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Original Articles

Distribution and Growth of *Vibrio parahaemolyticus* in Southern Chilean Clams (*Venus antiqua*) and Blue Mussels (*Mytilus chilensis*)

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Abstract

We evaluated the distribution and growth of *Vibrio parahaemolyticus* in the inland sea of southern Chile, where the world's largest foodborne gastroenteritis outbreak by the pandemic strain O3:K6 occurred in 2005. Intertidal samples of *Mytilus chilensis* and *Venus antiqua* were collected around port towns between 41°28'S and 43°07'S, during April to May 2011 and January to March 2012. We used most probable number real-time polymerase chain reaction (MPN-PCR) for enumeration of the *tlh*, *tdh*, and *trh* genes in freshly harvested bivalves and after a controlled postharvest temperature abuse. Pathogenic markers (*tdh*+ or *trh*+) were not detected. Total *V. parahaemolyticus* (*tlh*+) in freshly harvested samples reached up to 0.38 and 3.66 log MPN/g in 2011 and 2012, respectively, with values close to or above 3 log MPN/g only near Puerto Montt (41°28'S, 72°55'W). Enrichments by temperature abuse (>2 log MPN/g) occurred mainly in the same zone, regardless of the year, suggesting that both natural or anthropogenic exposure to high temperatures were more critical. Lower salinity and higher sea surface temperature in Reloncaví Sound and Reloncaví Estuary were consistent with our observations and allowed confirmation of the existence of a high-risk zone near Puerto Montt. Based on the results, a strategy focused on risk management inside this defined hazard zone is recommended.

Introduction

The bacterium *Vibrio parahaemolyticus* is recognized worldwide as a cause of foodborne gastroenteritis resulting from the consumption of raw or undercooked shellfish (DePaola *et al.*, 2010; Abd-Elghany and Sallam, 2013; Paydar *et al.*, 2013). Some of the main predictive risk factors for *V. parahaemolyticus* are water temperature (Baker-Austin *et al.*, 2013; Böer *et al.*, 2013; Vezzulli *et al.*, 2013), low-to-moderate salinity in estuarine harvest areas (Yamazaki and Esiobu, 2012; Rehnstam-Holm *et al.*, 2014), and postharvest handling (Gooch *et al.*, 2002).

In southern Chile, the pandemic serotype O3:K6 caused the world's largest outbreak of *V. parahaemolyticus*, with a peak of 10,984 cases in 2005 associated with consumption of shellfish from the inland sea near Puerto Montt (41°28′S, 72°55′W) in the Región de Los Lagos (González-Escalona *et al.*, 2005; Harth *et al.*, 2009). Based on the official database for fishery and harvesting, the zone has historically been the main source of bivalves in Chile and, in fact, the latest report indicates 56% of artisanal bivalve fisheries (mainly clams) and 97% of bivalve harvesting (mainly blue mussels) from the entire country comes from the inland sea between 41.5°S and 43.5°S (Anuario Estadístico de Pesca 2012, Sernapesca [www.sernapesca.cl]). Therefore, improving our understanding of the environmental and geographic factors involved in *V. parahaemolyticus* occurrence in this zone is essential.

The inland sea area of the Región de Los Lagos encompasses a transition zone between temperate–warm–wet and temperate–cold–wet climates (>2000 mm of rainfall per year). A large variability in maximum temperatures and duration of those maximum temperatures has been observed over recent years, with some unpredictable heat-wave events (close to or above 30°C). However, there is neither evidence of clear trends in this regard nor any long-term studies in the area. Moreover, the Reloncaví Estuary introduces a shallow warm-water plume (>13°C) with lower salinity (<30 ppt) into the Reloncaví Sound, which usually reaches the area around Puerto Montt, and contrasts with the conditions in deeper waters that are of oceanic origin, with temperatures <11°C and high salinity (~32.6 ppm) (Dávila *et al.*, 2002; Soto-Mardones *et al.*, 2009). In spite of the large southern Chilean

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outbreak, there are no reports linking the occurrence of *V. parahaemolyticus* with any oceanographic conditions or risk factors in the area. In this regard, based on remote sensing of sea surface temperature (SST) and records of the first poisoning during the beginning of the gastroenteritis outbreak in southern Chile, the government prohibited shellfish harvesting in the northern section of this inland sea (Rodriguez-Benito *et al.*, 2005) (Fig. 1), but probably due to the high economic and social impact of this prohibition, it was discontinued in 2005 and recommendation that raw bivalves are not to be consumed during the warmer months throughout the country was instead reinforced.

The aims of this study were (1) to evaluate the geographic differences in levels of *V. parahaemolyticus* in freshly harvested shellfish from the inland sea of the Región de Los Lagos, (2) to correlate those levels with remotely sensed SST and *in situ* water temperature and salinity, and (3) to determine whether loads of *V. parahaemolyticus* could be increased in worst-case scenarios of bivalve exposure to warm temperatures.

Materials and Methods

Collection and preliminary processing of samples

This study considered two edible bivalves of economic importance in Chile, the clam (*Venus antiqua*) and the blue mussel (*Mytilus chilensis*). Multiple sampling sites were selected from almost 200 km south of the main port town of the zone (Puerto Montt), between 41°28'S and 43°07'S (Fig. 1), where shellfish are currently farmed or harvested for local, national, or international markets.

Campaigns were carried out between April and May 2011 and January and March 2012. Sampling at each site was performed at low tide. Samples of bivalves (50 specimens of each species) were collected from the exposed intertidal zone, along 80 m transect parallel to the coastal line equidistant to the higher and lower tides. Each sample was washed with the local seawater, stored as separate sterile bags at 10 ± 2°C, and processed within 4 h. Water temperature and salinity were measured with a portable conductivity meter (EC 300, Merck). Each bivalve sample was split into two subsamples on arrival at the laboratory. One was processed directly in freshly harvested (FH) bivalves and the other was analyzed after an artificial postharvest temperature abuse (TA) to emulate any *Vibrio* multiplication in a worst-case scenario of bivalves exposure to warm environments. The TA consisted of an incubation at 35°C for 12 h in 2011. However, since samples were strongly affected by drainage and dehydration, abuse was later modified to 28°C for 18 h at the following sampling in 2012. Bivalves better withstood this new treatment with an increased intervalval fluid retention.

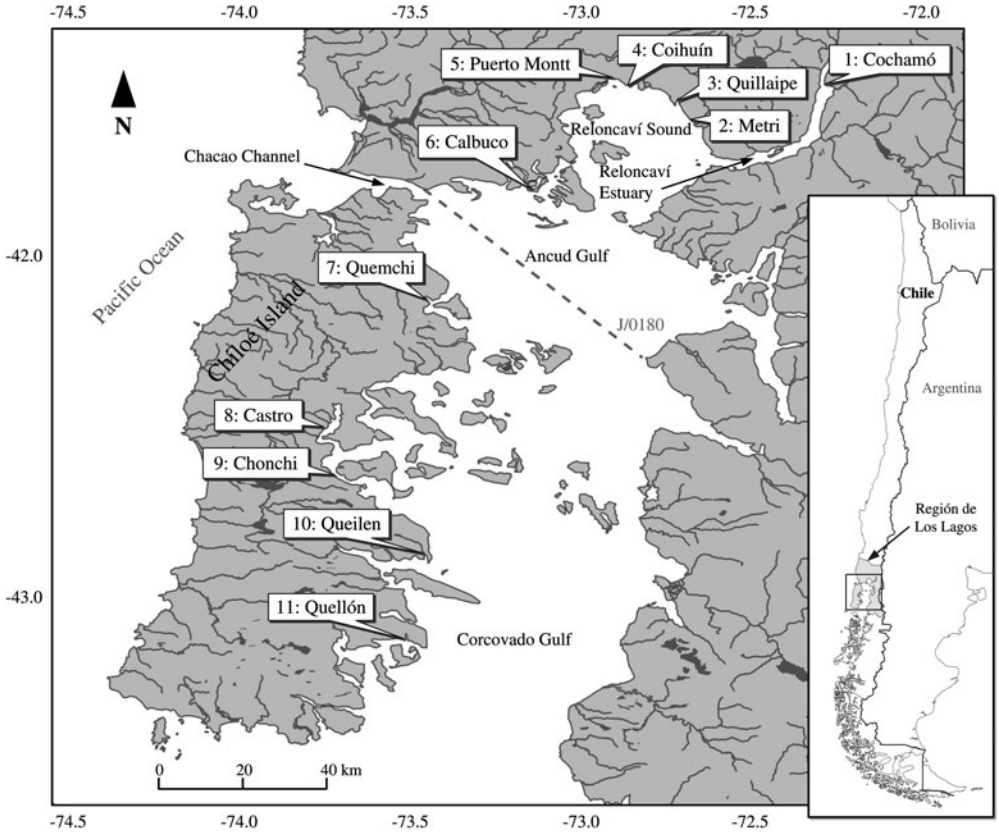

FIG. 1. Map of sampling sites in the inland sea of the Región de Los Lagos, southern Chile. J/0180 is a resolution from 2004 that had prohibited shellfish extraction to the north of the drawn transect (J/0180, January 30, 2004, Minsal [www.marearoja.cl]), which has been discontinued since 2005. Geographic projection: WGS 84. Inset indicates the position of the Región de Los Lagos within the country.

Vibrio parahaemolyticus *analysis*

Analyses were performed by most probable number (MPN) with a series of three tubes according to the U.S. Food and Drug Administration Bacteriological Analytical Manual (Kaysner and DePaola, 2004), followed by DNA extraction and real-time PCR with the following adaptations. The MPN enrichment series contained 10–0.001 g of sample. Since many of the temperature-abused samples from 2011 exceeded the upper range (1100 MPN/g), the sample dilution was extended in 2012, allowing up to 10^{-6} g incubation. After 18 h at 28°C, 200-μL aliquots of positive enrichments were centrifuged at $5000 \times g$ for 10 min. DNA from the pellets was extracted using E.Z.N.A.® Bacterial DNA Kit (Omega Bio Tek Inc., Norcross, GA) according to the manufacturer's instructions with genomic DNA elution in 100 μL of water PCR grade and stored at -20°C. Nucleic acid purities were verified by 260/280-nm absorbance ratios. Primers and Taqman probes were directed to *tlh*, *tdh*, and *trh* genes according to Nordstrom *et al.* (2007) and DePaola *et al.* (2010). Taqman probes were 5′ labeled with the 6-carboxyfluorescein reporter dye and 3′ labeled with the Minor Groove Binder quencher dye (Applied Biosystem). Mixture for reactions: 5 μL of 2X Brilliant II Master Mix (Agilent Technologies Inc.), 0.15 μL of diluted 1:50 Reference dye 1 mM, 0.45 μL of each primer 20 μM, 0.125 μL of each Taqman probe 20 μM, 2.825 μL of water PCR grade and 1 μL of DNA template. The real-time PCR simplex protocol consisted of denaturation at 95°C for 10 min and then 40 cycles of 95°C for 15 s and 60°C for 45 s. Runs were performed with Step One Plus (Applied Biosystem, Life Technologies Inc.). As positive control for PCR we use genomic DNA from the pathogenic *V. parahaemolyticus* strain RIMD 2210633.

Statistical analysis

Mann–Whitney tests were used in Statistica V7.0 (StatSoft Inc., Tulsa, OK, 2004). The level of significance was set at $p < 0.1$ upon MPN readings. Values below the detection limit (< 0.03 MPN/g) were substituted with 0.01 MPN/g, which is the next corresponding putative value if 100 g in tubes enrichment had been used. Similarly, values > 1100 MPN/g in 2011 were computed as 3600 MPN/g. The software QtiPlot 0.9.8.9 (Ion Vasilief, Craiova, Romania, 2011) was used in scatter plots and linear correlation involving water temperature, salinity, and direct loads of *V. parahaemolyticus* using log-transformed data.

Remote sensing of SST

Maps of SST were obtained from MODIS satellite database of US National Aeronautics and Space Administration (NASA), Sensors Aqua, and Terra. Data for SST were measured using the 11- and 12-μm channels at 1-km resolution and stored in Hierarchical Data Format. Data obtained at Level 2 (geophysical variable). Measurements, visualization, and geographic projection with the latest Word Geodetic System (WGS84) were performed by SeaDAS V7.0.2 (NASA, Greenbelt, MD, 2013).

Results

All sites on Chiloé Island and Ancud Gulf were sampled for both mussels and clams (*M. chilensis* and *V. antiqua*). However, collection of both bivalves was not possible in Reloncaví Sound sites, and only mussels could be sampled in Reloncaví Estuary (Table 1).

Pathogenic *V. parahaemolyticus* (*tdh*+ or *trh*+) was not detected in any FH or any controlled postharvest TA bivalves.

Total *V. parahaemolyticus* (*tlh*+) in FH bivalves was undetectable ($\leq$-1.52 log MPN/g) in the majority (82%) of samples collected in 2011 or detected at low levels (≤ 0.38 log MPN/g). By contrast, nearly all FH samples in 2012 had detectable *V. parahaemolyticus* levels (88%) (≤ 3.66 log MPN/g). The TA bivalves achieved loads of *V. parahaemolyticus* (*tlh*+) in a wider range, from < -1.52 log MPN/g for most samples from Chiloé Island to > 3.04 log MPN/g in samples from Reloncaví Sound and Reloncaví Estuary in 2011 and, in 2012, from < -1.52 log MPN/g to 6.04 log MPN/g with a similar distribution of loads as in 2011 (Table 2). Threshold cycle for *thl* in MPN-PCR ranged from 28.15 to 39.06, with a mode around 34 (not shown).

Based on Mann–Whitney tests on total *V. parahaemolyticus* (*tlh*+) measurements in FH or TA bivalves, we found no significant differences in *V. parahaemolyticus* levels between

Table 1. Details of Sampling Campaigns (Years 2011 and 2012) in the Inland Sea of the Región de Los Lagos, Southern Chile

Sampling site	*Zone*	*Latitude (°S)*	*Longitude (°W)*	*Sample*	*Date*	*Water T° (°C)*[a]	*Date*	*Water T° (°C)*[a]	*Salinity (ppt)*
1. Cochamó	ML-RE	41.49611	72.31070	*Mytilus chilensis*	10.05.11	11.8	28.01.12	16.2	19.4
2. Metri	ML-RS	41.59423	72.70616	*M. chilensis*	03.05.11	10.9	23.01.12	17.6	23.1
3. Quillaipe	ML-RS	41.54462	72.73768	*Venus antiqua*	03.05.11	11.3	23.01.12	17.2	21.0
4. Coihuín	ML-RS	41.49954	72.88630	*V. antiqua*	28.04.11	11.5	18.01.12	18.1	22.5
5. Puerto Montt	ML-RS	41.47570	72.92093	*M. chilensis*	28.04.11	11.6	18.01.12	17.5	21.7
6. Calbuco	ML-AG	41.80512	73.16934	*M. chilensis* and *V. antiqua*	13.05.11	12.1	01.03.12	13.2	29.3
7. Quemchi	ChI-AG	42.12486	73.47838	*M. chilensis* and *V. antiqua*	05.04.11	12.3	25.01.12	16.4	27.7
8. Castro	ChI	42.50575	73.78377	*M. chilensis* and *V. antiqua*	07.04.11	10.1	11.01.12	11.2	31.9
9. Chonchi	ChI	42.64181	73.74961	*M. chilensis* and *V. antiqua*	10.04.11	10.7	17.01.12	25.3	31.9
10. Queilen	ChI-CG	42.87575	73.47918	*M. chilensis* and *V. antiqua*	17.04.11	10.1	26.01.12	16.4	28.7
11. Quellón	ChI-CG	43.12203	73.54368	*M. chilensis* and *V. antiqua*	11.04.11	12.2	08.03.12	18.0	31.1

[a]Water temperature taken at different times of day with varying cloud cover.
AG, Ancud Gulf; ChI, Chiloé Island; ML, mainland; RE, Reloncaví Estuary; RS, Reloncaví Sound.

Table 2. Levels of Total *Vibrio parahaemolyticus* (*tlh*+) in Freshly Harvested (FH) and Controlled Postharvest Temperature Abused (TA) Intertidal Shellfish

	Total V. parahaemolyticus *(*tlh+*) (log MPN/g)*											
	2011						*2012*					
	M. chilensis			V. antiqua			M. chilensis			V. antiqua		
Sampling site (macro zone[a]*)*	*FH*	*TA*	*TA-FH*	*FH*	*TA*	*TA-FH*	*FH*	*TA*	*TA-FH*	*FH*	*TA*	*TA-FH*
1. Cochamó (ML)	<−1.52	>3.04	>4.56	NS	NS	—	3.38	5.66	2.28	NS	NS	—
2. Metri (ML)	<−1.52	>3.04	>4.56	NS	NS	—	3.66	4.88	1.22	NS	NS	—
3. Quillaipe (ML)	NS	NS	—	<−1.52	>3.04	>4.56	NS	NS	—	3.18	6.04	2.86
4. Coihuín (ML)	NS	NS	—	<−1.52	2.46	>3.98	NS	NS	—	2.88	5.38	2.50
5. P. Montt (ML)	−1.04	>3.04	>4.08	NS	NS	—	3.18	5.32	2.14	NS	NS	—
6. Calbuco (ML)	−0.37	2.46	2.83	<−1.52	<−1.52	0	1.97	3.04	1.07	0.66	2.46	1.80
7. Quemchi (ChI)	<−1.52	<−1.52	0	<−1.52	<−1.52	0	2.18	2.66	0.48	2.38	3.04	0.66
8. Castro (ChI)	<−1.52	1.63	>3.15	0.38	2.18	1.80	−0.03	1.88	1.91	0.30	2.66	2.36
9. Chonchi (ChI)	<−1.52	<−1.52	0	<−1.52	<−1.52	0	2.32	3.08	0.76	1.88	2.18	0.30
10. Queilen (ChI)	<−1.52	<−1.52	0	<−1.52	<−1.52	0	1.54	1.18	−0.36	2.32	3.04	0.72
11. Quellón (ChI)	<−1.52	<−1.52	0	<−1.52	<−1.52	0	<−1.52	<−1.52	0	<−1.52	<−1.52	0

Samples with more than 100 times enrichment by postharvest temperature abused are shaded [(TA-FH) > 2 log].
[a]See zone in Table 1.
MPN, most probable number; NS, not sampled.

bivalve species (*V. antiqua* vs. *M. chilensis*), even if comparisons were performed separately inside the groups for mainland or Chiloé Island ($p > 0.2$). These results gave us the support to combine data for both species and to perform the next Mann–Whitney tests upon the relative position of the sampling sites with respect to the risk zone defined at the beginning of the *V. parahaemolyticus* outbreak (Fig. 1) or upon treatment (FH or TA).

Segregated sampling data for *V. parahaemolyticus* (*tlh*+) in FH bivalves in 2012 (combining those for *V. antiqua* and *M. chilensis*) according to their location on the mainland (high-risk zone) or Chiloé Island (low-risk zone) showed that *V. parahaemolyticus* levels in FH bivalves of mainland (range: 0.66, 3.66 log MPN/g) were significantly higher than those of Chiloé Island (range: <-1.52, 2.38 log MPN/g) ($p = 0.09$). This difference was absent in the corresponding groups in 2011 when low or undetectable amounts of the bacterium were measured ($p = 0.62$).

On the other hand, TA measurements of total *V. parahaemolyticus* in mainland sites were significantly higher than FH in 2012 ($p = 0.09$), and the same was observed in 2011 ($p = 0.07$). In addition, readings for TA bivalves from Chiloé Island were significantly increased in 2012 ($p = 0.06$) but not in 2011 ($p = 0.37$), when total *V. parahaemolyticus* was undetected in FH bivalves and therefore probably absent and thus unavailable for laboratory enrichment.

The differences in sampling data for total *V. parahaemolyticus* after TA according to the relative position of sampling sites was still more evident, with TA values from the mainland significantly higher than values for Chiloé Island in both sampling campaigns ($p = 0.03$ in 2012 and $p = 0.01$ in 2011).

Loads of total *V. parahaemolyticus* in FH bivalves could be associated with sea water temperature and salinity. The low load levels in 2011 (April to May) coincided with *in situ* measured temperatures that ranged from 10.1 to 12.3°C (Table 1), while in 2012, all the highest values for *V. parahaemolyticus* (*tlh*+) (close to or above 3 log MPN/g) were associated with sea water temperatures ranging from 16.2 to 18.1°C. On the other hand, in January 2012, salinity in the estuary was 19 ppt (site 1) and an estuarine plume reached the sampling areas in Reloncaví Sound, resulting in readings < 23 ppt (sites 2–5). In fact, levels of total *V. parahaemolyticus* in bivalves were positively correlated with temperature ($R^2 = 0.79$) and negatively correlated with salinity ($R^2 = 0.64$) (Fig. 2), but it was not possible to determine which factor had more effect. At sites 11 (Quellón) and 9 (Chonchi), low levels of *V. parahaemolyticus* were detected, contrary to values than would be predicted based on water temperature on the day of sampling. These unexpected readings could be associated with the high salinity or low average temperature in the zone, especially considering oscillations within a day or even a few weeks. This last possibility was supported by the SST maps obtained by remote sensing, which revealed a dominant north–south gradient in temperature and consequently a SST distribution that largely fits the previously defined risk zone. Specifically, we observed SST > 15°C in the north during the entire sampling period of 2012 (Fig. 3, panels D, E, and F), or > 14°C during the start of the sampling period of 2011 (panel A). On the other hand, sampling sites in Corcovado Gulf (the southernmost sampled area) exhibited SST < 12°C in most observations; the only exceptions were the sampling sites located on the coastline of Chiloé Island with elevated SST at the beginning of January 2012 (panel D), a time at which an exceptionally high SST was observed in the entire region. Therefore, the SST maps seemed to explain why bivalves in the risk zone showed higher levels of bacteria in 2011 and 2012, and even why *V. parahaemolyticus* values were higher in 2012 than 2011. Interestingly, in May 2011 (autumn season) a different SST distribution was observed (panel C), with uniformly cool temperatures in the entire region generally associated with the dominant temperate climate and high rainfall.

Finally, the higher values (> 2 log) for total *V. parahaemolyticus* enrichment by TA (rated as TA-FH for log MPN/g) were mostly concentrated in the mainland (shaded areas in Table

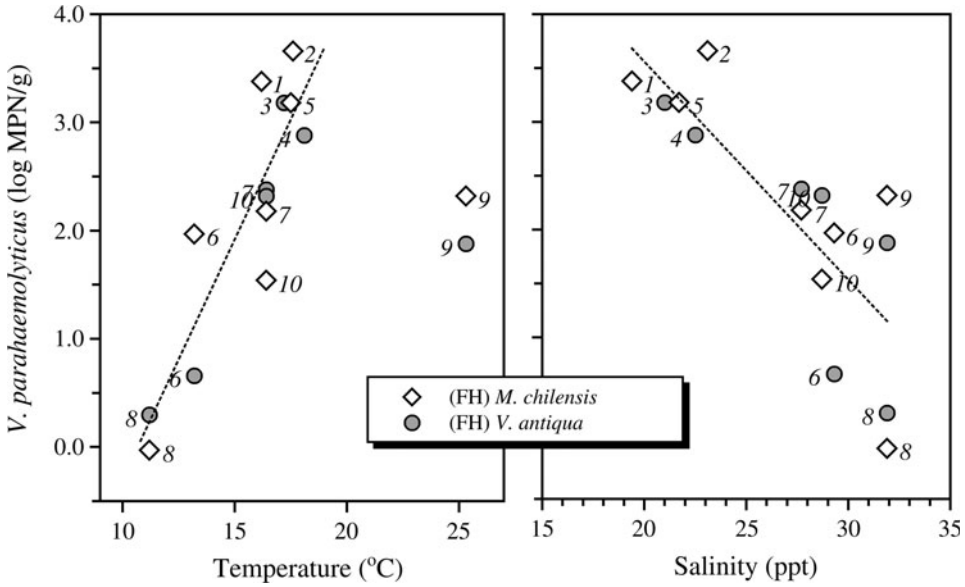

FIG. 2. Scatter plots and linear regressions between measurements of total *Vibrio parahaemolyticus* (*tlh*+) at 2012 in freshly harvested (FH) bivalves and temperature or salinity during sampling. Numbers next to symbols denote sampling sites. Site 11 with *V. parahaemolyticus* below the limit of detection was not considered. Temperature-based regression (excluding site 9): $R^2 = 0.79$, adjusted $R^2 = 0.74$, root mean squared error (RMSE) = 0.58, residual sum of squares (RSS) = 3.7. Salinity-based regression: $R^2 = 0.64$, adjusted $R^2 = 0.58$, RMSE = 0.70, RSS = 6.3. MPN, most probable number.

2). Differences in temperature could not support this apparent distribution in the enrichment behaviors because this parameter was kept constant during TA. On the other hand, this apparent distribution was not supported by the distribution of salinities measured in 2012 ($R^2 = 0.33$, Fig. 4).

Discussion

Our measurements of *V. parahaemolyticus* in freshly harvested intertidal shellfish are consistent with the minimal number of foodborne gastroenteritis cases from *V.*

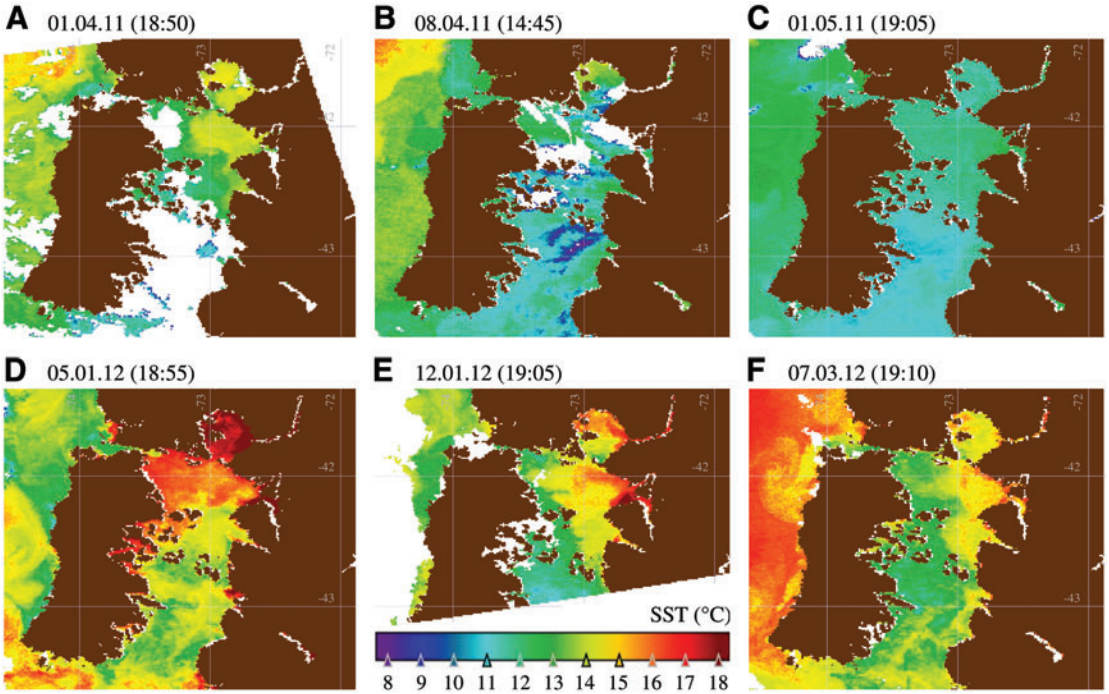

FIG. 3. Sea surface temperature (SST) maps of the region of interest. The criteria for selection of these images were the low-to-moderate percentage of cloud cover (white zones) and the temporal proximity to the *in situ* sampling dates. Numbers above panels denote dates and local hours. Maps have geographic projection that are identical to Fig. 1. Color images available online at www.liebertpub.com/fpd

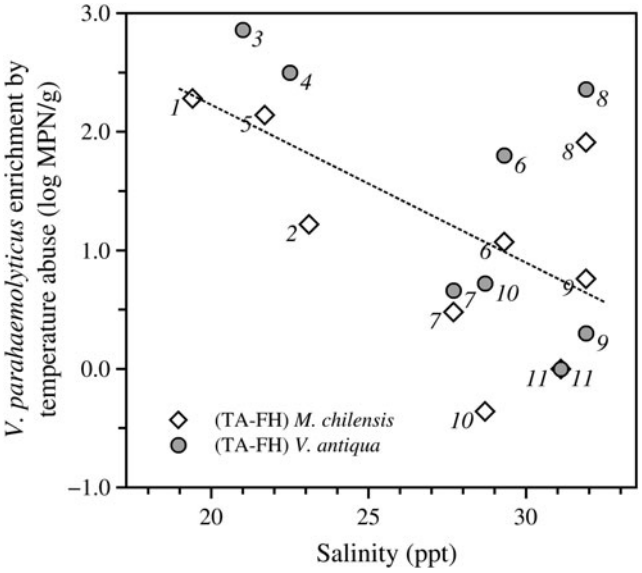

FIG. 4. Scatter plot and linear correlation between water salinity at sampling sites in 2012 and total *Vibrio parahaemolyticus* (*tlh*+) enrichment by controlled postharvest temperature abuse (TA-FH). Numbers in symbols denote sampling sites according to Tables 1 and 2. $R^2 = 0.33$, adjusted $R^2 = 0.24$, root mean squared error = 0.85, residual sum of squares (RSS) = 10.8. *M. chilensis*, *Mytilus chilensis*; *V. antiqua*, *Venus antiqua*.

parahaemolyticus reported during the study period, with 11 cases in Región de Los Lagos and 71 in the entire country in 2011, and only 1 case in the southern region and 36 in the entire country in 2012 (Informes *Vibrio parahaemolyticus*, Minsal [epi.minsal.cl]). The sampling years of this report thus appeared to be synchronized with the decline of the pandemic clone O3:K6 (García *et al.*, 2013). However, as had happened with the pandemic clone, we could speculate that other variants of this bacterium (Nair *et al.*, 2007) could find a favorable environment conducive to outbreaks from a nonpandemic clonal population. Therefore, measurements for total *V. parahaemolyticus* (*tlh*+) are useful for examining the possible occurrence, growth, or distribution of the bacterium.

Due to the wide tidal oscillation within the risk zone (around 7.5 m), it is possible to observe large intertidal platforms where abundant bivalves (such as *M. chilensis* and others mussels) could be heated to temperatures above 30°C as a result of exposure to solar radiation (García *et al.*, 2013) (Cochamó, Metri, and Puerto Montt are the best examples in this study). Within these intertidal platforms, there are also some large sand flats rich in burrowing bivalves (such as *V. antiqua* and other clams from Coihuín and Quillaipe), where waters arriving with incoming tides often reach temperatures of >25°C (and occasionally >30°C) during some summer days. These intertidal areas rich in bivalves are near rural settlements, so are therefore a priority for forecasting shellfishborne diseases.

In our study, the controlled temperature abuse during postharvest gave strong support to the predicted area of risk as previously defined (especially in Reloncaví Sound and Reloncaví Estuary). This method allowed us to assess not only the field burden of *V. parahaemolyticus* in bivalves, but also the growth potential of this bacterium due to natural or anthropogenic variables. On the other hand, some samples (especially from Chiloé Island) with considerable loads of total *V. parahaemolyticus* did not experience TA enrichment. At least in theory, the levels that could be reached by TA should depend on the initial load of this bacterium, but obviously other factors were also influencing the enrichment of *V. parahaemolyticus*. Although low salinity could be favoring higher levels of total *V. parahaemolyticus*, this parameter alone does not account for the variability observed during TA enrichment. Thus, we could speculate that the heterogeneity in *V. parahaemolyticus* multiplication by TA was partially determined by some uncontrolled biological factors that could also contribute to the local distribution of *V. parahaemolyticus*. Some of the possible biological factors that could be considered in future examinations are (1) the presence of antagonism inside the bivalve bacterial community (Aranda *et al.*, 2012) and (2) bacteriophages able to infect *V. parahaemolyticus* (Alagappan *et al.*, 2010; García *et al.*, 2013). Moreover, extrapolations of the findings and hypotheses to account for the environmental occurrence require further consideration of other biological factors not explored here, such as the abundance of zooplankton that interact with *V. parahaemolyticus* (Matz *et al.*, 2011; Rehnstam-Holm *et al.*, 2014) and other physical variables such as rainfall (Yamazaki and Esiobu, 2012), which is high in the region.

According to our observations, prediction and prevention of *V. parahaemolyticus* illnesses in Chile could be enhanced by an early warning system based on unusually high bacterial levels or changes in the climatic/environmental factors that could influence the presence and persistence of the bacterium. By using SST remote sensing, it is feasible to assist policy makers in assessing the risk by monitoring the environment on a synoptic scale at low cost (Schaeffer *et al.*, 2013). Due to the persistent cloud cover in the studied region, satellite-based monitoring could not replace the *in situ* monitoring of water temperature, and also salinity cannot be sensed remotely at the required resolution. However, synoptic *in situ* measurements require many resources and imply a high logistic cost due to the size of the area and the remote location of some zones. Thus, optimization of resources could be achieved by the combination of earth observing and meteorological satellites and the utilization of some strategic oceanographic monitoring buoys.

Conclusions

Our measurements of *V. parahaemolyticus* in intertidal freshly harvested and controlled postharvest TA bivalves from different areas of the inland sea of the Región de Los Lagos were consistent with the existence of an oceanographic province encompassing Reloncaví Sound and Reloncaví Estuary with favorable environmental conditions for the growth of this bacterium. Strengthening the control of harvested bivalves, prevention of TA, and the implementation of a risk-prediction model based on satellite records of SST coupled with *in situ* oceanographic monitoring and field measurements of *V. parahaemolyticus* in seafood is suggested.

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Disclosure Statement

No competing financial interests exist.

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