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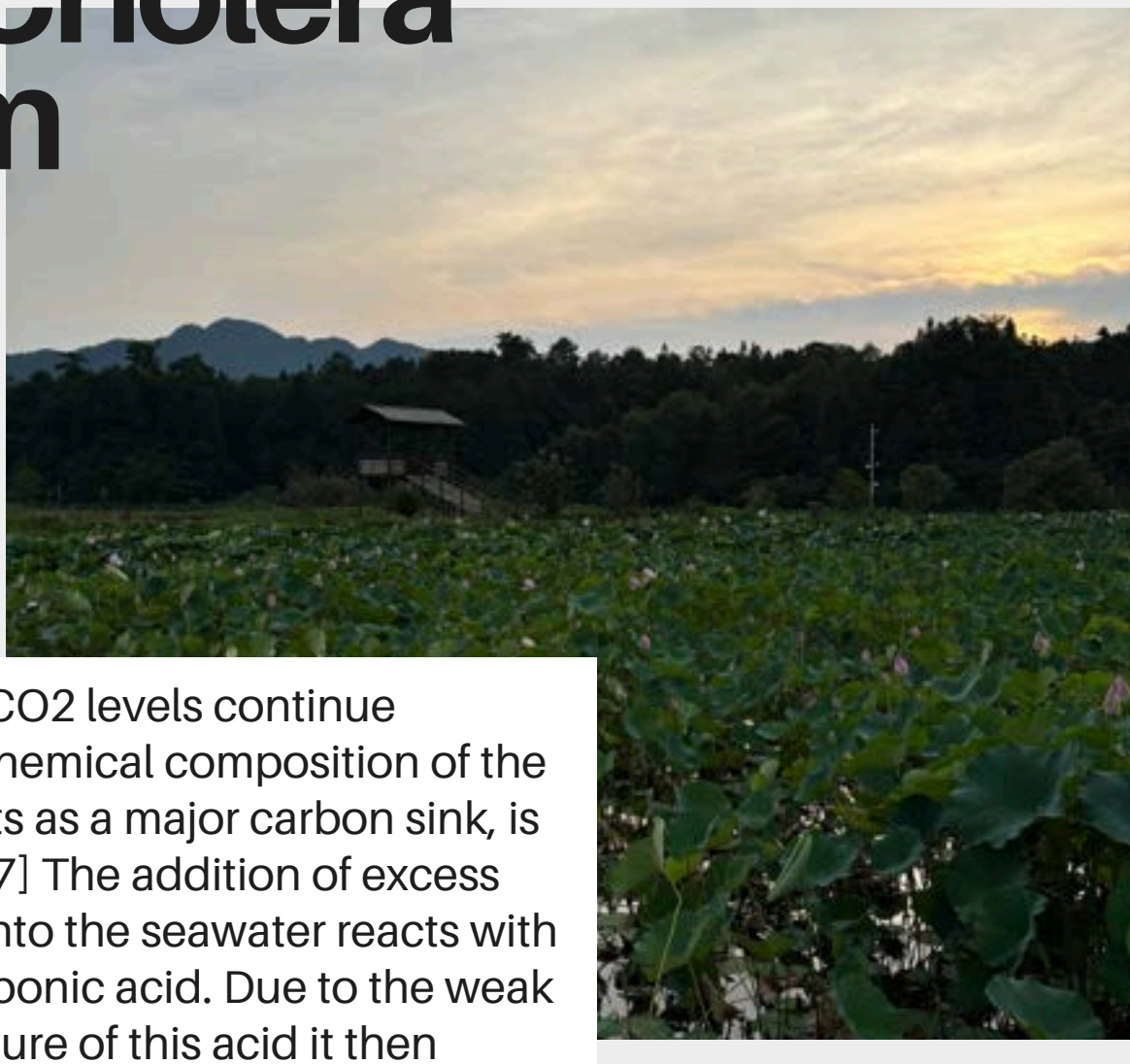
The Summer 2024 issue of GreenScience is a tale on environmental conservation told through an emphasis on the essential nature of both scientific discovery and dedicated people in the journey of bringing about change

Impacts of Climate Change on V. Cholera Biofilm



As atmospheric CO2 levels continue increasing, the chemical composition of the ocean, which acts as a major carbon sink, is heavily altered. [7] The addition of excess carbon dioxide into the seawater reacts with H20 forming carbonic acid. Due to the weak and unstable nature of this acid it then dissociates into bicarbonate and hydrogen ions. The acidic nature of these hydrogen ions triggers a decrease in the overall pH of seawater. Even slight changes in ocean acidity hold the potential to shape the behavior and survival of both marine organisms and people. [10] For example, decreasing pH affects aqueous bacteria and related communities of microorganisms, ultimately leading to potential negative impacts on human health.

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Vc is a gram-negative bacteria which lives primarily in aqueous habitats and is susceptible to the impacts of ocean acidification. Vc is represented by over 200 serotypes, among which are the Classical 0139 and El Tor 01 strains responsible for previous and currently ongoing global cholera pandemics[11].Previous estimates predict that there are up to 4 million cases of cholera yearly, with outbursts located primarily in countries with limited access to potable water[11].

Vc is among many bacterial species which utilize the production of biofilm in order to enhance resistance against stressful environmental factors. *Vc* biofilm is a formed by a community of microorganisms which interact and attach with VPS in its self-produced biofilm matrix. *Vc* biofilm develops primarily on chitin surfaces in its natural aquatic habitat. Through the use of flagellar propulsion coded for by the flaA gene loci, *Vc* approaches and attatches to suitable surfaces[9].

Ultimately, c-di-GMP regulates the transition from motile to sessile attachment through modulation of mannose sensitive hemagglutinin(mshA)[6]. mshA pili acts as a break, tethering to chitin surfaces and triggering irreversible attachment and secretion of VPS(vibrio polysaccharides)[15].

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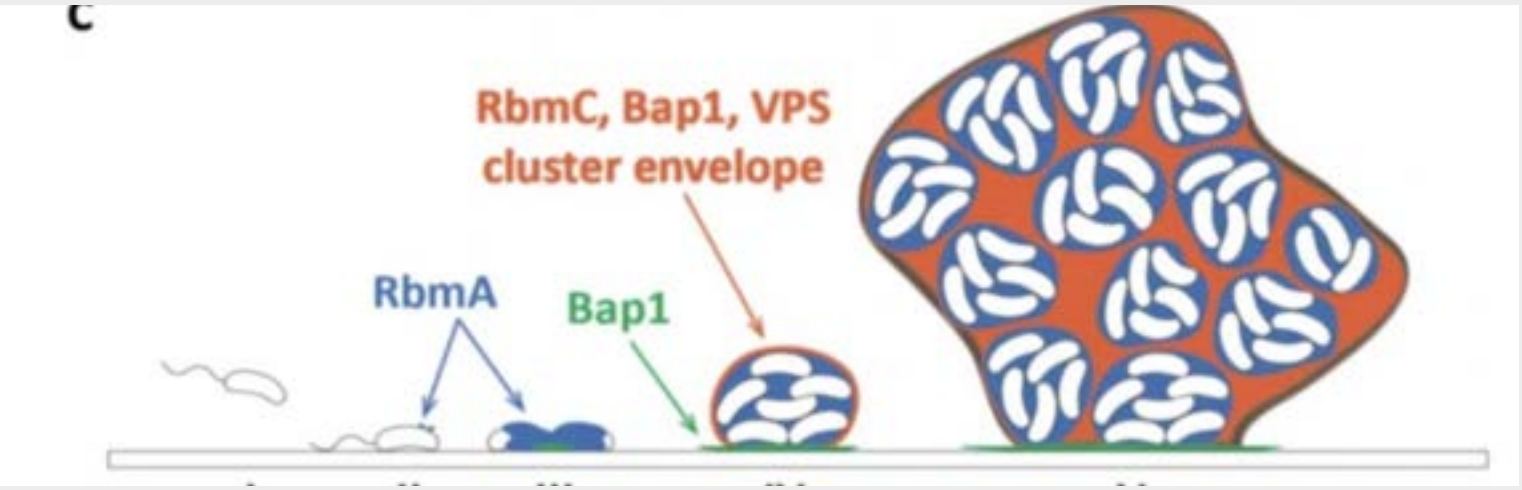


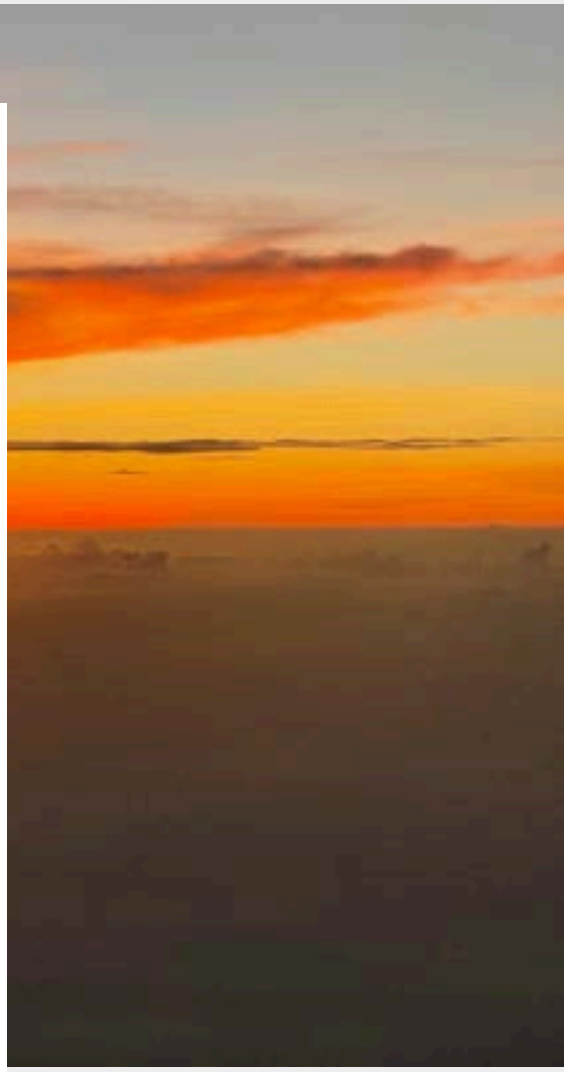
Following secretion, Vibrio Polysaccharides bind to matrix proteins, playing an essential role in the structure of biofilm in Vc. Monosaccharide analysis reveals that VPS structure is characterized primarily of the polysaccharides glucose and galactose[17]. The biosynthesis of VPS is controlled by gene clusters VPS-I and VPS-II, the presence of which is necessary for biofilm formation. [17] Following VPS biosynthesis, RbmA, an essential matrix protein which facilitates cell to cell interactions, is then expressed. [13]

This matrix protein crosslinks with VPS, binding sister cells as they divide and grow. [13] Other matrix proteins RbmC and Bap1 ensure surface adhesion in Vc biofilms through cell to substrate interactions facilitated by crosslinks with VPS. [13]

Among the many pH sensitive chemical structures essential to V. Cholera biofilm formation, carboxyl groups(a combination of carbonyl and hydroxyl groups attached to a single carbon atom), are highly reactive to changing acidities[12].

maging by Patel et al. shows the relationship between oxidation time(0-48 hours) and content (mmol kg^-1) of carbonyl or carboxyl groups[12]. Testing acidity levels varying between 5 and 7 they graphed the resulting fluctuations in carbonyl/carboxyl content. [12] Analysis of depolymerized VPS structure reveals the presence of carboxyl groups in VPS structure[8]. Monosaccharide analysis of depolymerized VPS reveals glucose, galactose, LD-heptose, glucosamine, and perosamine as the major building blocks of VPS [18].

However further studies reveal the presence of a free carboxyl group linked to a guluronic acid bound with a glycine adduct [8]. Thus, fluctuations in carboxyl group content will directly impact VPS structure and its ability to crosslink with matrix proteins Bap1, RmbA, and RbmC[18].
Vc which lacks proper biofilm structure and integrity will face increasing difficulty in resisting environmental stressors. Subsequently, anthropogenic pollution directly limits the favorability of vc survival in marine habitats.



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Square Dancing For Conservation

From within the heavy layers of dusk rose the hazy outline of the Huangshan mountain range. We stared out in silence as the tesla snaked quietly up the mountain road. Ahead a quaint village with sloping thatched roofs - crafted in signature Anhui fashion- and rolling fields of corn drew into view. The car engine whirred to silence as pulled into a well-lit roadside plaza.

Loud music, featuring a soprano singing Chinese Opera, seeped into the silence of the car. Zhang Zheng, a member of The Paradise nature conservation, turns around to face us from the driver's seat.

“Do we still remember the mission briefings?” He asks in Mandarin Chinese.

Today’s goal is to gather whether or not the village residents recognize the purpose of the square dancing we have set up. We are also here to check in on the engagement levels.” He continues.

Gesturing for us to follow with a lopsided smile he opens the car door and steps outside. The heat smothers the air, making each breath difficult as we make our way to the twenty or so elderly ladies dressed in brightly colored fabrics. With immense synchronization they perform a hypnotizing dance, moving to the right and swirling their arms in circular motions.

As we follow Zhang Zheng through the buzzing swarms of mosquitoes the opera singing playing from the antiquated television warbles to a stop and the ladies finish their dance with a flourish. Within seconds they had abandoned their neat columns and rows to swarm around us.

“How's the night going, ladies?”

“Good, good.” They chorused

A lady clothed head to toe in red fabric with her hair drawn back into a bun responds blithely,” No poachers sneaking into the nature reserve to report tonight!”

“All thanks to you ladies and your square dancing.” Zhang Zheng responds.

“We’re here from 8 to 10 pm every night.” proclaims another voice “I’d like to see poachers try sneaking in from this entrance now!”

Square dancing for conservation. Who would have thought such a common everyday practice could make such an impact. The ladies flocked back to their positions for the next dance. We watched as they hopped and clapped their ways a step closer to restoring HuangShan to what it once was.

Bioremediation- Key to a Greener Future?

Bioremediation broadly refers to the consumption or decomposition of pollutants through both naturally occurring and artificially induced microorganisms. Targeted polluted sites include air, marine ecosystems, soil, flue gasses, etc.

The microorganisms introduced through bioremediation break down harmful pollutants and chemical compounds into innocuous amounts of harmless gasses and water. The process of bioremediation requires specific environmental factors including temperature, nutrients, and food. To alter such factors amendments can be added through specific techniques under the umbrella term of bioremediation.

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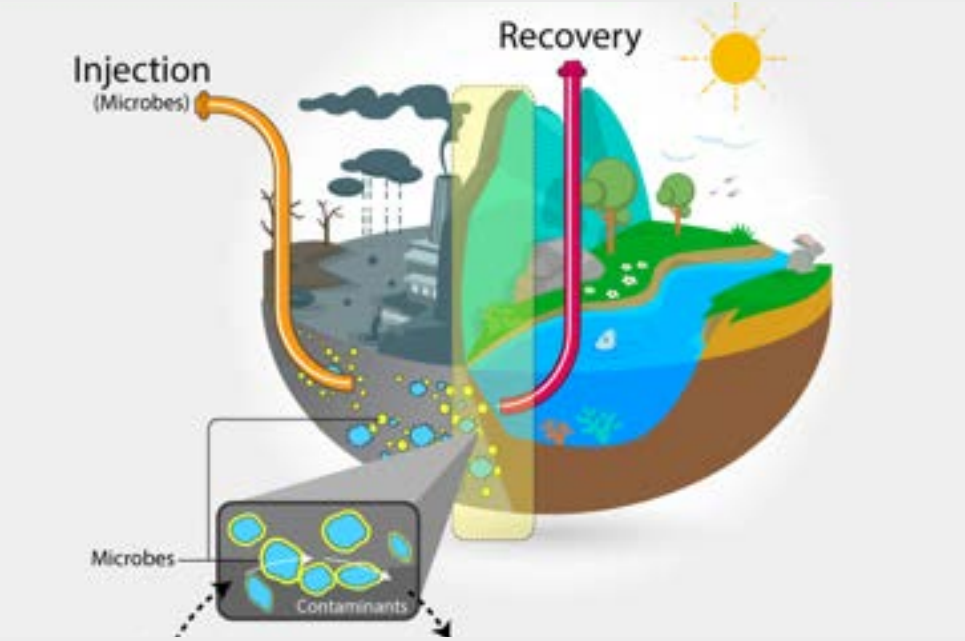
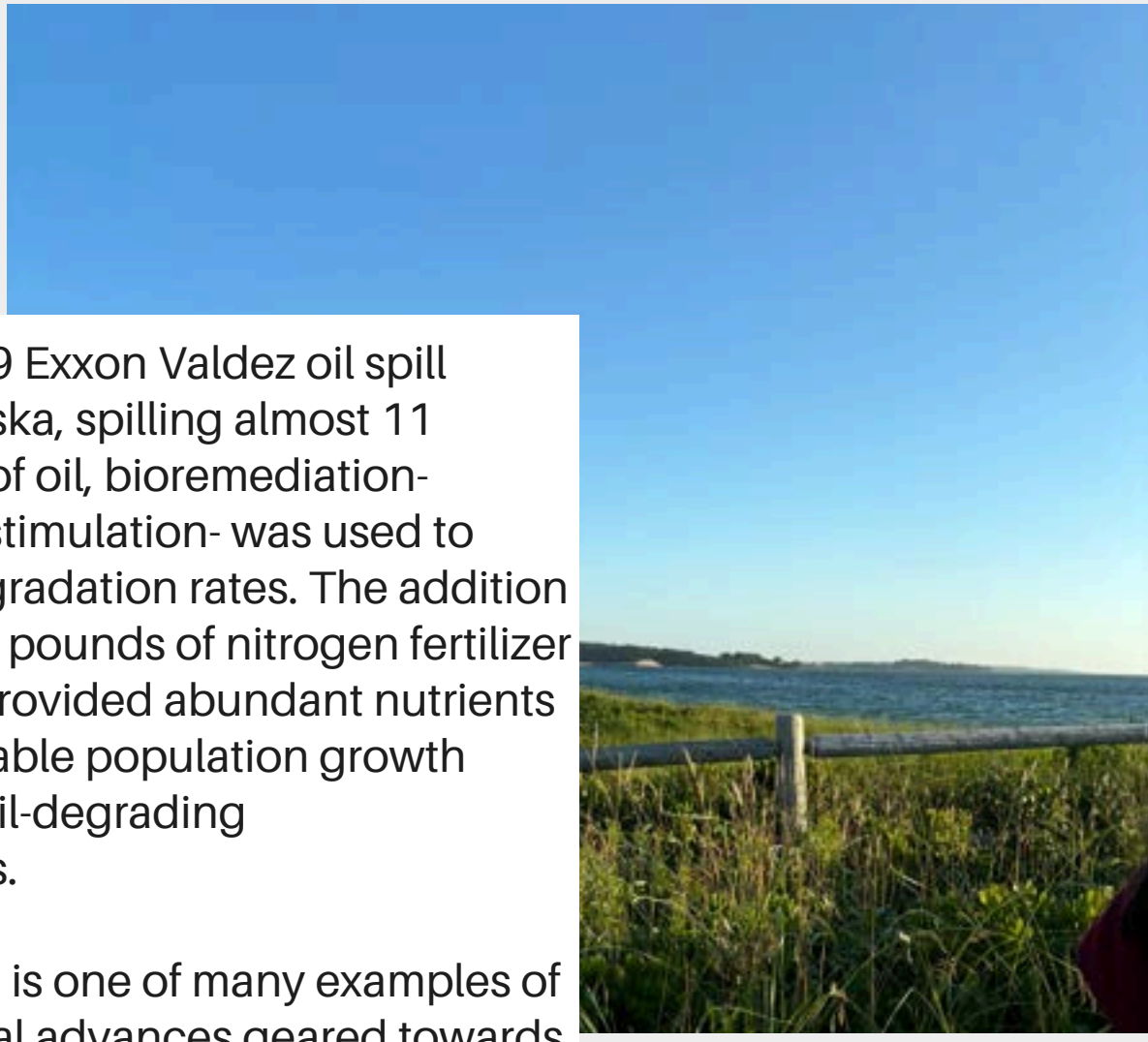


Such techniques include bioaugmentation/biostimulation which enhances pre existing microbial activity through the addition of amendments:organic substrates, electron donors/acceptors, nutrients. Biostimulation is commonly used to alter environmental conditions in order to increase favorability for the growth of certain microorganisms. Meanwhile bioaugmentation may be used to introduce necessary microorganisms which do not naturally occur at a site.

However, usage of bioaugmentation comes with a major drawback; it becomes difficult to control the growth of newly introduced microorganisms. Although these methods can be used to artificially enhance bioremediation, intrinsic bioremediation occurs without human help. Intrinsic bioremediation is akin to a nature's own method of cleaning up, occurring most commonly in soil and water.

In addition bioremediation can be done ex situ, offsite, as opposed to in situ, on site. Ex situ bioremediation is necessary when cleaning up pollutants in unsuitable climates for microorganism growth. Thus when a climate is too frigid or when the water is too acidic ex situ bioremediation is favored. A common example of modern bioremediation usage is the cleaning up of oil spills. As most petroleum hydrocarbons present in crude oils also occur naturally in marine ecosystems, oil degrading microorganisms are ubiquitous. Nevertheless, even under aerobic conditions, degradation, especially of less biodegradable compounds such as resin, occurs over extended periods of time.



When the 1989 Exxon Valdez oil spill occurred in Alaska, spilling almost 11 million gallons of oil, bioremediation- specifically biostimulation- was used to increase biodegradation rates. The addition of over 100,000 pounds of nitrogen fertilizer into the water provided abundant nutrients and more favorable population growth conditions for oil-degrading microorganisms.

Bioremediation is one of many examples of biotechnological advances geared towards tackling the ever growing issue of climate change. Despite its drawbacks, bioremediation allows for us to clean up the environment bit by bit.

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Saltwater Marsh Field Journal-A First Time Field Work Experience

The below is a description of a student's experience taking data at a salt marsh located on cape cod. Working with WHOI, their data was utilized to survey and document changes in the environment of the salt marsh. Salt marshes act as carbon sinks and have great capabilities to store carbon dioxide and other greenhouse gasses, however a surplus of such gasses may lead to ecosystem instability. Through testing such as the below detailed scientists gain a better understanding of the state of salt marshes and thus the overall wellbeing of our marine ecosystems.

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We arrived at the marshes at around 9:15 AM on the 26th of June. The air was sticky and humid although it wasn't sunny out. In silence we trekked through the long grass towards the shallow creek snaking through the terrain.

My group of four was assigned to a mud bank bordering the right fork of the creek. We took data on two locations near this location; a spot in the creek near a raised sandbank(41.575621, -70.640083) and the edge of a mud pond(41.575489, -70.639954).

We started with testing the temperature and pH of both locations(documented below) utilizing a thermometer and a digital pH meter.

	41.575621, -70.640083	41.575489, -70.639954
pH	7.7	7.2
Temperature (c)	23	26

Next we tested for nitrates and ammonia using the below procedure and testing kits.

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Nitrate:

1. Fill a clean test tube with 5ml of sample water
2. Add 10 drops of nitrate solution #1
3. Shake thoroughly
4. Add 10 drops of nitrate solution #2
5. Shake thoroughly
6. Wait for 5 minutes before matching sample water color with nitrate indicator strip

Ammonia:

1. Fill a clean test tube with 5ml of sample water
2. Add 8 drops of ammonia solution #1 and 8 drops of ammonia solution #2
3. Shake thoroughly
4. Wait for 5 minutes before matching sample water color with ammonia indicator strip

	41.575621, -70.640083	41.575489, -70.639954
ammonia(ppm)	0.25	0.25
nitrate(ppm)	0.3	0.25

We also tested for dissolved oxygen content in the water(an indicator of acidity) using the below methodology.

1. Rinse dissolved oxygen bottle three times before fully submerging under water and capping under water. Ensure that there are no air bubbles
2. Uncap bottle and add 8 drops each of manganous sulfate solution and alkaline potassium iodide azide
3. Cap and mix by inverting the bottle several times. Allow debris to settle at the bottom
4. Add 8 drops of sulfuric acid
5. Cap and gently shake dissolved oxygen bottle
6. Fill the test tube with 20 ml of solution from dissolved oxygen bottle
7. Insert titrator into Sodium Thiosulfate
8. Extract 10 ml with no air bubbles
9. Insert full titrator into hole in the cap of test tube
10. Titrate until liquid is clear
11. Add 8 drops of Starch Indicator solution
12. Repeat step 10
13. Record the number shown by the titrator(represents oxygen ppt)

	Oxygen (ppt)
41.575621, -70.640083	6.7
41.575489, -70.639954	6.4

Next we took data on 30 yards of the swamp observing the plant growth every 5 yards along the way. We used quadrats to estimate the approximate percent coverage of each sort of plant along the way.

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Location	Content
0m	5% Wrack, 49% SSA, 1% SAL
5m	90% SSA, 10% dead SSA
10m	80% SSA, 20% dead SSA
15m	60% SSA, 40% sediment + water
20m	40% SSA, 10% sediment + water, 50% bare ground
25m	65% SSA, 5% bare ground, 30% sediment +.water
30m	75% SSA, 25% sediment

Taking such data reveals present plant coverage of areas in the salt marsh helping to reflect the over all health of the salt marsh. In addition we monitor conditions such as pH, nitrogen, and dissolved oxygen to monitor the impacts of climate change and ocean acidification on the salt marsh.

Conclusion

Conserving our worlds natural environments is no easy task. However there are many ways to approach this daunting issue, ranging from scientific monitoring/research to people oriented solutions.

Tales of field work. Community conservation and outreach. Research. Biotech. There is no one way to tackle environmental conservation. Regardless of position every individual has the power to make an impact. Each unique conservation method, each advocate, and each activist brings us one step closer to the future we wish for.

Got feedback?

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