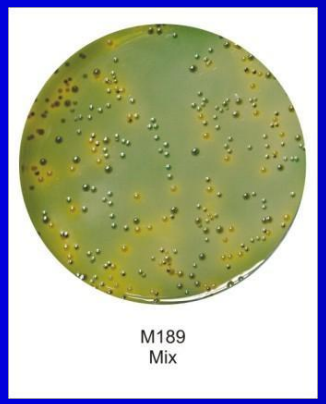
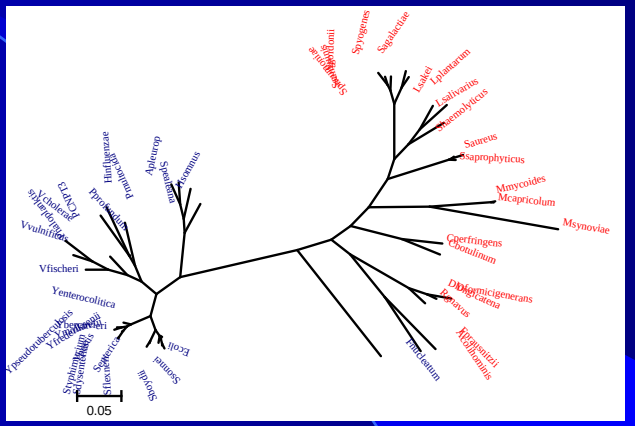
The Big Three Vibrio Pathogens: A Study in Contrast





Valerie J. Harwood, Ph.D.

Department of Integrative Biology

University of South Florida, Tampa FL

AMERICAN SOCIETY FOR MICROBIOLOGY

USF UNIVERSITY OF SOUTH FLORIDA

Northeastern Branch ASM October 20, 2015

Outline

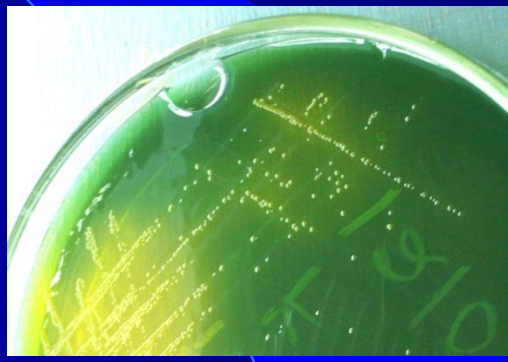
I. Phylogeny (Genetic Relatedness)

II. Morphology & Physiology

III. Ecology (where they live)

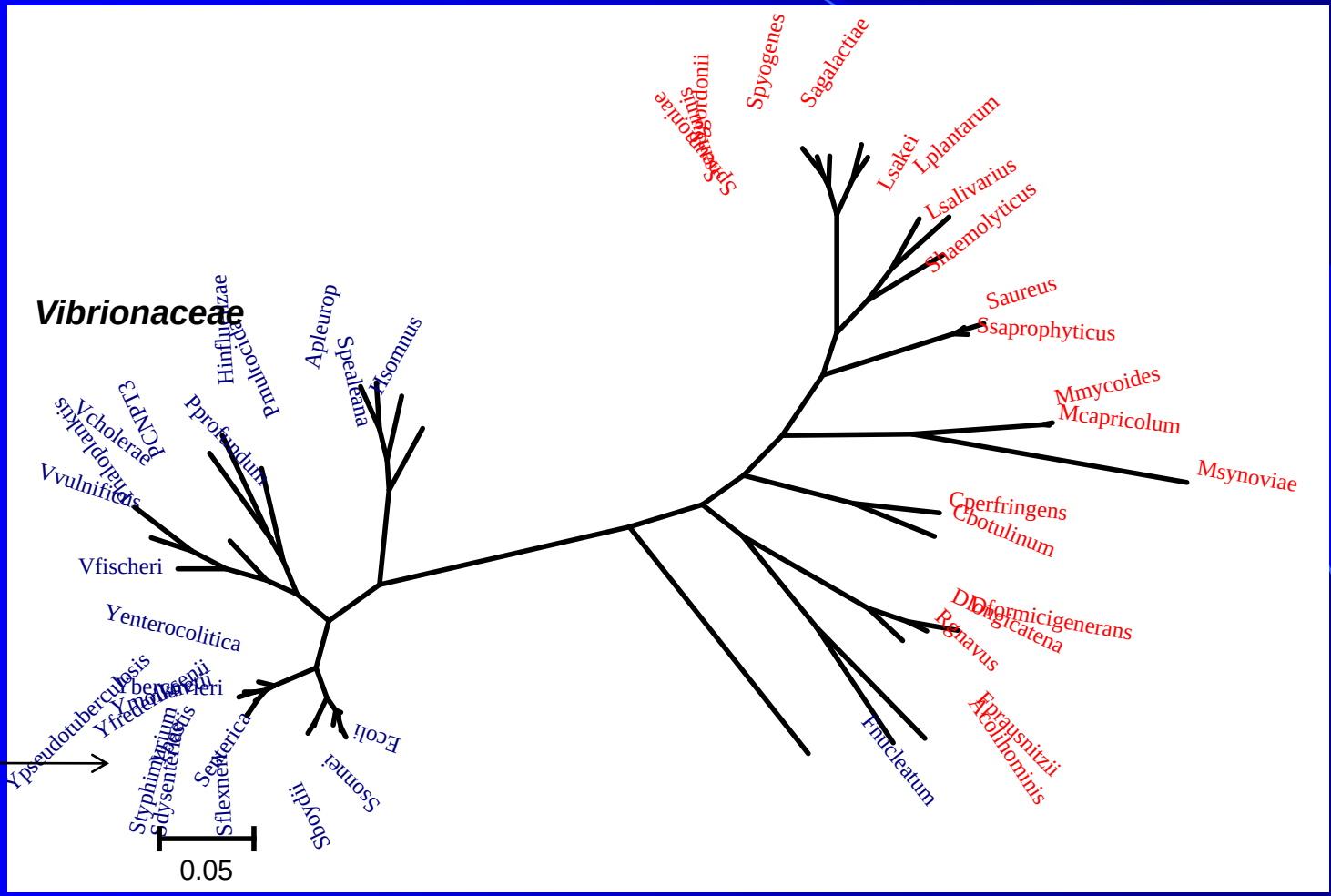
IV. Epidemiology & Pathogenesis

V. Focus on Cholera



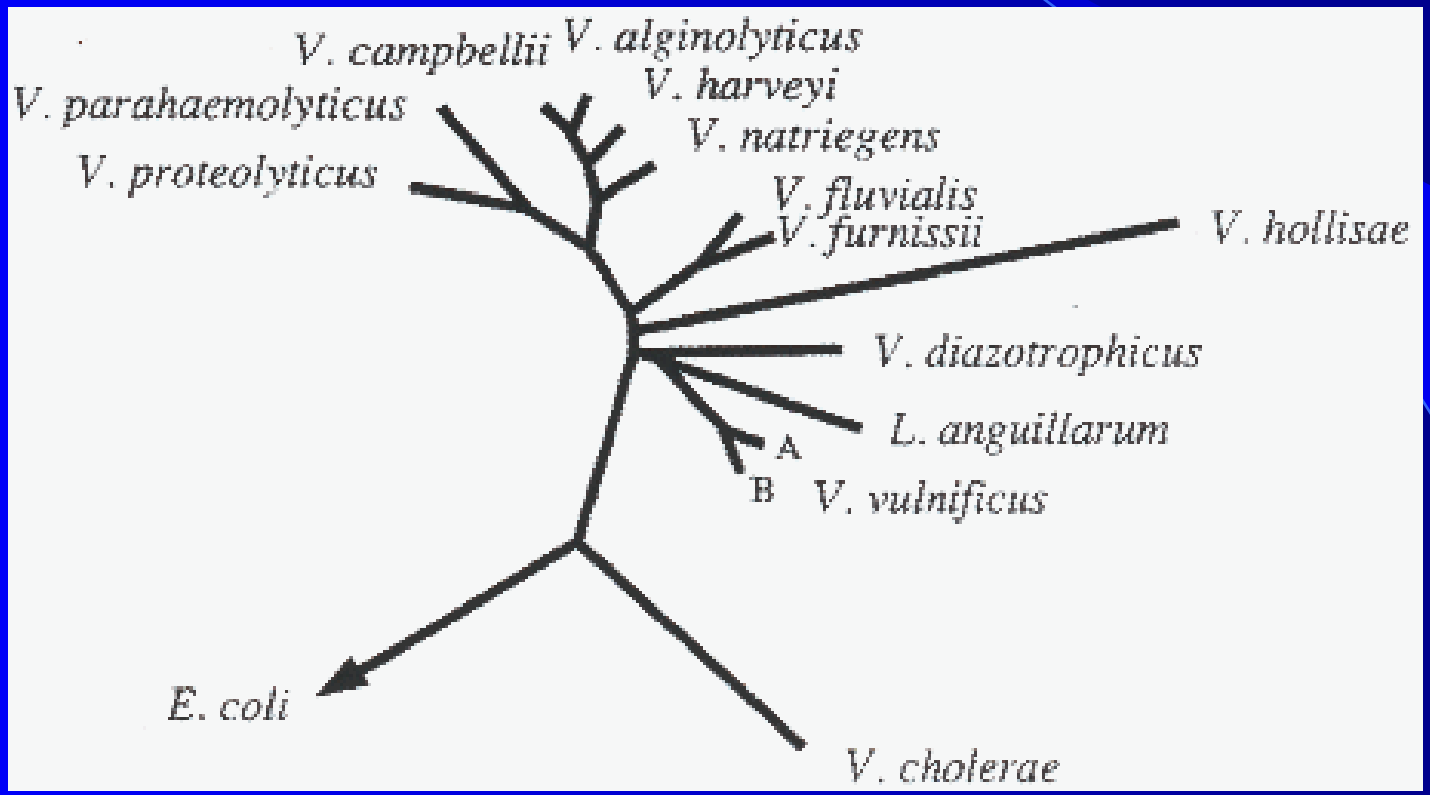
V. cholerae **colonies on TCBS agar**

Vibrio spp. Family *Vibrionaceae*



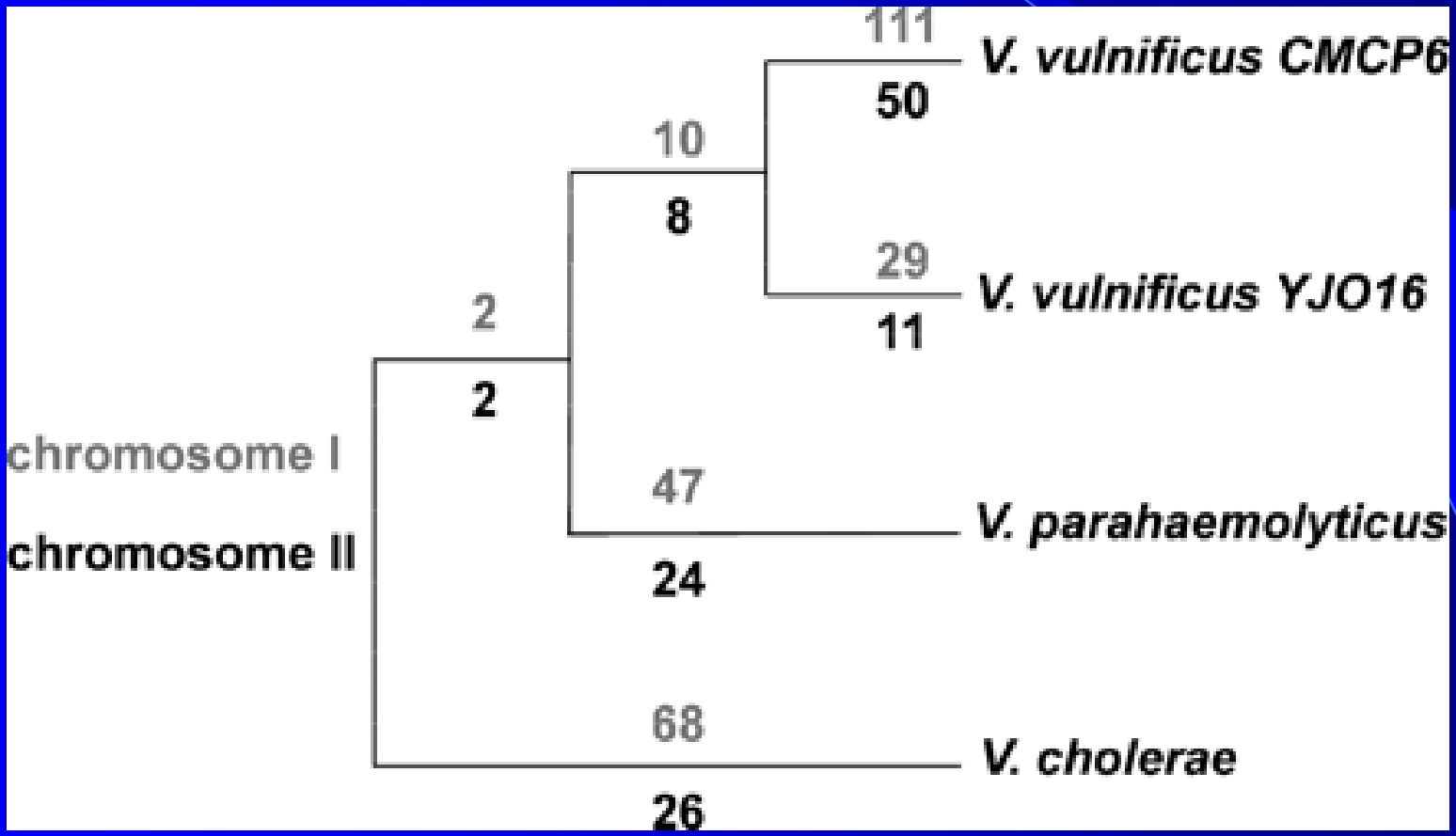
Enterobacteriaceae
i.e. E. coli, Salmonella

The Genus *Vibrio*



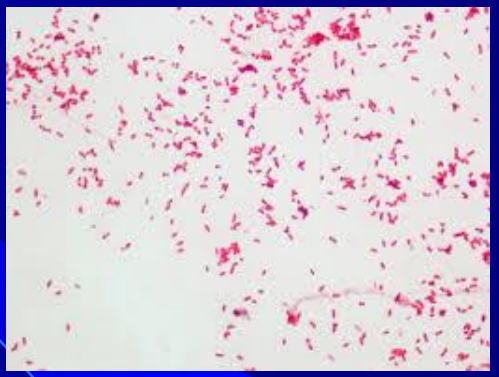
http://www.statensnet.dk/pligtarkiv/fremvis.pl?vaerkid=5079&reprid=0&filid=36&iarkiv=1

The Big Three Human Pathogenic *Vibrio* species

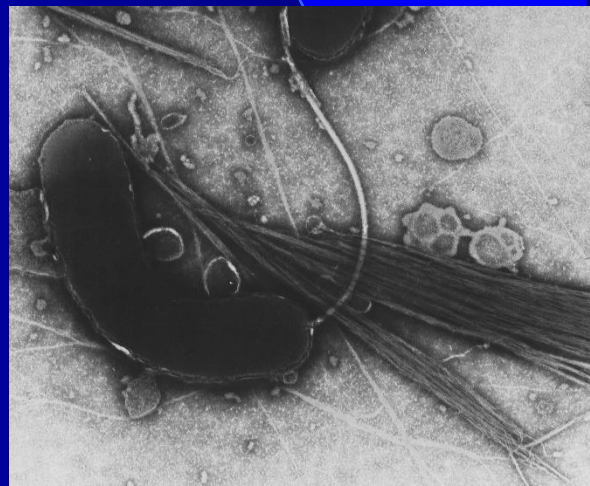


Lerat & Ochman 2010 Nuc Acid Res 33:3125

Shared *Vibrio* Characteristics



- Gram-negative, curved rods, oxidase +
- Adapted to estuarine conditions – euryhaline
- Natural inhabitants of estuarine waters
- Motile by flagella and pili
- Chemoorganoheterotrophic
- Facultative anaerobes
- Biofilm formation



Comparing the Big Three: Salinity

Vibrio cholerae

- Euryhalinne
- No salt required
- Uncommon above 10 ppt
- Survives in fresh water
- Threat to drinking water

Vibrio vulnificus

- Obligate halophile
- Optimum ~20 ppt
- Tolerates 5 ppt
- Not a freshwater pathogen

Vibrio parahaemolyticus

- Obligate halophile
- Optimum ~30 ppt
- Tolerates 20 ppt.
- Not a freshwater pathogen

Vibrio cholerae in the Environment is Constrained by Salinity

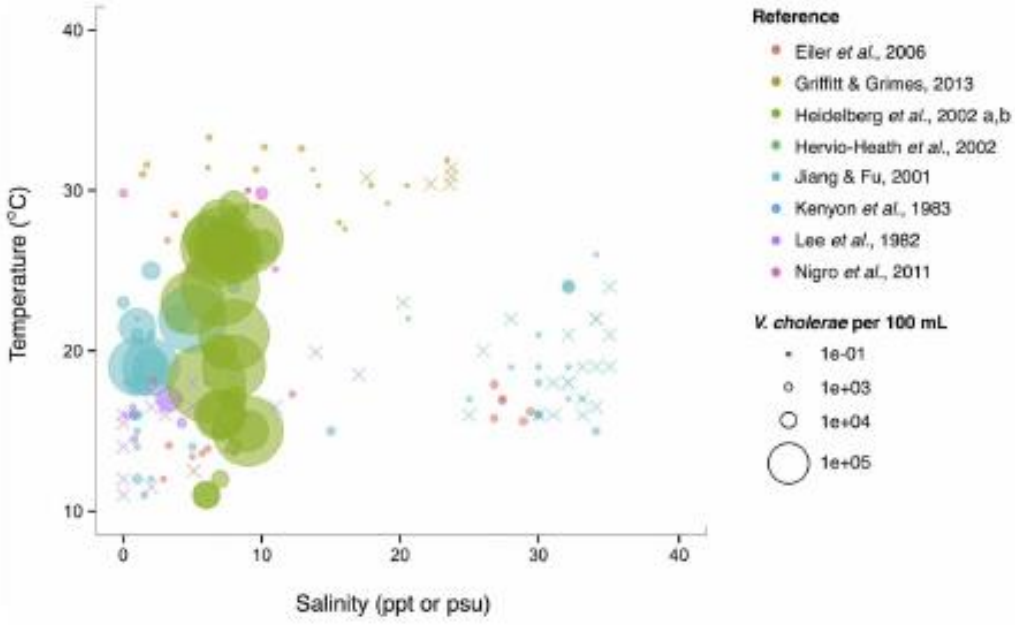


FIGURE 5 | *V. cholerae* favors lower salinity and occupies a broad temperature range. *V. cholerae* concentrations, i.e., MPN-estimated CFU or molecular marker gene copies per 100 mL, reported in different studies are plotted against the temperature (°C) and salinity values (ppt or psu) at which they were found. All studies report *V. cholerae*, including O1/O139 and non-O1/non-O139, except for Heidelberg et al. (2002a,b); DeLoney-Marino et al. (2003), whose genetic marker detected *V. cholerae*/*V. mimicus*. Circle (○) sizes correspond to concentrations, but note the breaks are scaled for clearer visualization, and not linearly. (×) indicates no *V. cholerae* found in that sample.

Takemura et al 2014. Frontiers in Microbiology 5:1-26

Comparing the Big Three: Temperature

Vibrio cholerae

- Broad range
- 10° C - 35° C in water
- 37 ° C in host

Vibrio vulnificus

- Grows poorly under 20° C
- Not culturable at less than 10-15 ° C
- 37 ° C in host
- Temp is determinant

Vibrio parahaemolyticus

- Grows poorly under 20° C
- Optimum >30 ° C
- 37 ° C in host
- Temp is determinant

Vibrio parahaemolyticus Is Constrained by Temperature

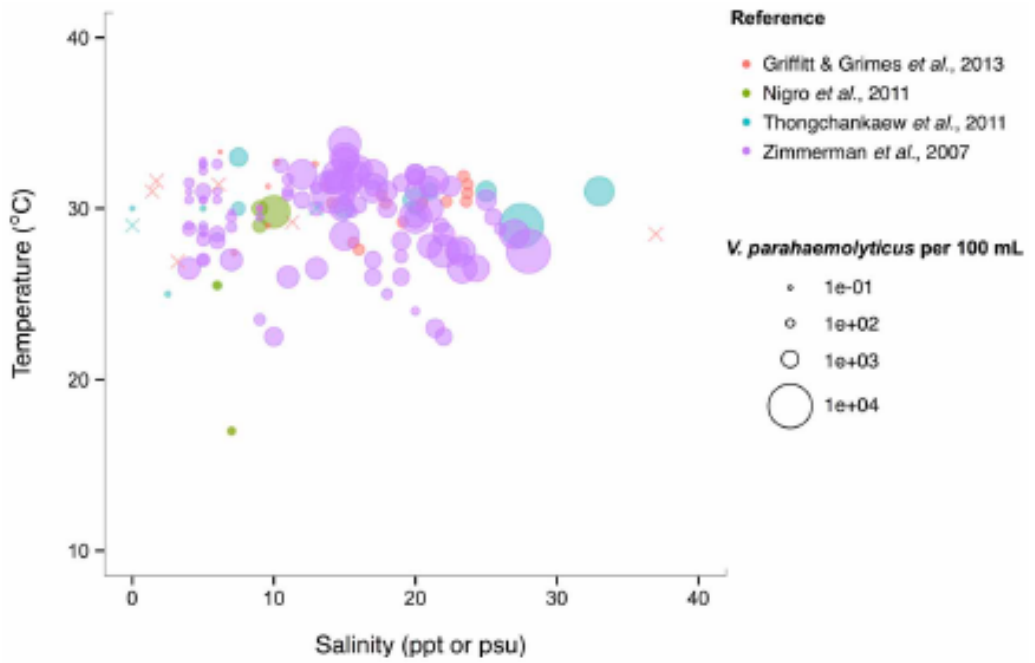


FIGURE 6 | *V. parahaemolyticus* favors high temperatures but is relatively unconstrained by salinity. Concentrations, i.e., MPN-estimated CFU or molecular marker gene copies per 100 mL, reported in different studies are plotted against the temperature (°C) and salinity values (ppt or psu) at which they were found in bulk water samples. Circle (○) correspond to concentrations, but note the breaks are scaled for clearer visualization, and not linearly. (×) indicates no *V. parahaemolyticus* found in that sample.

Takemura et al 2014. Frontiers in Microbiology 5:1-26

Comparing the Big Three: Virulence

Vibrio cholerae

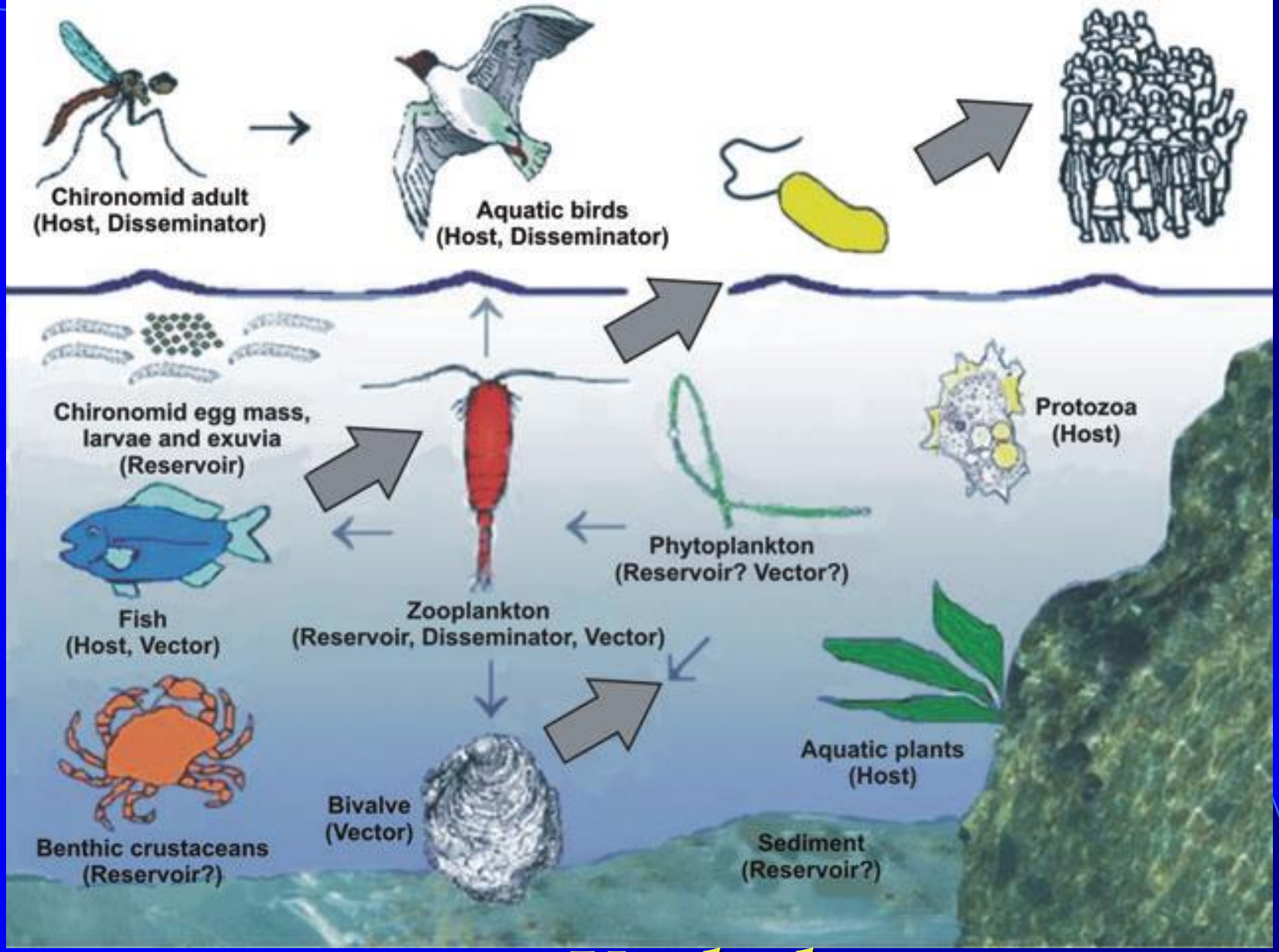
- Epidemic cholera
- O1 & O139
- Deadly diarrhea
- CTX Toxin
- Poor sanitation

Vibrio vulnificus

- Wound infections, AGI, septicemia
- Biotype I
- Capsule
- Raw shellfish consumption, open wound, liver dysfunction

Vibrio parahaemolyticus

- AGI
- O3:K6
- Infrequent outbreaks
- TDH, TRH
- Raw fish or shellfish consumption



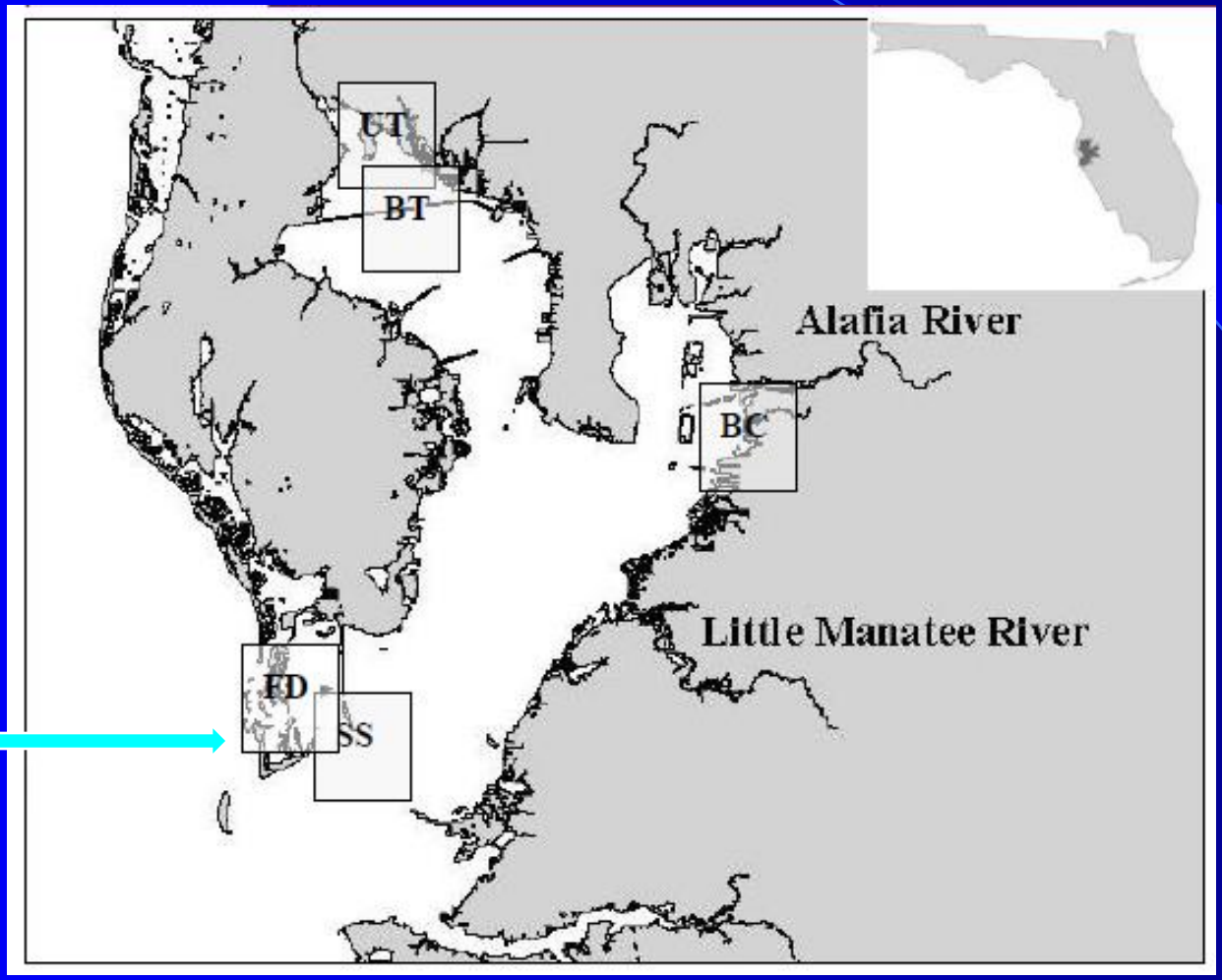
Environmental Reservoirs of ***V. cholerae***.... Vezulli et al

2010 Environmental Microbiology Reports 2:27-33

Vibrio spp. Can Become "Viable but Nonculturable"

- **VBNC represents a "dormant" or stressed state – but may still be infectious**
- **Hard to detect**
- **Makes surveillance more difficult – Quantitative PCR**

Focusing on *Vibrio vulnificus* Ecology in the Gulf of Mexico



Shellfishing permitted

Evidence for VBNC *V. vulnificus* in Tampa Bay Waters

Percent of water samples positive for *Vibrio vulnificus*

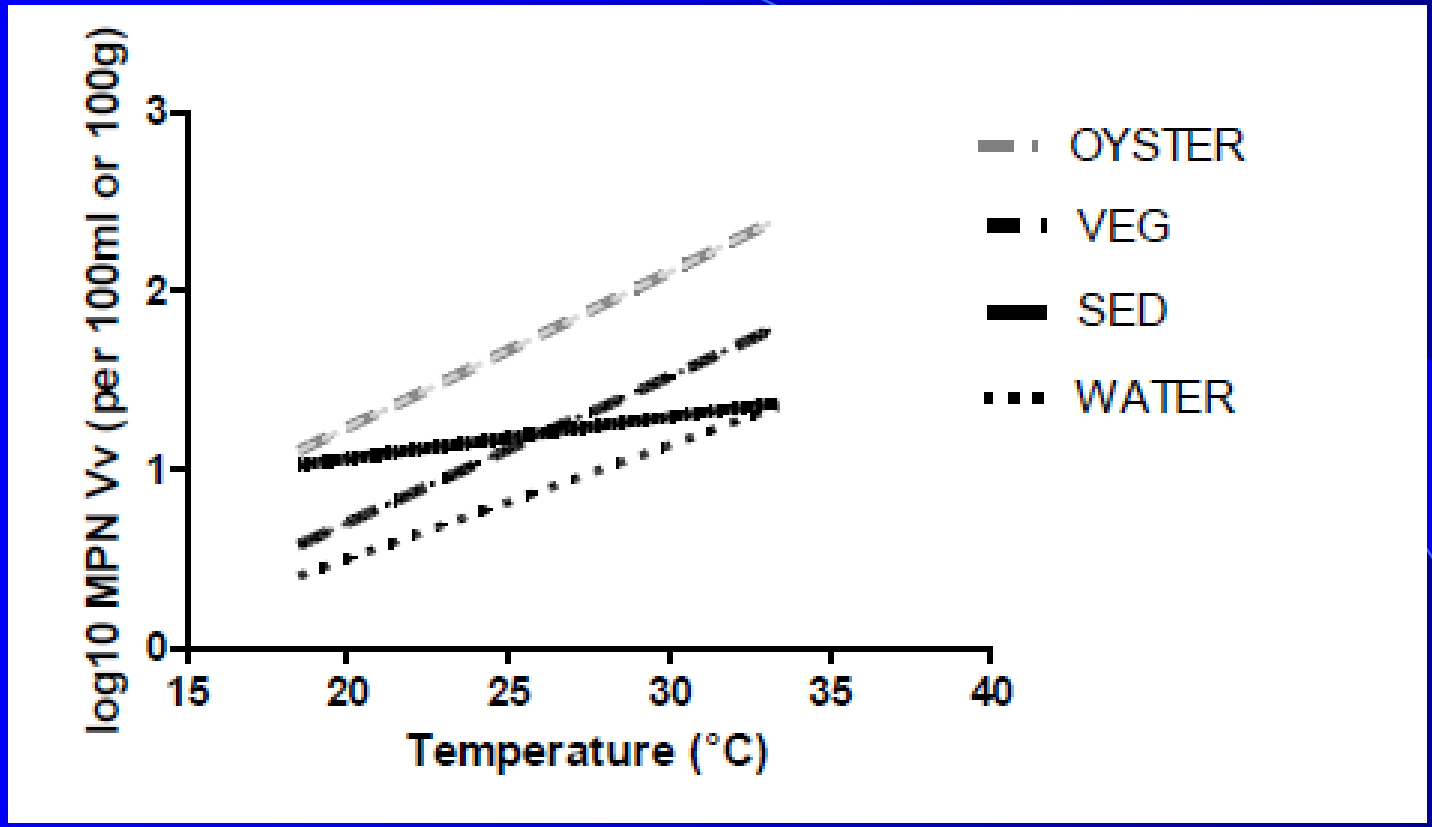
TABLE 1 Frequency of *V. vulnificus* detection by culture and qPCR per site and matrix

	Frequency of detection (%)[a]					
	Culture				qPCR	
Site/matrix	Water	Sediment	Oysters	Vegetation	(water)	Combined
BC	75	92	83	67	92	79
FD	42	33	50	50	92	44
SS	17	8	50	25	67	25
BT	50	58	92	58	83	65
UT	33	58	75	58	92	56
All sites combined ($n = 60$)	43	50	70	52	85	54

[a] Frequency is expressed as the percentage of positive detections ($n = 12$ sample events/site). The "Combined" column includes culture data from all matrices.

Chase, Young and Harwood 2015 Appl and Environ Microbiol

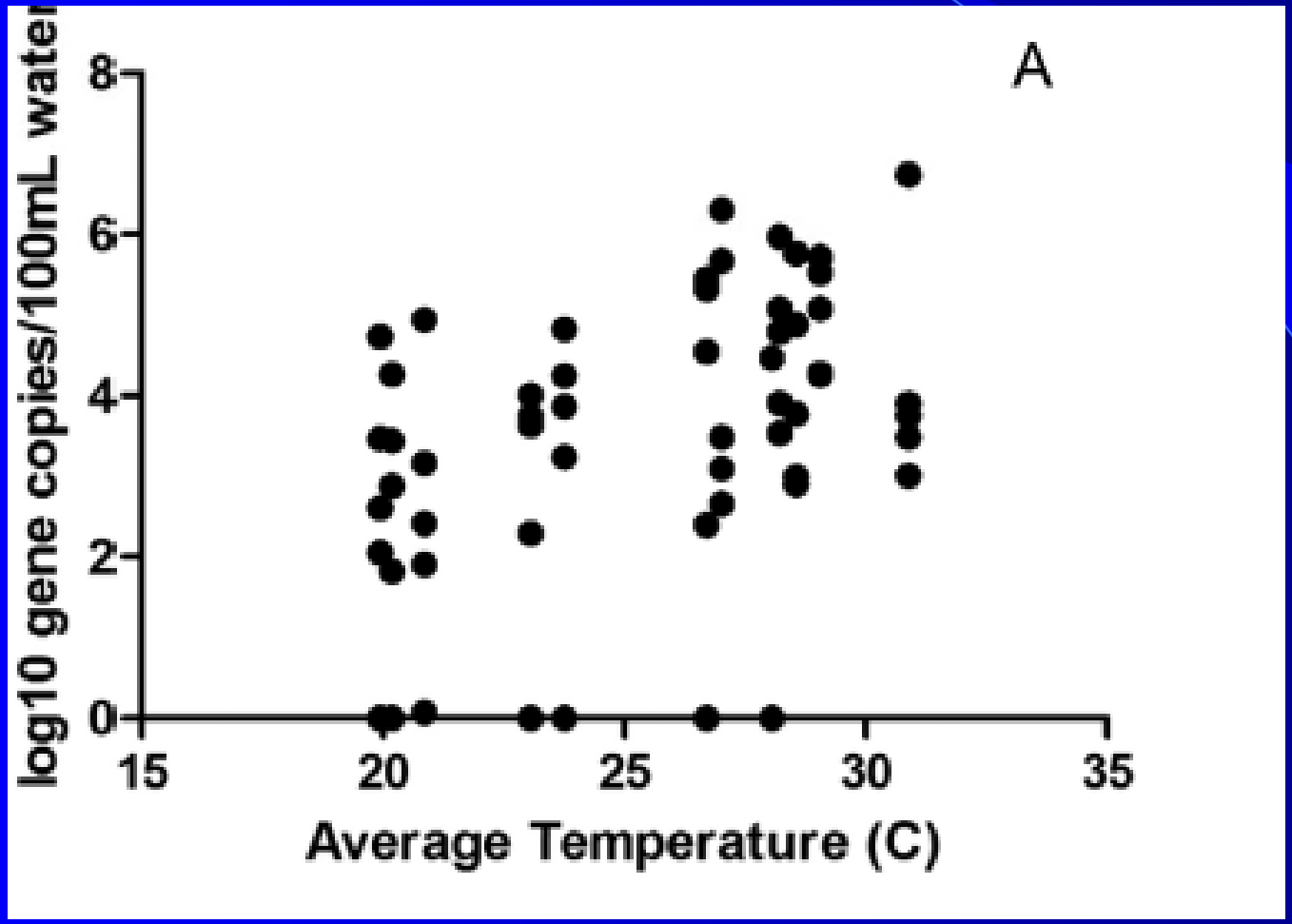
Culturable *V. vulnificus* vs. Temperature



Correlation Coefficient
Oyster 0.328
Vegetation 0.256
Sediment 0.122
Water 0.262

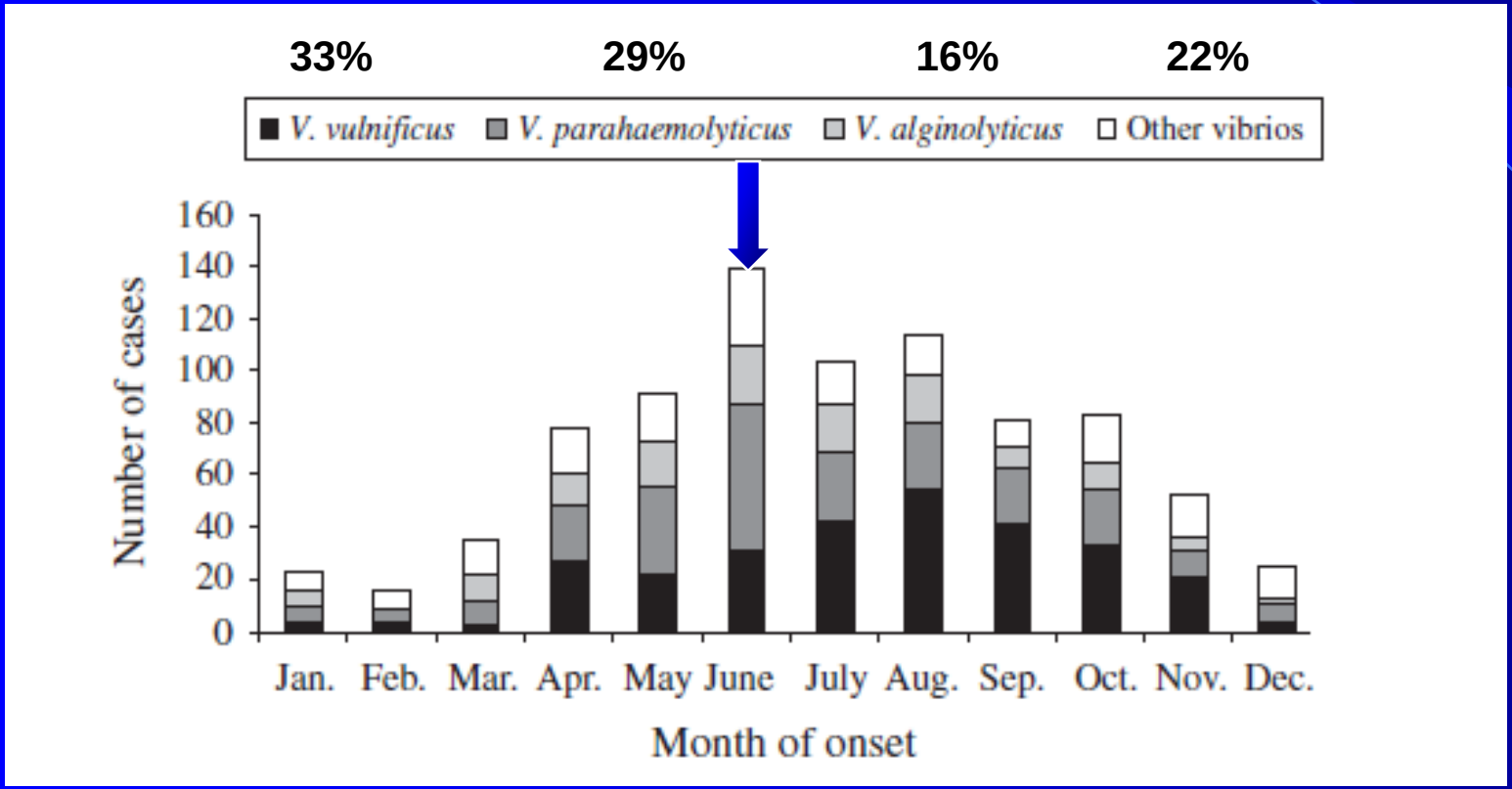
Significant interaction between sample type and temperature

QPCR of *vvhA* in Water Samples: Gene Copies vs. Water Temperature



P = 0.012
R = 0.321

Linking Ecology to Disease:
Temperature Matters in *Vibrio* Infection Incidence!



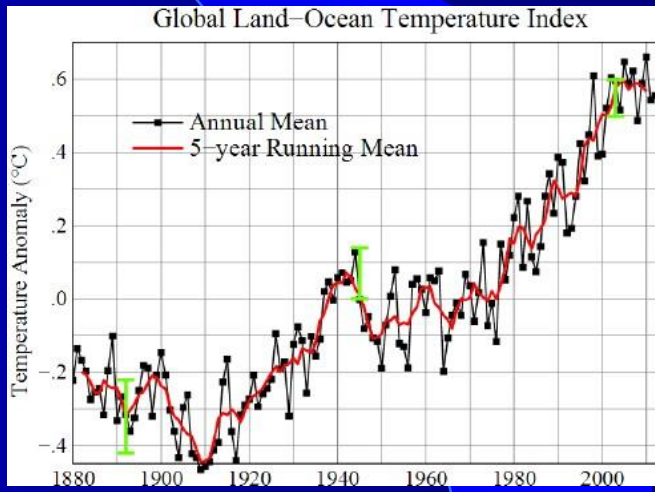
Florida
1998-2007
834 *Vibrio* Infections

***V. cholerae* non-O1 = 4th (Other vibrios)**

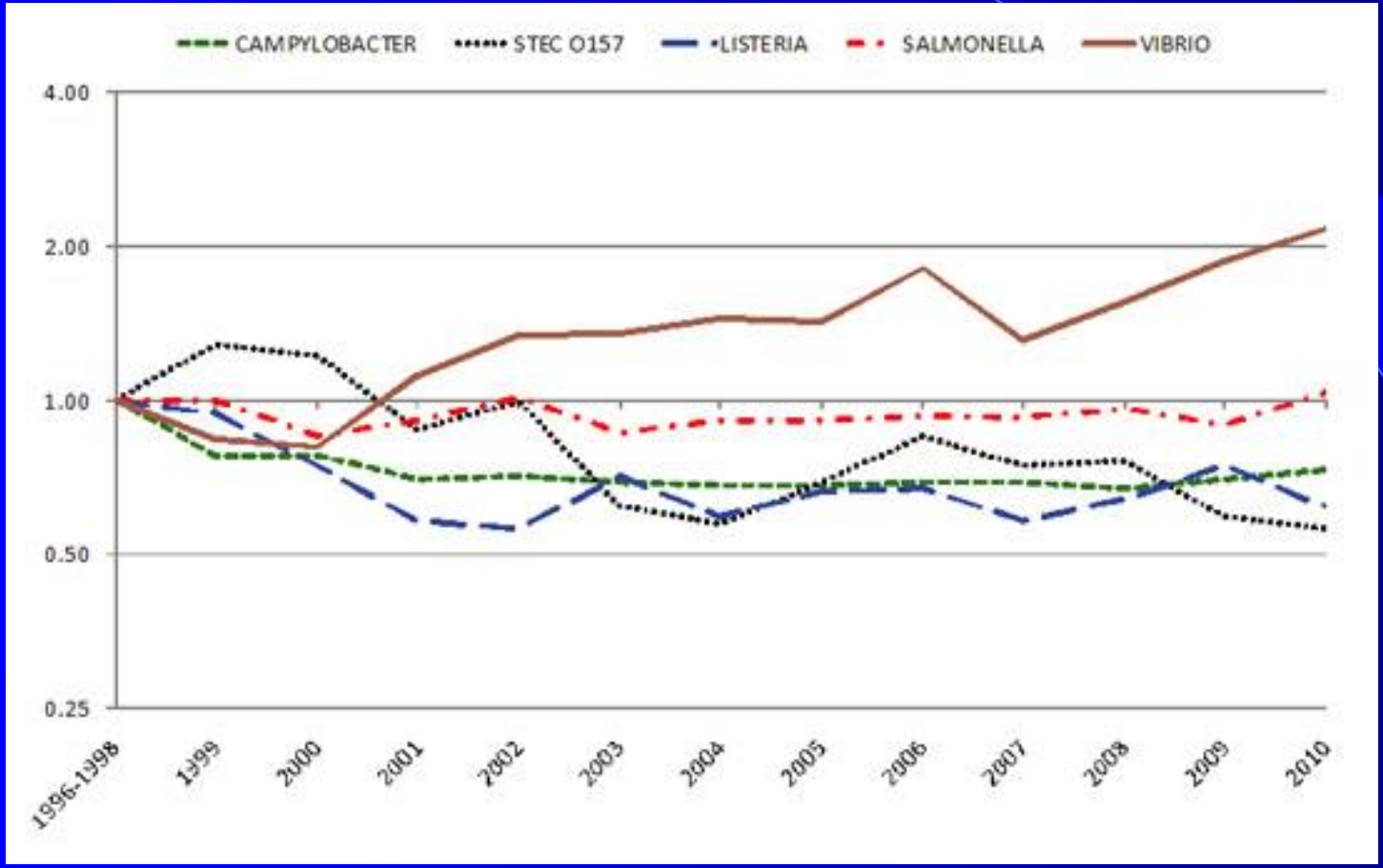
Weis et al 2011 Epidemiol Infect 139:591-598

Why Care About Vibriosis Other than Cholera?

- **Incidence of vibriosis in Florida is 0.4/100,000 annually (double national average)**
- **High case fatality rate (CFR) – *V. vulnificus***
- **National CFR for vibriosis is 3.6% (compare with ~0.4% for salmonellosis)**
- **Florida CFR for vibriosis is 10%!**

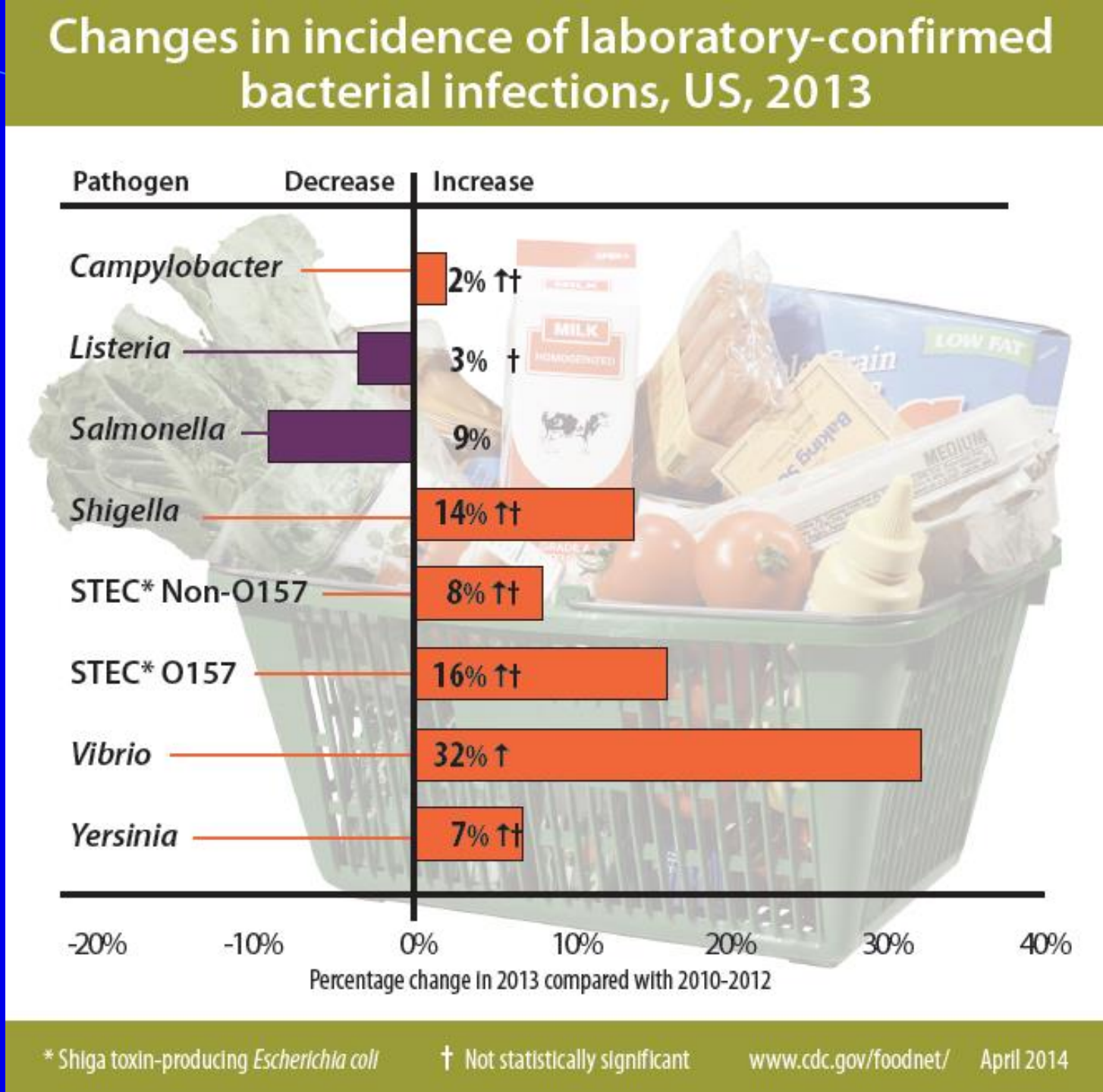


CDC – Trends in Foodborne Illness



http://www.cdc.gov/foodborneburden/trends-in-foodborne-illness.html

Incidence of vibriosis continues to increase; CDC estimates 8,000 cases in U.S. per year



Wound Infections and Acute Gastroenteritis Are Most Common

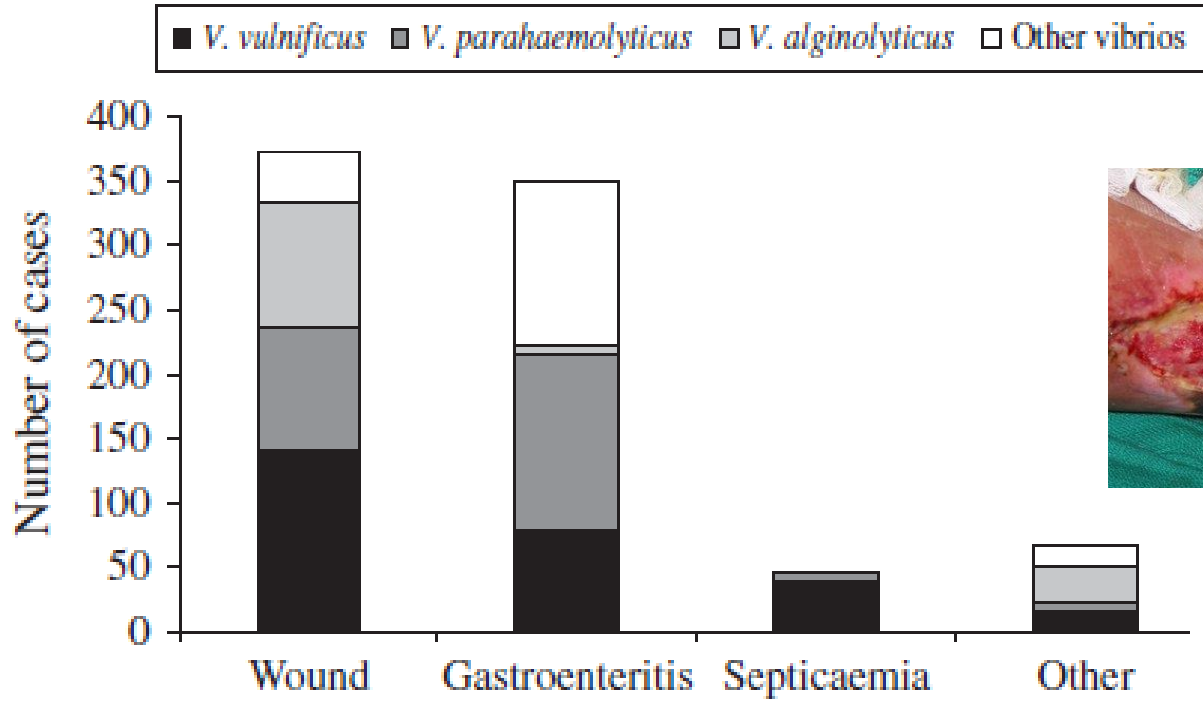


Fig. 3. Clinical syndromes of vibriosis by species, Florida, 1998–2007.

Exposure Factors for Vibriosis in Florida

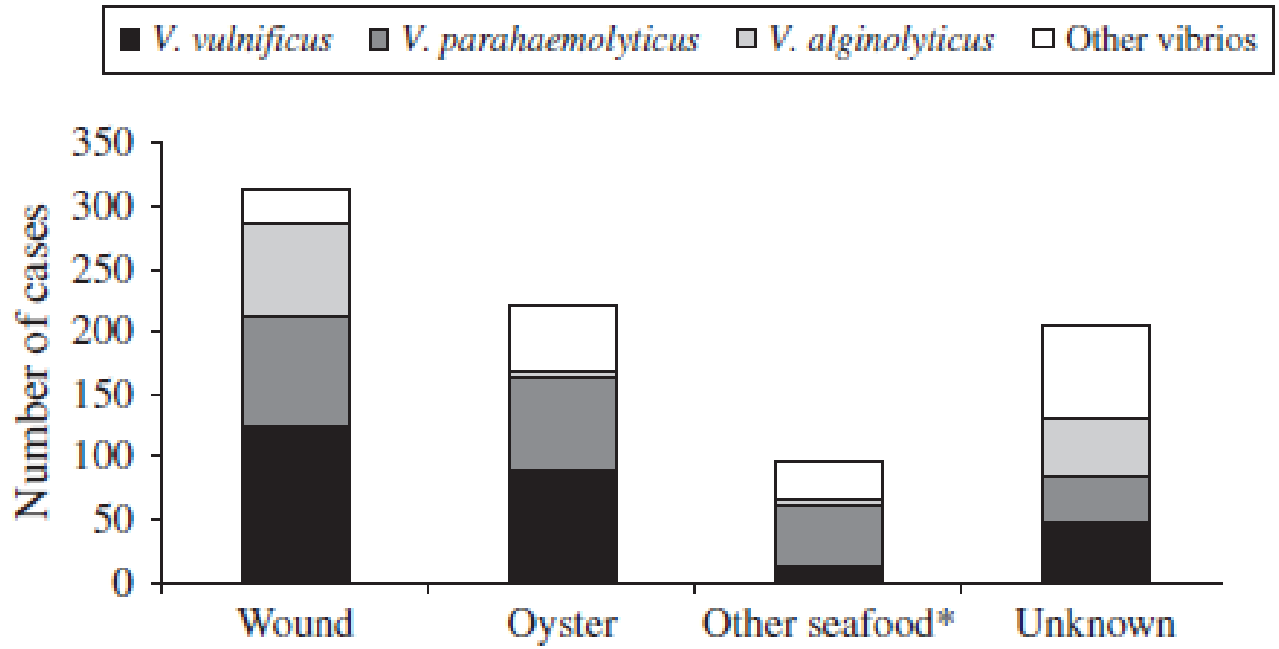
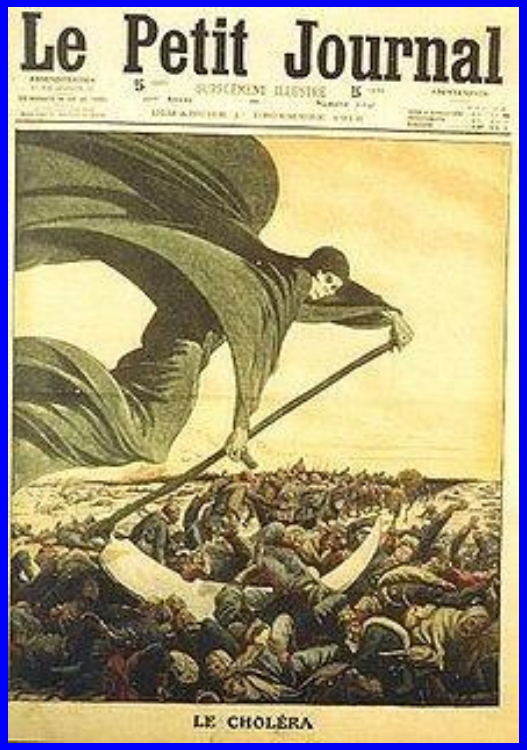
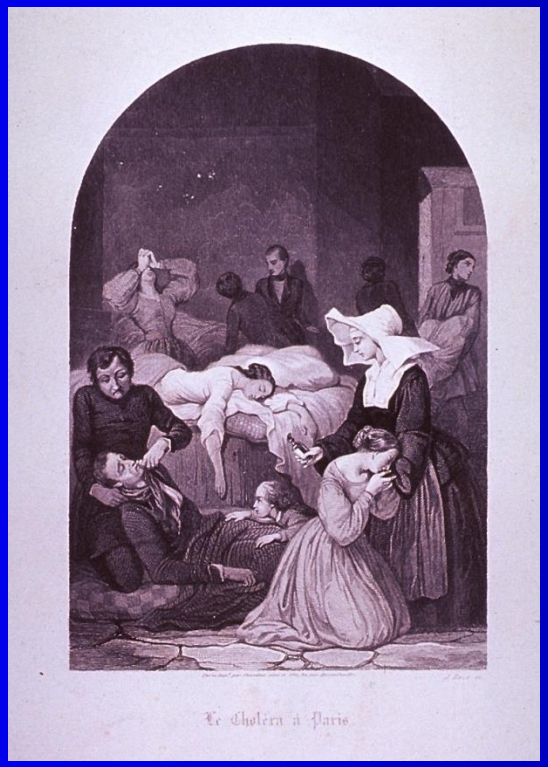


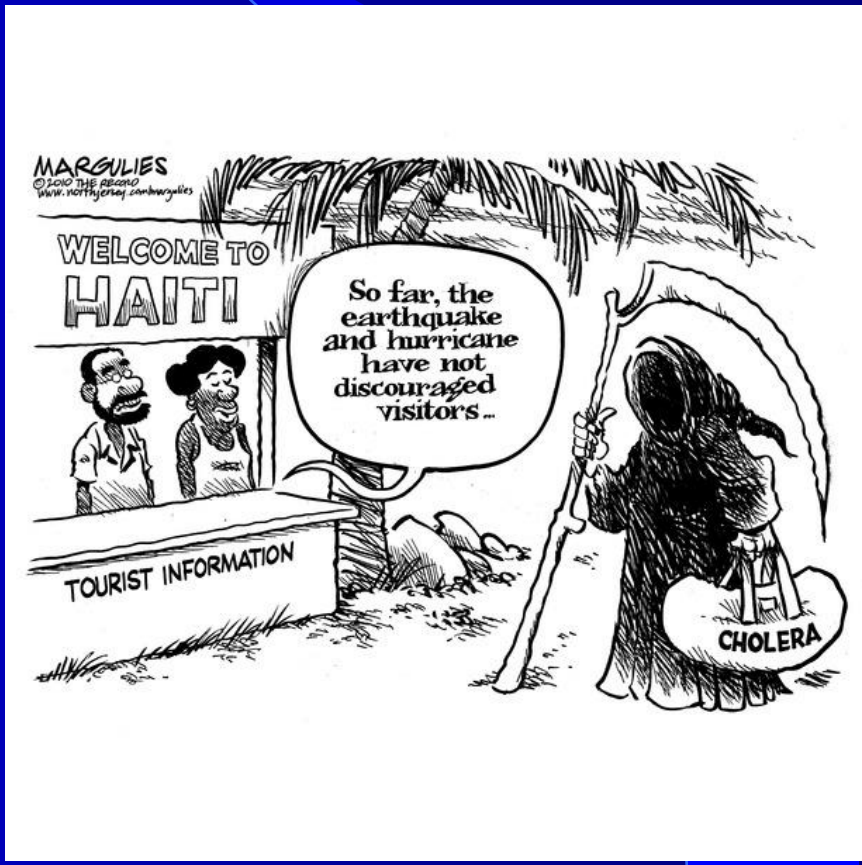
Fig. 4. Exposures associated with cases of vibriosis by species, Florida, 1988–2007. * Other seafood includes clams, mussels, shrimp, crab, fish, etc.

Illness from oysters has dropped but wound infections have increased

Cholera Has a Bad Reputation… for a Good Reason







Pandemic Cholera

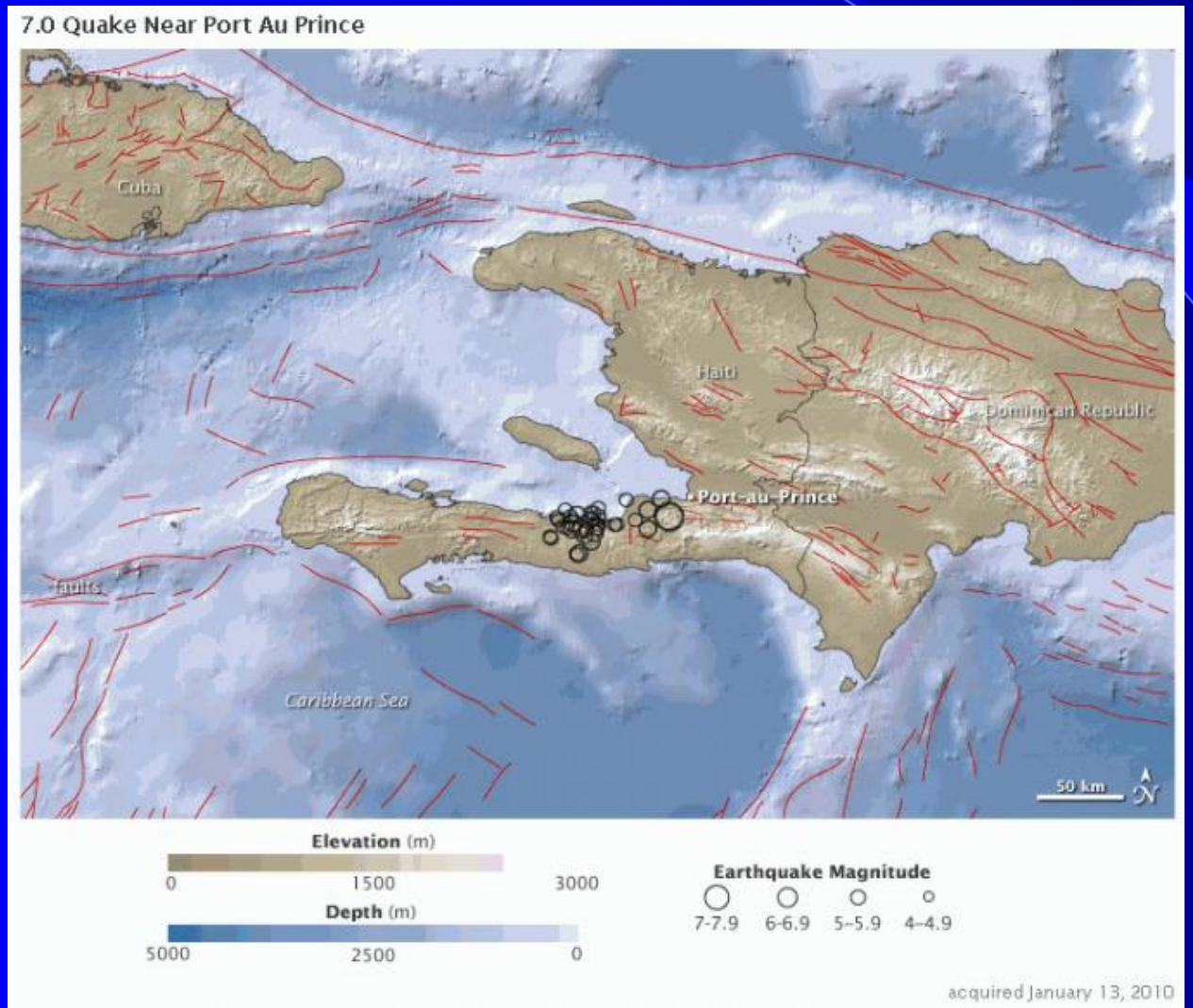
- The first cholera epidemic was documented in 1816 in Bengal
- The seventh pandemic is ongoing (2010 - ? epidemic in Haiti)

Haitian Cholera Epidemic 2010-?

First in Over a Century in Haiti

- **Oct 2015 750,000 cases, 427,000 hospitalized and over 9,000 deaths caused by a *V. cholerae* serogroup O1 (Ogawa) strain (Pan American Health Org).**

Cholera Epidemic Has Spread to Cuba and Dominican Republic



Epidemic & Pandemic Alert and Response (EPR)

- Overview
- Components
- Features
- **Outbreak News**
- Situation reports
- Publications
- Ebola virus disease in West Africa

Disease Outbreak News

Outbreak Bulletin - Vol. 4 Issue 4, 31 October 2014

In this issue, a general overview of outbreaks that occurred within the WHO African Region between **January - September 2014** is provided as well as a summary of ongoing outbreaks as reported by Member States.

Overview of reported outbreaks in WHO African Region

Based on data received from the Early Warning System through the Event Management System (EMS), 52 public health events were reported to the Regional Office between January and September 2014 of which 94% (49 / 52) were due to infectious diseases;

- Cholera being the most frequently reported infectious disease (33%)
- Dengue (11%)
- Ebola (11%) and
- Meningitis (11%)

Download the Disease Outbreak bulletin - Vol. 4 (1.41 MB)

Ebola virus disease – Mali

On 23 October 2014, WHO was notified by Mali's Ministry of Health of a laboratory-confirmed case of infection with Ebola Virus Disease (EVD). This is the first EVD case in Mali.

Details of the case are as follows:

search... Search

Cholera

Cholera fact sheet

Crimean-Congo haemorrhagic fever (CCHF)

CCHF fact sheet

Ebola virus disease

Fact sheet on Ebola virus disease [Français]

More on Ebola virus disease [Français]

Marburg

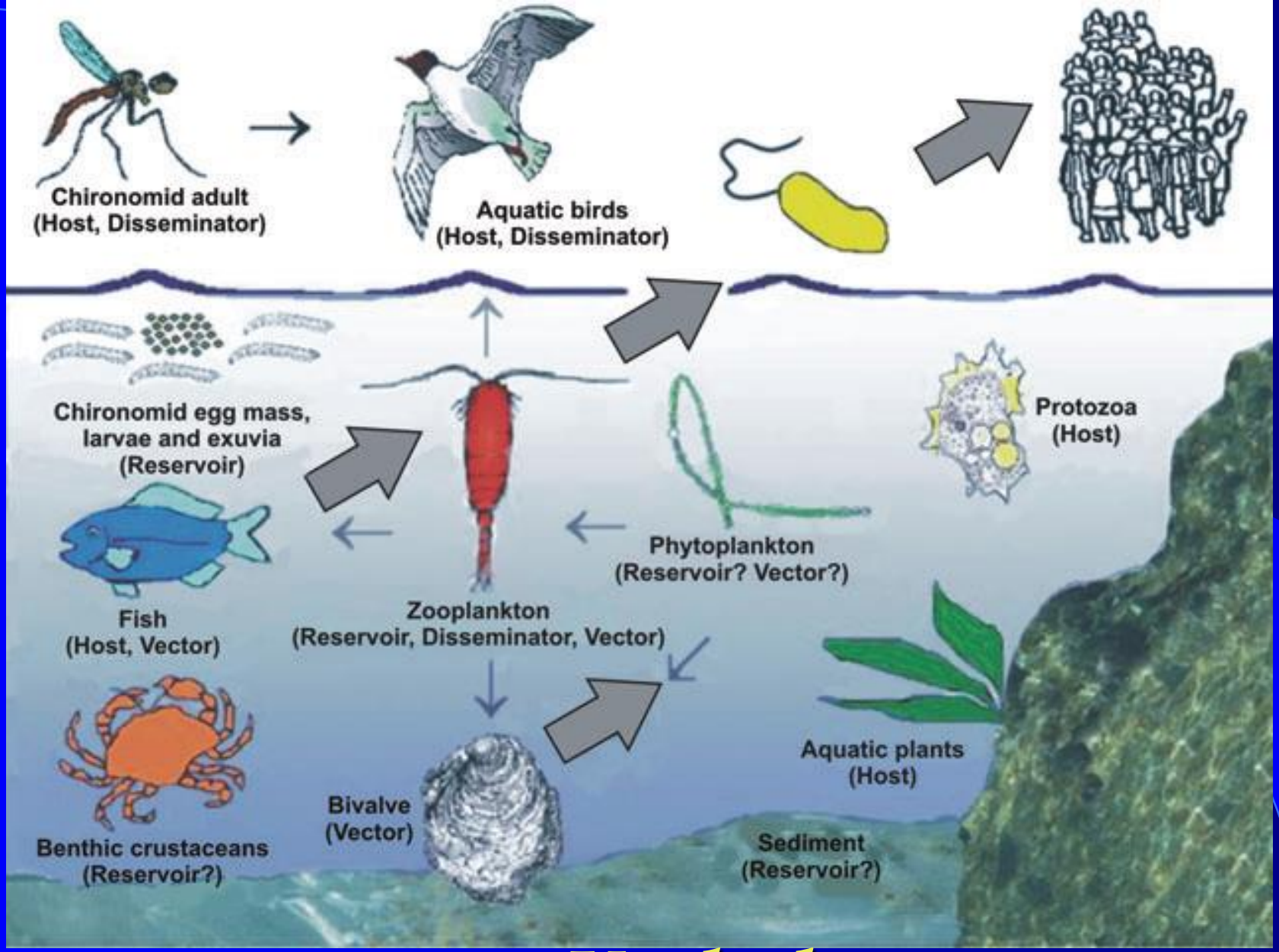
Marburg haemorrhagic fever fact sheet

Poliomyelitis

Poliomyelitis fact sheet

Transmission of *V. cholerae* & Cholera Treatment

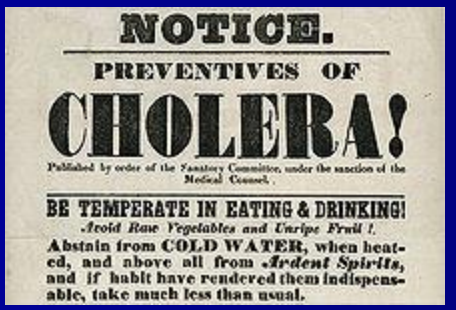
- Transmission from environmental reservoirs (next slides)
- Water or food consumption
- Person-to-person transmission (less likely)
- Minimum infectious dose = 10^3 – 10^6 cells
- Most effective treatment by oral or intravenous rehydration therapy
- Antibiotics, e.g. doxycycline, may be used



Environmental Reservoirs of ***V. cholerae***.... Vezulli et al

2010 Environmental Microbiology Reports 2:27-33

Cholera Is Rare in U.S.


NOTICE.

PREVENTIVES OF

CHOLERA!

Published by order of the Sanitary Committee, under the sanction of the Medical Council.

BE TEMPERATE IN EATING & DRINKING!

Avoid Raw Vegetables and Unripe Fruit!

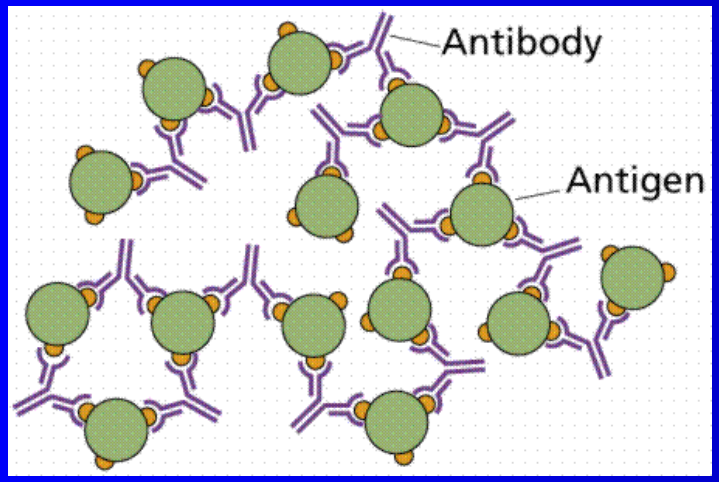
Abstain from COLD WATER, when heated, and above all from *Ardent Spirits*, and if habit have rendered them indispensable, take much less than usual.

NYC Board of Health, 1832

- Less than 10 imported cases/yr on average (data from last several decades).
- Occasional infections (sporadic occurrence, non-O1) acquired from uncooked Gulf Coast oysters.
- Several cases reported from Louisiana after Hurricanes Katrina and Rita (shellfish consumption).
- *V cholerae* serogroup 075 may be an emerging infectious strain in U.S.

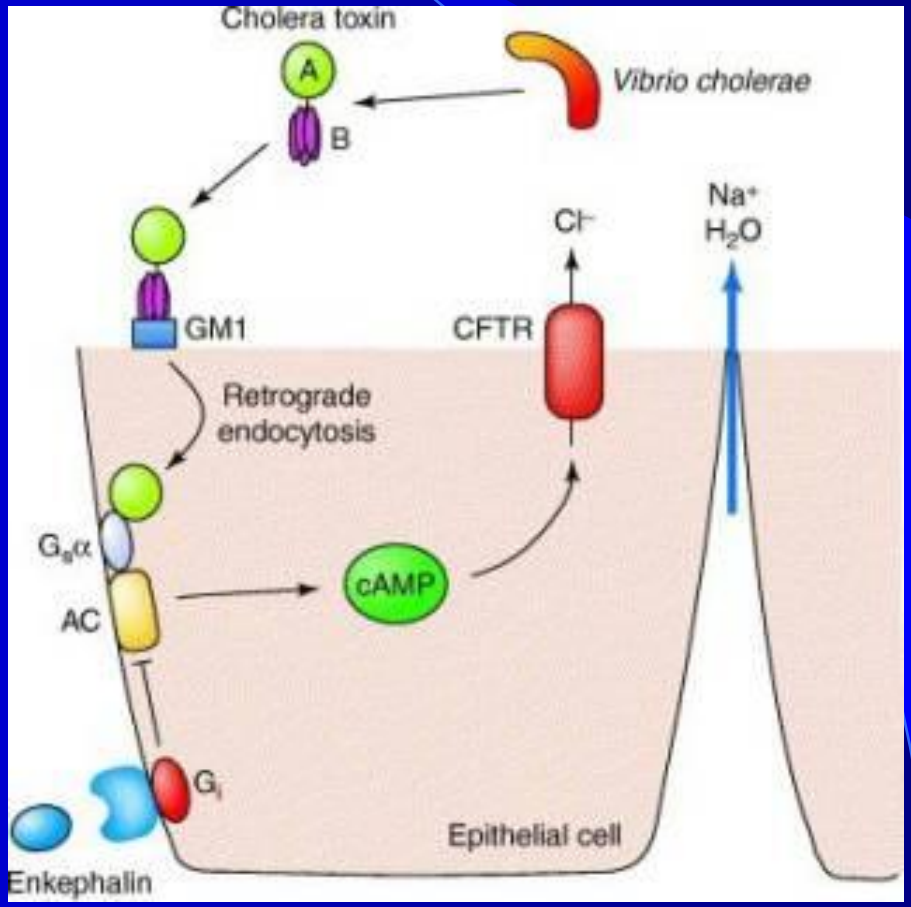
What's Up with the O?

- "O" in a bacterial strain name almost always designates serotype
- A way of discriminating among closely-related bacteria



What Makes the Epidemic *Vibrio cholerae* Strains More Virulent?

- Cholera toxin!
- Also known as CTX
- Hexamer (AB_5)
- Originates from CTXφ temperate bacteriophage



Wrapping Up

- ***Vibrio*** **spp. are autochthonous members of estuarine environments.**
- **Vibriosis from *Vibrio* spp. other than *V. cholerae* may become more common as global waters warm.**
- **Unlike other *Vibrio* spp., O1 and O139 (epidemic potential) strains of *V. cholerae* are generally contaminants from human fecal waste.**
- **Don’t eat raw shellfish!!**


Questions?
vharwood@usf.edu

Vaccines

- Two oral vaccines are available: Dukoral, ShanCol.
- Vaccination requires two oral doses a week apart.
- The U.S. Centers for Disease Control does not recommend vaccination for travelers.
- Protection is not complete, and tends to be of short duration.
- The vaccine is not available in the U.S.

THE Star
the people's paper

No. 15793 Friday September 27 2002 PP1641/3/2003
Peninsula RM1.00 / Sabah RM1.50 / Sarawak RM1.50

Vaccine for cholera strain discovered

KUALA LUMPUR: Three genetic engineers at the Universiti Sains Malaysia (USM) campus in Kubang Krian have succeeded in developing a prototype vaccine for the 0139 cholera strain also known as the Bengal strain.

The three scientists, Dr M. Ravichandran (group leader) Prof Madya Dr Zainuddin F Zainuddin and Dr P. Lalitha, have conducted preliminary trials with the vaccine on rabbits over a period of two years.

The prototype vaccine will now undergo more clinical trials for about three to five years before it is accepted as a fully-developed vaccine to be used on people.

There is at present no vaccine for the Bengal strain.

"The Bengal strain is more virulent and can cause large epidemics and has already spread to 11 Asian countries, including Malaysia," Dr Ravichandran said.

He said the new vaccine, referred to as VCUSM-1, was developed by applying genetic engineering techniques in which a vital gene of *V. cholerae* was isolated and characterised. The gene plays an important role in the growth and multiplication of the cholera bacterium.

The isolated gene was mutated to render it non-functional. The genetic manipulation studies were carried out USM researchers Nur Haslindawaty A R and Lai Chin Ting while the immunological evaluation studies were carried out by PhD candidate Atif Ali.

The Star, 27 Sept 2002