Investigating risk factors for mortality and reovirus infection in aquaculture production of soft-shell blue crabs (*Callinectes sapidus*)

Matthew I. Spitznagel\textsuperscript{a}, Hamish J. Small\textsuperscript{b}, Julie A. Lively\textsuperscript{c}, Jeffrey D. Shields\textsuperscript{b}, Eric J. Schott\textsuperscript{a*}

\textsuperscript{a}Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD 21202, USA. mspitznagel@umces.edu, schott@umces.edu

\textsuperscript{b}Virginia Institute of Marine Science, The College of William & Mary, P.O. Box 1346, Gloucester Point, VA 23062, USA. hamish@vims.edu, jeff@vims.edu

\textsuperscript{c}School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA. Janderson@agcenter.lsu.edu

*Corresponding author. Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, 701 East Pratt St., Baltimore, MD 21202, USA. Email: schott@umces.edu; phone 410-234-8881

Abbreviations

\textsuperscript{CsRV1:} *Callinectes sapidus* reovirus 1

\textsuperscript{RT-qPCR:} reverse transcriptase quantitative polymerase chain reaction
Abstract:
Crustacean aquaculture is prone to mortality from the combined effects of disease agents and the stresses associated with crowded, closed conditions. The culture practice of producing soft-shell blue crabs is no exception, suffering from mortality of about 25%. The virus, *Callinectes sapidus* reovirus 1 (CsRV1), has been reported at high viral loads in crabs dying in soft-shell shedding facilities. We investigated the relationship between crab mortality and CsRV1 prevalence and load in soft-shell crab production and whether death and virus infection correlated with identifiable aquaculture practices, environmental stresses, crab characteristics, or geographic regions. The patterns of CsRV1 prevalence, infection intensity, and mortality in blue crab aquaculture were studied in the Chesapeake Bay and Gulf of Mexico, USA. Using a genome-targeted assay, we compared virus loads in live and dead aquaculture crabs by individual sex and injury state from recirculating and flow-through systems of variable salinity, temperature, and crabs per aquaculture tank. Mortality was two-fold higher in flow-through aquaculture systems (33%) than in recirculating aquaculture systems (16%). Flow-through aquaculture systems had higher daily water temperature variability than recirculating aquaculture, and hypoxic events were observed only in flow-through systems during this study. Heavy CsRV1 intensity was found in 62% of all pre-molt mortalities in production compared with 7% of successfully molted soft-shell crabs. The CsRV1 virus load in dead crabs was elevated in higher salinity conditions. Using a mixed-effect model, the random effects of location and time were more significant than salinity in predicting CsRV1 load in all crabs and dead crabs. Our results support previous research showing that recirculating aquaculture has lower mortality in soft-shell production, and confirms the association of high viral loads of CsRV1 with crab mortality in these production systems. Moreover, the findings indicate that although CsRV1 is ubiquitous in these systems, management of culture conditions such as salinity and temperature may limit virus-associated mortality.

**Key words:** *Callinectes sapidus*, disease, CsRV1, molting, sustainability

**Highlights**
A collaborative study with US soft crab producers revealed that 22% of all pre-molt crabs die before molting, with 16% mortality in recirculating systems compared to 33% mortality in flow through systems.

The pathogenic virus, CsRV1, was present at high levels in the majority of crabs that died in soft-shell production but only in 7% of successfully molted crabs.

Virus prevalence in dead crabs was associated with lower salinity, but random effects of location and date were also associated with prevalence and load.

Although CsRV1 is ubiquitous, management of culture conditions such as salinity and temperature variation may limit virus-associated mortality.
1. Introduction:

Crustacean aquaculture supports extensive seafood industries worldwide, yet disease agents remain a major factor limiting production (Shields & Overstreet 2007; Stentiford et al., 2012; FAO, 2017). Many factors may exacerbate both disease susceptibility and mortality in aquaculture, such as high population density, stress of confinement, water quality, temperature fluxes, or hypoxia (Le Moullac & Haffner, 2000; Mohanty et al., 2018). Certain aquaculture practices, including disposal of dead aquaculture animals into neighboring marine waters, use of dead animals as bait, or untreated effluent release, can facilitate the spread of disease, yet these practices remain common in the industry (Lafferty et al., 2015; Shields 2017; Flowers et al., 2018).

The target of one of the world’s four largest crab fisheries, the blue crab, *Callinectes sapidus*, is at the center of a multi-million dollar aquaculture practice in the eastern United States (MD DNR, 2018; NOAA NMFS, 2016; FAO, 2018). This fishery-dependent practice involves holding pre-molt blue crabs, known colloquially as peelers, in shallow tanks until they molt into soft-shell crabs, which are a value added product consumed regionally and frozen for international trade (Oesterling, 1995; Chaves & Eggleston, 2003; Tavares et al., 2017). The molting process is inherently stressful for crabs (deFur et al., 1988; deFur, 1990). Combined with external stressors associated with harvest and aquaculture, molting stress may contribute to the reported 25% - 50% mortality in soft-shell crab production (Chaves & Eggleston, 2003; Oesterling, 1995). Despite numerous methods and manuals designed to help optimize culture conditions (e.g., Ogle et al., 1982; Oesterling, 1995), peeler crab mortality remains unpredictable and high. Recent studies have investigated the potential for disease to contribute to crab mortality in the shedding systems used by the industry in soft-shell production (Bowers et al., 2010; Rogers et al., 2015).

Across their US range and in Brazil, blue crabs are infected by the pathogenic virus *Callinectes sapidus* reovirus 1 (CsRV1, Bowers et al., 2010; Flowers et al., 2015). CsRV1 (previously identified as Reo-Like Virus, RLV) was identified as a cause of crab mortality in captive crabs in the 1970s (Johnson, 1977; Johnson, 1978), and subsequently in soft crab aquaculture production and a scientific blue crab hatchery (Bowers et al., 2010). The virus infects hemocytes and hemopoietic tissue (Johnson, 1977; Tang
et al., 2011). Injection of viral filtrate leads to paralysis and death of crabs in days or weeks and is associated with infiltration of hemocytes into neural tissues (Johnson, 1983; Bowers, et al., 2010).

Application of sensitive quantitative molecular assays for CsRV1 has shown a mean prevalence of 20% in wild crabs surveyed from the northeast United States, with most infected animals harboring $<10^4$ virus genomes per mg crab muscle tissue (Flowers et al., 2015). In contrast, an earlier study of soft-shell crab aquaculture in Maryland and Florida, USA, found CsRV1 in 71% of dead peeler crabs using an RNA electrophoresis assay that has an estimated detection limit of $10^5$-$10^6$ genome copies per mg muscle tissue (Bowers et al., 2010). This association of CsRV1 with peeler crab mortality suggests that CsRV1 may be an important contributor to mortality during blue crab molting and a source of considerable economic loss to the soft-shell crab industry (Johnson, 1983; Flowers et al., 2018).

Few studies have investigated how disease may interact with aquaculture practices to cause crab mortality during soft-shell production. It is not known how crab mortality and CsRV1 prevalence are affected by culture practices, environmental holding conditions, individual crab characteristics, or specific geographical location of facilities. To determine whether specific biological or environmental risk factors exist in soft-shell crab production, we partnered with soft crab producers in Maryland, Virginia, and Louisiana to measure different parameters used in shedding systems and correlate these with virus prevalence and loads using a quantitative real time polymerase chain reaction (RT-qPCR) assay (Flowers et al., 2015). Potential relationships between crab mortality, CsRV1 infection, aquaculture system type (flow-through vs. recirculation), salinity and water temperature, and the individual size, sex, and molting state of crabs were investigated using generalized linear mixed effect modelling (GLMM). Our findings may be useful for identifying management practices that reduce CsRV1-associated mortality and increase successful soft-shell crab production.
2. Materials and methods:

2.1. Crab collection and handling

Peeler crabs, culture system water, and culture practices were surveyed at soft-shell production facilities in Maryland, Virginia, and Louisiana from May to September, in 2016 and 2017. Freshly harvested live peelers that had not been placed in aquaculture, peelers that died in aquaculture, and successfully molted soft crabs were collected by participating watermen during one-week periods in each month, with 7-25 crabs of each type sampled. Live and dead crabs were transported on ice to the Virginia Institute of Marine Science (VIMS), Louisiana State University AgCenter (LSU AgCenter), or the Institute of Marine and Environmental Technology (IMET) depending on the location of the production facility. Crabs were either measured and dissected immediately or stored at -20°C for later analysis. All crabs were measured (carapace width) and assessed for obvious limb loss or puncture injuries prior to dissection. A 1-4 cm section of walking leg was removed from each crab and frozen, and those sourced from VIMS were preserved in 95% ethanol. In addition, crab sex, sample date, molt stage, type of shedding system, and location were recorded for all crabs on accompanying data sheets. Molt stage was assessed by the color along the margin of the propodus of the 5th walking leg and progressive splitting of the carapace. The red color along the margin typically indicates molting will occur within 3 days, pink with molting in 1-2 days, and splitting of the carapace epimal suture a sign that molting is imminent, and full molt indicating the full emergence of the new instar. All leg samples were transported to IMET for CsRV1 quantification.

Soft crab producers participating in the study were located in Pasadena, Patuxent, Tilghman Island, and Rock Hall, Maryland, West Point, Sarah’s Creek, and Chuckatuck Creek, Virginia, and Dulac and Franklin, Louisiana, USA (Figure 1). Two additional sites at Bear Creek, MD and Violet, LA were initially surveyed, but were excluded from statistical analysis as high-mortality outliers that were affected by hypoxia and toxic nitrite levels beyond the limits required for sustainable aquaculture operation. Sites were categorized by open (flow-through) or closed (recirculation) water circulation type. Both systems at Tilghman Island were flow-through sites located within 1 km of each other, but were independent
businesses and operations (Table A.1.) Water samples (10 ml) of all systems were collected daily by watermen, were assessed for salinity by refractometer, while nitrate, nitrite, general hardness, carbonate hardness, and pH were measured by aquarium test kit (5 in 1 Aquarium Test Strips, API®) for Maryland sites. Water temperatures in culture systems were measured hourly by automated HOBO™ dataloggers (Onset Corporation®). Mean water temperatures and salinities of each sampling period were computed from daily water samples for site comparison. Water temperature variability was defined as the maximum difference between any hourly point and the weekly period average. Participating watermen recorded whether they fished peeler crabs themselves or purchased peelers from other fishermen, the number of aquaculture tanks they used, average number of crabs per tank, and the number of crabs dying or molting to soft shell on each day of the survey. Mortality level was calculated by dividing the total number of reported crabs molting successfully by the sum of crabs dying or molting during a 7 day survey period.

2.2. Crab dissection and RNA extraction:

Crabs were dissected with sterile wooden rods and razor blades, and all handling and crab surfaces were cleaned with ELIMINase™. Aliquots of 25-100 mg of crab muscle and connective tissue were excised from a walking leg of each crab and homogenized in 1.0 mL RiboZol® (VWR Scientific) using ceramic beads in a MP® FastPrep24 homogenizer. RNA extraction methods were modified from those used by Flowers et al (2015). After RiboZol®-chloroform separation of RNA into isopropanol, two 12,000 g centrifuge washes with 500 µL 75% ethanol were carried out to increase RNA purity from levels obtained with earlier sampling methods. Resulting RNA pellets were then dissolved in 1 mM EDTA to decrease RNA degradation, and RNA purity and concentration were evaluated by NanoDrop™ spectrophotometry.

2.3. Quantification of CsRV1:

PCR primer selection and dsRNA standard preparation were adapted from Flowers et al (2015). The primer pair 5′-TGCCTTTGACGTCCAAAGAG-3′ (RLVset1F) and 5′-GCGCCATACCGAGCAAGTCAAAT-3′ (RLVset1R) are designed to detect an amplicon from the
ninth genome segment of CsRV1 (GenBank entry KU311716) (Flowers et al., 2016). Standard curves of the CsRV1 genome were produced by purifying viral dsRNA from crabs infected with greater than $10^{8}$ copies per mg muscle. Enrichment of dsRNA and verification of purity and quality followed the protocol of Bowers et al (2010). Standard concentrations ranging from $3.4 \times 10^{7}$ to 10 genomes per µL were dissolved in 25 ng per µL yeast tRNA and used for sample comparison.

The qPCR reagents, thermocycler parameters, and process for annealing primers to crab RNA were modified from Flowers et al. (2015). The qPCR reaction components included 1 x One-Step Master Mix, Low ROX (qScript™ One-Step qRT-PCR Kit, Low ROX, Quanta Bio), SYBR® Green (Quanta) and 500 nM of each primer. Primers were dissolved in 1mM ethylenediaminetetraacetic acid (EDTA).

Amplification was conducted by using 40 cycles of 5 s at 95 °C (melting) followed by 30 s at 61 °C (annealing and extension), followed by melting point analysis from 61 °C to 95 °C for verification of the correct amplification product.

2.4. Statistical analysis:

Statistical tests were conducted using JMP® Pro 13 (SAS, 2018) and the R 3.4.4 statistical package (R Core Team, 2018). Initial statistical correlations between mortality, CsRV1 prevalence and intensity, and specific aquaculture and crab variables were tested using correlation matrices. Significant correlations were defined as those where $p \leq 0.05$. In the case where any significant correlation was identified or more complex relationship noted, $\log_{10}(x+1)$ transformations were done prior to running ANOVA or GLM models. The Shapiro-Wilk test for normality and Levene’s test for homoscedasticity were applied when appropriate. When analyzing individual factor comparisons, parametric pairwise comparisons were tested via t-test, whereas one-way ANOVA with Tukey’s Honest Significant Difference test was used for multiple comparisons. Non-parametric comparisons used Wilcoxon rank-sum testing with Steel-Dwass pairwise comparison to determine significant differences. In cases where continuous data was being compared, a series of linear regressions were undertaken comparing appropriate data transformations with a regression t-test and sum-of-squares lack of fit analysis. Stepwise selection of best GLMM was
conducted using Akaike’s information criterion (AIC) in R (stepAIC function, MASS package, lmer, lme4 package, R Core Team, 2018) was used to determine the factors that best model crab mortality and CsRV1 prevalence and intensity of infection. In individual crab data models, date was used as a random effect in modelling because crab data was recorded daily rather than weekly, introducing potential sample clustering effects if uneven sample sizes and replication at different locations and times were not accounted for (Thorson & Minto, 2015). Due to geographic separation from the Chesapeake region and the disparate number of sites, the crab data from Louisiana was not included in the statistical models.

3. Results

3.1. Aquaculture mortality by site and date

The mean proportion of dead crabs among the eight culture facilities sampled in the Chesapeake Bay region was 21.7 ± 2.8% (n=12,172 crabs) (Table A.1). The mean salinity range was 3-20 psu in Virginia and 6-18 psu in Maryland. Mean temperature ranged from 67 to 86°F with a maximum peak of 99°F in Virginia and 73 to 85°F with a maximum peak of 90°F in Maryland. Mortality was significantly greater in flow-through water aquaculture systems (32.9 ± 4.3%; n_site = 3, n_time = 7) than in recirculating water systems (16.4 ± 3.1%; n_site = 5, n_time = 13) (Figure 2, Student’s t, d.f. = 18, t = -3.11, p = 0.0060). When we compared culture conditions between system types, salinity was significantly lower in recirculating systems (Table A.1; Student’s t; d.f. = 18, t = -2.55, p = 0.02). All systems surveyed with average salinities below 8 psu had mortality of 15.0% or less; however, there was no significant difference in mortality based on salinity alone (Regression ANOVA, F = 2.48, p = 0.13).

The final model for peeler crab mortality had significant system type (slope_recirculation = -21.8, p < 0.0001) and temperature variation fixed effects (slope = -1.20, p = 0.03; Model A, Table 1). Daily fluctuation in water temperature did not differ significantly between flow-through and recirculating systems (Figure 3, Student’s t, d.f. = 1, t = -1.52, p = 0.09). However, maximum temperature fluctuation measured at the flow through facility at Sarah’s Creek, VA, was more than twice as high as those at
recirculating sites. The Sarah’s Creek facility experienced 21.3% mortality on average: lower than the flow-through system average, but comparable to the overall shedding mortality in this study. No other significant relationships or variables correlated with crab mortality.

3.2. CsRV1 infection in aquaculture analyzed by individual crab

Throughout the aquaculture facilities surveyed in MD and VA, 75.4% of dead peelers (n = 305) were infected with CsRV1, compared with 23.9% of live soft-shell crabs (n = 184) and 33.3% of freshly harvested live peelers (n = 60; Figure 4a-c). The difference in survival was even more pronounced when considering infections of >10^6 CsRV1 genome copies per mg muscle tissue, with 62.3% of dead peeler crabs surveyed exceeding this infection intensity compared with 7.1% of live soft shell crabs (n = 288; Figure 4a-c, Table 1, Model B). Successful molting was the only significant fixed effect (slope = -3.16, p < 0.0001) with location (variation 1.28, p < 0.0001) and date (variation 1.88, p < 0.0001) as significant random effects associated with CsRV1 genome copy number (Table 1, Model B). No other factors, including salinity, system type, temperature, injury, or crab sex were significant factors influencing CsRV1 loads.

Considering specific environmental variables on a shedding system basis, higher salinity aquaculture sites experienced higher prevalence levels of the virus in dead peeler crabs (Figure 5). Prevalence in dead peelers best fit a reciprocal relationship to salinity (CsRV1 Prevalence (%) = 98.0 – 251.3/Salinity (psu); R^2 = 0.4374, ANOVA d.f. = 19, F = 13.99, p = 0.0015).

Salinity was the only fixed effect retained in the reduced mixed models of CsRV1 infection intensity (Table 1, Model C). The salinity effect was only marginally significant (p = 0.0498) with a low magnitude negative relationship (slope = -0.13) once other variables were accounted for in the total model. The random effects of date (variance = 1.05, p < 0.0001) and location (variance = 4.94, p < 0.0001) were more significant and higher magnitude. No other effects were significant, including injury to crabs.

3.3. Louisiana mortality and prevalence data
At two Louisiana shedding facilities surveyed in 2016, the percentage mortality of dead crabs was 14.5 ± 5.5% (n= 652 crabs) (Table A.2). In 2016 and 2017, the prevalence of CsRV1 in dead crabs was 21.9% (n = 82). Only one dead crab from the Franklin facility had a detectable CsRV1 infection. Sampling of live crabs in November 2017 found that both soft shell crabs (n=20) and live peelers (n=37) had a prevalence of CsRV1 of 5%, with only one heavily infected crab detected. Due to the low site and crab sample sizes from this distinct geographical region, Louisiana data was not included in the GLMM analyses (Table A.3). Aquaculture salinity was measured at 0-3 psu at Louisiana sites, while the mean temperature range was 70-85°F.

4. Discussion:

By collaborating with soft crab producers in three states, this study reconfirmed that soft crab production has variable and sometimes high mortality and that well-controlled recirculating aquaculture systems are crucial to minimize peeler crab mortality. Average peeler mortality was similar to that seen in a prior study in North Carolina, U.S.A., that reported 23% mortality in blue crab shedding systems (Chaves & Eggleston, 2003). In contrast to the North Carolina blue crab study, flow-through systems examined in the current study had twice the mortality of recirculating systems, where one in three peelers died on average. Despite the global importance of mortality and disease in crab aquaculture systems (Zhang et al., 2004, Deng et al., 2012; Oesterling 1995), information on mortality in crab aquaculture is scarce (FAO, 2018). Reports on aquaculture practices of Scylla spp. in southeast Asia and Africa indicate mortality levels similar to or higher than that observed in the short-term production of soft-shell blue crabs (Keenan, 1999; Mirera & Moksnes, 2015).

The lower mortality of crabs in recirculating systems was likely associated with better control of environmental variables compared to flow-through systems. For example, temperature variation was less in the recirculating systems, albeit the trend was not significantly different (p=0.08) between system types. Other environmental parameters may also contribute to crab mortality in poorly-controlled or flow-through systems. For example, we excluded one flow-through system in the Baltimore area from the
study because of separate events of hypoxia (<2 mg/L) and high nitrite (>10 mg/L) which were both associated with crab mortality of over 50%. In these latter examples, mortality was not likely a result of environmental variability *per se*, but the fact that one water quality parameter exceeded a biological threshold for crab survival.

Economically, peeler mortality represents a loss of time, effort and money to watermen. This amount of mortality appears to be a long-accepted cost of doing business for individual crab shedders, who may lose several thousand dollars’ worth of peeler crabs per week, depending on the size of their shedding operation. Estimates of peeler crab mortality have been identified as a critical need by the Chesapeake Bay Stock Assessment Committee (CBSAC, 2018). Based on 2016 data for peeler harvests, Maryland harvested 1225 metric tons of peeler crab (MD DNR, 2018, pers. comm.), while Virginia harvested 333 metric tons, and Louisiana 65 metric tons (NOAA NMFS, 2016). Based on the 22% mortality observed in this study, an estimated 356.2 metric tons of peelers (worth $2.58 million) died prematurely in these three states in FY 2016. Applied to the entire United States peeler harvest, this mortality represents a loss of 408.2 metric tons of blue crab. At 250g estimated average weight per peeler, this represents 1.63 million peeler crabs lost in FY2016.

This study revealed additional information about the association of CsRV1 infection with peeler crab mortality. When analyzed on the basis of individual crabs, CsRV1 infection was the most significant predictor of peeler crab death regardless of shedding system type. Heavy infections were found in almost two thirds of the dead peeler crabs, and was nine times higher than the prevalence of heavy infections in successfully molted soft-shell crabs. Estimating the economic consequences of the 62% of peelers that died with heavy CsRV1 infections in MD and VA suggests that the virus is associated with the loss of 212 metric tons of peeler crabs worth $1.53 million. CsRV1 is not the only reovirus to kill crabs in aquaculture: both *Eriocheir sinensis* (Zhang et al., 2004) and *Scylla serrata* (Deng et al., 2012) are reported to suffer from mortality associated with reoviruses.

Crabs with high virus loads likely did not acquire CsRV1 within the shedding systems. The prevalence of CsRV1 infections in live peeler crabs entering aquaculture (33%) was nearly the same as
the estimated prevalence of CsRV1 from the combined numbers of dead and live crabs processed in these systems (35%). That is, the overall prevalence of CsRV1 in peelers did not increase during the average of 5 days in short-term culture. This indicates that although virus replication within infected crabs may be accelerated during aquaculture, CsRV1 transmission between crabs in soft-shell crab production is minimal over the brief culture periods involved. We speculate that a certain fraction of crabs enter soft crab aquaculture with naturally-acquired CsRV1 infections which rapidly progress due to the additional stress of molting and sub-optimal conditions, and eventually contribute to mortality of peelers at the levels observed in this study.

Soft crab aquaculture conducted at lower salinities appeared to experience lower overall CsRV1 infection prevalence and intensity within the Chesapeake Bay than high salinity sites, but the difference was not significant. The current study documented that prevalence of CsRV1 was much lower in dead peelers from Louisiana shedding systems compared with dead peelers from Chesapeake systems. All Louisiana shedding facilities were at low salinity (0.6-6.5 psu), and also had lower dead loss than Chesapeake shedding facilities. While Louisiana sampling data were too sparse to permit powerful statistical comparison, the low mortality and CsRV1 prevalence found in Louisiana point to a need to better understand the effects of salinity on crab survival and CsRV1 infection in aquaculture. A 2002 North Carolina Fisheries Grant research report describes an intriguing study that shows very low peeler mortality in low salinity (2 psu) shedding systems (NC Fishery Resource Grant Program, 2002).

Together, our results and the referenced studies provide motivation to study whether low salinity (in harvest water or aquaculture systems) reduces CsRV1 prevalence and/or peeler mortality.

It is apparent that infection trends were affected by factors that we did not measure or control. First, strong random effects of site and date on CsRV1 prevalence were identified by GLMM. Second, CsRV1 prevalence levels at middle salinity sites did not fit well with the salinity regression, indicating that other factors influence overall infection rates in mesohaline conditions. The site and time factors in final modelling of CsRV1 prevalence suggest that the actual location of crab harvest, position in the estuary, or related factors may influence disease prevalence even more directly than salinity. This site-by-site
variation agrees with prior studies of CsRV1 prevalence in wild crabs, which showed wide variation by site, year, or month (Flowers et al., 2018; Flowers et al. 2015).

The association of high CsRV1 loads with crab mortality in aquaculture has implications for release of virus into the environment from aquaculture, particularly from flow-through systems. The 212 metric tons of heavily infected dead peelers estimated as discards from this study represents over 800,000 diseased crabs, which are potentially discarded into the Chesapeake Bay annually. This concern is supported by a prior study that documented elevated CsRV1 prevalence in blue crabs close to flow-through shedding facilities (Flowers et al., 2018). Although the transmission route of the virus remains unknown, many viruses in decapod crustaceans remain infective in carcasses (Oidtmann et al., 2018). Replacing flow-through systems with recirculating aquaculture and conscientious land-based disposal of dead crabs would interrupt the flow of CsRV1 to uninfected wild crabs, to the benefit of the fishery, watermen, and the environment.

Declarations of interest:

None.

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**References:**


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Table and Figure captions:

Table 1. Final reduced generalized linear mixed models (GLMM) predicting significant effects on crab mortality and CsRV1 infection intensity.

Figure 1. Map of soft-shell blue crab aquaculture facilities surveyed in 2016-2017.

Figure 2. Blue crab (*C. sapidus*) mortality (mean±s.e.) observed in flow-through and recirculating water aquaculture systems in the Chesapeake Bay soft shell crab industry.

Figure 3. Daily fluctuation from mean water temperature in flow-through and recirculating crab aquaculture systems (Student’s t, d.f. = 1, t = -1.52, p = 0.09).

Figure 4. Frequency histograms of log CsRV1 loads observed in Chesapeake a) live peelers, b) dead, and c) soft shell blue crabs from aquaculture.

Figure 5. Prevalence of CsRV1 infection in dead peeler crabs fit to a reciprocal regression (CsRV1 Prevalence (%) = 98.0 – 251.3/Salinity (psu); $R^2 = 0.4374$, ANOVA d.f. = 19, $F = 13.99$, $p = 0.0015$).

Appendices:

Table A.1. Record of sites, dates, water quality, system type, crab mortality, and CsRV1 prevalence data from surveyed Chesapeake soft shell crab aquaculture sites. Live soft and peeler crab sampling was progressively introduced to the experiment, and were not sampled at all sites.
Table A.2. Record of sites, dates, water quality, system type, crab mortality, and CsRV1 prevalence data from surveyed Louisiana soft shell crab aquaculture sites. Live soft and peeler crab sampling was progressively introduced to the experiment, and were not sampled at all sites. Mortality was not sampled in 2017.

Table A.3. Full generalized linear mixed model (GLMM) with potential effects on crab mortality and CsRV1 infection intensity.
Site Key:
1. Pasadena, MD
2. Rock Hall, MD
3. Tilghman & Philips Wharf, MD
4. Patuxent, MD
5. West Point, VA
6. Sarah’s Creek, VA
7. Chuckatuck Creek, VA
8. Franklin, LA
9. Dulac, LA

- Recirculation System
- Flow-through System
Dead Peeler Crab CsRV1 Infection Prevalence (%) vs. Salinity (psu)
Table 1. Final reduced generalized linear mixed models (GLMM) predicting significant effects on crab mortality and CsRV1 infection intensity.

<table>
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<th>Model</th>
<th>Predictor Variable</th>
<th>Slope Estimate</th>
<th>Standard Error</th>
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<th>Variance</th>
<th>Standard Deviation</th>
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<td>Date)</td>
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<td>C. Log(Dead Crab CsRV1 Load) ~ Salinity + (1</td>
<td>Site) + (1</td>
<td>Date)</td>
<td>Salinity (psu)</td>
<td>-0.13</td>
<td>0.07</td>
<td>0.0498</td>
<td>Location</td>
</tr>
<tr>
<td>d.f. = 320, AIC = 1601.30</td>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td>Date</td>
<td>1.05</td>
<td>1.03</td>
</tr>
<tr>
<td>Non-normal</td>
<td>Intercept</td>
<td>6.25</td>
<td>1.13</td>
<td>8.42E-05</td>
<td></td>
<td></td>
<td></td>
</tr>
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