Markus-Michalczyk, H.; Crawford, M.L. & Baldwin, A.H. (2020). Salinity tolerance and occurrence of *Salix nigra* Marshall (Black Willow) in tidal wetlands of Chesapeake Bay tributaries. *Northeastern naturalist*, 27, 229

Published version: https://dx.doi.org/10.1656/045.027.0205

NIOZ Repository: http://imis.nioz.nl/imis.php?module=ref&refid=328336

Salinity tolerance and occurrence of *Salix nigra* Marshall (Black Willow) in tidal wetlands of the Chesapeake Bay tributaries

Heike Markus-Michalczyk[ab], Michelle L. Crawford[a], Andrew H. Baldwin[a]

[a]University of Maryland, Department of Environmental Science and Technology, 423 Animal Science Building, College Park, MD 20742, USA

[b]NIOZ Royal Netherlands Institute for Sea Research, Department of Estuarine and Delta Systems, and Utrecht University, P.O. Box 140, 4400 AC, Yerseke, The Netherlands

Corresponding author: Heike Markus-Michalczyk[ab]

Phone number: 0049 40 712 15 10

e-mail: markus-michalczyk@naturundumwelt.info

Abstract

Salix nigra Marshall (Black Willow) is a pioneer tree species that establishes in North American (NA) river floodplains, and is widely used for bank stabilization. However, its salinity tolerance and occurrence in tidal wetlands of estuarine systems along the NA Atlantic coast is largely unknown. Climate change accompanied by land subsidence and changes in precipitation pattern induce salinity increase that may affect coastal vegetation. Alteration of hydromorphology of coastal plain estuaries causes additional salinity increase that affects tidal freshwater-forested wetlands. We investigated the salinity tolerance and occurrence of Black Willow in tidal wetlands of two Chesapeake Bay tributaries and in a greenhouse hydroponic experiment. A salinity of more than 1 part per thousand (ppt) salt limited the willows` occurrence in the field and the cuttings` performance in the experiment. Dry mass was significantly lower in salinity 2 and 3 ppt salt compared to the control and salinity 1 ppt salt. Cuttings originating from tidal freshwater wetlands developed more belowground biomass and leaves than cuttings from brackish wetlands. The better performance of cuttings originating from tidal freshwater wetlands may indicate a higher resilience to short-term salinity increase in tidal freshwater wetland forests with implications for tidal forest restoration in eastern NA.

Keywords

Black Willow, tidal wetlands, salinity increase, estuaries, Chesapeake Bay, North America

Introduction

Floodplain willows are adapted to physical disturbance, resprout from fragments and grow on exposed riverine sediments (Karrenberg et al. 2002). The NA *Salix nigra* Marshall (Black Willow) forms floodplain forest stands throughout the northeastern United States and Canada as well as south in the southeastern US (Argus 2007). The species dense root system allows it to colonize sand bars (Pitcher and McKnight 1990) and has application in bioengineering (Isebrands et al. 2014). Posts and cuttings are used to establish living revetments for river bank stabilization (Schaff et al. 2003, Martin et al. 2005) and for riparian buffers to reduce sediment loading to streams and filter runoff of nutrients and chemicals. The US government promotes the establishment of riparian buffers on qualifying agricultural areas (www.fsa.usda.gov). The possibility to use willows to mitigate potential environmental effects of global change (Isebrands et al. 2014) contributes to the focus on this NA woody species.

Potential environmental effects of global change are of particular concern where dense human populations and dynamic ecosystems co-occur as in coastal estuaries (McLusky and Elliott 2004). Tidal wetland creation is suggested to mitigate effects of climate change induced sea level rise (Temmerman et al. 2013) whereas salt marshes and willows are predicted to attenuate waves since they grow high in the intertidal (Borsje et al. 2011). In eastern NA, vast tidal freshwater wetlands occur along the Atlantic east coast from New Jersey to Georgia (Leck et al. 2009). Fluctuating salinity is the principal stress that tidal wetland organisms have to cope with (e.g., Odum 1988). Negative effects of salinity increase on tidal freshwater wetland plant communities are known (Sharpe and Baldwin 2012) and tidal freshwater wetlands are among the most vulnerable ecosystems to salinity increase (Pierfelice et al. 2016). Sea level rise is expected to lead to decline of tidal freshwater forested wetlands where soil accretion does not keep pace with sea level rise (Craft 2012, Swarth et al. 2013). Retreat of estuarine forest as a function of rising sea level is predicted for the Chesapeake Bay (Kirwan et al. 2007) where an average salinity increase of 0.5 ppt in the second half of the twentieth century was found (Hilton et al. 2008).

Black Willow is native to the NA Atlantic Coastal Plain Province (Argus 2007). However, compared to riverine forested floodplains, tidal forested wetlands have attained less attention, and NA tidal freshwater forests are understudied (e.g., Baldwin et al. 2009). In particular, knowledge on the increasing salinity in tidal wetlands and the salt tolerance and occurrence of Black Willow in northeastern NA tidal wetlands along an estuarine salinity gradient is scarce.

We thus studied the occurrence of Black Willow along the estuarine salinity gradient of Chesapeake Bay tributaries and investigated the salt tolerance experimentally. We hypothesized that (i) the salt tolerance differs intraspecifically (freshwater versus brackish origin); (ii) increased salinity would decrease the growth performance both in the field and under experimental greenhouse conditions; and asked (iii) whether Black Willow from brackish wetlands would be more salt tolerant than from tidal freshwater wetlands?

Methods

Field-site Description

The estuarine tidal wetlands along the NA east coast are included in the distribution map of the Black Willow (Argus 2007). However, the occurrence along eastern NA estuarine salinity gradients remains unclear. The Chesapeake Bay along with its tributaries is one of the biggest coastal plain estuaries on the Atlantic coast. According to a report on coastal wetlands in Maryland, the Smooth Alder/Black Willow shrub swamp vegetation type covers 524 acres (McCormick & Somes 1982). We selected the 60-70 km tidal stretch of the Patuxent River in Maryland, discharging into the western shore of the Chesapeake Bay, as our study area. We choose tidal freshwater wetlands (38°48'31"N; 76°42'38"W) and brackish wetlands (38°38'20"N; 76°41'42"W) as study sites. Furthermore, we studied the occurrence of Black Willow along the salinity gradient of the Nanticoke River, a tributary at the eastern shore in Delaware (Fig. 1).

Field observations of Black Willows in tidal wetlands

Forests were marked in aerial images of both field-sites. We studied these tidal forested wetlands with regard to the occurrence of Black Willow in July 2011 (Fig. 2). In the tidal freshwater wetland, ten individuals were selected due to the occurrence of straight shoots as sources for cuttings to be used experimentally. Unfortunately, we found only one individual at the brackish wetland. On each of the willows, tree height, and stem diameter at breast height were measured.

Soil salinity was measured under all selected individuals, on 27 July 2011 at the brackish wetland and on 29 July 2011 at the freshwater wetland, with a YSI-30 Multi-Parameter Analyzer. The measurements were taken after an extended drought period and high salinities were expected. Our data were supplemented by publically available salinity data collected by the Maryland Department of Natural Resources (MDNR) (Table 1). Since we found only one Black Willow in brackish wetlands of the Patuxent River, additional individuals were studied during a field study on 25 July in tidal wetlands along the Nanticoke River. We measured soil water salinity adjacent to these individuals to determine up to which salinity Black Willow occurs in tidal wetlands. Corresponding with our Patuxent River brackish wetland, the MDNR monitoring recorded at a downstream station indicated an average July salinity of 1.36 ppt and a salinity range between 0.04-13.45 ppt in 2003-2006. The monitoring station adjacent to our upstream Patuxent River tidal freshwater site recorded an average salinity of 0.13 ppt in July and a salinity range of 0.03-0.89 ppt in 2011. On the Nanticoke River at the Sharptown MDNR upstream station, the average July salinity was 0.86 ppt and the salinity range was 0.05-7.11 ppt (2012-2014). At the downstream Tyaskin (Patuxent River) station the average July salinity was 8.25 ppt and salinity range was 0.02-15.81 ppt (2012-2014).

Greenhouse hydroponic experiment with salinity treatments on cuttings

We conducted a hydroponic experiment on cuttings of Black Willows (i) from two origins (freshwater and brackish sites), (ii) in four salinities (0, 1, 2, and 3 ppt salt) during three weeks from 1 to 24 August 2011 in the greenhouse at the University of Maryland at College Park (US) under daylight and ambient temperature conditions. We sampled the individual on 27 July at the brackish wetland, and on 29 July 2011, the ten individuals at the tidal freshwater wetland along the Patuxent River. We harvested 200 cuttings at both sites, defoliated and trimmed them to 20 cm length and stored them at 20 °C in plastic bags in an incubator until the start of the experiment.

Wide-neck PET laboratory bottles (17.6 c 6.5 cm; experimental units) were filled with 500 ml tap water. Atlantic Sea Salt (Lima; Belgium) was added to reach the salinity levels of 1, 2 and 3 ppt salt. For the control, tap water was used (hereafter referred to as salinity 0 ppt salt). The volume of water and the salinity was kept constant by supplementing water and salt. Seven 20-cm long cuttings were placed per experimental unit. We set up seven replicates per each of the four salinity levels on cuttings from two origins, resulting in 336 cuttings in 48 experimental units. The experimental units were randomly arranged on plant tables in the greenhouse. We changed the arrangement weekly to account for possible spatial effects of environmental gradients.

At the beginning of the experiment, we determined initial fresh mass of cuttings. At the end of the experiments, we recorded the number of leaves and number of roots, harvested the shoot and root biomass of the cuttings, dried them separately at 60 °C until the mass remained constant and weighed them.

Data analyses

Since only one Black Willow individual at the Patuxent River brackish wetlands was found, it was not possible to apply statistical tests to compare the results of the soil water salinity at the tidal freshwater and the brackish wetland. Instead, we calculated the mean of the morphological and soil salinity data recorded at the tidal freshwater site and the supplemental measurements of salinity at Black Willow specimen along the salinity gradient at the Nanticoke River. We further calculated the mean of the soil water salinity data from the brackish wetland of the Patuxent River.

Final shoot, root and total biomass were analysed by two-factorial ANCOVA. We used initial fresh mass of cuttings as the co-variable to account for possible differences in cuttings' initial weight and tested for effects of salinity (level 0, 1, 2, and 3 ppt salt) and origin (freshwater vs. brackish site) on shoot, root and final biomass. Pairwise differences were tested by Tukey's post hoc tests. The dry mass data were log transformed to meet the assumptions of ANCOVA.

Shoot-root ratio of the final mass of cuttings was analysed by one-factorial ANOVAs including salinity (level 0, 1, 2, and 3 ppt salt) as factors. We did not include origin as a factor since final root dry mass was too low.

To analyse effects of salinity (0, 1, 2, and 3 ppt salt) and origin (freshwater vs. brackish) on growth (roots and leaves) of cuttings, we applied two-factorial ANOVAs. Pairwise differences were tested by Tukey's post hoc tests. All data analyses were carried out with STATISTICA 9.0.

Results

Field observations on the morphology of Black Willow and soil water salinity

The average height of Black Willows at the tidal freshwater site (Patuxent River) was 4.53 m (± 2.35 SD). We measured 9.1 m for the tallest and 1.3 m for the shortest willow. The average diameter was 0.24 m (± 0.16 SD). The only Black Willow at the brackish site (Patuxent River) was 7 m in height and 0.21 m in diameter.

The soil water salinity differed at the tidal freshwater site (mean 0.1 ± SD 0 ppt) compared to the brackish site (mean 1.54 ± SD 0.09 ppt). The mean of recorded data in supplemental salinity measurements at individuals along the salinity gradient at the Nanticoke River (0.53 ± SD 0.34 ppt) was lower compared to those at the Patuxent River (mean 1.54 ± SD 0.09 ppt). In the tidal wetlands of the Nanticoke River only one willow was found in a salinity higher than 1 ppt.

Greenhouse hydroponic experiment with salinity treatments on cuttings

Final dry mass, shoot and root dry mass, and shoot: root ratio. Shoot dry mass decreased significantly with increasing salinity ($p < 0.01$), root dry mass was highly decreased with increasing salinity ($p < 0.001$), and total dry mass decrease with increasing salinity was highly significant ($p < 0.001$) (Fig. 3; Table 2). Tukey tests showed that shoot dry mass (Fig. 3a) was significantly lower at salinity 2 and 3 ppt compared to the control, and at salinity 3 compared to 1 ppt salt. Root dry mass (Fig. 3b) was significantly lower at salinity 2 and 3 ppt compared to both the control and salinity 1 ppt salt. Total dry mass (Fig. 3c) was significantly lower at salinity 2 and 3 ppt compared to both salinity 0 and 1 ppt salt.

Significant effects of origin were found. Cuttings from freshwater sites developed more root dry mass and total dry mass compared to cuttings from the brackish site ($p < 0.05$) but no differences in shoot dry mass were found. Thus, results show a consistent pattern of salinity and effects of cuttings' origin: decreased performance with increasing salinity and with origin from brackish wetlands.

Growth performance: leaves and roots. The effect of salinity on leaves of cuttings was significant ($p < 0.01$) (Figure 4a; Table 3a). The cuttings in salinity 1, 2 and 3 ppt developed less leaves compared to the control. However, no significant differences were found among the salinities 1, 2 and 3 ppt salt.

The effect of cuttings' origin was highly significant ($p < 0.001$). Cuttings originating from the tidal freshwater site developed significantly more leaves.

The number of roots was not significantly affected by salinity (Figure 4b; Table 3b). To the contrary, a highly significant effect of cuttings' origin was found ($p < 0.001$). Cuttings originating from the tidal freshwater wetland developed more roots.

Discussion

For the first time, field studies on the occurrence of Black Willow in tidal wetlands in NA Chesapeake Bay tributaries combined with experimental salinity treatments on vegetative propagules (cuttings) were carried out. A salinity of more than 1 ppt limited occurrence and growth in the field and performance in the experiment, whereas propagules originating from tidal freshwater wetlands performed better compared to propagules from brackish wetlands. Our findings could inform estuarine restoration and flood control measures regarding optimal conditions where Black Willow can grow.

Field observations on Black Willow in tidal wetlands

Our observations at the Patuxent and Nanticoke Rivers, showed that Black Willow occurs in northeastern NA in both tidal freshwater and brackish wetlands. We found abundant Black Willows in tidal freshwater wetlands along the Patuxent River (Fig. 1) but the occurrence in brackish wetlands along the Patuxent and Nanticoke Rivers was limited to soil salinity up to 1.5 ppt. However, in the Maryland Wetlands Act of 1970, "Smooth Alder/Black Willow" was a recognized shrub swamp vegetation type of Maryland's tidal wetlands in elevated areas (McCormick and Somes 1982). However, modeling of marsh transect data from Maryland showed that lateral upland migration of marshes and deforestation during the transformation of forest soils into marsh soils increased during the last 150 years (Hussein 2009). Tidal wetlands comprised of Black Willow may have decreased in area. This is opposite to the former effects of sedimentation following upstream forest clearing, converting low marsh into high marsh (Khan and Brush 1994) when Black Willow may have been more abundant in tidal wetlands. Tidal freshwater wetlands are predicted to decline due to climate change induced salinity increase (e.g., Craft 2012, Swarth et al. 2013). At the Patuxent River site that is closest to our study sites, salinity spikes were recorded from 1995 to 2010 based on continuous sampling in summer

(NOAA 2011). Low riverine discharge reduces the dilution effect of estuarine water (Swarth et al. 2013). In summer 2011, the sea surface salinity even exceeded 5 ppt in most of the Chesapeake Bay tributaries so that only the water far upstream remained fresh (Fig. 4). These observations may explain our findings on Black Willow's limited downstream occurrence.

Experimental findings on cuttings in a salinity treatment

The effects on cuttings in our salinity treatment confirmed our field observations on the limited occurrence of Black Willow in brackish wetlands that may either originate from seeds or vegetative propagules. The decreasing growth performance with increasing salinity corresponds to experimental findings on two floodplain willow species in Australia (Kennedy et al. 2003): cuttings showed similar survivorship, growth rate and leaf development up to salinity 1.6 ppt, but decreased values for all of these variables in salinity 4 ppt salt. Dry mass of two European floodplain willow species approximated zero in salinity 4 and 6 ppt salt in a hydroponic greenhouse study (Markus-Michalczyk et al. 2014). In contrast to the studies mentioned above, we applied a salinity treatment on a finer scale (salinity 0, 1, 2, and 3 ppt salt). We first showed that salinity 1 ppt does not negatively affect Black Willow even when lasting for several weeks in hydroponics. This is in accordance with our findings on the occurrence of Black Willow in brackish wetlands and reduced growth in salinities higher than 1 ppt salt. Corresponding to Kirwan et al. (2007), estuarine forest retreat may be a function of rising sea level following limited tree recruitment ability in saturated soils. Composition of plant communities may change as a response to variation in vertical accretion, and increasing flooding and salinity (Swarth et al. 2013).

The genus *Salix* includes small shrubs (e.g., *Salix polaris* Wahlenb., Polar Willow) in arctic wetlands and large trees (e.g., *Salix alba* L., White Willow) in temperate floodplains.

Differences in mass may relate to our results that showed lower growth and dry mass of Black Willow cuttings compared to results of salinity treatment on white willow cuttings in a hydroponic experiment that lasted six weeks longer than our study (Markus-Michalczyk et al. 2014). In experiments on cuttings Kennedy et al. (2003) did not find differences between *Salix babylonica* L. (Weeping Willow) and *Salix fragilis* L. (Crack Willow) leaf number within salinity levels which may be explained by the fact that both belong to the same subgenus (subgenus *Salix*) from Eurasia, which also includes the White Willow (Skvortsov 1999). Black Willow belongs to subgenus *Protitea*, native to NA, including some species with a tropical distribution (Argus 2010). This classification reflects phylogenetic relationships and may indicate diverging ecological behavior in drought and salt tolerance (Pitta et al. 2005).

Experimental effect of origin

The effect of origin on shoot, root, and total dry mass was highly significant. Cuttings from freshwater sites developed more dry mass at salinity 0 and 1 ppt compared to 2 and 3 ppt salt, whereas performance of cuttings from brackish sites was significantly lower across all salinities. Intraspecific differences in Salicaceae salinity tolerance vary: differences were also found among experimentally tested willow varieties from clonal trial plots ranging from moderately saline conditions to extreme salinity (Hangs et al. 2011). White willow cuttings originating from freshwater wetlands performed better in salinity 2 ppt salt compared to those from a brackish origin (Markus-Michalczyk et al. 2014). Similarly, Black Willow originating from the Patuxent River tidal freshwater wetland developed more leaves and roots compared to that from the brackish wetland in our salinity treatment. Since occurrence was limited in the Patuxent and Nanticoke Rivers brackish wetlands, cuttings from brackish wetland may be negatively predisposed to a brackish environment. Black Willow reached a mortality up to 85% in areas with frequent salinity increase in coastal forests in the Mississippi Delta plain (Hoeppner et al. 2008) which is consistent with our findings of the poor performance of Black Willow in salt affected Chesapeake Bay tributaries.

Conclusions

Historically, humans settled in eastern NA primarily in tidal freshwater estuarine zones, and many tidal wetlands in the Chesapeake Bay have been lost due to human alteration (Baldwin et al. 2009). Today, tidal wetland restoration is often a management goal (e.g., the Chesapeake Bay Executive Order). Black Willow is a characteristic floodplain tree species in NA and widely used for bank stabilization (e.g., Isebrands et al. 2014). Thus, our findings of the limited salt tolerance (up to salinity 1 ppt salt) for both trees in the field and juveniles in the experiment, and the differences due to vegetative propagule origin, indicate the fragility of this natural resource. Our results have important implications for the selection of specimens in future restoration and flood protection measures.

Acknowledgments

We are very grateful to the members of wetland sciences working group at University of Maryland for fruitful discussions and Leah Hope-Menzies Beckett for hospitality and assistance in the fieldwork. We thank the Estuary and Wetland Research Graduate School (ESTRADE Hamburg) as a member of the German State Excellence Initiative (LExI) for financial support during the field work.

Literature cited

Argus, G.W. 2007. *Salix* (*Salicaceae*) distribution maps and a synopsis of their classification in North America, North of Mexico. Harvard Papers in Botany 12:335–368.

Argus, G.W. 2010. *Salix*. Pp 23-162, *In* D.E. Boufford et al. (Eds.). Flora of North America: North of Mexico. Vol.7: Magnoliophyta: Salicaceae to Brassicaceae. Oxford University Press, New York.

Baldwin, A.H., A. Barendregt, and D.F. Whigham. 2009. Tidal freshwater wetlands – an introduction to the ecosystem. Pp.1-10, *In* A. Barendregt, D.F. Whigham, and A.H. Baldwin (Eds.). Tidal Freshwater Wetlands. Backhuys Publishers, Leiden.

Borsje, B.W., B.K. van Wesenbeeck, F. Dekker, P. Paalvast, T.J. Bouma, M.M. van Katwijk, M.B. de Vries. 2011. How ecological engineering can serve in coastal protection. Ecological Engineering 37:113-122.

Craft, C.B. 2012. Tidal freshwater forest accretion does not keep pace with sea level rise. Global Change Biology 18:3615-3623.

Hangs, R.D., J.J. Schoenau, K.C.J. Van Rees, and H. Steppuhn. 2011. Examining the salt tolerance of willow (*Salix* spp.) bioenergy species for use on salt-affected agricultural lands. Canadian Journal of Plant Science 91: 509-517.

Hilton, T. W., R.G. Najjar, L. Zhong, and M. L. 2008. Is there a signal of sea-level rise in Chesapeake Bay salinity? Journal of Geophysical Research 113. doi: 10.1029/2007JC004247.

Hoeppner, S.S., G.P. Shaffer, and T.E. Perkins. 2008. Through droughts and hurricanes: Tree mortality, forest structure, and biomass production in a coastal swamp targeted for restoration in the Mississippi River Deltaic Plain. Forest Ecology and Management 256: 937-948.

Hussein, A. H. 2009. Modeling of Sea-Level Rise and Deforestation in Submerging Coastal Ultisols of Chesapeake Bay. Soil Science Society of America Journal 73:185-196.

Isebrands, J.G., P. Aronsson, M. Carlson, R. Ceulemans, M.Coleman, et al. 2014. Environmental Applications of Poplars and Willows. Pp.258-321, *In* J.G. Isebrands, and J. Richardson (Eds.), Poplars and Willows: Trees for Society and the Environment. CABI Publishing, Oxon, Rome, I. 634pp.

Karrenberg S., P.J. Edwards, and J. Kollmann. 2002. The life history of Salicaceae living in the active zone of floodplains. Freshwater Biology 47:733–748.

Kennedy, A.S., Ganf, G.G., and K.F. Walker. 2003. Does salinity influence the distribution of exotic willows (*Salix* spp.) at lower river Murray? Marine and Freshwater Research 54: 825-831.

Khan, H., and G. Brush. 1994. Nutrient and metal accumulation in a freshwater tidal marsh. Estuaries 17:345-360.

Kirwan, M. L., Kirwan, J. L., and C.A. Copenheaver. 2007. Dynamics of an estuarine forest and its response to rising sea level. Journal of Coastal Research 23:457–463.

Leck M.A., A.H. Baldwin, V.T. Parker, L. Schile, and D.F. Whigham. 2009. Plant communities of tidal freshwater wetlands of the continental USA and southeastern Canada. Pp. 41-58, *In* A. Barendregt, D.F., Whigham, and A.H. Baldwin (Eds.) Tidal Freshwater Wetlands. Backhuys, Leiden, NL. 318 pp.

McCormick, J., and H.A. Somes Jr. 1982. The coastal wetlands of Maryland. Report prepared for the Maryland Department of Natural Resources. Coastal Zone Management Program. Maryland, USA.

Markus-Michalczyk, H., D. Hanelt, K. Ludewig, D. Müller. 2014. Salt intrusion in tidal wetlands: European willow species tolerate oligohaline conditions. Estuarine, Coastal and Shelf Science 136:35-42.

Martin, L.T., S.R. Pezeshki, and F.D. Shields Jr. 2005. Soaking treatment increases survival of Black Willow posts in a large-scale field study. Ecological Restoration 23:748–763.

Maryland Department of Natural Resources (MDNR). Eyes on the Bay; Water Quality Data. eyesonthebay.dnr.maryland.gov/eyesonthebay/index.cfm; accessed 20 July 2019.

McLusky, D.S., and M. Elliott. 2004. The Estuarine Ecosystem. (3rd ed.), Oxford University Press, Oxford, UK. 309pp.

National Oceanic and Atmospheric Administration (NOAA). Chesapeake Bay Office in the sea nettle forecast via the sea nettle project. Available online at chesapeakebay.noaa.gov/products/salinity-model-for-sea-nettle-project. Accessed August 2011.

Odum, W.E. 1988. Comparative ecology of tidal freshwater and salt marshes. Annual Review of Ecology and Systematics 19:147-176.

Pierfelice, K.N., G. Lockaby, K.W. Krauss, W.H. Conner, G.B. Noe, and M.C. Ricker. 2016. Salinity Influences on Aboveground and Belowground Net Primary Productivity in Tidal Wetlands. Journal of Hydrological Engineering 22. D5015002.

Pitcher, J.A., and J.S. McKnight. 1990. *Salix nigra* Marsh. Black Willow. Pp. 768–772, *In* R.M. Burns, R.M. and B.H. Honkala (Eds.) Silvics of North America. Vol 2: Hardwoods. Agriculture Handbook 271. USDA Forest Service, Washington, DC, USA. 119pp.

Pitta, D.W., T.N. Barry, N. Lopez-Villalobos, and P.D. Kemp. 2005. Effect on ewe reproduction of grazing willow fodder blocks during drought. Animal Feed Science and Technology 120:217–234.

Schaff, S.D., S.R. Pezeshki, and F.D. Shields Jr. 2003. Effects of soil conditions on survival and growth of Black Willow cuttings. Environmental Management 31:748–763.

Sharpe, P.J., and A.H. Baldwin. 2012. Tidal marsh plant community response to sea-level rise: a mesocosm study. Aquatic Botany 101:34–40.

Skvortsov, A.K. 1999. Willows of Russia and Adjacent Countries. Taxonomical and Geographical Revision (English translation of 1968 Russian edition). University of Joensuu, Faculty of Mathematics and Natural Sciences, Report Series #39. Joensuu, Finland.

Swarth, C.W., P. Delgado, and D.F. Whigham. 2013. Vegetation Dynamics in a Tidal Freshwater Wetland: A Long-Term Study at Different Scales. Estuaries and Coasts 36:559-574.

Temmerman, S., P. Meire, T.J. Bouma, P.M.J. Herman, T. Ysebaert, and H.J. De Vriend. 2013. Ecosystem-based coastal defence in the face of global change. Nature 493:45–49.

United States Department of Agriculture Natural Resources Conservation Service (USDA). Plants Database. Available online at plants.usda.gov/java/. Accessed 23 January 2019.

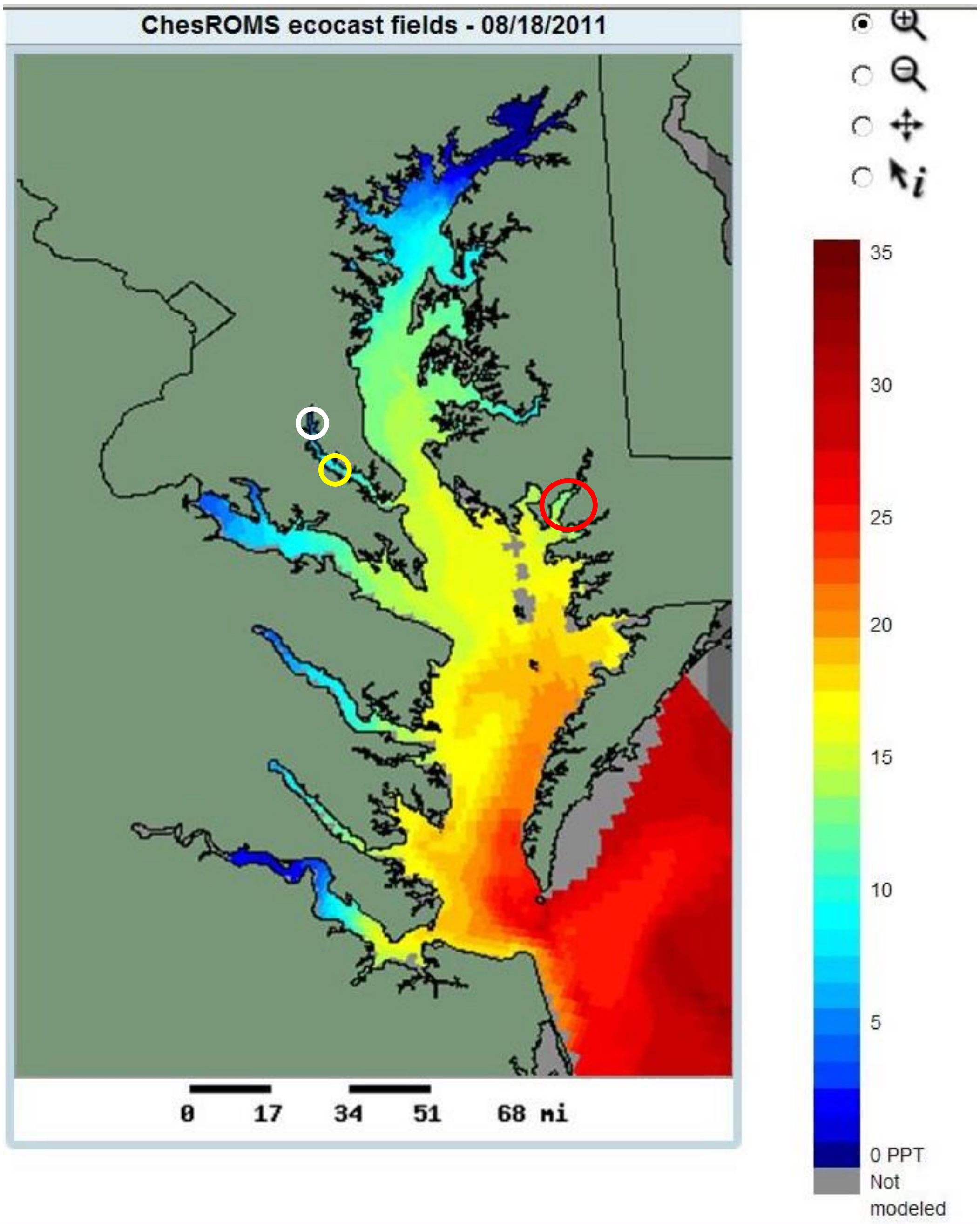

Figure 1 Location of the study area (tidal freshwater - white circle) (38°48'31"N; 76°42'38"W) and brackish (yellow circle) (38°38'20"N; 76°41'42"W) wetland site at the Patuxent River; study area along the Nanticoke River (red circle) and sea surface salinity in the Chesapeake Bay and its tributaries on 18 August 2011 during the study reported on herein (modified from National Oceanic and Atmospheric Administration (NOAA) Chesapeake Bay Office)

Figure 2 *Salix nigra* Marshall (Black Willow) in tidal freshwater wetland (a; b – both Patuxent River) and brackish wetland (c – Patuxent River; d – Nanticoke River) along northeastern North American estuaries (Chesapeake Bay tributaries).

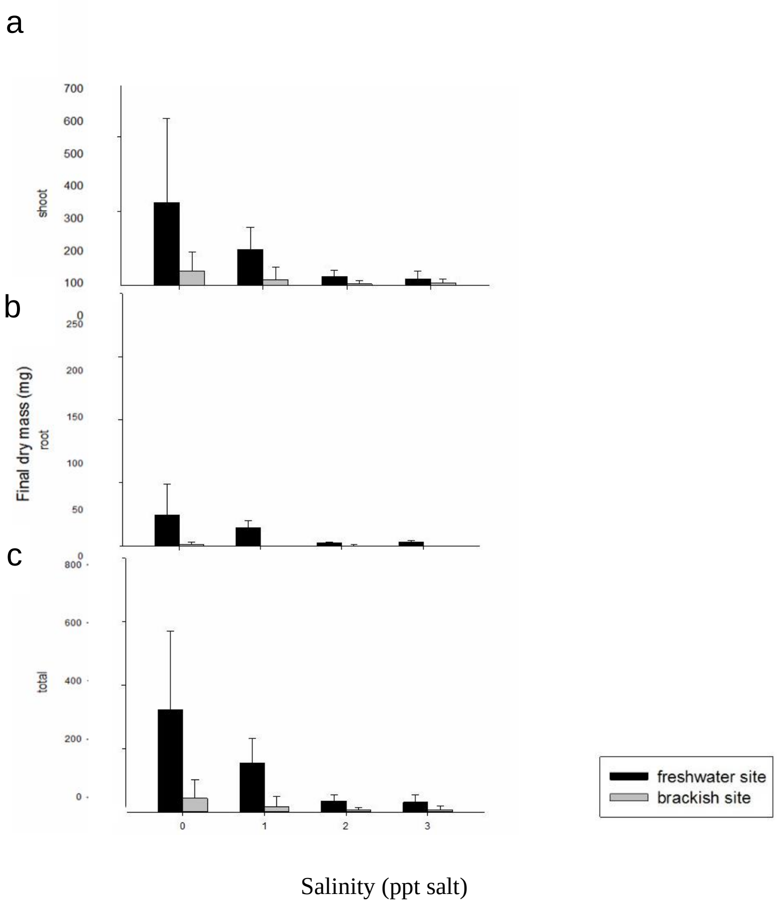

Figure 3 Salinity effects on final dry mass (mg) of shoot (a), root (b), and total dry mass (c) collected from Black Willow cuttings from two origins (tidal freshwater vs. brackish wetland) from a North American estuary (Patuxent River; tributary of Chesapeake Bay) in a hydroponic greenhouse experiment with salinity treatment (0 to 3 ppt salt) (bars represent mean ± SD; N = 6)

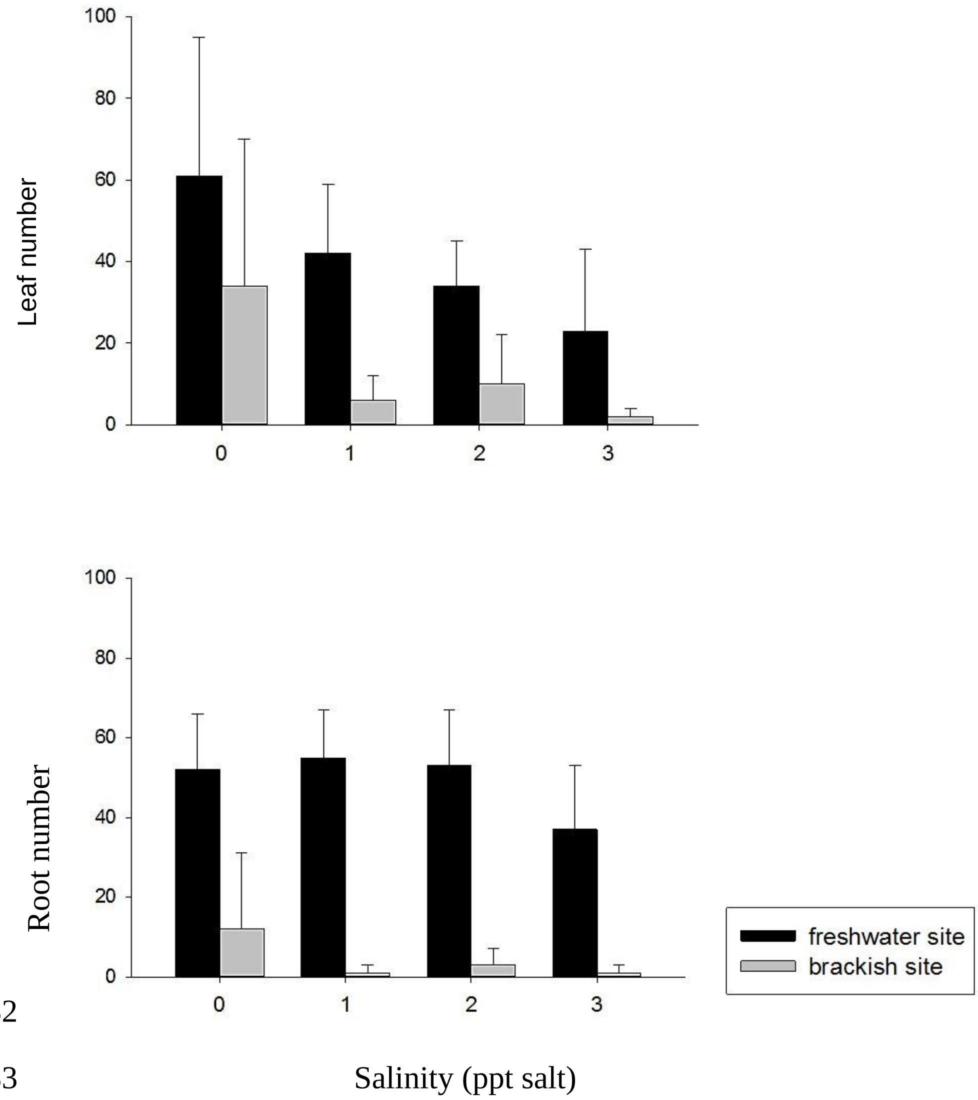

Figure 4 Salinity effects on number of leaves (graph above) and roots (graph below) of Black Willow cuttings from two origins (tidal freshwater vs. brackish) from a NA estuary (Patuxent River; tributary of the Chesapeake Bay) in a hydroponic greenhouse experiment in a salinity treatment (0 to 3 ppt salt) (bars represent mean ± SD; N = 6)

Table 1 Salinity (parts per thousand - ppt) recorded in MDNR monitoring stations in the Chesapeake Bay tributaries Patuxent (upstream: Jug Bay; July/average and range/year 2011; downstream: Kings Landing; July/average and range/year 2003-2006); and Nanticoke River (upstream: Sharptown; July/average and range/year 2012-2014; downstream: Tyaskin; July/average and range/year 2012-2014) (source: eyesonthebay.dnr.maryland.gov/eyesonthebay/index.cfm).

Chesapeake tributary	Patuxent		Nanticoke	
salinity in ppt	July/average	range/year	July/average	range/year
upstream	0.13	0.03 - 0.89	0.86	0.05 - 7.11
downstream	1.36	0.04 - 13.45	8.25	0.02 - 15.81

Table 2 Results of two-factorial ANCOVAs summarizing the effects of salinity (0, 1, 2, and 3 ppt salt) and origin (tidal freshwater vs. brackish site) on final dry mass (mg) of (a) shoot, (b) root, and (c) total dry mass of Black Willow cuttings in a greenhouse experiment with salinity treatment ranging from salinity 0 to 3 ppt salt (initial mass of cuttings (mg) served as covariate; recorded data were log transformed to meet assumptions of ANCOVA; N = 6). Cuttings were harvested in tidal wetlands of an eastern NA estuary (Patuxent River; tributary of the Chesapeake Bay).

	(a) shoot		(b) root		(c) total dry mass (mg)	
	F	*p*	F	*p*	F	*p*
Salinity	6.02	< 0.01	16.03	< 0.001	8.24	< 0.001
Origin	1.63	n.s.	6.48	< 0.05	6.71	< 0.05

Table 3 Results of two-factorial ANOVAs summarizing the effects of salinity (0, 1, 2, and 3 ppt salt) and origin (tidal freshwater vs. brackish site) on the number of (a) leaves, and (b) roots that Black Willow cuttings developed in a greenhouse experiment with salinity treatment ranging from salinity 0 to 3 ppt salt (cuttings' initial mass (mg) served as covariate; N = 6). Cuttings were harvested in tidal wetlands along an eastern NA estuary (Patuxent River; tributary of the Chesapeake Bay).

Source	(a) leaves		(b) roots	
	F	*p*	F	*p*
Salinity	6.10	< 0.01	2.61	n.s.
Origin	19.90	< 0.001	160.22	< 0.001
Salinity*origin	0.31	n.s.	1.47	n.s.