Title
Resisting Global Change: Oyster Aquaculture and Disease in an Era of Marine Heatwaves

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Publication Date
2023

Peer reviewed|Thesis/dissertation

Resisting Global Change:
Oyster Aquaculture and Disease in an Era of Marine Heatwaves

By

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Ecology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2023

*** * * DEDICATION * * ***

For Kendra Chan, Umihiko Hoshijima & Sarah White,
who deserved to have much longer lives and careers,
and who shaped the course of my own work
through their friendship, mentorship, and verve.

*** * * ACKNOWLEDGEMENTS * * ***

First and foremost, I want to thank the Hog Island Oyster Company (particularly Terry Sawyer, Gary Fleener, and Daniel Mancilla-Cortez), Bodega Bay Oyster Company (especially Whitt Strain and Sandford "Sandy" Jacques III), and Heidi Gregory at the Tomales Bay Oyster Company. This work and my own intellectual development would have been impossible without their magnanimity, curiosity, and involvement.

I want to express immense gratitude to my committee, who have provided me with support before and through my qualifying exams, in developing and designing experiments, and improving my ability to think and write as I wrote this dissertation. Thank you to Louie Yang, who constantly pushed the way I think about statistics, but also interpreting and visualizing data. I also have so much appreciation for Anne Todgham, whose generosity and physiological prowess helping me incorporate tools for better understanding how climate change impacts my system. Colleen Burge is someone I describe as being equal parts kind and brilliant. Without her, I could never have fully understood the impacts of OsHV-1. With her, I continue to learn the scientific context surrounding this virus, but also the cultural underpinnings of both the aquaculture industry and oysters themselves. Finally, I do not have enough words to describe how much I have appreciated Ted Grosholz's guidance. I am so grateful to Ted for giving me the space in his lab during these past five, very tumultuous, pandemic-ladden years. Ted's mentorship has truly been a gift, and I have carried a strange chip on my shoulder, wanting to prove myself worthy of being his student. Ted never seemed to worry about that – he has let me run loose and come back to him with questions and concerns both scientific and for the betterment of our community. Also, in moments of foolishness, Ted always grounded me and helped me see things with more clarity. Ted has this incredible ability to think quickly and thoughtfully, so that I left every meeting feeling better than I came in. He also has a deep well of empathy that meant I always felt comfortable being myself as both a scientist and person.

Upon starting my PhD, I challenged myself to vociferously apply for funding so that I could support myself throughout this self-growth journey and continued to be shocked that I was able to do so. Thus, I am deeply grateful for the UC Davis Graduate Group in Ecology Fellowship, Sustainable Oceans National Science Foundation Traineeship, UC Davis Coastal and Marine Science Institute Lead Mentor Position, California Sea Grant Fellowship, Russel J. and Dorothy S. Bilinski Fellowship, and Dennis & Patricia K. Salisbury Graduate Student Award for their support of my work.

I am so grateful for the multitude of communities I have belonged to throughout this process including the UC Davis Bodega Marine Lab as well as the Coastal and Marine Science Institute, where I not only did my research, but also developed my thinking. I am so appreciative of the Sustainable Oceans NRT and the Graduate Group in Ecology, through which I found friends and opportunities for peer mentorship. I am also thrilled that being a part of the Sustainable Oceans NRT program meant that I could fulfill my third-grade dreams of studying whales. I also want to thank the Scholars Strategy Network for their training and opportunities to connect my work with legislators, COMPASS Scientist Sentinels who helped me better configure how I grow into a leader, and Healthy Ocean Coalition Advocacy Academy which gave me reprieve from my own brain, but also a toolkit to increase the impact of my work. I am also eternally grateful to everyone doing Diversity, Equity, Inclusion, and Justice labor so that our field can be a more welcoming and prosperous place for marginalized individuals. It is difficult,

thankless work that often lands in the hands of junior and already overburdened people. While I cannot say I am excited, I *am* committed to continuing this work throughout my career. And yes, I did gift that book, hoping it would be received in the spirit it was intended. Instead, I learned once again that some people are not as far along in their journey as you might have hoped. But, I also learned that communities can come together to protect their own, and for that I am very thankful.

My friends and family have made this arduous process so much more bearable and, dare I say, fun. I am thankful to everyone who has been on this graduate school journey with me, and to everyone on the other side who understands how hard this is and has been rooting for me the along the way. Together we are all so weird and wonderful and I am so happy to have friends as an adult that are so kind and so smart. I am also grateful to my family. My mother, Sangeeta, and my brother, Manish, who have been by my side as I channeled myself and my grief into my career over the past decade. I want to thank my brother-in-law, Nikhil, and my mother-in-law, Kamala, for always finding ways to make me laugh as well as my father-in-law, Raghu, who channeled his own PhD experience into joy and support for me. And, my husband, Samaresh, who has made my hopes and dreams his own over the past decade, so that I can hone my scientific expertise towards the goal of helping our planet and its people. Finally, to my Pappa, who showed me what it means to have a deep love of nature and exploration, and without whom this work would have never been possible.

*** * * ABSTRACT * * ***

Resisting Global Change:

Oyster Aquaculture and Disease in an Era of Marine Heatwaves

Climate change is expected to increase heatwaves (MHWs) and disease transmission, potentially incurring losses of aquaculture stocks. Ostreid herpesvirus (OsHV-1) is a temperature-associated pathogen that predominantly affects the commercially important Pacific Oyster (*Magallana gigas*) and other species around the world, with some µVars causing > 95% mortality of oyster stocks. Over the course of three studies, we explored the utility of stress hardening (SH) in ameliorating the effects of MHWs (Chapter 1) and disease outbreaks (Chapter 2) by exposing multiple oyster species to a stressor before the onset of an event as well as a comprehensive overview of OsHV-1 detections in different locations and hosts (Chapter 3). In Chapter 1, we exposed juvenile Olympia oysters (*Ostrea lurida*), Kumamoto oysters (*Crassostrea sikamea*), and *M. gigas* to a two-week-long SH phase that involved exposure to a combination of temperature (15˚C v. 21˚C) and tide (immersion v. 6-hour tidal cycle) prior to a 72-hour simulated MHW at one of four temperatures (15˚C, 18˚C, 21˚C, 24˚C). Once the MHWs ended, a portion of *O. lurida* seed were grouped by their SH treatments, outplanted in Tomales Bay, CA, USA, and then assessed for mortality after nine months. Outplanted *O. lurida* that experienced tidal SH had perished by the time they were retrieved 279 days later. In contrast, 53.4% of *O. lurida* fully immersed during SH at 21˚C survived outplanting, while only 13.3% of those in the 15˚C SH treatment remained. Less than 10% of oysters across all species perished during the SH and MHW phases of the experiment, with *C. sikamea* experiencing the greatest losses. For Chapter 2, we investigated the effects of laboratory-based stress hardening (SH) via temperature (15˚C/16˚C v. 21˚C) and tide (tidal cycle vs. full submersion) on *M. gigas* and *C. sikamea* with the expectation that short-term exposure to warmer conditions and a simulated tidal regime would improve the performance of outplanted oysters. In 2021 and 2022, we exposed oysters to SH mechanisms before outplanting oysters at three sites spanning a thermal gradient in Tomales Bay, where they were exposed to OsHV-1 outbreaks in the field. We compared these outcomes to a baseline study completed in 2020, where *M. gigas* that had not been conditioned were exposed to OsHV-1 at two sites. *M. gigas* submerged in the warmer SH treatment had lower mortality in 2021, and those that experienced tidal fluctuations during SH had higher growth rates in 2022. In 2021, we saw no effect of SH on *C. sikamea* growth or mortality, but oysters were infected with OsHV-1. To our knowledge, this is the first study to provide OsHV-1 copy numbers (mg tissue-1) for *C. sikamea*. In Chapter 3, we used peer-reviewed literature, government reports, and unpublished data to describe the timeline of OsHV-1 variant detections, countries where *M. gigas* has tested positive, and other known hosts. We also summarized mean prevalence and peak infection intensity when that information was provided in the studies we assessed. Overall, SH has the potential to improve the resilience of aquaculture impacted by MHWs and disease, but it, along with the database of OsHV-1 detections we collated here, will likely be best used as part of an array of solutions addressing the multifaceted effects of climate change.

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*** * * CHAPTER 1 * * ***

Stress Hardening Selectively Influences the Responses of Three Commercially Important Oyster Species to a Simulated Marine Heatwave

Priya Shukla, Sarah Nancollas, Chelsey Souza, Audrey Deutsch, Sadie Small, Manuel Delgado, Samaresh Kowshik, Anne Todgham, Edwin Grosholz, Colleen Burge

ABSTRACT

Climate change is expected to increase the occurrence of marine heatwaves (MHWs), potentially incurring losses of aquaculture stocks. Stress hardening (SH) could ameliorate the effects of MHWs by exposing species to a stressor before the onset of an event. To test this approach, we exposed juvenile Olympia (*Ostrea lurida*), Kumamoto (*Crassostrea sikamea*), and Pacific (*Magallana gigas*) oysters to a two-week-long SH phase that involved exposure to a combination of temperature (15˚C v. 21˚C) and tide (immersion v. 6-hour tidal cycle) prior to a 72-hour simulated MHW at one of four temperatures (15˚C, 18˚C, 21˚C, 24˚C). Once the MHWs ended, a portion of *O. lurida* seed were grouped by their SH treatments, outplanted in Tomales Bay, CA, and then assessed for mortality after nine months. All outplanted *O. lurida* that experienced tidal SH had perished by the time they were retrieved 279 days later. In contrast, 53.4% of *O. lurida* fully immersed during SH at 21˚C survived outplanting, while only 13.3% of those in the 15˚C SH treatment remained. Further, SH temperature decreased condition index (CI) in *O. lurida* and *C. sikamea* after the MHW trials while *M. gigas* exposed to tidal regime during SH had higher CI. Additionally, oxidative stress (measured via protein carbonylation) was greater in *C. sikamea* than in *M. gigas*, though *M. gigas* that experienced 21˚C during SH exhibited greater protein carbonylation. Finally, less than 10% of oysters across all species perished during the SH and MHW phases of the experiment, with *C. sikamea* experiencing the greatest losses. Overall, SH has the potential to improve the resilience of aquaculture impacted by MHWs, but it will likely be best used as part of an array of solutions addressing the multifaceted effects of climate change.

KEYWORDS: Stress Hardening, Carryover Effects, Marine Heatwaves, Aquaculture, Oysters, Molluscs, *Ostrea*, *Crassostrea*, *Magallana*

INTRODUCTION

Marine heatwaves (MHWs) have increased in frequency and duration over the past century, with 87% of MHWs attributable to anthropogenic warming (Oliver et al. 2018, Frölicher et al. 2018, IPCC 2022). Defined as a discrete period of anomalously warm water (Hobday et al. 2016), MHWs have had immense biological impacts including coral bleaching in the Mediterranean Sea (Garrabou et al. 2009), disease outbreaks at Tasmanian shellfish farms (Oliver et al. 2017), and shifts in zooplankton availability that led to whale mortalities (Santora et al. 2020). With MHWs expected to increase in duration and frequency as climate change progresses (IPCC 2022), strategies for increasing the resilience of ecosystem services and coastal enterprises, including fisheries and aquaculture, are crucial (Smale et al. 2019, Smith et al. 2021).

MHWs are a persistent threat across the marine aquaculture sector (Callaway et al. 2012, Holbrook et al. 2020, Mugwanya et al. 2022, Smith et al. 2023), especially as these operations are often static with limited capacity for relocation (Sanchez-Jerez et al. 2016, Lester et al. 2018). Many species are cultivated at the edge of their thermal tolerance because warmer conditions often increase metabolism and accelerate growth (Hartog et al. 2023). Surpassing this threshold could be lethal (Mugwanya et al. 2022, Meng et al. 2022), but, perhaps more importantly, sublethal temperature challenges can also reduce the quality of the product as energy is diverted away from tissue quality to mechanisms of stress tolerance and repair (Callaway et al. 2012). Additionally, MHWs can trigger harmful algal blooms and result in economic losses because they prevent harvesting, sales, and consumption of filter-feeding bivalves that can concentrate the toxic algae (Griffith and Gobler 2020). Thus, exploring approaches for improving the responses of commercially cultivated species to MHWs is of critical importance.

One strategy for enhancing the resilience of organisms is to use stress hardening (SH), which involves acclimating them to a mild to moderate stressor (e.g., heat) prior to their exposure to a more severe stressor in the environment (Voolstra et al. 2021). SH has facilitated metabolic recovery in invertebrates (Malmendal et al. 2006) and assisted various coral species trying to cope with increased UV exposure (Ferrier-Pagès et al. 2007), elevated pCO_2 (Putnam et al. 2020), and high temperature (reviewed by Hackerott et al. 2021). In some cases, one stressor can foster cross-tolerance in another. For example, colonial stony corals (*Montastraea cavernosa*) bleached with the herbicide DCMU had greater symbiont retention under heat stress (Silverstein et al. 2015). Similarly, heat-shocked tidepool sculpins (*Oligocottus maculosus*) exhibited higher survival after subsequent exposure to low dissolved oxygen and increased salinity stress (Todgham et al. 2005). Given its utility across numerous taxa, it may be worth considering whether SH can also be a useful tool for conditioning farmed shellfish to MHWs.

Intertidal bivalves have demonstrated improved responses to heat stress following exposure to increased temperatures. In the Australian rock oyster (*Saccostrea glomerata*), individuals emersed at 50˚C for two hours after four weeks of periodic exposure to air at 30˚C and 40˚C had greater survival than those that remained submerged before the acute exposure (Scanes et al. 2023). Likewise, pearl oysters (*Pinctada maxima*) exposed to two three-day MHWs employed multiple physiological mechanisms to compensate for thermal stress (He et al. 2021). The suite of abiotic stressors that intertidal species are challenged with, such as increased temperatures from insolation and desiccation stress due to emersion (Helmuth 1998), can further

strengthen their adaptive capacity to environmental stress (Collins et al. 2023). Pacific oysters, *Magallana* (= *Crassostrea*) *gigas*, that experienced heat shock at 44˚C had better survival after being outplanted at higher tidal heights relative to heat shocked oysters at lower tidal heights (Hamdoun et al. 2003). Juvenile *M. gigas* deployed in the intertidal zone had greater metabolism and immunity than those in the subtidal zone (Corporeau et al. 2022). In addition, *M. gigas* exposed to warmer temperatures had higher antioxidant enzymatic activity (Rahman et al. 2019). Collectively, temperature and tide may be potential methods for SH in commercially farmed oysters.

In this study, we explored the effects of a simulated acute MHW following tidal and thermal SH on three oyster species: *Ostrea lurida*, *Crassostrea sikamea*, and *M. gigas*. To determine the effects of SH, we measured mortality and growth via condition index (CI) as well as protein carbonyl, a proxy for irreversible oxidative stress (Han et al. 2013). Across all species, we predicted that SH would result in lower mortality, higher CIs, and elevated protein carbonyl levels when oysters were subsequently exposed to a MHW.

METHODS

Oyster Cultivation History

The Olympia oyster (*Ostrea lurida*) is native to the eastern Pacific Ocean from Alaska, USA through British Columbia, Canada to Baja California, Mexico where it historically formed intertidal and subtidal estuarine reefs (Baker 1995). It can tolerate temperatures between 10.5˚C and 29˚C (Barber et al. 2016, Bible et al. 2020). Fishing,

pollution, sedimentation, and non-native predators have led to the species being nearly extirpated across its range (Brumbaugh and Coen 2009, White et al. 2009, Ridlon et al. 2022). Efforts using aquaculture infrastructure are underway to supplement natural *O. lurida* populations (Wasson et al. 2020, Ridlon et al. 2021) and the species is now being cultivated for human consumption (G. Fleener, Hog Island Oyster Company, pers. comm.).

The Kumamoto oyster (*Crassostrea sikamea*) originates from the western Pacific Ocean. Its range includes Japan (Hamaguchi et al. 2013), Korea (Hong et al. 2012), and China (Hu et al. 2018). A slow-growing species that takes nearly three years to reach market size, it is now commonly grown across the west coast of North America (Robinson 1992, Cáceres-Martínez et al. 2012). Its established reproductive range spans 20 ˚C to 28˚C (Fofonoff et al. 2018).

The Pacific oyster (*Magallana gigas*) is native to the Northwest Pacific including Russia, China, Korea, and Japan where it experiences temperatures from -1.8˚C to 35˚C. It has been intentionally introduced to establish new fisheries in 52 countries across the Northeast Pacific, Southwest Pacific, Northeast Atlantic-Mediterranean, Southwest Atlantic, and Indian Oceans (Fofonoff et al. 2018). Due to its capacity to tolerate a wide range of temperatures and environmental gradients, it is the most commonly farmed oyster species around the world (FAO 2022). It is also strongly associated with facilitating species introductions (Grosholz et al. 2015).

Temperature Manipulation

To generate the different SH temperature treatments, a titanium submersible heater with digital temperature control (Intelligent Heater) was deployed in one of two

400L sumps for one week prior to the beginning of the experiment to create a 15˚C and 21˚C treatment (Fig. 1.1). Each sump had fresh seawater circulating through and was connected to two five-channel manifolds that delivered water to ten tanks. The same system was employed during the 72-hour MHW phase for each temperature treatment (15˚C, 18˚C, 21˚C, and 24˚C). In addition to the digital temperature control, thermistors connected to a Raspberry Pi Model 3B+ and HOBO Loggers (Onset) deployed in each sump were used to monitor temperature. Four HOBO Loggers were also randomly assigned to one tank per tide-temperature treatment to confirm that within-tank temperatures matched the sump temperature.

The control SH/MHW temperature (15˚C) is representative of the mean annual temperature averaged across the whole year in Tomales Bay, California where both *O. lurida* is found and *C. sikamea* and *M. gigas* are cultured, while the elevated SH/MHW temperature 21˚C is an approximation of the higher range of summer seawater temperatures in this area (Smith et al. 1991, Hollarsmith et al. 2020, Shukla et al. *in prep*). The 18˚C and 24˚C MHW treatments represent intermediate and extreme seawater temperatures, respectively.

Tidal Simulation

Oysters in this experiment were either submerged for the entire two-week SH phase (“no tide” treatment) or experienced intermittent air exposure (“tide” treatment) (Fig. 1.1). For the “no tide” treatment, five tanks per temperature treatment remained full during SH. The “tide” treatment was created by draining (“low tide”) and re-filling (“high tanks”) tanks every six hours, resulting in two alternating “high tide” and two “low tide” periods.California experiences mixed semidiurnal tides (Nidzieko 2010),

wherein intertidal communities can still be submerged during “high low” tides. Thus, to standardize air exposure, oysters in the “tide” treatment were emersed for a total of 12-hours per day.

All tanks in the system were connected to the sumps via a manifold where flow rate could be controlled to allow the tank to remain full. A spigot at the base of each tank also allowed water to cycle through while maintaining the volume of water in the tank. To simulate tide, a Python program running on a Raspberry Pi 3 Model B+ connected to two electronically actuated ball valves (Asahi) controlled the movement of water into five tanks per temperature treatment (Fig. 1.1). Specifically, water flowed from each sump through one of two ball valves and then a manifold that determined the flow of water to five tanks per temperature treatment. Each day, the ball valve alternated between open (“high tide”) and close (“low tide”) for six-hour periods. The spigot at the base of each tank allowed water to rapidly drain at the onset of “low tide”.

Experimental Design

We used juvenile *O. lurida* (~4 mm) as well as juvenile *C. sikamea* (~25 mm) and *M. gigas* (~25 mm) from the Hog Island Oyster Company (HIOC) to test the effects of tidal and thermal SH on oysters’ responses to a 72-hour MHW (n = 1,600 per species). *C. sikamea* and *M. gigas* from the HIOC nursery in Samoa, CA and *O. lurida* from the HIOC farm in Marshall, CA were brought to the Bodega Marine Laboratory (University of California, Davis) in Bodega Bay, CA where all three oyster species were distributed across 20 18L tanks (n = 240 oysters per tank, n = 80 oysters per species per tank) that were connected to 400L sumps with flow-through seawater from the Pacific Ocean that

was passed through a clarifier and sand filter (Fig. 1.1). Within each tank, oysters were grouped by species into three separate mesh nylon bags; to prevent *O. lurida* seed (< 7 mm) from falling through the mesh they were wrapped in cheesecloth before being placed in the bag.

During SH, 20 tanks were split across two temperature treatments (15˚C v. 21˚C, n = 10 tanks per treatment). Oysters in the 21˚C treatment were brought up to temperature from 15˚C at a rate of 0.5˚C/hour where they then remained for two weeks. Within each temperature treatment, five tanks were assigned to either a "no tide" (fully submerged) or a "tide" (intermittently submerged or exposed for 6 hours each, two times per day) treatment. After SH, oysters were immersed in 15˚C seawater for 24 hours before being brought up to their MHW treatment at a rate of 0.5˚C/hour.

For the MHW, four conspecific oysters per tank from each combined SH temperature-tide treatment were mixed together and then randomly assigned to a new mesh bag (n = 20 conspecific individuals per bag). Oyster-ladden mesh bags went into five tanks per MHW treatment (15˚C, 18˚C, 21˚C, and 24˚C) (Fig. 1.1). During the MHWs, there was no tidal regime and oysters were fully submerged for three days (72 hours). With all oyster species and SH treatments accounted for, there were 12 mesh bags per MHW tank (n = 240 oysters per tank).

Throughout SH and the MHWs, oysters were fed daily by adding 10L *Nanochloropsis* and *Isochrysis* to each sump as well as a 20mL of undiluted Shellfish Diet 1800 (Reed Mariculture, ~2 billion algal cells/mL). All oysters were fed simultaneously when "tide" treatment tanks were full ("high tide"), but incoming seawater and drainage were shut off during feeding (~ 2 hours) to maximize oysters'

consumption of algae. Tanks and mesh bags were rinsed and *O. lurida* cheesecloths were swapped every other day to remove waste and pseudofeces.

O. lurida *Outplanting*

After the MHW trials, all surviving *O. lurida* seed were transported to the Hog Island Oyster Co. farm in Marshall, CA to be outplanted. *O. lurida* were grouped by their SH treatment combinations (tide x 15˚C: n = 392; tide x 21˚C: n = 389; no tide x 15˚C: n = 392; no tide x 21˚C: n = 393) and transferred into the chamber of a two-compartment SEAPA basket. The two baskets were then subtidally suspended at Sacramento Landing, Tomales Bay (38.15, -122.91) for nine months starting in March 2022. SEAPA baskets were cleaned monthly, but mortality was not measured until oysters were collected in December 2022.

Mortality

Upon conclusion of the MHW trials, mortality and growth were measured. To assess mortality, oysters were removed from their mesh bags and individuals whose valves were gaping or shells had separated at the hinge were considered dead. In the case of the outplanted *O. lurida*, mortality was evaluated across all SH treatments 279 days after the oysters were deployed.

Growth

Condition index (CI) was used to determine relative somatic tissue and shell growth for all species after the MHW trials. CI in *C. sikamea* and *M. gigas* was measured by separating the somatic tissue from the shell. Both tissue and shell were

dried in an oven for 24 hours at 80˚C before their dry weights were taken. For *O. lurida*, the shell was dried in an oven for 72 hours at 60˚C and weighed to get the dry weight of each oyster (Ricart et al. 2021); additional drying did not result in any further water loss. The somatic tissue was then measured by weighing the ash-free dry weight of their shells after 8 hours in a muffle furnace at 550˚C. CI was then calculated as:

$$\text{Condition Index (CI)} = \frac{\text{Somatic Tissue Weight (g)}}{\text{Shell Weight (g)}} \times 100 \quad (1)$$

Due to a malfunction with the muffle furnace, one set of *O. lurida* that experienced each SH combination and then a 15˚C MHW trial (n = 40 total; n = 10 per SH treatment) were lost and CI could not be evaluated.

Protein Carbonyl Content

After MHW trials were complete, a subset of *C. sikamea* and *M. gigas* (n = 3 per treatment per tank) were immediately frozen in liquid nitrogen and stored at -80˚C until they could be analyzed for protein carbonyl (Appendix 1.A), which was done using the Protein Carbonyl Content Assay Kit (Sigma Aldrich, MAK094). *O. lurida* seed were not assessed for protein carbonyl due to their small size and insufficient quantity of tissue for this assay.

Statistical Analyses

Statistical analyses were completed in R (v 4.2.3) using the *lme4* package (Bates et al. 2015) and post-hoc pairwise comparisons were done using the *emmeans* package (Lenth 2021). Generalized linear mixed models were fitted using maximum likelihood estimation, compared to a null model, and then the best model was chosen based on AIC scores ($P < 0.05$). For each species, the effects of SH and MHW treatments on oyster

mortality was estimated using logistic regression with a binomial distribution and a "logit" link function, while CI, and protein carbonyl were determined using generalized linear mixed models with either Gaussian or gamma distributions and an "identity" link function. In all cases, SH temperature, SH tide, and MHW temperature were included in models as fixed effects while tank was included as a random effect to account for non-independent samples. Model residuals were used to check for homoscedasticity and overdispersion, as well as normality in models run using a Gaussian distribution.

RESULTS

Mortality

Overall, less than 10% of replicates across all three species perished during the SH (Fig. 1.2) and MHW (Fig. 1.3) phases of this experiment, though there was substantial mortality among outplanted *O. lurida* (Fig. 1.4). Temperature and tide did not impact *O. lurida* mortality during the SH phase ($P > 0.05$, Table 1.1), nor did these factors or MHW temperature impact mortality after the MHW ($P > 0.05$, Table 1.1), and they did not interact. During SH, less than 1% of *O. lurida* that experienced 15˚C during SH as well as those in the 21˚C SH treatment with no tide died. Only a few more *O. lurida* (1.5 ± 0.6%, mean ± SE) in the 21˚C SH treatment with a tidal regime died (Fig. 1.2). During the MHW trials, *O. lurida* experienced as little as 0% and as much as 3% mortality (Fig. 1.3A). All outplanted *O. lurida* that experienced a tidal regime during SH died ($P < 0.01$, Fig. 1.4, Table 1.1), while individuals that were fully submerged at 15˚C had lower survival (13.3%) than those oysters fully submerged at 21˚C (53.4%).

C. sikamea had the highest mortality of all species during SH (Fig. 1.2), with the greatest losses in the 21˚C treatment with a tidal regime (2.5 ± 0.6%). SH temperature,

tidal regime, and MHW temperature did not influence mortality ($P > 0.05$, Table 1.1) except in the 24˚C MHW treatment where 5.5 ± 1% of oysters perished relative to 2.8 ± 1% at 15˚C ($P = 0.04$, Table 1.2), 3.3 ± 0.7% at 18˚C ($P > 0.05$, Table 1.2), and 2.0 ± 0.6% in the 21˚C treatment ($P < 0.01$, Table 1.2, Fig. 1.3B).

In *M. gigas*, all oysters survived the SH phase (Fig. 1.2) and between 0% and 2 ± 1% of oysters perished during the MHW trials (Fig. 1.3C). As a result, SH temperature ($P > 0.05$, Table 1.1), tide ($P > 0.05$, Table 1.1), and MHW temperature ($P > 0.05$, Table 1.1) did not significantly drive mortality.

Growth

SH temperature ($P < 0.01$, Fig. 1.5A, Table 1.3) influenced *O. lurida* CI, whereas tide and MHW temperature alone did not ($P > 0.05$, Tables 1.3, 1.4), with CI being 6.5% higher for oysters hardened at 15˚C (7.51 ± 0.21) compared to those at 21˚C (7.02 ± 0.21).

While tidal regime during SH did not influence CI in *C. sikamea* ($P > 0.05$, Fig. 1.5B, Table 1.3), SH and MHW temperatures ($P \leq 0.02$, Table 1.3) except for 21˚C ($P > 0.05$, Table 1.3) did; the two factors did not interact. *C. sikamea* stress hardened at 15˚C (4.26 ± 0.07) had a 6.1% higher CI than their counterparts at 21˚C (4.00 ± 0.07). And, oysters in the 15˚C MHW had the greatest CI (4.41 ± 0.10), while those in the 18˚C (4.08 ± 0.10), 21˚C (4.21 ± 0.12), and 24˚C (3.81 ± 0.08) treatments were 8.1%, 4.8%, and 15.7% smaller, respectively (Table 1.4).

Tidal regime during SH ($P < 0.01$, Fig. 1.5C, Table 1.3) and MHW temperature ($P = 0.01$, Table 1.3) determined CI in *M. gigas*, though they did not interact. In contrast, SH temperature did not affect CI ($P > 0.05$, Table 1.3). Interestingly, oysters exposed to a tidal cycle (3.57 ± 0.04) had a 10.1% larger CI than those that were fully submerged (3.97 ± 0.08) for the duration of the stress hardening period. Similar to *C. sikamea*, individuals in the 15˚C MHW (4.16 ± 0.14) had the highest CI, while oysters in 18˚C (3.98 ± 0.08), 21˚C (3.72 ± 0.05), and 24˚C (3.51 ± 0.08) treatments were 4.5%, 4.5%, and 18.5% lower, respectively (Table 1.4).

Protein Carbonyl Content

Neither SH temperature ($P > 0.05$) nor SH tide ($P > 0.05$) influenced protein carbonylation levels in *C. sikamea*, though MHW temperature did ($P < 0.01$; Table 1.5). Specifically, irrespective of SH treatment, protein carbonyl was greatest in oysters that experienced the 24˚C MHW (5.53 ± 0.50 nmole carbonyl mg protein^{-1}; Fig. 1.6A), while carbonylated proteins were 32.7% (3.72 ± 0.22 nmole carbonyl mg protein^{-1}), 28.9% (3.93 ± 0.43 nmole carbonyl mg protein^{-1}), and 34.4% (3.63 ± 0.18 nmole carbonyl mg protein^{-1}) lower in the 15˚C, 18˚C, and 21˚C, respectively.

SH temperature ($P < 0.05$) determined the quantity of carbonylated proteins in *M. gigas*, though SH tide ($P > 0.05$) and MHW temperature ($P > 0.05$) did not (Table 1.5). Oysters hardened at 15˚C (1.74 ± 0.09 nmole carbonyl mg protein^{-1}) had 19.1% fewer carbonylated proteins than those in the 21˚C treatment (2.15 ± 0.14 nmole carbonyl mg protein^{-1}; Fig. 1.6B).

DISCUSSION

This study demonstrates that SH does not always affect oyster growth and mortality after a MHW, but can have longer-term impacts. *M. gigas* exhibited no mortality during SH, unlike *O. lurida* and *C. sikamea*, with the latter experiencing the greatest mortality across SH and MHW phases and *O. lurida* exhibiting high mortality during outplanting. Increased temperature during SH reduced CI in *O. lurida* and *C. sikamea*, while CI decreased with increasing MHW temperature for *M. gigas* but increased with tidal exposure. SH temperature and MHW temperature influenced protein carbonyl levels in *M. gigas* and *C. sikamea*, respectively. Further, *O. lurida* that experienced tide during SH did not survive the nine-month outplanting, while those that were submerged at 21˚C had higher survival. Overall, SH temperature was a strong modulator of oyster responses during the subsequent MHWs and SH can influence immediate outcomes in oysters while also having lasting impacts.

SH temperature and tide influenced trends in CI across MHW temperatures. CI in *C. sikamea* (Fig. 1.5B) and *M. gigas* (Fig. 1.5C) generally decreased with MHW temperature, irrespective of temperature and tide treatment. Somewhat similarly, *O. lurida* CI was lowest for oysters in the 15˚C MHW treatment, but then was inversely related to MHW temperature (18˚C - 24˚C) for oysters that experienced either 15˚C with tide or 21˚C with no tide during SH (Fig. 1.5A). However, CI in *O. lurida* oysters exposed to 21˚C with tide rose with MHW temperature (18˚C - 24˚C) (Fig. 1.5A). Given that shell size does not change over short time scales, shifts in CI values are primarily due to differences in somatic tissue, which requires energy for protein synthesis. MHW

temperatures may have detrimentally affected oyster feeding, with the exception of *O. lurida* experiencing a tidal regime under 21˚C prior to MHW exposure, because food availability can affect growth (Brown and Hartwick 1988). Although daily feedings were standardized across treatments, oysters coping with MHW temperature stress may have attempted to feed after algae had been depleted. Additionally, CI in *M. gigas* exposed to tide was greater than in fully submerged oysters (Fig. 1.5C), indicating that the latter group may have also been feeding consistently while immersed during the SH phase. Thus, continuing to feed in a food-limited environment, especially during a MHW, may have been physiologically taxing. Despite the trends in *M. gigas* CI, it is important to consider the compounding effects of the stress that temperature and tide impose. Not only are oysters susceptible to thermal stress (Yang et al. 2016), but tidal exposure can also determine oysters' responses to temperature (Wang et al. 2021), which may have affected CI in each MHW temperature regime. Further, increased CIs of *O. lurida* exposed to tide and 21˚C during SH may capture a threshold at which growth can be temporarily maximized before prolonged warming becomes detrimental, as is expected under climate change (Sheridan and Bickford 2011). This also corroborates a well-established trade-off between growth and thermal tolerance, wherein faster-growing organisms have higher metabolic rates that keep them close to their physiological limits (McAfee et al. 2017, Li et al. 2018). As MHWs continue to propagate in the natural environment, they may create windows of rapid growth while repeat occurrences may bring oysters to the edge of their thermal tolerance. Aerial exposure during low tide may serve to further threaten oysters as global temperatures rise.

Tide during SH affected CI of juvenile *M. gigas*, but was otherwise not a major source of hardening. It had no effect on the CI of *C. sikamea* or on protein carbonyl volumes in *M. gigas* and *C. sikamea*. Nevertheless, tidal emersion is seen as a significant stressor in nearshore environments as aerial exposure alters the physiology of intertidal organisms in response to solar irradiance, desiccation, and ambient heat exchange (Truchot 1990, Helmuth 1998). This experimental system may have inadvertently simulated some of these effects during "low tide". Two temperature loggers that were only deployed in a 15˚C and 21˚C tank experiencing tidal regimes for the purposes of confirming that tank temperatures matched sump temperatures showed that air temperatures increased by 1-2˚C upon the water's egress, possibly due to respiration within the insulated tanks; this effect was not repeated in the oysters' absence (P. Shukla, unpublished data). Perhaps due to the physiological mechanisms that support their intertidal existence (Tomanek and Helmuth 2002), tidally exposed oysters did not exhibit any added stress during the experiment. While tide was not necessarily an effective SH mechanism for tolerating MHWs, increasing the resilience of emersed oysters is an important parallel endeavor. A severe heatwave in the Salish Sea that coincided with extreme low tides in June 2021 led to mass mortalities of several intertidal bivalve species, particularly in the low intertidal zone that is less frequently exposed. (Raymond et al. 2022). Thus, understanding which factors induce stress responses in oysters is crucial.

Protein carbonylation levels demonstrated that *C. sikamea* and *M. gigas* were experiencing stress during the experiment, though not in the same way. Specifically, oxidative damage of proteins was greatest for *M. gigas* in the warmer SH treatment and

for *C. sikamea* that experienced the 24˚C MHW. Additionally, the higher volume of carbonylated proteins across all treatments suggests that *C. sikamea* was stressed throughout the experiment, which may explain why it had the highest mortality rate. Thermal stress does not always influence protein carbonylation (Drake et al. 2017), but can be a useful metric for understanding the physiological effects of temperature on intertidal species (Han et al. 2013, Zhang et al. 2014). Thus, it would have been interesting to measure protein carbonylation in *O. lurida* during the experiment. Given that earlier life stages are generally more susceptible to environmental change (Thorson 1950, Underwood and Fairweather 1989), it would have been illuminating to see how oxidative stress in this species compares to the seemingly sensitive *C. sikamea* after both the lab experiment and outplanting were complete.

The mortality of outplanted *O. lurida* indicates that SH can have long-term effects. Oysters that were fully submerged for two weeks at 21˚C had greater survival than those at 15˚C, suggesting that exposure to warmer conditions contributed to their resilience (Fig. 1.4). Given that SH tide and temperature did not influence this species' mortality during the SH and MHW phases (Figs. 1.2, 1.3) and that CI was lower in the warmer SH treatment after the MHW trials were complete, this outcome was unexpected. Additionally, the variable environmental conditions of Tomales Bay (Smith and Hollibaugh 1997, Hollarsmith et al. 2019) over nine months could have counteracted the two weeks of SH that preceded outplanting. While there is evidence of short-term benefits following a heat challenge (Giomi et al. 2016, He et al. 2021), acute stressors have also been shown to impart benefits over longer time scales. For example, heat-shocked flat oysters (*O. angasi*) had higher survival than their unexposed

counterparts several months after experiencing thermal stress (Pereira et al. 2020). Because *O. lurida* populations have been decimated and their restoration is an ecological priority (Ridlon et al. 2021), it is valuable to explore how SH can be employed to improve their resilience.

Carryover effects from SH have the capacity to improve oyster responses to environmental change. However, it is clear this approach is not an overarching remedy for coping with MHWs. Periodic exposure to warmer temperatures can facilitate growth in the short-term and survival in the long-term, but it does not improve overall performance. Identifying specific outcomes that SH can enhance and the duration for which it will be used is critical. It also may be useful to test multiple methods of SH to determine which will be most effective. For example, static temperatures were used in this experiment, but fluctuating temperatures may be beneficial (Nancollas and Todgham 2022). Tide was not an effective SH mechanism for oysters in this study, but emersion has been shown to boost cardiac activity at warmer temperatures (Bjelde and Todgham 2013). And, exposure to warmer temperatures can increase survival in oysters subsequently infected with Ostreid herpesvirus (de Kantzow et al. 2019, Shukla et al. *in prep*). Thus, it is valuable to continue exploring contexts within which SH might be useful.

MHWs are expected to globally disrupt marine ecosystems (Weitzman et al. 2021, Traiger et al. 2022) as well as the production of finfish (Sánchez-Cueto et al. 2023) and shellfish (Lattos et al. 2022). Bivalve aquaculture, in particular, is seen as an opportunity for low-impact food production (Kumara et al. 2022), and developing tools that help aquaculture practitioners avoid loss of stocks to climate stressors is imperative

(de Burgh-Day et al. 2022). Understanding the impact of MHWs is especially valuable as disease events, such as Ostreid herpesvirus, are strongly associated with rising ocean temperatures (Harvell 1999, Burge et al. 2014, Green et al. 2019). SH is one strategy that may support aquaculture, but will not be a cure-all for the complex problems that climate change and MHWs introduce. Ultimately, it will likely be most effective when included in a suite of solutions that improve the resilience of aquaculture in a climate-affected ocean.

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FIGURES

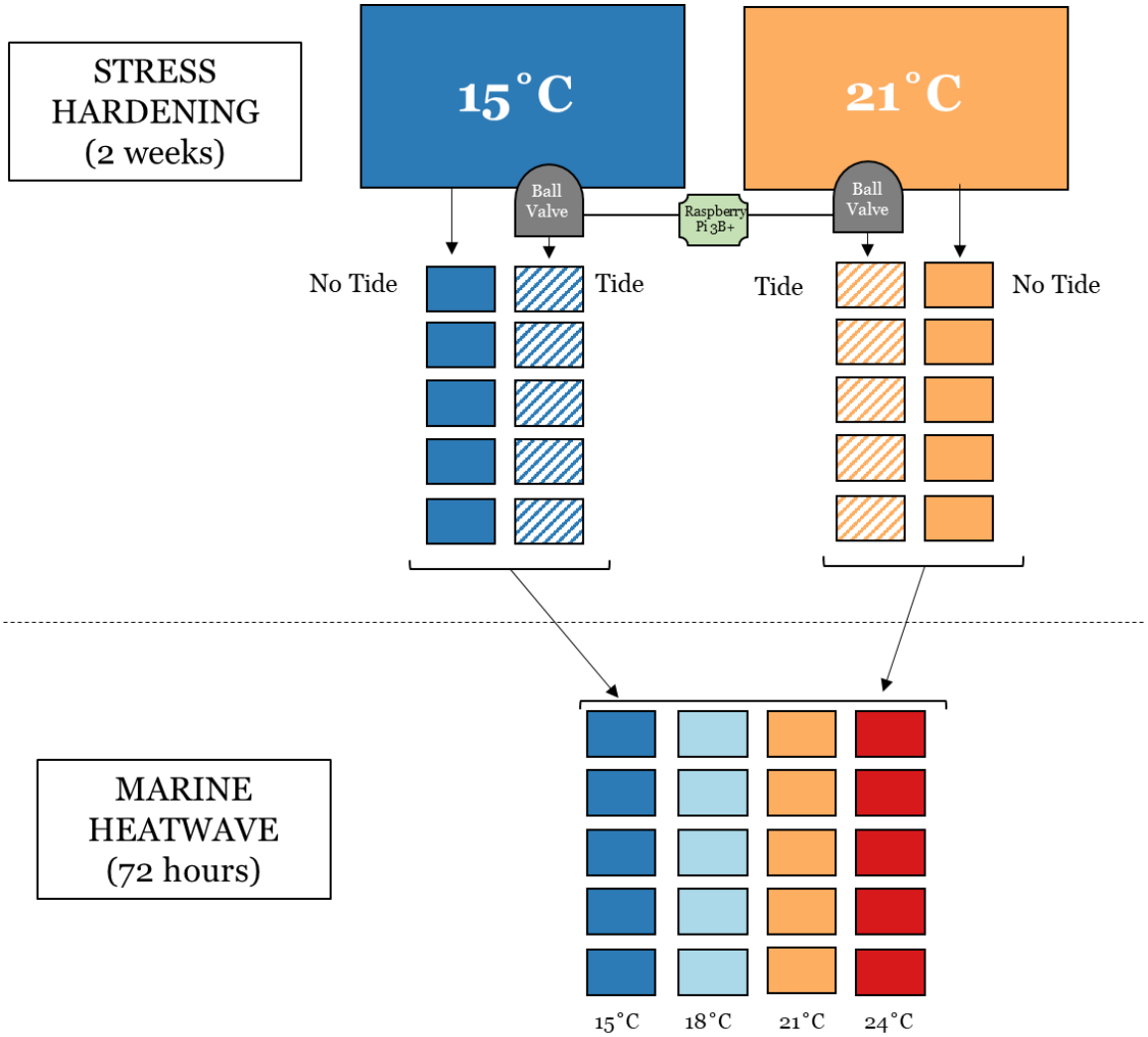

Figure 1.1. Two 400L sumps were brought to 15˚C and 21˚C that fed into 10 18L tanks each. Over a two-week stress hardening (SH) period, half of these thanks experienced a simulated tidal regime (indicated by hash-marked boxes below) via a Python program running on Raspberry Pi Model 3B+ that turned an electronically actuated ball valved every 6 hours to allow water flow in from the sumps when open or prevent submersion when closed. The remaining tanks experience full submersion for the duration of SH. After experiencing SH, oysters all oysters were orthogonally assigned to either a control treatment (15˚C) or one of the three marine heatwave treatments (18˚C, 21˚C, 24˚C) where they were immersed (i.e., no simulated tide) for 72 hours, prior to being assessed for mortality and growth metrics.

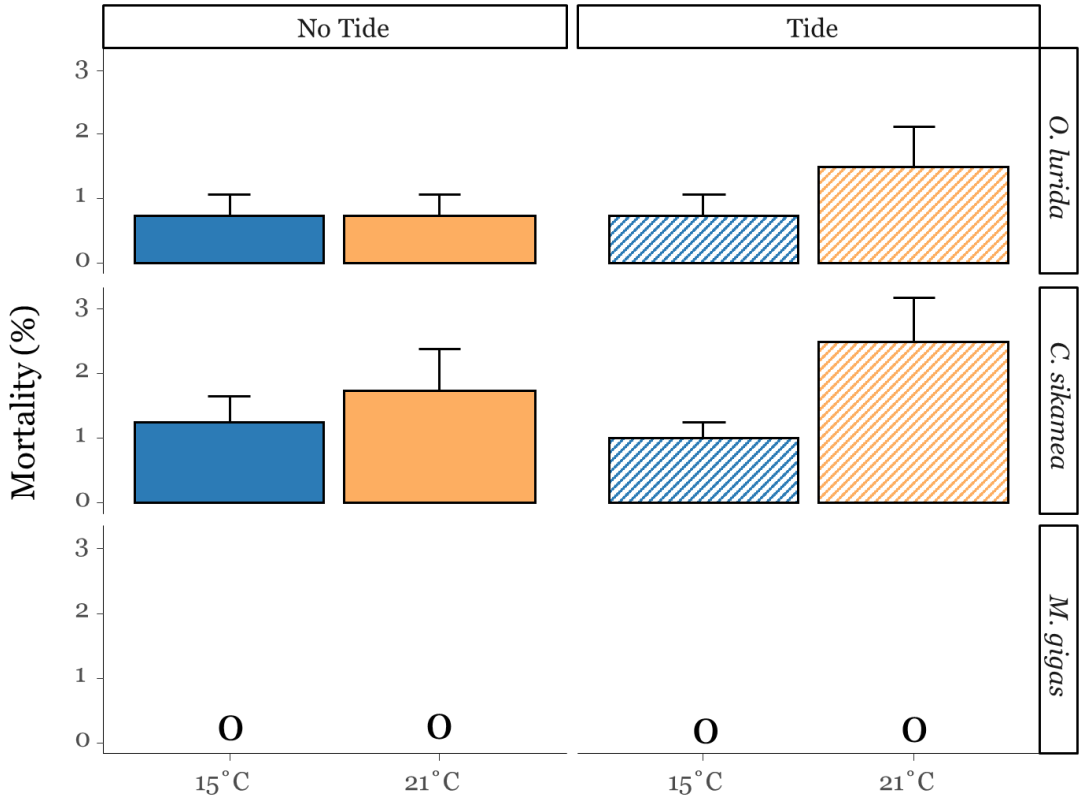

Figure 1.2. Mortality (mean ± SE) of *O. lurida*, *C. sikamea*, and *M. gigas* during the two-week stress hardening (SH) phase of the experiment. There were not statistically significant differences in mortality across SH treatments.

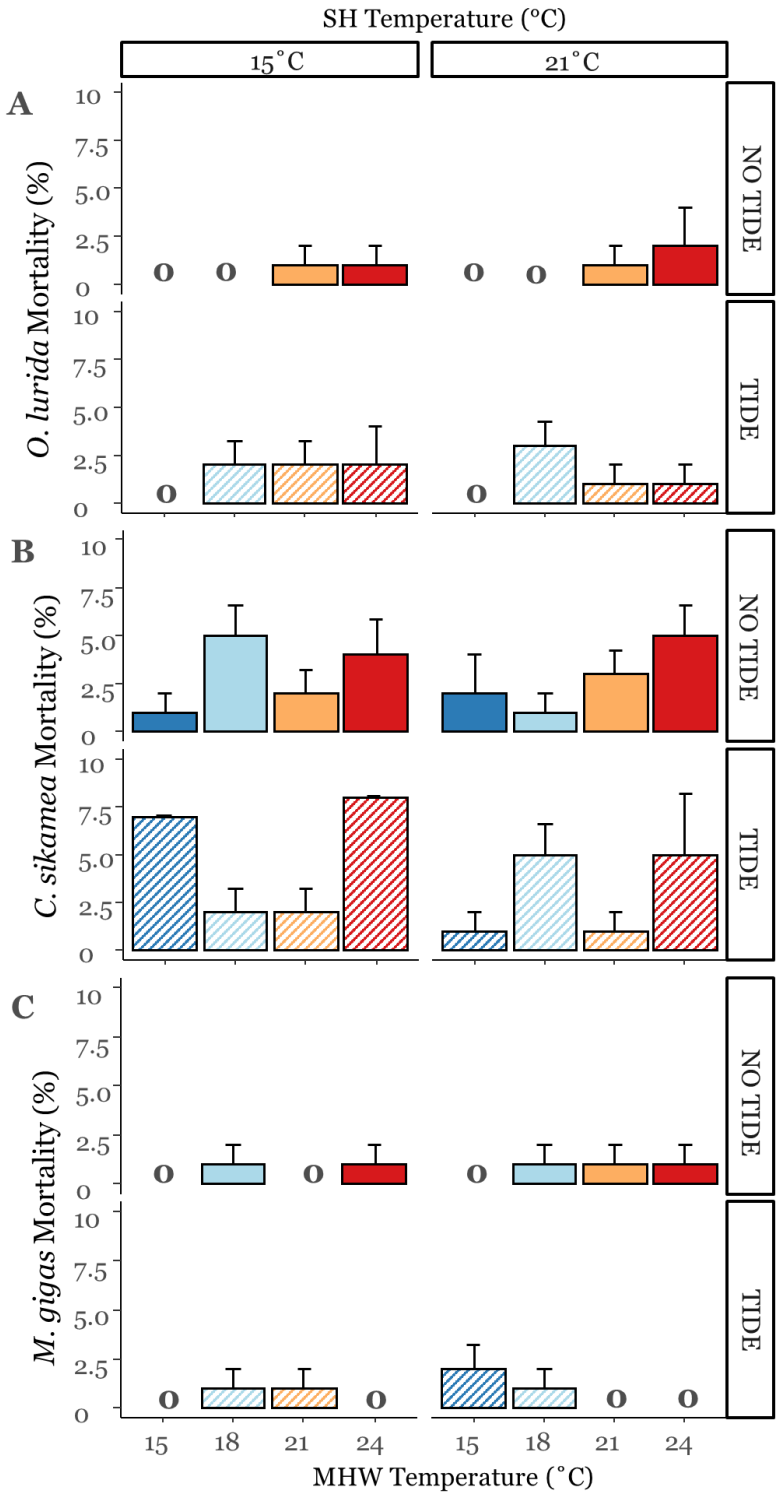

Figure 1.3. Mortality (mean ± SE) of *O. lurida* (A), *C. sikamea* (B), and *M. gigas* (C) after the 72-hour marine heatwave (MHW) phase of the experiment. There were no statistically significant differences in mortality across SH or MHW treatments.

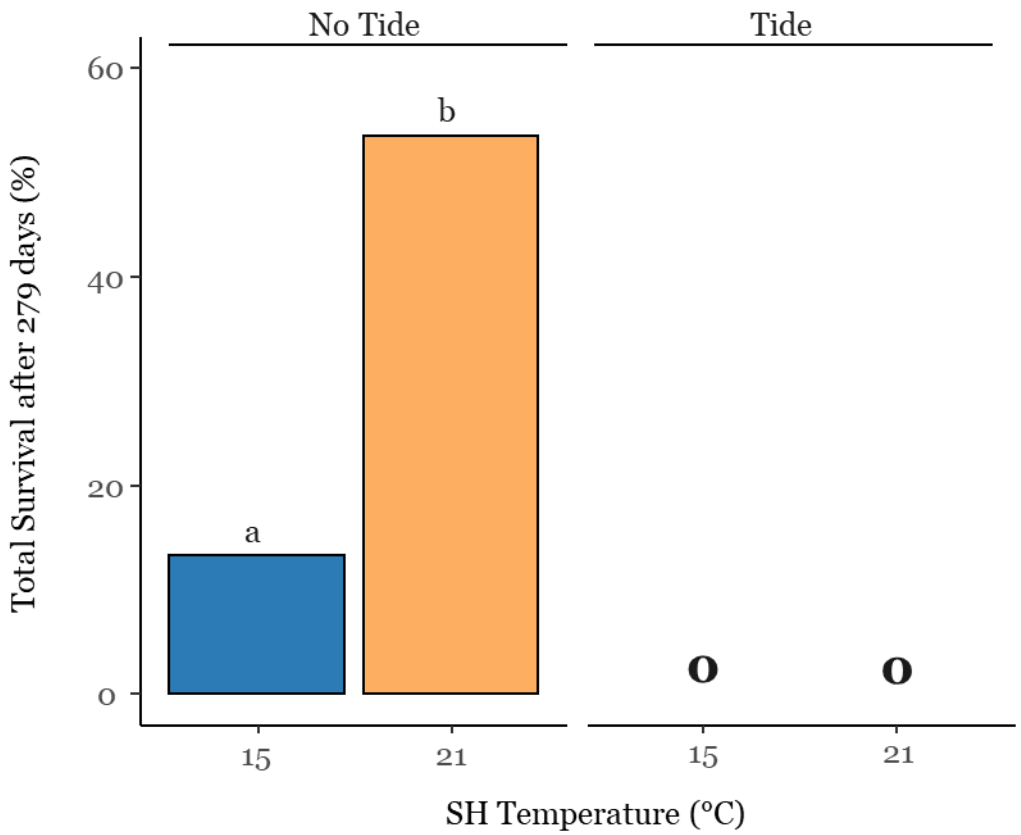

Figure 1.4. Percent survival of *O. lurida* that were outplanted in Tomales Bay from March – December 2022 after two weeks of stress hardening (SH) and a 72-hour marine heatwave. Total percent survival is displayed (as opposed to mean ± SE) because oysters were pooled by their SH treatment and could not be deployed in replicate. Letters indicate statistically significant differences in percentage survival.

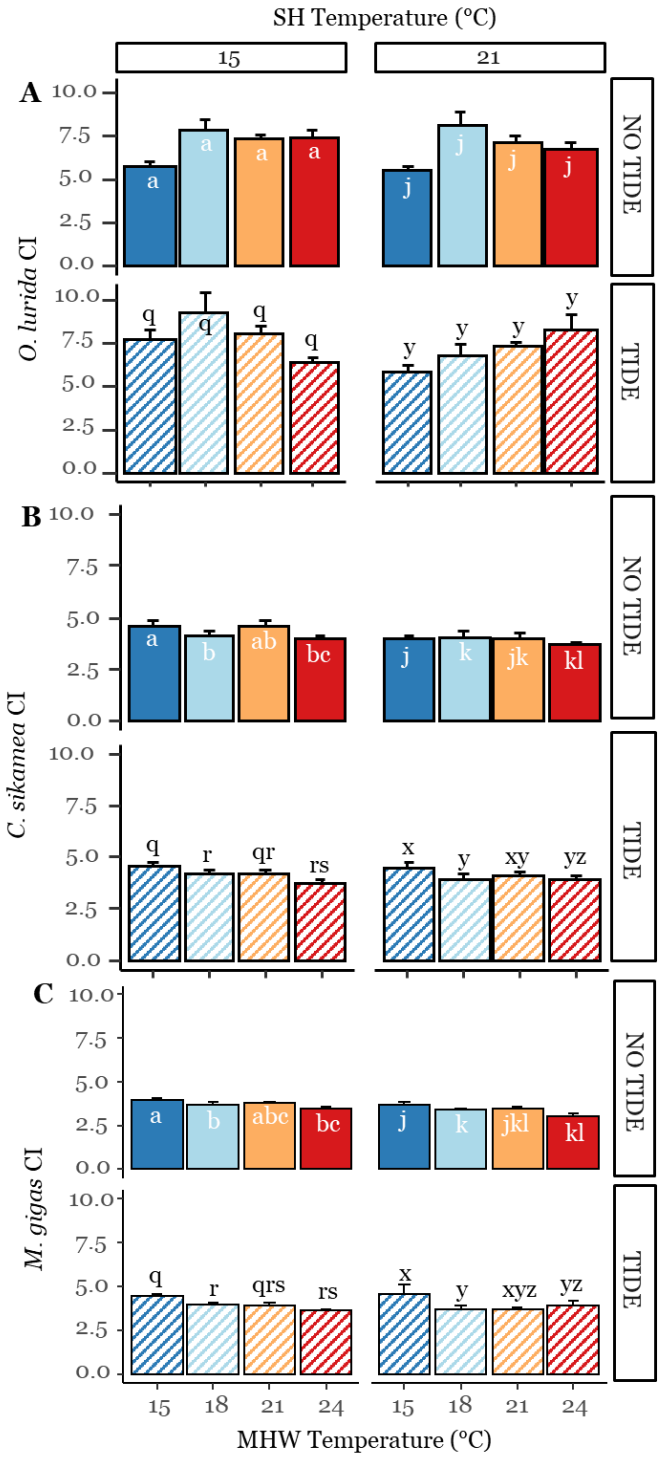

Figure 1.5. Condition Indices (CI, mean ± SE) of *O. lurida* (A), *C. sikamea* (B), and (C) *M. gigas* after the two-week stress hardening (SH) and 72-hour marine heatwave (MHW) phases of the experiment. Letters indicate statistically significant differences among MHW treatments within all four SH Temp x SH Tide treatment combinations. Statistical comparisons were made within species and not across them.

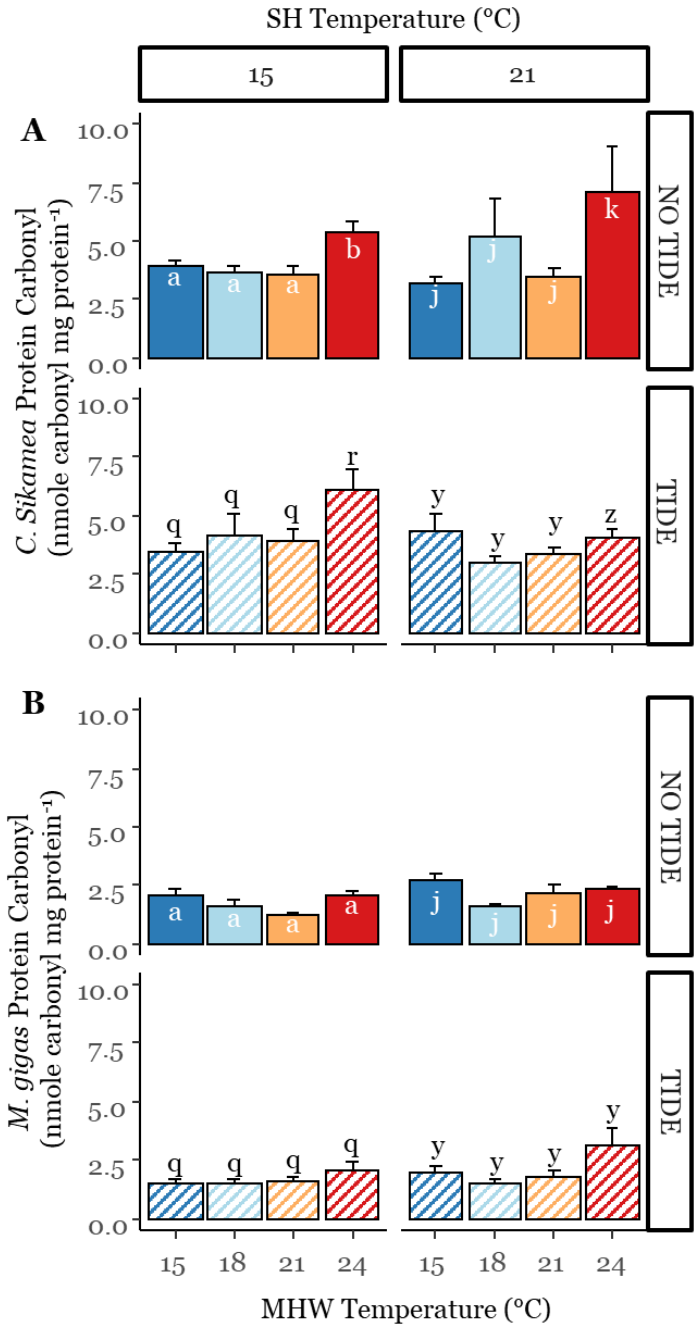

Figure 1.6. Protein carbonylation (mean ± SE) of *C. sikamea* (A) and *M. gigas* (B) after the two-week stress hardening (SH) and 72-hour marine heatwave (MHW) phases of the experiment. Letters indicate statistically significant differences among MHW treatments within all four SH Temp x SH Tide treatment combinations. Statistical comparisons were made within species and not across them.

APPENDIX 1.A - *Methods Used to Estimate Protein Carbonyl Content*

For *M. gigas*, 1 µL of MilliQ water was added for every milligram of somatic tissue, while 0.5 µL of MilliQ water was added for every milligram of *C. sikamea*'s somatic tissue. Following this, the sample was homogenized and then centrifuged at 13,000 x *g* for five minutes and 300 µL of supernatant was diluted with either 300 µL (*M. gigas*) or 900 µL (*C. sikamea*) of MilliQ so that the final proportion of MilliQ water was twice that of the somatic tissue for both species. DNPH solution was then added to each sample and incubated at room temperature for 10 minutes, followed by 30 µL of 87% TCA solution that was incubated on ice for five minutes and then centrifuged at 13,000 x *g* for two minutes. After the supernatant was removed while keeping the pellet intact, 500 µL ice-cold acetone was added and placed in a sonication bath for 30 seconds, followed by a five-minute incubation at -20˚C, and then centrifuged again at 13,000 x *g* for two minutes. Acetone was removed and 200 µL 6 M Guanidine solution was added to the pellet before briefly re-sonicating. Of this solution, 5 µL was taken to estimate protein content using a Bicinchoninic Acid Kit for Protein Determination (Sigma Aldrich, BCA1), while two 100 µL samples of this solution were transferred to a 96-well plate. Protein carbonyl content was quantified by measuring absorbance at 375 nm (A_{375}) and then determining the nmole of carbonyl per milligram of protein.

APPENDIX 1.B – *Statistical Tables*

Table 1.1.

Results for logistic regression to measure impacts of Stress Hardening (SH) Temperature and Tide as well as Marine Heatwave (MHW) Temperature on mortality for *O. lurida*, *C. sikamea*, and *M. gigas*. Absent values occur in this table because no random effect was included in the analysis for outplanted *O. lurida* and no *M. gigas* mortality was observed during SH (*ref* = reference variable).

Species	Variable	Fixed Effects	Random Effect	Estimate	SE	Z	P
O. lurida	SH	SH Temp	Tank	0.642	0.5917	1.085	0.278
		SH Tide	Tank	0.642	0.5917	1.085	0.278
	MHW	SH Temp	Tank	-0.254	0.5065	-0.501	0.616
		SH Tide	Tank	0.516	0.5188	0.994	0.32
		MHW Temp (15˚C)	Tank	*ref*	*ref*	*ref*	*ref*
		MHW Temp (18˚C)	Tank	18.20	37.34	0.487	0.626
		MHW Temp (21˚C)	Tank	18.20	37.34	0.487	0.626
		MHW Temp (24˚C)	Tank	18.38	37.34	0.492	0.623
	Outplanting	SH Temp	---	- 4.041	0.1772	-22.799	**< 0.01**
		SH Tide	---	- 1.359	0.1621	-8.384	**< 0.01**
C. sikamea	SH	SH Temp	Tank	0.584	0.4197	1.392	0.164
		SH Tide	Tank	0.081	0.40349	0.202	0.84
	MHW	SH Temp	Tank	-0.342	0.2781	-1.23	0.219
		SH Tide	Tank	0.3412	0.2781	1.23	0.219
		MHW Temp (15˚C)	Tank	*ref*	*ref*	*ref*	*ref*
		MHW Temp (18˚C)	Tank	0.172	0.4159	-11.662	0.6788
		MHW Temp (21˚C)	Tank	-0.326	0.4701	0.414	0.4879
		MHW Temp (24˚C)	Tank	0.769	0.3736	-0.694	**0.0396**
M. gigas	SH	SH Temp	Tank	---	---	---	---
		SH Tide	Tank	---	---	---	---
	MHW	SH Temp	Tank	0.408	0.6474	0.63	0.529
		SH Tide	Tank	$1.144 * 10^{-14}$	0.6344	0	1.0
		MHW Temp (15˚C)	Tank	*ref*	*ref*	*ref*	*ref*
		MHW Temp (18˚C)	Tank	0.6982	0.7088	0.804	0.422
		MHW Temp (21˚C)	Tank	$-2.806 * 10^{-13}$	1.002	0	1.0
		MHW Temp (24˚C)	Tank	$-2.132 * 10^{-13}$	1.002	0	1.0

TABLE 1.2. Pairwise comparisons of *C. sikamea* mortality after marine heatwave (MHW) trials.

MHW Comparison	Estimate	SE	Z	*P*
15˚C v. 18˚C	-0.172	0.416	-0.414	0.6788
15˚C v. 21˚C	0.326	0.470	0.694	0.4878
15˚C v. 24˚C	-0.769	0.374	-2.058	**0.0396**
18˚C v. 21˚C	0.498	0.455	1.095	0.2734
18˚C v. 24˚C	-0.597	0.354	-1.683	0.0923
21˚C v. 24˚C	-1.095	0.417	-2.628	**0.0086**

Table 1.3. Statistical output for generalized linear mixed effects models used to measure impacts of Stress Hardening (SH) Temperature and Tide as well as Marine Heatwave (MHW) Temperature on condition indices for *O. lurida*, *C. sikamea*, and *M. gigas*. The absence of degrees of freedom in the *O. lurida* condition index analysis is due to the use of a gamma distribution, instead of a gaussian distribution (*ref* = reference variable).

Species	Fixed Effects	Random Effect	Estimate	SE	df	t	P
O. lurida	SH Temp	Tank	-0.542	0.209	---	-2.600	**0.012**
	SH Tide	Tank	0.333	0.209	---	1.589	0.112
	MHW Temp (15˚C)	Tank	*ref*	*ref*	---	*ref*	*ref*
	MHW Temp (18˚C)	Tank	1.774	0.932	---	1.904	0.069
	MHW Temp (21˚C)	Tank	1.309	0.932	---	1.405	0.305
	MHW Temp (24˚C)	Tank	1.100	0.935	---	1.177	0.720
C. sikamea	SH Temp	Tank	-0.254	0.100	701	-2.542	**0.011**
	SH Tide	Tank	0.039	0.100	701	0.389	0.698
	MHW Temp (15˚C)	Tank	*ref*	*ref*	701	*ref*	*ref*
	MHW Temp (18˚C)	Tank	-0.327	0.141	701	-2.320	**0.021**
	MHW Temp (21˚C)	Tank	-0.204	0.141	701	-1.450	0.148
	MHW Temp (24˚C)	Tank	-0.591	0.142	701	-4.168	**< 0.01**
M. gigas	SH Temp	Tank	-0.154	0.091	753.326	-1.697	0.900
	SH Tide	Tank	0.390	0.091	753.395	4.307	**< 0.01**
	MHW Temp (15˚C)	Tank	*ref*	*ref*	*ref*	*ref*	*ref*
	MHW Temp (18˚C)	Tank	-0.470	0.226	16.177	-2.085	0.0532
	MHW Temp (21˚C)	Tank	-0.427	0.225	15.958	-1.900	0.0757
	MHW Temp (24˚C)	Tank	-0.649	0.225	16.092	-2.881	**0.0108**

Table 1.4. Pairwise comparisons of *O. lurida*, *C. sikamea*, and *M. gigas* condition indices after marine heatwave (MHW) trials.

Species	SH Treatment	MHW Comparison	Estimate	SE	z/t	*P*
O. lurida	SH Temp: 15˚C	15˚C v. 18˚C	-1.792	---	-1.816	0.069
	SH Temp: 15˚C	15˚C v. 21˚C	-1.008	---	-1.027	0.305
	SH Temp: 15˚C	15˚C v. 24˚C	-0.351	---	-0.359	0.720
	SH Temp: 15˚C	18˚C v. 21˚C	0.784	---	0.851	0.395
	SH Temp: 15˚C	18˚C v. 24˚C	1.441	---	1.577	0.1149
	SH Temp: 15˚C	21˚C v. 24˚C	0.657	---	0.722	0.471
	SH Temp: 21˚C	15˚C v. 18˚C	-1.697	---	-1.767	0.077
	SH Temp: 21˚C	15˚C v. 21˚C	-1.533	---	-1.593	0.111
	SH Temp: 21˚C	15˚C v. 24˚C	-1.695	---	-1.752	0.080
	SH Temp: 21˚C	18˚C v. 21˚C	0.163	---	0.182	0.856
	SH Temp: 21˚C	18˚C v. 24˚C	0.002	---	0.002	0.999
	SH Temp: 21˚C	21˚C v. 24˚C	-0.162	---	-0.178	0.8586
C. sikamea	SH Temp: 15˚C	15˚C v. 18˚C	0.327	0.141	2.319	**0.034**
	SH Temp: 15˚C	15˚C v. 21˚C	0.204	0.141	1.449	0.167
	SH Temp: 15˚C	15˚C v. 24˚C	0.591	0.142	4.167	**0.001**
	SH Temp: 15˚C	18˚C v. 21˚C	-0.122	0.141	-0.866	0.400
	SH Temp: 15˚C	18˚C v. 24˚C	0.264	0.142	1.861	0.081
	SH Temp: 15˚C	21˚C v. 24˚C	0.387	0.142	2.719	**0.015**
	SH Temp: 21˚C	15˚C v. 18˚C	0.327	0.141	2.319	**0.034**
	SH Temp: 21˚C	15˚C v. 21˚C	0.204	0.141	1.449	0.167
	SH Temp: 21˚C	15˚C v. 24˚C	0.591	0.142	4.167	**0.001**
	SH Temp: 21˚C	18˚C v. 21˚C	-0.122	0.141	-0.866	0.400
	SH Temp: 21˚C	18˚C v. 24˚C	0.264	0.142	1.861	0.081
	SH Temp: 21˚C	21˚C v. 24˚C	0.387	0.142	2.719	**0.015**
M. gigas	SH Tide: No Tide	15˚C v. 18˚C	0.470	0.226	2.085	**0.050**
	SH Tide: No Tide	15˚C v. 21˚C	0.427	0.225	1.900	**0.057**
	SH Tide: No Tide	15˚C v. 24˚C	0.649	0.225	2.881	**0.011**
	SH Tide: No Tide	18˚C v. 21˚C	-0.043	0.226	-0.192	0.850
	SH Tide: No Tide	18˚C v. 24˚C	0.179	0.226	0.790	0.441
	SH Tide: No Tide	21˚C v. 24˚C	0.222	0.225	0.985	0.339
	SH Tide: Tide	15˚C v. 18˚C	0.470	0.226	2.085	**0.050**
	SH Tide: Tide	15˚C v. 21˚C	0.427	0.225	1.900	**0.057**
	SH Tide: Tide	15˚C v. 24˚C	0.649	0.225	2.881	**0.011**
	SH Tide: Tide	18˚C v. 21˚C	-0.043	0.226	-0.192	0.850
	SH Tide: Tide	18˚C v. 24˚C	0.179	0.226	0.790	0.441
	SH Tide: Tide	21˚C v. 24˚C	0.222	0.225	0.985	0.339

Table 1.5. Protein Carbonyl of *C. sikamea* and *M. gigas* after the two-week stress hardening (SH) and 72-hour marine heatwave (MHW) phases of the experiment.

Species	Variable	Fixed Effects	Random Effect	Estimate	SE	*t*	P
C. sikamea	Protein Carbonyl	SH Temp	Tank	-0.177	0.374	-0.473	0.637
		SH Tide	Tank	-0.371	0.365	-1.015	0.311
		MHW Temp (15˚C)	Tank	*ref*	*ref*	*ref*	*ref*
		MHW Temp (18˚C)	Tank	0.210	0.438	0.478	0.633
		MHW Temp (21˚C)	Tank	-0.099	0.413	-0.240	0.811
		MHW Temp (24˚C)	Tank	1.805	0.535	3.377	**<0.01**
M. gigas	Protein Carbonyl	SH Temp	Tank	0.257	0.119	2.151	**0.032**
		SH Tide	Tank	-0.157	0.173	-0.912	0.363
		MHW Temp (15˚C)	Tank	*ref*	*ref*	*ref*	*ref*
		MHW Temp (18˚C)	Tank	-0.545	0.285	-1.909	0.056
		MHW Temp (21˚C)	Tank	-0.353	0.286	-1.235	0.217
		MHW Temp (24˚C)	Tank	0.296	0.286	1.035	0.301

*** * * CHAPTER 2 * * ***

Stress Hardening May Impact Pacific Oyster (*Magallana gigas*), but not Kumamoto Oyster (*Crassostrea sikamea*), Responses to an OsHV-1 Outbreak in Tomales Bay, CA

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ABSTRACT

As anthropogenic climate change causes ocean temperatures to rise, disease transmission is also expected to increase. Ostreid herpesvirus (OsHV-1) is a temperature-associated pathogen that predominantly affects the commercially important Pacific Oyster (*Magallana gigas*), with some µVars causing > 95% mortality of oyster stocks. Thus, exploring strategies for improving the species' response to a disease outbreak is of critical importance. In this study, we investigated the effects of laboratory-based stress hardening (SH) via temperature (15˚C/16˚C v. 21˚C) and tide (tidal cycle vs. full submersion) on commercially farmed oysters with the expectation that short-term exposure to warmer conditions and a simulated tidal regime would improve the performance of outplanted oysters. In 2021 and 2022, we exposed oysters to SH mechanisms before outplanting *M. gigas* and *Crassostrea sikamea* at three sites spanning a thermal gradient in Tomales Bay, CA, USA, where they were exposed to OsHV-1 outbreaks in the field. We measured mortality, growth, viral load, temperature, and chlorophyll *a* concentrations at all sites, and then glycogen and protein carbonyl content in stress hardened *M. gigas* in 2022 only. We compared these outcomes to a baseline study completed in 2020, where *M. gigas* that had not been conditioned were exposed to OsHV-1 at two sites. *M. gigas* submerged in the warmer SH treatment had lower mortality in 2021, and those that experienced tidal fluctuations during SH had higher growth rates in 2022. In 2021, we saw no effect of SH on *C. sikamea* growth or mortality, but oysters were infected with OsHV-1. To our knowledge, this is the first study to provide OsHV-1 copy numbers (mg tissue^{-1}) for *C. sikamea*. Additionally, glycogen content mirrored chlorophyll *a* concentrations, while protein carbonyl seemed to reflect OsHV-1 infection levels at the end of summer 2022. Further, *M. gigas* at the warmer South site had lower mortality rates despite being infected with OsHV-1. Ultimately, SH could be part of a larger approach to sustaining oyster aquaculture impacted by disease outbreaks in a changing ocean.

KEYWORDS: Stress Hardening, Carryover Effects, Aquaculture, OsHV-1, *Magallana*, *Crassostrea*

INTRODUCTION

Anthropogenic climate change is associated with global increases in infectious disease (IPCC 2022). Though there is some equivocation on which climatic factors may drive pathogen transmission (Khasnis and Nettleman 2005, Lafferty 2009, Karvonen et al. 2010), temperature is an established modulator of disease (Rohr and Cohen 2020). Thermal anomalies and variability during climatic events, such as El Niño, have been linked with increased disease activity (Rohr et al. 2013, Anyamba et al. 2019) and warmer conditions have facilitated infection across terrestrial and aquatic systems (Berger et al. 2004, Ward et al. 2007, Ciota and Keyel 2019). With global temperatures expected to rise (IPCC 2022), understanding the impacts of warming is especially critical in the lesser studied marine realm (Byers 2021).

With marine heatwaves increasing in frequency (Oliver et al. 2018), ocean habitats and marine aquaculture will likely become more susceptible to disease outbreaks (reviewed by Burge et al. 2014 and Byers 2021). Thermal stress has been tied to diseases degrading coral ecosystems, including aspergillosis, black band disease, and stony coral tissue loss disease (reviewed by Rosenberg and Ben-Haim 2002, Burge et al. 2014). Increased temperature has also been implicated in the decline of abalone (*Haliotis* spp.) populations because species that evolved in cooler regions are more vulnerable to withering syndrome, thus demonstrating that thermal history can drive species responses (Crosson and Friedman 2018). Aquaculture operations in tropical

regions generally experience greater disease severity and cumulative mortality than temperate areas (Leung and Bates 2013), though rising temperatures are expected to impact aquaculture across latitudes (Mugwanya et al. 2022). While it is valuable to understand how warming broadly influences disease, it is equally important to consider how those trends vary across different species, particularly when they are of economic importance.

Oyster cultivation, which makes up a significant portion of the 34.6 billion dollar (USD) worldwide shellfish aquaculture industry, is an economically important enterprise that is susceptible to a wide variety of diseases (Burge et al. 2014, Botta et al. 2020, FAO 2022). QX disease, caused by a protozoan parasite sensitive to changes in temperature and salinity, has decreased production of the Sydney rock oyster (*Saccostrea glomerata*) in the Hawkesbury River, AUS after widespread infections in 2005 (Rubio et al. 2013). Similarly, increases in water temperature and salinity are directly related to infections of Dermo, caused by *Perkinsus marinus*, in Eastern oysters (*Crassostrea virginica*) (Cook et al. 1998, La Peyre et al. 2010). Disease is especially well-studied in the Pacific oyster (*Magallana gigas*) because it is the most commonly farmed oyster worldwide (FAO 2022). Not only is *M. gigas* plagued by multiple pathogens (Elston 1993), such as *Vibrio spp.* (Dégremont et al. 2021, Wang et al. 2021), haplosporidians (Arzul et al. 2022), and protists (Elston et al. 2012), but the onset of one disease can overwhelm the immune system and lead to subsequent infections (de Lorgeril et al. 2018, Petton et al. 2021). Despite the propensity of etiological agents affecting *M. gigas*, Ostreid herpesvirus (OsHV-1) and its variants are arguably of greatest concern in *M. gigas* aquaculture due to their global distribution and lethality.

OsHV-1 has been documented in multiple oyster production regions over the past 50 years. Electron microscopy was initially used to identify herpes-like viral infections in *C. virginica* in the United States (Farley et al. 1972), *M. gigas* in France (Nicolas et al. 1992), as well as *Ostrea angasi* in New Zealand (Hine et al. 1992) and Australia (Hine and Thorne 1997). As herpesvirus continued to concomitantly occur with *M. gigas* mortality events (Deuff and Cochennec 1994, Renault et al. 1994, Le Deuff et al. 1996), sequencing of the OsHV-1 genome allowed for the development of PCR primers used to detect the reference strain of OsHV-1 at the end of the 20th century (Renault and Cecile 1998, Renault et al. 2000). Such molecular diagnostic tools have since been used to detect OsHV-1 variants across the globe (e.g., Arzul et al. 2002, Barbosa-Solomieu et al. 2005, Garcia et al. 2011), including the current study sites in Tomales Bay, CA, USA (Friedman et al. 2005, Fig. 2.1). Here, OsHV-1 was first detected in 1995 (C. Burge, pers. comm.), though summertime mortalities have been documented since 1993 (Friedman et al. 2005).

The association between OsHV-1 and mortality events has led to extensive research concerning how temperature affects disease transmission among oysters across different countries (Burge et al. 2006, de Kantzow et al. 2016, Delisle et al. 2018) and life stages. OsHV-1 has been implicated in oyster mortalities once seawater temperatures exceed 16˚C (Pernet et al. 2012, Petton et al. 2013), with some microvariants (µVars) becoming active above 18˚C (Paul-Pont et al. 2014, de Kantzow et al. 2016). Moreover, increases in mean daily seawater temperature (Burge et al. 2006, Garcia et al. 2011, Whittington et al. 2015b) as well as sudden shifts in temperature (Clegg et al. 2014, Renault et al. 2014) have been linked with subsequent outbreaks and

oyster mortality events. OsHV-1 DNA quantity tends to increase with temperature until it is sufficiently warm enough to inhibit viral propagation, with 24˚C as a suggested upper threshold for one variant (Pernet et al. 2012), though mortalities at 26˚C have been detected as well (de Kantzow et al. 2016). Infection does not always result in mortality and subclinically infected oysters, particularly those with low viral loads, are capable of surviving (Whittington et al. 2019). Given the tightly coupled relationship between temperature and OsHV-1, developing strategies for increasing oyster survival while grappling with both warming and disease is critical.

Exposing oysters to an environmental stressor prior to outplanting them may also decrease mortality rates. Termed stress hardening (SH), this method poses an opportunity to help oysters develop cellular mechanisms to cope with both elevated temperature and disease stress in the field. Increases in temperature are commonly used as a SH mechanism and can help inure organisms against impacts of more severe warming and other stressors (Todgham et al. 2005, Hackerott et al. 2021). Recently settled *C. virginica* immersed at 32˚C for one month that subsequently experienced salinity stress had higher survival than oysters without prior thermal exposure (Agrawal and Jurgens 2023). *M. gigas* that experience warmer waters prior to being infected with OsHV-1 can also exhibit lower mortalities despite increased virulence of OsHV-1 at higher temperatures (Delisle et al. 2018, de Kantzow et al. 2019), though this is not always the case (Camara et al. 2017). *M. gigas* adults, but not spat, that experienced warmer air temperatures by being deployed at higher tidal heights also had lower rates of OsHV-1 infection and greater survival (Whittington et al. 2015a). Thus, SH may have the capacity to reduce the susceptibility of *M. gigas* to OsHV-1.

To explore the potential benefits of SH on *M. gigas*, we exposed oysters to a combination of temperature and tidal hardening treatments in the lab before outplanting them at three oyster leases across a thermal gradient in Tomales Bay, CA during the summers of 2020-2022. The first year was a baseline study without SH, followed by an exploration of thermal hardening in 2021, and both thermal and tidal hardening in 2022. We also included temperature- and tidally hardened Kumamoto oysters (*C. sikamea*) in the 2021 deployment to understand (1) how SH impacts an oyster species not known to succumb to OsHV-1 and (2) whether it can serve as a viral reservoir. In addition to monitoring ambient temperatures and chlorophyll *a*, we measured oyster growth and mortality during each deployment as well as OsHV-1 quantity during mortality events. During the final year, we also measured protein carbonyl and glycogen content in *M. gigas* as indices of cellular protein damage and energy stores, respectively. We predicted that (1) the oysters experiencing the warmer temperature and a tidal regime during SH would have the lowest glycogen, mortality and viral load, and that (2) OsHV-1 quantity as well as protein carbonyl would be highest at the warmest site in Tomales Bay, CA.

METHODS

Site

Tomales Bay is a tectonically-formed, tidally-influenced estuary in northern California bisected by the San Andreas fault (Oberdorfer et al. 1990). It is considered a low-inflow drowned river estuary (Hearn and Largier 1997) with a Mediterranean climate and seasonally driven oceanography, including a thermal gradient that forms

each summer with cooler temperatures closer to the mouth of the bay and warmer conditions at the back (Smith and Hollibaugh 1997). Outplanting experiments occurred during Tomales Bay's upwelling season (April – July) and its relaxation season (August – November) (Smith et al. 1991), when OsHV-1 outbreaks have been detected in the bay (Friedman et al. 2005, Burge et al. 2006, 2007). The former is characterized by strong winds blowing surface waters offshore that cause cool, saline, nutrient-rich waters from the ocean's depths to emerge, while the latter has fewer wind events and increased stratification of water (Smith et al. 1991). Phytoplankton concentrations (the diatoms *Chaetoceros*, *Thalassiosira* and *Skeletonema* are most abundant during the summer) do not track summer temperatures in Tomales Bay, and instead peak in the middle (Hearn and Largier 1997), which is reflected in oyster growth throughout the bay (Kimbro et al. 2009, Hollarsmith et al. 2019). The globally cultured Pacific oyster (*M. gigas*) and slower-growing Kumamoto oysters (*C. sikamea*) were outplanted at aquaculture leases spanning Tomales Bay, with sites representing the "North" (experiences the lowest temperatures due to its proximity to the mouth of Tomales Bay, and therefore, the Pacific Ocean; 38.22,-122.95), "Middle" (38.21,-122.93), and "South" (furthest from the mouth of the Bay with the warmest temperatures; 38.12,-122.86) of the estuary (Fig. 2.1). Temperatures at the three sites in Tomales Bay followed a consistent thermal gradient with mean temperatures at the South site warmer than at the Middle site and coolest at the North site (Fig. 2.2).

Stress Hardening

Juvenile *M. gigas* (15-25 mm) and *C. sikamea* (25 mm) were shipped overnight on ice from the Hog Island Oyster Company nursery in Samoa, CA and delivered to the

UC Davis Bodega Marine Laboratory (BML), Bodega Bay, CA. They were immediately placed in tanks with flow-through seawater from the Pacific Ocean at 14˚C for 48 hours, which was identical to the temperatures at the nursery's floating upweller system each year (2020-2022). Oysters were then brought up to their respective SH temperature treatments at a rate of 0.5˚C per hour.

The system described in Shukla et al. *in prep* was used to generate SH treatments from 2021-2022 (see "Temperature Manipulation" and "Tidal Simulation" in Methods). Briefly, oysters that experienced thermal hardening experienced one of two consistent seawater temperature treatments and one of two tidal treatments. Oysters in the "tide" treatment were emersed during two alternating six-hour periods while those in the "no tide" treatment were submerged for the duration of SH. In 2021, both *M. gigas* and *C. sikamea* experienced SH in the lab for two weeks. While *C. sikamea* experienced both tidal ("tide" vs. "no tide") and temperature (16˚C v. 21˚C) hardening, *M. gigas* was in limited supply due to the COVID-19 pandemic and only experienced thermal hardening. Specifically, 10,000 25-mm *C. sikamea* were divided across 20 tanks (n = 500 oysters per tank) with five tanks assigned to each SH combination (16˚C and tide; 16˚C and no tide; 21˚C and tide; 21˚C and no tide). An additional 4,060 15-mm *M. gigas* were spread across four tanks (n = 1015 oysters per tank), two of which belonged to each SH temperature treatment (16˚C and no tide; 21˚C and no tide). In 2022, 10,000 25-mm *M. gigas* were spread across the same system used by *C. sikamea* in the previous year, but with a slightly different control temperature during SH (15˚C and tide; 15˚C and no tide; 21˚C and tide; 21˚C and no tide). Throughout SH, oysters were fed daily by adding 10L of *Nanochloropsis* and *Isochrysis* to each sump (reservoir of seawater used to create

thermal SH treatments) along with 20mL of undiluted Shellfish Diet 1800 (Reed Mariculture, ~2 billion algal cells/mL) during the "high tide" phase of SH (when all tanks were filled with water). During feeding, incoming seawater and drainage were suspended for ~ 2 hrs to facilitate algal consumption.

Outplanting Experiments

Outplanting experiments were conducted at leases belonging to the Hog Island Oyster Company (North), Bodega Bay Oyster Company (Middle), and Tomales Bay Oyster Company (South). *M. gigas* were outplanted at two sites (North and South) without SH to gather a baseline of OsHV-1 dynamics in Tomales Bay in 2020. Specifically, 250 25-mm oysters were deposited in one of four vexar baskets (10-mm mesh) at each site (n = 8 total baskets); oyster density was based upon standards used at the Hog Island Oyster Company (D. Mancilla-Cortez, pers. comm.). Each basket was clipped sequentially to a pre-existing line on the substrate courtesy of the associated oyster company from July – October. These oysters remained at their designated locations for the following two years and were sampled for OsHV-1 in December 2021 and August 2022; the baskets at the South site were clipped to a line that was severed during a winter storm in 2021 and thus were unavailable for sampling in 2022.

In 2021, *M. gigas* and *C. sikamea* from each of the four SH treatments were separately divided across nine baskets, with three baskets per treatment for both species (n = 18 baskets per site; n = 12 for *C. sikamea*, n = 6 for *M. gigas*) deployed at each site (North, Middle, and South) in a linear fashion on the substrate from July – September (Fig. 2.1). While 225 *M. gigas* were added to each basket prior to outplanting, *C. sikamea* mortalities during SH led to different starting amounts for each SH treatment,

with 250 oysters per basket for all oysters that experienced 16˚C (tide and no tide). In contrast, 245 and 175 oysters were put in all baskets representing the "21˚C and tide" as well as the "21˚C and no tide" SH treatments, respectively. A mortality event in a single tank within the latter SH treatment caused this low initial amount.

For 2022, 250 *M. gigas* were added to nine vexar baskets per four SH treatments (n = 3 baskets per treatment per site). Baskets were then dispersed across the same three sites (n = 12 baskets per site) used in the previous year. At each site, baskets were again arranged on the substrate in a single line from May - August.

Environmental Factors: Ambient Temperature and Chlorophyll a

Seawater (2020-2022) and air (2021-2022) temperature were measured using HOBO data loggers (Onset Inc.). Measurements were taken at regular intervals (2020: 1-hr; 2021-2022: 10 mins) until the end of the field portion of the experiment. To avoid insolation affecting air temperature readings, data loggers were housed within solar radiation shields (Onset Inc.).

Benthic (2021-2022) and seawater chlorophyll *a* (2022) were measured on a bimonthly basis at each site throughout the field portion of the experiment as a proxy for phytoplankton concentrations. To estimate benthic chlorophyll at each site, methods similar to Jacobs et al. (2021) were used. A 5-cc syringe was used to collect five 5-mm-deep sediment samples along the length of each line that baskets were clipped to. Each sample was taken in an area that was not inundated and devoid of visible macroalgae. Once extracted, each sample was deposited in a 15-mL falcon tube and then preserved in the dark at 0˚C (Appendix 2.A). Seawater chlorophyll *a* samples were taken by filling a 1-L opaque Nalgene bottle to the bottom of the neck with seawater flowing nearby that

was undisturbed with no visibly dissolved sediments. Samples were then frozen at -20˚C until they could be filtered and analyzed following methods outlined in Holm-Hansen and Riemann (1978) and Herbland et al. (1985) (Appendix 2.B).

Mortality

Mortality during the outplanting experiment was measured by counting the number of dead oysters in every basket on a bimonthly basis during low tides, when baskets were fully exposed and easily accessible. Specifically, oysters were removed from their respective baskets and examined to see if their valves were gaping or if shells had been separated from the hinge and were devoid of tissue. At the end of every deployment, cumulative mortality was calculated for each basket based on the total number of oysters lost during the outplanting period absent the amount of living oysters removed for PCR and physiology analyses.

Shell Length

Shell length was used to measure growth over the course of the outplanting experiment. Each year, a subsample of 10 oysters from each basket were selected during the first and final sampling time point to photograph next to a ruler for scale. These images were uploaded in ImageJ software (Schneider et al. 2012), where the line tool was used to determine the length between the hinge and posterior edge of the shell.

Glycogen Depletion

Glycogen samples were collected in June (before a mortality event) and August (during a mortality event) 2022. Five oysters were collected from each basket and transported on dry ice to the BML and stored at -80˚C until analysis. Glycogen content

analysis followed the protocol described by Nancollas and Todgham (2022), with a modification where glycogen (µmol glycosyl units) was standardized by the quantity of protein (g) instead of tissue weight, as the whole body was used in this analysis due to low cumulative glycogen levels in the gill and mantle tissues (Shukla and Nancollas, unpublished data) (Appendix 2.C).

Protein Carbonyl Content

Protein carbonyl samples were collected alongside glycogen samples in August 2022 when a mortality event occurred. Five oysters were collected from each basket and transported on dry ice to the BML and stored at -80˚C. Protein carbonyl was quantified using the Protein Carbonyl Content Assay Kit (Sigma Aldrich, MAK094) and followed the protocol used in Appendix 1.A of Shukla et al. *in prep*.

OsHV-1 Sample Preparation and Quantification

During each sampling, individual oysters were collected from each basket for the purposes of OsHV-1 quantification and stored at –80˚C until processed (2020: n = 20 oysters; 2021: n = 9 oysters; 2022: n = 20 oysters). However, only oysters collected during mortality events were used (two sampling time points per year). A fragment of gill and mantle tissue from each oyster was excised during dissection and pooled together (2020: n = 4 pools of 5 oysters each; 2021: n = 3 pools of 3 oysters each; 2022: n = 4 pools of 5 oysters each) to create a tissue sample weighing 15-25 mg (2020: n = 4 pools per basket per sampling period; 2021: n = 3 pools per basket per sampling period; 2022: n = 4 pools per basket per sampling period). The tissue was then digested and extracted using the Qiagen DNEasy Kit following the manufacturer's protocol. OsHV-1 was subsequently quantified in pooled samples (which is standard for large volumes of

OsHV-1 samples) using OsHV-1 specific qPCR following methods of Burge and Friedman (2012) as modified by Burge et al. (2020) and Agnew et al. 2020 (Appendix 2.D).

Statistical Analyses

Statistical analyses were conducted using maximum likelihood estimation methods via the *lme4* package in R (Bates et al. 2015). To test differences among chlorophyll *a* concentrations, sampling type (benthic versus seawater), site, and sampling period were input as fixed effects. For the baseline study in 2020, cumulative mortality of *M. gigas* was compared using site and sampling period as fixed effects with basket as a random effect to account for non-independence. SH temperature, SH tide, and site were included as fixed effects with basket as a random effect to interpret how they affected cumulative mortality of *C. sikamea* outplanted in 2021. Additionally, SH temperature, SH tide, and site were treated as fixed effects and basket as a random effect to estimate impacts on cumulative mortality of *M. gigas* that experienced only thermal hardening in 2021 and combined SH treatments in 2022. These same factors were used to determine changes in shell length for both species across all three years, with the addition of sampling period nested within year to account for differences in growth between the beginning and end of outplanting. Further, SH treatments, and site were used as fixed effects and basket as a random effect to predict protein carbonyl levels, while the same fixed and random effects along with sampling period as a fixed effect were used for glycogen content. For OsHV-1 quantity within oysters (data were log-transformed), SH treatments, site, and sampling period were treated as fixed effects with basket as a random effect for *M. gigas* in 2020 and *C. sikamea* in 2021. However,

sampling period was nested within year for stress hardened *M. gigas* outplanted in 2021 and 2022. An additional analysis from the mortality event in August 2022 that examined the difference in viral load between a random assortment of juvenile oysters outplanted in May 2022 and adult oysters originally outplanted in July 2020 (4 pools of 5 oysters each) used cohort as a fixed effect.

Linear mixed models with Gaussian distributions and an "identity" link function were used for the majority of analyses, except in the case of protein carbonyl and chlorophyll *a*, where a gamma distribution with an "identity" link function was employed. A logistic regression with a binomial distribution and "logit" link function was used for cumulative mortality. All models were compared to a null model and then AIC scores were used to select the best fitting model, with model residuals used to check for normality (if a Gaussian distribution was used), overdispersion, and homoscedasticity.

RESULTS

Oyster Temperature

Oysters at all three sites (North, Middle, South) experienced temperatures at or above 16˚C (Figs. 2.2, 2.3), when OsHV-1 is known to become active (Pernet et al. 2012, Petton et al. 2013). Oysters at the South site (2020: 97.6%; 2021: 87.4%; 2022: 89%) spent more time above this threshold than those at the Middle (2021: 86.6%; 2022: 78.6%) or North (2020: 71%; 2021: 69.8%; 2022: 59.5%) sites (Fig. 2.4A). Similarly, oysters at the North site (2020: 5.44%; 2021: 49.4%; 2022: 37.9%) spent less time at or

above 18˚C than oysters at the Middle (2021: 73.2%; 2022: 52.2%) and South (2020: 15.8%; 2021: 84.7%; 2022: 78.8%) sites in 2020 and 2021. This trend continued for the amount of time oysters spent at or above 21˚C in 2020 (North: 1.5%; South: 7.87%) and 2021 for all sites (North: 14.8%; Middle: 27.1%; South: 59%) and oysters at the South site (42%) spent the most time at or above 21˚C in 2022. But, oysters at the Middle site (12.5%) spent slightly less time at or above 21˚C than those in the North site (14.8%) in 2022. (Figs. 2.4B, 2.4C).

Benthic and Seawater Chlorophyll a

Across all sampling periods, chlorophyll *a* levels did not differ between benthic and seawater samples ($t = 0.349$, $P > 0.05$) nor did they vary from 2021 to 2022 ($t = 0.666$, $P > 0.05$). However, chlorophyll *a* concentrations were not consistent across sampling periods ($t = 3.544$, $P < 0.01$) and site ($t = -4.409$, $P < 0.01$). Overall, chlorophyll *a* was lowest at the South site (2.53 ± 0.24 mg m^{-3}; range: 0 - 7.68), while being relatively higher at the Middle (4.77 ± 0.43 mg m^{-3}; range: 1.38 – 13.2) and North (4.38 ± 0.44 mg m^{-3}; range: 1.18 – 15.8) sites (Figs. 2.5A-B, Table 2.1).

Mortality

During the initial baseline study in 2020, *M. gigas* mortality was higher at the North site (10.8% ± 5.5) than the South site (7.5% ± 3.7; $z = -2.2629$, $P < 0.01$) (Fig. 2.6A; Tables 2.2, 2.3). SH temperature affected *M. gigas* mortality ($z = -12.123$, $P < 0.01$) in both 2021 and 2022 ($z = -13.144$, $P < 0.01$), though not in the same way. There was an interaction between SH temperature and year ($z = 12.124$, $P < 0.01$), which reflected that relative to the oysters that experienced the cooler SH temperature treatment, those in the 21˚C SH treatment had lower cumulative mortality in 2021

(16˚C: 55.6% ± 12.4; 21˚C: 39.3% ± 8.5; Fig. 2.6B, 2.7A; Table 2.2), but higher cumulative mortality in 2022 (15˚C: 14.6% ± 3.3; 21˚C: 20.9% ± 3.3; Fig. 2.6C, 2.7B; Table 2.2). Both of these factors also interacted with site (z = -5.211, P < 0.01), as significantly lower mortality was observed at the South site (2021: 6.4% ± 1.9; 2022: 6.7% ± 1.0) compared with the Middle (2021: 66.7% ± 7.2; 2022: 24.8% ± 4.8) and North sites (2021: 69.2% ± 4.8; 2022: 21.8% ± 3.5) (z = -1.640, P > 0.05; (Fig. 2.6C, 2.7B; Table 2.2). Tidal exposure during SH did not affect mortality in 2022 (z = -1.434, P > 0.05).

Neither SH treatment influenced mortality for stress hardened *C. sikamea* outplanted in 2021 (z = -0.196/1.291, P > 0.05), However, mortality was significantly different among sites (z = 10.213, P < 0.01) with higher rates at the South site (20.1% ± 2.9), but no difference between the Middle (1.8% ± 1.3) and North sites (1.4% ± 1.2; z = -13.144, P > 0.05) (Fig. 2.6D, 2.7C; Tables 2.2, 2.3).

Shell Length

For *M. gigas* outplanted in 2020 as part of the baseline study, sampling period (t = 13.590, P < 0.01) was more important than site (t = -0.673, P > 0.05) in determining shell growth, with shells increasing 21.2% in length between July (4.21 ± 0.04 cm) and September 2020 (5.34 ± 0.07 cm) (Fig. 2.8A; Table 2.4). Although shell growth did vary between years (t = 6.840, P < 0.01), SH temperature did not influence the growth of *M. gigas* in 2021 or 2022 (t = -6.820, P > 0.05), but site did in both years (t = -2.966, P < 0.01). Oysters at the South site were 24.1% and 12.2% smaller (2.87 ± 0.05 cm) than those at Middle (3.78 ± 0.08 cm) and North (3.27 ± 0.07 cm) sites by August 2021 (Fig. 2.8B; Table 2.4), respectively. Similarly, *M. gigas* at the North (3.90 ± 0.0 cm) and

Middle (3.79 ± 0.07 cm) sites were each 6.9% and 4.2% larger than oysters at the South site (3.63 ± 0.04 cm) in August 2022. Tidal aerial exposure during SH also affected shell lengths (t = 2.017, P < 0.05), with oysters that experienced a tidal cycle in the lab (3.85 ± 0.05 cm) being 3.9% longer than those that were fully submerged for two weeks (3.70 ± 0.04 cm) (Fig. 2.8C; Table 2.4).

SH temperature and tidal treatments did not influence shell length in *C. sikamea* (t = -0.391, P > 0.05). However, sampling period (beginning versus end of outplanting) affected shell length (t = 19.096, P < 0.01) and interacted with site (t = 5.022/-10.111, P <0.01), though site alone did not affect shell length (t = -0.333/1.256, P > 0.05) (Fig. 2.8D; Table 2.4). Oysters were initially the same relative size (2.73 ± 0.02 cm), but *C. sikamea* shell length at the North site (3.53 ± 0.03 cm) differed significantly from both the Middle (P <0.01; 3.81 ± 0.04 cm) and South sites (2.98 ± 0.03 cm) by the end of outplanting.

Glycogen Depletion

Neither SH temperature (t = -0.809, P > 0.05) nor tidal treatments (t = 0.367, P > 0.05) influenced glycogen content. Glycogen did, however, vary between both sampling periods (t = -4.848, P < 0.05) and across site (t = -11.726/-9.101, P < 0.1), with both factors interacting (t = 9.566/4.321, P < 0.01; Table 2.5). When *M. gigas* were initially sampled in June 2022, glycogen was far higher at the North site (14.6 ± 1.22 μmol glycosyl units g protein^{-1}, t = 11.763 / 11.910, P < 0.01; Table 2.6) than at the Middle (2.35 ± 0.22 μmol glycosyl units g protein^{-1}) and South (2.20 ± 0.18 μmol glycosyl units g protein^{-1}) sites (t = 0.147, P > 0.05; Table 2.6). But, when sampled during the mortality event in August 2022, glycogen was greater in the Middle (11.0 ±

0.80 μmol glycosyl units g protein^{-1}) and North (9.83 ± 0.75 μmol glycosyl units g protein^{-1}) sites (t = -1.093, P > 0.05; Table 2.6) than in the South site (3.47 ± 0.50 μmol glycosyl units g protein^{-1}, t = 6.066 / 7.155, P < 0.01; Fig. 2.9; Table 2.6).

Protein Carbonyl Content

SH temperature (t = 1.576, P > 0.05; Table 2.4) and tidal exposure (t = 1.556, P > 0.05; Table 2.5) did not drive protein carbonyl content in *M. gigas* during a mortality event in August 2022 Rather, the amount of carbonylated proteins varied by site (t = 2.046, P < 0.05; Fig. 2.10; Table 2.4). The level of carbonylated proteins was higher at the North site (5.46 ± 0.63 nmole carbonyl mg protein^{-1}) than at the Middle (4.06 ± 0.25 nmole carbonyl mg protein^{-1}) and South (3.90 ± 0.32 nmole carbonyl mg protein^{-1}) sites (t > 2.200, P < 0.05; Fig. 2.10; Tables 2.5, 2.6), though the latter two sites did not differ significantly from one another (t = 0.299, P > 0.05; Fig. 2.10; Table 2.6).

OsHV-1 Load Across Three Years (2020 - 2022)

In 2020, oysters deployed at the North site had more copies (1.12×10^6 (mean) ± 8.92×10^5 (SE) copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 2.92×10^7) than those at the South site (1.03 ± 0.18 copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 3.01) (t = -2.330, P = 0.058; Fig. 2.11; Table 2.7), and this quantity did not vary significantly between sampling periods (t = -1.801, P > 0.05; Fig. 2.11; Table 2.7). When comparing viral loads in juvenile *M. gigas* outplanted in 2020 to adults from that cohort in 2021 and 2022, there continued to be a difference between the North and South sites (t = -5.472, P < 0.01; Table 2.8). OsHV-1 quantity did not differ between the two sampling periods in 2020 (t = -1.561, P > 0.05; Table 2.8), but it did change across the years. Specifically, mean viral load remained low at the South site in 2020 (1.76 ± 0.21 copies

of OsHV-1 DNA mg tissue^{-1}; range: 0 – 3.01) and 2021 (0.38 ± 0.17 copies of OsHV-1 DNA mg tissue $^{-1}$; range: 0 – 1.85) (t = -2.085, P < 0.05; Fig. 2.11; Table 2.7). In comparison, OsHV-1 viral quantity at the North site was highest at the beginning of the mortality event in 2020 ($1.89 \times 10^6 \pm 1.71 \times 10^6$ copies mg tissue^{-1}; range: 0 – 2.92×10^7), but lower in 2021 (39.9 ± 27.5 copies mg tissue^{-1}; range: 0.39 – 335) and 2022 (0.38 ± 0.171 copies mg^{-1} tissue; range: 0 – 1.85) (t = -2.135, P < 0.05; Fig. 2.11; Table 2.8). During a mortality event in August 2022, juvenile *M. gigas* ($1.57 \times 10^6 \pm 4.96 \times 10^5$ copies of OsHV-1 DNA mg^{-1} tissue; range: 62.5 – 7.00×10^6) outplanted at the North site earlier that summer had a much higher viral load than adult *M. gigas* (4.46 ± 3.15 copies mg tissue^{-1}; range: 0.45 – 13.8) that had been at the lease since 2020 (t = 4.026, P < 0.01).

Site (t = 47.931/-2.540, $P < 0.05$) influenced OsHV-1 load in *M. gigas*, though stress hardening did not (t = -0.103/-1.599, $P > 0.05$; Fig. 2.12; Table 2.9). Additionally, year and sampling period also influenced the quantity of viral copies (t = -7.720, P < 0.01). Specifically, in August 2021, viral copies were highest in the Middle ($3.19 \times 10^8 \pm 1.78 \times 10^8$ copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 2.67×10^9, t = -2.835 / 5.374, P < 0.05; Table 2.10) and North sites ($1.15 \times 10^7 \pm 9.19 \times 10^6$ copies of OsHV-1 DNA mg tissue^{-1}; range: 1.87 – 1.64×10^8, t = -2.835 / 2.539, P < 0.05; Table 2.10), but quite low in the South site (2258 ± 462 copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 5425, t = 2.539/5.374, P < 0.05; Table 2.10). However, by the subsequent sampling in September 2021, the quantity of OsHV-1 remained high in the North site ($2.63 \times 10^7 \pm 1.83 \times 10^7$ copies of OsHV-1 DNA mg tissue^{-1}; range: 332 – 33.4×10^8, t = -2.835 / 2.539, P < 0.05; Table 2.10), while declining in the Middle (165 ± 81.3 copies of OsHV-1 DNA mg tissue^{-1};

range: 0 – 1321, t = -2.835 / 5.374, P < 0.05; Table 2.10) and South (193 ± 16 copies of OsHV-1 DNA mg tissue^{-1}; range: 84.7 – 327, t = 2.539 / 5.374, P < 0.05; Table 2.10; Fig. 2.12). Viral load in July 2022 was greatest in the Middle site ($1.15 \times 10^{-6} \pm 4.59 \times 10^{-5}$ copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 1.30×10^{7}, t = -2.835/5.374, P < 0.05), followed by the South ($1.53 \times 10^{4} \pm 1.52 \times 10^{4}$ copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 7.31×10^{5}, t = 2.539 / 5.374, P < 0.05; Table 2.10) and North (15.7 ± 4.92 copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 141, t = -2.835/2.539, P < 0.05) sites. But, the number of OsHV-1 DNA copies in August 2022 was greatest in the North ($3.94 \times 10^{6} \pm 1.22 \times 10^{6}$ copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 3.45×10^{7}, t = -2.835/2.539, P < 0.05) and Middle ($2.50 \times 10^{6} \pm 1.15 \times 10^{6}$ copies of OsHV-1 DNA mg tissue^{-1}; range: 306 – 4.90×10^{7}, t = -2.835/5.374, P < 0.05) sites, while dropping at the South (143 ± 27.9 copies of OsHV-1 DNA mg tissue^{-1}; range: 0 – 1126, t = 2.539 / 5.374, P < 0.05; Table 2.10). As with *M. gigas*, SH did not influence the number of viral copies detected in *C. sikamea* (t = 0.07/-1.23, P > 0.05). However, site (t = 10.395/9.661, P < 0.01) and sampling period (t = 14.151, P < 0.01) determined OsHV-1 load in *C. sikamea*, with both factors interacting (t = 19.255/-18.353, P < *0.01*; Fig. 2.13; Table 2.11). In August 2021, OsHV-1 DNA quantity was highest at the Middle ($3.67 \times 10^{4} \pm 1.24 \times 10^{4}$ copies mg tissue^{-1}; range: 0 – 3.83×10^{5}) and South (2,917 ± 482 copies mg tissue^{-1}; range: 0 – 1.41×10^{4}, t = -0.719, P > 0.05; Table 2.12) sites, followed by the North site (8.23 ± 1.45 copies mg tissue^{-1}; range: 0.25 – 33.7, t = -9.659 / t = -10.385, P < 0.01; Table 2.12). But, by September 2021, OsHV-1 peaked at the North site ($3.81 \times 10^{4} \pm 1.29 \times 10^{4}$ copies mg tissue^{-1}; range: 0 – 4.48×10^{5}, t = 14.843 / t = 14.371, P < 0.01; Table 2.12), while being considerably lower at the Middle (7.27 ± 3.54 copies mg tissue^{-1}; range: 0 – 102) and

South (6.49 ± 2.38 copies mg tissue^{-1}; range: 0 – 63.1, t = -0.462, P > 0.05) sites (Fig. 2.13, Table 2.12).

DISCUSSION

We explored the potential for stress hardening (SH) by modifying temperature and tidal cycle to influence *M. gigas* responses to summertime OsHV-1 outbreaks in Tomales Bay, California. While SH did not affect oyster physiology or viral load, *M. gigas* immersed in the warmer water during the SH exposure in 2021 had greater survival after outplanting and those that experienced a tidal cycle during SH in 2022 experienced more shell growth. Site was arguably the most important factor, as where the oysters were outplanted in Tomales Bay determined their mortality, growth, and physiology as well as OsHV-1 and chlorophyll *a* concentrations. Specifically, *M. gigas* mortality and viral loads were greatest at the North and Middle sites while OsHV-1 quantities detected in *M. gigas* were lowest in the South site. During the August 2022 mortality event, energy stores, as measured by glycogen, in *M. gigas* was higher at the Middle and North sites and levels of carbonylated proteins, as an index of oxidatively damaged proteins, were greatest at the North site. Prior exposure to OsHV-1 may either reduce future infection levels or foster latency of the virus, as demonstrated by the adult *M. gigas* sampled in 2021 and 2022 that were originally outplanted in 2020. And, while *C. sikamea* mortality rates were highest at the South site and did not mirror OsHV-1 quantities, the species did contain OsHV-1 DNA at all three sites. Cumulatively, growing environments contributed significantly to *M. gigas* and *C. sikamea* responses during

OsHV-1 outbreaks, but SH still produced both positive and negative carryover effects that may further influence oyster performance during and after infection.

Outplanting location was a key driver of oyster responses throughout this experiment. The summer oceanographic dynamics of Tomales Bay are such that water within the inner bay had much higher residence time (Smith et al. 1991, Hearn and Largier 1997), which likely resulted in lower environmental chlorophyll *a* (Fig. 2.5) and whole body glycogen levels in *M. gigas* (Fig. 2.9) as well as reduced shell growth in both species (Fig. 2.8) at the South site. Low residence time did not prevent OsHV-1 from reaching this region of Tomales Bay, though it may have diminished infection intensity as the highest volumes of viral copies were detected in both species at the other two sites (Figs. 2.12 – 2.13). The distinct temperature regime of each site (Figs. 2.2 – 2.4) may have also played a role. Specifically, *C. sikamea* is sensitive to elevated temperatures (Shukla et al. *in prep*), which may have contributed to substantially higher mortality in this species at the South site in 2021 (Fig. 2.6D). Indeed, the North and Middle sites were far more similar in terms of oyster performance metrics despite their different temperature profiles. Cumulative mortality (Figs. 2.6B-D), shell lengths (Figs. 2.8B-D), and glycogen content (Fig. 2.9) of oysters did not vary much between these two sites. OsHV-1 load in both species and levels of carbonylated proteins in *M. gigas* did change across both sites over time, but likely reflect the intensity of the virus during the time at which oysters were sampled rather than local environmental conditions. Overall, site-specific dynamics were an important, but not the only, modulator of oyster outcomes.

SH was not consistently effective at improving the resistance or performance when outplanted of *M. gigas* responded to OsHV-1 in 2021 and 2022. Oysters that

experienced tidal fluctuations during SH had greater growth rates in the field (Fig. 2.8C), suggesting that exposure to a tidal cycle for two weeks during SH prepared them for aerial exposure during outplanting. Exposure to a warmer temperature during SH also improved *M. gigas* survival at the North and Middle sites in 2021 (Fig. 2.6B), but not 2022 (Fig. 2.6C). Size may have factored into the utility of thermal SH. Only 15-mm *M. gigas* were available in 2021 due to supply chain constraints during the COVID-19 pandemic. SH may have been more effective with these smaller juveniles than with the 25-mm *M. gigas* used in 2022, as higher metabolic rate accelerates growth (e.g., Johnson and Smee 2012) and acclimation in smaller organisms (e.g., Brown and Feldmeth 1971). The difference in SH efficacy may also be due to the delay between SH and onset of disease. In 2021, approximately four weeks elapsed between the end of SH and mortalities at the Middle site, whereas nearly eight weeks separated SH and mortality events in 2022. Further, the magnitude of SH may influence subsequent tolerance to heat and disease stress, as exposure to a warmer temperature during SH may have improved oyster outcomes after outplanting. There may also have been a mismatch between the temperature used for SH and the thermal regimes oysters experienced in the field such that an SH temperature of 21˚C was more effective in 2021 when mean temperature at each site was cooler than in 2022 (Fig. 2.2). Given that recent thermal history can affect physiological performance in molluscs (Drake et al. 2017), laboratory-based SH may only be beneficial for a limited time in terms of disease resistance.

Acclimation to temperature after outplanting could have directly influenced *M. gigas* responses to OsHV-1, as the quantity of OsHV-1 DNA copies at each site did not mirror

cumulative mortality. Small amounts of OsHV-1 were detected at the South site in 2020 (Fig. 2.11) where there was lower mortality than at the North site (Fig. 2.6A). In 2021 and 2022, *M. gigas* mortalities (Figs. 2.6B-C) and shell growth (Fig. 2.8B-C) were lower at the South site despite viral load approaching levels seen at the North and Middle sites (Fig. 2.12), though copy numbers did not approach the 10^7 -10^8 copies mg^{-1} representative of infection intensity in living oysters prior to mortality (Oden et al. 2011, de Kantzow et al. 2016) or the 10^6 copies mg^{-1} observed in other studies with mortalities (Dégremont 2011, de Kantzow et al. 2016, Hick et al. 2018, Burge et al. 2021), while they did at the North and Middle sites (Fig. 2.12). Given that the South site was the warmest and had the lowest chlorophyll *a* concentrations, oysters outplanted here may have been food-limited, and thus deprioritized growth to increase their thermal tolerance (McAfee et al. 2017, Li et al. 2018). This continued exposure to higher temperatures could have acted as a natural SH event, thereby increasing their survival while infected with OsHV-1. Acclimation at the South site may also explain why protein carbonyl content in *M. gigas* was similar between the South and Middle sites (Fig. 2.10), despite their differing thermal regimes as well as higher mortality rates (Fig. 2.6C) and OsHV-1 DNA copies (Fig. 2.12) at the Middle site. Protein carbonyl content was potentially highest at the North site due to the high viral load observed during this time relative to the previous sampling. Lower peak OsHV-1 loads in *M. gigas* could also be indicative of warmer temperatures triggering the transcription of genes concerning immunity, apoptosis, protein synthesis, and synaptic signaling that may have inhibited the progression of infection (Delisle et al. 2018). Together, SH and environmental hardening could have constrained growth and mortality rates as well as viral loads in *M. gigas* at the South

site, though thermal stress may not be the only means for increasing tolerance to disease.

Prior exposure to OsHV-1 may have also hindered re-infection in adult oysters and potentially caused latency of the virus. Progressively fewer viral copies were detected in *M. gigas* outplanted in 2020 when tested outside of a mortality event (December 2021) and during a mortality event (August 2022) (Fig. 2.11). The lower viral load in these asymptomatic adults, particularly in the winter when lower temperature may inhibit viral replication (Pernet et al. 2015), may demonstrate latency of OsHV-1 after surviving a prior infection (Arzul et al. 2002, Dégremont et al. 2013). Additionally, when adults from the 2020 cohort were compared to a random sample of juveniles recently outplanted near them at the North site in 2022, the adults experiencing their third outbreak had negligible quantities of OsHV-1 DNA while juveniles infected for the first time had substantially higher amounts. Similarly, the prevalence and quantity of OsHV-1 in older *M. gigas* that had been previously exposed to OsHV-1 in Woolaware Bay, AUS were lower than in naïve juveniles (Evans et al. 2017). Although these trends could be attributed to the adult oysters having a lower initial OsHV-1 load as juveniles that allowed them to survive or adults having lower susceptibility to infection compared with juveniles, immune priming from an initial infection is common across invertebrates (Little and Kraaijeveld 2004), including molluscs (Yao et al. 2021). In *M. gigas*, nucleic acid injections, such as poly(I:C), can stimulate the immune system and provide protection for up to five months from OsHV-1 (Lafont et al. 2017, 2020). And, *M. gigas* infected with an OsHV-1 µvar had high survival rates if re-infection occurred at a temperature <14˚C (Pernet et al. 2015). Thus, surviving an initial OsHV-1 infection can

foster some immunity to subsequent infections, though the growing environment plays an integral role in this process.

Understanding sources of viral transmission is imperative for protecting oysters from disease. It is not uncommon for viruses that predominantly affect one species to occur in others. For example, Denman Island disease is a parasitic infection caused by an intracellular protozoan that is largely associated with *M. gigas*, but has been detected in *C. sikamea* (Fallet et al. 2022). Although OsHV-1 primarily infects *M. gigas*, multiple other species have been infected with OsHV-1 (Shukla et al. *in prep*), including other bivalves (e.g., Burge et al. 2011, Bookelaar et al. 2020, Shukla et al. *in prep*). *M. gigas* are frequently co-cultured with other species, including fish, mussels, pearl oysters, sea cucumbers, and shrimp (Zamora et al. 2014, Omont et al. 2020, Chatzivasileiou et al. 2022). In Tomales Bay, *M. gigas* and *C. sikamea* are often cultivated within the same lease alongside one another. Given that OsHV-1 detection exceeded 10^3 copies mg^{-1} at all sites in 2021 (Fig. 2.13), our results corroborate that *C. sikamea* may be susceptible to OsHV-1 at a slower level and could serve as a reservoir (Burge et al. 2011, Friedman et al. 2020) and joins a handful studies that have demonstrated that *C. sikamea* can contain OsHV-1 DNA (Shimahara et al. 2012). To our knowledge, this is the first study to provide qPCR data for these detections, as prior studies have only been able to confirm positivity via conventional PCR, providing a baseline viral load for this species. Previously infected adult *M. gigas* may also serve as reservoirs, though there is limited evidence to suggest this occurs in the field (Evans et al. 2017). However, laboratory co-habitation studies have demonstrated that horizontal transmission from infected oysters to naïve ones is possible (Dégremont et al. 2013, Evans et al. 2015). Thus, it is important

to continue identifying potential infection pathways for OsHV-1, especially as outbreaks continue to occur within and beyond Tomales Bay.

OsHV-1 and its µVars threaten the Pacific oyster aquaculture industry as they continue to be associated with summer mortality events. OsHV-1 has coincided with high *M. gigas* mortality rates across multiple countries (Arzul et al. 2002, Burge et al. 2006, Paul-Pont et al. 2014, de Kantzow et al. 2016, Bookelaar et al. 2020, Shukla et al. *in prep*). Since 2008, several µVars of the reference strain have emerged that are associated with even greater losses of juvenile *M. gigas* stocks and sometimes become the dominant variant detected in infected oysters (Segarra et al. 2010, Martenot et al. 2012). For example, a newly identified OsHV-1 µVar in Australia was implicated in a mortality event between November 2010 and January 2011 that resulted in a loss of > 95% of *M. gigas* (Jenkins et al. 2013). Thus, mechanisms for sustaining oyster culture while coping with increasingly virulent strains of OsHV-1 are necessary. SH may have some potential, though its utility needs to be tested further. Experimenting with different stressors as well as the duration and magnitude of hardening may generate benefits for oysters infected with OsHV-1. Alongside SH and the farm management tools that growers already employ, physiological assessments, such as glycogen and protein carbonyl content, can also inform oyster performance in the field in terms of energy stores and protein damage. As these technical tools are not necessarily accessible to industry, their application will require increased collaborations between scientists and practitioners.

As ocean temperatures continue to rise due to anthropogenic climate change (Laffoley and Baxter 2016), marine disease is expected to continue proliferating (Harvell

1999, Burge et al. 2014). In addition to the impacts this will have on marine ecosystems (Byers 2021), the resilience of commercial industries, such as aquaculture, will also be tested (Lafferty et al. 2015, Pernet et al. 2016). It is increasingly important to develop complementary strategies for supporting such enterprises that will be dealing with both individual and synergistic consequences arising from the complexities of climate change and disease transmission. SH could potentially help address these challenges, though it should be treated as one option amongst a suite of solutions. Ultimately, further work is necessary to increase the oyster aquaculture industry's capacity to grapple with disease and science-industry partnerships can potentially provide a pathway for greater resilience in a changing ocean.

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FIGURES

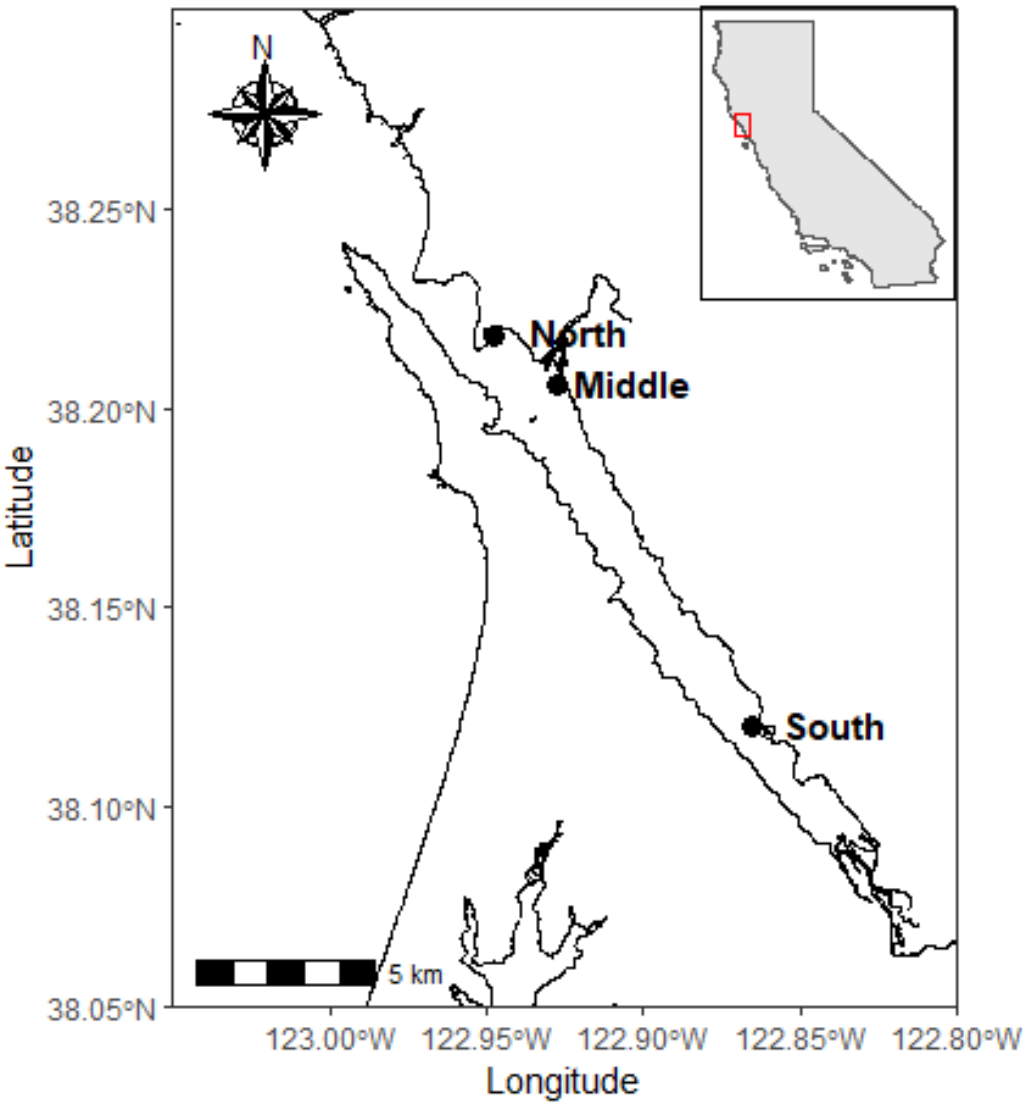

Figure 2.1. A map of Tomales Bay, California and the oyster aquaculture leases used during the experiment: "North" (Hog Island Oyster Company; 38.22,-122.95), "Middle" (Bodega Bay Oyster Company; 38.21,-122.93), and "South" (Tomales Bay Oyster Company; 38.12,-122.86).

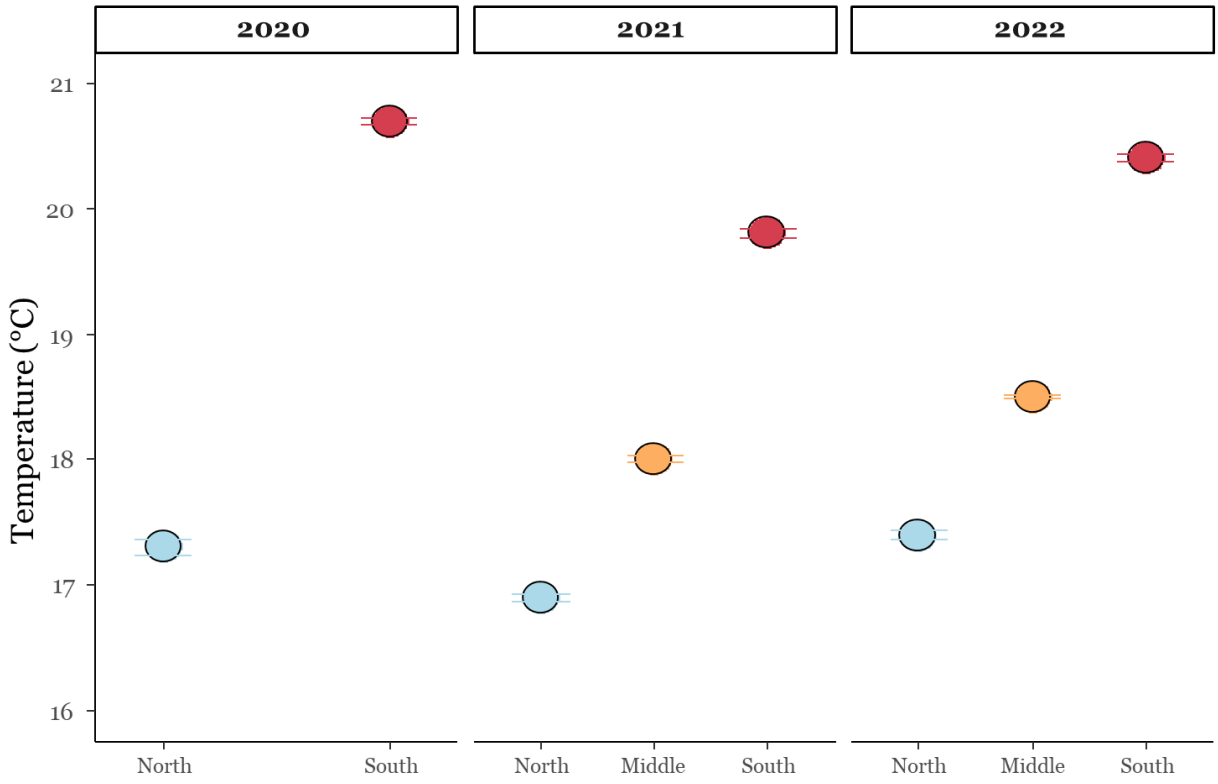

Figure 2.2. Seawater temperatures (mean ± SE) during summer outplantings at the North (blue), Middle (yellow), and South sites (red) across Tomales Bay, CA, USA from 2020 – 2022.

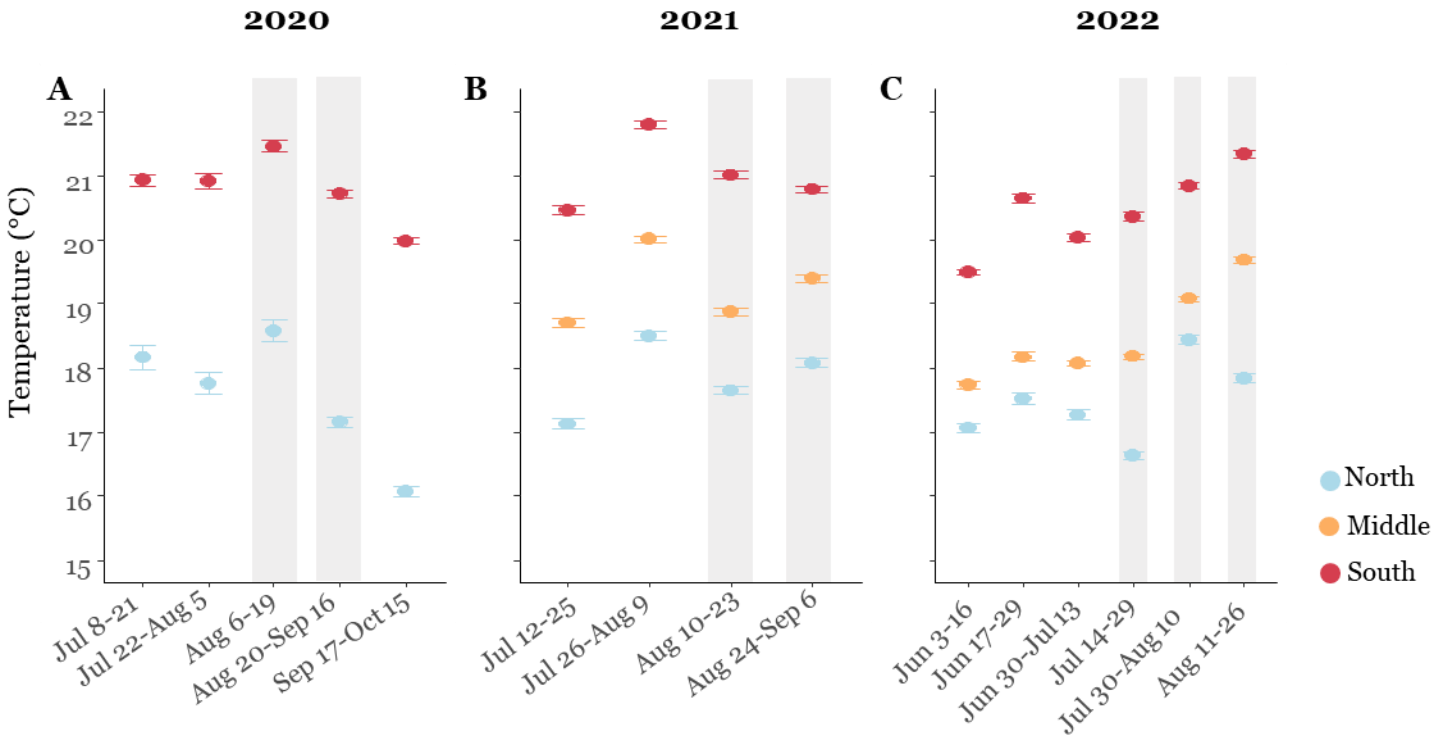

Figure 2.3. Seawater temperatures (mean ± SE) in the days preceding each sampling at North (blue), Middle (yellow), and South (red) sites from 2020 – 2022. Grey bars indicate when *M. gigas* mortality events occurred. Temperature data is absent from the Middle site in 2020 because no experimental oysters were deployed there that year.

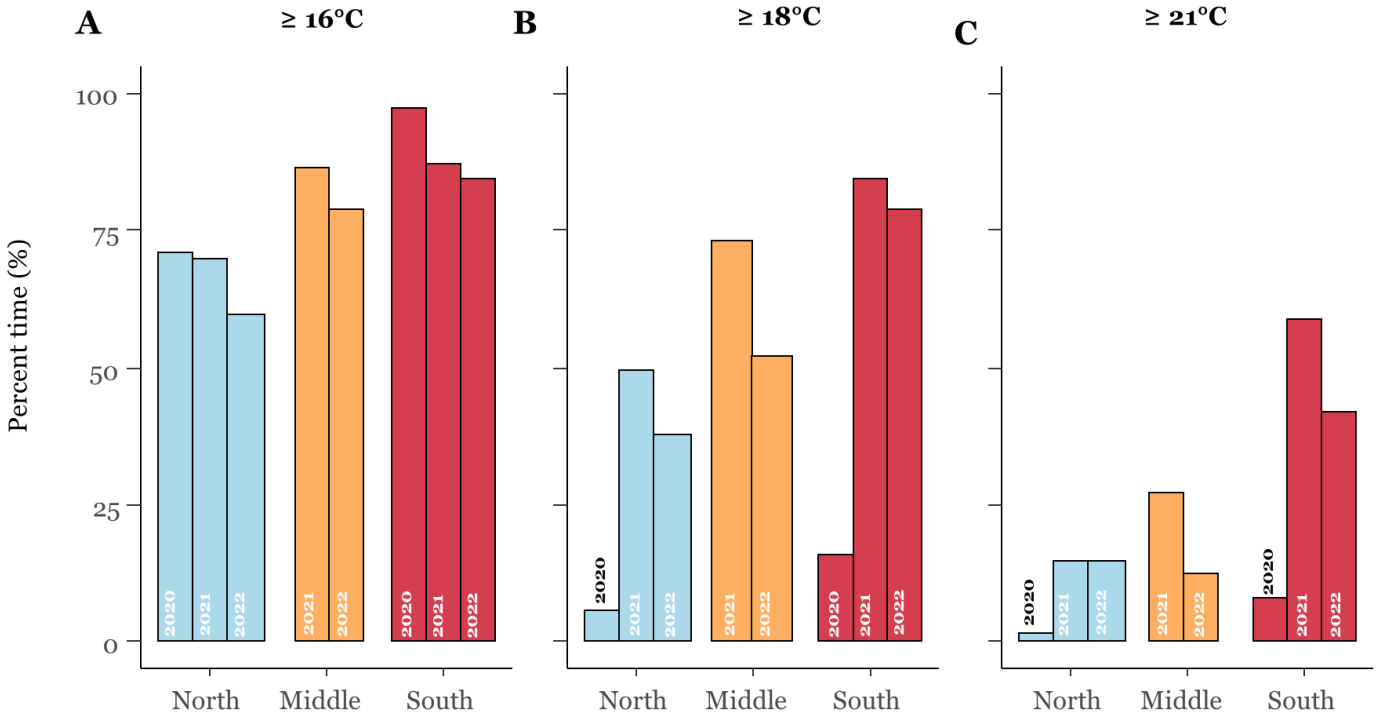

Figure 2.4. Percent of time oysters spent at or above (A) 16˚C, (B) 18˚C, and (C) 21˚C at each the North (blue), Middle (yellow), South (red) from 2020-2022. Temperature data is absent from the Middle site in 2020 because no experimental oysters were deployed there that year.

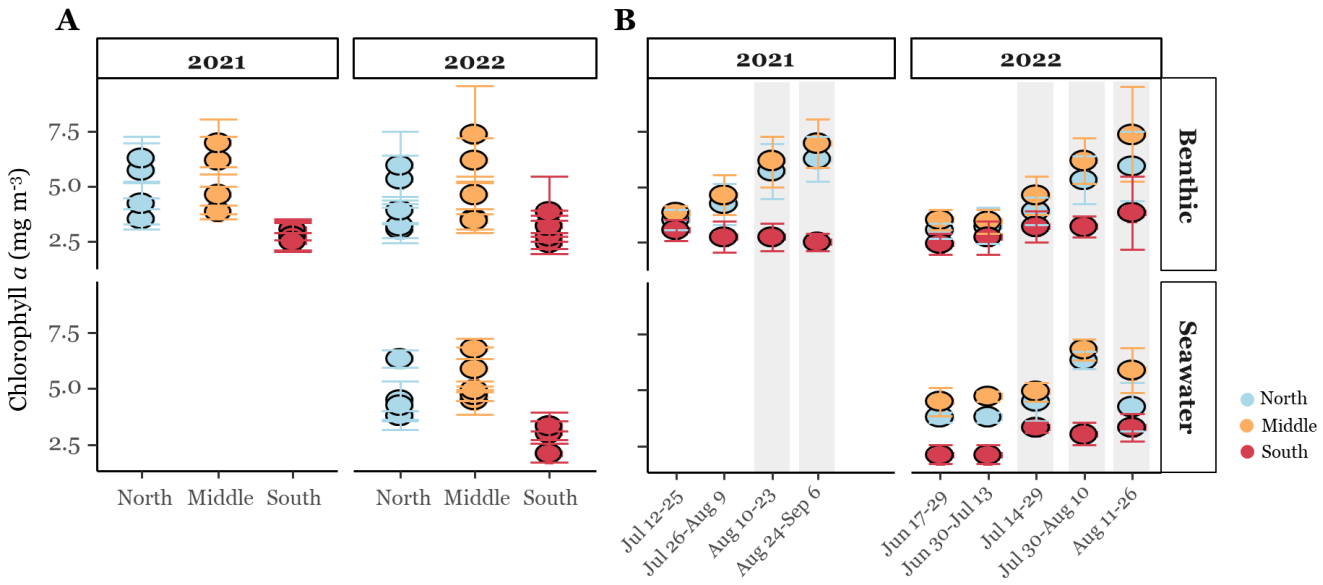

Figure 2.5. (A) Chlorophyll *a* concentrations (mean ± SE) from each sampling during deployments measured via benthic samples in 2021 (n = 36; 3 replicates x 4 samplings x 3 sites) and 2022 (n = 45; 3 replicates x 5 samplings x 3 sites) as well as seawater samples in 2022 (n = 45; 3 replicates x 5 samplings x 3 sites) at the North, Middle, and South sites spanning Tomales Bay, CA throughout the course of each deployment and (B) Chlorophyll *a* concentrations (mean ± SE) in benthic (2021, 2022) and seawater (2022) samples taken during each sampling (n = 3 per sample) at North, Middle, and South sites from 2021 – 2022. Grey bars indicate when *M. gigas* mortality events occurred.

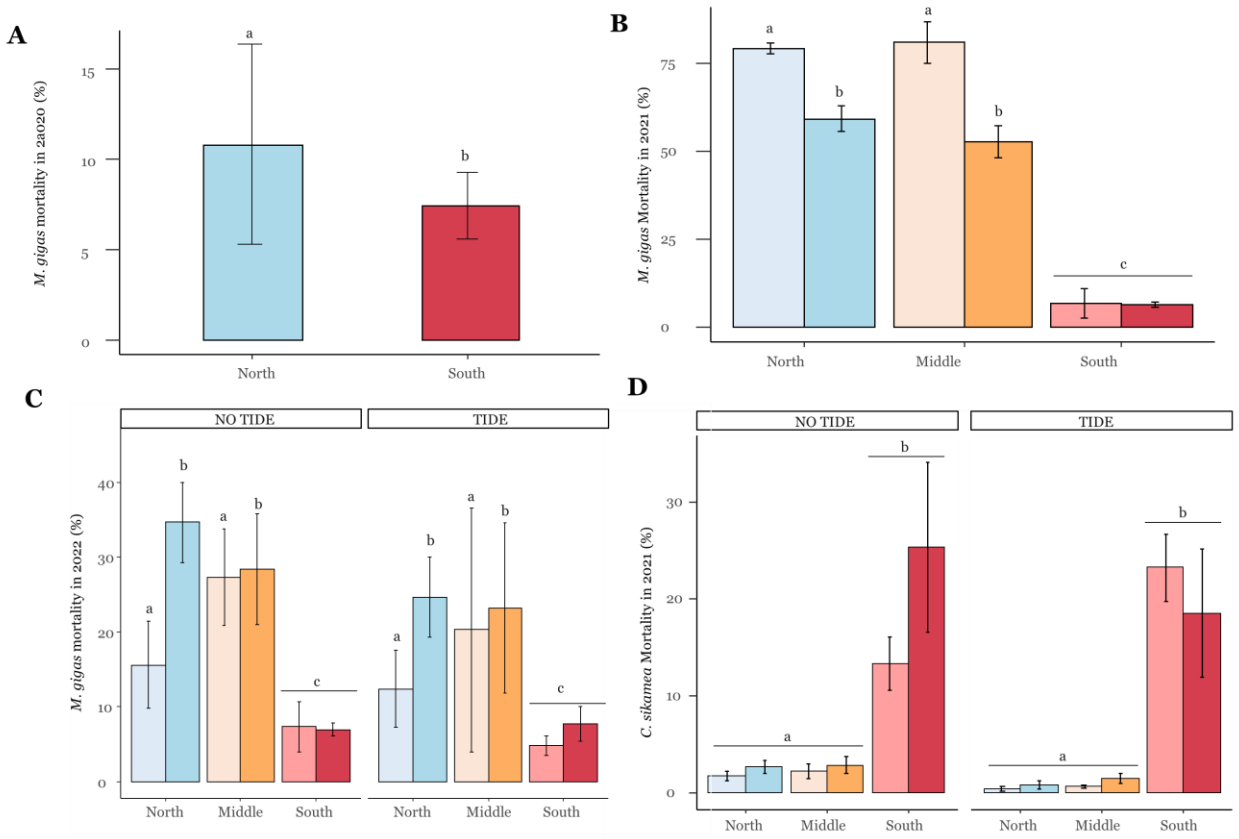

Figure 2.6. Cumulative mortality (mean ± SE) of (A) *M. gigas* in 2020 grouped by site (n = 182), (B) *M. gigas* in 2021 grouped by site and stress hardening (SH) temperature of either 16˚C (light) or 21˚C (dark) (n = 1922), (C) *M. gigas* in 2022 grouped by site, SH temperature of 15˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right) (n = 1600), and (D) stress hardened *C. sikamea* in 2021 grouped by site, SH temperature of 16˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right) (n = 627). Letters (a, b, c) indicate significant differences among treatments (SH temperature, SH tide, site) within each figure.

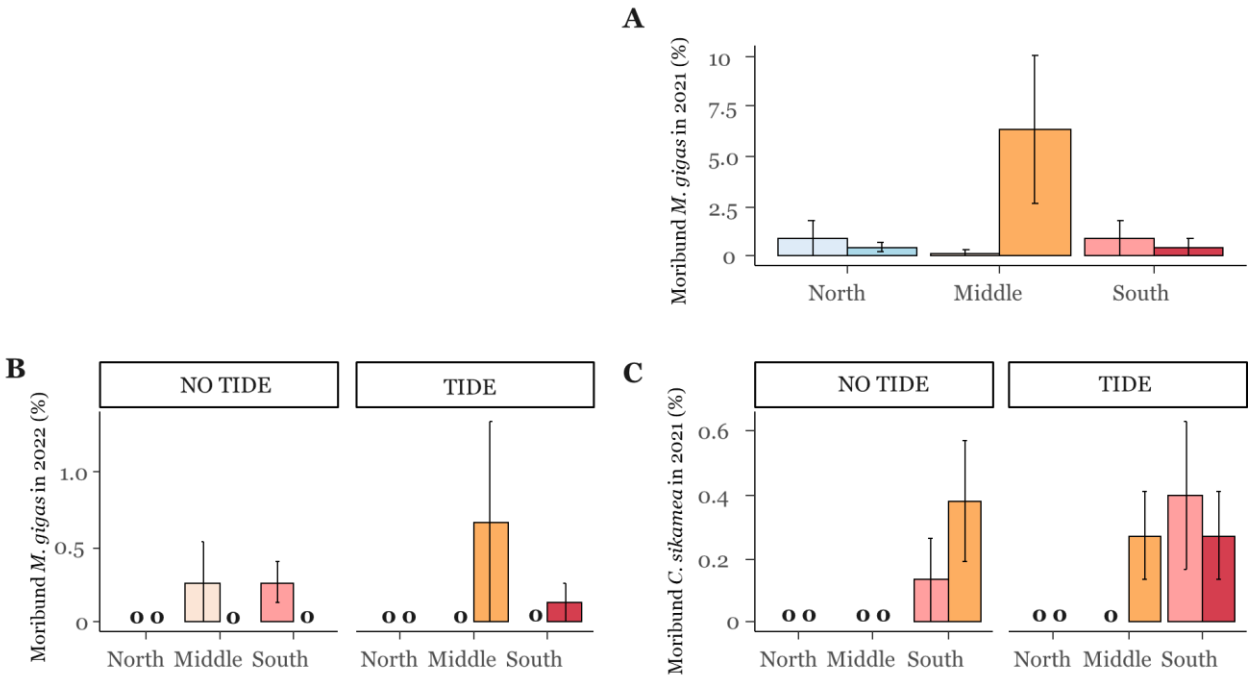

Figure 2.7. (A) Moribund (mean ± SE) *M. gigas* in 2021 grouped by site and stress hardening (SH) temperature of either 16˚C (light) or 21˚C (dark) (n = 62), (B) moribund *M. gigas* in 2022 grouped by site, SH temperature of 15˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right) (n = 10), and (C) moribund *C. sikamea* in 2021 grouped by site, SH temperature of 16 ˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right) (n = 10).

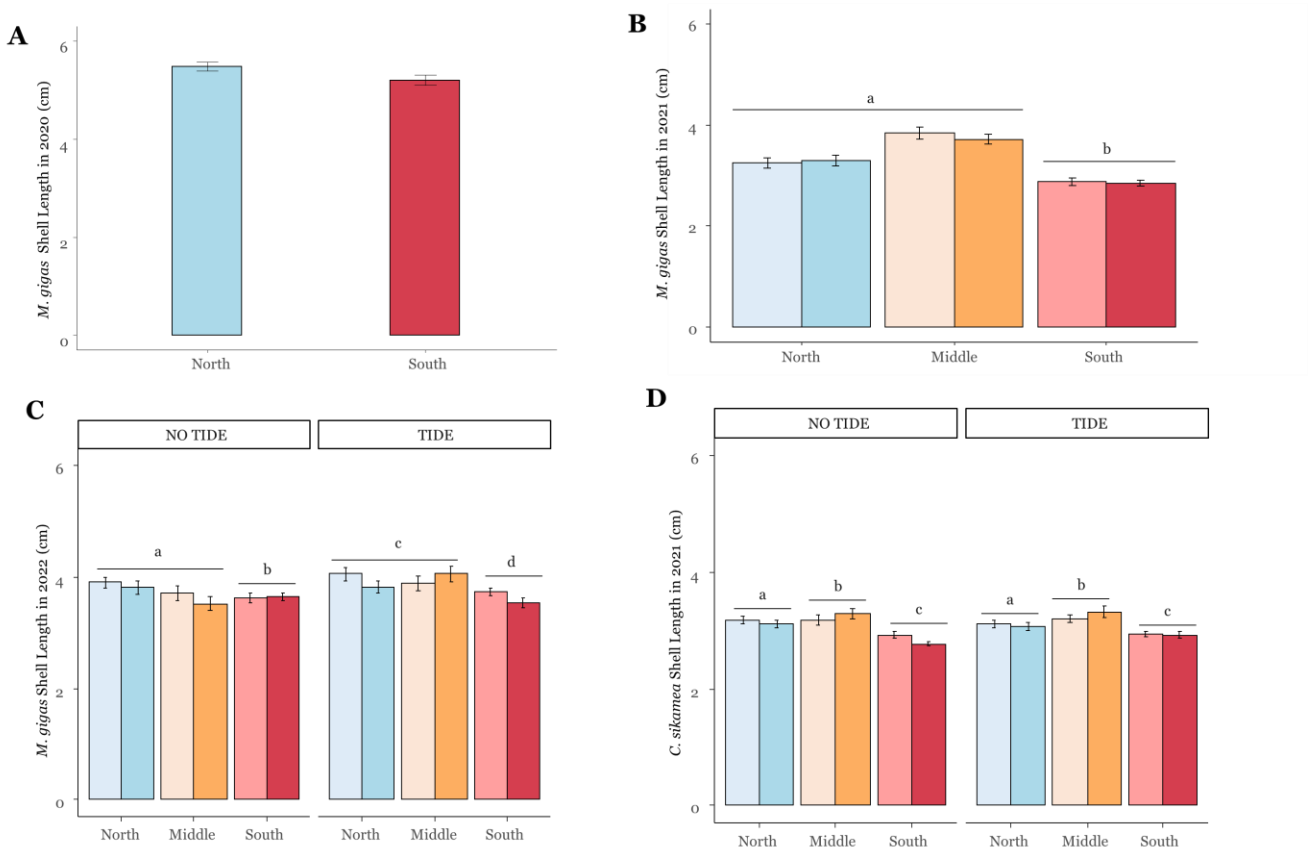

Figure 2.8. Shell lengths (mean ± SE) at the end of each outplanting for (A) *M. gigas* in 2020 grouped by site (n = 160), (B) *M. gigas* in 2021 grouped by site and stress hardening (SH) temperature of 16˚C (light) or 21˚C (dark) (n = 177), (C) *M. gigas* in 2022 grouped by site, SH temperature of 15˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right) (n = 356), and (D) stress hardened *C. sikamea* in 2021 grouped by site, SH temperature of 16˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right) (n = 361). Letters (a, b, c) indicate significant differences among treatments (SH temperature, SH tide, site) within each figure.

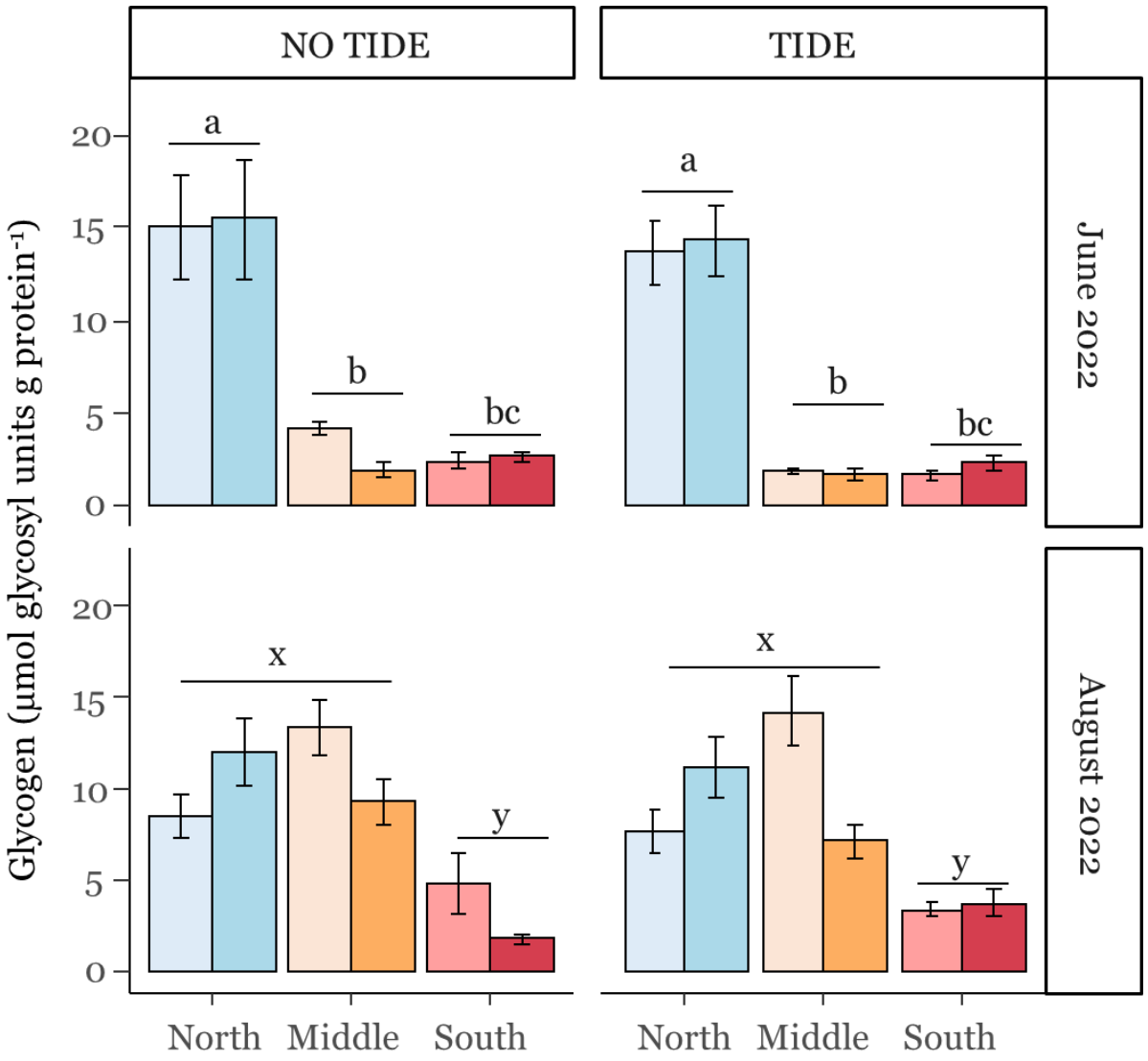

Figure 2.9. Glycogen content (mean ± SE) in *M. gigas* before (June 2022, n = 180) and during (August 2022, n = 180) a mortality event. Data are grouped by sampling time (June v. August 2022), site, stress hardening (SH) temperature of 15˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right). Letters (a, b, x, y) indicate significant differences among treatments (Sampling time, SH temperature, SH tide, site).

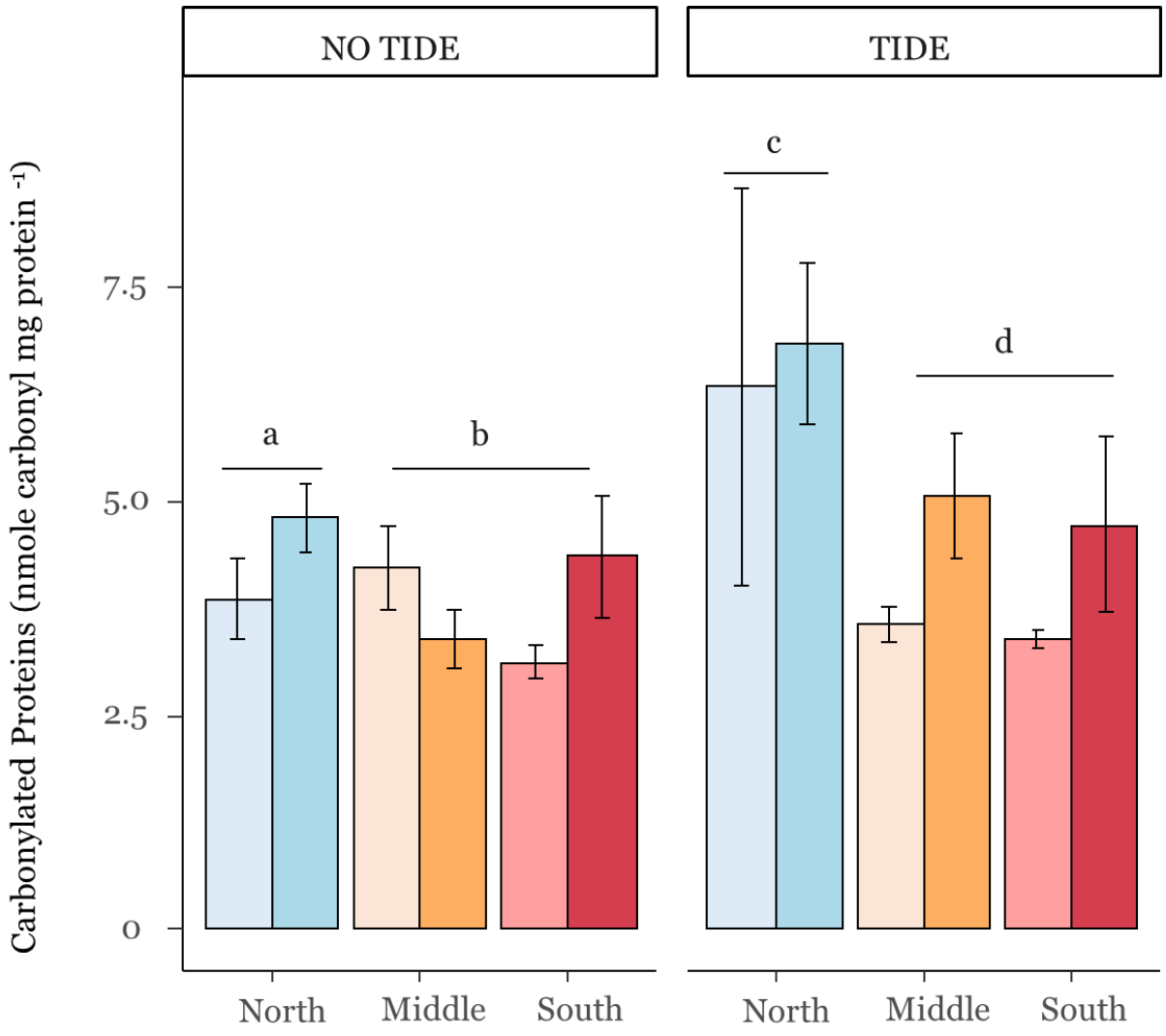

Figure 2.10. Protein carbonyl content (mean ± SE) in *M. gigas* during a mortality event in August 2022 (n = 177). Data are grouped by site, stress hardening (SH) temperature of 15˚C (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right)]. Letters (a, b, c, d) indicate significant differences among treatments (SH temperature, SH tide, site).

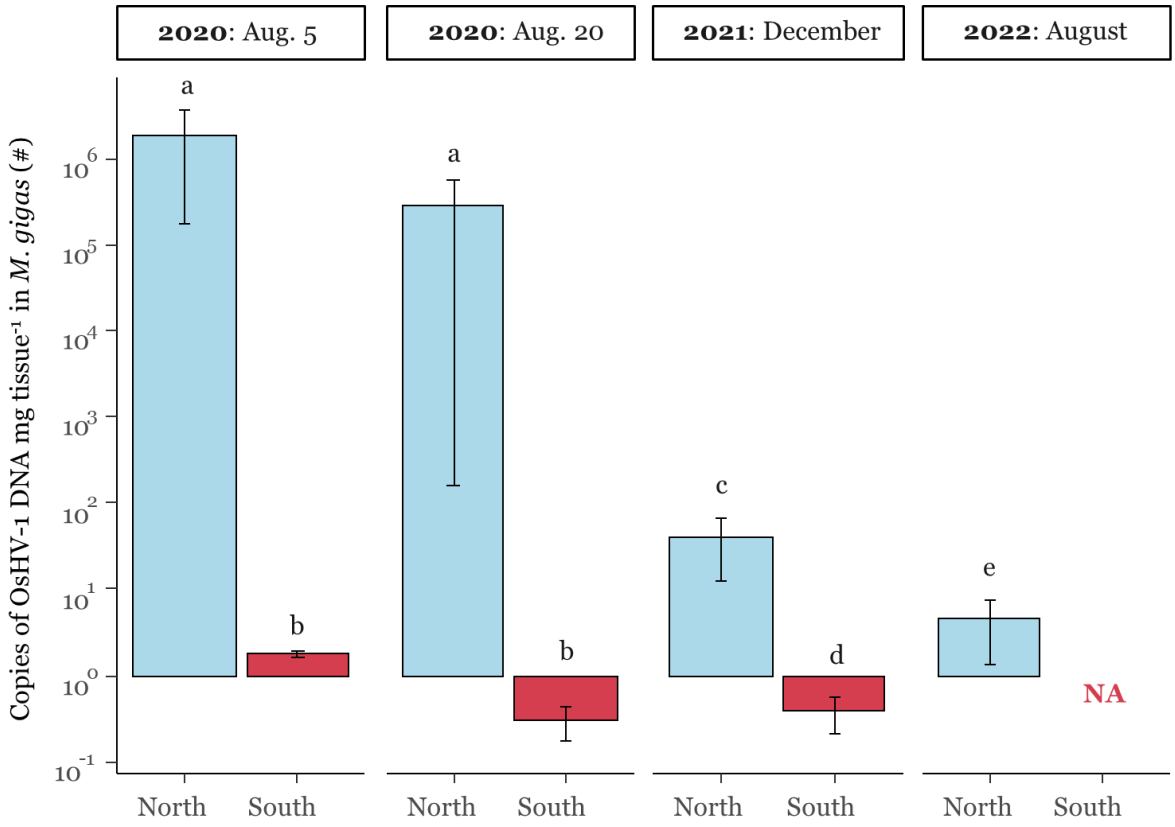

Figure 2.11. Quantity of OsHV-1 DNA copies (mean ± SE) in juvenile *M. gigas* during a mortality event in 2020 at the North and South sites (checked during two samplings, n = 65 pools) that were then re-tested as adults for OsHV-1 in December 2021 (n = 24 pools) and August 2022 (n = 4 pools). Due to equipment malfunction, oysters outplanted at the South site were not available for testing in 2022. Letters (a, b, c, d, e) indicate significant differences among treatments (Sampling time, SH temperature, SH tide, site).

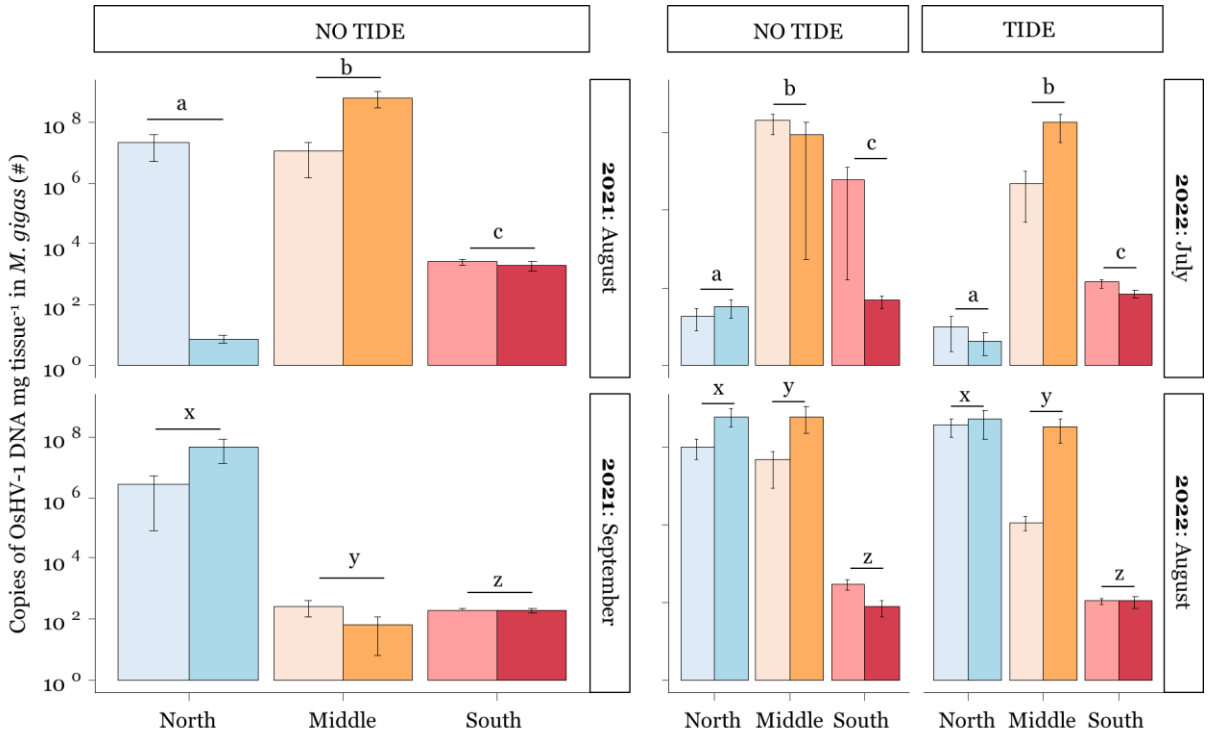

Figure 2.12. Amount of OsHV-1 DNA copies (mean ± SE) in stress hardened juvenile *M. gigas* during mortality events in 2021 (n = 108 pools) and 2022 (n = 325 pools). Data are grouped by sampling time (2021: August/September, 2022: July/August), site (North, Middle South), stress hardening (SH) temperature of 15˚C (2022)/ 16˚C (2021). (light) or 21˚C (dark), and SH tide treatments of "No Tide" (left) and "Tide" (right). Letters indicate significant differences among treatments. Letters (a, b, c, x, y, z) indicate significant differences among treatments (Sampling time, SH temperature, SH tide, site) within each year (i.e, 'a' in 2021 is not synonymous to 'a' in 2022).

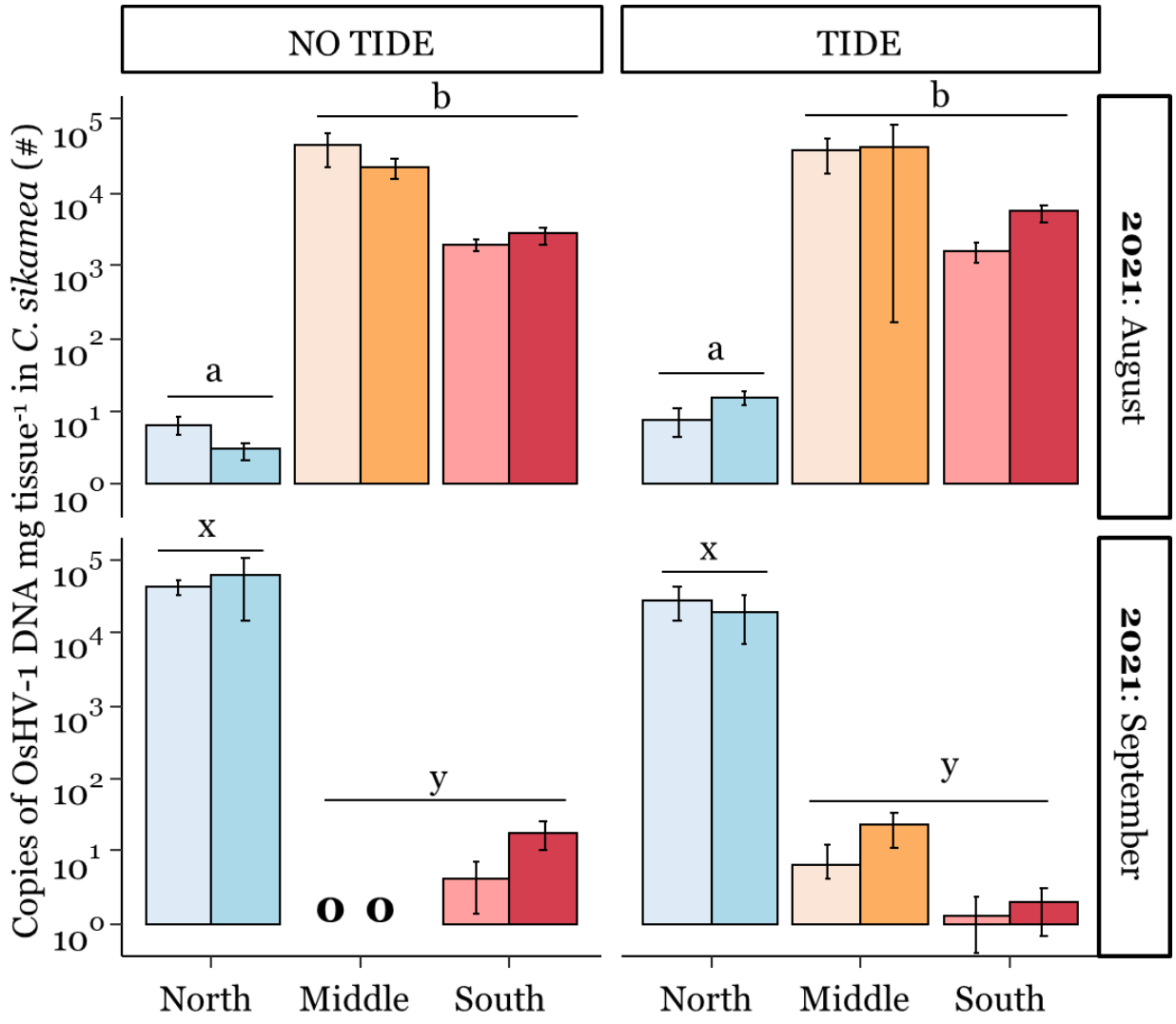

Figure 2.13. Amount of OsHV-1 DNA copies (mean ± SE) in stress hardened juvenile *C. sikamea* during a *M. gigas* mortality event in 2021 (n = 216 pools). Letters indicate significant differences among treatments.

APPENDIX 2.A – *Benthic Cholorphyll Methods*

Prior to analysis, samples were defrosted and 6-mL of 90% acetone was added to each sample, sonicated, and then placed in a 4˚C freezer overnight to extract chlorophyll. Samples were vortexed after 12 hrs to resuspend sediments and maximize extraction. Once extraction was complete, samples were centrifuged for 5 mins at 4 rpm and then returned to 4˚C until they could be run in the spectrophotometer. Each sample was pipetted into a cuvette with absorbance read on a spectrophotometer (Shimadzu) at 665 nm and 750 nm before and after a drop of 0.1 N HCl was added to the sample using a 1-mL pipette. Benthic Chl *a* concentation was then calculated using the following equation:

$$\text{Benthic Chlorophyll } a = (26.7 \times ((665_o - 750_o) - (665_a - 750_a)) \times V_e) \times A \quad [1],$$

where 665_o and 750_o represent absorbance before the addition of 0.1 N HCl, 665_a and 750_a are absorbances after the addition of 0.1 N HCl, V_e is volume of extract, and A is 10 × 1/(area of core in cm^2).

APPENDIX 2.B – *Seawater Cholorphyll Methods*

For spectrophotometric analysis, samples were defrosted and split into three 250-mL samples and passed through a 47 mm GF/F filter using a vacuum pump. This filter was then folded into quarters and added to a 15-mL falcon tube where 90% acetone was added. The sample was sonicated and then stored in the dark at -20˚C for 4 hrs to extract chlorophyll. Once extraction was complete, samples were vortexed, centrifuged for 5 mins at 4 rpm, and then carefully decanted into cuvettes. Absorbance was measured using a spectrophotometer as described above. Seawater Chl *a* concentration was then calculated using the following equation:

$$\text{Seawater Chlorophyll } a = 11.4 \times K \times (R \times (665_o - 750_o) - (665_a - 750_a)) \times V_e/L \times V_f \quad [2],$$

where 665_o and 750_o are the absorbances before the addition of 0.1 N HCl, 665_a and 750_a are the absorbances after the addition of 0.1 N HCl, V_e is the extraction volume, V_f is the volume filtered, L is the cuvette light path, K is the door factor from calibrations (2.43), and R is the maximum absorbance ratio of $665_o/665_a$ in the absence of phaeopigments (1.7).

APPENDIX 2.C – *Glycogen Content Methods*

Tissue was pulverized via a mortar and pestle in a liquid nitrogen jacket. Once the tissue had obtained a powdery consistency, it was weighed (mg) and then an equivalent volume of ice-cold 8% $HClO_4$ (µL) was added before the totality was homogenized on ice. The homogenate was then divided into two parts; 200 µL of the homogenate was placed in a microcentrifuge tube for estimating glycogen and frozen at -80°C. The remaining portion was centrifuged at 10,000 x *g* for 10 minutes at 4°C and the supernatant was neutralized using 3 mol L^{-1} K_2CO_3. It was again centrifuged at 10,000 x *g* for 10 minutes at 4°C before being preserved at -80°C to account for initial free glucose. Glycogen samples were then enzymatically digested as described by Hassid and Abraham (1957) and then analyzed for glucose by measuring absorbance at 340 nm before and after adding 2 µL hexokinase, and calculating the difference.

APPENDIX 2.D – *OsHV-1 qPCR Methods*

We targeted the OsHV-1 ORF 100/catalytic subunit of a DNA polymerase using the following forward (100 F: 5'-TGA TGG ATT GTT GGA CGA GA-3') and reverse (100 R: 5'-ATC ACA TCC CTG GAC GCT AC-3') primers and a standard curve from 3 to 3 x 10^7 copies per reaction to quantify viral DNA, a proxy for viral load. No template controls (n = 2) were included using PCR water as a template to each plate. Each 20 µL reaction contained 10 µl of Fast SYBR™ Green Master Mix, 5.9 µl of PCR water, 0.5 µL of 20 mg mL^{-1} BSA, 0.8 µl of each 10mM primer, and 2 µl of DNA. Each standard curve was run in triplicate and samples were run in duplicate using the Biorad CFX96 Touch Real-Time PCR Detection System with a detection limit of 3 copies per reaction. Cycling conditions for each qPCR run included: 95˚C for 2 minutes followed by 40 cycles of 95˚C (3 s) and 60˚C (30 s). A melt curve analysis for each qPCR reaction was performed by comparing the melting temperature peak of positive control DNA to that of the samples for each run. The melting curve profile consisted of denaturation at 95˚C for 10 s followed by an annealing step at 65˚C for 5 s. This was followed by a temperature ramp up to 95˚C in increments of 0.5 ˚C for 5 s. Copy numbers were standardized per mg of tissue.

APPENDIX 2.E – *Statistical Tables*

Table 2.1. Results for generalized linear mixed models regarding chlorophyll *a* concentrations in 2021 (benthic) and 2022 (benthic, seawater) across all three sites (*ref* = reference variable).

Fixed Effects	Estimate	SE	*t*	P
Sample Type	0.137	0.392	0.349	0.727
Site: *North*	*ref*	*ref*	*ref*	*ref*
Site: *Middle*	0.623	0.356	1.75	0.081
Site: *South*	-1.288	0.292	-4.409	**<0.001**
Year	-0.109	-0.252	-0.433	0.666
Year / Sampling Period	0.001	0.000	3.544	**<0.001**

Table 2.2. Results for generalized linear mixed models evaluating the effects of (1) site on cumulative mortality of *M. gigas* in 2020 as well as (2) stress hardening (SH) treatments and site on cumulative mortality of *C. sikamea* in 2021 and *M. gigas* in 2021 and 2022.

Year	Species	Fixed Effects	Random Effects	Estimate	SE	*z*	*P*
2020	***M. gigas***	Site	Basket	-0.416	0.158	-2.629	**0.009**
2021	***C. sikamea***	SH: Temp	Basket	0.2998	0.2323	1.291	0.197
		SH: Tide	Basket	-0.016	0.083	-0.196	0.845
		Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		Site: Middle	Basket	0.300	0.321	0.930	0.352
		Site: South	Basket	2.961	0.290	10.213	**<0.001**
2021 & 2022	***M. gigas***	Year	Basket	-7.160	0.545	-13.144	**<0.001**
		SH: Temp	Basket	-0.007	50.888	-12.123	**<0.001**
		SH: Tide	Basket	0.517	0.361	-1.434	0.1516
		Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		Site: Middle	Basket	-0.003	0.002	-1.909	0.056
		Site: South	Basket	-1577.04	2209.0	-7.140	**<0.001**
		SH: Temp * Year	Basket	-0.353	-0.029	12.124	**<0.001**
		SH: Temp * Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		SH: Temp * Site: Middle	Basket	136.22	82.571	1.649	0.0992
		SH: Temp * Site: South	Basket	615.502	118.12	5.211	**<0.001**
		Year* Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		Year* Site: Middle	Basket	1.487	0.779	1.910	0.056
		Year*Site: South	Basket	7.802	1.093	7.140	**<0.001**
		SH: Temp * Year * Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		SH: Temp * Year * Site: Middle	Basket	-0.007	0.004	-1.650	0.0989
		SH: Temp * Year * Site: South	Basket	-0.305	-0.006	-5.211	**<0.001**

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Table 2.3. Instantaneous Mortality of *M. gigas* (2020-2022) and *C. sikamea* (2021) during outplanting.

Year	Species	Stress Hardening: *Temperature*	Stress Hardening: *Tide*	Site	Time Point	Mean Mortality (%)	Std. Error
2020	*Magallana gigas*	No Stress Hardening	No Stress Hardening	North	1	0.6	0.1
					2	1.9	2.9
					3	6.5	3.8
					4	0.6	0.3
					5	0.2	0.1
				South	1	0.1	0.1
					2	0.3	0.2
					3	0.7	0.1
					4	4.3	1.4
					5	2	1.1
2021	*Magallana gigas*	16˚C	No Tide	North	1	4.6	0.1
					2	6.4	1.5
					3	74.8	13.1
					4	86.5	1.1
				Middle	1	5	0.1
					2	87.7	5.5
					3	67.6	24.1
					4	75.2	24.8
				South	1	5.6	0.5
					2	7.2	2.5
					3	5.8	0.3
					4	8.6	2.6
		21˚C	No Tide	North	1	4.6	0.01
					2	5.3	0.4
					3	39.2	16.5
					4	53	10.1
				Middle	1	4.9	0.3
					2	47.3	8.4
					3	29	6.9
					4	16.2	0.6
				South	1	4.9	0.3
					2	5.8	0.7
					3	6.9	1.2
					4	8.9	0.5

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	Crassostrea sikamea	16˚C	No Tide	North	1	4.3	0.1
					2	4.3	0.1
					3	4.7	0.1
					4	5.8	0.7
				Middle	1	4.3	0.3
					2	4.3	0.2
					3	5.4	0.2
					4	6	0.9
				South	1	5.9	0.5
					2	7.2	0.9
					3	9	1
					4	11.6	2.2
			Tide	North	1	4.1	0.1
					2	4.1	0
					3	4.5	0.1
					4	4.7	0.2
				Middle	1	4	0
					2	4.4	0.1
					3	4.7	0.1
					4	4.9	0.2
				South	1	9.5	0.9
					2	10.2	0.9
					3	12.9	0.4
					4	14.4	0.1
		21˚C	No Tide	North	1	7	0.5
					2	6.4	0.2
					3	6.6	0
					4	8.4	1
				Middle	1	6.3	0.3
					2	7.1	0.4
					3	7.4	1
					4	7.6	0.3
				South	1	13	0.3
					2	12.7	0.3
					3	13.8	0.6
					4	22.6	0.9
			Tide	North	1	4.3	0.3
					2	4.4	0.2
					3	4.9	0.3
					4	4.9	0.2

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				Middle	1	4.4	0.1
					2	5	0.1
					3	4.9	0.3
					4	4.9	0.2
				South	1	7.2	0.1
					2	7.7	0.2
					3	11.4	0.3
					4	15	0.4
2022	*Magallana gigas*	15˚C	No Tide	North	1	0	0
					2	0	0
					3	0	0.2
					4	0.2	0.2
					5	1.2	0.1
					6	21.3	8.4
				Middle	1	0	0
					2	0	0
					3	0	0
					4	14.8	12
					5	14.8	11.6
					6	7.6	5.9
				South	1	1.7	0.9
					2	1.3	0.4
					3	1.1	0.4
					4	0.8	0.2
					5	1.9	1.1
					6	2.5	2.5
			Tide	North	1	0	0
					2	0	0.1
					3	0	0
					4	0.2	0.2
					5	1.2	0.1
					6	16.7	7.8
				Middle	1	0	0.01
					2	0	0
					3	2.5	0.03
					4	19.7	18.1
					5	4.9	3.2
					6	1	0.7
				South	1	0.8	0.8
					2	1.1	0.9

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					3	0.6	0.4
					4	1.2	0.6
					5	1.3	0.7
					6	0.8	0.5
		21˚C	No Tide	North	1	0	0
					2	0	0.1
					3	1	0.2
					4	0.5	0
					5	20.3	10.7
					6	35.7	0.4
				Middle	1	0	0
					2	0	0
					3	0.1	0
					4	14.8	14.1
					5	7.4	5.1
					6	17.1	8.6
				South	1	0.5	0.3
					2	1.3	0.4
					3	1.5	0.3
					4	1.8	0.8
					5	2.6	0.9
					6	0.9	0.2
			Tide	North	1	0	0
					2	0.1	0
					3	1	0.5
					4	3.1	0.9
					5	4.5	2.9
					6	28.4	9.6
				Middle	1	0	0
					2	0	0
					3	0	0
					4	4	0.4
					5	21.5	11.9
					6	9.4	7.2
				South	1	2.1	0.9
					2	1.7	1
					3	2.6	0.7
					4	1.2	0.4
					5	0.7	0.5
					6	0.7	0.7

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Table 2.4. Results for generalized linear mixed models predicting the relationship between sampling period (beginning vs. end of outplanting) and site on *M. gigas* shell growth from 2020-2022, as well as the effects of stress hardening temperature and tide on *C. sikamea* (2021) and *M. gigas* (2021-2022) shell lengths (*ref* = reference variable).

Year	Species	Fixed Effects	Random Effects	Estimate	SE	*t*	*P*
2020	***M. gigas***	Sampling Period	Basket	1.134	0.083	13.590	**<0.001**
		Site	Basket	-0.071	0.105	-0.673	0.501
2021	***C. sikamea***	SH: Temp	Basket	-0.003	0.008	-0.391	0.696
		SH: Tide	Basket	0.0472	0.041	1.151	0.25
		Sampling Period		0.815	0.043	19.096	**<0.001**
		Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		Site: Middle	Basket	-0.017	0.051	-0.333	0.740
		Site: South	Basket	0.064	0.051	1.256	0.213
		Sampling Period * Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		Sampling Period * Site: Middle	Basket	0.304	0.060	5.022	**<0.001**
		Sampling Period * Site: South	Basket	-0.612	0.061	-10.111	**<0.001**
2021 & 2022	***M. gigas***	SH: Temp	Basket	-0.001	162.40	-6.820	0.240
		SH: Tide	Basket	0.144	0.071	2.017	**0.049**
		Site: North	*ref*	*ref*	*ref*	*ref*	*ref*
		Site: Middle	Basket	-0.004	0.007	0.626	0.534
		Site: South	Basket	-0.021	0.007	-2.966	**0.005**
		Year	Basket	0.055	0.008	6.840	**<0.001**
		Year: 2021 / Sampling Period: 1	*ref*	*ref*	*ref*	*ref*	*ref*
		Year: 2021 / Sampling Period: 4	Basket	0.003	0.003	12.291	**<0.001**
		Year: 2022 / Sampling Period: 1	*ref*	*ref*	*ref*	*ref*	*ref*
		Year: 2022 / Sampling Period: 6	Basket	0.005	0.002	27.446	**<0.001**

Table 2.5. Results for generalized linear mixed models concerning the physiology of *M. gigas* oysters before (glycogen only) and during (glycogen and protein carbonyl) an OsHV-1 outbreak in Tomales Bay as determined by a mortality event in 2022. Impacts of Stress Hardening (SH) Temperature and Tide as well as Sampling time and Site were estimated using a gamma distribution (*ref* = reference variable).

Physiology	Fixed Effects	Random Effect	Estimate	SE	*t*	*P*
Glycogen Content	SH Temp	Basket	-0.104	0.128	-0.809	0.419
	SH Tide	Basket	-0.694	0.768	-0.904	0.367
	Sampling	Basket	-1.603	0.331	-4.848	**<0.01**
	Site: *North*	*ref*	*ref*	*ref*	*ref*	*ref*
	Site: *Middle*	Basket	-21.229	1.810	-11.726	**<0.01**
	Site: *South*	Basket	-16.485	1.812	-9.101	**<0.01**
	Sampling * Site: *North*	*ref*	*ref*	*ref*	*ref*	*ref*
	Sampling * Site: *Middle*	Basket	4.474	0.468	9.566	**<0.01**
	Sampling * Site: *South*	Basket	2.026	0.469	4.321	**<0.01**
Protein Carbonyl	SH Temp	Basket	0.137	0.087	1.576	0.117
	SH Tide	Basket	0.804	0.516	1.556	0.120
	Site: *North*	Basket	1.291	0.631	2.046	**0.041**
	Site: *Middle*	Basket	0.1801	0.630	0.286	0.775
	Site: *South*	*ref*	*ref*	*ref*	*ref*	*ref*

Table 2.6. Results for pairwise comparisons between sites (North, Middle, South) during each sampling period (June v. August 2022) for glycogen and sites for protein carbonyl (North, Middle, South).

Physiology	Sampling	Site Comparison	Estimate	SE	*t*	*P*
Glycogen Content	June 2022	North v. Middle	12.281	1.04	11.763	**<0.01**
	June 2022	North v. South	12.434	1.04	11.910	**<0.01**
	June 2022	Middle v. South	0.153	1.04	0.147	0.884
	August 2022	North v. Middle	-1.141	1.04	-1.093	0.277
	August 2022	North v. South	6.358	1.05	6.066	**<0.01**
	August 2022	Middle v. South	7.499	1.05	7.155	**<0.01**
Protein Carbonyl	August 2022	North v. Middle	1.258	0.571	2.202	**0.029**
	August 2022	North v. South	1.401	0l560	2.500	**0.013**
	August 2022	Middle v. South	0.143	0.479	0.299	0.766

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Table 2.7. Results for OsHV-1 load for *M. gigas* outplanted in 2020 at the North and South sites (*ref* = reference variable).

Fixed Effects	Random Effect	Estimate	SE	*t*	*P*
Site: *North*	*ref*	*ref*	*ref*	*ref*	*ref*
Site: *South*	Basket	-1.666	0.715	-2.330	0.058
Sampling: *3*	*ref*	*ref*	*ref*	*ref*	*ref*
Sampling: *4*	Basket	-0.503	0.279	-1.801	0.077

Table 2.8. Results for comparison in OsHV-1 load (number of copies of OsHV-1 DNA mg tissue^{-1}) for *M. gigas* outplanted in 2020 at the North and South sites and then re-sampled for OsHV-1 in December 2021 and August 2022 (*ref* = reference variable).

Fixed Effects	Random Effect	Estimate	SE	*t*	*P*
Site: *North*	*ref*	*ref*	*ref*	*ref*	*ref*
Site: *South*	Basket	-1.432	0.262	-5.472	**<0.001**
Year: *2020* / Sampling Period: *3*	*ref*	*ref*	*ref*	*ref*	*ref*
Year: *2020* / Sampling Period: *4*	Basket	-0.478	0.306	-1.561	0.122
Year: *2021*	Basket	-0.691	0.331	-2.085	**0.040**
Year: *2022*	Basket	-1.421	0.666	-2.135	**0.036**

Table 2.9. Statistical output for analysis of viral load within *M. gigas* during mortality events in 2021 and 2022. These oysters were stress hardened using temperature and tide prior to being outplanted at three sites in Tomales Bay, California (*ref* = reference variable).

Species	Fixed Effects	Estimate	SE	*t*	*P*
M. gigas	SH: Temp	-0.023	0.224	-0.103	0.918
	SH: Tide	-0.458	0.287	-1.599	0.117
	Site: *North*	*ref*	*ref*	*ref*	*ref*
	Site: *Middle*	0.837	0.295	47.931	**0.007**
	Site: *South*	-0.749	0.295	-2.540	**0.015**
	2021 / Sampling: 3	*ref*	*ref*	*ref*	*ref*
	2021 / Sampling: 4	-0.690	0.364	-1.896	0.059
	2022 / Sampling: 4	-1.7218	0.2230	-7.720	**<0.001**
	2022 / Sampling: 5	*ref*	*ref*	*ref*	*ref*

Table 2.10. Pairwise comparisons for OsHV-1 load in *M. gigas* across sampling period and site (North, Middle, South) during mortality events in 2021 and 2022.

Species	Sampling	Site Comparison	Estimate	SE	*t*	*P*
M. gigas	August 2021	North v. Middle	-0.837	0.295	-2.835	**0.0100**
	August 2021	North v. South	0.749	0.295	2.539	**0.0144**
	August 2021	Middle v. South	1.586	0.295	5.374	**<0.001**
	September 2021	North v. Middle	-0.837	0.295	-2.835	**0.0100**
	September 2021	North v. South	0.749	0.295	2.539	**0.0144**
	September 2021	Middle v. South	1.586	0.295	5.374	**<0.001**
	July 2022	North v. Middle	-0.837	0.295	-2.835	**0.0100**
	July 2022	North v. South	0.749	0.295	2.539	**0.0144**
	July 2022	Middle v. South	1.586	0.295	5.374	**<0.001**
	August 2022	North v. Middle	-0.837	0.295	-2.835	**0.0100**
	August 2022	North v. South	0.749	0.295	2.539	**0.0144**
	August 2022	Middle v. South	1.586	0.295	5.374	**<0.001**

Table 2.11. Statistical output for OsHV-1 load in *C. sikamea* during an *M. gigas* mortality event in 2021. These *C. sikamea* experienced both SH temperature and tide before being outplanted at the North, Middle, and South sites in Tomales Bay, California (*ref* = reference variable).

Species	Fixed Effects	Estimate	SE	*t*	*P*
C. sikamea	SH: Temp	0.019	2.500	0.077	0.939
	SH: Tide	-0.306	0.249	-1.23	0.22
	Site: *North*	*ref*	*ref*	*ref*	*ref*
	Site: *Middle*	2.596	0.250	10.395	**<0.001**
	Site: *South*	2.416	0.250	9.661	**<0.001**
	Sampling: *3*	*ref*	*ref*	*ref*	*ref*
	Sampling: *4*	3.277	0.232	14.151	**<0.001**
	Site: Middle × Sampling: *4*	-6.307	0.3275	-19.255	**<0.001**
	Site: South × Sampling: *4*	-6.011	0.328	-18.353	**<0.001**

Table 2.12. Pairwise comparisons for OsHV-1 load in *C. sikamea* across sampling period and site (North, Middle, South) during an *M. gigas* mortality event in 2021.

Species	Sampling	Site Comparison	Estimate	SE	*t*	*P*
C. sikamea	August 2021	North v. Middle	-2.596	0.25	-10.385	**<0.001**
	August 2021	North v. South	-2.416	0.25	-9.659	**<0.001**
	August 2021	Middle v. South	0.180	0.25	-0.719	0.474
	September 2021	North v. Middle	3.711	0.25	14.843	**<0.001**
	September 2021	North v. South	3.595	0.25	14.371	**<0.001**
	September 2021	Middle v. South	-0.116	0.25	-0.462	0.645

* * * CHAPTER 3 * * *

Patterns of Global Spread for OsHV-1 Variants and Potential Reservoir Species

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ABSTRACT

Ostreid herpesvirus (OsHV-1) is a temperature-associated virus that has been detected in the Pacific oyster (*Magallana gigas*) and other species around the world. As *M. gigas* aquaculture continues to expand, there is a need to document locations where OsHV-1 and its variants have been detected as well as animal hosts other than *M. gigas*. Here, using peer-reviewed literature, government reports, and unpublished data, we describe the timeline of OsHV-1 variant detections, countries where *M. gigas* has been detected, and other known hosts. Additionally, we describe mean prevalence and peak infection intensity when that information was provided in the studies we assessed. The information in this review may support growers and managers in siting future aquaculture leases and understanding potential sources of virus.

INTRODUCTION

As atmospheric and oceanic temperatures rise due to climate change, disease transmission is expected to increase in terrestrial and marine systems alike (Harvell 2002, Lafferty 2009). While disease, like predation, plays a critical role in regulating wild populations (Scott 1988), anthropogenic interference, such as ballast water ferrying microbes (Hwang et al. 2018, Naik et al. 2019), has resulted in some pathogens having the capacity for the local and/or global extinction of species (Daszak et al. 2001). Mass mortality events have been occurring more frequently over the past several decades (Lessios et al. 1984, Garrabou et al. 2001, Fey et al. 2015) and infectious diseases are increasingly being implicated in them (Vezzulli et al. 2010, Sanderson and Alexander

2020). Thus, understanding both the historical trajectory of emerging diseases and their potential for causing population declines is of critical importance.

Marine disease has already adversely impacted natural systems (Harvell et al. 2019, Howells et al. 2020), fisheries (Groner et al. 2018), and aquaculture (Combe et al. 2023), particularly during periods of heat stress. Several coral diseases are associated with elevated temperatures (Burke et al. 2005), with black band disease infecting more than 60 scleractinian coral species across 22 countries spanning the Caribbean, Indo-Pacific, and Red Sea regions (Miller and Richardson 2015). Additionally, a host-agnostic rosette-like parasite threatens European fish species (Gozlan et al. 2005), as warming has contributed to declines in global fisheries (Free et al. 2019). And, disease progression occurs most rapidly for shellfish aquaculture in warmer, tropical regions (Leung and Bates 2013). Given the extent to which pathogens and temperature affect commercially-relevant species, it is important to determine when and where species are most susceptible to infections as well as prevalence of disease for transmission.

Disease is common in aquaculture environments and often has ecological and economic implications (Murray and Peeler 2005). Not only do pathogenic and parasitic loads rapidly increase due to the high densities at which animals are stocked (Krkosek 2010), but transmission between wild and cultivated species can also happen when operations occur in open water (Bouwmeester et al. 2021). Globally, losses from marine disease annually cost aquaculture operators billions of dollars (Lafferty et al. 2015). As aquaculture is considered a potential pathway to improving global food security (FAO 2022) and there is increasing interest in growing bivalves due to their low carbon

footprint (Krause et al. 2022), investigating how diseases may hinder their production is imperative.

Oysters comprise the largest portion of global bivalve aquaculture (34.1%; FAO 2022), leading to widespread concerns about diseases compromising production. Oyster production has increased rapidly over the past 30 years, with the Pacific oyster, *Magallana (= Crassostrea) gigas*, becoming the most frequently farmed commercial oyster in the world (Botta et al. 2020). As distribution of *M. gigas* has expanded, the geographic scope for disease has as well (Elston 1993). Among the suite of pathogens that afflict this species, Ostreid herpesvirus (OsHV-1) is arguably of greatest concern due to its association with mass mortality events (Paul-Pont et al. 2014) and frequent emergence of new variants (e.g., Burge et al. 2021). *M. gigas* summer mortality events have been reported across Asia, Europe, and North America over the past 70 years (Pathirana et al. 2019), with the movement of oysters and equipment from infected areas a potential cause of the virus' spread (Peeler et al. 2012). Herpes/herpes-like viruses were first observed in *C. virginica* from Maine, USA in 1970 via electron microscopy (Farley et al. 1972) and later observed in other oyster species as well (Hine et al. 1992, Comps and Cochennec 1993, Hine and Thorne 1997). The development of molecular tools permitted the purification, extraction, and partial (Le Deuff and Renault 1999)

and full sequencing (Davison 2002) of the Ostreid herpesvirus 1 (OsHV-1) from *M. gigas* larvae (GenBank accession no. AY509253). Since then, several variants of OsHV-1 have been described (Martenot et al. 2011, Trancart et al. 2023), including microvariants (μVars) (Segarra et al. 2010) and the closely related acute viral necrotic

virus (AVNV) found in the Chinese scallop, *Chlamys farreri* (Ren et al. 2013). Indeed, OsHV-1 has been reported in multiple animal hosts beyond *M. gigas* (e.g., O' Reilly et al. 2018, Bookelaar et al. 2018) and is often observed when seawater temperatures exceed 16˚C (Renault et al. 2014, Martenot et al. 2015), though there is variability in how shifts in seawater temperature affect different strains (e.g., de Kantzow et al. 2016). Overall, documenting water bodies where OsHV-1 has been detected and potential reservoirs for the virus is necessary as oyster aquaculture ramps up while climate change also progresses.

To inform ongoing and future oyster culturing efforts, there is a need to collate unique detections of OsHV-1 variants and known hosts. While locations and variants have been compiled in some studies (Mineur et al. 2015, Alfaro et al. 2019, Burge and Hershberger 2020) and white papers (e.g., USDA 2020), a comprehensive catalog of this information does not currently exist. Additionally, much of this information is in peer-reviewed literature that have not been holistically evaluated so as to better develop farm management strategies for OsHV-1. To address this gap, we reviewed published literature and government documents that reported OsHV-1 detections in the field and/or in alternate hosts and generated a database that includes detection location, variant type, and host species. We also included infection prevalence and intensity when that information was provided in the studies we evaluated.

METHODS

Literature Review

Our synthesis comprises peer-reviewed literature (full list in Appendix 3.A), white papers, and data from Shukla et al. *in prep* that report positive herpesvirus detections in animal hosts based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) protocols (Shamseer et al. 2015, PRISMA-P Group et al. 2015). From January - July 2023, two separate sets of search terms were used to identify global detections of Ostreid herpesvirus in *M. gigas* (Fig. 3.1) and other prospective hosts (Fig. 3.2) via Web of Science. All literature that fit those criteria was then imported into Covidence (www.covidence.org) screening software where two reviewers examined abstracts for inclusion based on relevancy. Literature found via cited reference searches, Google Scholar notifications, and reviewer suggestions were also incorporated when useful. The review includes studies that observed four variations of Ostreid herpesvirus ("OsHV", "OsHV-1", "OsHV-1 µVar", "OsHV-1 non-µVar variant"), and the closely related acute viral necrosis virus ("AVNV") (Ren et al. 2013). Studies from the late 20th century (1992-1998) that used light and transmission electronic microscopy to confirm that oysters exhibited "herpesvirus" and "herpes-like virus" that have been retroactively accepted as potential OsHV-1 occurrences prior to the advent of molecular tools, such as polymerase chain reaction (PCR) and *in situ* hybridization (ISH), were also included. Individual "detections" were categorized by host species, collection location, year of sampling, and variant type. To identify farming regions where OsHV-1 might recur, only *M. gigas* detections that occurred in the field were included. For all other hosts that may be potential reservoirs, both laboratory experiments and field detections were used. Once positive detections were identified, the host species, OsHV-1 variant, year of sampling, and location were extracted. If

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coordinates were not available in-text, we used other geographic information (e.g., maps, site descriptions) to estimate latitude and longitude.

If data concerning prevalence (proportion of individuals or pools testing positive for OsHV-1) and peak infection intensity (maximum quantity of viral copies measured via qPCR, standardized as amount of DNA per tissue weight) were available, that information was also extracted. Some studies provided generalized prevalence and viral load data across species, year, site, or variant, which prevented us from including that information in this analysis. Additionally, if prevalence was estimated at multiple time points during an individual year, a mean of those of values was taken to estimate overall prevalence. If data were presented in a figure, we used the analytical tool, WebPlotDigitizer (v. 4.6, Drevon et al. 2017), to estimate prevalence and maximum viral load (Zhu et al. 2019, Whited and Cervantes 2022).

RESULTS

Our review resulted in 116 total studies, with 75 of them providing information concerning OsHV-1 detections in *M. gigas* found in the field and 51 studies including data on infections in other animal hosts. OsHV-1 µVars (57.2%) and OsHV-1 (35.6%) comprised most detections, with the non-µVar (2.5%), herpes-like virus (1.8%), AVNV (1%), herpesvirus (<1%), and OsHV (<1%) making up the remainder. Herpesvirus and herpes-like viruses were detected from 1991-2002, before OsHV-1 detections began in 1999, and OsHV-1 µVars were first detected in 2008 (Fig. 3.3). Additionally, AVNV has been detected from 2007 to 2018, while some samples from 2011-2015 carried an OsHV-1 variant that differs from µVars. Older preserved samples were later analyzed via

PCR such that OsHV-1 detections date back to 1993 in France, while samples from France in 1998 and New Zealand in 1999 appeared to carry OsHV-1 µVars (Fig. 3.4). OsHV-1 and its variants were detected across multiple regions spanning Europe, Asia, Australia, North America, and South America. Most detections came from France (26.2%), Australia (21%), and Ireland (14.2%), with all other detections spread across 16 countries.

The majority of detections (71%) occurred in *M. gigas*, followed by the ark clam *Scapharca broughtonii* (4.7%), the European flat oyster *Ostrea edulis* (3.4%), and the Chinese scallop *Chlamys farreri* (3.3%); the remaining detections occurred in 39 other species. Most *M. gigas* detections happened in Australia (OsHV-1 µVar: 22%; OsHV-1 non-µVar: 3.5%; OsHV-1: <1%), France (OsHV-1 µVar: 18%; OsHV-1: 9.1%; Herpes-like virus: 2.7%; Herpesvirus: <1%), and Ireland (OsHV-1 µVar: 16.8%; OsHV-1: <1%) (Figs. 3.4, 3.5). AVNV has also been detected in *M. gigas* in Mexico (<1%). In contrast, other animals hosts most frequently tested positive for OsHV-1 (66.5%) or OsHV-1 µVar (26.3%) and spanned multiple taxa including crustaceans, annelids, and molluscs (Fig. 3.6). These detections largely occurred in China (35%), France (18%), Australia (8.8%), Spain (7.2%), Ireland (6.7%), and the USA (5.2%) (Figs. 3.4, 3.7).

Prevalence data were drawn from samples that tested positive for OsHV-1, OsHV-1 µVar, OsHV, herpesvirus, and herpes-like virus (Fig. 3.8). Here, we focus on prevalence data with more than a single measurement. Herpesviruses (87.5 ± 12.5% (mean ± SE)) and herpes-like viruses (61.3 ± 38.8%) had the greatest prevalence, but relatively low sample sizes (herpesvirus: 4; herpes-like virus: 2). Otherwise, OsHV-1 (58.4 ± 3.0%) was the variant with the greatest prevalence, followed by OsHV-1 µVar

(52.3 ± 3.2%), and OsHV (32.3 ± 13.7%). Prevalence was highest in *Octopus vulgaris* (93.2 ± 6.8%), *C. angulata* (80.3 ± 19.7%), *S. broughtonii* (76.9 ± 6.3%), *M. gigas* (63.9 ± 2.7%), *S. glomerata* (48.5 ± 9.4%), *Patinopectin yessoensis* (47.3 ± 21.3%), and *C. farreri* (47 ± 8.2%). Although the majority of these data were derived from France, China, and Australia, prevalence was greatest in Sweden (100 ± 0%) and Norway (100 ± 0%), followed by France (81.3 ± 3.6%), USA (65.3 ± 5.6%), and New Zealand (61.8 ± 18.3%). OsHV-1 µVar has appeared to increase in prevalence in France and Spain, while declining in Australia and Ireland (Fig. 3.9). Additionally, OsHV-1 prevalence remains high in China and France.

Maximum infection intensity data were only available for samples where OsHV-1 µVar and OsHV-1 were detected (Fig. 3.10); we again focused on infection data with more than a single measurement. DNA copy numbers for OsHV-1 µVar (1.89×10^8 mg tissue^{-1} ± 9.33×10^7 (mean ± SE)) OsHV-1 (1.66×10^8 mg tissue^{-1} ± 8.61×10^7) were similar. Peak infection intensity was greatest in *C. angulata* (1.11×10^9 mg tissue^{-1} ± 6.99×10^8), *M. gigas* (2.20×10^8 mg tissue^{-1} ± 9.44×10^7), *S. broughtonii* (9.99×10^6 mg tissue^{-1} ± 4.62×10^6), *O. edulis* (1.50×10^6 mg tissue^{-1} ± 1.50×10^6), *C. sikamea* (1.10×10^6 mg tissue^{-1} ± 8.20×10^5), and *Carcinus maenas* (9.60×10^5 mg tissue^{-1} ± 7.10×10^5). The countries with the highest maximal infection intensity were Portugal (9.49×10^8 mg tissue^{-1} ± 4.05×10^8), France (2.71×10^8 mg tissue^{-1} ± 1.66×10^8), USA (1.66×10^8 mg tissue^{-1} ± 1.40×10^8), Ireland (4.43×10^7 mg tissue^{-1} ± 2.50×10^7), and Italy (1.43×10^7 mg tissue^{-1} ± 1.43×10^7).

DISCUSSION

As interest in oyster cultivation continues to increase, there is a need for understanding where these commercially important species are most vulnerable to disease as well as areas susceptible to infection where OsHV-1 has potentially gone undetected. In this study, we synthesized peer-reviewed literature, government reports, and unpublished data (Shukla et al. *in prep*) to determine where *M. gigas* and other animal hosts have tested positive for OsHV-1 as well as the prevalence and intensity of those infections when that information was available. Across 116 studies, we identified that OsHV-1 and its variants have regularly been detected over the past 30 years across 18 countries and in 42 species other than *M. gigas*. Preserved samples from the end of the 20th century indicate that OsHV-1 occurred in France as early as 1993, while its µVars existed in France and New Zealand at the close of the 20th century. OsHV-1 was the variant with the highest prevalence, but infection intensity was similar for both OsHV-1 µVar and OsHV-1. OsHV-1 µVar prevalence has increased in France and Spain, while OsHV-1 prevalence is high in China and France. However, this subset of data may not accurately represent OsHV-1 prevalence overall, as farms can observe OsHV-1 mortalities that are not necessarily reported in white papers or peer-reviewed literature. Additionally, many species experienced prevalence and peak intensity comparable to or higher than *M. gigas*. Finally, France was the only country to exhibit both high prevalence and intensity.

This analysis provides a global and historical perspective on OsHV-1 variants that other studies have not. Prior studies that collated the geographic range and diversity of OsHV-1 variants and hosts have summarized information similar to what we report here

(USDA 2020) as well as information we do not, such as life stage (Alfaro et al. 2019) and GenBank accession numbers for different variants (Mineur et al. 2015). However, we build on these studies by including retroactive detections that demonstrate that OsHV-1 was active in France as early as 1993 (Renault et al. 2012), even though the virus was not sequenced until 1998 (Le Deuff and Renault 1999, Davison 2002). Similarly, the OsHV-1 µVar was first detected in 2008 (Segarra et al. 2010), but DNA was found in samples from France in 1998 (Garcia et al. 2011) and New Zealand in 1999 (Keeling et al. 2014). This is particularly valuable given that technological limitations in the 1990s prevented us from identifying OsHV-1, and infections were largely classified as "herpesvirus" and "herpes-like virus". By including both initial detections and those in older samples, we can more readily reconstruct viral activity and its influence on past mortality events. Similarly, once the OsHV-1 µVar sequence was available, it was detected both in older preserved samples (Garcia et al. 2011) and in *M. gigas* around the world (Mortensen et al. 2016, Barbieri et al. 2019, Burge et al. 2021), suggesting the variant had a wide geographic range and was present for nearly a decade before it was initially detected. Ultimately, this review provides an extensive summary of OsHV-1 variants over the past three decades that is not available elsewhere.

Prevalence and intensity data indicate the extent of infection across different variants, species, and locations. These data have not been aggregated in other reviews and provide a unique perspective on the effects of OsHV-1 and OsHV-1 µVar. Prevalence data suggest that OsHV-1 is more frequently detected in hosts than OsHV-1 µVar, but that the number of viral copies detected in samples is similar for both variants. The higher prevalence of the OsHV-1 reference variant may reflect its slower disease

progression, increasing the likelihood of OsHV-1 being detected in a host relative to the μVar. However, only 55% of the studies in this synthesis had prevalence data suitable for analysis, while only 36% of studies provided maximal infection intensity data. Thus, this analysis is only a subset of OsHV-1 studies and further investigation of these trends is necessary.

By knowing where OsHV-1 has occurred in the past and species within a farm's proximity that may be viral reservoirs, growers and managers can either avoid or carefully plan for prospective infections that lead to loss of stocks. While the majority of positive OsHV-1 occurrences were in commercially cultured bivalve species, a small subset of detections occurred in wild oysters, such as *S. glomerata* and *M. gigas*, as well as other molluscs in Australia's Georges River estuary (Evans et al. 2017). While *S. glomerata* is native to Australia, *M. gigas* has been introduced and outcompetes the native species at mid- and low intertidal heights (Krassoi et al. 2008). Nevertheless, the close proximity of both species to *M. gigas* aquaculture increases the likelihood of transmission from commercial leases to neighboring oyster populations during OsHV-1 outbreaks. Similarly, the common cockle, *Cerastoma edule*, is also susceptible to OsHV-1 because its distribution overlaps with cultivated *M. gigas* in Ireland (Bookelaar et al. 2020). It is unclear whether these wild populations succumb to OsHV-1 the way *M. gigas* does. But, because OsHV-1 can be latent in *M. gigas* (Dégremont et al. 2013), it is possible that the virus is latent in other bivalves as well. Thus, in addition to commercial oysters transmitting OsHV-1 to wild populations, these species may be responsible for future outbreaks that affect farmed *M. gigas*.

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Identifying hosts and countries that harbor OsHV-1 variants will hopefully aid growers and decision-makers in siting and increasing the productivity of oyster farms. Molluscs, particularly bivalves, are the most common animal hosts of OsHV-1 beyond *M. gigas*. Given their evolutionary similarities, it is not unsurprising that other oyster species (Moss et al. 2007, Shukla et al. *in prep*), scallops (Bai et al. 2015), clams (Xia et al. 2015), cockles (Bookelaar et al. 2020), and mussels (Evans et al. 2017) could potentially be OsHV-1 reservoirs that can transmit the virus to farmed *M. gigas*. However, given the global extent of OsHV-1 and the nascent exploration of alternate hosts, it is critical that other species – especially bivalves – within the proximity of ongoing aquaculture are tested for OsHV-1 variants. This is especially crucial given the geographic bias in OsHV-1 sampling thus far, which has largely occurred in the Mediterranean, United Kingdom, and Oceania. While the high volume of detections in these regions may be interpreted as locations where *M. gigas* is most vulnerable to OsHV-1 and OsHV-1 μVar, these might instead demonstrate where the virus is well-studied. Further, the countries in this study are not inclusive of all locations where *M. gigas* is cultured. Notably, there are no data from African countries despite operations in Algeria, Morocco, Namibia, and South Africa (FAO 2022). Surveys of OsHV-1 have also not been conducted in many countries where *M. gigas* has been introduced (Fofonoff et al. 2018, FAO 2022), such as Ecuador, Costa Rica, Fiji, Samoa, Israel, Romania, and Ukraine. To fully capture the global footprint of OsHV-1 variants, it may be helpful to test both *M. gigas* and native molluscan species near introduced populations as well as track mortality events in these areas. Moreover, as OsHV-1 continues to present challenges, cross-sectoral collaboration among scientists,

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managers, and growers in both developed and developing countries will become increasingly important.

Although OsHV-1 has only been detected over the past 30 years, it has a much larger global distribution than many other pathogens afflicting marine species (Burge et al. 2014). Diseases that affect bivalves are generally confined to specific regions (Guo and Ford 2016), while some finfish infections are more widespread. For example, vibriosis (*Vibrio spp.*) has been detected in the gilthead seabream (*Sparus aurata*) since 1990 and has been found in 10 countries (Sanches-Fernandes et al. 2022). Although OsHV-1 has been a concern in *M. gigas* for approximately the same timeframe, it occurs in nearly twice as many countries. In contrast, genetically distinct lineages of sea lice (*Lepeoptheirus spp.* and *Caligus spp.*) are found on wild and farmed salmonid species in the North Atlantic (Ford and Myers 2008), North Pacific (Godwin et al. 2022), and Southern Oceans (Kragesteen et al. 2018, Bravo and Treasurer 2023). The spread of sea lice is closely tied to the global expansion of salmon aquaculture, just as OsHV-1 is associated with increasing *M. gigas* mariculture. Indeed, as oyster aquaculture continues to grow, it is likely that that the footprint of OsHV-1 will, as well.

As climate change progresses, elevated ocean temperatures will foster increased disease transmission (Harvell 2002, Burge et al. 2014). Given that bivalve aquaculture production is expected to grow over the next decade (Froehlich et al. 2018), the footprint of *M. gigas* cultivation and OsHV-1 will likely expand. Thus, developing strategies for proactively coping with both climate and disease stress is imperative. Documenting viral sources will support this effort, though it is merely an initial step

towards developing a suite of resources that will fortify aquaculture operations in a changing ocean.

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FIGURES

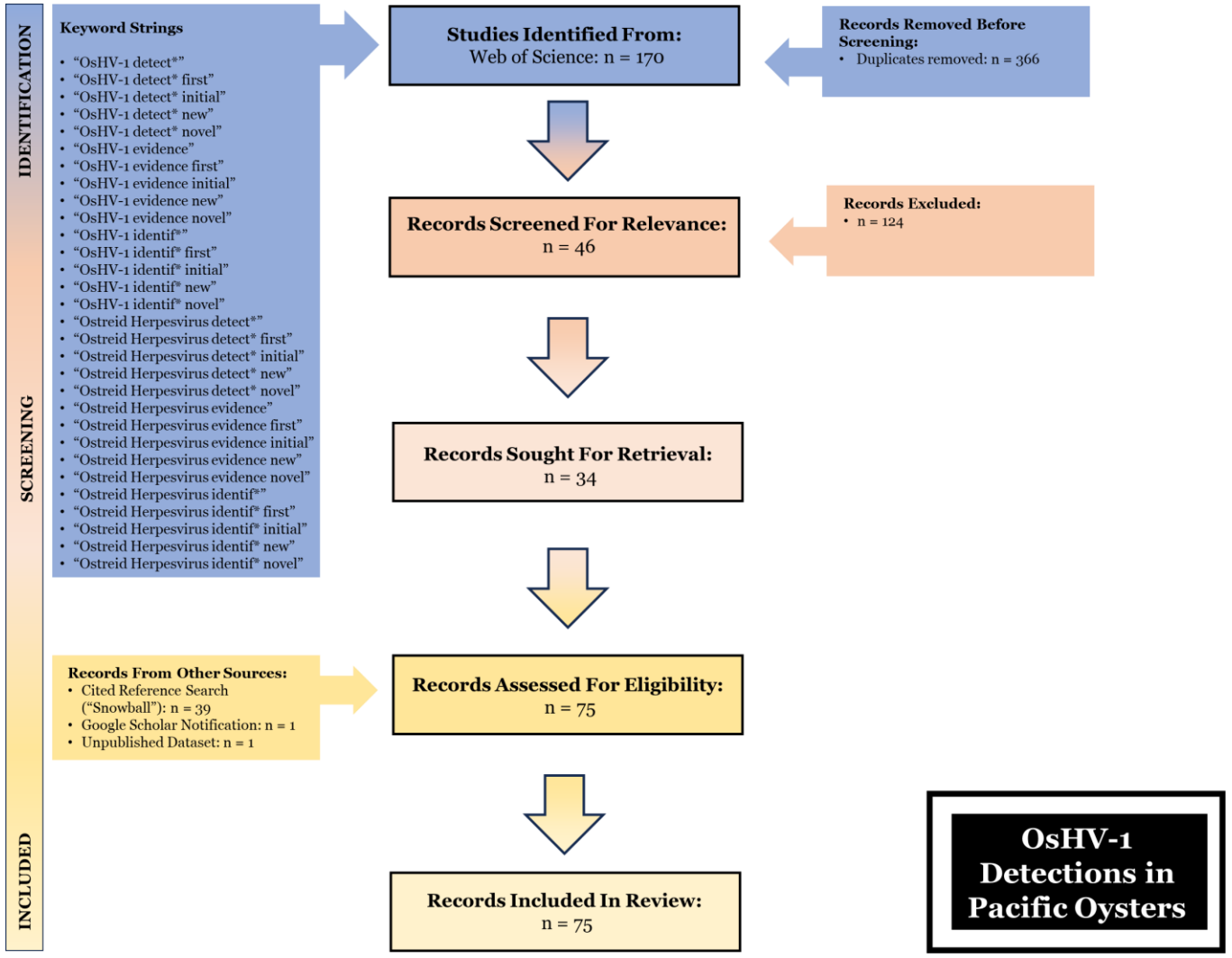

Figure 3.1. PRISMA flow diagram illustrating the outcomes of each phase of the system literature search for OsHV-1 detections in *M. gigas*. Modeled after Clements & George (2022).

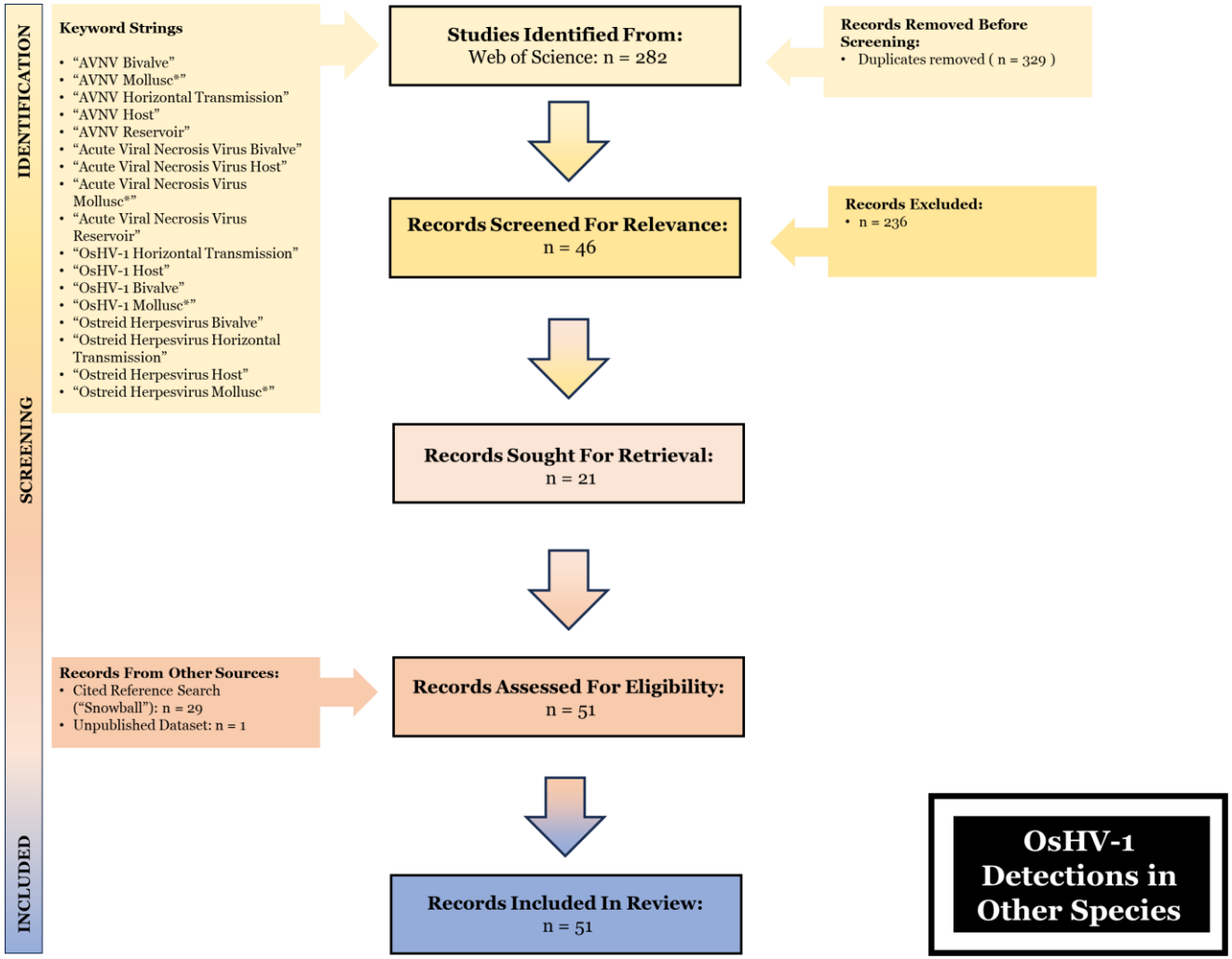

Figure 3.2. PRISMA flow diagram illustrating the outcomes of each phase of the system literature search for OsHV-1 detections in other species. Modeled after Clements & George (2022).

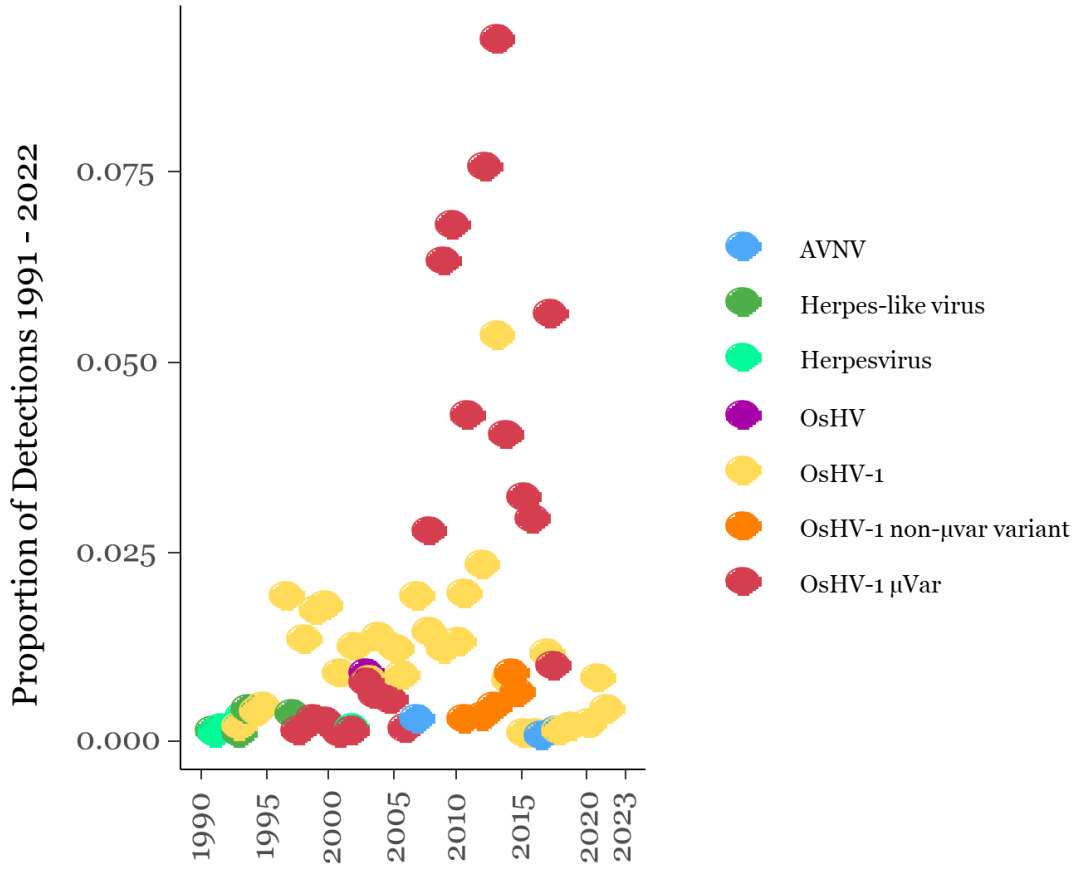

Figure 3.3. Detections of ANVN (blue), Herpes-like virus (dark green), and Herpesvirus (light green), OsHV (purple), OsHV-1 (yellow), OsHV-1 non- µVar (orange), and OsHV-1 µVar (red) variants detected in *Magllana gigas* samples from 1991 - 2022.

Figure 3.4. Map of OsHV-1 variant detections in *M. gigas* (left) and other species (right) from 1991 - 2022: ANVN (blue), Herpes-like virus (dark green), Herpesvirus (light green), OsHV (purple), OsHV-1 (yellow), OsHV-1 non- µVar (orange), and OsHV-1 µVar (red).

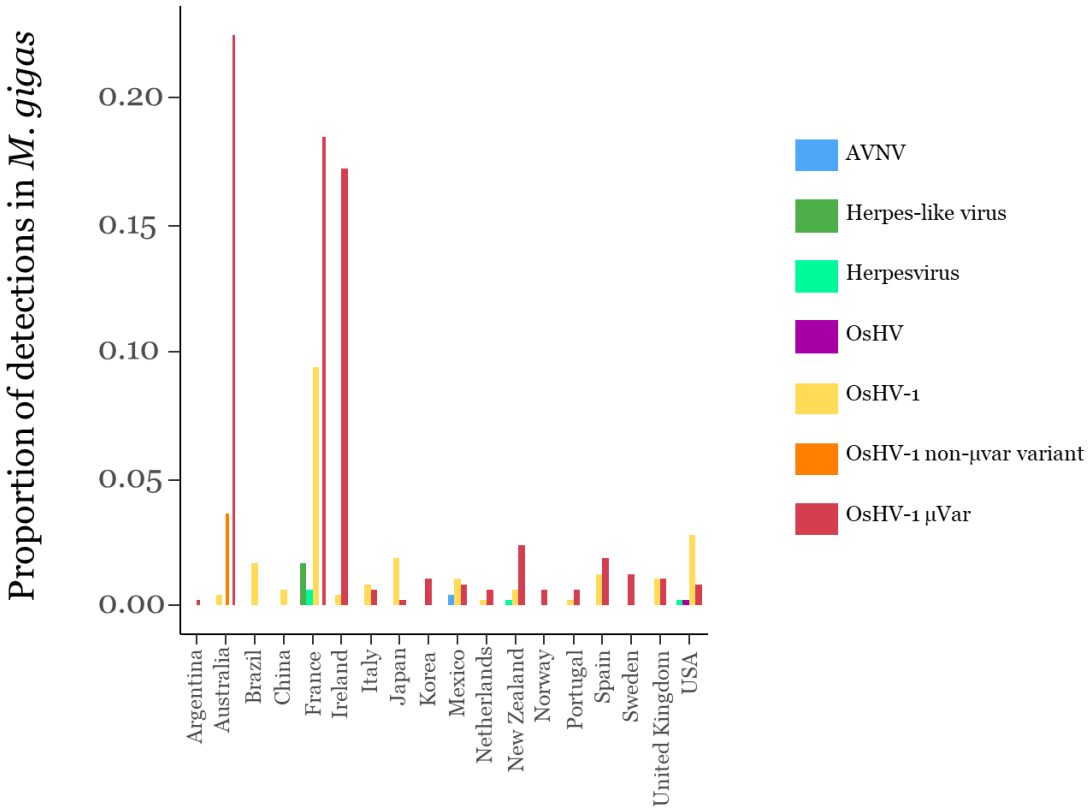

Figure 3.5. The proportion of OsHV-1 variants detected in *Magllana gigas* samples across 18 countries from 1991-2022.

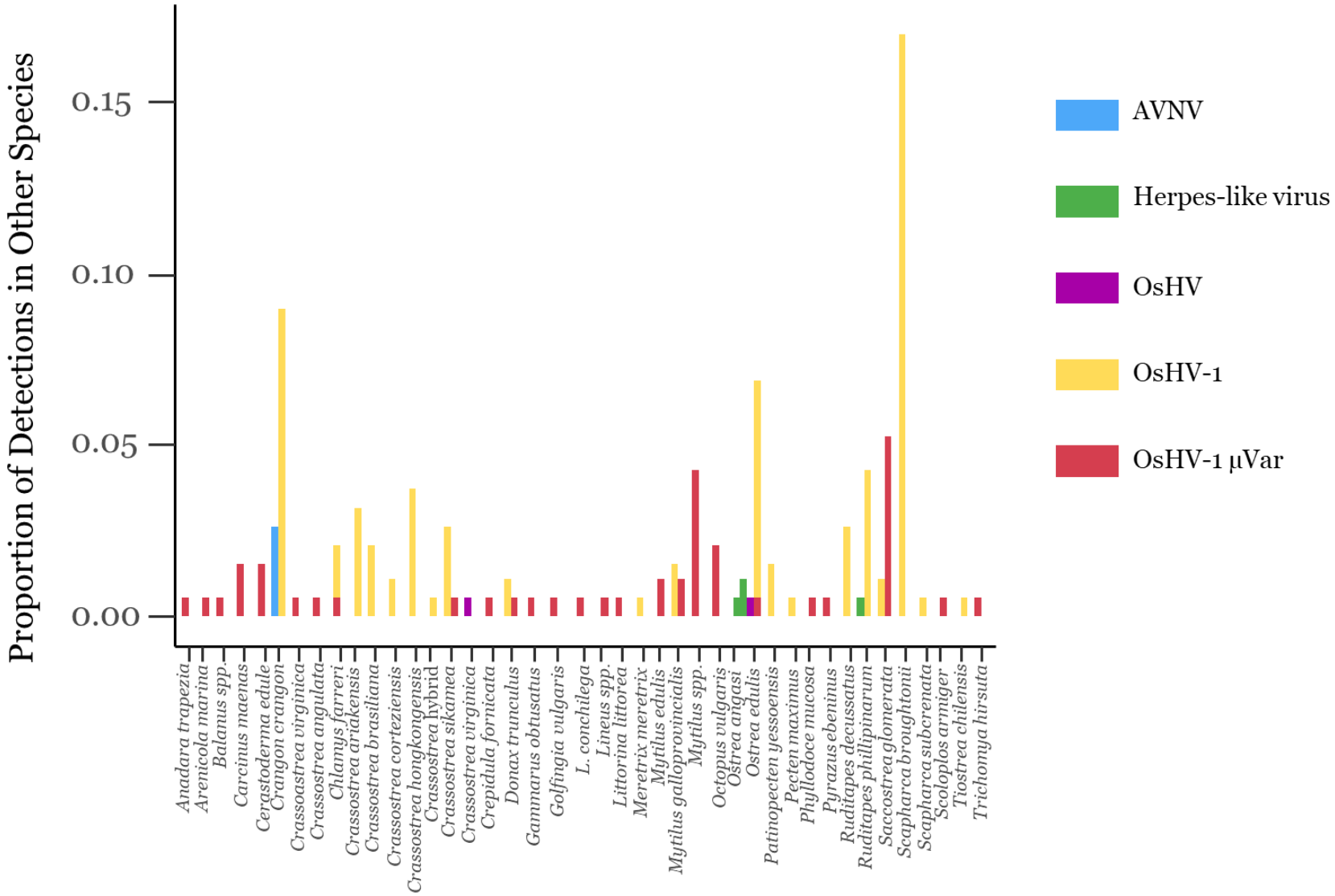

Figure 3.6. The proportion of OsHV-1 variants detected in 42 species other than *Magllana gigas*.

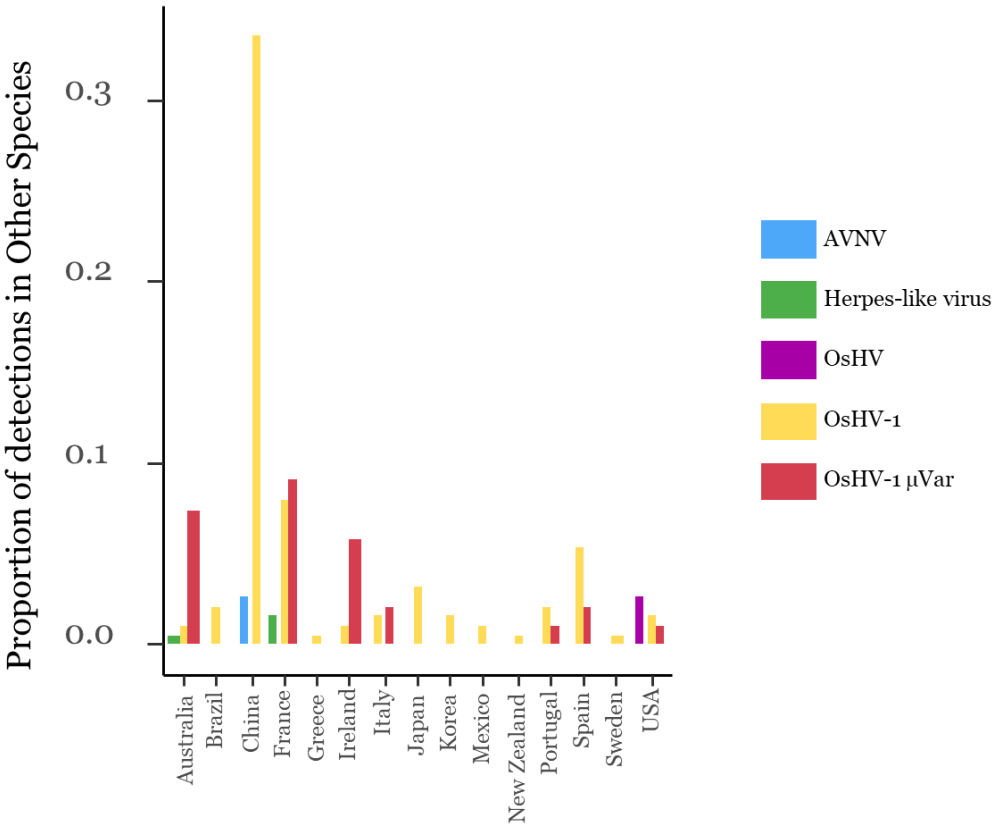

Figure 3.7. The proportion of OsHV-1 variants detected in species other than *Magllana gigas* across 15 countries.

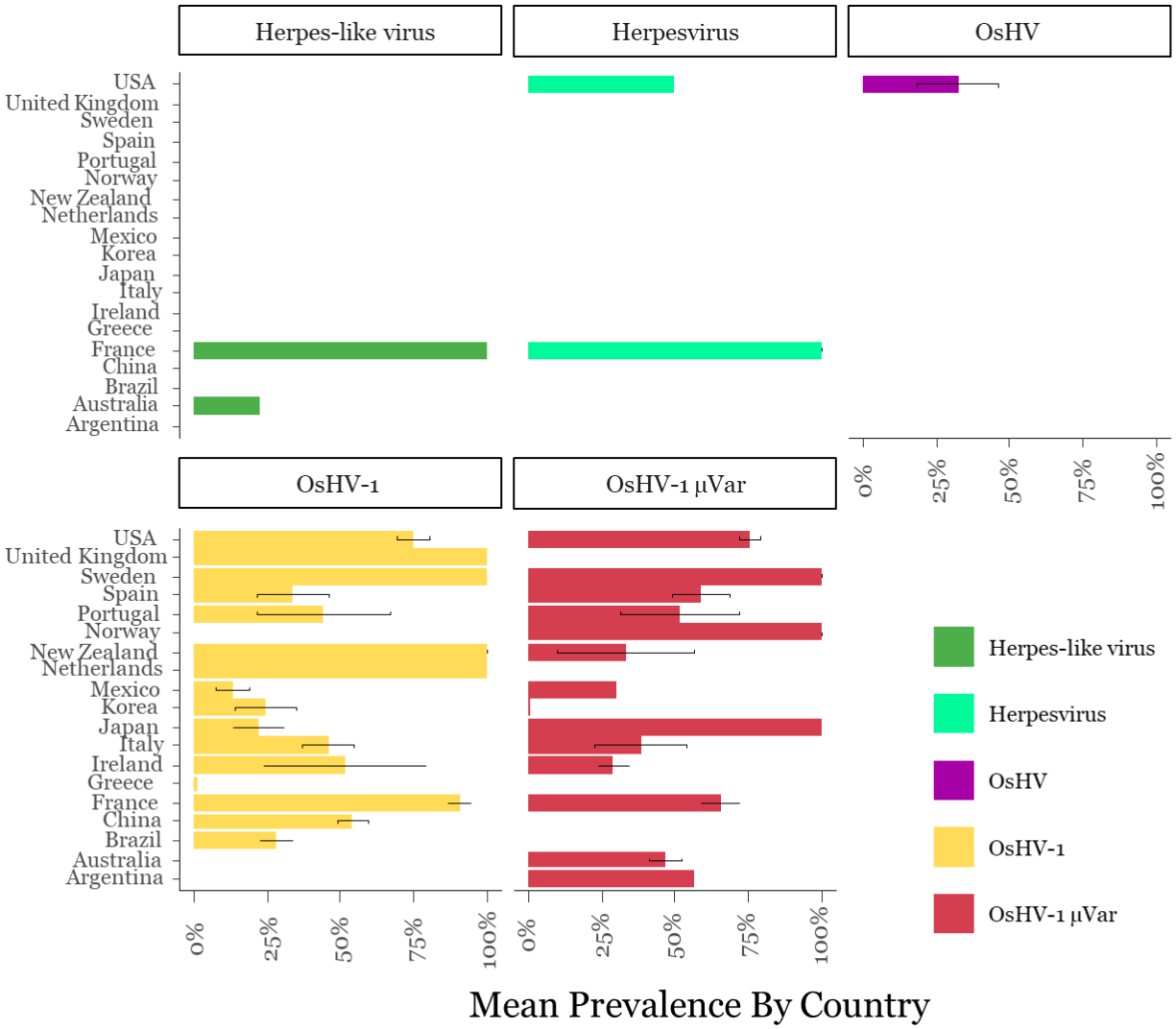

Figure 3.8. Mean prevalence of different OsHV-1 variants across 18 countries and all species including *M. gigas*.

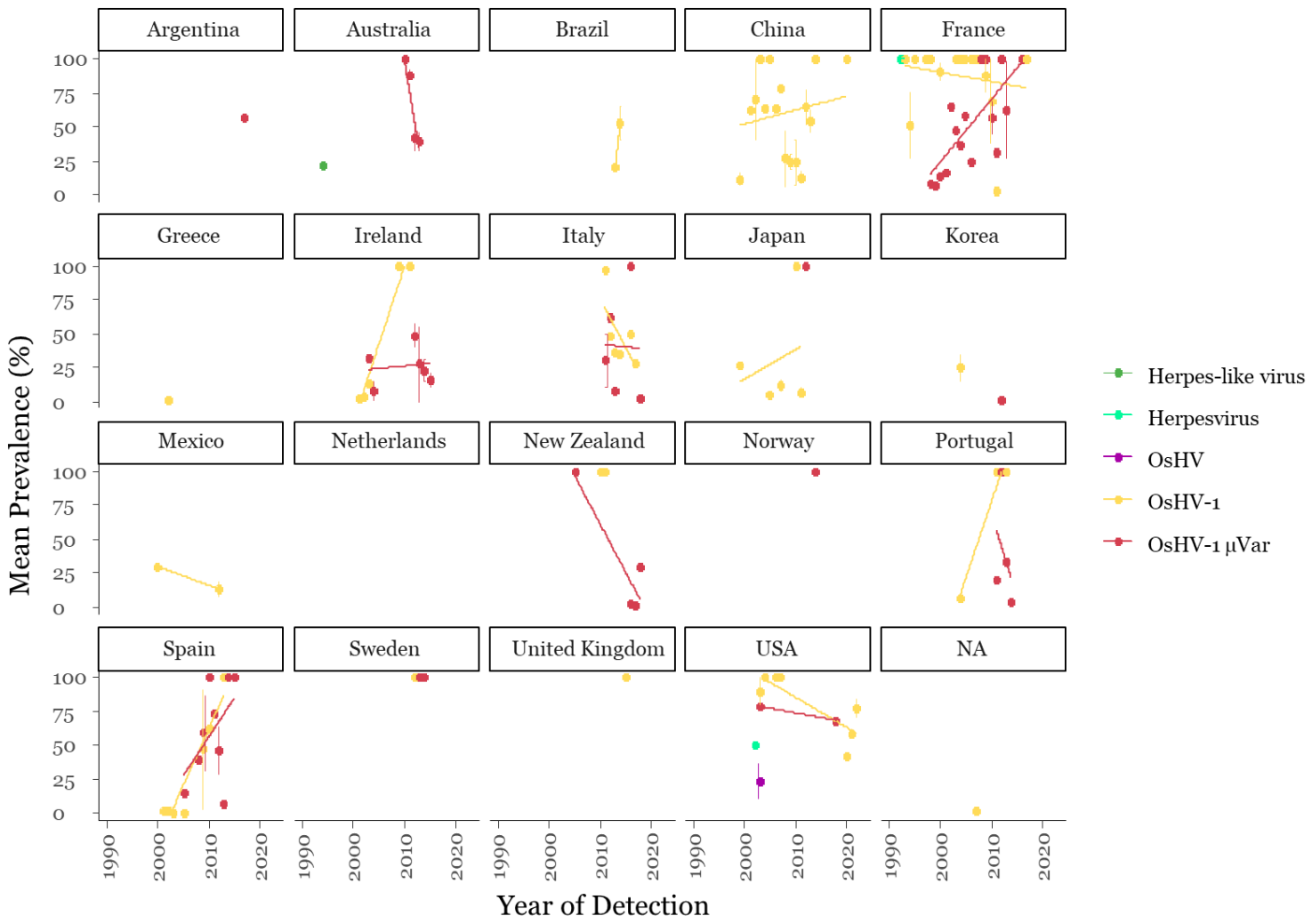

Figure 3.9. Mean prevalence of variants through time in each country.

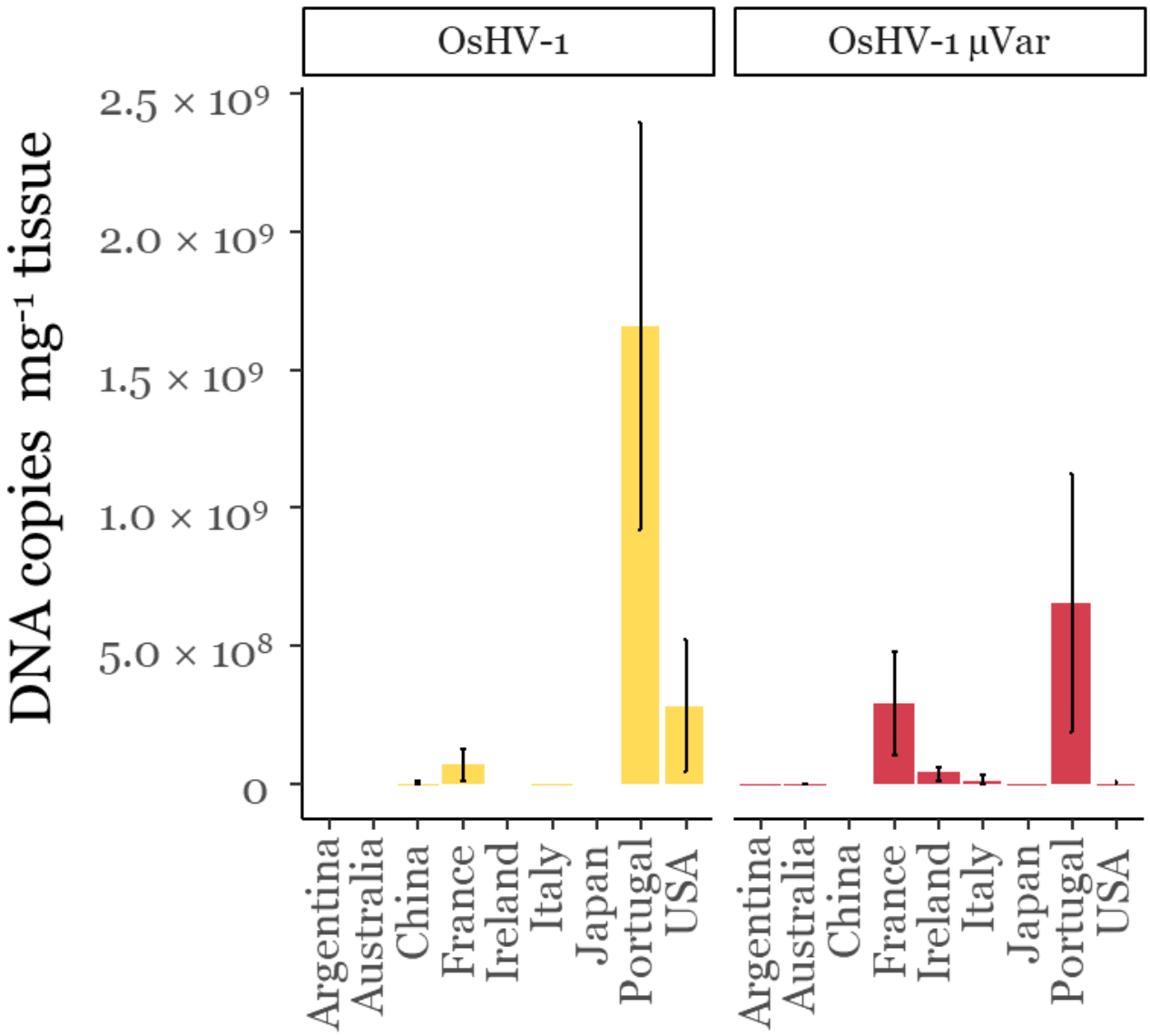

Figure 3.10. Mean peak infection intensity of different OsHV-1 variants across 9 countries.

APPENDIX 3.A - Table of studies with species and herpesvirus variants detected.

Year of Sampling	Species	OsHV Variant	Author & Year of Publication	Title
1991	*Magallana gigas*	Herpesvirus	Hine et al. 1992	Herpesviruses associated with mortalities among hatchery-reared larval Pacific oysters *Crassostrea gigas*
1991	*Magallana gigas*	Herpes-like virus	Nicolas et al. 1992	Herpes-like virus infecting Pacific-oyster larvae *Crassostrea gigas*
1992	*Magallana gigas*	Herpesvirus	Renault et al. 2000	Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France-Comparative study
1993	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
1993	*Magallana gigas*	Herpesvirus	Renault et al. 2000	Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France-Comparative study
1993	*Magallana gigas*	Herpes-like virus	Renault et al. 2000	Herpes-like virus infecting Japanese oyster (*Crassostrea gigas*) spat
1994	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
1994	*Magallana gigas*	OsHV-1	Barbosa-Solomieu et al. 2004	Diagnosis of Ostreid herpesvirus 1 in fixed paraffin-embedded archival samples using PCR and in situ hybridisation
1994	*Ostrea edulis*	OsHV-1	Barbosa-Solomieu et al. 2004	Diagnosis of Ostreid herpesvirus 1 in fixed paraffin-embedded archival samples using PCR and in situ hybridisation
1994	*Magallana gigas*	Herpes-like virus	Renault et al. 2000	Concomitant herpes-like virus infections in hatchery-reared larvae and nursery-cultured spat *Crassostrea gigas* and *Ostrea edulis*
1994	*Ostrea edulis*	Herpes-like virus	Renault et al. 2000	Concomitant herpes-like virus infections in hatchery-reared larvae and nursery-cultured spat *Crassostrea gigas* and *Ostrea edulis*
1994	*Ostrea angasi*	Herpes-like virus	Hine & Thorne 1997	Replication of herpes-like viruses in haemocytes of adult flat oysters *Ostrea angasi*: an ultrastructural study
1995	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves
1995	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
1995	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008
1997	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves

1997	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves
1997	*Ruditapes phillipinarum*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves
1997	*Magallana gigas*	Herpes-like virus	Renault et al. 2001	A herpes-like virus infecting *Crassostrea gigas* and *Ruditapes philippinarum* larvae in France
1997	*Ruditapes phillipinarum*	Herpes-like virus	Renault et al. 2001	A herpes-like virus infecting *Crassostrea gigas* and *Ruditapes philippinarum* larvae in France
1997	*Ruditapes phillipinarum*	OsHV-1	Renault et al. 2001	A herpes-like virus infects a non-ostreid bivalve species: virus replication in *Ruditapes philippinarum* larvae
1997	*Crassostrea angulata*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1997	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1997	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1997	*Ruditapes phillipinarum*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1997	*Magallana gigas*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1997	*Ruditapes decussatus*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1998	*Ruditapes decussatus*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves
1998	*Crassostrea angulata*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1998	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1998	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1998	*Ruditapes phillipinarum*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1998	*Magallana gigas*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1998	*Ruditapes decussatus*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1998	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with Crassostrea gigas spat mortality in France between 1998 and 2006
1999	*Crassostrea hongkongensis*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
1999	*Crassostrea ariakensis*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
1999	*Crassostrea angulata*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission

1999	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1999	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
1999	*Ruditapes phillipinarum*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1999	*Magallana gigas*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1999	*Ruditapes decussatus*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
1999	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
1999	*Magallana gigas*	OsHV-1µVar	Keeling et al. 2014	New Zealand juvenile oyster mortality associated with ostreid herpesvirus 1 - an opportunistic longitudinal study
2000	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves
2000	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Evidence for interspecies transmission of oyster herpesvirus in marine bivalves
2000	*Pecten maximus*	OsHV-1	Arzul et al. 2001	French Scallops: A New Host for Ostreid Herpesvirus-1
2000	*Crassostrea angulata*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
2000	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
2000	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
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2000	*Magallana gigas*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
2000	*Ruditapes decussatus*	OsHV-1	Renault & Arzul 2001	Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR
2000	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
2000	*Magallana gigas*	OsHV-1	Arzul et al. 2002	Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults
2000	*Magallana gigas*	OsHV-1	Vásquez-Yeomans et al. 2010	Gill erosion and herpesvirus in *Crassostrea gigas* cultured in Baja California, Mexico
2001	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2001	*Ostrea edulis*	OsHV-1	da Silva et al. 2008	Herpesvirus infection in European flat oysters *Ostrea edulis* obtained from brood stocks of various geographic origins and grown in Galicia (NW Spain)
2001	*Magallana gigas*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission

2001	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
2001	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
2002	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2002	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2002	*Crassostrea hongkongensis*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
2002	*Ostrea edulis*	OsHV-1	da Silva et al. 2008	Herpesvirus infection in European flat oysters *Ostrea edulis* obtained from brood stocks of various geographic origins and grown in Galicia (NW Spain)
2002	*Ostrea edulis*	OsHV-1	Arzul et al. 2001	Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission
2002	*Magallana gigas*	Herpesvirus	Friedman et al. 2005	Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes
2002	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
2003	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2003	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2003	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008
2003	*Ostrea edulis*	OsHV-1	da Silva et al. 2008	Herpesvirus infection in European flat oysters Ostrea edulis obtained from brood stocks of various geographic origins and grown in Galicia (NW Spain)
2003	*Crassostrea sikamea*	OsHV	Burge et al. 2011	Detection of the oyster herpesvirus in commercial bivalve in northern California, USA: conventional and quantitative PCR
2003	*Crassostrea virginica*	OsHV	Burge et al. 2011	Detection of the oyster herpesvirus in commercial bivalve in northern California, USA: conventional and quantitative PCR
2003	*Mytilus galloprovincialis*	OsHV	Burge et al. 2011	Detection of the oyster herpesvirus in commercial bivalve in northern California, USA: conventional and quantitative PCR
2003	*Ostrea edulis*	OsHV	Burge et al. 2011	Detection of the oyster herpesvirus in commercial bivalve in northern California, USA: conventional and quantitative PCR
2003	*Ruditapes phillipinarum*	OsHV	Burge et al. 2011	Detection of the oyster herpesvirus in commercial bivalve in northern California, USA: conventional and quantitative PCR
2003	*Magallana gigas*	OsHV	Burge et al. 2011	Detection of the oyster herpesvirus in commercial bivalve in northern California, USA: conventional and quantitative PCR
2003	*Magallana gigas*	OsHV	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with Crassostrea gigas spat mortality in France between 1998 and 2006

2003	*Magallana gigas*	OsHV	Burge et al. 2006	Mortality and herpesvirus infections of the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA
2003	*Magallana gigas*	OsHV-1µVar	Lynch et al. 2012	A previously undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the pacific oyster, *Crassostrea gigas*, in Ireland
2004	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2004	*Crassostrea ariakensis*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
2004	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008
2004	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
2004	*Crassostrea angulata*	OsHV-1	Batista et al. 2014	Insights on the association between somatic aneuploidy and ostreid herpesvirus 1 detection in the oysters *Crassostrea gigas*, *C. angulata* and their F1 hybrids
2004	*Crassostrea hybrid*	OsHV-1	Batista et al. 2014	Insights on the association between somatic aneuploidy and ostreid herpesvirus 1 detection in the oysters *Crassostrea gigas*, *C. angulata* and their F1 hybrids
2004	*Magallana gigas*	OsHV-1	Batista et al. 2014	Insights on the association between somatic aneuploidy and ostreid herpesvirus 1 detection in the oysters *Crassostrea gigas*, *C. angulata* and their F1 hybrids
2004	*Magallana gigas*	OsHV-1µVar	Lynch et al. 2012	A previously undescribed ostreid herpes virus 1 (OsHV-1) genotype detected in the pacific oyster, *Crassostrea gigas*, in Ireland
2004	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2012	Detection of the OsHV-1µVar in the Pacific oyster *Crassostrea gigas* before 2008 in France and description of two new microvariants of the Ostreid Herpesvirus 1 (OsHV-1)
2005	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2005	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2005	*Magallana gigas*	OsHV-1µVar	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2005	*Crassostrea ariakensis*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
2005	*Magallana gigas*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
2005	*Crassostrea sikamea*	OsHV-1	Moss et al. 2007	Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay
2005	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008

2005	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
2005	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2012	Detection of the OsHV-1µVar in the Pacific oyster *Crassostrea gigas* before 2008 in France and description of two new microvariants of the Ostreid Herpesvirus 1 (OsHV-1)
2005	*Magallana gigas*	OsHV-1µVar	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2005	*Magallana gigas*	OsHV-1	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2006	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2006	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2006	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008
2006	*Magallana gigas*	OsHV-1µVar	Garcia et al. 2011	Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006
2007	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2007	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2007	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008
2007	*Crassostrea ariakensis*	OsHV-1	Shimahara et al. 2012	Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) Variants in Japan
2007	*Crassostrea sikamea*	OsHV-1	Shimahara et al. 2012	Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) Variants in Japan
2007	*Magallana gigas*	OsHV-1	Shimahara et al. 2012	Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) Variants in Japan
2007	*Chlamys farreri*	AVNV	Ren et al. 2013	Complete genome sequence of acute viral necrosis virus associated with massive mortality outbreaks in the Chinese scallop, *Chlamys farreri*
2007	*Chlamys farreri*	AVNV	Tang et al. 2010	Physiological and immune responses of zhikong scallop *Chlamys farreri* to the acute viral necrobiotic virus infection
2008	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2008	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2008	*Magallana gigas*	OsHV-1µVar	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008

2008	*Magallana gigas*	OsHV-1	Segarra et al. 2010	Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008
2008	*Magallana gigas*	OsHV-1µVar	Schikorski et al. 2011	Experimental infection of Pacific oyster Crassostrea gigas spat by ostreid herpesvirus 1: demonstration of oyster spat susceptibility
2008	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2011	Detection of different variants of Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010
2008	*Magallana gigas*	OsHV-1	Morga et al. 2021	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species
2008	*Magallana gigas*	OsHV-1µVar	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2008	*Magallana gigas*	OsHV-1	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2008	*Magallana gigas*	OsHV-1µVar	Morrissey et al. 2016	An investigation of ostreid herpes virus microvariants found in Crassostrea gigas oyster producing bays in Ireland
2009	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2009	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas
2009	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2015	Detection of undescribed ostreid herpesvirus 1 (OsHV-1) specimens from Pacific oyster, *Crassostrea gigas*
2009	*Magallana gigas*	OsHV-1µVar	Pernet et al. 2012	Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean Thau lagoon, France
2009	*Magallana gigas*	OsHV-1µVar	Peeler et al. 2012	Investigation of mortality in Pacific oysters associated with Ostreid herpesvirus-1µVar in the Republic of Ireland in 2009
2009	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2011	Detection of different variants of Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010
2009	*Magallana gigas*	OsHV-1	Degremont 2011	Evidence of herpesvirus (OsHV-1) resistance in juvenile *Crassostrea gigas* selected for high resistance to the summer mortality phenomenon
2009	*Magallana gigas*	OsHV-1µVar	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2009	*Magallana gigas*	OsHV-1	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2009	*Magallana gigas*	OsHV-1µVar	Morrissey et al. 2016	An investigation of ostreid herpes virus microvariants found in *Crassostrea gigas* oyster producing bays in Ireland
2010	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2010	*Octopus vulgaris*	OsHV-1µVar	Prado-Alvarez et al. 2021	First detection of OsHV-1 in the cephalopod *Octopus vulgaris*. Is the octopus a dead-end for OsHV-1?
2010	*Magallana gigas*	OsHV-1	Renault et al. 2012	Analysis of Clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) Specimens by Sequencing Amplified Fragments from Three Virus Genome Areas

2010	*Saccostrea glomerata*	OsHV-1µVar	Jenkins et al. 2013	Identification and characterisation of an ostreid herpesvirus-1 microvariant (OsHV-1 µvar) in *Crassostrea gigas* (Pacific oysters) in Australia
2010	*Donax trunculus*	OsHV-1	Garcia et al. 2018	Descriptions of *Mikrocytos veneroïdes* n. sp. and *Mikrocytos donaxi* n. sp. (Ascetosporea: Mikrocytida: Mikrocytiidae), detected during important mortality events of the wedge clam *Donax trunculus* Linnaeus (Veneroida: Donacidae), in France between 2008 and 2011
2010	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2015	Detection of undescribed ostreid herpesvirus 1 (OsHV-1) specimens from Pacific oyster, *Crassostrea gigas*
2010	*Magallana gigas*	OsHV-1µVar	Degremont & Benabdelmouna 2014	Mortality associated with OsHV-1 in spat *Crassostrea gigas*: role of wild-caught spat in the horizontal transmission of the disease
2010	*Magallana gigas*	OsHV-1µVar	de Lorgeril et al. 2018	Inefficient immune response is associated with microbial permissiveness in juvenile oysters affected by mass mortalities on field
2010	*Donax trunculus*	OsHV-1µVar	Cochennec-Laureau et al. 2011	Les surmortalités des naissains d'huîtres creuses, *Crassostrea gigas*, : acquis des recherches en 2010
2010	*Mytilus edulis*	OsHV-1µVar	Cochennec-Laureau et al. 2011	Les surmortalités des naissains d'huîtres creuses, *Crassostrea gigas*, : acquis des recherches en 2010
2010	*Mytilus galloprovincialis*	OsHV-1µVar	Cochennec-Laureau et al. 2011	Les surmortalités des naissains d'huîtres creuses, *Crassostrea gigas*, : acquis des recherches en 2010
2010	*Magallana gigas*	OsHV-1µVar	Pernet et al. 2012	Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean Thau lagoon, France
2010	*Magallana gigas*	OsHV-1µVar	Petton et al. 2015	Factors influencing disease-induced mortality of Pacific oysters *Crassostrea gigas*
2010	*Magallana gigas*	OsHV-1µVar	Renault et al. 2014	Ostreid Herpesvirus 1 Infection among Pacific Oyster (*Crassostrea gigas*) Spat: Relevance of Water Temperature to Virus Replication and Circulation Prior to the Onset of Mortality
2010	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2011	Detection of different variants of Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010
2010	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2012	Detection of the OsHV-1µVar in the Pacific oyster *Crassostrea gigas* before 2008 in France and description of two new microvariants of the Ostreid Herpesvirus 1 (OsHV-1)
2010	*Magallana gigas*	OsHV-1	Morga et al. 2021	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species
2010	*Magallana gigas*	OsHV-1µVar	Keeling et al. 2014	New Zealand juvenile oyster mortality associated with ostreid herpesvirus 1 - an opportunistic longitudinal study
2010	*Magallana gigas*	OsHV-1µVar	Dundon et al. 2011	Detection of Type 1 Ostreid Herpes variant (OsHV-1µVar) with no associated mortality in French-origin Pacific cupped oyster *Crassostrea gigas* farmed in Italy
2010	*Magallana gigas*	OsHV-1µVar	Mortensen et al. 2016	Summer mortalities and detection of ostreid herpesvirus microvariant in Pacific oyster *Crassostrea gigas* in Sweden and Norway

2010	*Magallana gigas*	OsHV-1	Roque et al. 2012	First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain
2010	*Magallana gigas*	OsHV-1µVar	Morrissey et al. 2016	An investigation of ostreid herpes virus microvariants found in *Crassostrea gigas* oyster producing bays in Ireland
2011	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2011	*Crassostrea hongkongensis*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2011	*Magallana gigas*	OsHV-1	Serraca et al. 2016	Mortality and ostreid herpesvirus 1 infection in the pacific oyster *Crassostrea gigas* in the Gulf of La Spezia, Italy
2011	*Crassostrea angulata*	OsHV-1	Batista et al. 2015	Sequence variation in ostreid herpesvirus 1 microvar isolates detected in dying and asymptomatic *Crassostrea angulata* adults in the Iberian Peninsula: Insights into viral origin and spread
2011	*Octopus vulgaris*	OsHV-1µVar	Prado-Alvarez et al. 2021	First detection of OsHV-1 in the cephalopod *Octopus vulgaris*. Is the octopus a dead-end for OsHV-1?
2011	*Saccostrea glomerata*	OsHV-1µVar	Jenkins et al. 2013	Identification and characterisation of an ostreid herpesvirus-1 microvariant (OsHV-1 µ-var) in Crassostrea gigas (Pacific oysters) in Australia
2011	*Magallana gigas*	OsHV-1	Shimahara et al. 2012	Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) Variants in Japan
2011	*Donax trunculus*	OsHV-1	Garcia et al. 2018	Descriptions of *Mikrocytos veneroïdes* n. sp. and *Mikrocytos donaxi* n. sp. (Ascetosporea: Mikrocytida: Mikrocytiidae), detected during important mortality events of the wedge clam *Donax trunculus* Linnaeus (Veneroida: Donacidae), in France between 2008 and 2011
2011	*Magallana gigas*	OsHV-1µVar	Batista et al. 2015	Sequence variation in ostreid herpesvirus 1 microvar isolates detected in dying and asymptomatic *Crassostrea angulata* adults in the Iberian Peninsula: Insights into viral origin and spread
2011	*Magallana gigas*	OsHV-1µVar	Hwang et al. 2013	Ostreid herpesvirus 1 infection in farmed Pacific oyster larvae *Crassostrea gigas* (Thunberg) in Korea
2011	*Magallana gigas*	OsHV-1µVar	Carrasco et al. 2017	A Production Calendar Based on Water Temperature, Spat Size, and Husbandry Practices Reduce OsHV-1 µVar Impact on Cultured Pacific Oyster *Crassostrea gigas* in the Ebro Delta (Catalonia), Mediterranean Coast of Spain
2011	*Magallana gigas*	OsHV-1µVar	Petton et al. 2015	Factors influencing disease-induced mortality of Pacific oysters *Crassostrea gigas*
2011	*Magallana gigas*	OsHV-1µVar	Grijalva-Chon et al. 2013	Detection of a new OsHV-1 DNA strain in the healthy Pacific oyster, *Crassostrea gigas* Thunberg, from the Gulf of California
2011	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2012	Detection of the OsHV-1µVar in the Pacific oyster *Crassostrea gigas* before 2008 in France and description of two new microvariants of the Ostreid Herpesvirus 1 (OsHV-1)
2011	*Magallana gigas*	OsHV-1	Morga et al. 2021	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species

2011	*Magallana gigas*	OsHV-1 non-µVar variant	Trancart et al. 2023	Diversity and molecular epidemiology of Ostreid herpesvirus 1 in farmed *Crassostrea gigas* in Australia: Geographic clusters and implications for "microvariants" in global mortality events
2011	*Magallana gigas*	OsHV-1µVar	Paul-Pont et al. 2013	Influence of husbandry practices on OsHV-1 associated mortality of Pacific oysters *Crassostrea gigas*
2011	*Magallana gigas*	OsHV-1µVar	Keeling et al. 2014	New Zealand juvenile oyster mortality associated with ostreid herpesvirus 1 - an opportunistic longitudinal study
2011	*Magallana gigas*	OsHV-1	Whittington et al. 2013	Final Report: 2011/053 Aquatic Animal Health Subprogram: Pacific oyster mortality syndrome (POMS) - understanding biotic and abiotic environmental and husbandry effects to reduce economic losses
2011	*Saccostrea glomerata*	OsHV-1	Whittington et al. 2013	Final Report: 2011/053 Aquatic Animal Health Subprogram: Pacific oyster mortality syndrome (POMS) - understanding biotic and abiotic environmental and husbandry effects to reduce economic losses
2011	*Mytilus galloprovincialis*	OsHV-1µVar	Domeneghetti et al. 2014	Mortality occurrence and pathogen detection in *Crassostrea gigas* and *Mytilus galloprovincialis* close-growing in shallow waters (Goro lagoon, Italy)
2011	*Magallana gigas*	OsHV-1µVar	Domeneghetti et al. 2014	Mortality occurrence and pathogen detection in *Crassostrea gigas* and *Mytilus galloprovincialis* close-growing in shallow waters (Goro lagoon, Italy)
2011	*Magallana gigas*	OsHV-1µVar	Morrissey et al. 2016	An investigation of ostreid herpes virus microvariants found in *Crassostrea gigas* oyster producing bays in Ireland
2012	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2012	*Patinopecten yessoensis*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2012	*Scapharca broughtonii*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2012	*Magallana gigas*	OsHV-1	Serraca et al. 2016	Mortality and ostreid herpesvirus 1 infection in the pacific oyster *Crassostrea gigas* in the Gulf of La Spezia, Italy
2012	*Crassostrea corteziensis*	OsHV-1	Martínez-García et al. 2020	OsHV-1 and notifiable protozoa in healthy *Crassostrea corteziensis* cultured in two distant areas of the Gulf of California
2012	*Scapharca broughtonii*	OsHV-1	Bai et al. 2016	Identification and characterization of ostreid herpesvirus 1 associated with massive mortalities of *Scapharca broughtonii* broodstocks in China
2012	*Scapharca broughtonii*	OsHV-1	Xia et al. 2015	Complete genome sequence of Ostreid herpesvirus-1 associated with mortalities of *Scapharca broughtonii* broodstocks
2012	*Magallana gigas*	OsHV-1µVar	Prado-Alvarez et al. 2015	Occurrence of OsHV-1 in *Crassostrea gigas* Cultured in Ireland during an Exceptionally Warm Summer. Selection of Less Susceptible Oysters
2012	*Magallana gigas*	OsHV-1µVar	Jee et al. 2013	Detection of Ostreid Herpesvirus 1 from adult Pacific Oysters *Crassostrea gigas* Cultured in Korea
2012	*Saccostrea glomerata*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia

2012	*Anadara trapezia*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2012	*Magallana gigas*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2012	*Mytilus spp.*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2012	*Pyrazus ebeninus*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2012	*Trichomya hirsuta*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2012	*Magallana gigas*	OsHV-1µVar	Martenot et al. 2015	Detection of undescribed ostreid herpesvirus 1 (OsHV-1) specimens from Pacific oyster, *Crassostrea gigas*
2012	*Magallana gigas*	OsHV-1µVar	Carrasco et al. 2017	A Production Calendar Based on Water Temperature, Spat Size, and Husbandry Practices Reduce OsHV-1µVar Impact on Cultured Pacific Oyster *Crassostrea gigas* in the Ebro Delta (Catalonia), Mediterranean Coast of Spain
2012	*Magallana gigas*	OsHV-1µVar	Morrissey et al. 2017	Mortality in *Crassostrea gigas* oysters in Ireland during 2012
2012	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2015	Further observations on the influence of husbandry practices on OsHV-1µVar mortality in Pacific oysters *Crassostrea gigas*: Age, cultivation structures and growing height
2012	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2019	Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management
2012	*Ostrea edulis*	OsHV-1	Morga et al. 2021	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species
2012	*Magallana gigas*	OsHV-1 non-µVar variant	Trancart et al. 2023	Diversity and molecular epidemiology of Ostreid herpesvirus 1 in farmed *Crassostrea gigas* in Australia: Geographic clusters and implications for "microvariants" in global mortality events
2012	*Magallana gigas*	OsHV-1µVar	Paul-Pont et al. 2013	Influence of husbandry practices on OsHV-1 associated mortality of Pacific oysters *Crassostrea gigas*
2012	*Magallana gigas*	OsHV-1µVar	Keeling et al. 2014	New Zealand juvenile oyster mortality associated with ostreid herpesvirus 1 – an opportunistic longitudinal study
2012	*Magallana gigas*	OsHV-1	Whittington et al. 2013	Final Report: 2011/053 Aquatic Animal Health Subprogram: Pacific oyster mortality syndrome (POMS) - understanding biotic and abiotic environmental and husbandry effects to reduce economic losses
2012	*Saccostrea glomerata*	OsHV-1	Whittington et al. 2013	Final Report: 2011/053 Aquatic Animal Health Subprogram: Pacific oyster mortality syndrome (POMS) - understanding biotic and abiotic environmental and husbandry effects to reduce economic losses

2012	*Magallana gigas*	OsHV-1µVar	Domeneghetti et al. 2014	Mortality occurrence and pathogen detection in *Crassostrea gigas* and *Mytilus galloprovincialis* close-growing in shallow waters (Goro lagoon, Italy)
2012	*Magallana gigas*	OsHV-1µVar	Gittenberger et al. 2016	Ostreid herpesvirus OsHV-1 µVar in Pacific oysters *Crassostrea gigas* (Thunberg 1793) of the Wadden Sea, a UNESCO world heritage site
2012	*Magallana gigas*	OsHV-1µVar	Morrissey et al. 2016	An investigation of ostreid herpes virus microvariants found in *Crassostrea gigas* oyster producing bays in Ireland
2013	*Chlamys farreri*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2013	*Ruditapes phillipinarum*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2013	*Crassostrea hongkongensis*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2013	*Scapharca broughtonii*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2013	*Magallana gigas*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2013	*Meretrix meretrix*	OsHV-1	Bai et al. 2015	Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China
2013	*Magallana gigas*	OsHV-1	Serraca et al. 2016	Mortality and ostreid herpesvirus 1 infection in the pacific oyster *Crassostrea gigas* in the Gulf of La Spezia, Italy
2013	*Crassostrea angulata*	OsHV-1	Batista et al. 2015	Sequence variation in ostreid herpesvirus 1 microvar isolates detected in dying and asymptomatic *Crassostrea angulata* adults in the Iberian Peninsula: Insights into viral origin and spread
2013	*Scapharca broughtonii*	OsHV-1	Bai et al. 2016	Identification and characterization of ostreid herpesvirus 1 associated with massive mortalities of *Scapharca broughtonii* broodstocks in China
2013	*Mytilus spp.*	OsHV-1µVar	O'Reilly et al. 2018	The role of the mussel Mytilus spp. in the transmission of ostreid herpesvirus-1 microVar
2013	*Magallana gigas*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2013	*Saccostrea glomerata*	OsHV-1µVar	Evans et al. 2017	Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia
2013	*Magallana gigas*	OsHV-1	Mello et al. 2018	First evidence of viral and bacterial oyster pathogens in the Brazilian coast
2013	*Crassostrea brasiliana*	OsHV-1	Mello et al. 2018	First evidence of viral and bacterial oyster pathogens in the Brazilian coast
2013	*Magallana gigas*	OsHV-1µVar	Batista et al. 2015	Sequence variation in ostreid herpesvirus 1 microvar isolates detected in dying and asymptomatic *Crassostrea angulata* adults in the Iberian Peninsula: Insights into viral origin and spread
2013	*Magallana gigas*	OsHV-1µVar	Fleury et al. 2020	Latitudinal drivers of oyster mortality: deciphering host, pathogen and environmental risk factors

2013	*Magallana gigas*	OsHV-1µVar	Gangnery et al. 2019	Connectivities with shellfish farms and channel rivers are associated with mortality risk in oysters
2013	*Magallana gigas*	OsHV-1µVar	Pernet et al. 2018	Determination of risk factors for herpesvirus outbreak in oysters using a broad-scale spatial epidemiology framework
2013	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2015	Further observations on the influence of husbandry practices on OsHV-1µVar mortality in Pacific oysters *Crassostrea gigas*: Age, cultivation structures and growing height
2013	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2019	Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management
2013	*Magallana gigas*	OsHV-1 non-µVar variant	Trancart et al. 2023	Diversity and molecular epidemiology of Ostreid herpesvirus 1 in farmed *Crassostrea gigas* in Australia: Geographic clusters and implications for "microvariants" in global mortality events
2013	*Magallana gigas*	OsHV-1µVar	Paul-Pont et al. 2014	Descriptive epidemiology of mass mortality due to Ostreid herpesvirus-1 (OsHV-1) in commercially farmed Pacific oysters (*Crassostrea gigas*) in the Hawkesbury River estuary, Australia
2013	*Magallana gigas*	OsHV-1µVar	Domeneghetti et al. 2014	Mortality occurrence and pathogen detection in *Crassostrea gigas* and *Mytilus galloprovincialis* close-growing in shallow waters (Goro lagoon, Italy)
2013	*Magallana gigas*	OsHV-1µVar	Mortensen et al. 2016	Summer mortalities and detection of ostreid herpesvirus microvariant in Pacific oyster *Crassostrea gigas* in Sweden and Norway
2014	*Scapharca broughtonii*	OsHV-1	Bai et al. 2018	Dual transcriptomic analysis of Ostreid herpesvirus 1 infected *Scapharca broughtonii* with an emphasis on viral anti-apoptosis activities and host oxidative bursts
2014	*Octopus vulgaris*	OsHV-1µVar	Prado-Alvarez et al. 2021	First detection of OsHV-1 in the cephalopod *Octopus vulgaris*. Is the octopus a dead-end for OsHV-1?
2014	*Mytilus spp.*	OsHV-1µVar	O'Reilly et al. 2018	The role of the mussel *Mytilus spp.* in the transmission of ostreid herpesvirus-1 microVar
2014	*Crassostrea brasiliana*	OsHV-1	Mello et al. 2018	First evidence of viral and bacterial oyster pathogens in the Brazilian coast
2014	*Magallana gigas*	OsHV-1	Mello et al. 2018	First evidence of viral and bacterial oyster pathogens in the Brazilian coast
2014	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2019	Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management
2014	*Magallana gigas*	OsHV-1	Burioli et al. 2017	First description of a mortality event in adult Pacific oysters in Italy associated with infection by a *Tenacibaculum soleae* strain
2014	*Magallana gigas*	OsHV-1 non-µVar variant	Trancart et al. 2023	Diversity and molecular epidemiology of Ostreid herpesvirus 1 in farmed *Crassostrea gigas* in Australia: Geographic clusters and implications for "microvariants" in global mortality events
2014	*Magallana gigas*	OsHV-1µVar	Mortensen et al. 2016	Summer mortalities and detection of ostreid herpesvirus microvariant in Pacific oyster *Crassostrea gigas* in Sweden and Norway
2014	*Ostrea edulis*	OsHV-1µVar	López Sanmartín et al. 2016	Experimental infection of European flat oyster *Ostrea edulis* with ostreid herpesvirus 1 microvar (OsHV-1µVar): Mortality, viral load and detection of viral transcripts by in situ hybridization

2015	*Octopus vulgaris*	OsHV-1µVar	Prado-Alvarez et al. 2021	First detection of OsHV-1 in the cephalopod *Octopus vulgaris*. Is the octopus a dead-end for OsHV-1?
2015	*Carcinus maenas*	OsHV-1µVar	Bookelaar et al. 2018	Role of the intertidal predatory shore crab *Carcinus maenas* in transmission dynamics of ostreid herpesvirus-1 microvariant
2015	*Mytilus spp.*	OsHV-1µVar	O'Reilly et al. 2018	The role of the mussel Mytilus spp. in the transmission of ostreid herpesvirus-1 microVar
2015	*Cerastoderma edule*	OsHV-1µVar	Bookelaar et al. 2020	Host plasticity supports spread of an aquaculture introduced virus to an ecosystem engineer
2015	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2019	Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management
2015	*Magallana gigas*	OsHV-1	Morga et al. 2021	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species
2015	*Magallana gigas*	OsHV-1 non-µVar variant	Trancart et al. 2023	Diversity and molecular epidemiology of Ostreid herpesvirus 1 in farmed *Crassostrea gigas* in Australia: Geographic clusters and implications for "microvariants" in global mortality events
2016	*Magallana gigas*	OsHV-1µVar	de Kantzow et al. 2017	Risk factors for mortality during the first occurrence of Pacific Oyster Mortality Syndrome due to Ostreid herpesvirus - 1 in Tasmania, 2016
2016	*Mytilus galloprovincialis*	OsHV-1	Battistini et al. 2020	Microbiological and Histological Analysis for the Evaluation of Farmed Mussels (Mytilus galloprovincialis) Health Status, in Coastal Areas of Italy
2016	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2019	Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management
2016	*Magallana gigas*	OsHV-1µVar	Burioli et al. 2018	A novel divergent group of Ostreid herpesvirus 1µVar variants associated with a mortality event in Pacific oyster spat in Normandy (France) in 2016
2016	*Magallana gigas*	OsHV-1µVar	Pande & Andel 2018	Ostreid herpesvirus and Vibrio species pilot surveillance study farmed Pacific Oysters (*Magallana gigas*) in Croisilles Harbour, Marlborough Sounds, New Zealand.
2016	*Magallana gigas*	OsHV-1µVar	Abbadi et al. 2018	Identification of a newly described OsHV-1µVar from the North Adriatic Sea (Italy)
2017	*Magallana gigas*	OsHV-1µVar	Ugalde et al. 2018	Analysis of farm management strategies following herpesvirus (OsHV-1) disease outbreaks in Pacific oysters in Tasmania, Australia
2017	*Scapharca subcrenata*	OsHV-1	Gao et al. 2018	Real-time quantitative isothermal detection of Ostreid herpesvirus-1 DNA in *Scapharca subcrenata* using recombinase polymerase amplification
2017	*Mytilus galloprovincialis*	OsHV-1	Battistini et al. 2020	Microbiological and Histological Analysis for the Evaluation of Farmed Mussels (*Mytilus galloprovincialis*) Health Status, in Coastal Areas of Italy
2017	*Magallana gigas*	OsHV-1µVar	Whittington et al. 2019	Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management
2017	*Magallana gigas*	OsHV-1	Martínez-García et al. 2020	Prevalence and genotypic diversity of ostreid herpesvirus type 1 in *Crassostrea gigas* cultured in the Gulf of California, Mexico

2017	*Magallana gigas*	OsHV-1µVar	Martínez-García et al. 2020	Prevalence and genotypic diversity of ostreid herpesvirus type 1 in *Crassostrea gigas* cultured in the Gulf of California, Mexico
2017	*Magallana gigas*	AVNV	Martínez-García et al. 2020	Prevalence and genotypic diversity of ostreid herpesvirus type 1 in *Crassostrea gigas* cultured in the Gulf of California, Mexico
2017	*Magallana gigas*	OsHV-1	Martínez-García et al. 2020	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species
2017	*Magallana gigas*	OsHV-1µVar	Pande & Andel 2018	Ostreid herpesvirus and Vibrio species pilot surveillance study farmed Pacific Oysters (*Magallana gigas*) in Croisilles Harbour, Marlborough Sounds, New Zealand.
2017	*Magallana gigas*	OsHV-1µVar	Barbieri et al. 2019	First detection of Ostreid herpesvirus 1 in wild *Crassostrea gigas* in Argentina
2017	*Magallana gigas*	OsHV-1	Friedman et al. 2020	Unraveling concordant and varying responses of oyster species to Ostreid Herpesvirus 1 variants
2017	*Crassoastrea virginica*	OsHV-1µVar	Friedman et al. 2020	Unraveling concordant and varying responses of oyster species to Ostreid Herpesvirus 1 variants
2017	*Crassostrea sikamea*	OsHV-1µVar	Friedman et al. 2020	Unraveling concordant and varying responses of oyster species to Ostreid Herpesvirus 1 variants
2017	*Magallana gigas*	OsHV-1µVar	Kim et al. 2019	Mass mortality in Korean bay scallop (*Argopecten irradians*) associated with Ostreid Herpesvirus-1µVar
2017	*Arenicola marina*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Crangon crangon*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Cerastoderma edule*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Crepidula fornicata*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Golfingia vulgaris*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*L. conchilega*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Lineus spp.*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Balanus spp.*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Phyllodoce mucosa*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Carcinus maenas*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Gammarus obtusatus*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak

2017	*Littorina littorea*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Mytilus edulis*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Scoloplos armiger*	OsHV-1µVar	Vanhuysse et al. 2021	Changes in benthic macrofauna in oyster parks during an OsHV-1µVar oyster spat mortality outbreak
2017	*Ostrea edulis*	OsHV-1	Pernet et al. 2021	Competition for food reduces disease susceptibility in a marine invertebrate
2017	*Mytilus spp.*	OsHV-1	Pernet et al. 2021	Competition for food reduces disease susceptibility in a marine invertebrate
2018	*Magallana gigas*	OsHV-1µVar	Burge et al. 2021	The first detection of a novel OsHV-1 microvariant in San Diego, California, USA
2018	*Magallana gigas*	OsHV-1µVar	Corporeau et al. 2022	Harsh intertidal environment enhances metabolism and immunity in oyster (*Crassostrea gigas*) spat
2018	*Magallana gigas*	OsHV-1µVar	Mosca et al. 2021	*Crassostrea gigas* (Thunberg 1793) cultivation in southern Adriatic Sea (Italy): A one-year monitoring study of the oyster health
2018	*Magallana gigas*	OsHV-1	Martínez-García et al. 2020	Prevalence and genotypic diversity of ostreid herpesvirus type 1 in *Crassostrea gigas* cultured in the Gulf of California, Mexico
2018	*Magallana gigas*	OsHV-1µVar	Martínez-García et al. 2020	Prevalence and genotypic diversity of ostreid herpesvirus type 1 in *Crassostrea gigas* cultured in the Gulf of California, Mexico
2018	*Magallana gigas*	AVNV	Martínez-García et al. 2020	Prevalence and genotypic diversity of ostreid herpesvirus type 1 in *Crassostrea gigas* cultured in the Gulf of California, Mexico
2018	*Magallana gigas*	OsHV-1µVar	Pande & Andel 2018	Ostreid herpesvirus and Vibrio species pilot surveillance study farmed Pacific Oysters (*Magallana gigas*) in Croisilles Harbour, Marlborough Sounds, New Zealand.
2018	*Magallana gigas*	OsHV-1µVar	Divilov et al. 2019	First evaluation of resistance to both a California OsHV-1 variant and a French OsHV-1 microvariant in Pacific oysters
2018	*Magallana gigas*	OsHV-1µVar	Kim et al. 2019	Mass mortality in Korean bay scallop (*Argopecten irradians*) associated with Ostreid Herpesvirus-1µVar
2019	*Scapharca broughtonii*	OsHV-1	Bai et al. 2022	Paired miRNA and RNA sequencing provides a first insight into molecular defense mechanisms of *Scapharca broughtonii* during ostreid herpesvirus-1 infection
2020	*Magallana gigas*	OsHV-1	Zhang et al. 2023	Identification and Characterization of Infectious Pathogens Associated with Mass Mortalities of Pacific Oyster (*Crassostrea gigas*) Cultured in Northern China
2020	*Magallana gigas*	OsHV-1	Shukla et al. in prep	Stress Hardening May Impact Pacific Oyster (*Magallana gigas*), but not Kumamoto Oyster (*Crassostrea sikamea*), Responses to an OsHV-1 Outbreak in Tomales Bay, CA
2021	*Magallana gigas*	OsHV-1	Shukla et al. in prep	Stress Hardening May Impact Pacific Oyster (*Magallana gigas*), but not Kumamoto Oyster (*Crassostrea sikamea*), Responses to an OsHV-1 Outbreak in Tomales Bay, CA

021	*Crassostrea sikamea*	OsHV-1	Shukla et al. in prep	Stress Hardening May Impact Pacific Oyster (*Magallana gigas*), but not Kumamoto Oyster (*Crassostrea sikamea*), Responses to an OsHV-1 Outbreak in Tomales Bay, CA
2022	*Magallana gigas*	OsHV-1	Shukla et al. in prep	Stress Hardening May Impact Pacific Oyster (*Magallana gigas*), but not Kumamoto Oyster (*Crassostrea sikamea*), Responses to an OsHV-1 Outbreak in Tomales Bay, CA
NA	*Crassostrea angulata*	OsHV-1µVar	Sanmartín et al. 2016	Evidence of vertical transmission of ostreid herpesvirus 1 in the Portuguese oyster *Crassostrea angulata*
NA	*Scapharca broughtonii*	OsHV-1	Xin et al. 2018	Ostreid Herpesvirus-1 Infects Specific Hemocytes in Ark Clam, *Scapharca broughtonii*
NA	*Scapharca broughtonii*	OsHV-1	Xin et al. 2018	Validation of housekeeping genes for quantitative mRNA expression analysis in OsHV-1 infected ark clam, *Scapharca broughtonii*
NA	*Scapharca broughtonii*	OsHV-1	Wang et al. 2022	Characterization of a Novel C-type Lectin against OsHV-1 infection in *Scapharca broughtonii*
NA	*Tiostrea chilensis*	OsHV-1	Hine et al. 1998	Replication of a herpes-like virus in larvae of the flat oyster *Tiostrea chilensis* at ambient temperatures
NA	*Ostrea edulis*	Herpes-like virus	Comps & Cochennec 1993	A Herpes-like Virus from the European Oyster *Ostrea edulis* L.
NA	*Chlamys farreri*	AVNV	Chen et al. 2013	A preliminary study of differentially expressed genes of the scallop *Chlamys farreri* against acute viral necrobiotic virus (AVNV)
NA	*Chlamys farreri*	AVNV	Chen et al. 2011	Haemocyte protein expression profiling of scallop *Chlamys farreri* response to acute viral necrosis virus (AVNV) infection
NA	*Scapharca broughtonii*	OsHV-1	Huang et al. 2022	Iron Regulatory Protein 1 Inhibits Ferritin Translation Responding to OsHV-1 Infection in Ark Clams, *Scapharca Broughtonii*
NA	*Scapharca broughtonii*	OsHV-1	Xin et al. 2020	Influence of temperature on the pathogenicity of Ostreid herpesvirus-1 in ark clam, *Scapharca broughtonii*
NA	*Chlamys farreri*	AVNV	Chen et al. 2014	Bioinformatics analysis of hemocyte miRNAs of scallop *Chlamys farreri* against acute viral necrobiotic virus (AVNV)
NA	*Magallana gigas*	OsHV-1	Morga et al. 2021	Genomic Diversity of the Ostreid Herpesvirus Type 1 Across Time and Location and Among Host Species
NA	*Magallana gigas*	OsHV-1	Arzeta-Pino et al. 2018	Herpes virus OsHV-1 and the protist *Perkinsus marinus* modify the expression of the Down syndrome cell adhesion molecule gene in gill and mantle of *Crassostrea spp.*
NA	*Magallana gigas*	Herpes-like virus	Le Deuff et al. 1994	Experimental transmission of a Herpes-like virus to axenic larvae of Pacific oyster, *Crassostrea gigas*
NA	*Magallana gigas*	Herpes-like virus	Le Deuff et al. 1996	Effects of temperature on herpes-like virus detection among hatchery-reared larval Pacific oyster *Crassostrea gigas*
NA	*Scapharca broughtonii*	OsHV-1	Bai et al. 2017	Experimental infection of adult Scapharca broughtonii with Ostreid herpesvirus SB strain
NA	*Scapharca broughtonii*	OsHV-1	Xin et al. 2019	OsHV-1 infection leads to mollusc tissue lesion and iron redistribution, revealing a strategy of iron limitation against pathogen