

3.2.1 Survival of Staggered Redeployment Treatments

Mortality observed during CDS was relatively low, with oyster death predominantly detected the day of redeployment or later in the field. Only one oyster in Redep 2 and Redep 6 was observed to be dead in CDS. Day-of-redeployment mortality in Redep 1, 2, and 3, was not observed. Day-of-redeployment mortality for Redep 4, 5, and 6 was 33%, 5%, and 29%, respectively. While in the field, there were a few oysters (N=4) that lost their tag; these oysters were removed from each cohort prior to survival analysis. Accounting for the removal of oysters that died in CDS, lost tags, or died the day of redeployment, sample sizes for each redeployment cohort used in the survival analysis were N=22 (Redep 1), N=16 (Redep 2), N=20 (Redep 3), N=12 (Redep 4), N=19 (Redep 5), and N=10 (Redep 6). Similarly, several tagged oysters in the control treatment lost tags, and these were removed from the cohort, bringing the sample for the survival analysis to N=93.

Survival differed significantly among control and redeployment treatments (log-rank test: $\chi^2 = 106$, $df = 6$, $p < 0.001$), with survival probability generally decreasing with time spent in CDS. Pairwise log-rank comparisons with a Benjamini-Hochberg correction showed the survival of the control treatment was significantly higher than all redeployment treatments ($p < 0.001$). Amongst redeployment treatments, survival in the Redep 5 and Redep 6 treatments was significantly lower than in Redep 1 (adjusted $p = 0.008$ and 0.026, respectively) and Redep 2 (adjusted $p = 0.015$ and 0.027). No other pairwise differences were detected. Median survival, the time at which 50% mortality was detected, varied widely amongst treatments. It was not reached in the control or Redep 1 treatment, and was 61 days in Redep 2, 23.5 days in Redep 3, and 35 days in Redep 4. Median survival was the shortest in Redep 5 and Redep 6 (14 days and 10.5 days, respectively) (Figure 5). The accumulated mortality (i.e., mortality in CDS + day-of-

redeployment + in the field) was 18.2% (Control), 40.2% (Redep 1), 52.9% (Redep 2), 70% (Redep 3), 66.7% (Redep 4), 84.2% (Redep 5), 86.7% (Redep 6) for each treatment.

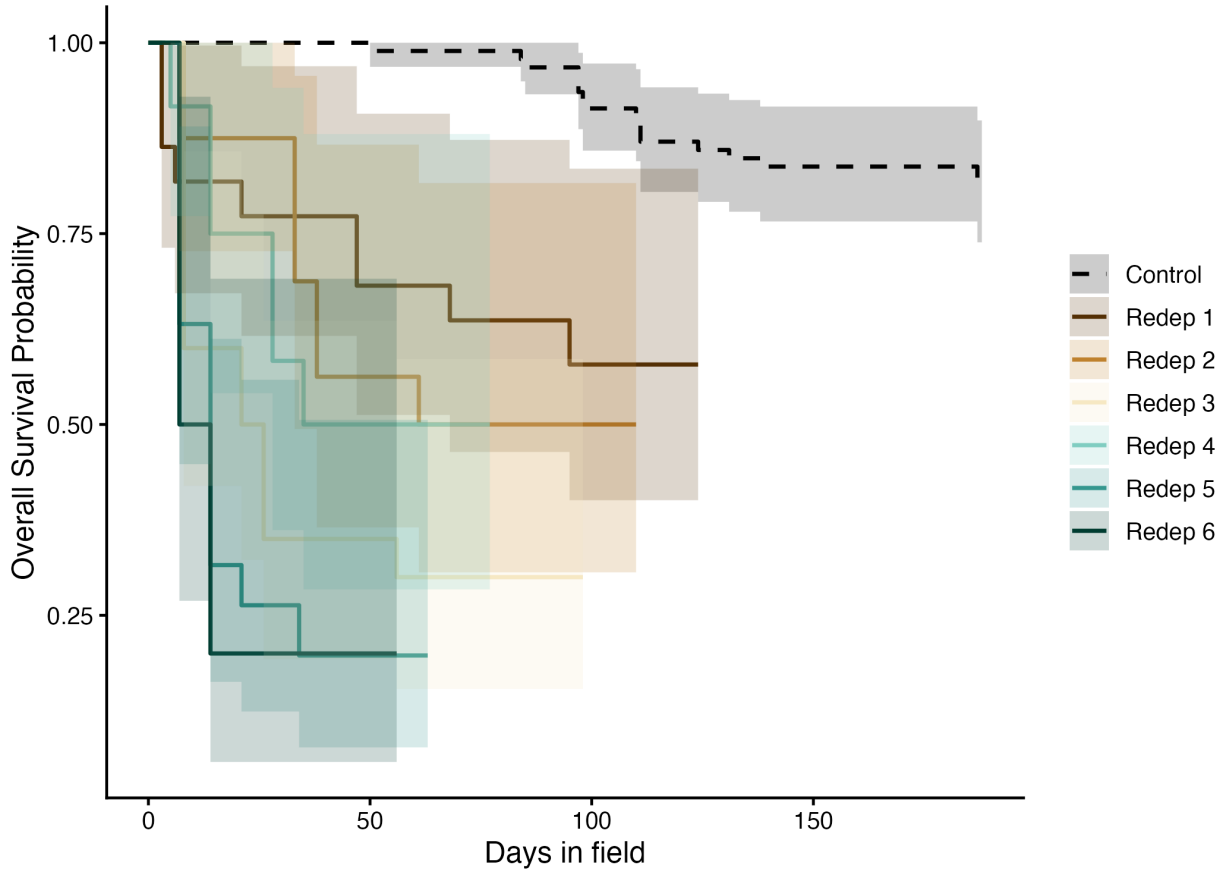


Figure 5. Kaplan-Meier Plot. Kaplan-Meier curves displaying survival probability for the control (dashed line) and redeployment treatments (colored solid lines). The x-axis represents days in the field following transfer from CDS.

3.2.2 Survival Measurements from Farm Overwintering

Survival among oysters stored subtidally on the farm was 80%, $N = 615.5$. Slightly higher mortality was observed on our second farm sampling date (W23; mortality = 22%; $N = 314$) than on our first farm sampling date (W21; mortality = 19%; $N = 301.5$). However, there was not a significant difference in mortality recorded between the two dates ($\chi^2 = 0.4$, $df = 1$, $p = 0.545$).

4. DISCUSSION

The overarching goal of our study was to investigate the effectiveness of CDS as an overwintering strategy for oyster farmers in the Northeast while simultaneously gaining insight

into oyster physiology during this quiescent period. In the first year of our study, Experiment 1 assessed short-term oyster survival, experienced while in CDS or shortly after being returned to seawater. We found high survival, with no significant impact of size and storage orientation. Cardiac activity was monitored in a subset of oysters to characterize oyster physiology during CDS. We found that heart rate decreased with time spent in CDS, with a periodic heartbeat becoming undetectable five weeks into CDS even in alive oysters. Because short-term survival measurements may not capture delayed effects of storage, our objective for Experiment 2 was to determine if CDS duration impacted long-term survival in the field, thus providing a more reliable measure of CDS effectiveness. Our findings suggest that long-term survival in the field decreases with time after CDS, with oysters redeployed at later dates tending to have lower survival rates.

4.1 Oyster Survival in CDS

In Experiment 1, oyster survival was high (87.5%) following the 16-week winter storage. While mortality was tracked during the CDS period, mortality was not detected until the oysters were returned to seawater at the end of dry storage. Additional mortality occurred when oysters were subsequently held in seawater at 13 ºC for an extended period, reducing overall survival to 77.1%. Similarly, in Experiment 2, mortality in CDS was low, with mortality predominantly observed once oysters were redeployed in the field. The delayed mortality observed in seawater but not CDS may reflect sampling difficulties during CDS and/or oysters exceeding physiological limits following re-immersion. During quiescence, oysters remain tightly shut, which can make it difficult to assess viability without compromising the animals. In a similar study, Hidu and colleagues addressed this issue by periodically transferring a subset of oysters to seawater throughout the CDS period, which may provide a more reliable assessment of oyster viability (Hidu et al., 1988).

Delayed observation of mortality may also be due to physiological limits that occur during re-immersion. When exposed to air, many bivalves close their shells to reduce water loss and prevent desiccation. As the animal respires, oxygen is quickly depleted from the internal shell environment. To cope with the decreased oxygen levels, organisms transition from aerobic to anaerobic metabolism, which is less energetically efficient (McMahon, 1988). When re-immersed, bivalves often employ higher aerobic metabolism to recover from this “oxygen debt”.

Thus, the combined energy expenditure of anaerobic and aerobic metabolism during emersion and re-immersion may have overwhelmed energy stores following long-term CDS, subsequently leading to mortality in seawater (Yin et al., 2017). The increase in mortality with time spent in CDS observed in Experiment 2 is consistent with the hypothesis that oxygen debts accumulate during storage and ultimately overwhelm energy stores, although we did not measure energy reserves or metabolites. Further, other factors such as starvation and toxic metabolite accumulation may have contributed (Byrne et al., 1991; Mayrand et al., 2017)

Across both experiments, short-term mortality (i.e., mortality measured within a day of re-immersion) was low when oysters were re-immersed in seawater before 16 weeks of CDS. After week 18 (Redep 4), however, mortality on the day of redeployment increased, indicating a potential threshold for storage duration under these conditions. Contrary to these findings, Hidu and colleagues found high survival (>80%) up to six months in CDS (Hidu et al., 1988). In our study, water temperature upon re-immersion may explain differences in mortality. Oysters in Redep 4, 5, and 6 were re-immersed in water temperatures near or above 5°C (Figure 3). The temperature at which previous studies have found that oysters begin to enter quiescence (Comeau et al., 2012). This may have queued rapid reawakening and elevated metabolic rates, further overwhelming potentially limited energy stores. With regard to long-term survival in the field, food availability during re-immersion in the spring will also likely impact survival. High food availability may allow oysters to meet energy demands, while limited availability may increase mortality risk (Caillon et al., 2025).

The present study examined factors influencing survival in two-year old (Experiment 1) and one-year old (Experiment 2) oysters. In Experiment 1, oyster size (i.e., weight) and storage orientation were not significant predictors of oyster survival (Figure 4). These results differ from previous studies that found that larger oysters had higher survival in CDS. Research has shown that overwintering physiology can differ with life stage along with size. In an examination of overwintering physiology in water, Bridier and colleagues found that adult and seed Eastern oysters varied in their use of energy stores to cope with near-freezing conditions. While adults maintain lipid membrane fluidity by increasing the proportion of highly unsaturated fatty acids in their lipid membranes, seed ceases this process, as it can be an energy-intensive mechanism that challenges limited energy reserves (Bridier et al., 2026). How oysters utilize energy reserves

long-term through CDS, and how this may impact survival differences, is unknown. Although our two experiments examined different age classes, which overlapped considerably in size, differences in experimental design prevent direct comparisons of survival between groups. Thus, depending on the needs of farmers, future studies may consider examining the survival of different age classes in CDS rather than focusing only on size.

The overall effectiveness of CDS will depend on how oyster survival directly compares to oysters held in the field. Survival of oysters overwintered subtidally at the farm was not measured experimentally in Experiment 1; however, the farmer where oysters were sourced estimated survival at approximately 50% (*personal comm*). In Experiment 2, oyster survival rates on the farm were greater than those held in CDS (80% vs. > 59%). Indicating large survival differences between oysters overwintered subtidally on the farm and in CDS across years. Collaborative research efforts between farmers and state agencies in different regions have shown that CDS may be an effective option for maintaining oysters if environmental conditions become more variable, and winter ice conditions become less predictable (Haskin, 2021). Choosing between CDS and field overwintering may ultimately come down to feasibility for farmers. Purchasing and operating a CDS container in the winter months has considerable upfront costs, and the required time and labor, both of which scale with farm size, are critical factors for determining the financial efficacy of CDS. Further study and analysis could weigh the costs and benefits of this system, how it scales, and how it compares to other overwintering strategies.

4.2 Oyster Physiology During CDS

Heart rate is a primary tool for assessing cardiac activity and understanding organismal physiology. For ectothermic species, such as marine mollusks, it can serve as an effective indicator of metabolic rate and proxy for whole-organism stress (Braby and Somero, 2006). In Experiment 1, we monitored and estimated heart rate in a subset of oysters held in CDS. Prior to entering CDS, oyster heart rate ranged from 8.2 to 10.4 bpm, which is consistent with the heart rate measured for bivalves held at similar temperatures (de Carvalho et al., 2022). Once in CDS, we found that the heart rate decreased over time, though the time at which a heartbeat became undetectable was specific to each individual (Supplementary Figure 1). However, across all oysters, the latest time at which a periodic heartbeat was detected was five weeks into CDS.

Unfortunately, we were unable to confidently estimate heart rate for oysters during the spring awakening portion of the experiment, as periodic sequences could not be visually confirmed. This may have been due to the displacement of sensors over their hearts once they were transferred back into seawater.

A reduction in heart rate, known as bradycardia, is often considered a stress response triggered by environmental conditions such as extreme temperature, hypoxia, and pollutant exposure (Boroda et al., 2020; Davis et al., 2023; Liu and Wang, 2016; Paynter et al., 2024). However, decreased metabolic rate and accompanying bradycardia are also fundamental traits of animals entering dormancy (Wilsterman et al., 2021). To our knowledge, this is the first study examining oyster heart rate under long-term cold and dry conditions. While our goal was to gain insight into cardiac physiology in conditions relevant to aquaculture storage, our findings are most comparable to studies examining heart rate under naturally occurring immersion and emersion cycles, such as those associated with the intertidal environment.

For bivalves, and specifically Eastern oysters, bradycardia is a common response to air exposure (Coleman and Trueman, 1971; Helm and Trueman, 1967; Willson and Burnett, 2000). As the internal shell environment becomes depleted of oxygen, it is predicted that the onset of hypoxia induces a reduction in metabolic rate with a transition from aerobic to anaerobic metabolism (Peterson et al., 2025). Here, the decreased heart rate of oysters in CDS may provide evidence for this transition, but future studies can confirm this by measuring end byproducts of anaerobic metabolism throughout storage, providing insight into metabolic processes underlying quiescence in CDS (Bridier et al., 2026). Bivalves have also adapted to consistent air exposure through behavioral changes (Hicks and McMahon, 2003; McMahon, 1988; Nicastro et al., 2012). Some bivalve species rely on atmospheric oxygen uptake during long-term emersion, where shell gaping vents the internal cavity airspace, allowing surface tissues to maintain low levels of aerobic metabolism (Deaton, 1991; McMahon, 1988). Even when bivalves engage in anaerobic respiration, as oysters likely did during CDS, air gaping can release waste products and prevent acidosis similar to when bivalves open their valves in water (Littlewood and Young, 1994; Lombardi et al., 2013; Mayrand et al., 2017). However, this behavior can also increase bivalve susceptibility to desiccation. Air gaping in bivalves is primarily influenced by temperature and humidity, two key controllable factors in CDS, where increased relative humidity and reduced

ambient temperature reduce water loss and thus susceptibility to desiccation. Therefore, if oysters engage in air breathing during long-term storage, behavioral analysis can provide meaningful insight into specific conditions needed to support their survival in CDS.

4.3 Challenges in Long-term Cardiac Monitoring

In this study, heart rates were estimated from data sequences that were classified as periodic using the previously described algorithm (Hellicar et al., 2015). Period estimates, which were used to calculate heart rate, were determined using biologically relevant thresholds described in the literature for mollusks held in similar temperature and air conditions (Bakhmet, 2017; de Carvalho et al., 2022; Liang et al., 2021). Here, our minimum threshold for heart rate was 1.7 bpm. During long-term CDS, several oysters had a documented minimum heart rate of 1.7 bpm (Table 1). It is likely that oyster heart rate may have been lower than this but was not identified due to the algorithm's thresholds. Further, heart rate values were only calculated from periodic sequences, but it is likely that oysters in CDS experienced high variability in the time between consecutive heartbeats (aperiodicity). This aperiodic heartbeat is seen in other mollusks during emersion and under cold temperatures (Bakhmet, 2017; Liang et al., 2021). In the present study, an oyster with high heartbeat variability would have had a data sequence that would be classified as aperiodic and thus missed by our algorithm. Depending on the questions being asked, further refinement of the algorithm may be necessary.

While we could not identify the exact time of death, survival of oysters outfitted with heart rate monitors was 60%. This value was slightly lower than the subset of oysters used to examine survival in Experiment 1, which may be attributed to the small holes that were drilled in the shells to attach heart rate monitors. While numerous studies have been successful in measuring cardiac response to abiotic and biotic stressors using similar methods (Davis et al., 2023; de Carvalho et al., 2022), there is evidence to suggest that drilled holes can compromise survival in the long term (Paynter et al., 2024). Biosensors such as the cardiac monitors used in our study are becoming increasingly adopted due to their accessibility, ease of use, and ability to generate high-resolution datasets (Burnett et al., 2013; Miller, 2022). However, for long-term deployments, careful attention to attachment methods and signal processing techniques will be essential to ensure data reliability and the capacity to answer targeted research questions.

5. CONCLUSION

In this study, our goal was to add to the limited but growing research examining oyster overwintering practices employed by farmers in the Northeast. Overwintering oysters in CDS offers farmers the opportunity to maintain their stock in a highly controlled setting, unlike the field, where oysters have the potential to be impacted by freezing temperatures, ice scour, sedimentation, and predators. While previous research has reported high survival rates associated with CDS (Hidu et al., 1988), we found survival rates to be highly variable and dependent on storage duration. Further work is needed to test if CDS duration inherently increases mortality risk and if other factors such as temperatures before or after CDS may adjust risk independent to duration. Moreover, additional survival monitoring, and subsequent growth, following field deployment and later in the growing season will provide information on potential long-term effects of CDS.

We provided insight into oyster physiology during CDS by measuring cardiac activity. As seen in the literature, we found that oyster heart rate decreased with time spent in CDS, showing a transition to a quiescent state. Further studies examining underlying behavioral and physiological processes during this state will provide information on how oysters use energy to maintain this state and conditions that will yield the highest survival, such as the amount of time in CDS. Overall, this study generates informed questions about the underlying physiological processes that govern survival and ultimately dictate what CDS conditions will yield the best survival rates for farmers in the Northeast.

CRediT authorship contribution statement

Jannine D. Chamorro: Writing – original draft, Formal analysis, Visualization, Writing – review and editing; , **Kaila J. Frazer:** Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing – review and editing, **Josephine C. DeMerit:** Conceptualization, Investigation, Methodology, Data curation, Writing – review and editing, **Andrew R. Villeneuve:** Investigation, Methodology, Data curation, Writing – review and editing, **Selina L. Cheng:** Investigation, Writing – review and editing, **Joseph A. Rankin:** Conceptualization, Resources, Writing – review and editing; **Brittany M. Jellison:** Conceptualization, Writing – review and editing; **Easton R. White:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review and editing

Data statement

Oyster survival data can be found at github.com/QuantMarineEcoLab/oyster-cds-2024-26. Data related to cardiac activity is available upon request.

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Declaration of competing interests

Co-author, Joseph A. Rankin, owns the oyster farm from which oysters were sourced.

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