Evaluation of Low Temperature Depuration for Reducing *Vibrio parahaemolyticus* in Oysters

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Vibrio parahaemolyticus

- Occurs naturally in the marine environment.
- Leading cause of foodborne infections associated with seafood consumption in U.S.
- Major outbreaks of *V. parahaemolyticus* infections associated with raw oyster consumption:
  - Pacific Northwest, Gulf of Mexico, and Atlantic Northeast (1997- 1998).
  - First outbreak in Alaska (2004).
  - Washington and Oregon (2006).

Oyster Post-harvest Treatments

- Low temperature pasteurization
- Rapid chilling
- Freezing
- High pressure processing
- Irradiation
- Most of these processes require significant amounts of initial investment
- Oysters are often killed during the process

Depuration

- A controlled process allowing shellfish to purge sand and grit from the gut into clean seawater.
- Usually leads to bacterial reduction in shellfish and increased shelf life of refrigerated products.
- Process with clean seawater at ambient temperature is not effective in reducing *Vibrio* in shellfish.
- Usage for reducing *Vibrio* in oysters is limited.
- Replacing seawater with a solution exhibiting strong antibacterial activities, such as electrolyzed oxidizing (EO) water, or lowering water temperatures may result in better reduction of *Vibrio* in oysters.

Water Electrolysis

- First reported in 1970s to possess antimicrobial properties.
- Conducted by subjecting dilute salt (0.05-0.2% NaCl) solution to electrodes.
- Acidic oxidizing water (EO water, pH < 2.7)
  - high oxidation-reduction potential (ORP) (1,100 mV)
  - free chlorine (10 - 100 ppm)
  - strong bactericidal effects against pathogens

Antibacterial Effects of EO Water

Bacterial reduction (log CFU/ml):

- *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Venkitanarayanan *et al*., 1999).
  - > 9 (30 sec)
- *Enterobacter aerogenes* and *Staphylococcus aureus* (Park *et al*., 2002)
  - > 8 (30 sec)
- *Campylobacter jejuni* (Park *et al*., 2002)
  - > 8 (10 sec)
- *Bacillus cereus* (Kim et al., 2000)
  - cell: > 7 (60 sec)
  - Spore: > 3 (120 sec)

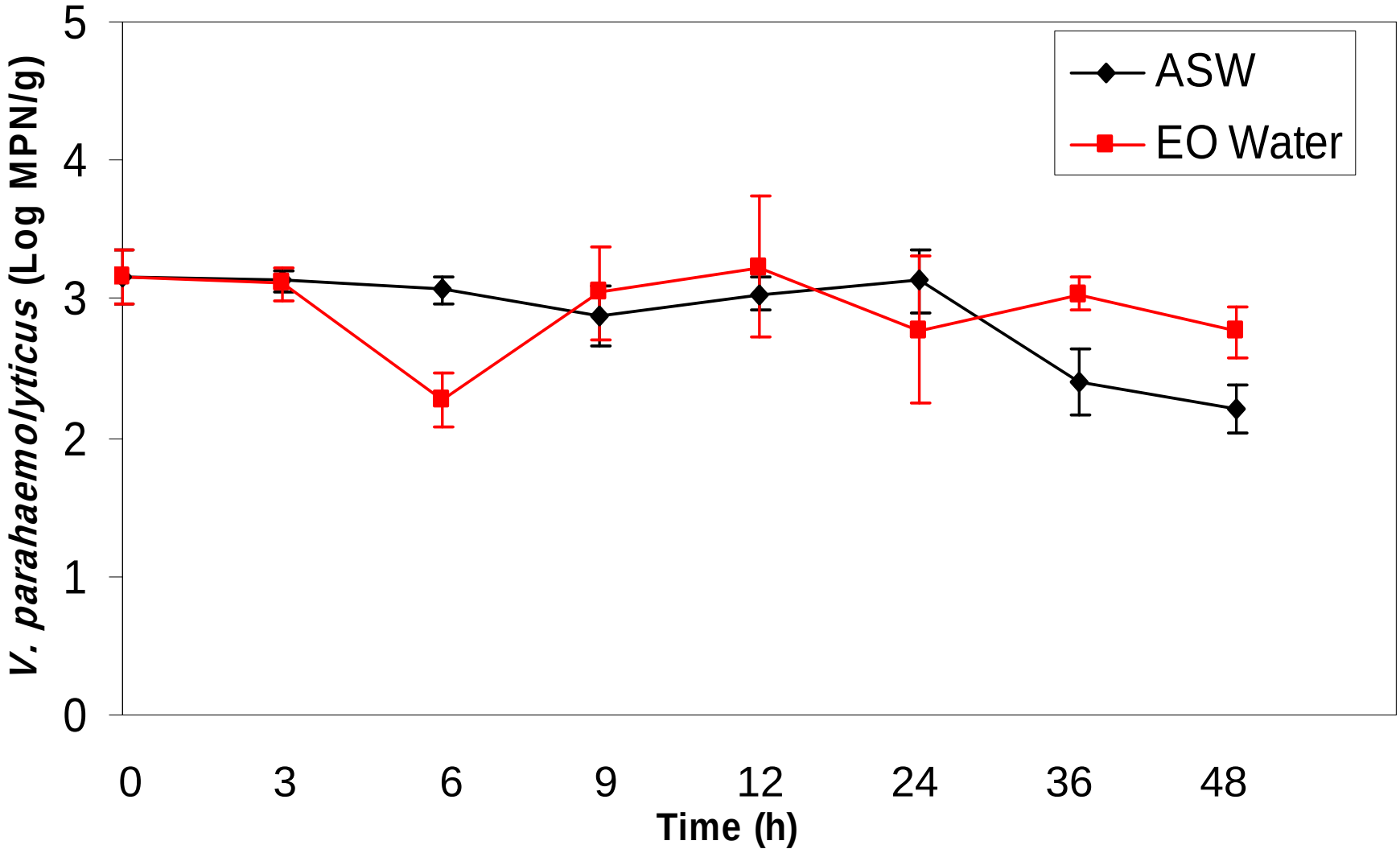
EO Water Application

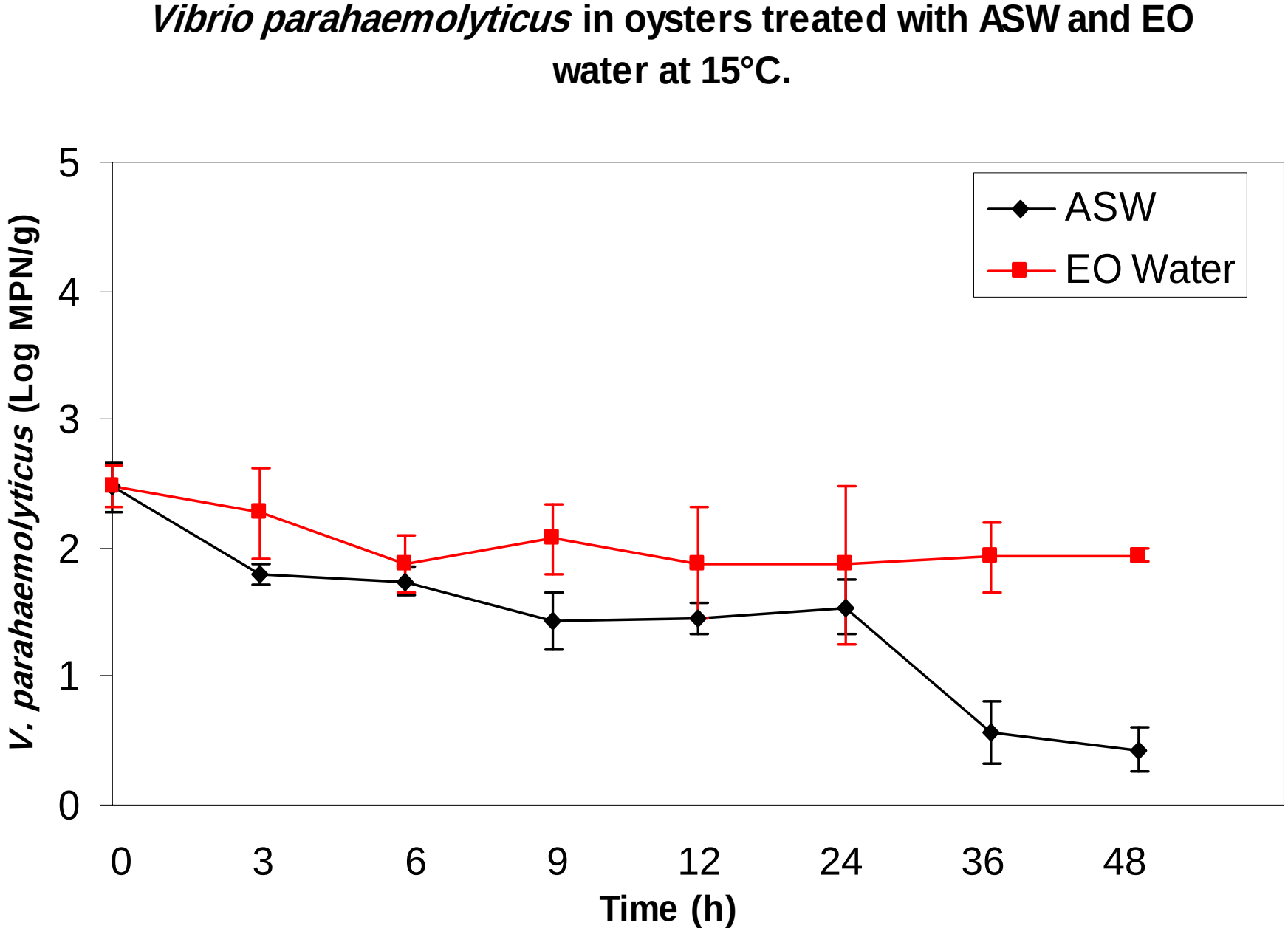
- Medical and dental professionals
  - treat wounds
  - disinfect medical equipments
- Restaurant for disinfecting utensils
- Washing fresh fruits and vegetables
- Poultry carcass washing
- Household disinfections of cutting boards and dishes
- Sanitizing food processing equipments and surfaces
- Water and sewage treatment

Depuration Studies

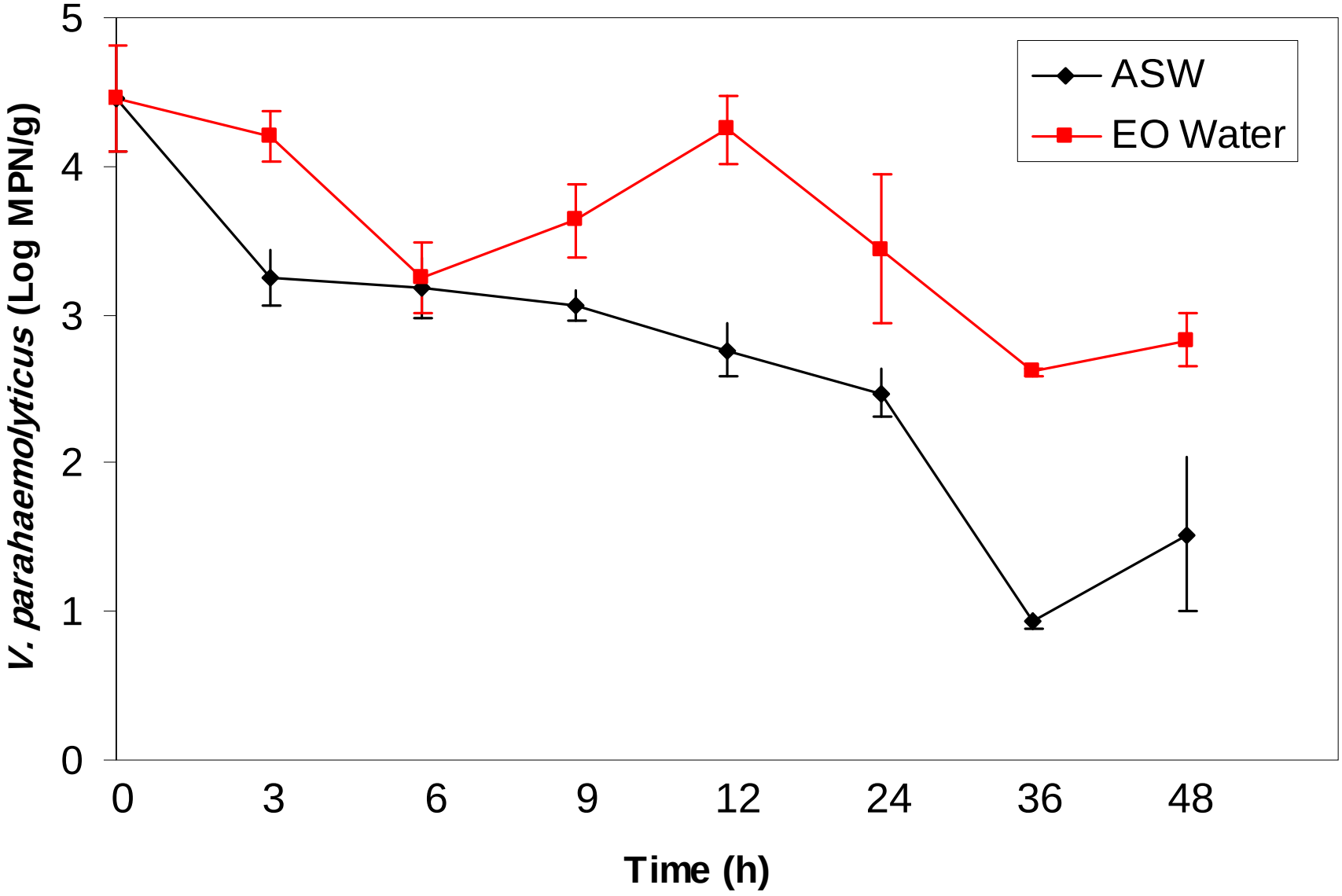
- Effects of EO water and low temperature depuration on reducing *V. parahaemolyticus* in oysters.
- Pacific oysters were inoculated with 5-strain cocktail of *V. parahaemolyticus* (10^{2-4} MPN/g).
- Oysters were placed in circulating EO water (25-30 ppm chlorine) containing 3% NaCl or artificial seawater (salinity: 29.6 ppt) at 22, 15, 10, and 5°C.
- Populations of *V. parahaemolyticus* in oysters were analyzed at 6, 12, 24, 36, and 48 h.

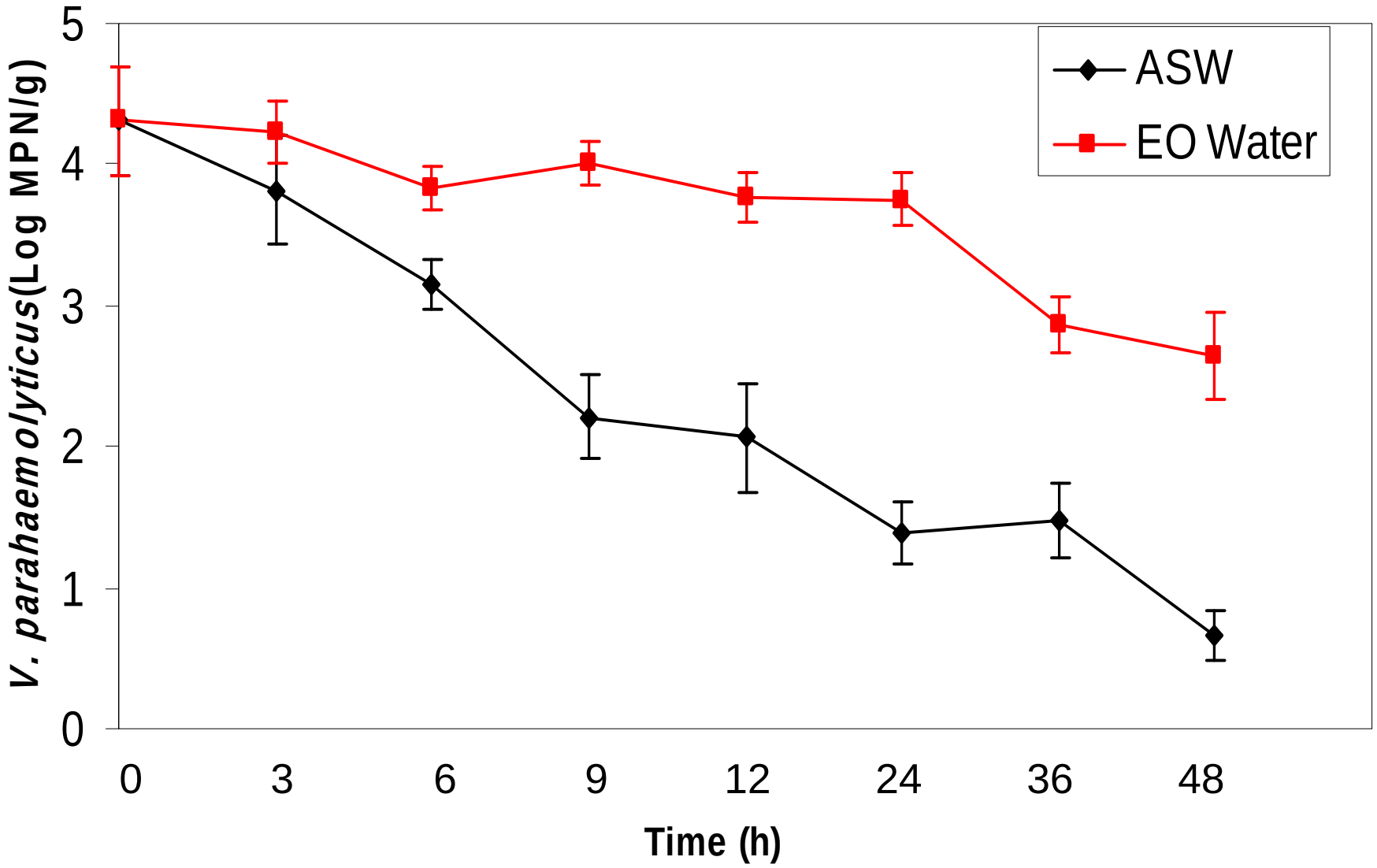
Vibrio parahaemolyticus in oysters treated with ASW and EO water at 22°C.

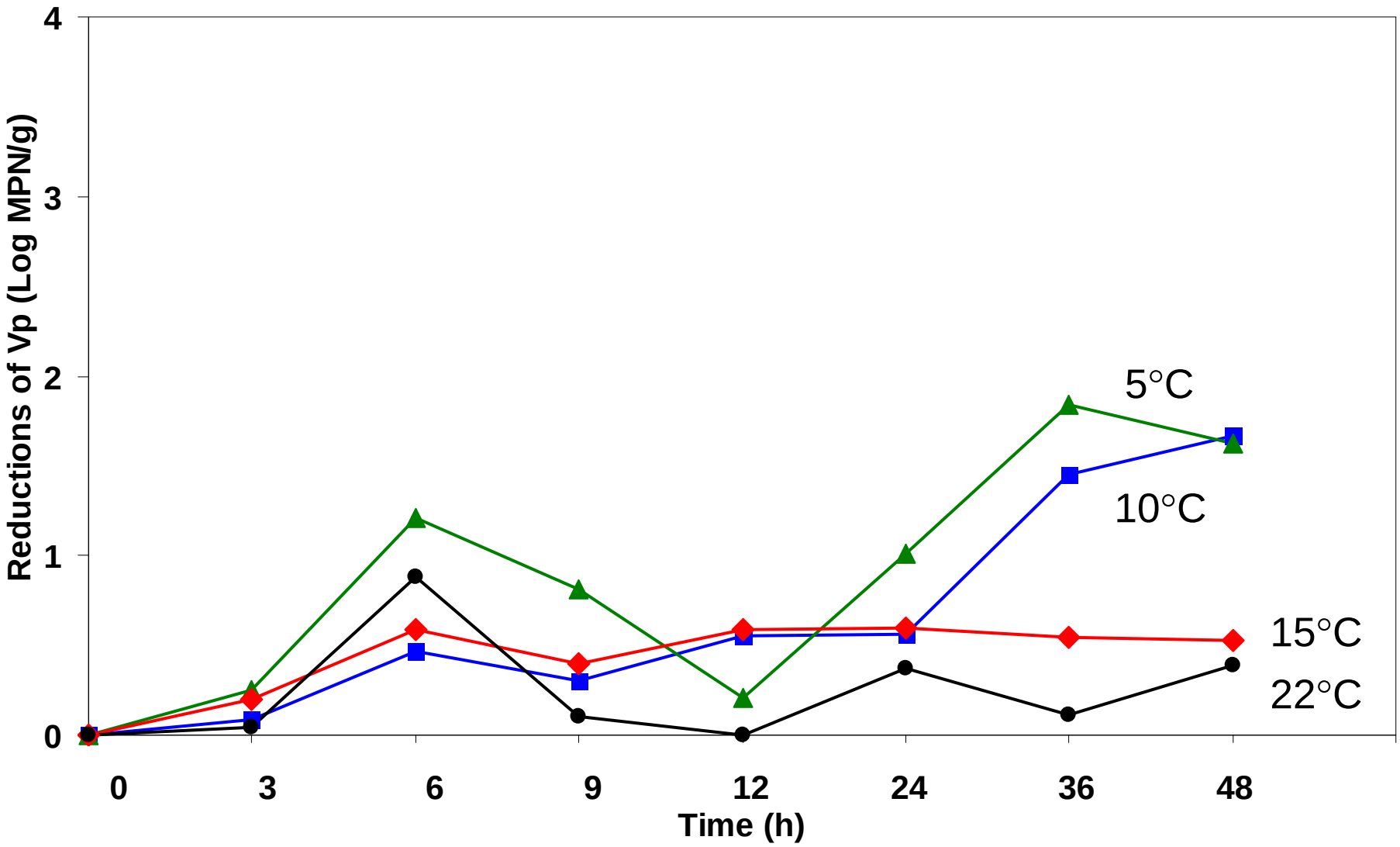



Vibrio parahaemolyticus in oysters treated with ASW and EO water at 15°C.
V. parahaemolyticus (Log MPN/g)
0
1
2
3
4
5
0
3
6
9
12
24
36
48
Time (h)
ASW
EO Water

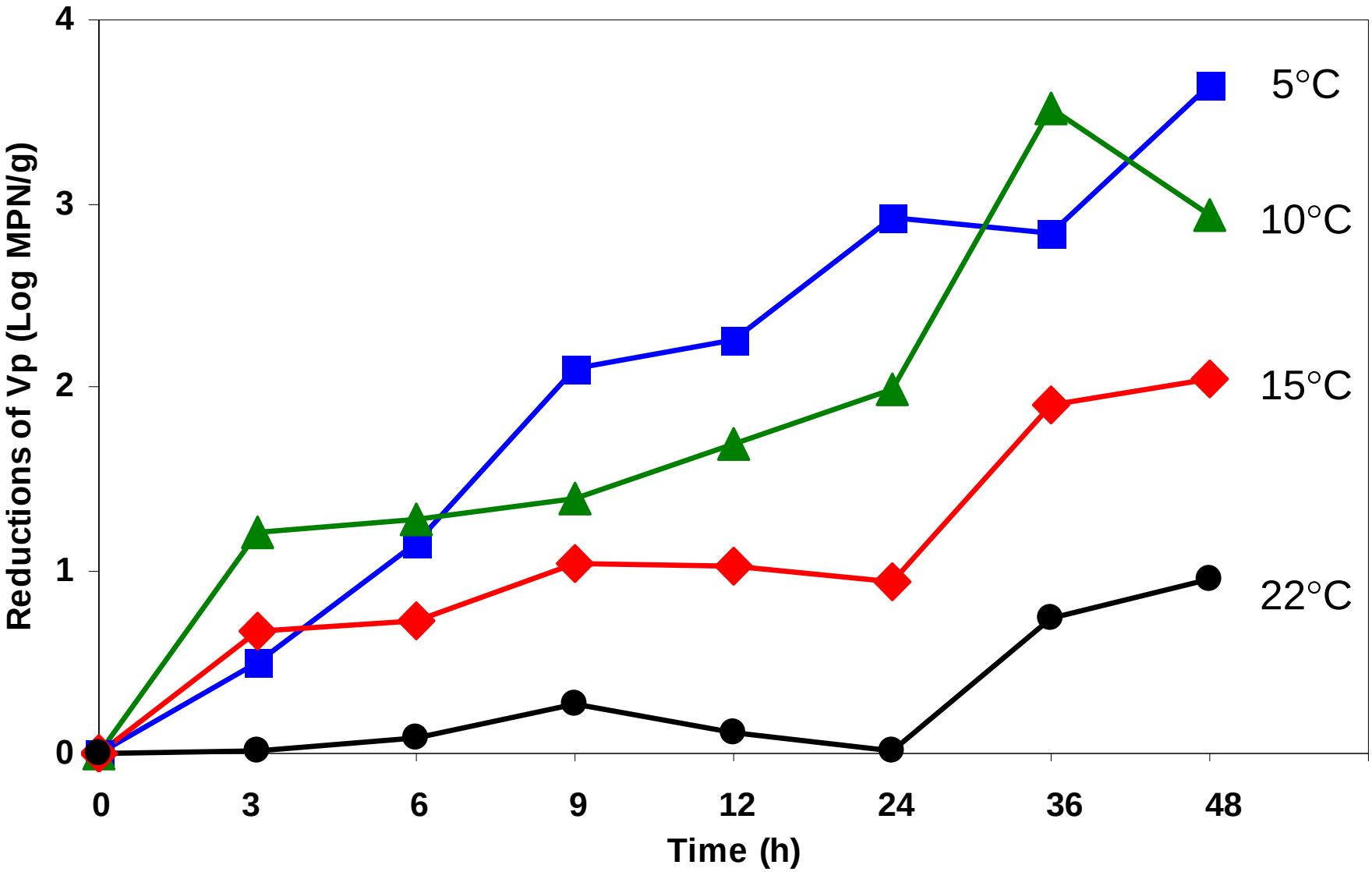
Vibrio parahaemolyticus in oysters treated with ASW and EO water at 10°C.




Vibrio parahaemolyticus in oysters treated with ASW and EO water at 5°C.
V. parahaemolyticus(Log MPN/g)
0
1
2
3
4
5
0
3
6
9
12
24
36
48
Time (h)
ASW
EO Water



Reductions of *V. parahaemolyticus* in laboratory-inoculated oysters depurated in EO water at various temperatures.



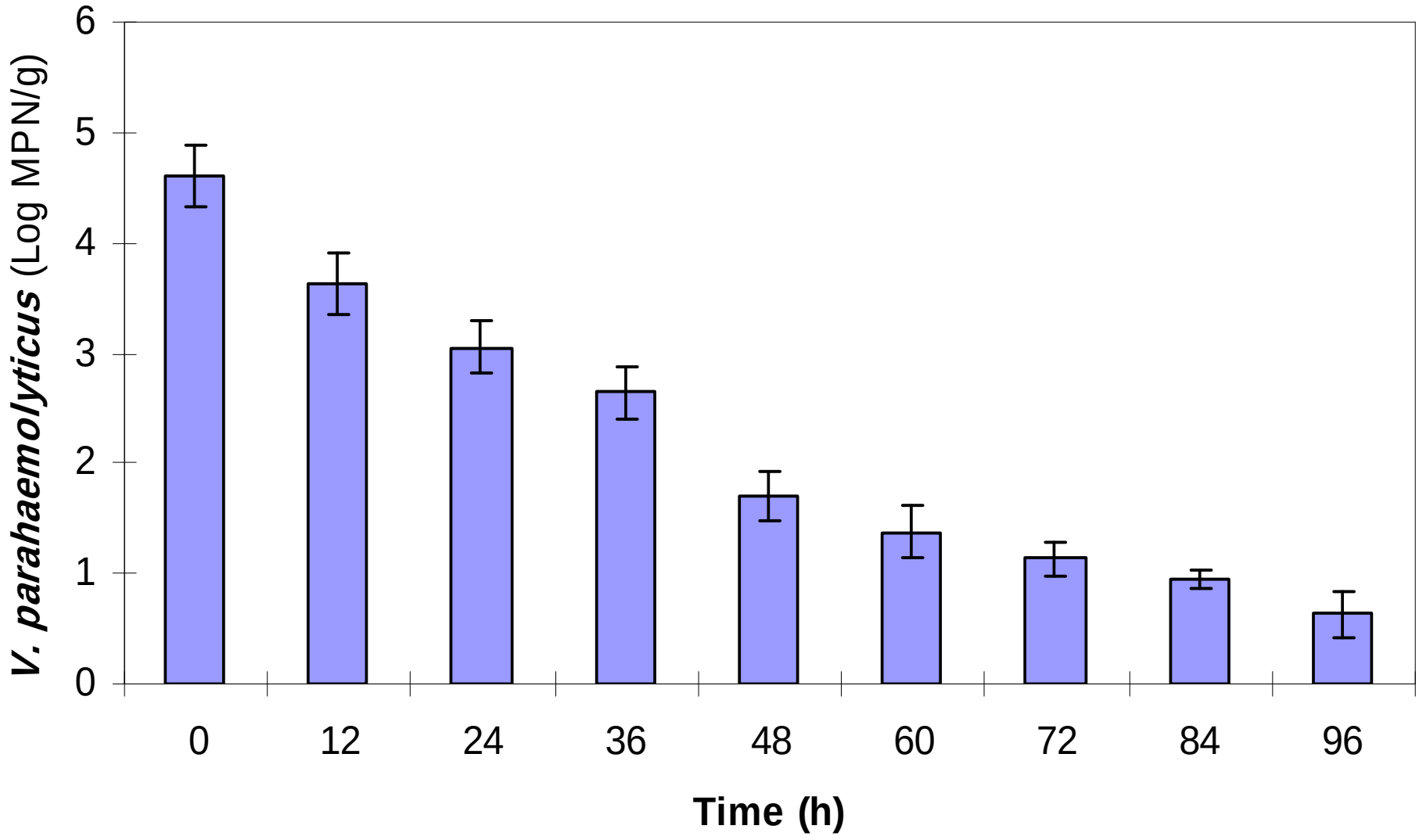
Reductions of *V. parahaemolyticus* in laboratory-inoculated oysters depurated in artificial seawater at various temperatures.

Summary

- EO water creates an unfavorable growth environment for oysters - oysters stop water-filtering activity after exposure to the water.
- Depuration with EO water (30 ppm chlorine and 3 % NaCl) was capable of reducing 90% (1.0 log MPN/g) *V. parahaemolyticus* in oysters at room temperature.
- Decreasing water temperatures did not enhance the efficacy of EO water in reducing *V. parahaemolyticus*.
- Depuration with ASW at temperatures below 15°C greatly increased the efficacy in decontamination.

Low Temperature Depuration of Oysters with Naturally Accumulated *V. parahaemolyticus*

- Oysters were collected on July 24, 2006 from Hood Canal of Washington after the recent outbreak.
- Oysters were left at ambient temperature for about 18 h to allow growth of *V. parahaemolyticus* in oysters before being depurated in artificial seawater at 5°C.



V. parahaemolyticus in naturally contaminated oysters depurated in artificial seawater at 5°C.

Conclusions

- *V. parahaemolyticus* in oysters could be reduced by about 3.0 log MPN/g after 48 h of ASW depuration at 5°C.
- Increasing depuration at 5°C to 96 h increased the reduction of *V. parahaemolyticus* in oysters to nearly 4.0 log MPN/g.
- More studies are needed to validate the efficacy of low temperature depuration in reducing naturally accumulated *V. parahaemolyticus* in oysters in a commercial scale operating system.

Acknowledgements

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