Sediment Variation and Occurrence of the Milky Ribbon Worm (*Cerebratulus lacteus*) in the Gulf of Maine

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Abstract

Average seawater temperatures have been steadily increasing for the past four decades, creating drastic ecosystem shifts for many aquatic species. Commercial landings of softshell clams across the state have decreased by almost 75%, as a result high rates of predation due to warming temperatures (Beal et al., 2018). The mortality rate of soft-shell clams is known to correlate with higher densities of milky ribbon worms (*Cerebratulus lacteus*) (MRW). Past studies have found that 100% of soft-shell clams died with MRW present and 0% died in the absence of MRW. While the milky ribbon worm is known to significantly impact ecosystems, the reason of the species' recent increase is unknown. The main goal of this study is to test whether sediment variation across various sites can shed light on the occurrence of milky ribbon worms. To test the impact of sediment variation on the increase in milky ribbon worms, three plots were placed along each tidal zone at all six sites along the Casco Bay. Samples were collected once in July and once in August. One of the sites, Cousin's Cove had the highest abundance of softshell clams out of all the sites and no MRW presence in July; while in August, Cousin's Cove had MRW presence at all tidal zones and no softshell presence. The presence of MRW at Cousin's cove seems to have wiped out the softshell clam population at the site within a month's time. This study determined that MRW occurs at sites with the highest concentrations of "clayey silts". The softening of mudflats in coastal Maine may be attributed to historic human activity and/or low wave energy. Both of which promote the deposition of fine sediments, which support the tunneling of microorganisms like MRW. Further research over a longer time period as well as assessing LOI, nitrate and conductivity of all samples is necessary to provide a more detailed insight on how sediment variation impacts the occurrence of the milky ribbon worm.

Introduction

Globally, marine aquaculture production has substantially increased the past three decades as additional ocean areas have been deemed ‘suitable’ for further development of the industry (Britsch et al., 2021). The aquaculture expansion in Maine has become a central focus within the United States. Aquaculture in Maine began in the late 1970s when the Darling Marine Center (DMC) aquaculture hatchery was built for research and education purposes. The DMC was the first facility to raise soft-shell clams on such a large scale, producing triploid shellfish of four species (Eastern oysters in 1979, soft-shell clams and bay scallops in 1981 and hard-shell clams in 1986) (*Darling Marine Center - Aquaculture*, 2021). In 2020, there were 179 aquaculture leases in Maine representing a total of 1,430 acres: 690 of which for shellfish aquaculture. The economic potential of aquaculture is exceedingly high while the biological potential has yet to be fully accessed and utilized (Britsch et al., 2021).

The shellfish industry is one of the largest contributors to Maine’s overall economy. The soft-shell clam (*Mya arenaria*) fishery is the second highest commercial fishery to the lobster in both landings and value (McClenachan et al., 2015). In 2019, 7.8 million pounds of soft-shell clam landings valuing to approximately $18.2 million. The total economic benefit of the soft-shell clam industry estimates to $67.5 million (Webber et al., 2020). Most of the revenue from this industry is sold out of state, boosting sales and expanding Maine’s economy. Dispersed among Maine’s eight coastal counties, there are 2,000 residents who own fishing licenses, 56 aquaculture leaseholders and 107 certified shellfish dealers. Aquaculture leases are given by the state but clamming licenses, intended for harvesting clams in the wild, are given by municipalities. Clamming licenses are given out by the municipal shellfish co-management program, allowing each coastal community to manage the soft-shell clam fishery with a greater capacity to respond to change (Webber et al., 2020).

Ocean acidification, as a result of climate change, has been studied to significantly alter species development and reproduction. is By the end of the 21st century, increasing levels of carbon dioxide will cause an increase of ocean acidification by lowering pH by 0.2-0.5 pH units (Salisbury et al., 2008). Acidic river water mixing into the ocean can have serious effects on the development of soft-shell clams. Shellfish have a shell made up of calcium carbonate and are known to be among the most vulnerable species in an increasingly acidic ocean because the process of calcification becomes more difficult (Clements & Chopin, 2017). Clements et al conducted a study to address the effects of sediment acidification on juvenile soft-shell clams, finding that burrowing behavior in addition to post-settlement dispersal of these species are altered by acidification (Clements et al., 2016). Ocean acidification can pose threats to the shellfish industry as a whole, leading to declines in commercial soft-shell clam populations and “unproductive” intertidal areas for clammers ("Maine Clammers Association Newsletter Winter 2016," 2016).

In addition to ocean acidification, average seawater temperature, due to climate change, have been steadily increasing for the past four decades, creating drastic ecosystem shifts for many aquatic species. Average seawater temperatures have been steadily increasing for the past four decades, creating drastic ecosystem shifts for many aquatic species. The softshell clam is an infaunal, suspension-feeding bivalve that lives in the soft-bottom intertidal zone in the state of Maine (Beal et al., 2018). Commercial landings of softshell clams across the state have decreased by almost 75%, as a result of warming temperatures and high rates of predation (Beal et al., 2018). There is a strong correlation between the severity of winters and subsequent summer abundance of softshell clams (Beukema & Dekker, 2014). Cold winters are known to reduce the abundance of both the softshell clam as warming temperatures persisting throughout the fall and into the winter months will lead to a higher abundance of softshell clam recruits. As a result, there has been an increase in predation rates on clams by crustaceans, including the invasive milky ribbon worm (*Cerebratulus lacteus*) (MRW) and European green crab (*Carcinus maenas*) (Beal et al., 2009; Webber et al., 2020). The apparent decrease in soft-shell clams can be detrimental to the communities and individuals within Maine. As over-harvesting occurs, more restrictions are placed on clammers such as conservation or pollution closures (Flatebo, 1996). While this is a positive for the species, it forces Maine clammers out of their jobs.

While climate change has had a negative effect on softshell clams, it has also been correlated with a large population explosion of the green crab in northern New England (Beal et al., 2016). Green crabs have been present in Maine for over a century but have only recently been identified as a threat due to the impending effects of climate change. A green crab population boom has occurred in history before, when seawater temperatures rose in the 1950s, negatively affecting clam fisheries at the time. Fortunately, shortly after in the 1960s, a series of cold winters occurred allowing for the clam fisheries to rebound (McClenachan et al., 2015). As the coldest nights of the year increase with climate change, the likelihood for clam fisheries to rebound post green crab population explosion significantly decreases.

Climate conditions directly affect the life cycle of this species, with higher reproductive success linked to larvae exposure at higher salinities, and warming water during the winter leading to green crab higher densities (Monteiro et al., 2021).Within the past 5 years, green crab populations have significantly increased, coinciding with these climate change related changes in the Gulf of Maine (McClenachan et al., 2015; Monteiro et al., 2021). Beal et al conduced field trials to assess the experimental effects of predator exclusion on the softshell clam along three tidal estuaries in southern Maine, determining that green crab growth rates were much faster in

2018 than they were at a time when coastal seawater temperatures were much cooler. During the warmer months, predators are likely to consume most 0-y class clams. Results from these field experiments suggest that predation, when affected by climate change, is so severe that >0.01% of 0-y class individuals of softshell clams survive beyond their first year (Beal et al., 2018). This suggests that increasing temperatures along the Maine cost have led to an increase in the growth rate of green grabs, and that the physiological plasticity has allowed green grabs to better adapt to warming conditions (Beal et al., 2018). While they continue to be a concern, there have been many studies conducted focusing on the ecological impacts of the green grab as an invasive species.

The milky ribbon worm, another predator of the milky ribbon worm, is substantially less studied than the green crab. The overall impact the animal has on other species has not been thoroughly studied while knowledge about the milky ribbon worms' general biology and ecology is limited. It is known that this Nemertea lives in the intertidal and subtidal burrows of sand and mud along the Atlantic coast of the United States and Canada (McDermott, 2001). Unique in comparison to other nemertean's, MRW are slow moving, nocturnal predators that rely on their highly potent toxins and rabidly everted proboscis to feed. These neurotoxins enable MRW to immobilize and kill their prey easily. Due to the flexible body structure of this species, they can access habitats and prey that many other predators cannot by squeezing themselves into the smallest of crevices. Such habitats include seagrass beds, algal holdfasts and mussel clumps (Thiel & Kruse, 2001).

There are two types of feeding patterns for MRW*:* suctorial and microphagous. Suctorial feeding is the process of 'sucking' the fluids and organs of prey from the inside, while microphagous feeding is the consumption of the entire prey organism at once. Using its siphons, MRW consume the soft-shell clam by inserting its head into the clam's mantle cavity. This method of killing prey is visually distinguishable in comparison to other species (i.e. Green Crab) (Bourque et al., 2001). A wide range of studies have found that the mortality rate of soft-shell clams increases with higher densities of MRW. By conducting field and laboratory studies, Bourque et al tested certain control measures that reduce predation on MRW to better understand the relationship between the two species. Habitat modifications were used as control measures in order to evaluate the abundance of MRW and soft-shell clams. They found that 100% of soft-shell clams died with MRW present and 0% died in the absence of MRW.

Due to the lack of studies on the milky ribbon worm, the reason for the species' recent increase as well as an explanation for the presence or absence of MRW are unknown. Some potential explanations include temperature changes, competition, presence of blood or sand worm, and freshwater flow. One potential explanation that we are focusing on is the sediment variation in mudflats on the coast of Maine. There are many studies that have measured sediment variation by identifying biological and physical factors that influence mudflat porosity, water content, particle size distribution, and soil characteristics.

Porosity is the ratio of pore volume out of the total sediment volume (Wheatcroft et al., 2013). Mudflat porosity is very relevant to the recent sediment variation in the Casco Bay because porosity affects "mudflat trafficability", a term clammers refer to when saying that they can no longer drive their trucks on the flats (Elfstrom, 2020). Additionally, the amount of porosity can affect the ability for certain animals, like MRW, to burrow (Wheatcroft et al., 2013). Prior research on the factors that influence mudflat porosity suggest that porosity increases with the percentage of "clayey silts" (Wheatcroft et al., 2013). Porosity has been higher when sedimentation occurs quickly, because the sediment does not have time to consolidate. Erosion, time above tide and exposure to the sun have been considered to increase consolidation, which therefore decreases porosity. Factors like the migration of micro-organisms in the sediment and macro-organism burrowing counteract this consolidation (Wheatcroft et al., 2013). MRW are an example of a micro-organism that requires tunneling to migrate. Although this study showed a small-scale variation in porosity across sites (based on factors like grain size, tunneling organisms, range of seagrass, etc.), Wheatcroft et al concluded that biotic and abiotic factors counteract one another to maintain an equilibrium porosity over time. This study, along with the changes the clammers have observed (Elfstrom, 2020), suggest a disruption to the natural equilibrium of the ecosystem. Changes in the microbenthic community, range of seagrass, tunneling organisms, and exposure time between tides are other factors that influence sediment porosity on a temperate mudflat (Wheatcroft et al., 2013). Increased porosity due to tunneling should be of special concern, given that MRW spend much of their life burrowing through the sediment.

Another study assessed the success of mudflat restoration in the Canadian Squamish Estuary after a two-year period. Success was determined by comparing sediment characteristics (percent loss-on-ignition, water content, and particle size distribution) and the macroinvertebrate community from the restored area to other sites within the estuary (Roberts et al., 2020b). This study concluded that depth and site location were the greatest determinants of sediment variation. Most of the variation was attributed to the difference in sediment grain size: an inverse relationship was determined to exist between percentage of coarse sand in the sediment, fine sand and silt, loss-on-ignition, and water content. The restored site showed signs of recovery, but the macroinvertebrate still had not respond to restoration efforts. Sediments from the restored sites were mainly silts and fine sands. Clams were only present in reference sites, while bloodworms (*Glycera americana*) were present at restored sites (Roberts et al., 2020b). These relationships will be necessary to consider when assessing the validity of the data collected in relation to the clammers observations that the mudflats have become softer. Softer sediment makes it harder for softshell clams to burrow, leaving the species more vulnerable to predation by MRW. Understanding the reason for the recent change in sediment characteristics may provide insight on why MRW is present as certain sites vs others.

A study involving 18 mudflats from North-west Europe established the classification of intertidal mudflats (Dyer et al., 2000). They determined that mudflat classification based on biological community is difficult because of spatial and temporal variation (Barnes. & Hughes, 1988; Dyer et al., 2000). Barnes and Hughes determined that grain size and percentage of organic material are important when distinguishing habitats within the same site, but not across sites. They describe two main difficulties of classifying community structure and zonation of a mudflat. The first is that the date in which surveys are conducted is crucial, since the community structure of mudflats varies from summer to winter months. The second problem is the natural variation in sediment between local areas. One area may contain sediment that supports certain species, while a nearby area may have sediment that supports merely opposite species (Barnes. & Hughes, 1988). This implies that across multiple towns, there probably will not be a strong correlation between sediment profile and MRW presence, but within the same estuary or /town site, it might be significant enough to warrant categorization.

While the milky ribbon worm is known to significantly impact ecosystems, the reason of the species' recent increase is unknown. The objective of this paper is to provide insight for these unanswered questions. The main goal of this study is to test whether sediment variation across various sites can shed light on the occurrence of milky ribbon worms. Additionally, this study aims to determine if sediment variation can help explain the overall presence or absence of MRW on mudflats and discover the nature of variation in sediment characteristics across multiple sites.

Methodology

Study Sites

To test the impact of sediment variation on the increase in milky ribbon worms, four geographically distinct towns along the Gulf on Maine (Figure 1) were selected. Within each town, one to two intertidal mudflats were chosen based on the presence of soft-shell clams and recommendation from local clammers. All data were collected at high, medium, and low tides parallel to ocean line. Maps of plot locations at each of the six sites are in Appendix A.

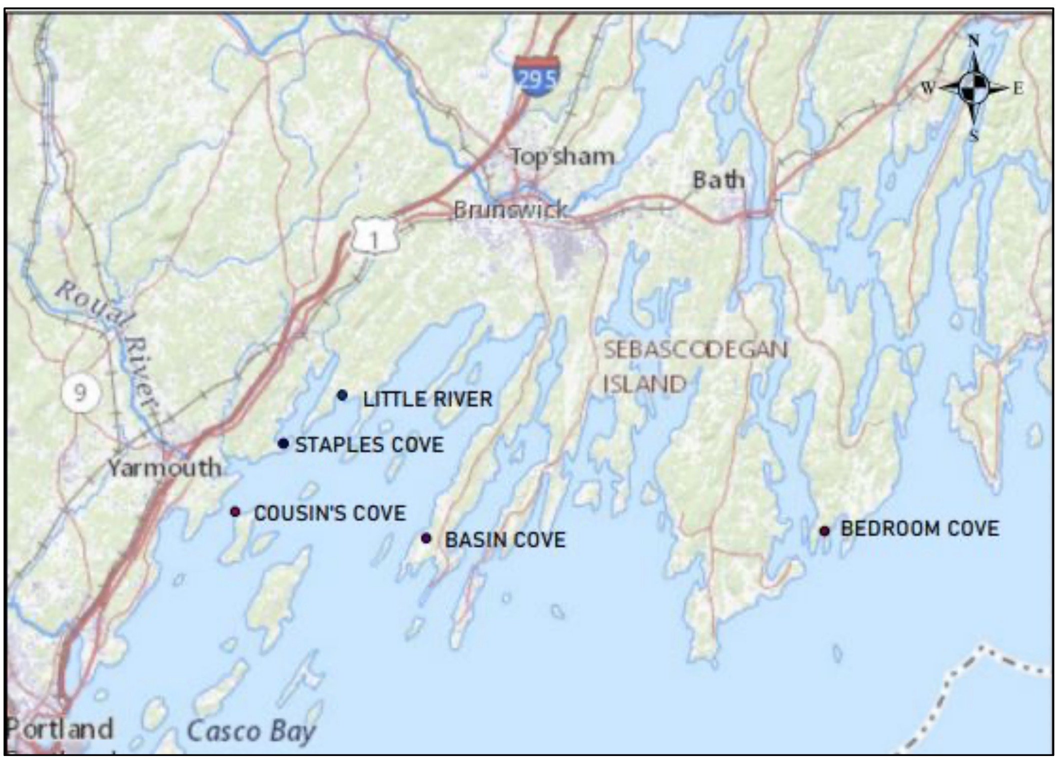

Figure 1: Site Location Map

Field Sampling

To test the sediment variation at each intertidal flat, three randomly placed 1 ft x 2 ft plots, following the Department of Maine Resources Soft Shell Clam Population Survey Field Guide, were established at each tidal zone (Resources, 2021). Tide predictions were used to select sampling days when the tide would be no more than .5 above or below the average. Sampling sites varied widely in total area; placement of high, medium, and low plots was not affected by the differences in size. Samples were taken in the first round of samples at each site on 14 July (Staples Cove), 15 July (Little River), 22 July (Bedroom Cove), 23 July (Strawberry Cove), 28 July (Basin Cove), and 29 July (Cousin's Cove). In the second round, samples were taken at each site on 11 August (Staple's Cove), 13 August (Little River), 14 August (Basin Cove), 16 August (Bedroom Cove), and 17 August (Cousin's Cove). Strawberry Cove was only sampled in July.

Each entire plot was excavated with a clam hoe, to measure and record the number of softshell clams and quahogs present. To follow clammer protocol, softshell clams were measured by their width, and quahogs were measured by their height. The width of the quahogs was also measured to allow for species comparison. All shellfish were then returned to the sediment. Any MRW present were pulled out and placed into labeled plastic bags. Additionally, the number of blood worms and sand worms present at each plot were counted. PVC cores were inserted into the soil (depth of 20 cm) to extract sediment samples at each location and plots. Cores were cut at 10 cm depth from the top, regardless of core length, to establish the top and bottom of each core.

Lab Processing and Statistical Analysis

A series of variables were measured from the sediment core samples: pH, soil texture by particle size using the hydrometer method (Gee & Or, 2002), loss on ignition (LOI), and moisture. The hydrometer method measures the density of each sample at appropriate times. Based on those measurements, we calculated the amount of silt or clay remaining in suspension and the particle size distribution of the sample (Gee & Or, 2002). Tests were done on the top and bottom samples of each core collected. Statistical analysis tests were performed, such as a Principal Component Analysis, to assess the relationship between pH, % moisture, % organic matter, and % silt at each site.

Results:

Plot Data

The research revealed that there was generally little overlap between softshell clam presence and milky ribbon worm presence. An overview of the presence or absence of soft-shell clams, MRW, and other worms in July and August respectively is presented in Table 1a and 1b. In July, there was softshell presence at Staple's Cove, Little River, and Bedroom Cove, while there was MRW presence at Staple's Cove and basin Cove. In August, there was softshell presence at Little River and Bedroom Cove, but no longer at Cousin's Cove. Additionally, there was MRW presence Staple's Cove, Little River, Basin Cove, and Cousin's Cove. Overall, softshell clams were never found at low tide, and MRW were found most frequently at medium and low tide, which only occasional occurrence at high tide. Cousin's Cove in July had the highest abundance of softshell clams out of all the sites and no MRW presence, but in August, Cousin's Cove had MRW presence at all tidal zones and no softshell presence.

SITE	PLOT LOCATION	SOFTSHELLS	MRW	OTHER WORMS
STAPLE'S COVE	HIGH	X		X
STAPLE'S COVE	MID			
STAPLE'S COVE	LOW		X	X
LITTLE RIVER	HIGH	X		
LITTLE RIVER	MID	X		X
LITTLE RIVER	LOW			X
BASIN COVE	HIGH		X	X
BASIN COVE	MID		X	X
BASIN COVE	LOW		X	X
BEDROOM COVE	HIGH	X		X
BEDROOM COVE	MID	X		X
BEDROOM COVE	LOW			
COUSINS COVE	HIGH	X		X
COUSINS COVE	MID			X
COUSINS COVE	LOW			X

SITE	PLOT LOCATION	SOFTSHELLS	MRW	OTHER WORMS
STAPLE'S COVE	HIGH		X	X
STAPLE'S COVE	MID			X
STAPLE'S COVE	LOW		X	X
LITTLE RIVER	HIGH	X		X
LITTLE RIVER	MID	X		X
LITTLE RIVER	LOW		X	X
BASIN COVE	HIGH			X
BASIN COVE	MID		X	X
BASIN COVE	LOW		X	X
BEDROOM COVE	HIGH	X		X
BEDROOM COVE	MID	X		X
BEDROOM COVE	LOW			X
COUSINS COVE	HIGH		X	X
COUSINS COVE	MID		X	X
COUSINS COVE	LOW		X	X

Table 1a & b: July and August data (respectively) of presence and absence of softshell clams, milky ribbon worms, and other worms by site and plot location

Sediment Characteristics

Figure 2 displays particle size distribution data with the percentage of the sample that is smaller than any particle diameter. At all sites, most of the samples are in the smaller size fraction categories that classify as silt or clay (.05-.1 to .001-.002). The data aligned with observations from the field, as most beaches visited had noticeably higher silt and clay content. The exception was Bedroom cove, which was the sandiest site visited.

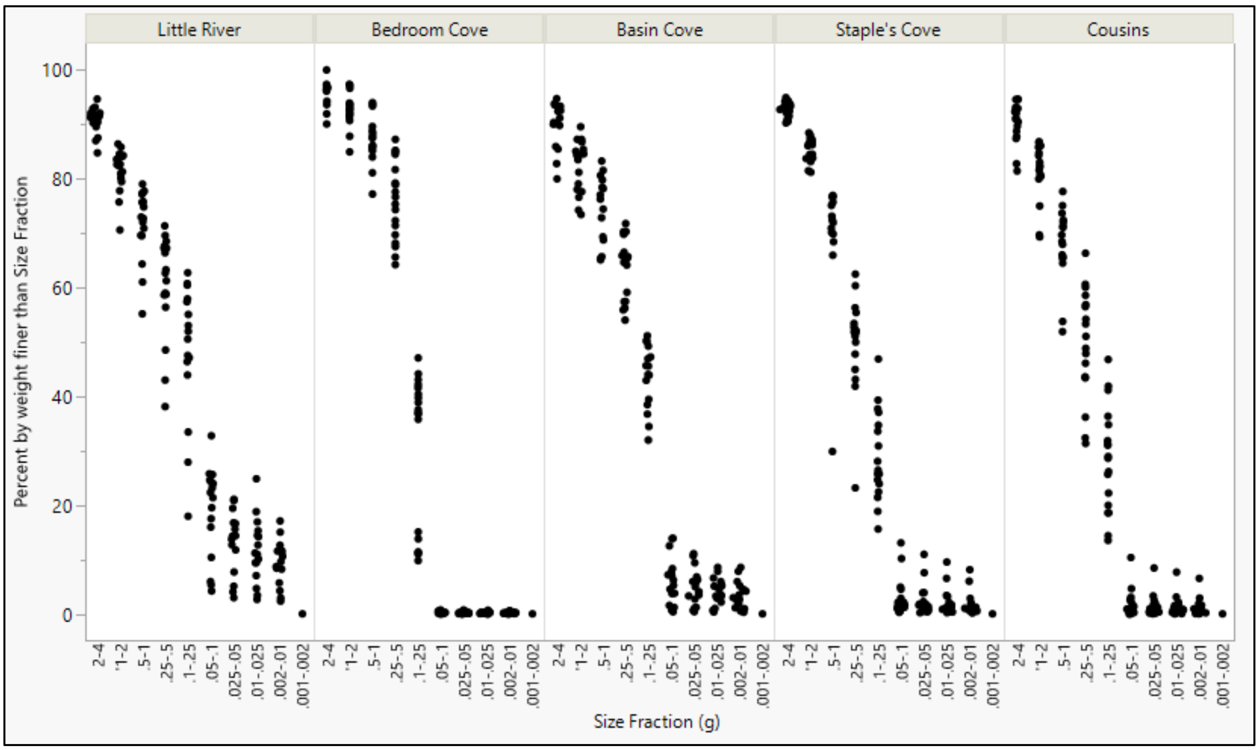

Figure 2: Size fraction data calculated from the settling method for texture is displayed above. Each dot represents the percentage of the sample that is finer than the given size fraction.

A principal component analysis (PCA) was completed to assess the relationship between pH, % moisture, % organic matter, and % silt at each site. The PCA indicated that the first principal component (PC1) contributed to 48.12% of variation among sites, whereas the second principal component (PC2) accounted for 25.4% of variation (PC1 + PC2 = 73.484%; Fig 3). Percent silt and % moisture have a strong relationship with positive values along PC1. Similarly, pH has a strong relationship with the negative values of PC1. Percent organic matter has a strong relationship with the positive values of PC2. There does not seem to be any separation between MRW presence and absence within the PCA.

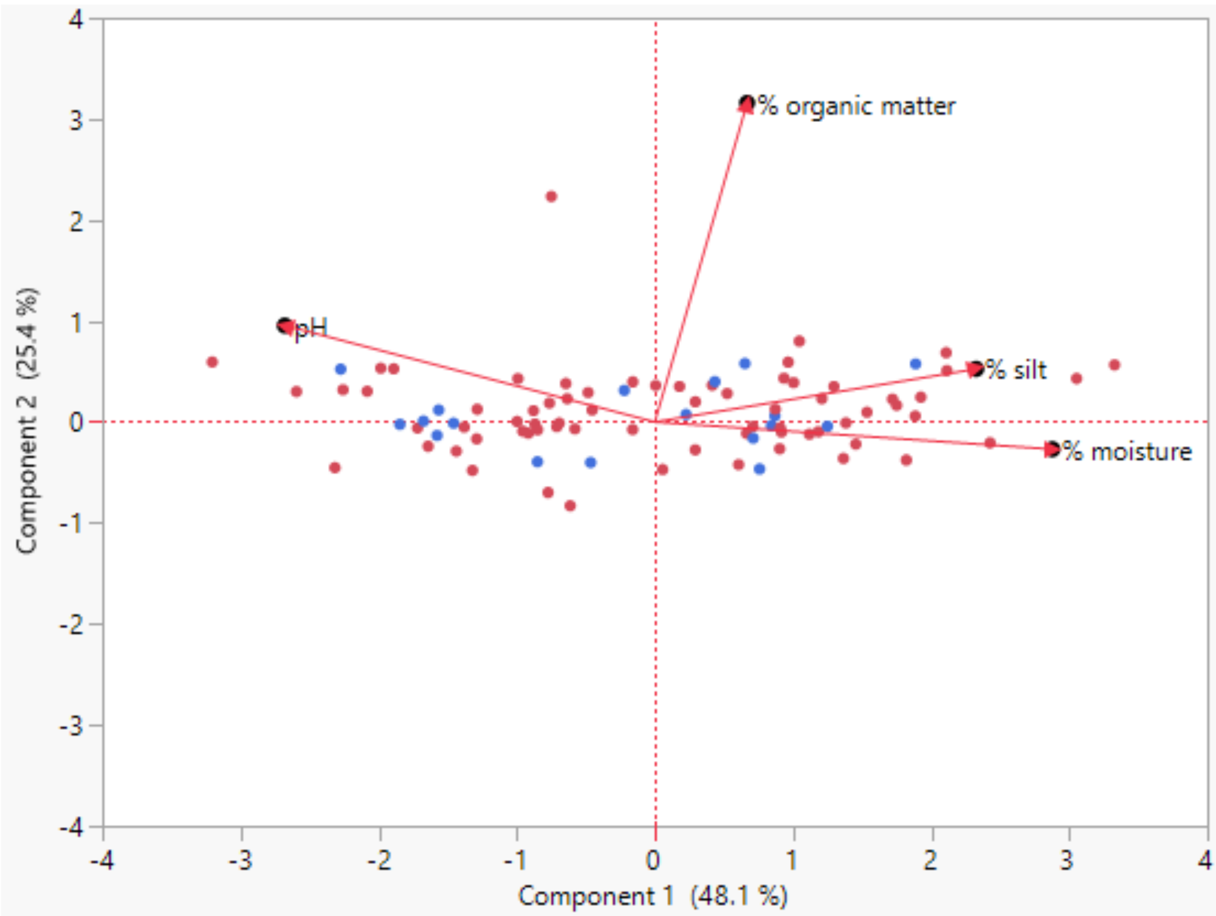

Figure 3: Principal component analysis (PCA) of sediment variables: pH, % moisture, % organic matter and % silt for each site. The first principal component accounts for 48.12% of variation among samples (based on eigenvalue calculations). Ellipses delineate based on MRW presence and absence. Red dots represent the absence of MRW, and the blue dots represent the presence of MRW at a given sample.

Number	Eigenvalue	Percent	Cum Percent
1	1.9248	48.12	48.12
2	1.015	25.374	73.484
3	0.6824	17.061	90.555
4	0.3778	9.445	100

Table 2: Eigenvalue table associated with the Principal Component Analysis

Discussion:

Average seawater temperatures have been steadily increasing for the past four decades, creating drastic ecosystem shifts for many aquatic species. Commercial landings of softshell clams across the state have decreased by almost 75%, as a result of high rates of predation due to warming temperatures (Beal et al., 2018). The mortality rate of soft-shell clams is known to correlate with higher densities of MRW. For example, Bourque et al tested certain control measures that reduce predation on MRW and used habitat modifications as control measures in order to evaluate the abundance of MRW and soft-shell clams. They found that 100% of soft-shell clams died with MRW present and 0% died in the absence of MRW. Based on the results of this paper, Cousin's Cove in July had the highest abundance of softshell clams out of all the sites and no MRW presence; while in August, Cousin's Cove had MRW presence at all tidal zones and no softshell presence. The presence of MRW at Cousin's cove seems to have wiped out the softshell clam population at the site within a month's time. While the reason of the species recent increase may be unknown, MRW occurrence may be attributed to the softening of mudflats in coastal Maine due to historic human activity and/or low wave energy. The introduction of MRW predators may also provide more information on the occurrence of the species in the Casco Bay.

Mudflat trafficability

Based on the results and Tables 1a and 1b, most MRW presence is at three of the sites: Staple's Cove, Basin Cove, and Cousin's Cove. This aligned with the size fraction data (Figure 2) because there were the highest concentrations of "clayey silts" at these three sites in comparison to the others. The presence of MRW in combination with the high levels of "clayey silts" at these three sites may be what is preventing the sediment from consolidating. The clammers have observed changes in the "mudflat trafficability" and porosity of the sediment, preventing them from driving their trucks onto the mudflats. The presence of MRW and high levels of ‘clayey silts’ may be the cause of the changes the clammers observed, and what is affecting the "mudflat trafficability". Previously, clammers were able to drive out onto the mud in the summers low tide, and drive out again as the tide came in. They note that the mud exposed at low tides is getting softer, making it increasingly difficult to walk on the mudflats without sinking in the mud up to your knees (Elfstrom, 2020).

Studies have found that relative sea-level rise, climatic variations, and human effects are interplaying determinants of sediment accretion and the type of sediment accreted (Ward et al., 2008). A study involving 18 sites from North-west Europe established the classification of intertidal mudflats (Dyer et al., 2000). The primary grouping characteristics that emerged in the correlative analysis were tidal range, exposure to waves, and slope. Mean density, defined by the depth of a footprint impression, was used to describe bulk density and a proxy for porosity. Dry density was determined to be a significant variable within the meso/macro tidal flats (tidal range of 2-6 meters) and categorized into “hard” (sinking of less than 10 cm), “medium,” (10-30 cm) and “soft” (greater than 30 cm). This parameter of density may be useful when classifying the sediment based on the clammers’ descriptions (Dyer et al., 2000; Elfstrom, 2020). Density was also found to be significant in Dyer’s 1998 classification of mudflats (Dyer, 1998). These two studies suggest that meso/macro tidal flats are most common in areas of low wave energy which leads to high rates of sedimentation and low rates of compaction and density. The low wave energy promotes deposition of fine sediments, which support microorganisms. These microorganisms then stabilize the sediment and increase porosity and water content through bioturbation. In areas of high wave energy, the opposite occurs. These interactions of variables may be helpful when explaining the clammers' observations (Elfstrom, 2020).

Research suggests that sediment delivery on sites where anthropogenic influence has occurred, cause coarse sediments to settle earlier in the foothills and then the finer silts and clays go all the way down the bay (Jaffe et al., 2007; Roberts et al., 2020a). Jaffe et al conducted a study on the anthropogenic influence on sedimentation and intertidal mudflat change in San Pablo Bay, California from 1856 to 1983 (Jaffe et al., 2007). The study determined that the shape, composition and extent of the mudflats significantly changed over this time period in response to sediment-delivery fluctuations. The abundance of sediment as a result of hydraulic mining led to a 60% increase in intertidal mudflat area and deposition in the shallows (Jaffe et al., 2007). The results suggested a positive correlation between mudflat area and the rate of sediment delivery, and an inverse correlation between erosion severity and the rate of sediment delivery (Jaffe et al., 2007). There were two periods in this time frame where natural sedimentation patterns were disrupted by human activity. The first was due to hydraulic mining washing finer particulates into the bay by tributary rivers and the second was a period of net erosion when dams were built on the tributary rivers thus interrupting sediment delivery to the bay (Jaffe et al., 2007). This study demonstrates that it is important to examine historic human activity, not only in the Casco Bay but also up-river. Little River, one of the sites visited, has a similar history. In 1683, a sawmill was built on Little River that was originally destroyed during the Indian wars. The reconstruction of the mills was built on these waterways, causing significant amounts of erosion (Butler, 2019). As anthropogenic influences lead to increases in erosion, the delivery of sediment to the bay is interrupted. Coarser grains dislodged during these activities settle first, and the finer particles wash into the bay. Additionally, porosity increases with the percentage of "clayey silts" and is higher when sedimentation occurs because the sediment cannot consolidate. Factors that contribute to this are the migration of microorganisms in the sediment, since this tunneling counteracts consolidation (Wheatcroft et al., 2013). Our results show that while Little River only had some MRW presence, it did have softshell presence in both July and August. If finer sediment is more porous and thus ‘softer’, then perhaps such activities on Little River are partially responsible for the change in mudflat trafficability. This may explain the softer sediment accruing in the Casco Bay area, therefore attracting MRW presence as well.

Based on the PCA, % silt and % moisture have a strong relationship with positive values along PC1. Similarly, pH has a strong relationship with the negative values of PC1. These results suggest that PC1 increases with higher amounts of % silt and % moisture; as % silt increases, % moisture tends to increase as well. This indicates that sites with higher amounts of silt and moisture make up most of the variation within the samples. Mudflats are formed by a buildup of sediment carried in by tides and rivers. This may be attributed to the suggested increase in sediment delivery in areas affected historically by human activity (Jaffe et al., 2007). Percent organic matter has a strong relationship with the positive values of PC2. The four variables analyzed with the PCA, pH, % moisture, % organic matter, and % silt, do not appear to influence MRW presence or absence. MRW are sulfide tolerant and have the ability to survive in sediment composed of 50% or more organic content (Veisel, 2020). Soils that are collecting organic matter are sealed by sapropel; such soils have shallow sulfide deadline and digging more than 12 in releases sulfide smell into the air. As this sulfide deadline approaches the surface, habitat conditions for milky ribbon worms improve. These are characteristics of declining soil for softshell clams especially because high silt and organic matter content slows down softshell clam growth (Veisel, 2020). While the occurrence of MRW does not seem to be influenced by these variables, it appears the opposite is true for softshell clams.

The clammers observations that the porosity of sediment in the intertidal mudflats has changed impacts softshell clam populations as softer sediment pose new risks to the species. A study assessing the influence of sediment type on antipredator response of softshell clams discovered that the species burrowed more deeply in mud than in coarser sand (Thomson & Gannon, 2013). Thomson and Gannon found that sediment grain size impacted the magnitude of softshell burrowing response, the smaller the sediment grain size, the stronger the response. The softer sediment in the Gulf of Maine may increase the strength of softshell burrowing response because coarser sediment slows development and constricts feeding capabilities (Thomson & Gannon, 2013).

Opposingly, our results show that clams were frequently preyed upon regardless of burrowing depth. MRW live under rocks, and burrow in the sand of shallow water areas about 6-8 inches from the surface (Carter & Moon, 2020). The ability of this Nemertean to burrow deep below the surface, allows for predation in areas where softshell development and feeding abilities may be dampened. As Thomson and Gannon determined, softshell clams are increasingly vulnerable to predation in areas with softer sediment. These factors may be the reason for the presence of MRW and absence of softshell clams at Cousin's Cove, Staple's Cove and Basin Cove. These were the sites studied containing the highest size fraction of silt and clay (.05-.1 to .001-.002). To really assess the variability in sediment, conducting research for more than 2 months is necessary. We were able to see some variation but taking samples every month for a year would give more accurate results. We planned to look at LOI, nitrate and conductivity of all samples, but ran out of time. Measuring these variables may provide more insight on the variation as well.

Predation

Soft shells were collected mostly high tidal zones, which is plausible because predators, such as MRW, have been studied to eradicate softshell clams these areas. The first-year post birth is the most vital for the survival of softshell clam. Since they are small at this stage of development, this makes them increasingly vulnerable to predators such as MRW and Green Crabs. Typical female clams reproduce 120,000 to 3 million eggs a year, but the survival rate is .1 percent due to the intensity of predation on the species (Jett, 2019). Due to this, the species has had to adapt over the years and can be found in sandy areas (Baker & Mann, 1991). Anecdotally, our results show that Bedroom Cove, the sandiest site visited, was one of the few sites with softshell clam presence.

Infaunal predators are important in structuring soft-bottom communities (Bourque et al., 2001). Examples of infaunal predators that are important in intertidal areas include nemerteans and polychaetes. These predators can significantly decrease prey populations, such as the soft-shell clam. Bloodworms, a polychaete, is the 6th most important commercial bait worm fishery in Maine. Bloodworm landings are worth between $5 million and $8 million each year (Sypitkowski et al., 2010). Ambrose et al found that bloodworm digging exposed 6% of softshell clams, influencing rates of survivorship as well (Ambrose, 1986). Both bloodworms and sandworms (*Nereis virens*) are important because of their harvest as part of the bait worm fishery. Collecting data on the bait worms is interesting because these species' joint interactions with MRW are unknown.

One study used bloodworms (*Glycera dibranchiata*) to assess if the presence of polychaetes would affect MRW predation on soft shell clams. Bloodworms are commonly found in the same habitat as MRW, and have been demonstrated to increase the abundance of infaunal species by predating on their predator (Ambrose, 1986). They discovered that the presence of bloodworms significantly reduced MRW predation on clams. There was approximately a 33% mortality rate when softshell clams and MRW were placed alone, and a 20% mortality rate when bloodworms were present with softshell clams and MRW. But, a 9% mortality rate was found when bloodworms were alone with the clams still occurred, making its use as a control measure uncertain (Bourque et al., 2001). Attempts to duplicate this experiment outside from the lab have been unsuccessful because the use of mudflat sediment introduced green crabs. This made it difficult to determine the direct impact of bloodworms on MRW predation of softshell clams (Bourque et al., 2001; Carter & Moon, 2020).

The sandworm (*Nereis virens)* is another bait worm that has been studied to control nemertean predation on softshell clams. In addition, the relationship between MRW and sandworms has revealed a migratory response of sandworms in the presence of MRW. Studies have indicated that infauna will move into the water column in response to the presence of infaunal predators. This observed escape response may be in response to MRW. Baroque et al found that there was no significant reduction in clam mortality when sandworms were present, confirming that sandworms will be ineffective in suppressing nemertean predation on softshell clams (Bourque et al., 2001).

Bloodworms typically predate on sandworms; experimental exclusion of bloodworms results in enhanced survivorship of sandworms (Wilson, 1990). Sandworms are known to negatively impact many infaunal species in soft-sediment communities. Thus, as bloodworms predate on sandworms, infaunal populations that are typically threatened by predation from sandworms, will increase (Sypitkowski et al., 2010) Our results show varied bloodworm and sandworm presence at each site. Further research on the interactions of multiple predator-prey relationships, such as isolating bloodworms and sandworms with MRW and softshell clams, is necessary to shed light on the recent increase of MRW presence in the Casco Bay.

Overall, the results of this study determined that MRW occurs at sites with the highest concentrations of "clayey silts". Literature review indicates that the softening of mudflats in coastal Maine may be attributed to historic human activity and/or low wave energy. Both of which promote the deposition of fine sediments, which support the tunneling of microorganisms like MRW. Additionally, increased predation rates due to climate warming plays a significant role in the relationships of commercial bait worms and MRW. Further research over a longer time period in addition to testing for LOI, nitrate and conductivity on all samples is necessary to provide a more detailed insight on how sediment variation impacts the occurrence of the milky ribbon worm. A longer study may also provide more information on the interactions of multiple predator-prey relationships that have yet to be fully studied previously.

References

Ambrose, W. G. (1986). Estimate of removal rate ofNereis virens (Polychaeta: Nereidae) from an intertidal mudflat by gulls (Larus spp.). *Marine Biology*, *90*(2), 243-247. https://doi.org/10.1007/BF00569134

Baker, P., & Mann, R. L. (1991). Soft Shell Clam Mya arenaria. *VIMS Books and Book Chapters*, *19*.

Barnes., R. S. K., & Hughes, R. N. (1988). *An Introduction to Marine Ecology*. Blackwell Scientific Publications.

Beal, B., Protopopescu, G., Yeatts, K., & Porada, J. (2009). Experimental Trials on the Nursery Culture, Overwintering, and Field Grow-Out of Hatchery-Reared Northern Quahogs (Hard Clams), Mercenaria mercenaria (L.), in Eastern Maine. *Journal of shellfish research*, *28*, 763-776. https://doi.org/10.2983/035.028.0405

Beal, B. F., Coffin, C. R., Randall, S. F., Goodenow, C. A., Pepperman, K. E., Ellis, B. W., Jourdet, C. B., & Protopopescu, G. C. (2018). Spatial Variability in Recruitment of an Infaunal Bivalve: Experimental Effects of Predator Exclusion on the Softshell Clam (<i>Mya arenaria</i> L.) along Three Tidal Estuaries in Southern Maine, USA. *Journal of shellfish research*, *37*(1), 1-27, 27. https://doi.org/10.2983/035.037.0101

Beal, B. F., Nault, D.-M., Annis, H., Thayer, P., Leighton, H., & Ellis, B. (2016). Comparative, Large-Scale Field Trials Along the Maine Coast to Assess Management Options to Enhance Populations of the Commercially Important Softshell Clam, Mya arenaria L. *Journal of shellfish research*, *35*(4), 711-727. https://doi.org/10.2983/035.035.0401

Beukema, J. J., & Dekker, R. (2014). Variability in predator abundance links winter temperatures and bivalve recruitment: correlative evidence from long-term data in a tidal flat. *Marine Ecology Progress Series*, *513*, 1-15. https://www.int-res.com/abstracts/meps/v513/p1-15/

Bourque, D., Miron, G., & Landry, T. (2001). Predation on soft-shell clams (Mya arenaria) by the nemertean Cerebratulus lacteus in Atlantic Canada: Implications for control measures. *Hydrobiologia*, *456*, 33-44. https://doi.org/10.1023/A:1013061900032

Britsch, M. L., Leslie, H. M., & Stoll, J. S. (2021). Diverse perspectives on aquaculture development in Maine [Article]. *Marine Policy*, *131*, Article 104697. https://doi.org/10.1016/j.marpol.2021.104697

Butler, J. (2019). History of Kennebunk Maine. *University of Maine Digital Commons*.

Carter, J., & Moon, N. (2020). Milky Ribbon Worm (Cerebratulus lacteus) Predation and Mitigation: A Review. *Casco bay regional shellfish working group*.

Clements, J. C., & Chopin, T. (2017). Ocean acidification and marine aquaculture in North America: potential impacts and mitigation strategies. *Reviews in Aquaculture*, *9*(4), 326-341. https://doi.org/https://doi.org/10.1111/raq.12140

Clements, J. C., Woodard, K. D., & Hunt, H. L. (2016). Porewater acidification alters the burrowing behavior and post-settlement dispersal of juvenile soft-shell clams (Mya arenaria). *Journal of Experimental Marine Biology and Ecology*, *477*, 103-111. https://doi.org/https://doi.org/10.1016/j.jembe.2016.01.013

Darling Marine Center - Aquaculture. (2021). https://dmc.umaine.edu/welcome/history/history-aquaculture/

Dyer, K. R. (1998). The typology of intertidal mudflats. *Geological Society, London, Special Publications*, *139*(1), 11-24. https://doi.org/10.1144/gsl.Sp.1998.139.01.02

Dyer, K. R., Christie, M. C., & Wright, E. W. (2000). The classification of intertidal mudflats. *Continental Shelf Research*, *20*(10), 1039-1060. https://doi.org/https://doi.org/10.1016/S0278-4343(00)00011-X

Elfstrom, O. (2020). The Natural History of the Milky Ribbon Worm: As Told by Maine Clam Diggers. *Bates College*.

Flatebo, G. (1996). Clamming Money in the Mud Estuary Project Fact Sheet. *Casco Bay Estuary Project*.

Gee, G. W., & Or, D. (2002). 2.4 Particle-Size Analysis. In *Methods of Soil Analysis* (pp. 255-293). https://doi.org/https://doi.org/10.2136/sssabookser5.4.c12

Jaffe, B. E., Smith, R. E., & Foxgrover, A. C. (2007). Anthropogenic influence on sedimentation and intertidal mudflat change in San Pablo Bay, California: 1856–1983. *Estuarine, Coastal and Shelf Science*, *73*(1), 175-187. https://doi.org/https://doi.org/10.1016/j.ecss.2007.02.017

Jett, D. (2019). The good, the bad and the muddy. *Findings from the Field*, *2*(1), 2.

Maine Clammers Association Newsletter Winter 2016. (2016). *Maine Clammers Association*.

McClenachan, L., O'Connor, G., & Reynolds, T. (2015). Adaptive capacity of co-management systems in the face of environmental change: The soft-shell clam fishery and invasive green crabs in Maine. *Marine Policy*, *52*, 26–32. https://doi.org/10.1016/j.marpol.2014.10.023

McDermott, J. J. (2001). Status of the Nemertea as prey in marine ecosystems [Conference Paper]. *Hydrobiologia*, *456*, 7-20. https://doi.org/10.1023/A:1013001729166

Monteiro, J. N., Pinto, M., Crespo, D., Pardal, M. A., & Martinho, F. (2021). Effects of climate variability on an estuarine green crab Carcinus maenas population. *Marine Environmental Research*, *169*, 105404. https://doi.org/https://doi.org/10.1016/j.marenvres.2021.105404

Resources, S. o. M. D. o. M. (2021). *Soft Shell Population Survey Field Guide*.

Roberts, E. M., Stroshein, S. D., & Bendell, L. I. (2020a). Change in sediment features and the macroinvertebrate community within an estuarine ecosystem two years post-restoration. *Ecosphere*, *11*(7), e03206. https://doi.org/https://doi.org/10.1002/ecs2.3206

Roberts, E. M., Stroshein, S. D., & Bendell, L. I. (2020b). Change in sediment features and the macroinvertebrate community within an estuarine ecosystem two years post-restoration [Article]. *Ecosphere*, *11*(7), Article e03206. https://doi.org/10.1002/ecs2.3206

Salisbury, J., Green, M., Hunt, C., & Campbell, J. (2008). Coastal Acidification by Rivers:A Threat to Shellfish? *Eos, Transactions American Geophysical Union*, *89*(50), 513-513. https://doi.org/https://doi.org/10.1029/2008EO500001

Sypitkowski, E., Bohlen, C., & Ambrose Jr, W. G. (2010). Estimating the frequency and extent of bloodworm digging in Maine from aerial photography. *Fisheries Research*, *101*(1-2), 87-93.

Thiel, M., & Kruse, I. (2001). Status of the Nemertea as predators in marine ecosystems. *Hydrobiologia 456, 21-32*.

Thomson, E., & Gannon, D. P. (2013). Influence of Sediment Type on Antipredator Response of the Softshell Clam, Mya arenaria. *Northeastern Naturalist*, *20*(3), 498-510. http://www.jstor.org/stable/43287133

Veisel, T. (2020). *The Cultivation and Chemistry of Marine Soils*. The Blue Crab Forum.

Ward, L. G., Zaprowski, B. J., Trainer, K. D., & Davis, P. T. (2008). Stratigraphy, pollen history and geochronology of tidal marshes in a Gulf of Maine estuarine system: Climatic and

relative sea level impacts [Article]. *Marine Geology*, *256*(1-4), 1-17. https://doi.org/10.1016/j.margeo.2008.08.004
Webber, M. M., Stocco, M., & Schmitt, C. (2020). *Maine Shellfish Handbook*.
Wheatcroft, R. A., Sanders, R. D., & Law, B. A. (2013). Seasonal variation in physical and biological factors that influence sediment porosity on a temperate mudflat: Willapa Bay, Washington, USA. *Continental Shelf Research*, *60*, S173-S184. https://doi.org/https://doi.org/10.1016/j.csr.2012.07.022
Wilson, W. H. (1990). Competition and predation in marine soft-sediment communities. *Annual Review of Ecology and Systematics*, *21*(1), 221-241.

Appendix A: Map of Plot Locations at each Site


Bedroom Cove Tidal Zones
N
W
E
S
High Tide
Mid Tide
Low Tide
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Staple's Cove Tidal Zones
N
W
E
S
Low Tide
Mid Tide
High Tide
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Cousin's Cove Tidal Zones
N
W
E
S
Low Tide
Mid Tide
High Tide
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community


Little River Tidal Zones
N
W
E
S
High Tide
Mid Tide
Low Tide
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community


Basin Cove Tidal Zones
N
W
E
S
High Tide
Mid Tide
Low Tide
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community