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Mortalities of Eastern and Pacific Oyster Larvae Caused by the Pathogens *Vibrio coralliilyticus* and *Vibrio tubiashii*

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***Vibrio tubiashii* is reported to be a bacterial pathogen of larval Eastern oysters (*Crassostrea virginica*) and Pacific oysters (*Crassostrea gigas*) and has been associated with major hatchery crashes, causing shortages in seed oysters for commercial shellfish producers. Another bacterium, *Vibrio coralliilyticus*, a well-known coral pathogen, has recently been shown to elicit mortality in fish and shellfish. Several strains of *V. coralliilyticus*, such as ATCC 19105 and Pacific isolates RE22 and RE98, were misidentified as *V. tubiashii* until recently. We compared the mortalities caused by two *V. tubiashii* and four *V. coralliilyticus* strains in Eastern and Pacific oyster larvae. The 50% lethal dose (LD_{50}) of *V. coralliilyticus* in Eastern oysters (defined here as the dose required to kill 50% of the population in 6 days) ranged from 1.1×10^4 to 3.0×10^4 CFU/ml seawater; strains RE98 and RE22 were the most virulent. This study shows that *V. coralliilyticus* causes mortality in Eastern oyster larvae. Results for Pacific oysters were similar, with LD_{50}s between 1.2×10^4 and 4.0×10^4 CFU/ml. *Vibrio tubiashii* ATCC 19106 and ATCC 19109 were highly infectious toward Eastern oyster larvae but were essentially nonpathogenic toward healthy Pacific oyster larvae at dosages of $\geq 1.1 \times 10^4$ CFU/ml. These data, coupled with the fact that several isolates originally thought to be *V. tubiashii* are actually *V. coralliilyticus*, suggest that *V. coralliilyticus* has been a more significant pathogen for larval bivalve shellfish than *V. tubiashii*, particularly on the U.S. West Coast, contributing to substantial hatchery-associated morbidity and mortality in recent years.**

Vibrio tubiashii has been associated with major mortality events in shellfish hatcheries on the U.S. Pacific Coast and in other locations worldwide (1–4). It was first reported as a pathogen of larval oysters by Haskell S. Tubiash (5) in 1965 and was later named *V. tubiashii* by Hada et al. (6). In 2006 and 2007, *V. tubiashii* was implicated in high mortalities of larval Pacific oysters (*Crassostrea gigas*), Kumamoto oysters (*Crassostrea sikamea*), and geoduck clams (*Panope abrupta*) on the West Coast, where production losses were reported to be 59% (3). Losses in larval production caused financial hardships for the two largest hatcheries on the Pacific Coast and resulted in shortages of oyster and clam seed needed for commercial planting by the shellfish industry. On the U.S. East Coast, major outbreaks of *V. tubiashii* in larval Eastern oysters (*Crassostrea virginica*) have been observed (1, 5).

Another bacterium, *Vibrio coralliilyticus*, is best known as a coral pathogen responsible for coral bleaching and has been associated with significant losses to coral reefs worldwide (7, 8). Recently, *V. coralliilyticus* was shown to be infectious to a variety of fish and shellfish, including Pacific oyster larvae (9, 10), the great scallop (*Pecten maximus*) and the European flat oyster (*Ostrea edulis*) (10), rainbow trout (*Oncorhynchus mykiss*), and larval brine shrimp (*Artemia* spp.) (11). Around the time that *V. coralliilyticus* was shown to be pathogenic to fish and shellfish, it was also learned through DNA sequencing that some marine isolates thought to be *V. tubiashii* were actually *V. coralliilyticus* (7, 12). *V. coralliilyticus* and *V. tubiashii* are closely related phylogenetically (7, 13). Although some major hatchery crashes have been linked to *V. tubiashii* (3), it is now known that some of the etiological agents reported to be *V. tubiashii* are actually *V. coralliilyticus*. The strains formerly known as *Vibrio tubiashii* ATCC 19105, maintained by the American Type Culture Collection (ATCC, Manassas, VA), and *V. tubiashii* RE22, isolated from Pacific oyster larvae from a hatchery in Oregon (3), have been shown by sequencing to be *V. coralliilyticus* strains (12). Whole-genome sequencing was recently completed on RE98 (22), which was previously identified as a highly pathogenic strain of *V. tubiashii* obtained from a shellfish hatchery in Oregon (3). The results show that it is also a *V. coralliilyticus* strain (22).

One of the most productive estuaries on the U.S. West Coast, Willapa Bay, WA, suffered dramatic losses in natural spat set of oysters from 2005 until recently (14). Although the causes are not fully understood, it seems likely that *V. coralliilyticus* and/or *V. tubiashii* played some role in this phenomenon. These losses in natural spat set correlated with losses in hatcheries in Oregon and Washington, suggesting a common etiology. There is a need to readjust conventional thinking about the role of these two pathogens in hatchery outbreaks, disruptions of natural spat set, and coral diseases.

In this paper, we (i) examine the relative pathogenicities of two strains of *V. tubiashii* and four strains of *V. coralliilyticus* in larval Eastern and Pacific oysters, (ii) show the infectibility of Eastern oysters by *V. coralliilyticus*, and (iii) suggest that *V. coralliilyticus* is a more significant pathogen for larval Pacific oysters than previously recognized.

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MATERIALS AND METHODS

Bacterial cultures. The *Vibrio tubiashii* strains used were ATCC 19109 (type strain) and ATCC 19106. The strains of *V. coralliilyticus* consisted of ATCC BAA-450; ATCC 19105 and RE22, both originally listed as *V. tubiashii* but found to be *V. coralliilyticus* (12); and RE98, a highly pathogenic strain that was originally thought to be *V. tubiashii* but was shown by complete genome sequencing to be *V. coralliilyticus* (22). All isolates were routinely grown on Luria-Bertani broth or agar (Becton, Dickinson and Co., Sparks, MD) containing 2% added NaCl (3% NaCl total). Incubations were carried out at 26°C, and broth cultures were incubated with shaking at 250 rpm.

Sources of larvae and algae. Larval Pacific oysters were obtained from Taylor Shellfish Farms in Quilcene, WA, where they, along with algae and seawater, were shipped via FedEx to the USDA ARS Laboratory in Dover, DE; they were received the next day. Larvae were shipped in dry packs. A dry pack consists of several thousand larvae wrapped in a small piece of bolting cloth and surrounded by damp paper towels. They are shipped with an ice pack in a Styrofoam box. This is a normal method for shipping larvae to commercial shellfish buyers. The seawater accompanying the larvae had a salinity of approximately 28 ppt and was filtered through a 0.22-μm membrane and aerated for 1 h before resuspension of the larvae. Larvae were maintained at approximately 26°C for the duration of the experiments. Algae (which served as food for the larvae) were a mixture of *Chaetoceros*, *Isochrysis*, *Pavlova*, *Nannochloropsis*, and *Tetraselmis* species.

Larval Eastern oysters, seawater, and algae were provided by Rutgers University's Aquaculture Innovation Center, North Cape May, NJ. The larvae were shipped in approximately 28- to 30-ppt salinity seawater via the Cape May-Lewes Ferry and were picked up at the Lewes Ferry terminal and transported to the USDA ARS laboratory in Dover, DE. The total travel time was less than 4 h from departure from the hatchery to arrival at the laboratory. Live algae consisted of *Pavlova lutheri* and a *Nannochloropsis* sp. Larvae were maintained in the water in which they were received at 26°C throughout the study.

Eastern oyster larvae were also obtained from the University of Maryland Horn Point Hatchery in Cambridge, MD. These were shipped in dry packs and were received the next day along with algae and seawater. These larvae were raised, maintained, and challenged with vibrios in low-salinity (9.6-ppt) seawater. The Eastern and Pacific larvae were 1 to 2 weeks old at the time of receipt. All algae were maintained at room temperature (about 22°C) for the duration of each experiment under bright daylight, with constant aeration, and with the occasional addition of sterile seawater to feed the cultures and to prevent them from becoming too dense.

Larval oyster challenge. Larval oysters were dispensed at approximately 15 to 30 larvae per well into 3 rows of 24-well plates (18 wells/plate were used), and the final volume of seawater was adjusted to 950 μl/well by using the seawater that was provided with the larvae after filtering it through a 0.22-μm membrane. The larvae in the wells were enumerated under a stereo zoom microscope, and counts were recorded. Plates, with covers on, were maintained under a biosafety hood at approximately 26°C. Oxygen transfer was sufficient under these conditions so that the larvae did not require further aeration. Larvae were fed 50 μl of the appropriate algal culture daily. The day before larval challenge, a small portion of a colony of *V. coralliilyticus* or *V. tubiashii* was picked to 20 ml of LB broth with 2% added NaCl and was incubated at 26°C and 250 rpm overnight. The overnight cultures were then used to reinoculate fresh tubes of LB broth on the morning of the assay, followed by incubation at 26°C and 250 rpm for several hours until the optical density at 600 nm (OD_{600}) reached 0.80 ± 0.02. Cultures were then diluted (maximum dilution, 10^{-5}) in sterile seawater at 30-ppt salinity. The wells of 24-well plates were inoculated with 1 μl of *V. coralliilyticus* or *V. tubiashii*, either undiluted or at various dilutions. Uninoculated wells served as *Vibrio*-negative controls, and another set of control wells was inoculated with 1 μl of LB broth or dilutions of LB broth in sterile seawater to serve as medium controls. Duplicate plates were prepared for each pathogen, and dilutions of the pathogens, as well as negative controls, were inoculated in triplicate onto each plate. At the same time that the wells were inoculated with the vibrios, titers of the *Vibrio* inocula were determined using a quantitative pour plate assay (15) on dilutions (10^{-3} through 10^{-5}, inclusive) of the vibrios in sterile seawater, with each dilution plated in triplicate. Colonies were enumerated after incubation at 26°C for 48 h. Dead and live larvae were enumerated microscopically at 0 and 6 days. Dead larvae were easily recognized under a stereomicroscope by the lack of movement in their velar cilia and their characteristically light (somewhat bleached) appearance compared with the darker brown or green color of viable larvae. Most live larvae remained free-swimming throughout each 6-day experiment. Assays were performed on both Eastern and Pacific oyster larvae during May and June 2014.

Statistics. Linear regression analysis was performed by plotting the percentage of mortality against the pathogen concentration, from which each 50% lethal dose (LD_{50}) and correlation coefficient of the regression line was calculated and reported. The LD_{50}, defined here as the dose of *V. coralliilyticus* or *V. tubiashii* required to kill 50% of the larvae in 6 days, was calculated to include mortality that had been observed at a dosage of zero. In instances when multiple dilutions elicited 100% mortality for any given pathogen, only the lowest dilution giving 100% mortality was used in the analysis.

RESULTS

The relative mortalities and LD_{50}s of Eastern and Pacific oyster larvae after infection with varying levels of two strains of *V. tubiashii* and four strains of *V. coralliilyticus* are shown in Table 1. In Eastern oyster larvae, all four strains of *V. coralliilyticus* produced 100% mortality at concentrations between 2.9×10^4 and 7.8×10^4 CFU/ml (Table 1). The LD_{50}s of the *V. coralliilyticus* isolates ranged from 1.1×10^4 CFU/ml to 3.0×10^4 CFU/ml. To our knowledge, this is the first time that *V. coralliilyticus* has been shown to cause mortality in larval Eastern oysters. In comparison, Pacific oyster larvae experienced 100% mortality when exposed to 2.7×10^4 to 6.8×10^4 CFU/ml of *V. coralliilyticus*, except in one of the duplicate assays for ATCC strain BAA-450, where the highest dilution tested (4.6×10^4 CFU/ml) gave only 56.5% mortality. The LD_{50}s for the *V. coralliilyticus* strains in Pacific larvae ranged from 1.2×10^4 to 4.0×10^4 CFU/ml (Table 1). Based on the LD_{50} data, *V. coralliilyticus* strain BAA-450 was twice as infectious to Eastern oyster larvae as it was to Pacific oyster larvae, and strain 19105 was 30% more infectious toward Pacific larvae, whereas strains RE22 and RE98 were nearly equally infectious to Eastern and Pacific larvae. The lowest LD_{50}s for both Eastern and Pacific oysters were obtained with *V. coralliilyticus* RE98, suggesting that this strain is the most pathogenic among the isolates tested. Strain RE22 was a close second in infectivity.

In comparison, the two *V. tubiashii* strains gave more varied results (Table 1). In Eastern oysters, strain 19106 was highly pathogenic, with an LD_{50} of 3.8×10^3 CFU/ml, making it the most infectious bacterium among the six that were tested on Eastern oysters. Strain 19109 had an LD_{50} of 1.2×10^4 CFU/ml, comparable to the LD_{50}s of the *V. coralliilyticus* strains in Eastern oysters. In Pacific oysters, however, neither of the *V. tubiashii* strains showed significant pathogenicity. In fact, there was ≤2.5% mortality in Pacific oysters challenged with as much as 1.6×10^4 CFU/ml of *V. tubiashii* strain 19109. With strain 19106, mortality was also very low (2.4% at a dosage of 1.1×10^4 CFU/ml). Overall, these results indicate that *V. coralliilyticus* is pathogenic to both Pacific and Eastern oyster larvae, whereas the *V. tubiashii* strains are infectious principally to Eastern oyster larvae.

TABLE 1 Mortality and LD_{50}s of Eastern oyster (*Crassostrea virginia*) and Pacific oyster (*Crassostrea gigas*) larvae after challenge with various levels of *Vibrio coralliilyticus* or *Vibrio tubiashii*

Larvae	Pathogen	Assay date (mo/day/yr)[a]	Concn of pathogen (CFU/ml seawater[b])	Mortality of larvae (%)	LD_{50} (CFU/ml seawater)[c]	Correlation coefficient (*r*)
Eastern oysters	*V. coralliilyticus* ATCC BAA-450	6/4/14	4.6×10^4	100.0	2.1×10^4	0.970
			2.3×10^4	76.1		
			4.6×10^3	5.8		
			2.3×10^3	1.0		
			4.6×10^2	1.9		
			NC	0		
	V. coralliilyticus ATCC 19105	6/11/14	7.8×10^4	100.0	3.0×10^4	0.950
			3.9×10^4	85.0		
			7.8×10^3	28.2		
			3.9×10^3	10.7		
			7.8×10^2	7.7		
			NC	1.0		
	V. coralliilyticus RE22	6/11/14	6.6×10^4	100.0[d]	1.4×10^4	0.987
			3.3×10^4	100.0		
			6.6×10^3	38.2		
			3.3×10^3	12.5		
			6.6×10^2	14.8		
			NC	8.0		
	V. coralliilyticus RE98	6/11/14	5.8×10^4	100.0[d]	1.1×10^4	0.925
			2.9×10^4	100.0		
			5.8×10^3	61.4		
			2.9×10^3	16.5		
			5.8×10^2	14.5		
			NC	10.6		
	V. tubiashii ATCC 19106	6/4/14	6.0×10^3	70.7	3.8×10^3	0.962
			3.0×10^3	55.6		
			6.0×10^2	7.2		
			3.0×10^2	0		
			NC	2.3		
	V. tubiashii ATCC 19109	6/4/14	1.6×10^4	66.1	1.2×10^4	0.989
			8.0×10^3	40.7		
			1.6×10^3	3.4		
			8.0×10^2	9.0		
			1.6×10^2	0.8		
			NC	1.4		
Pacific oysters	*V. coralliilyticus* ATCC BAA-450	6/4/14	4.6×10^4	56.5	4.0×10^4	0.925
			4.6×10^3	19.0		
			4.6×10^2	0		
			NC	0		
		6/26/14	6.8×10^4	100.0		
			3.4×10^3	16.5		
			6.8×10^3	0		
			3.4×10^2	0		
			6.8×10^2	0		
			NC	0.8		
	V. coralliilyticus ATCC 19105	5/15/14	5.2×10^4	100.0	2.1×10^4	0.940
			2.6×10^4	52.9		
			5.2×10^3	36.7		
			2.6×10^3	17.5		
			5.2×10^2	16.0		
			NC	0.6		
		6/11/14	7.8×10^4	100.0[d]		
			3.9×10^4	100.0		
			7.8×10^3	44.8		
			3.9×10^3	0		
			7.8×10^2	1.0		
			NC	0		

(Continued on following page)

TABLE 1 (Continued)

Larvae	Pathogen	Assay date (mo/day/yr)[a]	Concn of pathogen (CFU/ml seawater[b])	Mortality of larvae (%)	LD_{50} (CFU/ml seawater)[c]	Correlation coefficient (*r*)
	V. coralliilyticus RE22	5/15/14	5.4×10^4	100.0[d]	1.4×10^4	0.927
			2.7×10^4	100.0		
			5.4×10^3	36.6		
			2.7×10^3	50.8		
			5.4×10^2	3.1		
			NC	7.4		
		6/11/14	6.6×10^4	100.0[d]		
			3.3×10^4	100.0		
			6.6×10^3	19.4		
			3.3×10^3	4.1		
			6.6×10^2	2.2		
			NC	0.7		
	V. coralliilyticus RE98	6/11/14	5.8×10^4	100.0[d]	1.2×10^4	0.881
			2.9×10^4	100.0		
			5.8×10^3	68.3		
			2.9×10^3	12.7		
			5.8×10^2	0		
			NC	0.8		
	V. tubiashii ATCC 19106	6/26/14	1.1×10^4	2.4	Not done[e]	Not done
			5.3×10^3	0		
			1.1×10^3	3.7		
			5.3×10^2	0		
			1.1×10^2	0		
			NC	0		
	V. tubiashii ATCC 19109	6/4/14	1.6×10^4	2.0	Not done[e]	Not done
			8.0×10^3	1.0		
			1.6×10^3	1.1		
			8.0×10^2	0.9		
			NC	1.0		
		6/26/14	1.1×10^4	2.5		
			5.3×10^3	0		
			1.1×10^3	0		
			5.3×10^2	0		
			1.1×10^2	0		
			NC	0		

[a] In cases where two assay dates are given, the two sets of data were combined to calculate the LD_{50} and correlation coefficient.
[b] NC, negative control.
[c] Results were reported 6 days after inoculation with vibrios.
[d] Data point not included in regression analysis or calculation of LD_{50}.
[e] Regression analysis was not performed because of insufficient range in the data due to low or negligible mortality rates.

Mortalities for controls of the Pacific oyster larvae after 6 days (the duration of each experiment) were very low (≤1%) in all but one trial, where *V. coralliilyticus* RE22 gave 7.4% mortality in one of the duplicate independent assays and 0.7% in the other (Table 1). In Eastern oyster larvae, negative controls showed low mortalities (0 to 2.3%) for *V. coralliilyticus* ATCC strains BAA-450 and 19105 and for both *V. tubiashii* strains after 6 days (Table 1). Interestingly, the same batch of larvae (from 11 June 2014) was used in experiments with strains RE22 and RE98, giving negative-control mortalities of 8.0% and 10.6%, respectively. The reasons for these different mortality rates are unknown. Higher levels of mortality in controls often suggest that the larvae may be contaminated or stressed; however, this does not appear to be the case, since all Eastern oyster larvae were from the same batch, were dispensed at the same time, and were maintained similarly.

A comparison of Eastern oyster mortalities was also performed at two salinities (28 ppt and 9.6 ppt). Larvae raised at 28 ppt were obtained from Rutgers University's Aquaculture Innovation Center, while larvae grown at 9.6 ppt were from the University of Maryland Horn Point Hatchery. Larvae were challenged with two strains of *V. coralliilyticus* (ATCC BAA-450 and RE98) and one strain of *V. tubiashii* (ATCC 19106). The LD_{50} results for the oysters maintained at 28-ppt salinity are shown in Table 1 as 2.1×10^4, 1.1×10^4, and 3.8×10^3 CFU/ml, respectively. Interestingly, the larvae maintained at 9.6-ppt salinity gave dramatically different results. No mortality was detected after 6 days with *V. coralliilyticus* levels as high as 3×10^5 CFU/ml for BAA-450 and 3.6×10^5 CFU/ml for RE98, or with *V. tubiashii* ATCC 19106 at 5.1×10^4 CFU/ml. Thus, it appears that at 9.6-ppt salinity, *V. coralliilyticus* and *V. tubiashi* were less infectious to the larvae than they were at 28-ppt salinity.

DISCUSSION

This paper shows the relative differences in larval oyster pathogenicity for two *V. tubiashii* and four *V. coralliilyticus* strains in two commercially valuable oyster species. In the recent past, *V. tubiashii* has been associated with mortalities of Pacific oysters along

the U.S. West Coast; however, some of the strains thought to be *V. tubiashii* were actually *V. coralliilyticus*. Of the two strains of *V. tubiashii* maintained by the ATCC (ATCC 19106 and ATCC 19109), neither showed significant pathogenicity toward Pacific oyster larvae. Strain 19109 was recently confirmed to be an authentic *V. tubiashii* strain by complete genomic sequencing (23), whereas strain 19106 was previously confirmed by sequencing to be *V. tubiashii* (13). Whole-genome sequencing of strain RE98, previously thought to be a *V. tubiashii* strain, recently identified it as *V. coralliilyticus*, and it was shown here to be pathogenic toward larval Eastern and Pacific oysters. These results raise the question of whether *V. tubiashii* has been problematic in West Coast hatcheries or whether the problems in hatcheries were principally or exclusively associated with *V. coralliilyticus* strains, such as RE98 and RE22, both of which were originally isolated from a West Coast shellfish hatchery during high-mortality events (3). Both RE98 and RE22 were previously shown to be highly pathogenic toward Pacific oysters but had not been tested against Eastern oysters (3, 16). Comparison of the LD_{50} results from Table 1 provides a simple means to assess the relative effects of these pathogens on healthy oyster larvae under the conditions of these assays (28- to 30-ppt salinity, 26°C).

It is clearly possible that different results would have been obtained if the larvae had been maintained in water of a different salinity, temperature, or pH. In fact, when we compared Eastern oyster larvae that had been raised at salinities of 9.6 and 28 ppt against two *V. coralliilyticus* strains and one *V. tubiashii* strain, no mortality was recorded at the lower salinity, in contrast to substantial mortality at the higher salinity. It is also noteworthy that a salinity of 9.6 ppt is much lower than that used in most hatchery operations and that later in the season, the low-salinity hatchery experienced high mortalities from unknown causes. Clearly, further investigation is required to identify environmental factors that may influence the invasiveness of different strains of *V. coralliilyticus* and *V. tubiashii* in larval shellfish.

Problems with high shellfish mortalities on the U.S. West Coast have been associated not only with vibrios but also with acidification of the seawater, upwelling of oxygen-depleted seawater, and increasing seawater temperatures. Acidification of waters due to an ever-growing adsorption of atmospheric CO_2 into the world's oceans is changing the marine ecosystem (17). Ocean acidification and rising seawater temperatures affect shellfish growth, immune response, and shell formation (18–20). West Coast shellfish have also been negatively affected by the upwelling of water from an oxygen-depleted zone (sometimes referred to as a "dead zone") making its way into productive shellfish beds. These deep-ocean anoxic zones result from the consumption of dissolved oxygen by bacteria as they degrade organic materials associated principally with decaying phytoplankton. An enlarged or previously unrecognized anoxic zone was reported off the Oregon coast in 2004 (21) and may have contributed to the introduction of higher levels of vibrios, as well as nutrient-rich, acidified deep waters, onto the continental shelf. The presence of acidified seawater, anoxic seawater, or seawater at temperatures higher than those normally encountered by shellfish larvae may, alone or combined, make larvae more susceptible to infection with opportunistic pathogens, such as *V. coralliilyticus* and *V. tubiashii*.

Whatever the stress, it is likely that the health status of oyster larvae will affect the LD_{50} for various pathogens. The present study used shellfish early in the spawning season in an effort to minimize *Vibrio* contamination, which, we know from personal experience, generally increases over the summer months, when *Vibrio* levels are at their peak in environmental waters. Therefore, the LD_{50} data presented here are representative of the effects of selected *V. coralliilyticus* and *V. tubiashii* strains on apparently healthy oysters. As summer progresses, the LD_{50}s would be expected to change, perhaps radically, depending on the overall stresses placed on the oyster larvae (e.g., acidification, high temperatures, low oxygenation, or over- or underfeeding), as well as the levels of endogenous vibrios and other organisms that may be present. Consequently, Pacific larvae that were resilient to *V. tubiashii* in these studies might be highly susceptible to the same pathogens when stressed. Therefore, it is possible that *V. tubiashii* ATCC 19106 and ATCC 19109 could be infectious to stressed Pacific oyster larvae or to larvae dosed with higher concentrations of *V. tubiashii*.

Previous confusion about the identity of some presumptive *V. tubiashii* strains led to the conclusion that *V. tubiashii* was a significant pathogen in Pacific Coast hatcheries. The revelation that some of the strains previously identified as *V. tubiashii* were actually *V. coralliilyticus* and the fact that our LD_{50} assays indicate that *V. coralliilyticus* is more pathogenic than *V. tubiashii* in Pacific oyster larvae suggest that *V. coralliilyticus* is a more substantial pathogen than *V. tubiashii* and that *V. coralliilyticus* may have been problematic in Pacific hatcheries for many years. *Vibrio coralliilyticus* is not only relevant to the culturing of Pacific and Eastern oyster larvae but may be responsible for high mortalities in commercial larval production of other shellfish species worldwide. Likewise, *V. coralliilyticus* could be responsible for reductions in natural spat set leading to occasional reductions in shellfish populations in many locations, potentially accounting for the lack of spat set observed in Wallapa Bay, WA (14).

Further study of *V. coralliilyticus* and *V. tubiashii* is needed to (i) determine their infectivities in shellfish stressed by increased temperatures, low oxygen levels, acidified seawater, etc., (ii) examine the combined effects of environmental stresses and bacterial exposure on larval mortalities, (iii) quantify the prevalence and persistence of *V. coralliilyticus* and *V. tubiashii* in Atlantic and Pacific coastal environments and within hatcheries, (iv) better identify their virulence mechanisms, and (v) determine if shellfish, corals, or other marine life are reservoirs supporting either the prevalence or the persistence of these pathogens in the marine environment. Since *V. coralliilyticus* is better known as a coral pathogen, research is also needed to identify potential causal relationships between coral disease and shellfish disease. It is unclear whether larval shellfish or corals are more susceptible to *V. coralliilyticus*, but such a determination would be within easy reach for researchers today. It would also be worthwhile to determine if *V. tubiashii* can infect corals, too, much as some strains of *V. coralliilyticus* are known to infect both corals and larval shellfish. The information provided in this paper clarifies the levels of *V. coralliilyticus* and *V. tubiashii* required to cause mortalities in apparently healthy Eastern and Pacific oyster larvae. It is hoped that this information will lead to better water quality monitoring within hatcheries to prevent outbreaks of shellfish disease.

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