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Research Paper

Performance of Cold Chains for Chesapeake Bay Farmed Oysters and Modeled Growth of *Vibrio parahaemolyticus*

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ABSTRACT

Temperature-controlled supply chains (cold chains) require an unbroken chain of refrigeration to maintain product quality and safety. This study investigated cold chains for farmed oysters raised in the Chesapeake Bay, one of the largest shellfish-growing regions in the United States, and sold live to the half-shell market in surrounding states. Temperature sensors were used in boxes of oysters from February to September 2017, which generated 5,250 h of temperature data. Thirty-nine businesses participated in the temperature sensor study, and 26 of those businesses participated in interviews to further understand how cold chains function. Internal oyster temperatures were measured above 50°F (10°C) for over 1 h in 19% (7 of 36) of shipments, which is a temperature that exceeds National Shellfish Sanitation Program criteria. The highest internal oyster temperature recorded in any shipment was 54.5°F (12.5°C). Some parts of the cold chain had difficulty maintaining storage temperatures below 45°F (7.2°C) in warmer months when *Vibrio* control plans were in effect. We modeled the effects of temperature on *Vibrio parahaemolyticus*. The model predicted moderate bacterial growth before oysters were under temperature control, but cold chains prevented further bacterial growth and provided a moderate drop-off in *V. parahaemolyticus* abundance.

Key words: Cold chain; Oyster; Shellfish; Supply chain; Temperature; *Vibrio*

Seafood is made available to consumers via a network of producers, processors, freight carriers, wholesalers, food retailers, and restaurants. This network is called a supply chain, which creates economic opportunities for businesses and is a critical component of the food system. Temperature-controlled supply chains (i.e., cold chains) require an unbroken chain of refrigeration to maintain product shelf life and quality, as well as food safety *(1)*. Another feature of supply chains is the level of traceability—whether finished products can be tracked from the farm to the consumer *(2)*. A high level of traceability can support a wide range of functions, including product differentiation, identification of origin and production methods, fraud prevention, waste reduction, facilitation of food recalls, and improvement in consumer trust *(2, 3, 13, 16)*. Some seafood products have considerable traceability issues due to the degree of complexity tied to such factors as international supply chains, species substitution, and other factors. The U.S. government will implement a traceability program in 2018 for 14 priority species of seafood *(22)*.

Seafood supply chains are complex, and very little is known about them outside of the seafood industry. There are 2,500 businesses in the United States that are certified to harvest, process, and distribute molluscan shellfish *(33),* and to our knowledge, there has never been an effort to map these supply chains. Understanding the size, shape, and performance of supply chains is critical for implementing traceability programs and ensuring food safety.

There are specific regulations that pertain to certain shellfish species that are often eaten raw, owing to the associated foodborne illness risks. The U.S. shellfish regulations include a tag system to track key attributes about shellfish from harvest to consumers *(24)*. These tags are used by government agencies, while performing shellfish-borne disease tracebacks and product recalls, and by consumers to aid in product differentiation.

There are also regulations related to handling of product during harvest, processing, and distribution to reduce some risks associated with shellfish-borne diseases *(10, 24, 29)*. States with confirmed cases of *Vibrio vulnificus* or *Vibrio parahaemolyticus* or both must develop *Vibrio* control plans (VCPs) for warm seasons to further mitigate risks associated with these bacteria *(24)*. *V. vulnificus* and *V. parahaemolyticus* are gram-negative bacteria naturally found in estuarine and marine environments, which are becoming more abundant and spreading to new geographic areas due to climate change *(34)*. *V. vulnificus* is the leading

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cause of death related to seafood consumption in the United States *(5)*. *V. parahaemolyticus* causes gastroenteritis that is usually self-limiting but can result in business losses devastating to the oyster industry. From 1996 to 2010, infections associated with *Vibrio*-contaminated seafood, including oysters, tripled (0.09 to 0.28 cases of vibriosis per 100,000 people calculated by Cholera and Other *Vibrio* Illness Surveillance and 0.15 to 0.42 cases of vibriosis per 100,000 people calculated by the Foodborne Diseases Active Surveillance Network) *(25)*.

Temperature control of shellfish during harvest, processing, and throughout the distribution is essential to control *Vibrio* growth. Storing oysters at ambient conditions allows *V. vulnificus* and *V. parahaemolyticus* to multiply inside the oyster shell *(8, 12)*. One approach to reduce risk of *Vibrio* growth involves postharvest processing methods, such as pasteurization, high pressure processing, or rapid freezing, which have been developed to reduce pathogen risks in shellfish *(4)*. These methods, however, can negatively affect the taste of raw oysters *(6)*. Icing is a method to control *Vibrio* growth in lieu of postharvest processing *(15, 20, 27)*. In comparing cooling methods, ice slurries perform slightly better at rapidly cooling oysters than layered ice, and both methods achieve cooling faster than refrigeration alone *(15, 18)*. Cooling oysters too quickly with ice may lead to a negative side effect of gaping (e.g., open shells), which leads to loss of oyster liquor, reduced shelf life, and possibly oyster death *(20)*. Additionally, storing oysters below freezing could kill the animals, whereas prolonged storage at or above 50°F (10°C) can degrade product appearance, odor, and texture *(21)*. Previous oyster temperature studies have primarily focused on the harvest or production level and have not adequately investigated the role of cold chains in maintaining product quality and safety.

The aim of this study was to gain a better understanding of temperature control in shellfish cold chains within the Chesapeake Bay region to support modeling efforts and inform policy development. Oysters are the most valuable marine aquaculture species in the United States, and the Chesapeake Bay is the largest estuary in the United States and one of the top oyster-producing regions of the country *(23, 31)*. We asked seafood businesses about their protocols and practices for handling oysters and their perceptions of cold chains. Then, we sought to answer two research questions: (i) Are oyster temperatures different across the stages of the supply chain? (ii) What is the modeled abundance of *V. parahaemolyticus* and the associated risk of gastroenteritis in the supply chain? Finally, we identified areas in which the seafood industry can improve cold-chain performance for oysters.

MATERIALS AND METHODS

Study design. We assessed the performance of regional cold chains for molluscan shellfish by using a mixed methods approach involving temperature sensor measurements and interviews with businesses. We focused on farmed Eastern oysters (*Crassostrea virginica*) sold live to the half-shell market. Our rationale for this focus was that this product form is a growing segment of the shellfish industry and maintaining proper temperature control for live oysters is challenging. The study area included farmed oysters raised in the Chesapeake Bay and distributed to surrounding states: Virginia, Maryland, Delaware, Washington, DC, and Pennsylvania. We excluded all wild-caught oysters, shucked oysters, and frozen half-shell oysters from the study, as well as any product harvested outside the Chesapeake Bay and sold outside the study region. The study was reviewed by the Johns Hopkins School of Public Health Institutional Review Board.

Participant recruitment. Participants were recruited into the study on the basis of chain sampling methods, starting with local and regional seafood wholesalers in February 2017. Wholesalers then referred us to other participants in their own supply chains (oyster producers, freight carriers, food retailer, and restaurants). Participants were contacted by phone or e-mail and given a one-page description of the study and a consent form. As an incentive, participants were provided data about the performance of their own cold chain. The inclusion criteria were being an active business in the regional shellfish supply chain, being over 18 years of age, speaking English, and agreeing to participate in the study.

Survey tool. We developed a survey tool to collect information from businesses in the supply chain, and a subset of these questions pertained to cold-chain performance. The responses to this subset of questions are reported with our temperature data analyses to provide important context. The survey was administered in parallel with the temperature sensor study and was performed as an in-person or phone interview. Survey responses were shared with participants to check accuracy.

Temperature sensors. Temperature sensors (SmartButton, ACR Systems Inc., Surrey, British Columbia, Canada) were used to track oyster temperature and ambient air temperature at 1-min or 10-min intervals starting at harvest and through the supply chain. The manufacturer reported that the sensors have a working range of –40 to 185°F (–40 to 85°C), which is within the range of temperatures we expected to observe in our cold chains. The reported accuracy of the sensors was ±1.8°F from –22 to 113°F (±1.0°C from –30 to 45°C). We independently tested the interbutton variability in our laboratory by using simulated field conditions and determined it was 1°F (0.6°C). We used a pilot study with wholesalers to determine the variability among three oyster boxes shipped to the same final destination and determined that a single box was adequate to make generalizations about temperatures in warehouses and trucks (see Supporting Information [Supplemental Material] Fig. S1). Handheld analog thermometers, calibrated in an ice water bath, were used for spot readings on farms.

Harvest and on-farm processing. We visited six oyster aquaculture operations to monitor the harvest and on-farm processing. Each farm was visited once during months in which VCPs were in effect. Virginia producers are required to follow *V. vulnificus* and *V. parahaemolyticus* plans from 1 May to 30 September, while Maryland producers are required to follow *V. parahaemolyticus* plans from 1 June to 30 September. Three of the six farms were visited again in March when VCPs were not in effect, and the ambient temperature in the region ranged from the middle 30s to middle 50s in degrees Fahrenheit. To protect the anonymity of participants, we do not report farm origin or state in our results.

The temperatures of the harvest water, ambient air, wash water, and the walk-in refrigerator were measured. Notes were also taken about sun exposure and processing methods. After oysters were harvested and unloaded at the dock, SmartButton sensors were inserted into oysters to measure temperature at 1-min intervals during washing, grading, boxing, and storage. All oysters containing temperature sensors were wrapped in red duct tape to prevent their introduction into the food supply. Just before the product was shipped, the sensors reading at 1-min intervals were removed, and new sensors reading at 10-min intervals were inserted inside one oyster per 100-count box. (Sensors set at 10-min intervals increased sensor operating life up to 2 weeks.) Farms that sell products in mesh bags were asked to place the bags inside a box. One SmartButton sensor was taped to the outside of each 100-count wax box to measure ambient air temperature in shipments. Between four and eight boxes were tracked from each farm. Stamped envelopes, a study description, and a note card to record arrival times were enclosed in each box to allow the final recipient (food retail or restaurant) to return the sensors to the Virginia Seafood Agricultural Research and Extension Center at Virginia Tech. Producers were offered financial compensation for boxes of oysters used in the study.

Statistical analyses. Temperature sensor data were downloaded by using manufacturer software (TrendReader, ACR Systems Inc.), analyzed in Excel (Microsoft Corporation, Redmond, WA) and graphed in Prism (Version 6, GraphPad, La Jolla, CA). One-way analyses of variance (ANOVAs) with repeated measures and Greenhouse-Geisser corrections were used to compare the mean temperature among groups. Shipments with incomplete data were removed from the one-way ANOVAs. If significance was observed in an ANOVA, Tukey's multiple comparison test was used with individual variances computed for each comparison. The *t* tests were used to compare mean temperatures by step of supply chain in VCP months versus non-VCP months.

To better understand temperature outliers, we classified each shipment by the number of times temperature sensor values exceeded certain National Shellfish Sanitation Program (NSSP) criteria. These criteria were oysters with internal temperatures >50°F (10°C) or a shipping environment >45°F (7.22°C). We also added another criterion for cold abuse, oysters or their environment held at <35°F (1.67°C). This is not part of the NSSP; however, oysters held below 35°F are susceptible to gaping. We noted if shipments were above or below the criteria for 1 h or more (on the basis of readings taken at 10-min intervals).

***V. parahaemolyticus* modeling.** We modeled the expected abundance of *V. parahaemolyticus* in oysters and the associated risk of gastroenteritis by using internal oyster temperature data. Statistical models were based on the U.S. Food and Drug Administration's risk assessment of *V. parahaemolyticus* in raw oysters *(11)*. The models first estimated the abundance of bacteria at the point of harvest and determined bacterial growth rate when exposed to temperatures higher than a refrigeration threshold of 41.7°F (5.4°C), as well as bacterial die-off rate when stored at or below the refrigeration threshold. The *V. parahaemolyticus* abundance estimation was modeled iteratively so that the previous estimation informed future abundance, whereas the calculation of risk was based on the level of bacteria at a given time point. The abundance at the point of harvest was estimated as a function of harvest water temperature by using the following equation:

$$V = -0.63 \times 0.1W$$

where V is the log-transformed number of *V. parahaemolyticus* bacteria per gram of oyster meat (Vp/g) at the time of harvest, and W is surface water temperature (°C) measured at the time of harvest. *Vibrio* abundance postharvest was calculated by using a growth or die-off model, which can be expressed as follows:

$$A_i = \begin{cases} A_{i-1} + 0.00372(T_i - 5.4) & \text{if } T_i \geq 5.4°\text{C} \\ A_{i-1} - 0.0003 & \text{if } T_i < 5.4°\text{C} \end{cases}$$

where A_i is the log-transformed Vp/g at time point *i,* A_{i-1} is abundance at the previous time point (10 min prior) and its value at the point of harvest is V, and T is the ambient air temperature (°C) measured at time point *i*. Risk was calculated at each time point by using a beta-Poisson dose-response model, which can be expressed as

$$R_i = 1 \times 10^{-5} \times \left(1 - \left(1 + \frac{D_i}{3.54 \times 10^7}\right)^{-0.6}\right)$$

where R_i is the expected number of gastroenteritis cases per 100,000 servings of one dozen oysters, and D_i is the dose of pathogenic *V. parahaemolyticus* that is estimated by using the following equation:

$$D_i = 36 \times 10^{A_i}$$

Given that the observable doses only occurred on the linear portion of the estimated beta-Poisson slope, a linear version of the model was approximated by using the Taylor series:

$$R_i = 1 \times 10^{-5} \left(\frac{0.6}{3.54 \times 10^7}\right) \times 36 \times 10^{A_i} = 0.0061 \times 10^{A_i}$$

For all modeling efforts, air temperature at the time of harvest was held constant in the model until sensors were placed in oysters; water temperature at harvest was substituted if air temperature was not measured. All modeling was performed in R statistical software, version 3.4.3 *(28)*.

RESULTS

Overview of study population. We recruited 39 participants for the temperature-tracking portion of the study, and 64% of participants agreed to be interviewed. Participants' roles in the supply chain are reported in Table 1. Most groups (i.e., producers, freight carriers, wholesalers, wholesale delivery companies, and food retailers) had high enrollment for the interview, with the exception of restaurants.

Figure 1 provides a simplified diagram of the supply chain on the basis of interviews and our observations. Most products were transported from producers to wholesalers by a third-party freight carrier. Wholesalers operated their own fleets of refrigerated trucks and delivered products to local food retail and restaurant customers. Four oyster producers in the study used direct sales, which typically used direct to consumer third-party freight carriers.

Oyster producers: site visits. We visited six farms during VCP months and collected interview and temperature data related to harvest and on-farm processing (Tables 1 and 2 and Fig. 2, and Tables S1 and S2 contain values in degrees Celsius). On average, the harvest water temperature was 73.2 ± 4.0°F (range: 70 to 80°F) (22.9 ± 2.2°C; range: 21.1 to 26.7°C) and the air temperature at harvest was 70.3 ± 1.5°F (range: 68 to 72°F) (21.3 ± 0.8°C; range: 20.0 to 22.2°C). Processing steps were similar among the producers

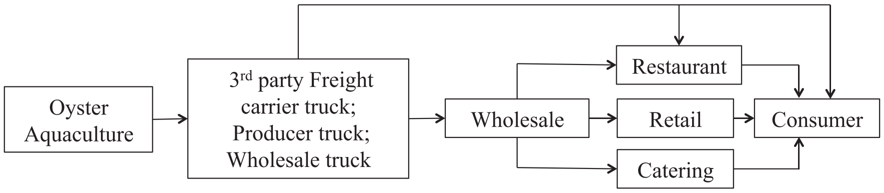

FIGURE 1. *Supply chains for oysters produced in the Chesapeake Bay and sold in surrounding states. Data on supply chains were collected from interviews with participants.*

and involved different combinations of washing, grading, hand sorting, boxing, and mechanical refrigeration.

Mechanical refrigeration was the primary means of cooling among producers in this study; even producers who used layered ice or ice slurries did so to prevent the product from warming in ambient conditions and not in place of mechanical refrigeration. Half of the producers used ice during processing: one producer used an ice-filled hopper, one producer used an ice bath to draw out worms, and two producers used ice to chill bagged products before mechanical refrigeration. Two producers did not use same-day shipment and preferred to instead chill products overnight in walk-in refrigerators.

Oyster producers: time to temperature control. Important benchmarks for *Vibrio* control are the time to temperature control and the time to reach an internal oyster temperature ≤50°F. The NSSP defines temperature control as maintaining the environment at ≤45°F by using ice, mechanical refrigeration, or other approved means *(27)*. To compare across all farms, we used the time to mechanical refrigeration as the starting point for temperature control. Producers in this study achieved temperature control on average in 2.5 ± 1.0 h (range: 1.2 to 4.0 h; Table 2). Products reached an internal oyster temperature ≤50°F within 5.7 ± 3.0 h (range: 3.1 to 10.2 h) after harvest (Table 2).

Oyster producers: temperature profiles. Figure 2 presents annotated temperature profiles for each of the six producers during VCP months of June to September. Several details are important to note in Figure 2. Figure 2A and 2B are harvests that occurred on the same day at one farm—the first, a large morning harvest, and the second, a smaller harvest to fulfill last-minute orders. Figure 2C has a notable point of inflection in temperature when box lids were added during refrigeration that slowed product cooling, which produces a similar insulating effect as putting a lid on a cup of hot coffee. The producer in Figure 2C appeared to have an undersized refrigerator chiller, which also increased the time to cool the product. Producers in Figure 2D and 2G processed oysters the day before shipment. In Figure 2E, the producer washed and refrigerated the product immediately after harvesting and then later rewashed the product with 60°F (15.56°C) tap water, which created a temperature spike. In Figure 2F, the producer used liberal amounts of ice at every stage of processing and achieved rapid, staged cooling.

Supply chain: study population. We measured internal oyster temperature and environment temperature throughout supply chains with temperature sensors. A total of 156 sensors were used from February to September 2017, including 34 sensors implemented as part of a pilot study (see Fig. S1). The overall return rate for sensors with usable data was 81%, which generated 5,250 h of temperature data. The sensors without usable data (19%) included sensors that were knocked off of boxes, tampered with, lost, discarded, or did not contain usable data due to sensor malfunction or human error in programing. (Five boxes of Chesapeake Bay oysters with temperature sensors were shipped to California [$n = 1$], Illinois [$n = 2$], Minnesota [$n = 1$], and North Carolina [$n = 1$] and were not included in this data set because they shipped to destinations outside of the Chesapeake Bay region.)

Supply chain: temperature by stage of supply chain. Oysters entered the supply chain warmer in VCP months than a non-VCP month; however, the final oyster temperature at the retail level of the supply chain was the same

TABLE 1. *Study population in the Chesapeake Bay and self-reported cold-chain temperatures*

	Sample size		Median cold-chain temp (°F; range)[a]			
Supply chain	Temp study	Interviews	Receiving room	Live room/refrigerator	Truck	Ice use (%)[b]
Producer	6	6	—	42 (38–50)[c]	—	50
Freight carrier	5	4	—	—	34 (33–36)	25
Wholesale	2	2	34.5 (34–35)	40 (38–42)	37 (36–38)	50[d]
Food retail/restaurant	26	13	—	38 (29–40)	—	58
Total	39	25	—	—	—	52

[a] Table S1 contains values in degrees Celsius. To convert degrees Fahrenheit to degrees Celsius, subtract 32, multiply by 5, and divide by 9. —, participants did not use this form of refrigeration.
[b] The denominator is the interview sample size.
[c] Measured by researchers.
[d] Ice used only in delivery.

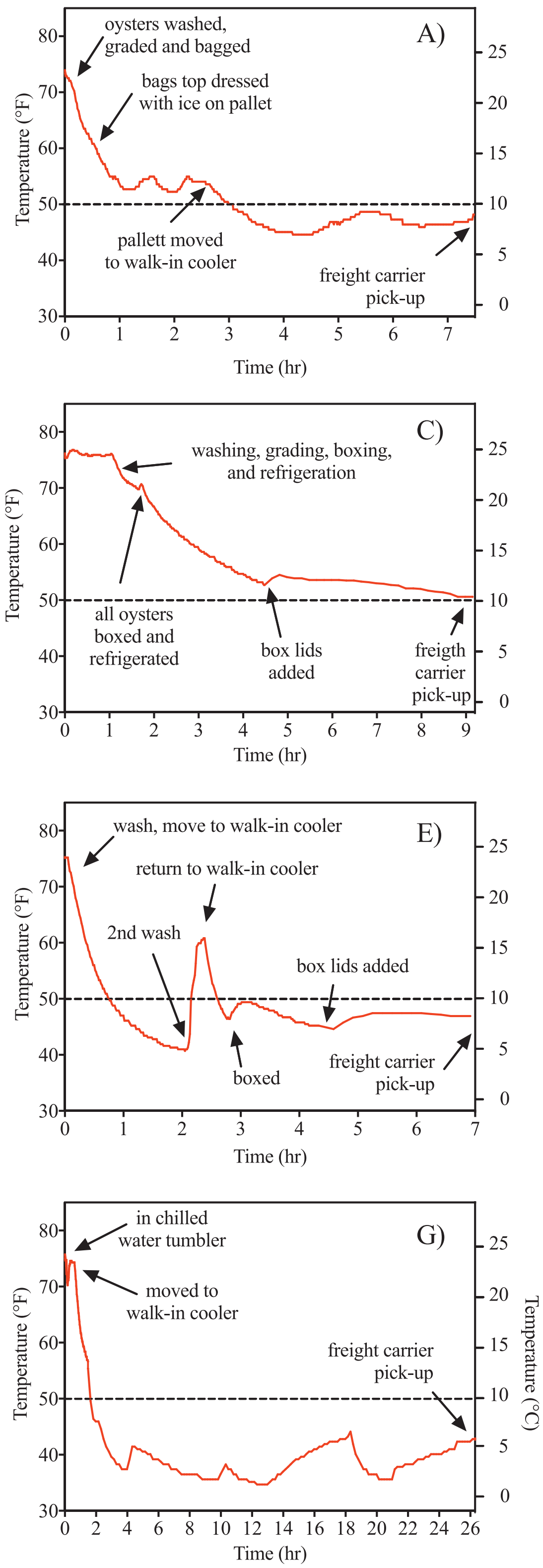

FIGURE 2. *Internal oyster temperature during harvest and on-farm processing at six farms in the Chesapeake Bay. (A and B) Producer 1 at two time points on the same day (A:* n = 2, *7 a.m. harvest; B:* n = 2, *12 p.m. harvest), (C) producer 2 (*n = 4*), (D) producer 3 (*n = 4*), (E) producer 4 (*n = 3*), (F) producer 5 (*n = 4*), and (G) producer 6 (*n = 2*). The dashed line represents the NSSP temperature criteria. The red line is the mean.*

TABLE 2. *Temperature control during oyster harvesting and on-farm processing during six farm visits, June to September 2017*

Producer (P) code	Harvest temp (°F)		Ice (yes/no)	Refrigerator temp (°F)[a]	Time (h) to achieve:		Oyster internal temp at pickup (°F)	Ideal product temp (°F)[b]
	Water	Air			Environment temp <45°F	Oyster internal temp <50°F		
P1	80	70	Yes[c]	44	4.0	4.5	47.3	40–45
P2	70	72	No	50	2.5	10.2	50.6	37–40
P3	71	70	Yes[d]	38	1.8	8.9	41.5	41
P4	70	70	No	40	3.0	3.1	46.9	<50
P5	76	72	Yes[c,d]	38	2.4	4.4	39.0	<45
P6	72	68	No	45	1.2	3.3	42.8	40

[a] Table S2 contains values in degrees Celsius. To convert degrees Fahrenheit to degrees Celsius, subtract 32, multiply by 5, and divide by 9.
[b] On the basis of interviews with producers.
[c] Layered ice.
[d] Ice slurry.

(Supporting Information text and Table S3). After determining that VCP was a significant factor, we pooled the temperature sensor data by VCP status and by stage of supply chain (Fig. 3).

We hypothesized that internal oyster temperatures would decrease as the product moved through the cold chain, a concept that agrees with self-reported cold-chain temperatures in Table 1. In a non-VCP month, there was no significant difference in internal oyster temperature comparing all supply chain groups (producers, freight carriers, wholesale, wholesale delivery, food retailers or restaurants; ANOVA: $F = 2.4$, $P = 0.18$). There was a significant difference in the box temperature among all groups (ANOVA: $F = 6.1$, $P = 0.03$), but these differences were not statistically significant when comparing neighboring groups, such as producers to freight carriers, freight carriers to wholesalers, or wholesalers to food retailers or restaurants.

In VCP months, there were significant differences in both the internal oyster temperature (ANOVA: $F = 76.4$, $P < 0.0001$) and the box temperature (ANOVA: $F = 38.8$, $P < 0.0001$). Pairwise comparisons suggest that producers, when holding product under temperature control, maintain boxes at cooler temperatures than freight carriers ($P < 0.05$), but producers and freight carriers had similar internal oyster temperatures. Freight carriers maintained boxes at warmer temperatures than wholesalers ($P < 0.0001$), which led to warmer internal oyster temperatures ($P < 0.0001$). Box temperatures were not different between wholesale and wholesale delivery or between wholesale delivery and food retailers or restaurants, suggesting that a relatively uniform environmental temperature was maintained along this portion of the supply chain. Internal oyster temperatures were cooler in wholesale delivery than wholesale ($P < 0.01$), perhaps due to the use of ice during delivery. There was no difference between internal oyster temperatures during wholesale delivery and at food retailers or restaurants. (See Table S4 for P values from all tests.)

Supply chain: outlier analysis. In addition to comparing mean values, it is also useful to analyze outliers when the temperature was warmer or colder than expected for sustained periods of time. Overall, 7 (19%) of 36 of shipments had internal oyster temperatures greater than 50°F for more than 1 h (all were in VCP months). The

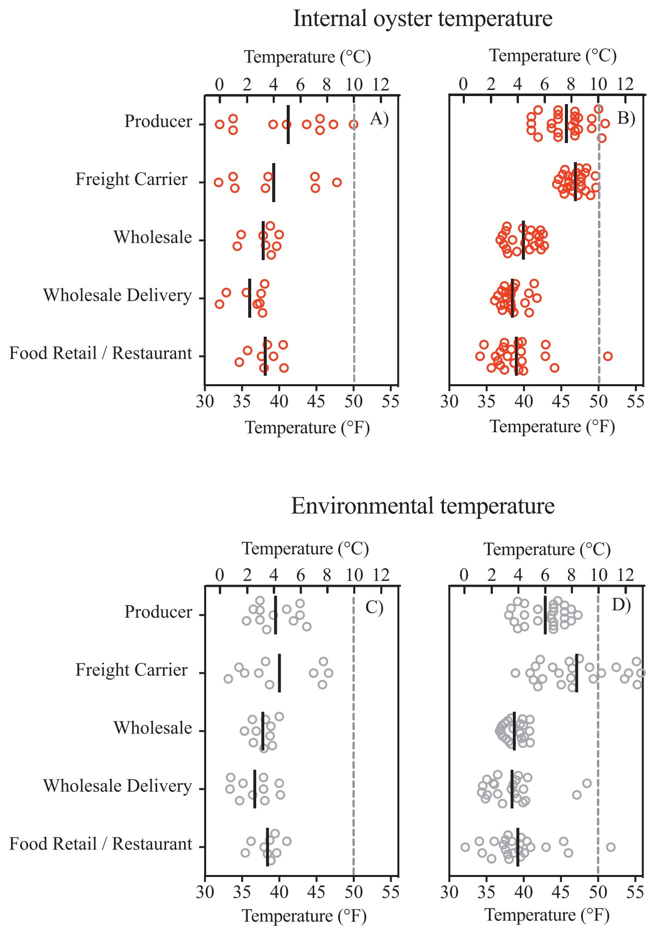

FIGURE 3. *Strip plots of (A and B) internal oyster temperature and (C and D) environmental temperature for shipments in* Vibrio *control plan (VCP) months (B and D:* n = 26*) and a non-VCP month (A and C:* n = 12*). The black bar is the mean value, red or grey circles are individual samples, and the dashed line represents the NSSP temperature criteria. Producer values are point estimates for the temperature just before pickup, while all other values are the average temperature reading for each sample.*

product temperature in these seven shipments exceeded NSSP criteria; the maximum internal oyster temperatures were 50.9 (for five shipments), 52.7, and 54.5°F (10.5 [for five shipments], 11.5, and 12.5°C). Over four-fifths of shipments (21 [81%] of 26) in VCP months were held in storage conditions above 45°F for over 1 h.

Internal oyster temperatures were less than 35°F for more than 1 h in 10 (28%) of 36 shipments, which put products at risk for freezing. Cooler internal oyster temperatures were more common in a non-VCP month than in VCP months.

Supply chain: exemplar temperature profiles. We plotted temperature profiles of six shipments to show typical examples of temperature control issues (Fig. 4). (Temperature profiles for all shipments are available in Fig. S2.) Figure 4A depicts a 100-count waxed box shipped in March 2017. The box was iced by the producer just before freight carrier pickup, and the product remained at near freezing temperatures for ~24 h, which could kill oysters or reduce shelf life (we did not visually inspect the box for gaping or mortalities). Figure 4B shows a 50-count box of oysters shipped direct to a consumer in July 2017. The packaging was a polystyrene cooler containing gel packs nested inside a cardboard box. The box was shipped by using a direct to consumer freight carrier with 2-day ground delivery. The product temperature slowly climbed from the middle 30s to the low 40s in degrees Fahrenheit during shipment, but remained well below 50°F, indicating that 2-day shipment under these circumstances was acceptable. Figure 4C and 4D show short and long periods of time when 100-count boxes of oysters were outside of temperature control. The internal oyster temperature slowly rose and then fell once temperature control was recovered by the wholesaler. Figure 4D shows one of five shipments in which internal oyster temperatures exceeded 50°F for more than 1 h (as described previously). We suspect that these issues could be due to the product being stored on a loading dock between trips in a refrigerated truck. Figure 4E depicts a spike in temperature during delivery of a 100-count box of oysters to a food retailer or restaurant in March 2017. The product temperature stabilized after the box was moved to the walk-in refrigerator. Figure 4F was more severe and shows a food retailer or restaurant in June 2017 whose refrigerator was either malfunctioning or set at an unsafe temperature for storage of shellfish.

Modeling *V. parahaemolyticus* abundance and health risks. Models of *V. parahaemolyticus* abundance and associated risks for all shipments are displayed in Figure 5. Temperature abundance profiles for each shipment are available in the Supporting Information. *V. parahaemolyticus* models estimated initial abundance at the time of harvest, which ranged from 30 to 108 counts per g. The model predicted internal growth of *V. parahaemolyticus* on the farm and before temperature control was no more than 25 counts per g overall. After temperature control was achieved, there was a gradual decline in abundance across all shipments, except one (Fig. 4F), which remained at temperatures above the refrigeration threshold throughout the majority of sensor measurements. The mean estimated *V. parahaemolyticus* abundance at the end of all shipments' cold chains (when the product was delivered) was 50.5 counts per g, with a range of 17.8 to 115.13 counts per g. The expected risk of gastroenteritis was relatively low across all shipments, with a mean risk of 0.03 cases per 100,000 servings of raw oysters and with a range of 0.01 to 0.07 cases per 100,000 servings. (Modeled *V. parahaemolyticus* abundance and associated risks for each shipment are available in Fig. S3.)

DISCUSSION

Main findings. To our knowledge, this is the first study in the United States to assess the performance of cold chains for shellfish. During summer months when VCPs were in effect, internal oyster temperatures were higher among producers, freight carriers, and wholesalers than those same groups in March, a month with cooler weather and no VCP. We attribute this difference to warmer harvest waters and higher storage temperatures among some producers and freight carriers in summer months. The NSSP advises that shellfish should be held below 50°F internal temperature and maintained in an environment that is 45°F or below by using ice or mechanical refrigeration *(24)*. When modeling the effects of temperature on *V. parahaemolyticus,* we found moderate bacterial growth before oysters entered the cold chain. Once oysters were under temperature control, models suggest no further *V. parahaemolyticus* growth occurred, and there was a moderate drop-off in bacterial abundance. This indicates that the cold chain was effective in mitigating the risk of *V. parahaemolyticus,* even when oysters were stored slightly above the NSSP limits. Exposure to warm temperatures immediately following harvest and before temperature control is achieved appears to be the period of greatest risk for *V. parahaemolyticus* growth in Chesapeake Bay oyster supply chains. Our findings are supported by a validation study confirming that the model agrees with *V. parahaemolyticus* growth rates in postharvest Chesapeake Bay oysters *(26)*.

Comparison to previous work. Performance of shellfish cold chains is relatively understudied; we identified only one report from Australia that used temperature sensors to assess cold chains *(19)*. In that study, 42 to 50% of shipments were not in compliance with Australian Shellfish Quality Assurance Programs due to a combination of time and temperature issues, including cold abuse *(19)*. Supply chains in the Chesapeake Bay faced similar challenges related to temperature control; however, it is difficult to compare between the studies because different regulations are used in each country. Similar to the Australian study, we found each group within the supply chain had aspects of temperature control that could be improved. In fact, several participants noted that continual improvement is what they seek in their own business and for businesses they deal with. One participant in our study noted, "selling oysters is a team effort up and down the

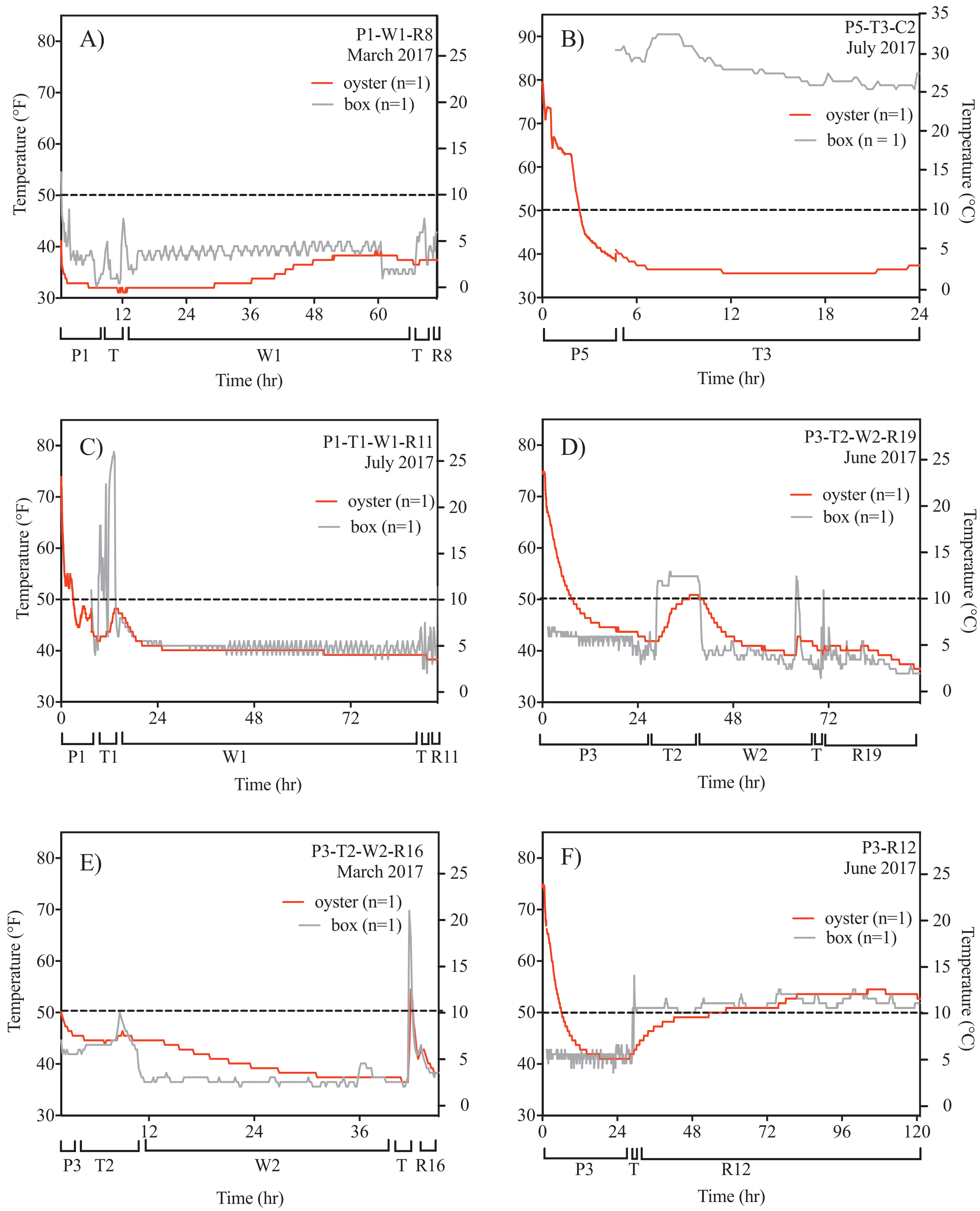

FIGURE 4. *Six examples of temperature-related issues in Chesapeake Bay–farmed oyster cold chains. Temperature profiles for all oyster shipments are provided in the Supporting Information. Temperature profiles of internal oyster temperatures and environment temperatures from harvest to food retailers or restaurants. The sensor sampling interval was 1 min at the oyster producer and 10 min in the supply chain. The dashed line represents the NSSP temperature criteria. P, producer; T, freight carrier (truck); W, wholesale; R, food retailer or restaurant; C, consumer. The number following P, T, W, or R was assigned to each participant to provide anonymity.*

supply chain." What follows is a discussion of temperature control at each step of the cold chain and identified areas for improvement.

Oyster production. Achieving rapid temperature control of harvested oysters is critical for food safety. Our findings were comparable to mechanical refrigeration used by Jones et al. *(15)* but slower than using ice for cooling. Differences between studies may be due to the experimental design, because our study tracked the temperature of the entire harvest with multiple sensors. Producers who wish to improve product cooling may consider using temperature sensors for internal validation studies with the help of local seafood extension agents or state regulators. Several state regulators in the northeast have implemented temperature sensors at farms to learn more about shellfish harvest and

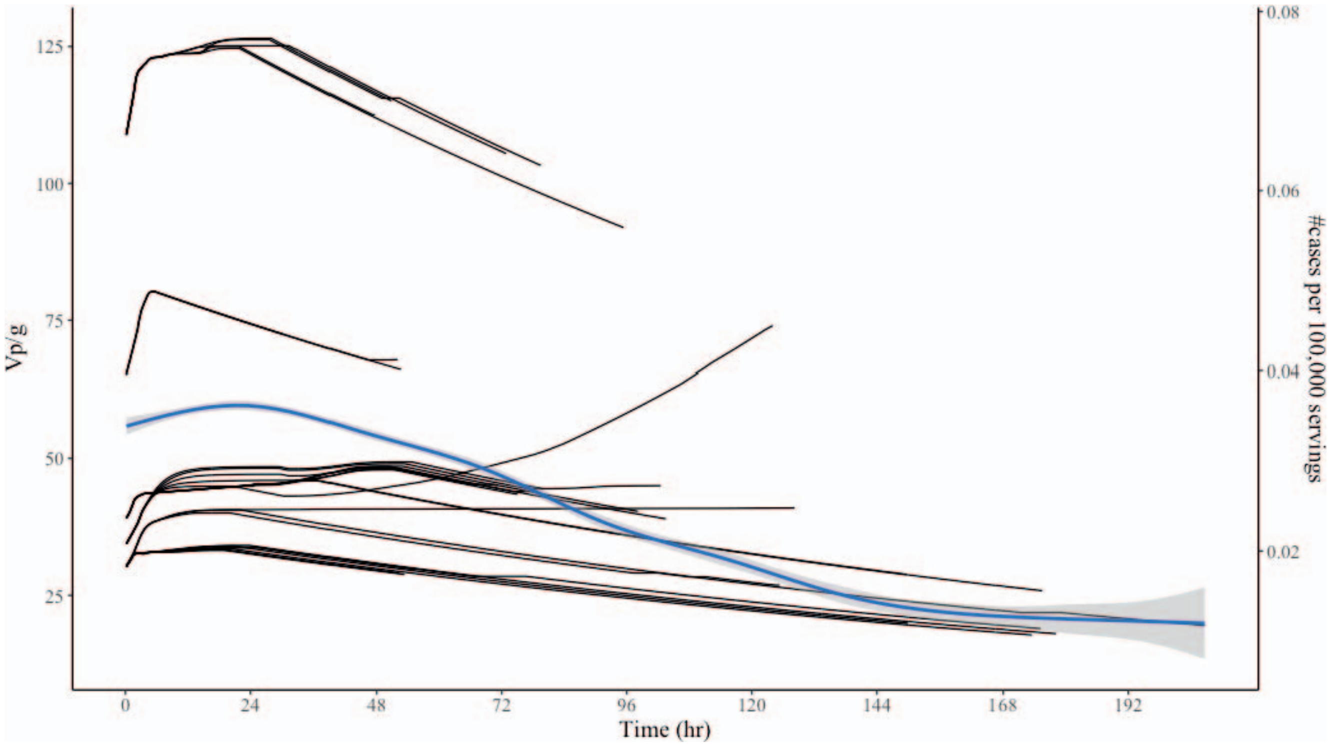

FIGURE 5. *Estimated* Vibrio parahaemolyticus *abundance per gram oyster tissue (left* y *axis) and the risk of illness as cases per 100,000 servings (right* y *axis) from oysters produced in the Chesapeake Bay and shipped to surrounding states. Estimations of both can be displayed simultaneously due to the linear approximation of the beta-Poisson dose-response model.* Vibrio *abundance at harvest was estimated on the basis of water temperature, and growth in supply chains was calculated by using iterative temperature-based models. The black lines represent the abundance or risk of individual oyster shipments. The blue line depicts the mean abundance or risk across all oysters estimated by using a generalized additive model, and the grey band displays the corresponding 95% confidence interval. Vp/g,* V. parahaemolyticus *bacteria per gram. This figure only presents data from shipments made in* Vibrio *Control Plan months.*

processing. If cooling the product for same-day shipping is challenging, oysters can be harvested and stored overnight in refrigeration to ensure adequate cooling has been achieved.

Transportation. Freight carriers in our study transported products from producers to wholesalers by using refrigerated trucks maintained at a median self-reported temperature of 34°F (1.4°C). As one freight carrier described it, "my job is to maintain temperature only, not to bring the temperature down." A certain amount of time outside refrigeration is expected, as products are loaded and unloaded at facilities. In some cases, we measured environmental temperatures above 45°F, and when prolonged, they affected internal oyster temperatures. Two areas that could be improved are reducing the time products spend unrefrigerated at loading docks or transfer stations and adding supplemental ice if products are stored at loading docks or transfer stations for extended periods of time.

Wholesale. Wholesalers purchased oysters from producers and sold them to food retailers and restaurants. Wholesalers had the most advanced mechanical refrigeration systems of any group in the supply chain and provided a stabilizing force on the cold chain with self-reported temperatures between 38 and 42°F (3.3 and 5.6°C). Our sensor data suggest environmental temperatures in storage and delivery were not an issue for wholesalers. Holding oysters at near freezing temperatures in cooler months was an issue that could be improved upon and could have been caused by excessive icing during delivery to food retailers and restaurants. Wholesalers could contribute to improving overall performance of their cold chains by conducting their own or third-party quality control audits, which some participants indicated as an area for future work.

Retailers and restaurants. For food retailers and restaurants, oysters are just one of many products they purchase. Oysters can be ordered most days of the week, and product turnover is high. Large food retailers, such as supermarket chains, specify product temperatures in their hazard analysis and critical control point plans and can influence the practices of upstream businesses. Oysters in this study were maintained in refrigerators set to a self-reported temperature of 38°F, and our sensor data indicate that temperature control was not an issue for restaurants or food retailers. There was no consistent method for processing and storing oysters; this is an area in which education, training, and cross talk between the restaurant industry and seafood industry could be useful. Participants were aware of food safety practices specific to shellfish, such as maintaining tags for 90 days, and few had been involved in product recalls. We did not track oyster mortality; however, some participants noted that on occasion boxes had to be returned or discarded due to gaping. Addressing the underlying reasons behind gaping could be a topic for future studies.

***Vibrio* modeling.** The cold-chain temperature data supported models of *Vibrio* growth and illness risks, and these models indicate a very low risk of gastroenteritis from consuming the oysters in this study. The models, derived from the U.S. Food and Drug Administration's risk assessment of *V. parahaemolyticus* in raw oysters *(32),* have been widely incorporated into cold-chain supply regulations in shellfish *(24),* and its framework continues to be used when estimating the risk of *V. parahaemolyticus* *(30)*. In this study, abundance and risk estimates were restricted to values of central tendency. Further incorporating the known variability (e.g., abundance across oyster samples) and parameter uncertainty into these models would likely increase the estimated range for the risk of gastroenteritis from consuming oysters in the sampled supply chains. Future work will incorporate such variability and uncertainty to provide a probabilistic risk assessment of these cold chains. Future work could also improve upon the existing risk models. For example, the current model framework currently relies solely on water temperature to determine bacterium abundance at the point of harvest *(32)*. However, many studies have indicated that additional environmental parameters, such as salinity and turbidity, can improve risk estimation *(14),* particularly in the Chesapeake Bay *(9)*. Furthermore, the risk assessment was completed in 2005, and since then a number of studies have further investigated the population dynamics of *V. parahaemolyticus* during a variety of harvest and post-harvesting practices *(7, 9, 15, 17, 21)*. Incorporating these studies into future modeling efforts of the supply chain will likely further improve our understanding of *V. parahaemolyticus* populations and the risk of foodborne illness from consuming raw oysters. Others have developed predictive models of *V. parahaemolyticus* growth in Pacific oyster supply chains under different scenarios *(11)*.

Strengths and limitations. There are several strengths and limitations of the study. The temperature sensors used in the study had significant benefits in their small size and ability to fit inside an oyster and take readings over several weeks. The sensors, however, required manual inspection, did not measure humidity, and were relatively expensive (US$50 each). The sensors did not have global positioning system or radio frequency identification capabilities, so we contacted participants to ascertain the time that boxes arrived or departed facilities or both. Our study included a limited number of visits to producers; repeated visits were performed at half of the study farms, and visiting more farms would improve our ability to generalize about temperature control at the farm level. Our farm visits were observational, and future research could manipulate variables to assess the effectiveness of different practices related to harvesting and handling. Many freight carriers, wholesalers, and retailers and restaurants were repeatedly sampled during the study period, which increased our ability to generalize about this part of the supply chain. The oyster supply chains in the study did not involve international shipments, and future cold-chain studies should include international seafood supply chains.

Farmed oysters are an environmentally sustainable product requiring no feed inputs, and opportunities to refine food safety in production, processing, and supply chains can support growth within the industry. For many, oysters are a luxury food; however, lessons learned from oyster cold chains can be applied to other refrigerated seafood products in the United States and elsewhere. We hope the findings of this study provide a better understanding of cold chains for shellfish, provide baseline data for modeling *Vibrio* growth, and support industry and government regulations and policy development.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-18-044.s1.

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