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

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ABSTRACT

In the northernmost part of the Eastern oyster (*Crassostrea virginica*) range, oyster farmers face challenges maintaining stock through freezing winters. To avoid leaving oysters exposed to variable field conditions, many farmers overwinter oysters outside of the water in cold-dry storage (CDS). Here, we sought to add to the limited but growing empirical research examining the effectiveness of CDS and factors contributing to oyster survival (i.e., size and storage orientation). 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 4.6 ºC and 81.8% humidity for four months (Nov – Mar). To gain insight into oyster physiology during CDS and the transitions in and out of storage, we monitored oyster cardiac activity over the course of the experiment. While oyster mortality was monitored throughout the storage period, it was not observed until oysters were re-immersed in seawater at the end of the experiment. We found high survival in CDS (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. 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 [1]. Across this range, the aquaculture of Eastern oysters is a rapidly growing industry, contributing to local economies and providing numerous ecosystem services [2,3]. As a result of covering such a broad latitudinal range, oysters experience diverse environmental conditions that can impact physiological performance [4], 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 [5,6]. 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 [7,8].

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 [7,9]. During this period of inactivity, or quiescence, oysters minimize valve gaping by completely closing themselves off to the surrounding environment or remaining slightly open [7,9]. However, 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 function [10,11]. As energy reserves are exhausted through the winter season, vulnerability to potential disturbance increases [9,12]. This is especially true for oysters in the field. 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 [9,12]. 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 [13]. Due to the uncontrolled variability of field conditions, farmers and researchers have maintained oysters outside of the water during winter [14]. 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 [15]. 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 [9,16]. 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 [14,17,18] and decreasing pest abundance [19]. A CDS approach termed "pitting" involves storing oysters in underground pits (e.g., vegetable cellars), layered in burlap [18]. 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 [14,17].

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 [14,17]. 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 specific storage treatment [17]. In contrast, Hidu and colleagues found higher rates of survival, ~80%. Here, oyster size and storage duration, but not temperature (0-6 ºC), seemed to impact survival in CDS. Across two consecutive years, they found larger oysters had higher survival. Further, three to five months seemed to be the optimal storage time [14]. Another factor to consider is the oysters' storage orientation [18]. 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 are 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 forms at the surface from December to March [20]. We measured oyster survival over four months in a temperature and humidity-controlled chamber. Specifically, we were interested in determining if oyster size and storage orientation impacted survival during 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. Lastly, to gain insight into oyster physiology during CDS and the transitions in and out of storage, we fitted a subset of oysters with cardiac activity monitors throughout the experiment. As cardiac monitors become increasingly prevalent for long-term monitoring [21], 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.

2. METHODS

2.1 Organism Collection and CDS Monitoring

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). On November 26th, 2024, 3,000 oysters (two years of age, average length ~ 35-65 mm) 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 ºC and 81.8% humidity (Figure 1). While in CDS, oysters were held in 12 3/8” mesh bags at an approximate concentration of 1,000 oysters per bag.

On December 16th, 2024, monitoring for survival commenced. Eight oysters from each bag were taken as representative samples to monitor survival over the CDS period. All oysters (*N*=96) were weighed to examine the influence of size on survival. To determine if storage orientation influenced survival, four oysters from each bag were stored cupped shell up, and the other four were stored cupped shell down. 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 16th, 2024, to March 17th, 2025. 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 in line with previous studies [14,17]. 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 20-gallon 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 are reported descriptively and were not included in formal statistical analyses but are intended to inform future targeted studies.

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 [22]) 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 [23].

2.3 Monitoring Cardiac Activity

To further investigate oyster physiology during CDS, cardiac activity was monitored in a separate subset of individuals. On December 18th, 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 [24]. 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 a short-term acclimation and ensure post-drilling survival. Following 26 hours in seawater, all oysters were placed back in CDS at 4 ºC on December 19th, 2024. After approximately 16 weeks in CDS, on March 18th, 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 monitored for 14 days. The temperature and salinity during this time were 6.7 ± 0.8 ºC and 16.3 ± 1.5 (mean ± standard deviation), respectively.

2.4 Cardiac Signal Processing

We investigated changes in cardiac activity during four distinct periods of 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), 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 (different from those with heart rate sensors attached; [25]). Awakening behavior is synchronous among oysters exposed to the same conditions, so we assumed those with heart rate sensors attached also emerged from quiescence [7,12]. 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 [26]. 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 [26]. Saturated sequences are those where a significant proportion of the recorded values reach a maximum threshold, often the sensor maximum, usually caused by external factors such as light interference (Figure 2A). Aperiodic sequences are defined as having a lack of periodicity, which may be due to the absence of a strong signal reflected off the heart surface or the absence of a heartbeat (Figure 2B). Lastly, periodic sequences result because a heartbeat modifies 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) [26].

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 at similar temperatures or air exposure conditions [27,28]. 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.

3. RESULTS

3.1 Oyster Survival

Mortality was not observed during the CDS period but was documented post 24-hour transfer 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 3). 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 interval at these size extremes likely reflects the small sample sizes at 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.

3.2 Cardiac Analysis

We examined cardiac activity during four distinct periods in our 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), 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.

Seawater: The percentage of sequences classified as saturated, aperiodic, and periodic was 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 rate was variable across individuals and time (Supplemental Fig 1A). However, averaged over the entire acclimation period, heart rates were relatively similar, ranging from 8.2 - 10.4 bpm (Table 1).

First 48 hours in CDS: The percentage of sequences classified as saturated, aperiodic, and periodic was <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 Fig 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: There were no sequences classified as saturated. The percentage 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 Fig 1C).

Spring Awakening: 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	
	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	8.97
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 that were used to calculate period estimates.

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. We found high survival in CDS, with no significant impact of size and storage orientation. All mortality was documented once oysters were placed back in seawater. Cardiac activity was monitored in a subset of oysters to characterize oyster physiology during CDS. Here, 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.

4.1 Oyster Survival in CDS

Oyster survival was high (87.5%) following the four-month winter storage. While mortality was tracked during the period of CDS, mortality was not observed 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%. The delayed mortality observed in seawater but not CDS may reflect sampling difficulties during CDS 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 [14]. However, their methods yielded similar results to those presented here overall. In the second case, 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 more energetically taxing [29]. 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 [30]. While we cannot confirm either case in the current study, it is likely that this delayed mortality will occur in aquaculture settings as well. Thus, measurements of post-immersion survival on farms rather than immediately following CDS are necessary to accurately determine the efficacy of CDS. Future studies may also investigate time in dry storage as a predictor of post-CDS survival. If oxygen debts are cumulative during CDS, then oysters exiting a longer period of CDS would be more likely to overwhelm their energy stores during re-immersion than oysters exiting a shorter period of CDS. 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 [31].

The present study examined factors influencing oyster survival in adults two years of age. Here, oyster size (i.e., weight) and storage orientation were not significant predictors of oyster survival (Figure 3). These results differ from previous studies that found that larger oysters had higher survival in CDS [14]. However, 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 utilization 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 [11]. How oysters utilize energy reserves long-term through CDS, and how this may impact survival differences is unknown. 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.

While not directly addressed in this study, the overall effectiveness of CDS will depend on how oyster survival directly compares to oysters held in the field. Anecdotally, survival of oysters that overwintered at the site of collection was approximately 50% (*personal comm*), suggesting that CDS may be a more effective option. Collaborative research efforts between farmers and state agencies in different regions have shown that CDS may be an effective option for maintaining oysters as environmental conditions become more variable, and winter ice conditions become less predictable [32]. Choosing between CDS and field overwintering may ultimately come down to feasibility for farmers. While purchasing and operating a CDS container in the winter months has considerable upfront costs, the lower mortality rates could make this overwintering strategy profitable in the long run. However, consideration of 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 [33]. Here, 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 [28]. Once in CDS, we found that the heart rate decreased over time. The time at which a heartbeat became undetectable was specific to each individual (Supplementary Fig 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 [34–37]. However, decreased metabolic rate and accompanying bradycardia are also fundamental traits of animals entering dormancy [38]. 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 [39–41]. 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 [42]. 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 [11]. Bivalves have also adapted to consistent air exposure through behavioral changes [29,43,44]. 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 [29,45]. 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 [10,46,47]. 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 reduces 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 [26]. 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 [27,28,48]. 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 [27,48]. 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 refinements to 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, 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 utilizing similar methods [28,35], there is evidence to suggest that drilled holes can compromise survival in the long term [37]. 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 [21,49]. 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. We found that holding oysters in CDS yielded generally high survival rates. However, direct comparisons to survival in field conditions are needed to make accurate comparisons between both methods. Further, our survival measurements were conducted immediately following CDS, revealing only short-term impacts of this method. 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 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; **Josephine 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, **Kaila J. Frazer:** 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-2024-25. 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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FIGURES

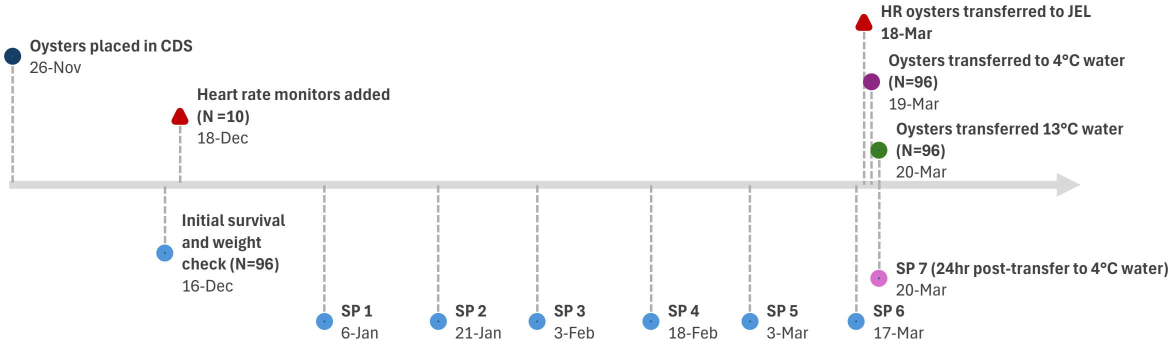

Figure 1. Experimental Timeline. Oysters were transferred to cold-dry storage on Nov 26th; survival monitoring commenced on Dec 16th. Oyster viability was assessed approximately every two weeks, depicted by sampling points (SP) on the timeline. On March 19th, oysters used to monitor survival were transferred to 4 °C seawater for 24 hours, then assessed for mortality (SP 7). Following 24 hours, surviving oysters were transferred to seawater held at 13 °C. Red triangles depict when a separate subset of oysters were outfitted with cardiac monitors and transferred to the Jackson Estuarine Laboratory for spring awakening.

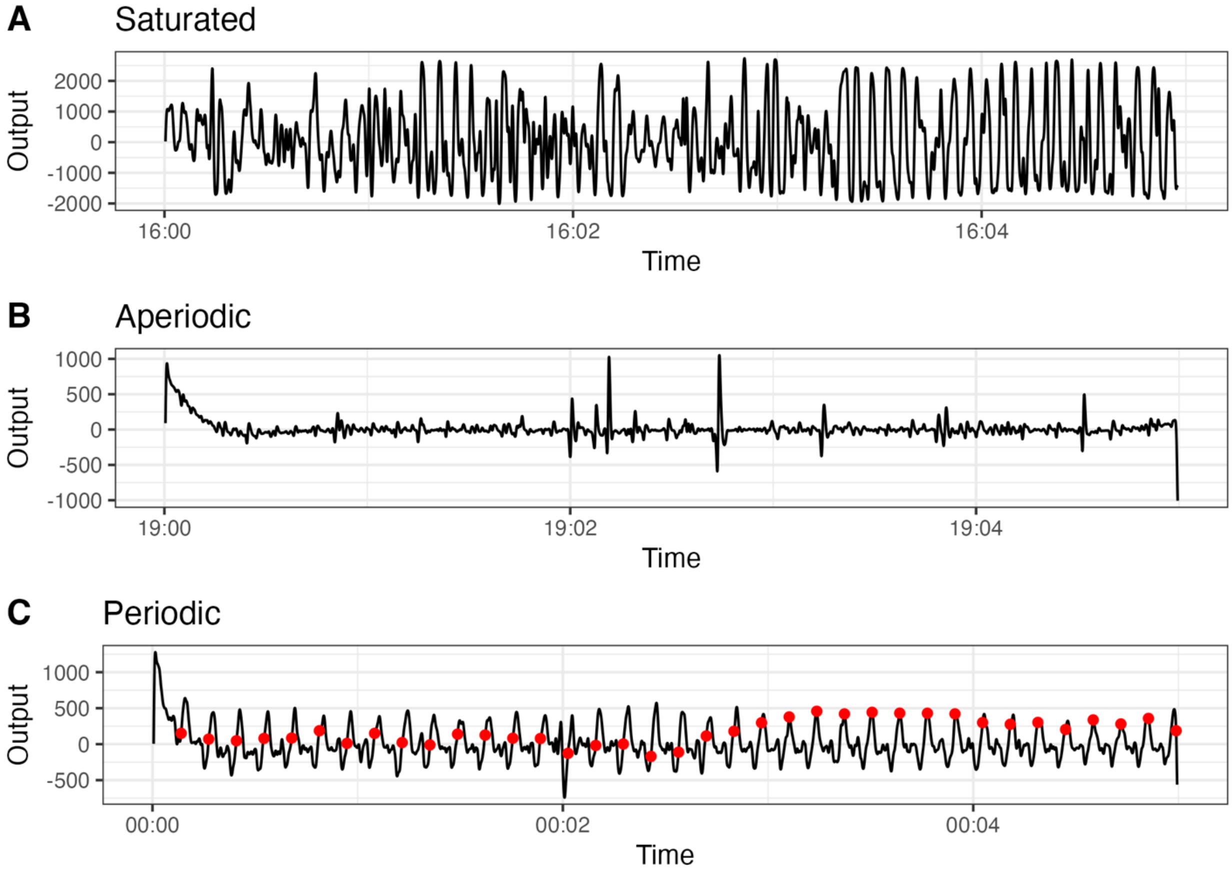

Figure 2. Classification of Cardiac Data. Examples of data sequences classified as A) saturated, B) aperiodic, and C) periodic. The black line represents the filtered data. The x-axis displays an interval of five minutes. The output on the y-axis is a unitless value representing the relative intensity 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.

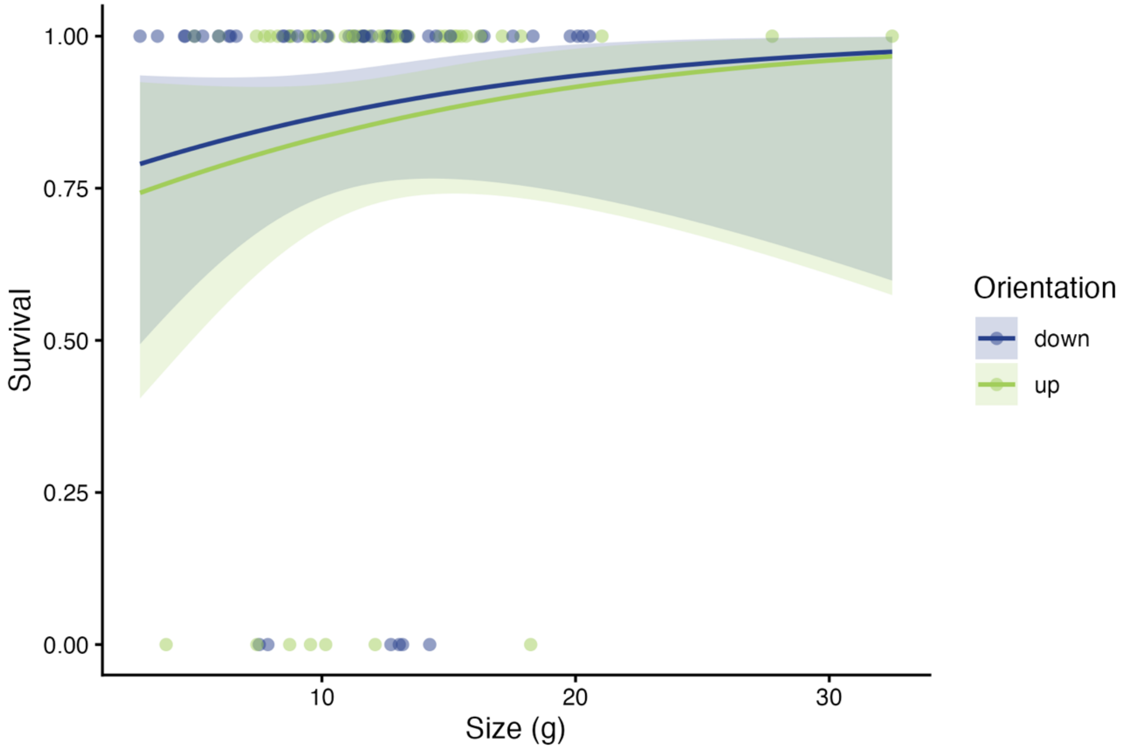

Figure 3: Cold-Dry Storage Survival (CDS). Survival of oysters held in CDS (*N*=96) following transfer to 4 ºC seawater. Size is represented on the x-axis and probability of survival on the y-axis, with points representing survival status (alive = 1, dead = 0). Storage orientation is represented by color (blue: cupped side down; green: 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.