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First comparison of French and Australian OsHV-1 µvars by bath exposure

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ABSTRACT: Economically devastating mortality events of farmed and wild shellfish due to infectious disease have been reported globally. Currently, one of the most significant disease threats to Pacific oyster *Crassostrea gigas* culture is the ostreid herpesvirus 1 (OsHV-1), in particular the emerging OsHV-1 microvariant genotypes. OsHV-1 microvariants (OsHV-1 µvars) are spreading globally, and concern is high among growers in areas unaffected by OsHV-1. No study to date has compared the relative virulence among variants. We provide the first challenge study comparing survival of naïve juvenile Pacific oysters exposed to OsHV-1 µvars from Australia (AUS µvar) and France (FRA µvar). Oysters challenged with OsHV-1 µvars had low survival (2.5% exposed to AUS µvar and 10% to FRA µvar), and high viral copy number as compared to control oysters (100% survival and no virus detected). As our study was conducted in a quarantine facility located ~320 km from the ocean, we also compared the virulence of OsHV-1 µvars using artificial seawater made from either facility tap water (3782 µmol kg^{-1} seawater total alkalinity) or purchased distilled water (2003 µmol kg^{-1}). Although no differences in survival or viral copy number were detected in oysters exposed to seawater made using tap or distilled water, more OsHV-1 was detected in tanks containing the lower-alkalinity seawater, indicating that water quality may be important for virus transmission, as it may influence the duration of viral viability outside of the host.

KEY WORDS: Ostreid herpesvirus 1 · Microvariant · OsHV-1 µvars · POMS · Pacific oyster · *Crassostrea gigas* · Viral disease · qPCR · Alkalinity · Emerging infectious diseases

1. INTRODUCTION

A robust aquaculture industry is dependent on understanding risks, such as disease, that affect production. Cultured oysters contribute billions of dollars to the global economy, with the key species cultured, the Pacific oyster *Crassostrea gigas*, alone generating over 1 billion USD in 2016 (FAO 2018). Over the past several decades, devastating losses in farmed and wild shellfish due to infectious diseases have been reported (reviewed by Carnegie et al. 2016). Of the current diseases impacting Pacific oys-

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ter aquaculture, the ostreid herpesvirus 1 (OsHV-1) and its variants are considered one of the most significant threats to culture of this species globally (Barbosa-Solomieu et al. 2015). OsHV-1 is a double-stranded DNA virus (207 kb; ~116 nm in size) in the order *Herpesvirales* (Davison et al. 2005, 2009).

OsHV-1 and its variants, particularly the OsHV-1 microvariants (µvars), are virulent and problematic viruses of larval, seed, and adult Pacific oysters (reviewed by Barbosa-Solomieu et al. 2015, Pernet et al. 2016, Burge et al. 2018). In the 1990s, losses of larval and juvenile oysters associated with OsHV-1 were first described in France, New Zealand, and the USA (reviewed by Burge et al. 2018). One variant, known as the OsHV-1 reference strain, was purified, described, and fully sequenced from infected oyster larvae in France (Davison et al. 2005). Since 2008, an economically devastating increase in *C. gigas* mortality has been associated with new genetic variants of OsHV-1, the OsHV-1 µvars, as defined by OIE (2018). OsHV-1 µvars have spread within Europe and have caused losses in Australia, New Zealand, and Asia (reviewed by Pernet et al. 2016, Burge et al. 2018). Sequence variations in some genomic regions have been observed among the different OsHV-1 µvars (Barbosa-Solomieu et al. 2015, Abbadi et al. 2018), although a complete understanding of strain variation and how this impacts virus phenotype remains unknown.

Given the emergence and spread of the OsHV-1 µvars over the past decade, it may be only a matter of time before currently uninfected Pacific oyster culture areas such as the US West Coast are impacted. Laboratory-based transmission studies in quarantine make it possible to test the susceptibility of Pacific oysters to OsHV-1 µvars (Schikorski et al. 2011 a,b, Kirkland et al. 2015). However, no study to date has compared the susceptibility of Pacific oysters to more than 1 OsHV-1 variant. In this study, OsHV-1 µvars from France (FRA µvar) and Australia (AUS µvar) were used to challenge naïve juvenile Pacific oysters. Using a laboratory infection model (Kirkland et al. 2015) in quarantine, our primary objective was to compare these 2 viral variants with respect to host survivorship and infection using viral load (determined by quantitative PCR, qPCR), as a proxy of infection. Our research was conducted in a quarantine facility ~320 km from the ocean, requiring us to make artificial seawater (ASW) for these studies. Therefore, our secondary objective was to compare survivorship, infection, and virus copy numbers available in tank water using seawater made from either well water that was available at the facility or purchased distilled water.

2. MATERIALS AND METHODS

2.1. Oysters and holding

Diploid Pacific oysters (~9 mm), which had never been selected for resistance to OsHV-1 infection, were produced by a supplier in Kona, Hawaii (from a mixed pod of Molluscan Broodstock Program broodstock held in Bay Center, Washington) and grown at a floating upwelling system ('flupsy,' a nursery system) in Humboldt Bay, California, prior to shipment (on ice) to the University of Arizona, Aquaculture Pathology Laboratory (UA APL) in Tucson, Arizona. On arrival, oysters were brought up to ~18°C at 30–31 ppt and held for 2 d prior to the beginning of the experiment. All ASW was made with Crystal Sea® MarineMix 150 gallon (~567 l) mixture (Marine Enterprises International) dissolved in well water available at the UA APL (ASW_{Tap}).

2.2. OsHV-1 homogenate prep

Stocks of inoculum of the AUS µvar (a derivative of the Australian prototype strain; Kirkland et al. 2015) and FRA µvar (tissue prepared from infected oysters collected in Marennes-Oléron Bay following protocols of Schikorski et al. 2011a,b) were shipped directly to the UA APL with permission from the USDA-APHIS and the Arizona state veterinarian. In order to conduct multiple experimental challenges with the same inoculum, we produced a larger stock of 0.22 µm filtered homogenate that was cryopreserved using 10% fetal bovine serum/10% glycerol and stored at −80°C at the UA APL (Kirkland et al. 2015). Briefly, 0.22 µm filtered homogenate was produced by injecting susceptible naïve sub-adult Pacific oysters (n = 10). At 48 h post-injection, gill and mantle were excised, homogenized, and filtered (0.22 µm) to create inocula (Burge & Friedman 2012, Kirkland et al. 2015). Viral copy numbers were quantified by extracting 200 µl of each inoculum using a Zymo Quick DNA Mini-Plus Kit and the Biological Fluids and Cells method, followed by OsHV-1 specific qPCR (see Section 2.4).

For each inoculum, a genetic characterization was made by amplifying DNA from 2 regions of the OsHV-1 genome, the 'C' and 'IA' regions, using the C2/C6 primers (targeting open reading frame [ORF] 4) and IA1/IA2 primers (targeting ORF 43), respectively (Renault & Arzul 2001). Each inoculum was run in duplicate with 2 negative control reactions. Briefly, 50 µl reactions contained 1× PCR buffer, 0.2 mM

each dNTP, 1.5 mM $MgCl_2$, 0.3 µm of each primer, 2.5 U DNA polymerase (Promega GoTaq 9PIM300), and 4 µl of DNA using PCR cycling conditions described by Segarra et al. (2010) and OIE (2018). Following PCR amplification, PCR products were run on a standard agarose gel (Segarra et al. 2010) to ensure 1 band at the appropriate size was obtained before submitting to the IMET Bioanalytical Services lab (Baltimore, MD) for direct sequencing. Briefly, 10 µl of each PCR reaction were cleaned (Ampure, Beckman Coulter) and sequenced bi-directionally using the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific) and run on the 3130 XL Genetic Analyzer from Life Technologies. Sequence base pairs were called using Sequencher (Gene Codes), and alignments of the AUS µvar and FRA µvar were created using Clustal Omega (EMBL-EBI).

2.3. Experimental challenges

Challenges were conducted in 3 l tanks with aeration at 21–22°C with 10 oysters (~9 mm) per tank. For each OsHV-1 µvar, 2 tanks contained artificial seawater made using well water (ASW_{Tap}) and 2 tanks contained ASW made with distilled water (ASW_{Dist}). Similarly, control tanks contained either ASW_{Tap} (n = 2) or ASW_{Dist} (n = 2). The alkalinity of ASW_{Tap} and ASW_{Dist} was assayed using end-point titration according to the methods of Wippel (2017).

Prior to addition to the challenge experiment, oysters were brought up to 22°C and relaxed using $MgCl_2$ (50 g l^{-1} in 3 l for ~100 oysters) to ensure comparative exposure of each virus for 2 h before 10 animals were added to each tank containing 2.5 l of the appropriate seawater. Aliquots (500 ml) of 1.00×10^7 virus copies ml^{-1} homogenate were added to each tank to obtain a final concentration of 1.67×10^6 virus copies ml^{-1}. Each day, oysters were monitored for mortality by lightly tapping the tank and watching for oyster valve closure; dead animals and 1 ml of seawater from each tank were collected on each day of the experiment and frozen at −80°C for future qPCR analysis. Tank water was changed every 48 h. After 6 d, all survivors were collected and frozen at −80°C.

2.4. OsHV-1 quantification in oysters and seawater

OsHV-1 specific qPCR was used to determine the viral load in all oysters and from seawater collected from each tank on each day of the experiment. Samples were processed in a BSL3 facility. The Zymo Quick-DNA Mini Plus kit was used following the manufacturer's protocol to extract 200 µl of tank water (Biological Fluids and Cells method) or ~25 mg of gill and mantle tissue (Solid Tissues method) from each oyster. Using methods of Burge & Friedman (2012) with some modifications, we targeted the OsHV-1 ORF 100/catalytic subunit of a DNA polymerase δ using the following forward (100 F: 5′-TGA TGG ATT GTT GGA CGA GA-3′) and reverse (ORF 100R: 5′-ATC ACA TCC CTG GAC GCT AC-3′) primers. A standard curve was generated from a dilution of a plasmid from 3 to 3×10^6 copies per reaction. Briefly, each 20 µl reaction included 10 µl of the Fast SYBR™ Green Master Mix, 15 µg of BSA, 300 nM of each primer, and 2 µl of DNA (~50 ng). The plasmid standard curve was run in triplicate, and samples in duplicate. All qPCR reactions used the Applied Biosystems 7500 Fast Real-time PCR system or Applied Biosystems QuantStudio 3 with a cut-off of 3 copies per reaction. qPCR runs were done with standards and random samples on both platforms to demonstrate consistency (data not shown). Cycling conditions for each qPCR run were 95°C for 20 s followed by 40 cycles of 95 and 60°C. Following each run, a melting curve analysis was performed to confirm the specificity of each qPCR reaction by comparing the melting temperature peak of the positive control DNA to that of the experimental samples. The melting curve profile consisted of denaturation at 95°C for 15 s followed by an annealing step for 15 s at 60°C. This was followed by continuous fluorescence monitoring during a 20 min temperature ramp to 95°C that was held for 15 s. A limit of ±1°C for melting temperature peak shift was set as the cutoff for species-specific amplifications.

2.5. Data analysis

All analyses were conducted in JMP 14.0 (SAS). Survivorship was analyzed with a Kaplan-Meier survival analysis using log-rank chi-squared statistics ($p < 0.05$). First, water type (ASW_{Dist} vs. ASW_{Tap}) was tested for each virus using all data; results indicated no difference in survival. Second, differences in survivorship were compared between AUS µvar and FRA µvar (pooling the water type). The qPCR data was $\log_{10}(x+1)$ transformed prior to analysis to normalize data. Least-squares regression was employed to determine differences in qPCR loads in tissues and tank water between AUS µvar and FRA µvar in each water type (overall data, ASW_{Tap} or ASW_{Dist}, and

water type by day [Days 0–5 nested within water type]). Differences in tissue mean virus copies between survivors and dead oysters for each virus were tested using least-squares regression. Post hoc testing to identify differences between or among groups were conducted using a Tukey HSD for 3 or more factors.

3. RESULTS

Survival was low in oysters exposed to either OsHV-1 µvar and was significantly lower in those exposed to the AUS µvar (2.5%) than to the FRA µvar (10%) ($p = 0.004$, log-rank test statistic = 8.50, df = 1, Fig. 1). The mean survival time was 3.58 ± 0.17 d for oysters exposed to the AUS µvar, and 4.33 ± 0.23 d for oysters exposed to the FRA µvar. All control oysters survived.

Tissue viral loads (Table 1) were similar between oysters exposed to AUS µvar and FRA µvar ($p > 0.05$; see Table 2 for tissue statistical outcomes). No differences in viral loads were detected between dead oysters or those that survived ($p > 0.05$), although few survivors (n = 5) were available. In oysters exposed to the FRA µvar, tissue levels differed among experimental days ($p < 0.001$) with levels higher on Days 3 (ASW_{Tap}), 4 (ASW_{Dist}), and 5 (ASW_{Dist}) compared to Day 2 (ASW_{Dist}) ($p < 0.05$); viral loads from all other days and water types were similar to all others ($p > 0.05$). No differences in viral loads among days and water types were observed for oysters exposed to AUS µvar. When all days were pooled, no significant effect of water type on tissue loads was detected (i.e. ASW_{Tap} vs. ASW_{Dist}; $p > 0.05$) for either variant. No viral DNA was detected in control oysters.

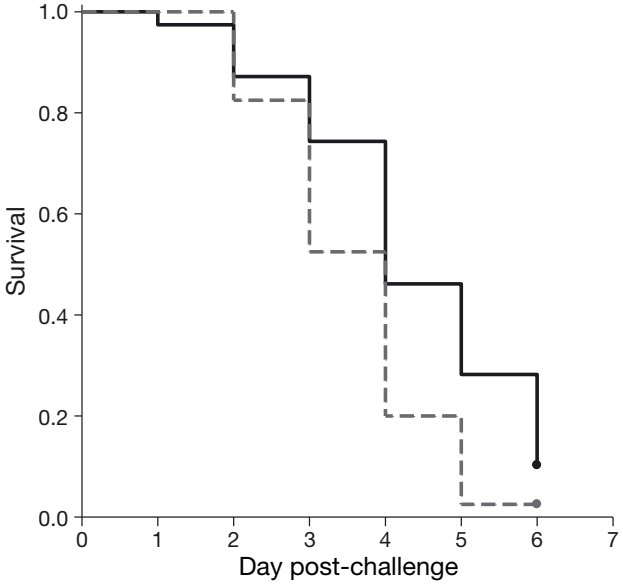

Fig. 1. Kaplan Meier survivorship curves for *Crassostrea gigas* seed exposed to OsHV-1 µvars from France (solid line) and Australia (dashed line)

The alkalinity of the ASW_{Tap} was 3782 µmol kg^{-1} at 30 ppt vs. 2003 µmol kg^{-1} ASW_{Dist}. More AUS µvar DNA was detected in water from exposure tanks with ASW_{Dist} than in those with ASW_{Tap} ($p < 0.05$; Table 3); water from exposure tanks with FRA µvar showed a similar but non-significant trend ($p = 0.062$) with the different seawaters. An increase in OsHV-1 µvar DNA was detected in tanks exposed to AUS µvar on Day 3 ($p < 0.01$) but not in tanks exposed to FRA µvar. Trace amounts of OsHV-1 µvar DNA were detected in water from the control tanks, but at significantly lower levels ($p = 0.001$).

PCR amplification produced a single band for each assay of the expected sizes for the C2/C6 (~709 bp) and IA1/IA2 assays (~607 bp) with the AUS µvar and the FRA µvar. Direct sequencing of the amplicons produced sequences of the same length (after trimming primer sequences) for AUS µvar and the FRA µvar, i.e. 651 bp (C2/C6) and 576 bp (IA1/IA2). Sequence alignments indicated a high degree of similarity between variants of 99.82% (IA1/IA2) and 100% (C2/C6) (Figs. S1 & S2 in the Supplement at www.int-res.com/articles/suppl/d138p137_supp.pdf). Sequence data were deposited in GenBank under accession numbers MT157286–MT157289.

4. DISCUSSION

This is the first study to compare survival and infection of naïve Pacific oysters from the West Coast of the USA to 2 OsHV-1 µvars (AUS µvar and FRA µvar), a first step in the much-needed characterization of the effects of the newly observed multiple variants of OsHV-1 in susceptible hosts. Overall survival of oysters was low (2.5–10%), although trends varied statistically, and infection levels in tissues as measured by proxy using qPCR was high (>10^{10} mean copies g^{-1}) and similar between OsHV-1 variants. Thus, the American oyster industry based on the Pacific oyster could be heavily impacted if OsHV-1 µvar from France or Australia is unintentionally introduced to the west coast of North America. Further experiments should be conducted using other oyster stocks to more fully characterize susceptibility or resistance of Pacific oyster stocks or specific family lines present in the USA (Divilov et al. 2019).

Mortality levels and high OsHV-1 copy number are consistent with highly susceptible stocks or family

Table 1. OsHV-1 copy number for all samples collected in *Crassostrea gigas* tissue (copies g^{-1} of tissue) or artificial seawater (copies ml^{-1} of seawater) made with either well water (ASW_{Tap}) or distilled water (ASW_{Dist}). SEM: standard error of mean

Exposure	Sample type	Water	OsHV-1 copies			N
			Range	Mean	SEM	
Control	Tissue	ASW_{Dist}	0.00–0.00	0	0	19
Control	Tissue	ASW_{Tap}	0.00–0.00	0	0	12
Control	Water	ASW_{Dist}	$(2.53–6.01) \times 10^3$	4.63×10^3	3.07×10^3	11
Control	Water	ASW_{Tap}	$(1.33–5.15) \times 10^3$	3.09×10^3	3.69×10^2	11
AUS	Tissue	ASW_{Dist}	$(0.53–6.59) \times 10^{10}$	2.79×10^{10}	3.98×10^9	20
AUS	Tissue	ASW_{Tap}	$(0.00–4.05) \times 10^{11}$	8.12×10^{10}	2.81×10^{10}	20
AUS	Water	ASW_{Dist}	$(0.05–4.22) \times 10^5$	1.65×10^5	4.39×10^4	10
AUS	Water	ASW_{Tap}	$(0.43–7.08) \times 10^4$	2.49×10^4	6.02×10^3	11
FRA	Tissue	ASW_{Dist}	$1.05 \times 10^5–1.07 \times 10^{11}$	3.60×10^{10}	7.78×10^{10}	20
FRA	Tissue	ASW_{Tap}	$1.04 \times 10^5–2.83 \times 10^{11}$	4.40×10^{10}	1.47×10^{10}	20
FRA	Water	ASW_{Dist}	$3.40 \times 10^3–3.21 \times 10^5$	6.75×10^4	2.79×10^4	14
FRA	Water	ASW_{Tap}	$1.01 \times 10^3–5.60 \times 10^4$	1.49×10^4	5.21×10^3	12

Table 2. Variations in seed oyster tissue levels of OsHV-1 µvars from Australia (AUS) and France (FRA) when held in seawater made with distilled water (ASW_{Dist}) or well water (ASW_{Tap}). ns: not significant

Virus	Time period	Model effects (fixed factors)	ANOVA Source	df	SS	MS	*F*-ratio	p	Effect magnitude: η^2	Outcome
AUS	Overall	Water	Model	1	16.4	16.4	0.941	>0.05	ns	No effect
			Error	38	662.1	17.43				
			Total	39	678.5					
FRA	Overall	Water	Model	1	2.53	2.53	0.109	>0.05	ns	No effect
			Error	38	883.9	23.26				
			Total	39	886.43					
AUS	Days 0–5	Water/Day	Model	8	89.33	11.17	0.588	>0.05	ns	No effect
			Error	31	589.12	19				
			Total	39	678.45					
FRA	Days 0–5	Water/Day	Model	10	525.88	52.59	4.23	<0.01	0.59	
			Error	29	360.56	12.43				
			Total	39	886.43					
		Water	ASW_{Tap} or ASW_{Dist}	1	3.12		0.251	>0.05	ns	$ASW_{Dist} = ASW_{Tap}$
		Day	Day	9	523.35		4.68	<0.001	1.00	Day 3 ASW_{Tap} = Days 4 & 5 ASW_{Dist} > Day 2 ASW_{Dist}; rest = all
Both	Mortalities	Virus	Model	1	197.086	197.086	1.00	>0.05	ns	No effect
			Error	73	1432.99	196.299				
			Total	74	1452.69					

lines (Dégremont 2011, Oden et al. 2011, Hick et al. 2018). This study was terminated after 6 d when even the few survivors contained high tissue copy numbers (1.85×10^6 to 1.84×10^{10} copies g^{-1}; n = 5), suggesting that higher mortality might have occurred over subsequent days. Longer trials may be necessary to determine long-term survival potential and whether survivors represent resistant genotypes. No viral DNA was detected in control oysters, and to date, OsHV-1 has not been detected at the source nursery (C. A. Burge unpubl. data, R. Elston pers. comm). Low virus levels were observed in control water samples, likely as a result of aerosolization of viral DNA in the wet lab; no virus was amplified from control oyster tissues and no control oysters died.

Differential survival of Pacific oyster stocks and/or family lines to OsHV-1 variants has been well-documented in previous field and laboratory studies (reviewed by Dégremont et al. 2015). Further testing of oyster stocks and/or family lines with multiple µvars is necessary to examine if resistance or susceptibility to one variant confers resistance to other variants. In France, oysters bred for high survival to 'summer mortality' and exposed to OsHV-1 were also

Table 3. Variations in water levels of OsHV-1 µvars from Australia (AUS) and France (FRA) when exposed *Crassostrea gigas* seed was held in seawater made with distilled water (ASW_{Dist}) or well water (ASW_{Tap}). ns: not significant

Virus	Time period	Model effects (fixed factors)	ANOVA Source	df	SS	MS	*F*-ratio	p	Effect magnitude: η^2	Outcome
AUS	Overall	Water	Model	1	12.32	12.32	7.55	<0.05	0.28	$ASW_{Dist} > ASW_{Tap}$
			Error	19	31	1.63				
			Total	20	43.32					
FRA	Overall	Water	Model	1	8.81	8.81	3.83	0.062	ns	ns, but trend for $ASW_{Dist} > ASW_{Tap}$
			Error	24	55.19	2.3				
			Total	25	64					
AUS	Days 0–5	Water/Day	Model	9	40	4.44	14.7	<0.0001	0.92	
			Error	11	3.32	0.302				
			Total	20	43.32					
		Water	ASW_{Tap} or ASW_{Dist}	1	0.014		0.048	>0.05	ns	$ASW_{Dist} = ASW_{Tap}$
		Day	Day	8	27.68		11.45	<0.01	0.692	Day 3 ASW_{Dist} > rest
FRA	Days 0–5	Water/Day	Model	11	36.57	3.32	1.7	>0.05	ns	No effect
			Error	14	27.43	1.96				
			Total	25	64					

more resistant to OsHV-1 µvar (Dégremont 2011, Dégremont et al. 2015). In addition, resistant oysters were able to both limit infection loads and eliminate virus from their tissues (Dégremont 2011, Dégremont et al. 2015).

In challenge studies, water quality, such as alkalinity, may be an important consideration. In our study, more OsHV-1 µvar DNA was detected in tanks containing ASW_{Dist}, significantly so for the AUS µvar and a trend for FRA µvar. Thus, water quality (e.g. solutes) may be important for experimental challenges. The ASW_{Tap} at the UA APL has a high alkalinity so that when used to make artificial seawater, the total alkalinity was nearly double that made with ASW_{Dist} (~1800 µmol kg^{-1} water higher). In the NE Pacific, ocean acidification (declining seawater pH and carbonate saturation) has been linked to oyster hatchery larval culture failures (Barton et al. 2012), with the industry responding by buffering the seawater with sodium carbonate to an alkalinity in excess of 3000 µmol kg^{-1} seawater (Barton et al. 2015) similar to that used in our ASW_{Tap} treatment. Further testing of how seawater alkalinity influences the ability of OsHV-1 viruses to retain viability and transmissibility may provide useful information for hatcheries impacted by these viruses.

In the natural environment, the temperature necessary for OsHV-1 µvar outbreaks may be variable (Pernet et al. 2012, Jenkins et al. 2013, Paul-Pont et al. 2014, Renault et al. 2014, de Kantzow et al. 2016). Similar to many marine diseases (Burge et al. 2014), temperature is considered an important driver for OsHV-1 outbreaks and may be important in understanding the relationship between OsHV-1 genotype and phenotype. For example, thermal thresholds for disease outbreaks are 16°C in France and 21°C in Australia (Pernet et al. 2012, Jenkins et al. 2013, Paul-Pont et al. 2014, Renault et al. 2014, de Kantzow et al. 2016). Importantly, differences in outbreak temperature thresholds may be related to several factors, including methods to measure, analyze, and report water temperatures, other co-varying environmental factors, and oyster physiological condition and/or genetics (de Kantzow et al. 2016). In our study, we maintained oysters at 22°C, a similar temperature profile to studies showing disease transmission and high levels of mortality in experimental studies in France (Schikorski et al. 2011 a,b, Delisle et al. 2018) and Australia (Kirkland et al. 2015, de Kantzow et al. 2016), which may be an optimal temperature for full disease expression (de Kantzow et al. 2016, Delisle et al. 2018). However, to fully understand the oyster–virus relationship, a next step is to test oyster susceptibility to these viruses across a thermal gradient. Thermal thresholds may differ among viruses and hosts and are important to identify in order to effectively manage these viruses. Additionally, recent studies have indicated that although OsHV-1 is necessary for initiating mortalities, opportunistic bacteria often colonize oysters infected with OsHV-1 (de Lorgeril et al. 2018); therefore, the role of both temperature and host microbiome, as well as the microbial environment, may be important factors to examine in future experiments.

In conclusion, we have provided the first comparison of the highly virulent French and Australian

OsHV-1 µvars. We suggest that many key questions remain to be answered in order to effectively manage OsHV-1 µvars: What is an appropriate length of study to determine long-term survival potential of an oyster species, family line, or stock? How does water quality impact long-term viral viability, virulence, and transmissibility of OsHV-1? How might water temperature impact host susceptibility based on the thermal optima of both host and pathogen (i.e. virulence)? By answering these questions, we will enable aquaculturists and resource agencies to better manage our aquatic resources.

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