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LABORATORY MESOCOSMS AND GEOLOGICAL STUDIES FOR MONITORING WETLANDS DIVERSITY

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Abstract

Anthropogenic impacts have destroyed many salt marshes and mangroves world-wide, and now they must be rebuilt to restore their valuable ecosystem services. Laboratory mesocosms provide important information on impacts from sea-level rise, climate change, pollution and biological invasions. We illustrate these principles using examples from Chezzetcook Estuary, a boreal **mesotidal** wetland in northeastern Canada. This estuary is the location of classical paleoecology studies using foraminifera, testate amoebae and pollen to reconstruct sea level changes to ±1 cm precision and to determine natural versus anthropogenic pollution effects. We have developed a replicable laboratory mesocosm system to examine various responses under controlled conditions at all seasons. This mesocosm experiment provides the first high-resolution data on population dynamics and compositions of foraminifera in conjunction with the meiofauna (nematodes, ostracods, copepods) coexisting in the tidal marsh sediments. We are also beginning to use stable isotopes to understand the specific trophic roles of foraminifera and the meiofauna that comprise most of the small food-web in the highly productive salt marsh ecosystem. These studies, combined with palynological and geochemical records of human impacts, are important for managing salt marsh systems and for interpreting foraminiferal records of paleo-sea level and paleoclimate change. We also report on new studies of surface sediments from the boreal **microtidal** wetlands of the Danube Delta and adjacent continental shelf. Initial results reveal 20 phytoplankton taxa and >50 zooplankton or benthic meiofauna with organic remains that mark the pathways of particulate organic carbon to the delta plain and nearshore. In 2013, the amounts of freshwater algal spores and dinocysts in the surface layer did not indicate strong eutrophication in the river channel, Razim Lake and plume environments; however, they show a potential for *Gloeotrichia* blooms nearshore, and outbreaks of *Botryococcus* on the prodelta front. Dinocysts in prodelta sediments could also be seeded into the Danube lakes by storm waves and cause serious damage where summer water salinity is above 9. We recommend EU partnership to study sediment cores that record changes in the Delta paleosalinity, plankton and meiofaunal populations since Roman occupation time. More global collaboration efforts is required to reduce wasted time, energy and finances on overlapping 'trial-and-error' experiments and evaluators of success. There is also need for exchange of microfossil and palynological data to standardize laboratory methods and taxonomy.

Keywords: foraminifera, meiofauna, palynology, Chezzetcook Estuary, Danube Delta

1 INTRODUCTION AND OBJECTIVES

It is widely known that coastal tidal wetlands and saltmarshes throughout the world perform an essential role in stabilizing coastlines and acting as both carbon storehouses and sources of ocean "blue carbon" (Scott et al., 2014). Unfortunately, anthropogenic impacts such as dredging, land drainage, river dams, climate warming, increased storminess, and rising sea levels have destroyed coastal wetlands world-wide, and now they must be rebuilt to restore their valuable ecosystem services. As part of this effort to plan for marsh restoration, laboratory mesocosms can provide important information on the probable impacts of continued sea-level rise, climate change, pollution and biological invasions within a specified geographical region. The mesocosm data in turn can be applied to better understanding wetland geology.

Mesocosms, experimental systems from m^2 to hectares in size, have been used for decades in ecological research. They are important for testing a parameter of interest within a controlled setting. Although they cannot fully replicate a natural setting, mesocosms are important for understanding natural ecosystems, as certain environmental parameters can be controlled (Lawton, 1995). Most salt marsh studies are restricted to intermittent field sampling and manipulations, as it is difficult to replicate these low-energy tidal systems in the laboratory. Northern temperate salt marshes pose an additional challenge of being frozen and snow-covered for part of the year, making regular field sampling for seasonal comparison studies difficult. The travel distance limitations are a particularly challenging for vast wetlands like that of the Danube Delta.

In this paper, we illustrate the value of mesocosm experiments using an example from Chezzetcook Estuary, a boreal **mesotidal** wetland in northeastern Canada, and then we show how data might be useful for Danube Delta **microtidal** wetlands studies (Figure 1). The Canadian Chezzetcook site is selected because it is the location of classical paleoecology studies using foraminifera, testate amoebae and pollen to reconstruct sea level changes to ±1 cm precision, and past studies have been successful in determining natural versus anthropogenic pollution effects (e.g. Chagué-Goff et al, 2001; Sabean et al., 2009). These studies provide models that could refine ongoing studies of geological and anthropogenic impacts in the microtidal Danube Delta and wetlands (e.g. Giosan et al., 2012; Sanchi et al., 2014).

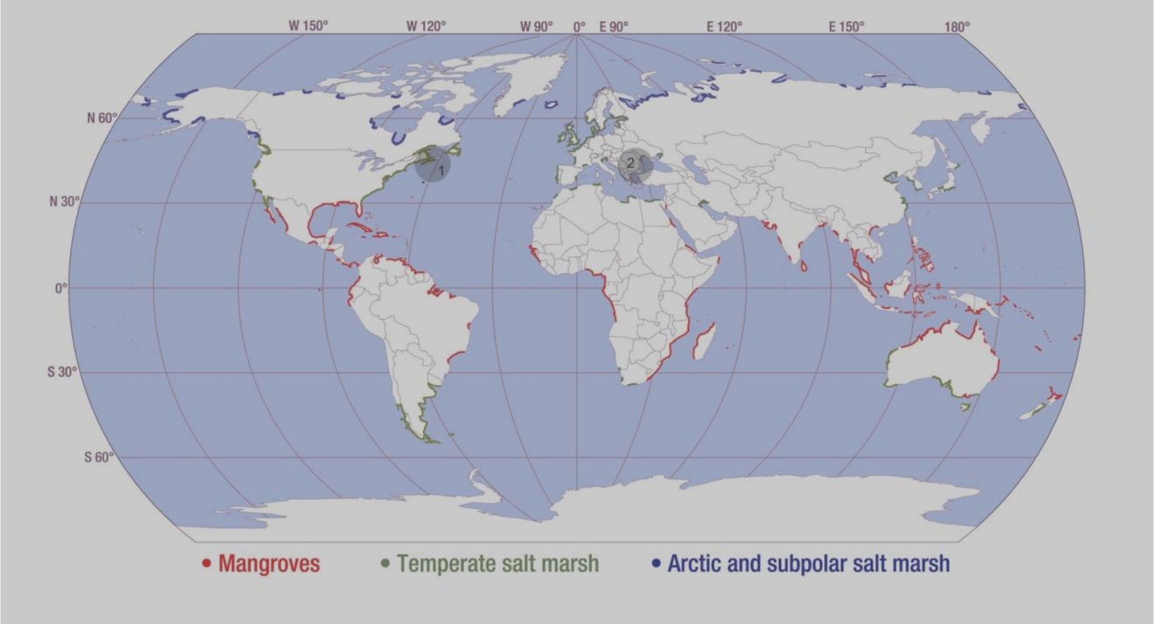

Figure 1. Global coastal wetland distributions and locations of study areas in this paper. 1 Chezzetcook Inlet; 2 Danube Delta (from Scott et al., 2014).

1.1 Chezzetcook Marsh background information

Chezzetcook Marsh, southeastern Canada (44.70°N, 63.25°W) is about 7 km long by 2 km wide and is located in a cold temperate Boreal region. Temperatures average from -6°C in January to about 19°C in August, with about 1400 mm precipitation (rain and snow) per year. This estuary formed in a partially drowned drumlin field at the mouth of the Chezzetcook River >6,000 years ago and salt marsh infill is currently growing seaward (Figure 2). Semi-diurnal tides range from 0.4 m to 2 m, flooding the marshes at high tide. Salinity is highest in the summer when evaporation is high and also in winter when the marsh is ice-covered; salinities are lower during the spring snowmelt and in the autumn when rainfall is higher (Scott et al., 2014). The salinity range of 25 psu in the lower marsh to 0-5 psu at the head of the marsh influences not only the vertical zonation of the flora and fauna but also the duration of tidal submergence.

The Chezzetcook marshes have been studied for over 35 years and used to develop ultra-sensitive methods for monitoring of sea-level change. Scott et al. (2001) show how benthic foraminifera record paleo-sealevel curves to an accuracy of ±1cm elevation. Gehrels et al. (2005) derived quantitative transfer functions from foraminiferal transect data to apply in high-resolution records of changes over the past ~1200 years. They show that the rapid sea level rise in the western North Atlantic Ocean is closely linked to recent global warming: the pre-1900s rate of 1.6 mm/yr doubled to 3.2 mm/yr from 1900−1920.

Sedimentological, pollen and dinoflagellate cyst (dinocyst) data were used as proxies of geological and vegetation changes in the outer estuary in a 2000-year sediment record of Chezzetcook marsh development. The paleoecological records show that natural sedimentation processes shape the regressive marsh-sediment complexes, despite the 3.8 mm/year rapid sea level rise. In contrast, at the inner estuary, the salt marsh developed rapidly over the last 200 years because of sediment in-wash from European land use. These paleoecological studies highlight the differences in prevailing sediment sources (natural versus anthropogenic) and distribution mechanisms throughout the estuary. Additionally, other sediment cores reveal recent geochemical changes from both natural and anthropogenic influences. For example, on the landward edge of the marsh, high concentrations of Fe, Ni, Co, Mo and As reflect anthropogenic inputs from recent road construction adjacent to Chezzetcook (Chagué-Goff et al., 2001).

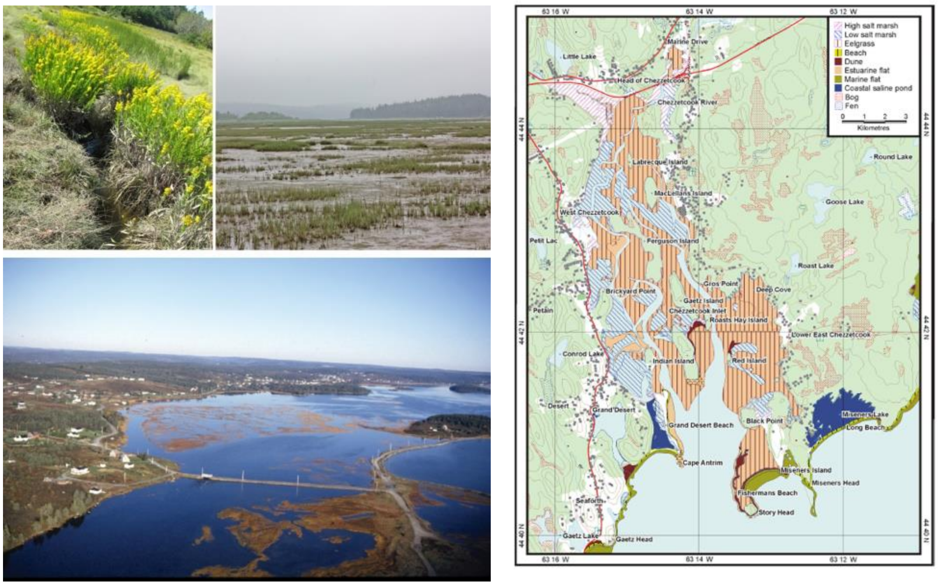

Figure 2. Chezzetcook marsh, Nova Scotia, Canada. Left: high and middle marsh (top left); low marsh (top right); outer estuary (bottom). Right: map of the inlet (from McCullough et al., 2005).

1.2 Danube Delta background information

Most of the past paleoecological studies at Chezzetcook focus on foraminifera and pollen as proxies for sea level and anthropogenic change, respectively. In the Black Sea and Caspian Sea regions, however, powerful new proxy-data tools are being developed using a much wider array of organic-walled microfossils called Non-Pollen Palynomorphs (NPP). These microfossils include the organic linings of microforaminifera, ostracod shell linings and mandibles, insect and crustacean exoskeletal remains and egg cases, the egg capsules of annelid worms, the resting spores of tintinnids and dinoflagellates and various fungal remains, including spores of crop diseases (Mudie et al., 2011). In 2012, a new study commenced that uses surface sediments from the boreal **microtidal** wetlands of the Danube Delta and adjacent continental shelf as part of the EU Black Sea ERA.NET WAPCOAST project *“Water pollution prevention options for coastal zones and tourist areas: application to Danube Delta front”*. This project maps distributions of the NPP in relation to water depth, salinity and distance from shore (Figure 3).

The Danube Delta plain, delta front and prodelta are located between about 44.5° and 45.5° N and from 28.5° to 30.5° E, hence including the latitude of Chezzetcook Inlet. However, the location of this vast wetland (~ 700 x 500 km) at the mouth of the longest river system in W. Europe, on the shore of the microtidal Black Sea, creates a very different environmental setting. The climate of the semi-enclosed western Black Sea is more continental, with lower annual precipitation (300-400 mm), high evaporation (~1000 mm/yr), and higher mean summer temperature of 22° C in July, including periods of drought and maxima up to ~37° C. Average winter annual temperature is also warmer (-1°C in January) because there are no polar currents like those cooling the shores of Eastern Canada; however, the ground freezes in November and temperatures can drop to -21.5° C (Gâsteşcu, 2009). The semi-diurnal tidal range is very small (~7-11 cm) but strong northwesterly winds result in frequent storms during spring and autumn, with storm waves that erode and transport sand into the delta. Surface water salinity ranges from a summer maximum of ~18 in Black Sea to less than 1 psu at Tulcea on the fluvial delta plain. Summaries of the recent history of the tidal freshwater wetlands are given by Gâsteşcu (2009) and by Scott et al. (2014).

Several classical models have been developed to delimit characteristic palynological facies which distinguish the transition from coastal lakes across delta front, delta foreset, prodelta, shelfal and delta fan sub-environments (Mudie et al., 2014). These models aim at understanding the fate of fluvial sediments in marine environments and they are the basis for interpreting how sea-level change shapes delta evolution. In the Black Sea, these models have been used to interpret radiocarbon-dated cores from the Danube Fan (e.g. Giosan et al., 2012) and to back-track the evolution of the delta plain tributaries and shoreline over the past 5,000 years of sea level changes from −2 m to +1.5m.

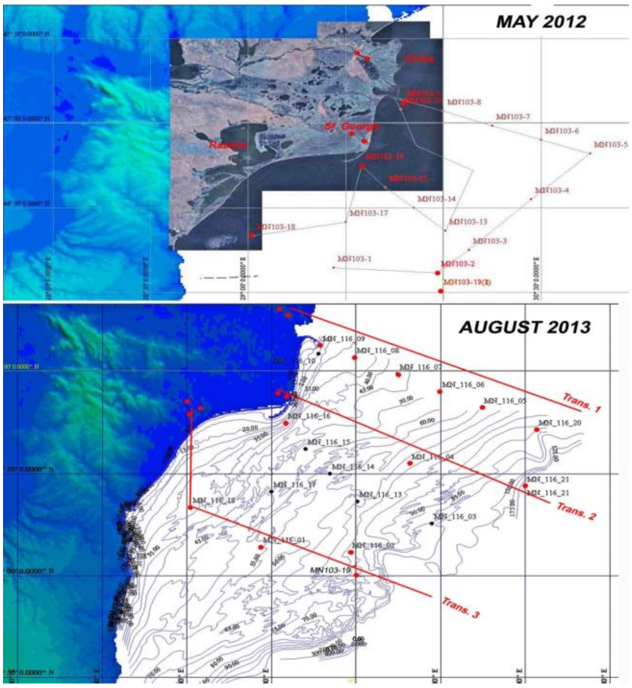

Figure 3. Locations of Danube Delta surface sediment samples sampled for palynology.

The accuracy of models for conditions before and after the recent changes (dam- and channel-construction, nutrient discharge) are limited, however, by 1) uncertainties in dating of molluscs and bulk sediments, 2) imprecise knowledge about the linkages between microfossils, and modern conditions in the delta environments, and 3) uncertainties about NPP biology and taxonomy. The WAPCOAST study aims to refine future paleocological studies through better understanding of the relation between living meiofaunas and fossil palynological assemblages as inferred from our studies of the Chezzetcook mesocosm data, including the way that stable isotope values (δ^{13}C and δ^{15}N) are altered by complex foodchain linkages.

2 METHODS

2.1 Indoor laboratory marshes: using mesocosms to study salt marshes

We have developed a replicable laboratory mesocosm system to examine various responses of the Chezzetcook salt marshes under controlled conditions at all seasons (Figure 4). This system was designed by Frail-Gauthier et al. (2011) in the salt water laboratory at Dalhousie University, using pumps and filtered seawater from Halifax Harbour, an inlet of the Atlantic Ocean (Scott et al., 2014). To represent the natural Chezzetcook inlet hydrology, 6 hour diurnal tide cycles, continuous freshwater input, 8-12 hour UV light cycles, and ambient lab temperatures control as many environmental parameters as possible. However, temperature of the freshwater and sea water entering the system varies by season, from 23°C in summer to no less 10°C in winter. Mudflats, low marsh, middle marsh and high marsh environments are scaled into a 2.5 m long x 0.5 m wide tank, with heights of the marsh segments (40 cm for high marsh to 5 cm high for mudflat) reflecting their elevations about sea level and approximate exposure time during tidal cycles. The mesocosm uses previously studied transects at Chezzetcook Inlet: a high salinity transect near the entrance and low salinity transect near the head (Figure 2). The low salinity transect receives a continuous freshwater inputs. Overall, lab salinities ranges from 23 to 29 psu in the high salinity transect and from 20 psu (mudflat) to approximately 4 psu (high marsh) in the low salinity transect.

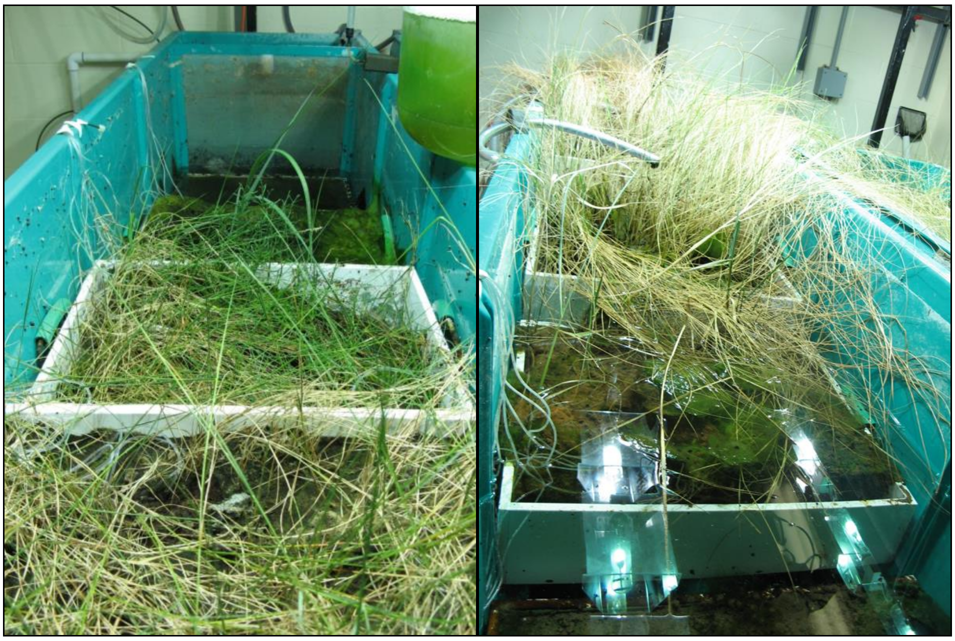

Figure 4. Laboratory mesocosms of Chezzetcook Inlet marsh at Dalhousie University. Left: side-view of the high salinity (outer estuary) transect at low tide, with high marsh in foreground. Right: low salinity (inner estuary) transect during rising tide with mudflat in foreground (photos by J. Frail-Gauthier).

This indoor mesocosm experiment collects high-resolution data on population dynamics and compositions of foraminifera in conjunction with the meiofauna (nematodes, ostracods, copepods) coexisting in the tidal marsh sediments. Biweekly, 10cm^3 surface sediment samples are taken using a pseudo-replication technique (Morvan et al., 2006); these samples are washed over meshes of 500 and 45 microns, stained with rose Bengal (Walton, 1952) and preserved in Borax®-buffered ethanol. Samples are counted by wet-splitting (Scott&Hermelin, 1993) into subsamples and by examining under binocular microscopy at magnifications of 160 and 300x. All foraminifera, living and dead, and all meiofauna (mainly nematodes, ostracods, gastropods, errant polychaetes, tube polychaetes, oligochaetes, insect larvae, mites, amphipods, copepods, isopods) are counted. The study also examines the specific trophic roles of foraminifera and the meiofauna by stable C and N isotope analysis of the grasses, sediment organic matter, algae, foraminifera, meiofauna and invertebrate macrofauna from the natural inlet, and by predator-prey laboratory feeding trials. Samples from each zone of the marsh were analysed for C and N stable isotopes at University of California, Davis (stableisotopefacility.ucdavis.edu); $\delta^{13}C$ and $\delta^{15}N$ values were calculated based on Vander Zanden &Rasmussen (2001). Using these data, it is possible to determine the food energy sources of each taxonomic and/or functional group from the $\delta^{13}C$ values, and the relative trophic positions of the groups can be tracked by examining the $\delta^{15}N$ values.

2.2 Danube Delta palynology

In May 2012 and August, 2013, samples of Delta front and foreset surface sediment (MN103-1 to 19 and MN116-02 to -21) were collected for palynology study from the Romanian vessel R/V Mare Nigrum (MN), together with CTD, water chemistry measurements for each site. Our Delta plain surface samples include four sites in the Razim-Sinoe Lakes, two on the southern Sfantu Gheorghe delta-lobe, and two on the Chilia (= Kilia) delta-lobe (Figure 3). The total sample set covers water depths from 3−196 m, at distances from 21 km upstream on the Delta Plain to about 130 km offshore on the outermost continental shelf. The surface water salinity range in spring 2012 was 5.5 – 18.1 psu; in summer 2013, the range was 16.96 to 21.01 psu. In the delta tributaries, salinity is ~1-3 psu; Razim Lake salinity is ~ 1 – 13 psu (Bretcan et al., 2009).

In the laboratory, the sediment samples were weighed and processed for palynology study using the standard technique for marine and brackish water sediment (Mudie et al., 2011). Air-dried sediment samples (1.5 – 8 g dry weight) are disaggregated in distilled water with 0.04% Calgon solution, and sieved at 10 microns to remove the inorganic fine silt, clay and coarse particles. *Lycopodium* tablets were added to allow quantitative estimates of palynomorph concentrations. Inorganic carbonate was removed by digestion with 10% HCl, without heating, and silicates were removed by cold 52% HF. The resulting organic residues represent the silt- to fine sand-sized refractory POC (particulate organic carbon), such as freshwater algal spores, fungal spores, and animal remains, including microforaminiferal organic linings, ostracod mouthparts and chitinous egg capsules of Cladocera and Copepoda. The organic residues were mounted on glass slides in glycerine gel and examined by transmitted light microscopy using a Zeiss Universal research microscope with interference light objectives, at a magnification of x400. Particle counts were to totals of 200 – 400 palynomorphs per slide.

3 Results

3.1 Chezzetcook Laboratory mesocosm

The Dalhousie University mesocosm experiment provides the first high-resolution data on population dynamics and compositions of foraminifera in conjunction with the meiofauna coexisting in the tidal marsh sediments (Frail-Gauthier et al., 2011). The replicable laboratory mesocosm system allows examination of ecosystem responses under controlled conditions during all seasons without the hardships and expenses of field work during the winter freeze-up and during summer insect invasions. Although the mesocosm is environmentally controlled, the marshes still replicate seasonal cycles of vegetation: *Spartina* dies off during winter (November to March) and re-grows during spring and summer. Foraminiferal changes have also followed these seasonal trends. After two years, seasonal foraminiferal and floral assemblages resemble the seasonal data collected from the natural Chezzetcook marsh, despite use of filtered water in the laboratory.

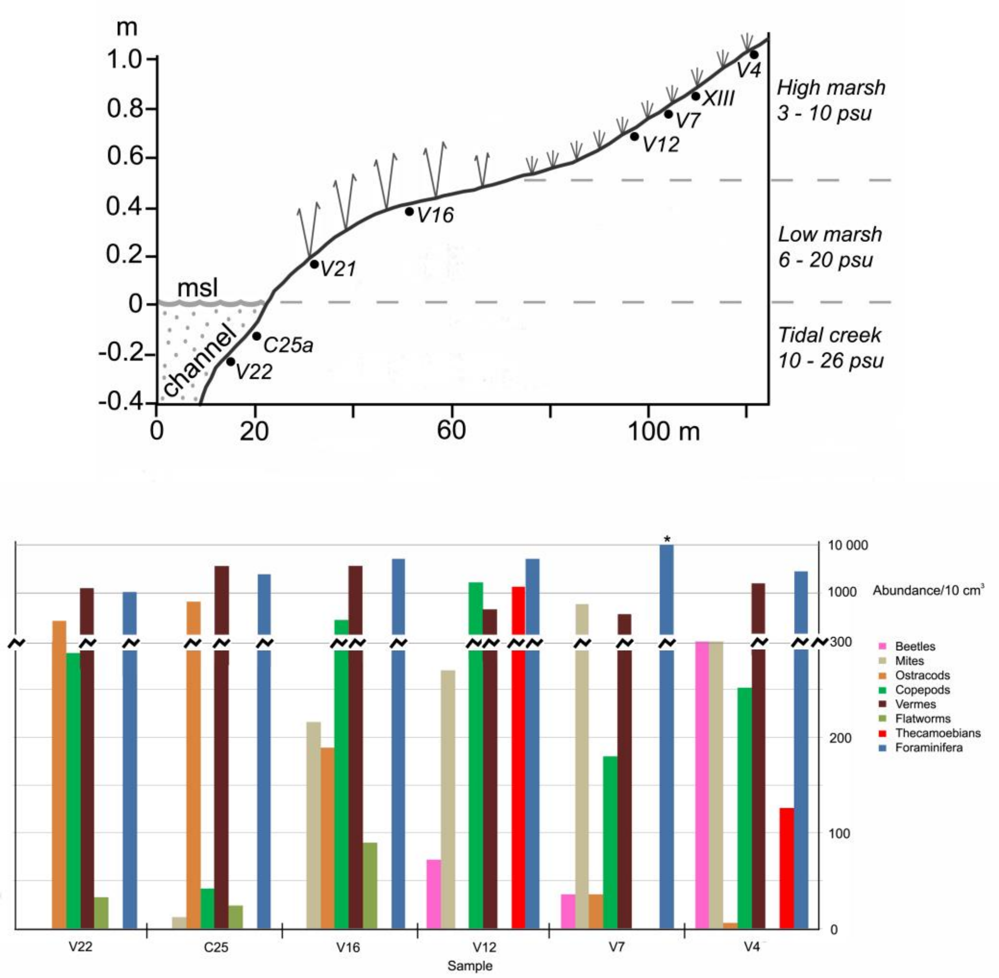

Figure 5. Profile of Chezzetcook marsh, showing zonation (top) and corresponding meiofaunal diversities per 10 cm^3 sediment sample (bottom). Note scale-break from 300-950.

For the first time, we have tabulated foraminiferal assemblages and distributions alongside meiofauna; preliminary compositions showing meiobenthos diversity are given in Figure 5 and in Table 1. Preliminary stable isotope analyses (April 2014) show C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ values for macroscopic and microscopic plants, foraminifera, meiofauna and sediment, revealing the importance of sediment organic matter (mostly as decomposing *Spartina* grasses). Trophic interactions of meiofaunal consumers can be interpreted through these stable isotope analyses (Figure 6). Initial results (Table 1) of NPP in palynological studies of the mesocosm samples show that a comparable array of taxa are preserved as organic remains, although some conspicuous differences in abundances are evident – in part reflecting the lower counts for the initial palynology data, and in part, reflecting the presence of many juvenile forms (<45 µm) of taxa seen in the palynology preparations but not easily visible under reflected light microscopy.

Table 1. Estimated abundances of protist and meiofaunal specimens in 10 cm^3 of surface sediment taken from the Chezzetcook laboratory mesocosm in summer (August).

Taxa	**Mudflat**	→			**High marsh**	
	V22	C25	V16	V12	V7	V4
			Meiobenthos Counts (per 10cm^3)			
Testate Protozoans						
Calcareous foraminifera	96	324	234	36	378	42
Agglutinated foraminifera	945	2946	7056	7362	16812	3978
Thecamoebians	0	0	0	1962	0	126
Invertebrate Meiofauna						
Flatworms (3 spp.)	33	24	90	0	0	0
Nematodes (vermes)	1506	4502	4878	594	504	876
Annelid worms (vermes) (10+ spp.)	153	894	558	90	90	312
Ostracods (2 spp.)	486	876	189	0	36	6
Copepods (3 spp.)	288	42	441	2574	180	252
Isopods	21	0	0	0	0	0
Amphipods (5 spp.)	0	0	0	0	0	0
Grass shrimp	0	18	0	0	0	0
Collembolan	0	0	0	36	0	0
Insect larvae (5+ spp.)	21	12	9	0	0	6
Beetle (2 spp.)	0	0	0	72	36	342
Mite (3 spp.)	0	12	216	270	792	312
			Palynological Sample Equivalents (per 10 cm^3)			
Testate Protozoans						
Foraminifera organic linings (4 taxa)	2394		7504			8736
Thecamoebian (*Arcella)*	399		300			624
Tintinnid	0		560			390
Invertebrate Meiofauna						
Flatworm egg capsules	4788		600			0
Nematodes (vermes)	3192		17,109			2496
Annelid worms (vermes)	325		1200			624
Ostracod lining/mandible	1596		300			250
Copepod eggs (3 taxa) & instars	4390		2700			1560
Cladoceran exoskeleton	399		2700			0
Radiolarian egg (*Filinia*)	300		300			0
Insect larvae	20		250			31
Beetle	0		0			312
Mite	80		600			312
Gastropod radula	1197		0			31
Microalgae						
Anaboena algal strands	260		350			1248
Spirogyra spores	0		0			312
Phytoplankton			**Field Data (1980) (#/g sediment)**			
Marine dinocysts (16 taxa)	2191	647	1890	1519	737	555
Freshwater dinocysts (4 taxa)	156	13	100	31	91	95

The palynology mesocosm marsh samples reveal some foraminifera with nematodes inside their organic shell linings and they record additional biodiversity within the <45 micron size fraction that is not easily seen within the raw matrix of sediment and coarse particulate organic matter (POC). Overall, the mesocosm samples representing the marsh zones (Figure 5) include 12 major faunal groups: foraminifera (protists, 24 species), "vermes" (nematodes, oligochaetes/polychaetes) and ostracods (2 species) predominate; there are common harpacticoid copepods, flatworms (4 egg-capsule types) and thecamoebians (2 species), and smaller numbers of gastropods, beetles, mites, insects and isopods. In addition to abundant leaf fragments of angiosperms, plants include diatoms and blue-green algae. The palynology samples reveal the additional presence various small crustacean instars, eggs of a *Filinia*-type rotifer, two types of copepods eggs and a cladoceran. Other protists found in the palynology samples are dinoflagellates (19 species), including reservoirs of *Alexandrium tamarense* cysts which cause paralytic shellfish poisoning). Pollen (mainly from forest trees and marsh grasses) is also an abundant component of the POC.

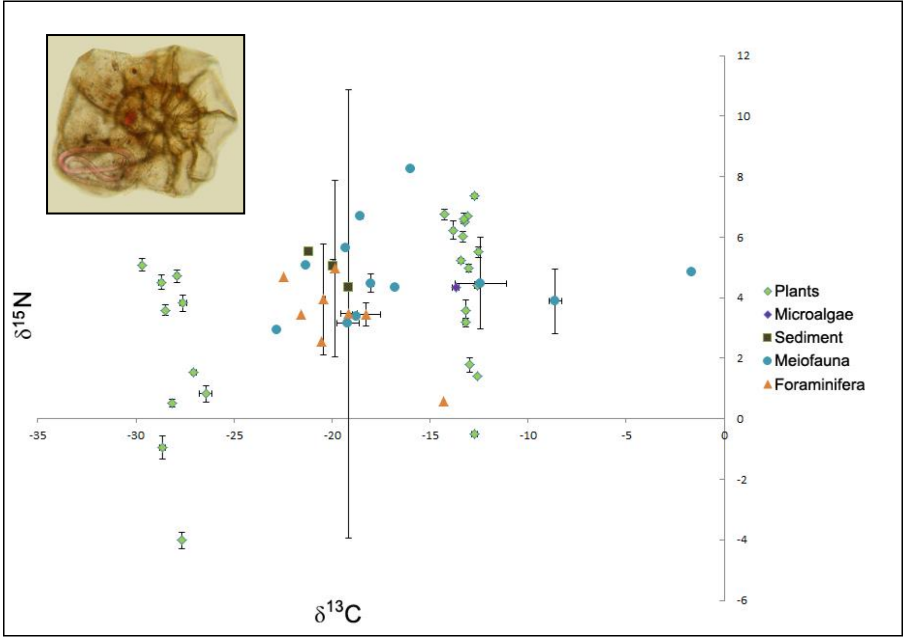

Figure 6. Stable isotope analysis data (δ^{13}C and δ^{15}N values) for main food web components in Chezzetcook marsh. Inset shows a microforaminiferal lining encompassing a nematode worm. This interaction is evident in the stable isotope data, with nematodes being at a higher trophic position (δ^{15}N = 4.32; SD= 1.52) than their presumed foraminiferal prey (δ^{15}N= 3.44; SD= 0.38). Standard deviations are for means of three replicates; large deviations indicate samples combined to achieve minimum weight for stable isotope analyses and may represent a mix of trophic levels (δ^{15}N).

3.2 Danube Delta palynology

The initial studies were made along three transects (Figure 3) extending from channels of the three Danube Delta lobes (Chilia, Sulina, Sf. Gheorghes) across the delta front and the prodelta, west of the deep-sea delta fan where previous palynological core studies were made (Giosan et al., 2012; Sanchi et al,. 2014). All samples contain an abundance of well-preserved refractive POC, including large amounts of pollen from *Ambrosia* ragweed. The abundance of this recently introduced weed pollen is important because it establishes that the surface sediments were deposited *after 1990 AD* when this allergy-causing weed became an important health problem in the region (Kiss&Béres, 2006). This weed pollen shows that our surface samples record contemporary events and are not palimpsest deposits which are widespread in the outer lakes. In general, the Delta Plain and Razelm Lake samples are clearly distinguished from the delta front, prodelta and shelf sediments by 1) kerogen consisting of abundant coarse plant fragments and phytoliths (grass and other herbs); 2) presence of common organic remains of small cladocerans, juvenile ostracodes, arcellaceans, chironomid fly mandibles and insect pupae; 3) common to abundant fungal bodies, hyphae and large, multicellular fungal spores; and 4) by the absence or extreme sparseness of dinoflagellate cysts (dinocysts) and foraminiferal linings. The offshore MN sample suites contain more diverse palynological assemblages which vary with distance offshore and are all distinguished by predominance of amorphogenic kerogen rather than plant fragments. This amorphogen probably represents carbon residues derived from marine algae which are represented in most of the MN samples by dinocysts and *Cymatiosphaera* algal spores. These characteristics – amorphogen and dinocysts – are the keymarks distinguishing the seaward end of cross-estuary profiles, as shown by Yanko-Hombach et al. (2013). The offshore samples also contain variable numbers of microforaminiferal linings, ostracod mandibles, gastropod radulae and copepod eggs.

Spring-summer differences among five categories of Danube Delta NPP are weakly expressed. At the two near-shore MN sites, wood and charcoal particles are about 50% higher in spring, presumably reflecting the relatively cooler conditions and the need to burn more wood for heating. Within the Delta Plain, variable amounts of charcoal and wood fragments could indicate sub-regional shifts in agricultural practices – e.g burning of reed beds or fallow crop fields. Spring-summer dinocyst concentrations vary greatly from site to site, probably reflecting patchiness in phytoplankton blooms as evident from satellite imagery showing complexes of coccolith blooms over the shelf margin. Within the Delta Plain, variable amounts of charcoal and wood fragments also reflect sub-regional shifts in agricultural practices – e.g burning of reed beds or fallow crop fields. Overall, the NPP results show a diversity of phytoplankton (~20 taxa) and zooplankton or benthos (>50 taxa) which leave organic remains in the sediments and can be used to mark the pathways of particulate organic carbon to the delta plain and nearshore (Table 2). Comparison with published reports for plankton and meiobenthos from Kilia Delta (Guliekova, 2012) and the Razim-Sinoe lake complex (Vadineau et al., 1997) indicate that these NPP represent the fossilised remains of only about one-third of the total meiofaunal biodiversity, but this is a significant increase over the sparse number of zoomorphs previously reported in Danube Delta paleoecological studies.

Table 2. Estimated abundances per gram sediment of pollen and NPP in surface samples from the Danube Delta plain and the transect sites on the delta front and foreset. Numbers in parentheses show numbers of taxa recognised. All data are initial results of ongoing work.

		→						→				→			
Station No.	**Ch2**	**9**	**9'**	**8**	**7**	**6**	**Sf G**	**16**	**16'**	**R02**	**R21**	**18**	**1**	**2**	**2'**
Water depth (m)	**9.0**	**17.4**	**17.4**	**33.6**	**45.7**	**54.8**	**5.0**	**24.3**	**24.6**	**2.8**	**1.8**	**33.1**	**58.5**	**71.4**	**72**
km from land	**0**	**3**	**3**	**15**	**35**	**50**	**0**	**13**	**13**	0	**0**	**28**	**68**	**110**	**110**
Pollen+spore /g (x1000)	**6.8**	**14.2**	**7.8**	**5.2**	**38.5**	**48.5**	**12.4**	**38.3**	**14.0**	**34.2**	**3.6**	**7.4**	**39.2**	**48.1**	**24.2**
Fungi/g (x1000)	**3.4**	**3.1**	**5.0**	**0.54**	**1.6**	**6.2**	**7.9**	**7.4**	**6.3**	**4.4**	**0.81**	**0.48**	**9.2**	**9.1**	**1.9**
Charcoal/g	**143**	**372**	**837**	**30**	**600**	**0**	**1254**	**1799**	**1169**	**1648**	**188**	**239**	**1154**	**1182**	**968**
Animal remains: Total	**1713**	**483**	**930**	**904**	**3230**	**5796**	**1447**	**6168**	**5111**	**13122**	**1909**	**1672**	**6600**	**9456**	**4452**
Protozoans/g															
Microforam. (6 taxa)	0	60	93	316	969	0	0	2056	2839	160	94	398	300	394	581
Thecamoebian (*Arcella*)	280	65	0	136	646	0	289	0	0	0	0	0	300	0	194
Rotalaria *(Filinia)*	80	120	0	24	0	0	0	0	0	1296	94	80	0	0	0
Tintinnid (5 taxa)	41	100	280	45	600	2070	0	3341	1150	1620	125	637	1200	3152	968
Invertebrate Meiofauna/g															
Flatworm- *Neorhabdocoel*	164	0	90	0	0	0	0	0	0	320	157	0	0	0	0
Flatworm egg capsule	0	0	0	135	300	0	0	0	0	0	376	0	0	0	0
Nematode	0	70	0	0	0	0	96	0	0	486	219	0	0		
Ostracod-mandible	163	120	0	0	0	0	100	0	0	810	125	239	300	1	194
-carapace	0	0	300	0	0	400	0	0	0	486	140	0	300	1	0
Copepod egg	0	0	0	90	330	2009	0	0	167	1134	0	0	0	1182	581
Cladoceran *(Bosmina)*	245	0	0	0	0	0	288	0	0	1944	188	0	0	0	0
Tardigrade/*Bosmina*	204	70	186	0	0	0	180	0	3	1782	188	159	2700	0	0
Crustaceans - claw	41	0	0	0	0	400	96	514	0	0	0	0	0	394	193
Crustacean fragment	0	0	90	0	0	0	0	0	334	4536	312	0	0	0	193
Crustacean egg (white)	85	0	0	90	250	1200	0	0	160	0	30	0	1800	0	387
egg (orange)	0	0	0	0	0	2800	0	0	120	0	0	159	300	1476	968
Chitin fragment indet.	245	0	0	0	0	0	193	0	0	0	0	0	0	0	0
Arachnid	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Insect -claw	160	0	0	40	0	350	96	0	167	0	60	0	600	394	0
Insect wing-scale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastropod -mandible	0	0	0	0	0	400	90	0	0	0	91	0	600	0	196
Phytoplankton/g															
Marine dinocysts x1000 (31)	0	0.96	0.28	4.1	67.8	113.0	0	6.7	6.0	0.16	0	1.67	75.0	30.7	29.0
Freshwater dinocyst (2)	80	140	651	0	0	0	0	1285	334	810	91	0	0	0	0
Freshwater algae (15)	569	345	930	1130	5814	2070	2016	2570	2505	2754	453	1149	5100	3940	3291
Hexasterias problemat.	0	0	0	40	0	0	100	0	0	0	0	0	0	1576	0

4 DISCUSSION

Salt marsh mesocosms, both in indoor and outdoor settings, help answer many research questions (see Scott et al., 2014). The importance of using salt marsh mesocosms in restoration projects and future recommendations are explained in detail by Callaway et al. (1997). An indoor marsh creation project by Padgett and Brown (1999) used a very similar design to our Chezzetcook laboratory marsh, including continuous sea water inflow and a timed drain to simulate tidal cycles. That study examined the effect of soil drainage and the amount of organic content on the growth of *Spartina alterniflora*. Instead of using segments from a local salt marsh, they used clean sand and peat moss in varying percentages to manipulate substrates for *Spartina* seedlings. The study monitored the growth of the vegetation and recruitment of invertebrate infauna to gain insight to marsh creation and restoration. Results showed that soil organic content is crucial for the recruitment and growth of a new salt marsh (Gleason et al. 1979). These studies emphasize the importance of using experimental systems for the development and growth of a salt marsh; however, salt marsh mesocosms are also important for examining *in situ* processes and interactions of species.

We are beginning to understand the specific trophic roles of foraminifera and the meiofauna that comprise most of the small food-web in the highly productive salt marsh ecosystem. Most of the meiofauna (including foraminifera) in the marsh use sediment-derived primary sources, which are a mixture of both terrestrial- and marine-derived phytodetritus. As expected, foraminifera occupy a lower trophic position than other meiofauna (such as nematodes), validating their role as important primary-level consumers that help support the small food web of a mesotidal salt marsh. These studies, combined with pollen and geochemical records of human impacts, are important for managing salt marsh systems and for interpreting foraminiferal records of paleo-sea level and paleoclimate change. Previously, distributions of foraminifera were thought to only be physical— grain size, salinity, tidal inundation—but biological interactions (such as competition and predator-prey interactions) may play an important role in the long-term abundances across marsh zones. For example, in the Chezzetcook mudflats, we often see a much lower number of foraminifera compared to high numbers of meiofauna and the same feature is evident in the palynological data (Table 1). The fact that this trend is evident on both the highly dynamic field setting exposed to periodic severe storm impacts and in the sheltered laboratory points strongly to a biotic rather than a physical (abiotic) cause. The detailed marsh assemblage data also show us the transition from terrestrial zones to the marine mudflat, which are clearly visible in the salt marsh grasses, but not so easily discerned in the plant macrofossils (Yanko-Hombach et al., 2013; Mudie et al., 2014). Assemblages may also record changes in relation to disturbance—i.e. as test deformations (Kravchuk, 2006; Sabean et al., 2009) and shifts between calcareous and agglutinated foraminifera (Dabbous&Scott, 2012).

In our fluvial-marine Danube Delta study area, the amounts of freshwater algal spores and dinocysts in the 2012-2013 surface sediment samples do not indicate strong eutrophication of the delta channel, lake and plume environments. This result is in contrast to the concerns of previous years reported by Giosan et al. (2012), Coolen et al.(2013), and evidence of eutrophication in the upper delta lakes (Török&Teodorof, 2013). Part of the difference may lie in the greater bioturbational mixing of the sediment samples previously studied, hence integration of a longer sediment time interval than in our surface samples and pointing to possibly important interannual variations. However, relative few delta lake samples were available for our initial study and we seek to expand our delta plain sample set. There is also need to standardise the taxonomy of NPP zooplankton and meiobenthos remains by collaboration with specialists studying the delta lakes. In other Black Sea studies, we have correlated concentrations of the red tide dinocyst *Lingulodinium machaerophorum* with historical changes in deforestation and nutrient inputs, and Bradley et al. (2013) found that the discharge of shipping ballast may continue to spread harmful algal blooms (HABs), at least in the southwestern Black Sea. Our new WAPCOAST data do reveal that there is potential for Cyanophyte (*Gloeotrichia* blooms in the nearshore area and for outbreaks of *Botryococcus* blooms on the prodelta front. Cysts of toxic dinoflagellates (*Alexandrium* spp., *L. machaerophorum*) which are present in vast numbers within some prodelta sediments (Table 2) could also be seeded into the lower Danube lakes by suspension in storm waves and/or cross-shelf mixing. Within the lakes, these HABs might cause serious damage in areas where the summer water salinity is above 9, the salinity at which *L. machaerophorum* can form thick-walled diploid resting spores. Studies of ostracods, foraminifera and nematodes (Kravchuk, 2006) show correlation between deformities and toxic metal concentrations but it remains to be determined if these can be traced in the fossilised organic linings. Mesocosm experiments will provide a valuable source of material to trace potential deformations in the microforaminiferal lining of NPP in addition to refining our identification of the animal remains. By coupling high-resolution mesocosm food-chain studies with paleoecological studies, we are now beginning to make unique contributions to coastal wetland ecology studies which use palynology to trace temporal changes in vegetation associated with anthropological stresses (e.g. deforestation, eutrophication and fires), and which use organic-walled NPP as proxies for responses in marsh microbiota.

Comparison of the mesotidal, higher salinity (>25 psu) environment of lower estuarine areas of Chezzetcook Inlet (Table 1) to the microtidal, brackish (<18 psu) environment of Danube Delta (Table 2) shows similar taxonomic groups and relative abundances, despite the large differences in wetland sizes and fluvial regimes. However, in the Danube Delta, reports (e.g. Kiss, 2012) indicate there is a higher percentage of freshwater taxa, such as freshwater hydrozoans (3 spp.), oligochaetes and hirudineans (25 spp.), cladocerans (38 spp.) and rotifers (70+ spp.) than in the mesotidal saline Chezzetcook inlet where the fluvial input is small and the entire marsh system is periodically flooded by water of near-normal marine salinity (26-32 psu). These results also point to the fact that overall, much of the Danube wetlands area is at least periodically more stagnant and eutrophic compared to the tidally flushed and less polluted watershed area of Chezzetcook. In Chezzetcook, the majority of macrobenthos is also marine (polychaete worms, crustaceans and gastropods). It is not possible to compare the planktonic populations directly because the Chezzetcook mesocosm and field samples are taken during low tide with no water cover; however, the 1980 palynological data indicate a greater abundance of dinoflagellates and fewer freshwater algae than in the Danube plain ecosystems.

Our ongoing studies show the importance of combining quantitative biological information and geological records. Understanding the biological and ecological limitations of various taxonomic groupings can help to refine interpretations of the paleoenvironmental changes resulting from shifts in delta geomorphology and pollution (Scott et al., 2001). In the Razim-Sinoe lakes, for example, in 10 years between 1980 and 1990, there was a significant reduction in the number of taxa (100 to 47 zooplankton groups; 16 to 10 zoobenthos) related to human impacts (Vadineanu et al., 1997). However, the long-term importance of these biodiversity changes is best evaluated by comparison with a longer time-series of data extending back to before the trophic collapse of the Black Sea ecosystems (e.g. Giosan et al., 2012). More detailed knowledge of the way living meiofauna, macrobenthic fauna, and plankton are recorded as fossil assemblages can help in the planning for management and recovery of human-impacted influences in this important estuarine system.

5 CONCLUSION

Our initial results show that mesocosms like ours at Dalhousie University have multiple benefits to both ecologists and geologists for gaining in-depth understanding of marsh species interactions and their geological finger-prints as proxies for changes in space and time. A laboratory salt marsh can also replicate natural variations, such as migrations and dispersal, storms, intense weather (rain or drought), invasions and pollutants. Additionally, other parameters can be manipulated to understand specific causes and effects, and to avoid damage to the natural environment. For example, pollution and remediation, and invasive species experiments, can be carried out without either polluting the estuary or risk of spreading foreign invaders. Physical parameters, such as temperature, salinity, and tidal heights, can easily be changed and their resulting effects monitored, which is useful in predicting effects of climate change, sea level rise and shoreline erosion. The new ultra-high resolution stable isotope data also point towards the need for refined interpretations of paleosalinities (hence sea-levels) as determined from fossil peat and shell deposits in paleodelta settings (e.g. Giosan et al., 2012; Yanko-Hombach et al., 2013 and Mudie et al., 2014).

The vast size of the Danube Delta and accompanying range of microhabitats presents a formidable challenge to engineering design and scale of laboratory mesocosms. However, multiple experiments could examine differences in responses in the fluvial delta versus fluvial-marine areas, and could test different responses to sedimentation, nutrient enrichments and salinization-desalinization. New understanding of the responses and interactions of ecosystem components to forced stresses, e.g. hotter summers, colder winters, more frequent droughts and storms, would open up better understanding of paleoenvironmental data recorded in sedimentary archives from the delta and delta front environments. We recommend work with EU partners to study sediment cores that record changes in the Delta paleosalinity and responses in plankton and microfaunal populations since Roman occupation time. In general, more global collaboration is required to reduce wasted time, energy and finances on overlapping ‘trial-and-error’ experiments and evaluators of success. We also recommend exchange of microfossil and palynological data for international standardization of laboratory methods and taxonomy.

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