Water Chemistry in Shellfish Hatcheries and Nurseries:

The Role of pH, Alkalinity, and Calcium Carbonate

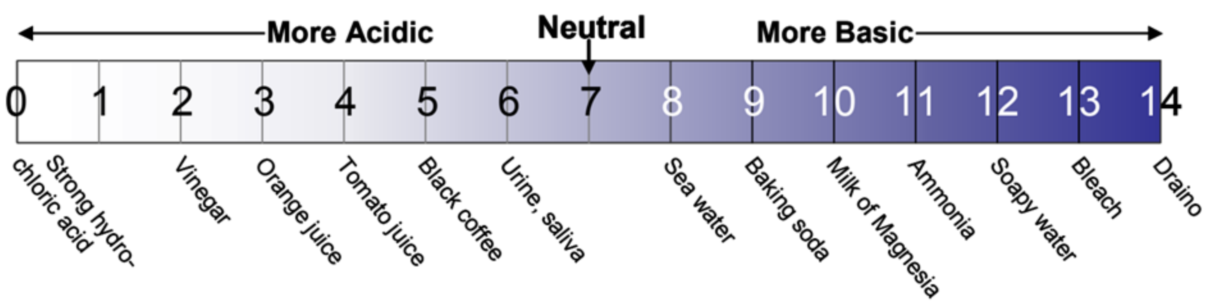
HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY

Sea Grant

UF | IFAS
UNIVERSITY of FLORIDA

What are pH and alkalinity?

- **pH** – Measure of concentration of hydrogen (H+) in water
  - Logarithmic scale from 0 to 14 with 7 a neutral value
  - Scale inverse of hydrogen ion concentration, more H+ = higher acidity = lower pH
  - Rapid changes causes stress to aquatic animals

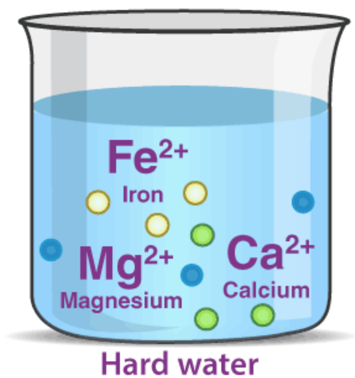


- **Alkalinity** – Measure of water to buffer pH and neutralize acids
  - Alkaline compounds (bicarbonates, carbonates) remove H+ by combining with them making new compounds, lowering acidity of water, and increasing pH
  - Proper levels prevent stress in aquatic organisms due to rapid pH changes

What are calcium carbonate and hardness?

- **Calcium carbonate ($CaCO_3$)** – Source of alkalinity present in rocks (limestone)
  - Most abundant mineral on Earth
  - Primary calcium carbonite minerals are calcite and aragonite
  - Created in water when calcium ions react with carbonate ions

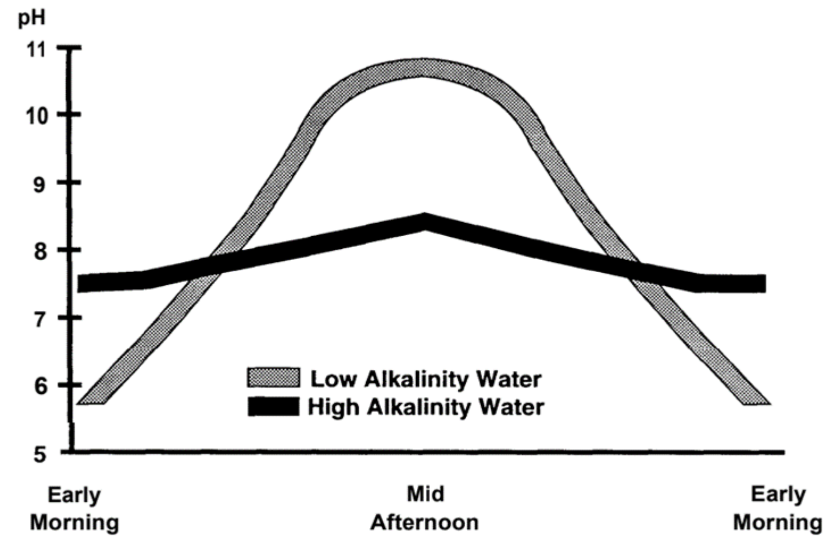
- **Hardness ($CaCO_3$)** – Amount of calcium along with other minerals like magnesium and iron dissolved in water
  - Calcium (Ca), an alkaline earth metal
  - Calcium ion (Ca^{2+}) binds with negatively-charged alkalinity anions -- bicarbonate (HCO_3^{2-}) and carbonate (CO_3^{2-}) – to form calcium carbonate ($CaCO_3$)
  - Expressed as $CaCO_3$ instead of calcium



Why do these parameters vary?

- **Daily** due to photosynthesis of phytoplankton
  - Photosynthesis takes place during day, using CO_2 which increases pH
  - At night, photosynthesis stops, respiration occurs, which creates CO_2 and decreases pH
- **Seasonally** due to temperature and rainfall
  - Temperature influences dissolved CO_2
  - As T ↑, pH and alkalinity ↓ due to ↑ CO_2
  - Rainwater is slightly acidic and contributes to lowering (dissolution) of $CaCO_3$

Dilution of salt water by rainfall, freshwater, anthropogenic inputs, and other sources may cause pH, alkalinity, calcium carbonate to vary



How are these parameters measured?

- Multiple ways to measure these parameters – aquarium or field test kits, sensors or probes, meters – inexpensive, readily available, and easy to use
- Monitor these parameters at same time during day due to normal pH changes
- Consider accuracy of test kits and meters

Equipment used in Project (parameters measured weekly, biweekly)

Oakton Handheld Meter
for pH

Hannah Marine Kit
for Alkalinity

Hach Spectrophotometer
for Calcium Hardness

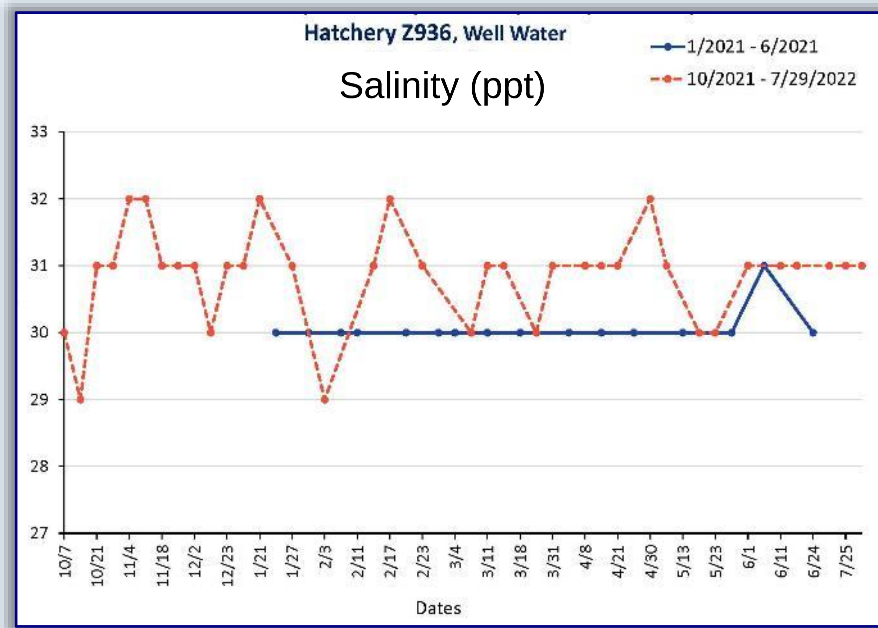
Why are these parameters important for shellfish seed production?

- $CaCO_3$ used by mollusks to form and build their shells and necessary for proper shell development
- Inadequate pH and alkalinity levels cause larval shell deformities, poor growth and survival
- Hatchery rearing water should resemble natural seawater chemistry

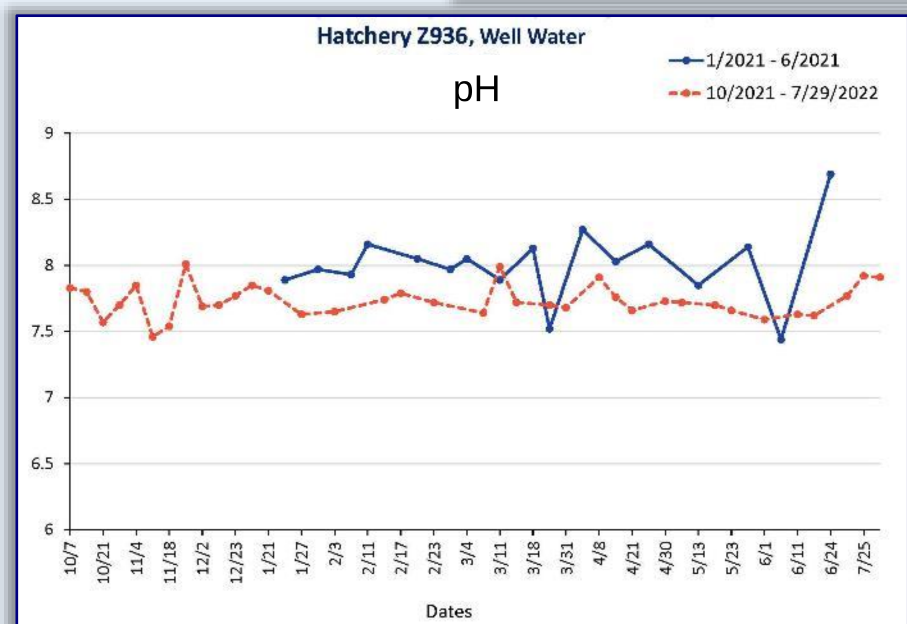
Recommendations for Shellfish Hatcheries

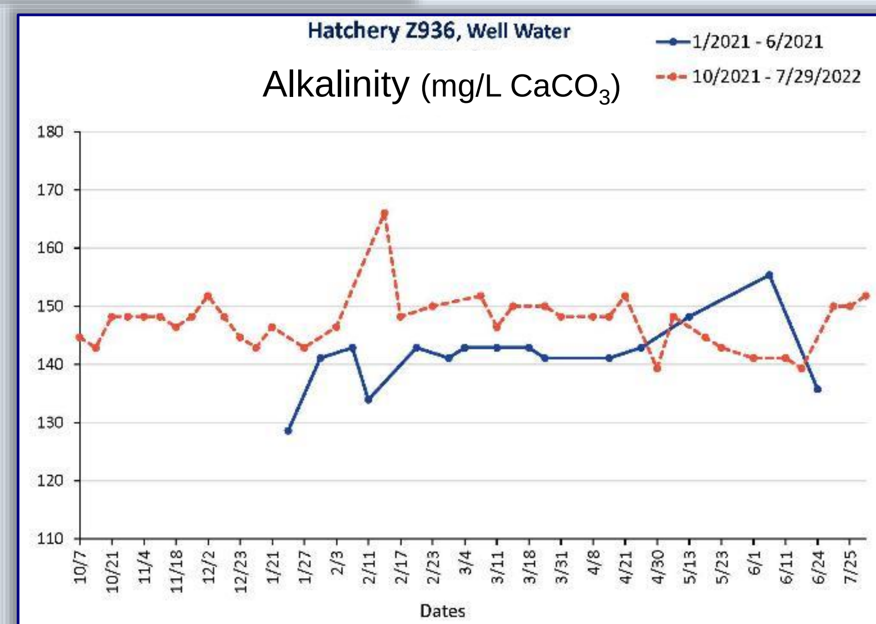
Parameter	pH	Alkalinity mg/L	Hardness mg/L
Recommended level	8.2	150-180	200
Acceptable range	7.8-8.4	110-200	-

Water Chemistry at Florida Clam Hatchery, 2021-2022

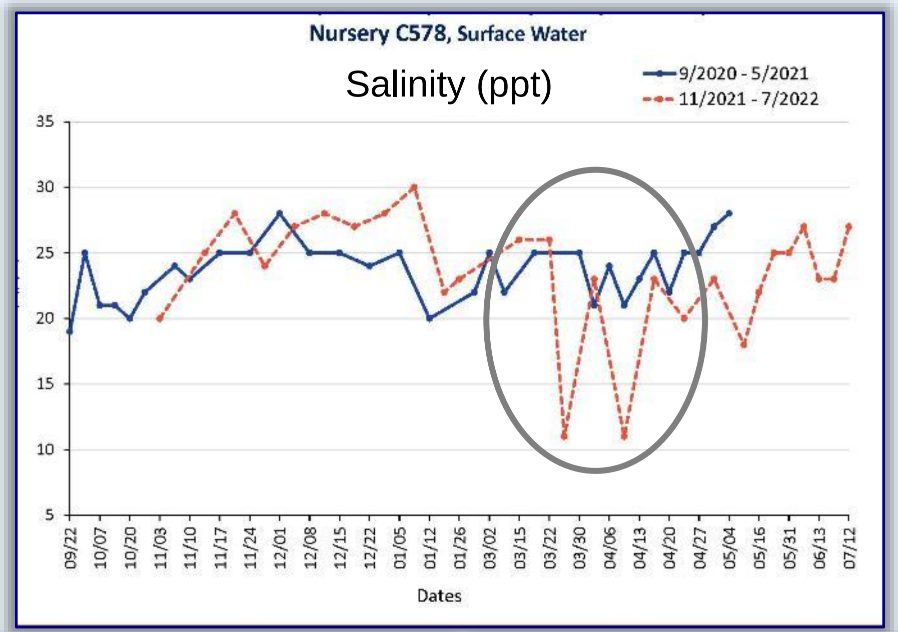


1/2021 - 6/2021
10/2021 - 7/29/2022

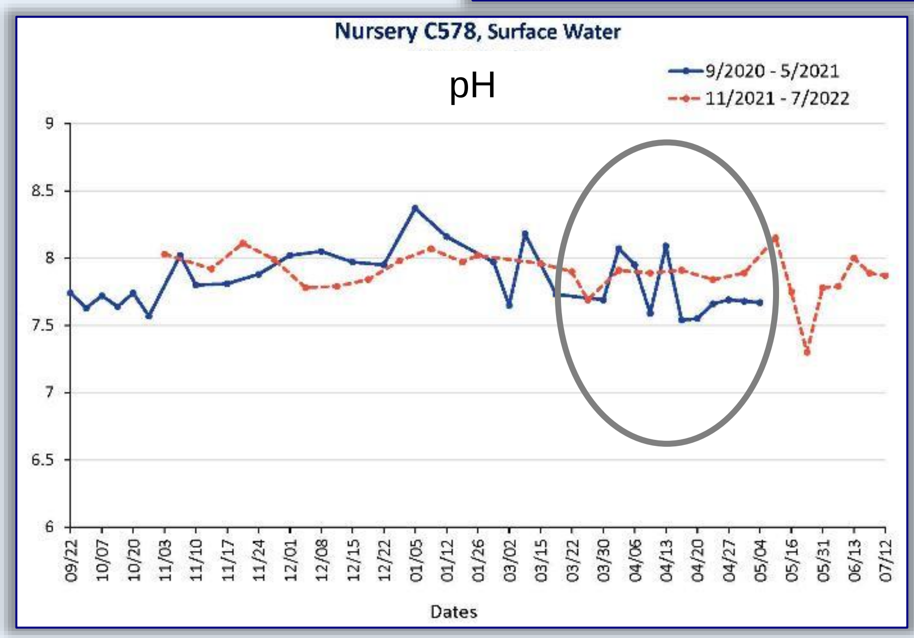


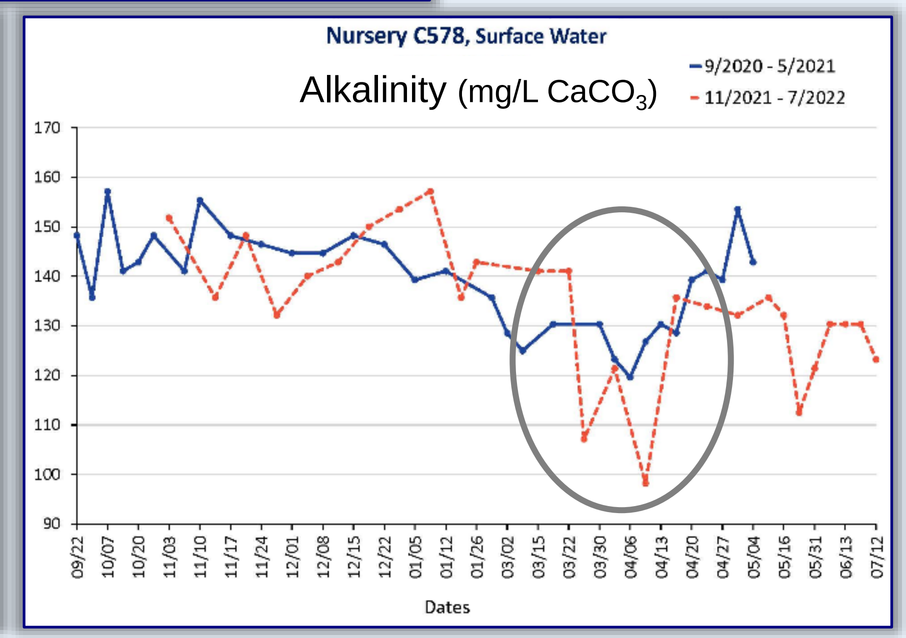


Water Chemistry at Florida Clam Nursery, 2020-2022



9/2020 - 5/2021
11/2021 - 7/2022





pH and Alkalinity Values at Florida Clam Seed Production Facilities, 2020-2022

Facility Code	pH					
	Year 1 (2020-21)			Year 2 (2021-22)		
	average	min	max	average	min	max
e232 (H,TW)	7.9	7.7	8.1	-	-	-
e232 (H,SW)	8.0	7.8	8.1	-	-	[illegible]
f626 (H,TW)	7.8	7.5	8.1	-	-	[illegible]
f626 (H,SW)	7.8	7.6	8.1	-	-	[illegible]
g109 (H,TW)	7.9	7.5	8.4	7.7	7.0	[illegible]
g109 (H,WW)	7.0	6.7	7.2	7.0	6.4	[illegible]
K885 (H,TW)	-	-	-	8.1	8.1	[illegible]
K885 (H,WW)	-	-	-	8.3	7.7	[illegible]
n185 (H,WW)	7.8	6.8	8.7	7.9	7.1	[illegible]
u537 (H,WW)	7.5	7.4	8.0	7.4	7.1	[illegible]
y378 (H,SW)	7.8	7.3	8.1	7.9	[illegible]	[illegible]
z936 (H,WW)	8.0	7.4	8.7	7.7	[illegible]	[illegible]
c578 (N, SW)	7.8	7.5	8.4	7.9	[illegible]	[illegible]
j737 (N, SW)	7.8	7.4	8.0	7.8	[illegible]	[illegible]
o613 (N, SW)	7.7	6.6	8.9	7.9	[illegible]	[illegible]
t383 (N, SW)	7.8	7.2	8.2	8.0	[illegible]	[illegible]
w329 (N, SW)	7.8	7.6	8.1	7.7	[illegible]	[illegible]

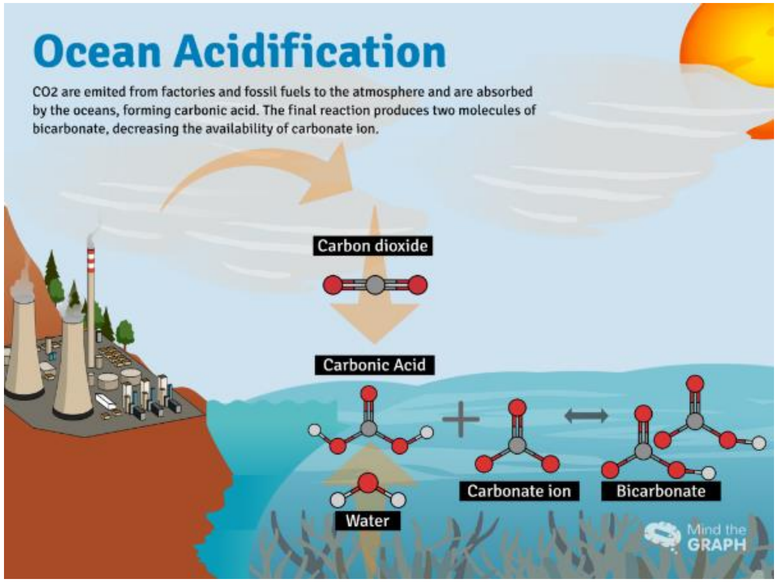
Facility Code	Alkalinity (ppm $CaCo_3$)					
	Year 1 (2020-21)			Year 2 (2021-22)		
	average	min	max	average	min	max
e232 (H,TW)	122	84	138	-	-	-
e232 (H,SW)	132	121	146	-	-	-
f626 (H,TW)	130	111	152	-	-	-
f626 (H,SW)	131	120	145	-	-	-
g109 (H,TW)	160	64	212	121	71	209
g109 (H,WW)	213	207	229	210	136	220
K885 (H,TW)	-	-	-	253	151	268
K885 (H,WW)	-	-	-	258	232	266
n185 (H,WW)	138	123	150	113	68	146
u537 (H,WW)	167	146	179	171	155	179
y378 (H,SW)	162	130	195	155	130	182
z936 (H,WW)	142	129	155	147	139	166
c578 (N, SW)	139	120	157	134	98	157
j737 (N, SW)	139	120	177	136	100	164
o613 (N, SW)	140	127	152	122	79	146
t383 (N, SW)	133	118	154	134	118	161
w329 (N, SW)	137	121	152	133	93	216

Legend:

Facility: H-Hatchery N-Nursery

Source Water: SW-Surface water TW-Tank Water WW-Well Water

Ocean Acidification and Shellfish Hatcheries



- When atmospheric CO_2 is absorbed by the ocean, chemistry of the seawater is changed
- Water and CO_2 form H^+ and bicarbonate ions (HCO_3^-) lowering pH
- As CO_2 dissolved levels increase, excess H^+ results in fewer available carbonate ions (CO_3^{-2})

The Whiskey Creek Shellfish Acid Tests

Ocean acidification and its effects on Pacific oyster larvae

BY NATHAN GILLES

Alan Barton drains seawater from a large tank, filtering its oyster larvae as he goes. He will then sort the larvae by size, using custom-made screens.

The sun chips away at the marine layer on this swiftly warming May morning in the bay. On the estuary's muddy banks, clammers dressed in knee-high rubber boots dig in the dark sludge, while throughout the bay other aquatic farmers dredge for their prize: oysters.

The oysters are *Crassostrea gigas*, commonly called the Pacific oyster. These "giant oysters" measure from 3 to 15 inches long. They're huge moneymakers for global aquaculture, and they have a special relationship to this place. This is Netarts Bay, Oregon, the center of the state's oyster industry and home to the Whiskey Creek Shellfish Hatchery, one of the nation's largest producers of Pacific oyster larvae. Hatcheries such as Whiskey

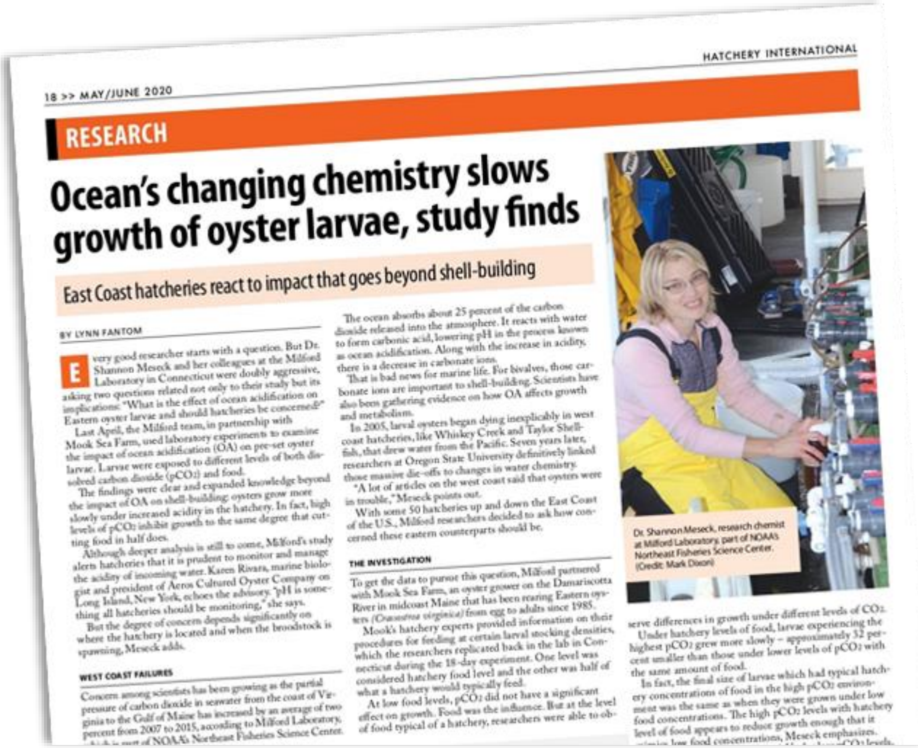
handful of places. For oyster growers from California to Canada to succeed, hatcheries must raise larvae. Unfortunately, as an incident at Whiskey Creek proved, the larvae are under siege.

In 2007, Pacific oyster larvae at Whiskey Creek started dying en masse. Oregon State University scientists later pinned the crime on ocean acidification. This is the term many are using to

ocean, CO_2 forms carbonic acid, which lowers the water's pH level, making it more acidic. The consensus is that ocean acidification is just getting started. As CO_2 is continually pumped into the air, the world's oceans are expected to slide further toward the acid side of the spectrum, and that, say researchers, won't be good for animals like the Pacific oyster. That's because oysters and other mollusks make their shells from calcium carbonate, which is becoming increasingly susceptible to breaking down in our ever-more corrosive seas. This is what happened at Whiskey Creek: the seawater in which the hatchery was raising its larvae had

- Hatcheries in Pacific NW began experiencing oyster larvae mortalities in 2007
- Upwelling of CO_2-rich oceanic waters resulted in lower pH and aragonite saturation levels
- Aragonite, most soluble form of $CaCO_3$, necessary for molluscan larvae to build shell
- Adding buffering system to hatchery water supply to increase pH was the solution

Coastal Acidification and Shellfish Hatcheries



RESEARCH

Ocean's changing chemistry slows growth of oyster larvae, study finds

East Coast hatcheries react to impact that goes beyond shell-building

BY LYNN FANTOM

Every good researcher starts with a question. But Dr. Shannon Meseck and her colleagues at the Milford Laboratory in Connecticut were doubly aggressive, asking two questions related not only to their study but its implications: "What is the effect of ocean acidification on Eastern oyster larvae and should hatcheries be concerned?"

Last April, the Milford team, in partnership with Mook Sea Farm, used laboratory experiments to examine the impact of ocean acidification (OA) on pre-set oyster larvae. Larvae were exposed to different levels of both dissolved carbon dioxide (pCO_2) and food.

The findings were clear and expanded knowledge beyond the impact of OA on shell-building: oysters grow more slowly under increased acidity in the hatchery. In fact, high levels of pCO_2 inhibit growth to the same degree that cutting food in half does.

Although deeper analysis is still to come, Milford's study alerts hatcheries that it is prudent to monitor and manage the acidity of incoming water. Karen Rivara, marine biologist and president of Aeros Cultured Oyster Company on Long Island, New York, echoes the advisory. "pH is something all hatcheries should be monitoring," she says.

But the degree of concern depends significantly on where the hatchery is located and when the broodstock is spawning, Meseck adds.

WEST COAST FAILURES

Concern among scientists has been growing as the partial pressure of carbon dioxide in seawater from the coast of Virginia to the Gulf of Maine has increased by an average of two percent from 2007 to 2015, according to Milford Laboratory, which is part of NOAA's Northeast Fisheries Science Center.

The ocean absorbs about 25 percent of the carbon dioxide released into the atmosphere. It reacts with water to form carbonic acid, lowering pH in the process known as ocean acidification. Along with the increase in acidity, there is a decrease in carbonate ions.

That is bad news for marine life. For bivalves, those carbonate ions are important to shell-building. Scientists have also been gathering evidence on how OA affects growth and metabolism.

In 2005, larval oysters began dying inexplicably in west coast hatcheries, like Whiskey Creek and Taylor Shellfish, that drew water from the Pacific. Seven years later, researchers at Oregon State University definitively linked those massive die-offs to changes in water chemistry.

"A lot of articles on the west coast said that oysters were in trouble," Meseck points out.

With some 50 hatcheries up and down the East Coast of the U.S., Milford researchers decided to ask how concerned these eastern counterparts should be.

THE INVESTIGATION

To get the data to pursue this question, Milford partnered with Mook Sea Farm, an oyster grower on the Damariscotta River in midcoast Maine that has been rearing Eastern oysters (*Crassostrea virginica*) from egg to adults since 1985.

Mook's hatchery experts provided information on their procedures for feeding at certain larval stocking densities, which the researchers replicated back in the lab in Connecticut during the 18-day experiment. One level was considered hatchery food level and the other was half of what a hatchery would typically feed.

At low food levels, pCO_2 did not have a significant effect on growth. Food was the influence. But at the level of food typical of a hatchery, researchers were able to observe differences in growth under different levels of CO_2.

Under hatchery levels of food, larvae experiencing the highest pCO_2 grew more slowly – approximately 32 percent smaller than those under lower levels of pCO_2 with the same amount of food.

In fact, the final size of larvae which had typical hatchery concentrations of food in the high pCO_2 environment was the same as when they were grown under low food concentrations. The high pCO_2 levels with hatchery level of food appears to reduce growth enough that it mimics low food concentrations, Meseck emphasizes.

Dr. Shannon Meseck, research chemist at Milford Laboratory, part of NOAA's Northeast Fisheries Science Center. (Credit: Mark Dixon)

In the Northeast US…

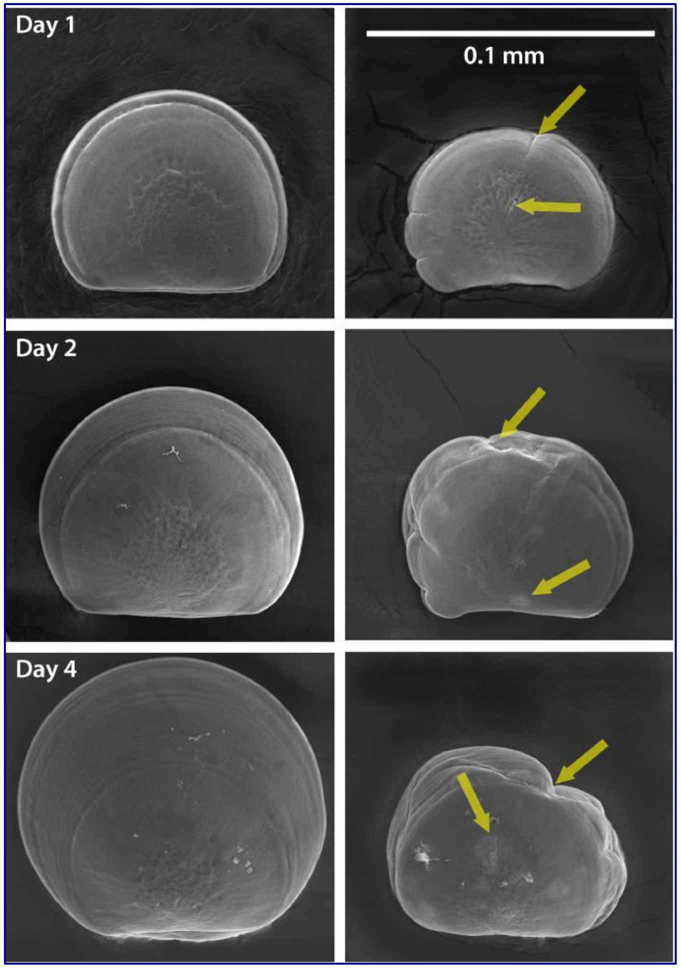
- Hatcheries began experiencing larval production problems in 2009
- Decreased $CaCO_3$ saturation states due to increased atmospheric CO_2 and freshwater runoff from heavy rain events
- Buffering incoming hatchery water to increase carbonate saturation state has resulted in better production

What about the Southeastern US?

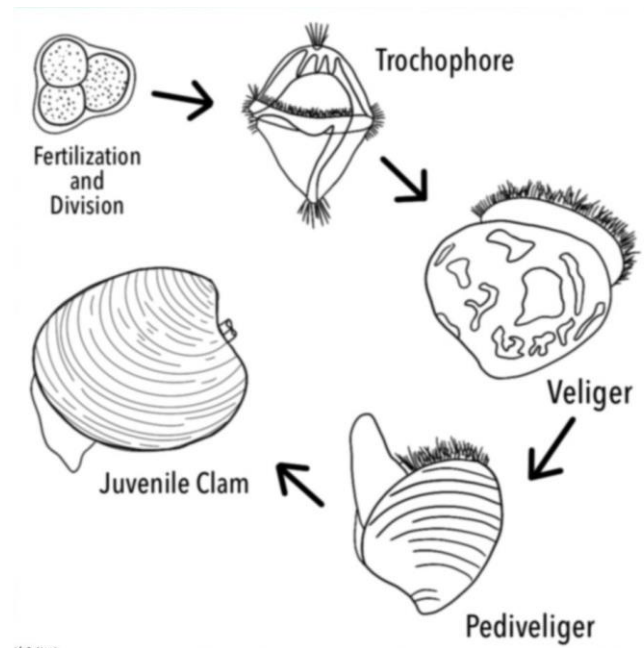
- Coastal waters tend to be well buffered, with lower CO_2 and higher alkalinity levels
- River water discharges due to rain events, tropical storms and hurricanes are driving factors in carbonate chemistry
- Impacts of climate change likely to affect coastal water chemistry

Shell Development of Larval Bivalves

- Concentration of carbonite ions and dissolved calcium are building blocks of shell
- Post-fertilization, larvae capture ions from seawater, combine with calcium to form aragonite
- Energetically expensive as larvae accrete more shell mass than organic mass
- Post-metamorphosis, juvenile shell is comprised of calcite, aragonite or both minerals



Pacific oyster larvae from the same spawn, raised in waters exhibiting favorable (LEFT: pCO_2=403 ppm, Ωarag=1.64, pH=8.00) and unfavorable (RIGHT: pCO_2=1418 ppm, Ωarag=0.47, pH=7.5) carbonate chemistry during the spawning period. Scanning Electron Microscopy images show larval shells at 1, 2, and 4-days post-fertilization. Arrows show defects (creases) and features (light patches) suggestive of shell dissolution.



What are saturation states?

- Measure of tendency of mineral to form or dissolve in water
- Calculated by ratio of mineral dissolved ions to solubility (equilibrium) product (Ksp)
- Levels of $CaCO_3$ minerals can be measured by saturation state (omega Ω)
  - Calcite – most common $CaCO_3$ mineral and most stable form
  - Aragonite – second most common $CaCO_3$ mineral and most soluble form

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}^{*}}$$

- Ca^{2+} and CO_3^{2-} are concentrations of ions in solution
- K_{sp} is solubility product at a given temperature, salinity, and pressure

- If $\Omega < 1$, $CaCO_3$ can readily dissolve, if $\Omega > 1$, then $CaCO_3$ mineral formation is favored
- Calcifying bivalves live in conditions where $\Omega > 1$, thrive in conditions $\Omega \geq 1$
- Corals need Ω levels >3 to form and grow

Saturation State Levels at Florida Clam Seed Production Facilities, 2020-2022

Ω Calcite Saturation State [1]

Facility Code	Year 1 (2020-21)			Year 2 (2021-22)		
	average	min	max	average	min	max
e232 (H,TW)	1.24	0.72	2.03	-	-	[illegible]
e232 (H,SW)	1.15	0.48	1.93	-	-	[illegible]
f626 (H,TW)	1.13	0.47	1.73	-	-	[illegible]
f626 (H,SW)	1.09	0.47	1.72	-	-	[illegible]
g109 (H,TW)	2.48	0.35	3.85	1.69	0.10	[illegible]
g109 (H,WW)	0.35	0.24	0.60	0.58	0.08	[illegible]
K885 (H,TW)	-	-	-	6.07	[illegible]	[illegible]
K885 (H,WW)	-	-	-	4.53	[illegible]	[illegible]
n185 (H,WW)	2.35	0.12	6.82	2.68	[illegible]	[illegible]
y378 (H,SW)	1.97	0.54	3.03	2.22	[illegible]	[illegible]
z936 (H,WW)	2.36	1.65	3.15	1.36	[illegible]	[illegible]
c578 (N, SW)	1.16	0.58	2.66	1.32	[illegible]	[illegible]
j737 (N, SW)	1.86	0.54	2.76	1.26	[illegible]	[illegible]
o613 (N, SW)	3.18	0.74	8.49	2.90	[illegible]	[illegible]
t383 (N, SW)	1.75	0.31	3.61	1.99	[illegible]	[illegible]
w329 (N,SW)	1.02	0.62	1.52	1.26	[illegible]	[illegible]

[1]**Reference:** CO2SYS Program developed for CO_2 system calculations, Oak Ridge National Laboratory, US Department of Energy

Ω Aragonite Saturation [1]

Facility Code	Year 1 (2020-21)			Year 2 (2021-22)		
	average	min	max	average	min	max
e232 (H,TW)	0.76	0.29	1.30	-	-	-
e232 (H,SW)	0.72	0.30	1.23	-	-	-
f626 (H,TW)	0.70	0.30	1.08	-	-	-
f626 (H,SW)	0.68	0.29	1.07	-	-	-
g109 (H,TW)	1.60	0.22	2.47	1.09	0.06	3.90
g109 (H,WW)	0.23	0.15	0.39	0.38	0.05	2.73
K885 (H,TW)	-	-	-	3.85	2.32	5.64
K885 (H,WW)	-	-	-	2.87	1.18	4.33
n185 (H,SW)	1.53	0.08	4.48	1.74	0.15	3.81
y378 (H,SW)	1.28	0.34	2.01	1.44	0.49	3.27
z936 (H,WW)	1.55	1.08	2.08	0.90	0.26	1.63
c578 (N, SW)	0.73	0.36	1.65	0.83	0.22	1.53
j737 (N, SW)	1.19	0.34	1.76	0.80	0.25	1.56
o613 (N, SW)	2.09	0.47	5.62	1.88	0.20	3.96
t383 (N, SW)	1.14	0.19	2.40	1.30	0.26	2.73
w329 (N, SW)	0.64	0.39	0.96	0.80	0.10	2.54

Legend:

Facility: H-Hatchery N-Nursery

Source Water: SW-Surface water TW-Tank Water WW-Well Water

Buffering System for Shellfish Hatcheries

Delivering a buffering solution into a water storage tank using a pH controller and pump

- Buffering system using inexpensive meters and controllers to increase and maintain pH and alkalinity levels

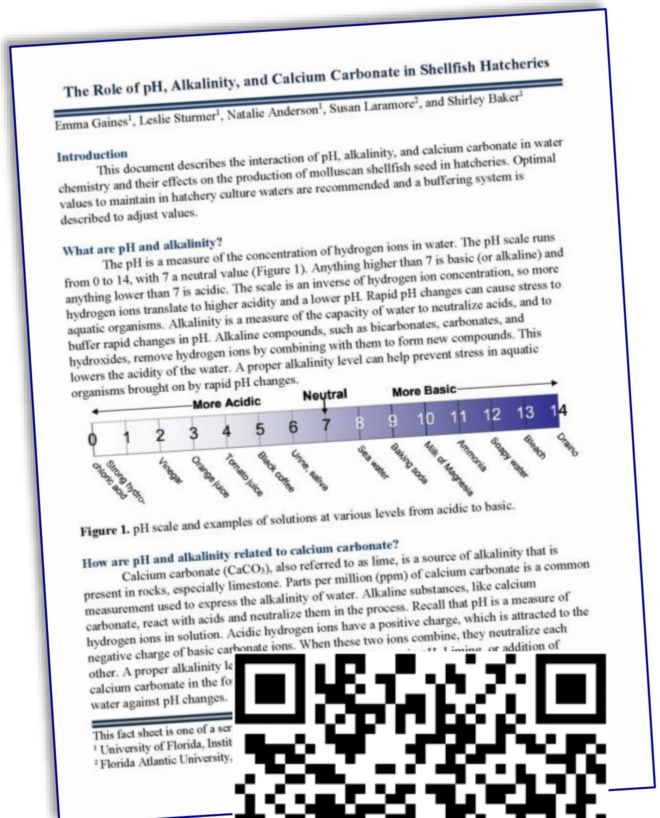
Water storage tank used to fill larval rearing systems

pH meter/controller and pump with long cords to reach other tanks

55-gal polypropylene tank with saturated solution of soda ash (98 lbs)

- A meter measures pH of tank water, adds buffer (soda ash) to achieve optimal pH via an injection pump
- A controller calculates the required injection rate and activates the system

Additional Information



The Role of pH, Alkalinity, and Calcium Carbonate in Shellfish Hatcheries

Emma Gaines[1], Leslie Sturmer[1], Natalie Anderson[1], Susan Laramore[2], and Shirley Baker[1]

Introduction

This document describes the interaction of pH, alkalinity, and calcium carbonate in water chemistry and their effects on the production of molluscan shellfish seed in hatcheries. Optimal values to maintain in hatchery culture waters are recommended and a buffering system is described to adjust values.

What are pH and alkalinity?

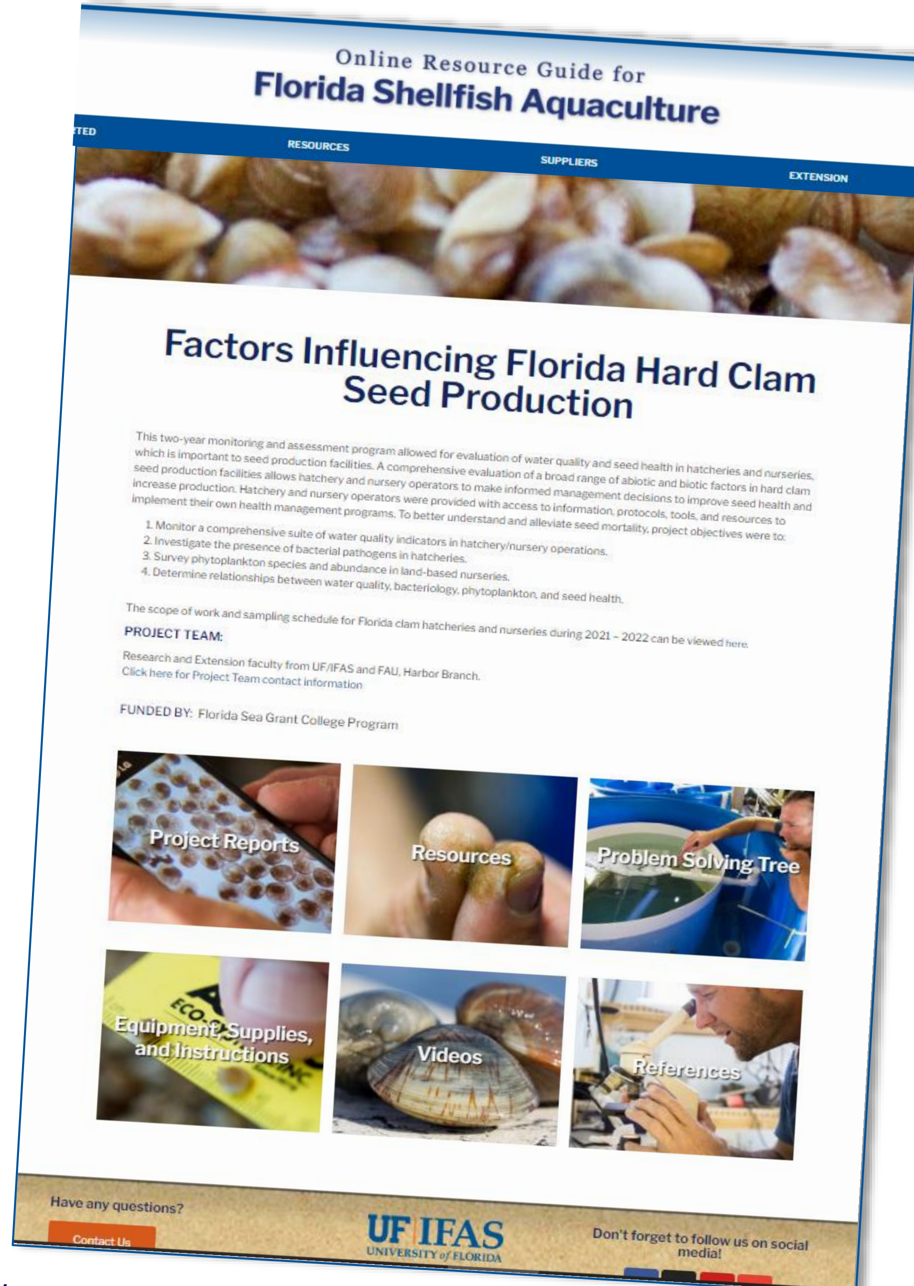
The pH is a measure of the concentration of hydrogen ions in water. The pH scale runs from 0 to 14, with 7 a neutral value (Figure 1). Anything higher than 7 is basic (or alkaline) and anything lower than 7 is acidic. The scale is an inverse of hydrogen ion concentration, so more hydrogen ions translate to higher acidity and a lower pH. Rapid pH changes can cause stress to aquatic organisms. Alkalinity is a measure of the capacity of water to neutralize acids, and to buffer rapid changes in pH. Alkaline compounds, such as bicarbonates, carbonates, and hydroxides, remove hydrogen ions by combining with them to form new compounds. This lowers the acidity of the water. A proper alkalinity level can help prevent stress in aquatic organisms brought on by rapid pH changes.

Figure 1. pH scale and examples of solutions at various levels from acidic to basic.

How are pH and alkalinity related to calcium carbonate?

Calcium carbonate ($CaCO_3$), also referred to as lime, is a source of alkalinity that is present in rocks, especially limestone. Parts per million (ppm) of calcium carbonate is a common measurement used to express the alkalinity of water. Alkaline substances, like calcium carbonate, react with acids and neutralize them in the process. Recall that pH is a measure of hydrogen ions in solution. Acidic hydrogen ions have a positive charge, which is attracted to the negative charge of basic carbonate ions. When these two ions combine, they neutralize each other. A proper alkalinity le… calcium carbonate in the fo… water against pH changes.

This fact sheet is one of a ser…
[1] University of Florida, Instit…
[2] Florida Atlantic University, …



Fact sheet and website page

https://shellfish.ifas.ufl.edu/clam-seed-project-2020-22/