

1 Ecological Applications: *Article*

2

3 Running head: Coastal squeeze on oyster reefs

4

5 Coastal squeeze on temperate reefs: long-term shifts in salinity, water quality, and oyster-
6 associated communities

7

8 Maxwell Tice-Lewis^{1,2}, Y. Stacy Zhang¹, S. Gray Redding³, Niels L. Lindquist¹, Antonio B.
9 Rodriguez¹, Clare M. Fieseler^{4,5}, Quentin A. Walker^{6,7}, F. Joel Fodrie^{1,*}

10

11 ¹Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC
12 28557

13 ²Present Address: Confederated Tribes of Siletz Indians, Siletz, OR 97380

14 ³National Fish and Wildlife Foundation, Washington, DC 20005

15 ⁴Environment, Ecology, and Energy Program, University of North Carolina at Chapel Hill,
16 Chapel Hill, NC 27514

17 ⁵National Museum of Natural History, Smithsonian Institution, Washington, DC 20013

18 ⁶National Centers for Coastal Ocean Science, Beaufort Laboratory, Beaufort, NC 28516

19 ⁷CSS-Inc., Fairfax, VA 22030

20 *Corresponding author: jfodrie@unc.edu; 252 726 6841 (ext 149)

21

22 Open Research: We agree to the Ecological Society of America's Open Research Policy. Data
23 are available on Dryad (<https://doi.org/10.5061/dryad.sj3tx9665>).

24 **Abstract**

25 Foundation species such as mangroves, saltmarshes, kelps, seagrasses, and oysters thrive within
26 suitable environmental envelopes as narrow ribbons along the land-sea margin. Therefore, these
27 habitat-forming species and resident fauna are sensitive to modified environmental gradients. For
28 oysters, many estuaries impacted by sea-level rise, channelization, and municipal infrastructure
29 are experiencing saltwater intrusion and water-quality degradation that may alter reef
30 distributions, functions, and services. To explore decadal-scale oyster-reef community patterns
31 across a temperate estuary in response to environmental change, we resampled reefs in the
32 Newport River Estuary (NRE) during 2013-2015 that were previously studied during 1955-1956.
33 We also coalesced historical NRE reef distribution (1880s-2015), salinity (1913-2015), and
34 water-quality driven shellfish closure boundary (1970s-2015) data to document environmental
35 trends that could influence reef ecology and service delivery. Over the last 60-120 years, the
36 entire NRE has shifted toward higher salinities. Consequently, oyster-reef communities have
37 become less distinct across the estuary, manifest by 20-27% lower species turnover and
38 decreased faunal richness among NRE reefs in the 2010s relative to the 1950s. During the 2010s,
39 NRE oyster-reef communities tended to cluster around a euhaline, intertidal-reef type more so
40 than during the 1950s. This followed faunal expansions farther up-estuary and biological
41 degradation of subtidal reefs as NRE conditions became more marine and favorable for
42 aggressive, reef-destroying taxa. In addition to these biological shifts, the area of suitable bottom
43 on which subtidal-reefs persist (ultimately regulated by up-estuary intrusion of marine waters)
44 and support human harvest (driven by water quality, eroding from up-estuary) has decreased by
45 >75% since the natural history of NRE reefs was first explored. This “coastal squeeze” on
46 harvestable subtidal oysters (reduced from a 4.5-km to a 0.75-km envelope along the NRE’s

47 main axis) will likely have consequences regarding the economic incentives for future oyster
48 conservation, as well as the suite of services delivered by remaining shellfish reefs (e.g.,
49 biodiversity maintenance, seafood supply). More broadly, these findings exemplify how
50 “squeeze” may be a pervasive concern for biogenic habitats along terrestrial or marine ecotones
51 during an era of intense global change.

52

53 **Key words:** coastal narrowing; community assembly; ecotones; environmental stress gradients;
54 saltwater intrusion; sea-level rise; shellfish closures; water quality

55

56 **Introduction**

57 Predicting responses of ecosystems to global change is bolstered by understanding how the
58 distributions of habitat-forming foundation species will shift across depths, elevations, or
59 latitudes in response to changes in the position of suitable abiotic (fundamental niche) and biotic
60 (realized niche) environmental conditions (Snedaker 1995, Parmesan and Yohe 2003). In
61 particular, the global redistribution of more mobile terrestrial and marine taxa, manifest as
62 whole-community shifts, may be linked to the distribution and prevalence of specific biogenic
63 structures that serve as foraging, mating, refuging, and resting habitats (Asch and Erisman 2018,
64 Morley et al. 2018).

65 Over the next several decades, the impacts of global and local environmental shifts on biogenic
66 habitats and associated communities may be magnified in dynamic coastal and estuarine
67 environments where steep physiochemical and biological gradients exist between terrestrial and
68 marine biomes (i.e., concentrated over meter-to-kilometer scales; Gunter 1956). For example, in
69 response to relative sea-level rise (RSLR), temperate saltmarshes must either accrete vertically or

70 transgress landward to persist (Morris et al. 2002). For saltmarshes otherwise capable of
71 landward retreat in response to RSLR, human development/infrastructure can obstruct natural
72 migration corridors. As such, these saltmarshes become “squeezed” between rising water on the
73 seaward edge and coastal development on the landward margin that truncate the zone of suitable
74 environmental niche space (for saltmarshes, primarily vertical position relative to tidal
75 inundation; Doody 2004, Pontee 2013). Broadly, the potential for “squeeze” may be a
76 fundamentally relevant concern for all coastal biogenic habitats existing along strong
77 physiochemical gradients of temperature (rocky intertidal mussels, Barry et al. 1995; kelp,
78 Dayton 1985), light (seagrass, Ochieng et al. 2010), dissolved oxygen (oysters, Lenihan and
79 Peterson 1998), and storm-generated physical disturbance (coral, Fabricius et al. 2008).

80 Estuaries are classically defined as the mixing zones between rivers and the sea; thus, salinity
81 gradients are first-order drivers of local floral and faunal distributions (Odum 1988). The eastern
82 oyster (*Crassostrea virginica*) typically occupies subtidal zones within brackish estuarine waters
83 and intertidal zones in the lower estuary where salinities are characteristically marine (Bahr and
84 Lanier 1981). Brackish water and aerial exposure ultimately provide these bivalves with refugia
85 from enemies such as predators, space competitors, and bioeroders across these prominent
86 environmental stress gradients (Fodrie et al. 2014, Walles et al. 2016).

87 Many temperate estuaries have been significantly modified by local development and global
88 change, including pervasive bathymetric modifications to accommodate commercial ports,
89 development of an intracoastal waterway network for boating/shipping transit, and marina
90 construction, as well as RSLR and human population growth throughout most watersheds
91 (Kennish 2002). These activities are profoundly altering salinity and related water-quality
92 regimes that may have cascading impacts on biogenic habitats such as oyster reefs. For instance,

93 dredging/channelization generally increases tidal prism, and combined with RSLR can lead to
94 saltwater intrusion up estuary (Ralston and Geyer 2019). The Newport River Estuary (NRE),
95 North Carolina (NC), USA, is a model system for exploring the consequences of these dynamics
96 as many of these anthropogenic alterations define the NRE. Additionally, long-term place-based
97 research--often linked to the presence of field stations (Sagarin et al. 1999, Able 2016)--is a
98 critical requisite for detailed investigations of multidecadal ecosystem change in response to
99 human influences (e.g., fishing, urbanization, climate change). Owing to the extended presence
100 of university and government marine labs nearby, studies documenting the distribution and
101 natural history of eastern oyster reefs in the NRE date back to the 1880s (e.g., Winslow 1889,
102 Grave 1901, 1905, McDougall 1943). In conjunction with these biological reports, there is a
103 substantial library of environmental data collected from the NRE over the last 100+ years.
104 Regulated by the salinity regime of the NRE at the start of the 20th century, the transition
105 between primarily intertidal reefs (lower estuary) to chiefly subtidal reefs (upper estuary)
106 occurred 12-km upstream of Beaufort Inlet (the nearest ocean connection) a century ago (Grave
107 1901, 1905), and was characterized for both economic and ecologic purposes. Grave (1905)
108 postulated that subtidal oyster cultivation was feasible only upstream of this “line” due to
109 periodic freshets that limited the distribution of common oyster enemies, such as predatory
110 marine gastropods and bioeroders. Additionally, Grave (1901) qualitatively noted the
111 enhancement of biodiversity in the NRE due to the distinct invertebrate assemblages supported
112 by subtidal versus intertidal reefs. Building from these observations, Wells (1961) sampled both
113 subtidal and intertidal oyster reefs of the mesohaline and euhaline (5-35 practical salinity units)
114 regions of the NRE and quantified the distribution of 300+ reef-associated fauna across this
115 salinity gradient.

116 To assess how oyster-reef communities in the NRE have changed in nature or distribution over
117 decadal scales, potentially in response to human-driven salinity shifts, we resampled reefs during
118 2013-2015 that were previously sampled in 1955-1956 by Wells (1961). We made direct
119 comparisons of reef-associated faunal communities between 1955-1956 and 2013-2015 to
120 evaluate taxon- and assemblage-level distributions across the NRE during this six-decade
121 interval. We combined these faunal data with multidecadal NRE salinity records to address the
122 following questions: (1) Has the structure and distribution of NRE oyster-reef communities
123 changed over time? (2) Which faunal groups account for differences among reefs or through
124 time? And (3) for any changes we observed across estuary or time, did faunal shifts correspond
125 with spatiotemporal patterns of NRE salinity gradients? We also incorporated ancillary data,
126 such as presence/absence of intertidal and subtidal reefs during the 1880-2015 period and water-
127 quality closures throughout the historical record to consider: (4) Has the transition line between
128 subtidal and intertidal reefs shifted up or down estuary in response to changes in salinity
129 regimes? And (5) how have water-quality closures interacted with the subtidal-intertidal reef
130 transition line to determine the extent of NRE bottom suitable for restoration, maintenance, and
131 cultivation of subtidal reefs.

132

133 **Methods**

134 *Study System and Field Collections*

135 Despite modifications, the NRE remains a shallow (1-m mean depth at mean low water)
136 drowned-river system covering 134 km² and defined by extensive intertidal mudflat and
137 saltmarsh areas (Kirby-Smith and Costlow 1989). The NRE extends 16.5 km from Beaufort Inlet
138 to the bayhead delta, receives freshwater from a relatively small watershed (250 km²) via a 15-

139 km long main-stem river (~20-m wide), and has a 0.75-m tidal range (Ensign et al. 2013). In
140 historical surveys, subtidal reefs dominated the upper 4.5 km of the NRE across broad shallow
141 flats, although the crests of these reefs could/can infrequently (as a proportion of total reef
142 extent) extend intertidally along raised bars given the tidal range in this section of the NRE
143 (Winslow 1889, Grave 1901, 1905). Conversely, intertidal reefs with shallow subtidal flanks
144 dominated the lower 12 km. Wells (1961) sampled oyster-reef-associated communities at five
145 sites throughout the NRE during 1955-1956, with the specific focus of relating the composition
146 and distribution of fauna with salinity. In the lower, euhaline portion of the estuary (based on
147 measurements made at the time of study), Wells (1961) sampled Shark Shoal (SS) and Pivers
148 Island (PI)(Fig. 1). In the polyhaline NRE, Wells (1961) sampled Gallants Point (GP) and White
149 Rock (WR)(Fig. 1). Near the mesohaline-polyhaline transition in the upper NRE, Wells (1961)
150 sampled Cross Rock (CR)(Fig. 1). We conducted visits to all reefs specifically described in
151 Winslow (1889), Grave (1905), and Wells (1961) to qualitatively determine if reefs remained
152 extant as of 2013 (Table 1). Since Wells (1961), the SS and GP sites have been dramatically
153 altered with near-total loss of oyster reefs: SS has been transformed into Radio Island via dredge-
154 spoil deposits from the adjacent channel serving the Port of Morehead City, while GP is now
155 occupied by multiple boat marinas and a bridge span over the former oyster-reef sites (Table 1).
156 Therefore, we focused our 2010s sampling revisits on Wells' (1961) PI, WR, and CR sites,
157 which nearly span the entire NRE (Fig. 1). Based on our interpretation of Wells' (1961) site
158 descriptions, the specific patch reef he sampled for WR was absent at the time of our 2013-2015
159 revisits; therefore, we moved 1 km to the south-southwest to sample a comparable reef in the
160 middle region of the estuary termed White Rock Replacement (WRR)(Fig. 1).

161 To sample oyster-reef associated communities, Wells (1961) employed a simple sampling
162 methodology: “*During each station visit, a gallon [3.78 liter] jar was filled with oysters, their*
163 *associates, and shell. An effort was made to collect from each of the minor variations in the*
164 *habitat, such as tidal zones, and to have the sample reflect the faunal diversity of the bed.*”
165 During 1955-1956, Wells (1961) collected 13, 6, and 15 samples at PI, WR, and CR,
166 respectively. Collections from PI and CR spanned all seasons, while WR collections were made
167 only during summer (Table 1). Samples were returned to the laboratory, where Wells (1961)
168 identified all fauna at the lowest taxonomic level possible, which was ultimately published as a
169 taxon-specific presence-absence matrix across reefs and over time. To generate data comparable
170 with Wells (1961), we followed the same collection approach and collected material from the
171 reef crest down to the reef base (including subtidal fringe as possible) to capture faunal
172 composition and diversity on each reef. We also collected a comparable number of samples as
173 did Wells (1961): 16 trips to both PI and CR that spanned all seasons, and 14 trips to WRR
174 spanning all seasons (Table 1). Generally, 2013-2015 sampling followed a bimonthly schedule (a
175 single 3.78-liter sample per reef per interval), with some deviations due to factors such as storm
176 events (e.g., Hurricane Arthur in 2014). Similarly, sampling during 1955-1956 occurred
177 coincident with storm landfalls (Hurricanes Connie, Diane, and Ione 1955)(Wells 1961). All
178 2013-2015 samples were transported to the laboratory where we identified fauna to the lowest
179 taxonomic level possible.

180 *Historical Records of Salinity, Water Quality Closures, and Reef Longevity*

181 Given the central role of salinity in regulating across-estuary distributions of reef types
182 (subtidal versus intertidal) and associated fauna (reviewed in Baggett et al. 2015, Walles et al.
183 2016), we explored if and how salinity gradients have shifted in the NRE over time by

184 coalescing a 1913-2015 time series from multiple published and regulatory agency sources. To
185 leverage these historical salinity data and provide greater context for our faunal investigations,
186 we evaluated all known records taken from within 0.5 km of PI, WR/WRR, or CR reefs
187 (separately for each site). For PI, sources included: (1) Hoyt (1920), who reported monthly
188 salinity recordings during 1913-1914 (N=42). (2) Wells (1961), who reported monthly salinity
189 observations during 1955-1956 (N=124 across all sites). (3) The North Carolina Division of
190 Marine Fisheries Shellfish Sanitation (NCDMFSS) program, which provided point-measure
191 salinities at fixed NRE sampling sites during 1965-2015 (N=1900 across all sites) (NCDEQ
192 2021). Coarsely, NCDMFSS sampling near each reef site followed a biweekly schedule,
193 although sampling frequency ranged from 0-60 observations in any given year based on
194 management needs and weather patterns throughout the survey. And (4) the present study, which
195 provided point-measure salinities taken during faunal sampling efforts at each reef site (N=44
196 across all sites). For WR/WRR and CR, salinity data were limited to sources 2-4 as described
197 above. We could not completely standardize these salinity data in terms of sampling seasonality,
198 storm events, diel periodicity, and water-level context. As a check against potential biases
199 associated with this low degree of standardization, we also explored salinity patterns in the NRE
200 using only the NCDMFSS database. Conclusions drawn from that more-standardized subset of
201 salinity data regarding spatiotemporal patterns are entirely consistent with conclusions drawn
202 from the complete, coalesced NRE salinity record.

203 NCDMFSS also provided records regarding the boundaries of permanent shellfish harvest
204 closure areas in the upper NRE in response to changes in water quality over time. These closures
205 are mandated by the Food and Drug Administration when counts of indicator fecal coliform

206 bacteria exceed a geometric mean of 14 most probable number (MPN) per 100 ml or a 90th
207 percentile reading >43 MPN per 100 ml (National Shellfish Sanitation Program 2017).

208 *Statistical Analyses*

209 We used several multivariate statistical approaches to examine patterns in oyster-reef
210 associated faunal communities across the NRE in the 1950s and 2010s. All multivariate analyses
211 were based on the presence/absence of taxa in individual samples. For Wells (1961) to include a
212 species in his publication, that taxon had to be present in $\geq 20\%$ of all samples. To achieve as
213 accurate comparison as possible, we also excluded ‘rare’ or transient taxa observed in $< 20\%$ of
214 samples collected during 2013-2015. To not overestimate differences between the 1950s and
215 2010s, however, all species reported by Wells (1961) were included in the 2013-2015 database
216 regardless of their occurrence frequency in our sampling. Oysters were themselves excluded
217 from the community analysis because they were present in every sample, and thus would have no
218 impact on multivariate differences among communities. To avoid reporting spurious differences
219 between communities over time driven by differences in taxonomic expertise among researchers
220 or shifts in accepted phylogenetic relationships at the species or genus level, we conducted all
221 analyses at the family level, except Actiniaria (anemones) and Nemertea (ribbon worms), which
222 were grouped at the Order and Phylum levels, respectively. At this resolution, we are confident
223 that faunal identities were conserved over time.

224 To evaluate evidence of statistical differences among samples, we used a crossed permutational
225 MANOVA (PERMANOVA) with NRE site (PI, WR/WRR, CR), period (1950s, 2010s), and
226 site*period as fixed factors. PERMANOVA post-hoc procedures were used to evaluate pairwise
227 differences as necessary based on statistical results in PERMANOVA. To determine which
228 families were driving differences among NRE sites and between study periods, we calculated

229 similarity percentages (SIMPER) among samples. We also used nonmetric multidimensional
230 scaling (nMDS) of reef-associated faunal presence/absence to explore similarity in samples
231 collected across the NRE during 1955-1956 and 2013-2015. Environmental and temporal
232 variables of salinity, Julian day (a proxy for seasonal effects), and year were fitted to the data
233 using ENVFIT to determine how these variables correlated with observed community groupings
234 in nMDS space. The salinity value assigned to a particular sample was taken from the
235 corresponding spot measures taken by Wells (1961) or our research team at the time of sampling
236 at each reef.

237 To assess community turnover (beta-diversity) at multiple scales, we calculated multivariate
238 dispersions for within-reef samples, across the NRE, and between study periods (Anderson et al.
239 2006). To further quantify taxonomic turnover among NRE sites (i.e., beta-diversity across PI,
240 WR/WRR, CR) as an indicator of the strength of environmental/community gradients across
241 these reefs and through time, we calculated Jaccard distances (*1-Jaccard index*) for all PI-
242 WR/WRR, PI-CR, and WR/WRR-CR pairwise combinations of samples collected in the 1950s
243 and 2010s, with time periods handled separately. Using each sample-by-sample dissimilarity
244 score as a replicate for site-pair comparisons, we assessed statistical differences in between-site
245 turnover during the 1950s versus the 2010s using the Mann-Whitney U test (separate tests for PI
246 versus WR/WRR, PI versus CR, WR/WRR versus CR). This nonparametric approach was used
247 because variances of site-versus-site Jaccard scores were heteroscedastic between study periods
248 for all three site-pair comparisons.

249 To further evaluate the statistical correlation between NRE salinity gradients and the level of
250 taxonomic turnover across reefs, and through time, we conducted a single Mantel test. This
251 correlation paired two variables drawn from separate but identically structured matrices (i.e.,

252 site-pair*period): Jaccard dissimilarity in faunal communities and euclidean differences in mean
253 salinities across sites and time (i.e., 1950s versus 2010s). Mean salinities for each site*period
254 were calculated from the replicate point-based measurements taken during each sampling visit at
255 each site in Wells (1961) or the present study, separately.

256 To evaluate multidecadal salinity patterns across the NRE, we calculated annual minimum,
257 maximum, and mean (as possible) salinities from monthly values provided by each data source
258 for PI, WR/WRR, and CR. For PI, least-squares regressions were conducted for annual minimum
259 maximum, and mean salinities during 1913-2015. For WR/WRR and CR, least-squares
260 regressions were conducted for annual minimum, maximum, and mean salinities during 1955-
261 2015.

262 We used the following statistical packages to explore spatiotemporal dynamics of NRE oyster
263 reef communities: nMDS , PERMANOVA, SIMPER, ENVFIT, PERMDISP, Jaccard distances,
264 and the Mantel test were conducted using the R package, ‘vegan’ version 2.5-7 (Oksanen et al.
265 2019). Least squared regressions and Mann-Whitney U tests were conducted using R version
266 4.04 (R Core Team 2020). Patterns of water-quality closures and reef persistence through time in
267 the NRE did not require quantitative statistical tests.

268

269 **Results**

270 *Spatiotemporal patterns of reef-associated fauna*

271 There were notable differences in NRE reef-associated faunal communities over time. Fewer
272 taxa (families or lowest possible taxonomic resolution) of reef-associated fauna were collected in
273 2013-2015 (N=36) than in 1955-1956 (N=54). Wells (1961) collected 52, 44, and 33 taxa at PI,

274 WR, and CR, respectively. By comparison, during 2013-2015 we collected 27, 26, and 26 taxa at
275 PI, WRR, and CR, respectively.

276 Given these community level differences, 1955-1956 and 2013-2015 faunal samples were
277 distinct in multivariate space (SIMPER=56% different; PERMANOVA df=1, F=18.67, p<0.001;
278 Fig. 2). Indeed, sample year (nMDS axis 1) and salinity (nMDS axis 2), but not Julian day, were
279 significantly correlated with community variability (Year: $R^2=0.700$, $P<0.001$; Salinity:
280 $R^2=0.366$, $P<0.001$; Julian: $R^2=0.002$, $P=0.91$). Taxa contributing most to the community
281 difference between 1955-1956 and 2013-2015 (50% cumulative contribution) were hydrozoa
282 (Campanulariidae), bryozoans (Electridae, Victorellidae, and Bugulidae), sedentary polychaetes
283 (Sabellariidae and Spionidae), burrowing amphipods (Corophiidae), sessile protists
284 (Folliculinidae), anemones (Actiniaria spp.), errant polychaetes (Phyllodocidae and Eunicidae),
285 bivalves (Veneridae), Ribbon worms (Nemertea), gastropods (Pyramidellidae), isopods
286 (Sphaeromatidae), and parasitic barnacles (Sacculinidae).

287 We documented up-estuary movement of several taxa from 1955-1956 to 2013-2015. Four
288 families (Tellinid clams, Tubificid worms, Sacculinid barnacles, and Muricid snails) were
289 observed at WRR in 2013-2015 that were not at WR in 1955-1956. Notably, Atlantic oyster
290 drills (*Urosalpinx cinerea*, Muricidae), an important predator of oyster spat (Chestnut and Fahy
291 1956), were collected in 21% of samples at WRR in 2013-2015 while absent in Wells' (1961)
292 surveys. Wells (1961), however, did record oyster drills in 66% of samples at Gallants Pt., 7-km
293 downstream from WRR, and 86% of samples at PI, 10-km downstream from WRR. Together,
294 these data indicate an upriver shift in drill distribution. We also documented the up-estuary
295 migration of 5 taxa from WR in 1955-1956 to CR in 2013-2015, including Caprellid amphipods
296 and Leptocheliids, Venerid clams, and Eunicid and Terebellid polychaetes.

297 Despite these overarching differences between study periods, some things appeared consistent
298 between the 1950s and 2010s. Common reef-associated fauna in the NRE across all sites and
299 both study periods included: barnacles, Balanidae (*Amphibalanus eburneus*); amphipods,
300 Melitidae (*Melita nitida* and *Dulichchiella appendiculata*); xanthid crabs, Panopeidae (*Panopeus*
301 *herbstii* and *Eurypanopeus depressus*); Gastropods, Calyptraeidae (*Crepidula spp.*) and
302 Pyramidellidae (*Boonea impressa*); polychaetes, Nereididae (*Alitta succinea* and *Nereis falsa*);
303 and Mytilid bivalves (*Geukensia demissa* and *Brachidontes exustus*) (Table 2). Spionid
304 polychaetes (*Polydora websteri*) were abundant at all sites across both studies except PI in 1955-
305 1956 (25% of samples). Anemones (*Actiniaria spp.*) and Venerids (*Mercenaria spp.*) were
306 similarly abundant at all sites in both studies except CR in 1955-1956 (13.3% and 0%,
307 respectively). Notably, PI, WR/WRR, and CR could be distinguished from each other in
308 multivariate community space during both 1955-1956 and 2013-2015 (Fig. 2). Moreover, during
309 both the 1950s and 2010s (as with sampling overall), community composition was significantly
310 correlated with salinity, primarily across nMDS axis 2. Unsurprisingly, PI samples were
311 typically associated with higher salinities, CR samples were generally associated with lower
312 salinities, and WR/WRR samples fell in between PI and CR extremes.

313 Fundamentally related to our core questions, among-site faunal differences for PI, WR/WRR,
314 and CR truncated over time. Indeed, between-reef dispersions (mean distance of samples to
315 group centroid) during 2013-2015 were 14% smaller than during 1955-1956 ($F=0.952$, $P<0.001$).
316 Relatedly, 2013-2015 samples/sites appeared to cluster more tightly in nMDS space that was
317 correlated with higher salinities, relative to 1955-1956 analogues (Fig. 2).

318 These patterns were also clear in species-turnover metrics among reef sites (Fig. 3). Jaccard
319 distance between PI and WR in 1955-1956 (52%) was nearly 1.4x greater than PI-versus-WRR

320 distance in 2013-2015 (38%) ($U=-6.62$, $P<0.001$). Between PI and CR, Jaccard distance was
321 1.25x greater in 1955-1956 (60%) than in 2013-2015 (48%) ($U=-7.23$, $P<0.001$). Similarly, WR-
322 versus-CR Jaccard distance in 1955-1956 (49%) was ~ 1.3 x higher than for WRR-versus-CR in
323 2013-2015 (37%) ($U=-5.89$, $P<0.001$). Notably, the degree of faunal turnover (Jaccard distances)
324 among reefs and over time was significantly correlated with the magnitude of salinity difference
325 recorded between reef-sampling events (Mantel $R=0.16$, $P<0.001$).

326 *Salinity, water quality, and reef persistence*

327 NRE salinity regimes have shifted over multidecadal scales in terms of extremes and averages.
328 Across all sites, only PI, the site nearest to Beaufort Inlet, did not experience a shift in annual
329 mean and maximum salinities over time (Mean: $R^2=0.02$, $P=0.37$; Max: $R^2=0.02$, $P=0.18$); both
330 metrics consistently approached full marine salinity throughout the entire record (Fig. 4A).
331 Conversely, annual minimum salinities recorded at PI increased notably across the 1913-2015
332 time span ($R^2=0.21$, $P=0.01$), from annual minimum readings <20 ppt in the early-to-mid 20th
333 century toward >25 ppt by 2015 (Fig. 4A). At the mid-estuary WR/WRR reefs, annual mean,
334 maximum, and minimum salinities all increased across 1955-2015 (Mean: $R^2=0.26$, $P<0.001$;
335 Max: $R^2=0.20$, $P<0.01$; Min: $R^2=0.097$, $P<0.05$). Early in the record, annual salinities at
336 WR/WRR typically ranged between 10-25 ppt, while by the end of the record salinities
337 consistently ranged between 15-30 ppt (Fig. 4B). At CR, our farthest upstream NRE study site,
338 annual mean, maximum, and minimum salinities also rose through time, but only mean and
339 maximum trends were statistically unambiguous (Mean: $R^2=0.27$, $P<0.001$; Max: $R^2=0.21$,
340 $P<0.01$; Min: $R^2=0.07$, $P=0.07$). Salinities ranged between 5-20 ppt early in the record at CR,
341 and shifted toward 15-30 ppt by 2015 (Fig. 4C).

342 Prior to the 1970s, the entire 16.5-km (along main stem) NRE was open to oyster harvest.
343 Following a failure of the Newport Municipal Sewage Treatment plant in 1969, and subsequent
344 standardized water-quality sampling by NCDMFSS, shellfish harvest was permanently
345 prohibited just upstream of CR in 1972. This closure line, running perpendicular to the NRE
346 main-stem axis, was located 15.5 km from Beaufort Inlet. In 1998, as water-quality testing
347 continued, this closure line moved 0.9 km down estuary. In 2005, the closure line moved an
348 additional 0.25 km down estuary. Finally, as we were concluding our reef sampling in 2015, the
349 closure line moved once more, 0.1 km farther down estuary. By 2016, only the lower 14.25 km
350 of the NRE was open to oyster harvest and cultivation. Finally, reefs such as “Green Reef” and
351 “Turtle Rock” mapped by Grave (1901), as well as “Shark Shoal”, “Gallants Point”, and “White
352 Rock” sampled by Wells (1961) were extirpated by 2013 (Table 1). In addition, across 2013-
353 2015, the subtidal fringes of Pivers Island no longer matched the spatial extent as described in
354 Wells (1961).

355

356 **Discussion**

357 Temperate oyster reefs and associated fauna in the NRE have exhibited remarkable changes in
358 composition and distribution during the last 60-120 years. These changes have been driven
359 primarily by shifts in underlying abiotic environmental gradients (i.e., salinity). These dynamics
360 have occurred coincident with NRE water quality degradation, resulting in a “squeeze” on
361 subtidal, harvestable oyster reefs along the river-to-ocean axis. This oyster reef “squeeze”
362 phenomena is analogous to the compression of suitable habitat for saltmarsh plants along
363 developed estuarine shoreline shorelines in an age of accelerated sea-level rise (Pontee 2013).
364 Building from these examples, we stress that “coastal squeeze” is likely to broadly impact

365 shallow-water and intertidal biogenic habitats that exist along steep environmental gradients and
366 are subject to multiple anthropogenic perturbations. More broadly, we speculate that the
367 distribution and extent of many foundation species existing primarily along ecotones in terrestrial
368 or aquatic systems may be relatively sensitive to anthropogenic environmental perturbations.

369 The “squeeze” on NRE oyster reefs has manifest in two ways: (1) reef communities along the
370 NRE main axis have become less distinct from each other since the 1950s, with between-reef
371 faunal turnover (beta-diversity) and whole-estuary richness (alpha-diversity for the entire NRE)
372 decreasing over time; and (2) the area within the NRE in which subtidal-reef communities can
373 persist (ultimately driven by higher salinity waters intruding at the down-estuary margin) and
374 support human harvest (driven by expanding water quality degradation, impinging along the up-
375 estuary margin) has decreased by >75% since the distribution and natural history of reefs in this
376 system were described a century ago (Winslow 1889, Grave 1901). These community shifts
377 corresponded with a long-term decrease in NRE environmental heterogeneity (i.e., estuarine
378 salinity gradient) that is reported to fundamentally drive spatial community turnover in diverse
379 marine and terrestrial contexts (Chase and Myers 2011). Throughout the NRE, reefs have
380 become more marine and intertidal in physical nature and faunal composition. Notably, this
381 homogenization of reef communities decreased NRE-scale species richness, contrary to the
382 paradigm of increased species diversity associated with higher salinities, versus brackish waters,
383 in estuarine systems (Odum 1988).

384 Without planned manipulative experiments at whole-estuary scales to evaluate (i.e., serially
385 discount) a suite of oyster stressors, the links we have drawn between oyster-reef community
386 patterns and salinity through time are somewhat correlative by nature. In the context of a broader
387 literature that greatly informs our observations, however, we consider it reasonable to

388 acknowledge salinity as a first-order driver of oyster reef dynamics in the NRE. Indeed, for >100
389 years the distribution of oyster reefs in the context of salinity regimes has been realized (e.g.,
390 Winslow 1996; Baggett et al. 2015) with mechanisms documented at physiological (La Peyre et
391 al. 2013) through community (reviewed in Bahr and Lanier 1981) levels. Over geologic
392 horizons, the seaward-landward migration reefs is also thought to be driven by RSLR and
393 salinity regimes (Goff et al. 2015). Moreover, many of the reef-associated community members
394 shifting their distribution in the NRE are defined by well-described salinity tolerance envelopes
395 that match observed environmental shifts (detailed below). In this regard, the mechanistic link
396 we draw between shifts in salinity and reef communities based on long-term observations and
397 well-documented organism-environmental relationships is analogous with the direct connections
398 made between poleward range shifts and global warming (sensu Morley et al. 2018).
399 Furthermore, existing data do not support other known oyster stressors such as disease,
400 hypoxia/anoxia, or over-sedimentation as limiting in this system (note, harvest impacts are also
401 considered below, separately). Despite active wild-harvest and shellfish farming operations in
402 the NRE throughout the last century, there are no reports of pervasive disease outbreaks in this
403 system. The NRE is relatively shallow and well mixed, and bottom-water oxygen concentrations
404 remain above 6 ml L⁻¹ (>75% saturation) year-round in the mesohaline and euhaline portions of
405 the system (Kirby-Smith and Costlow 1989). Finally, high sediment-accumulation rates over the
406 last 50 years are confined to the head of the NRE (9.7 mm yr⁻¹) and decrease downstream
407 approaching RSLR in the vicinity of CR (<5.0 mm yr⁻¹; Mattheus et al. 2009), and therefore
408 would not explain the disproportionate loss of reefs farther down-estuary.

409 While we also readily acknowledge the limits and dangers of extrapolating findings from a
410 single study system too broadly (Hurlbert 1984), we also emphasize that the basic relationship

411 between salinity and the intertidal-versus-subtidal distribution of eastern oyster reefs is robust
412 (Baggett et al. 2015, Walles et al. 2016). Therefore, any system experiencing natural or human-
413 driven shifts in salinity might be informed by lessons drawn from multidecadal patterns in the
414 NRE. In particular, we anticipate that other small drowned-river estuaries and tidal creeks with
415 relatively small watersheds and little freshwater input, or estuaries in low-lying coastal plains
416 particularly vulnerable to RSLR, are mostly likely to see upstream migration of eastern oyster
417 reefs, as those systems are most prone to saltwater intrusion (excluding reverse estuaries).
418 Additionally, even some large estuarine embayments, such as San Francisco Bay, are subject to
419 saltwater intrusion given the system-specific levels of ocean connectivity and freshwater inflows
420 (Cloern and Jassby 2012). Conversely, some estuaries with large watersheds and high freshwater
421 inputs, such as Mobile Bay, may see increased frequency, duration, or magnitude of freshets
422 (sensu Park et al. 2007) that could potentially drive the area suitable for subtidal reefs seaward.
423 Notably, the extent of bottom habitat suitable for subtidal reef persistence in these deeper, river-
424 dominated systems may be more significantly regulated by the development and expansion of
425 low-oxygen conditions (Lenihan and Peterson 1998).

426 Multiple taxa contributed to the differences we observed across time and space, indicative of
427 major community level changes. For instance, we documented fewer desiccation-intolerant fauna
428 during 2013-2015 across the NRE than did Wells (1961) and observed several species with
429 distributions shifted up-estuary between sampling in the 1950s and 2010s. For instance, the
430 majority (17 of 25) of families that drove community level differences at PI between 1955-1956
431 (Wells 1961) and 2013-2015 are characteristically subtidal (Ruppert and Fox 1989), and were
432 less frequently observed in recent sampling relative to six decades ago. Most notably, these
433 included taxa such as boring sponges (Clionaidae), gastropods (3 families, including oyster

434 drills), mossy and encrusting bryozoans (Bugulidae, Membraniporidae, Schizoporellidae),
435 tunicates (Molgulidae), and sedentary polychaetes (Sabellariidae and Terebellidae). Similarly, 10
436 out of 23 families that drove community level shifts between WR (1950s) and WRR (2010s)
437 were characteristically subtidal, and decreased in abundance over time, including mossy and
438 encrusting bryozoans (Bugulidae, Electridae, and Alcyonidiidae), tunicates (Molgulidae and
439 Styelidae), hydrozoans (Campanulariidae), and sedentary polychaetes (Sabellidae, Sabellariidae,
440 and Serpulidae).

441 In practical terms, the loss of subtidal habitat at PI and WR/WRR is a proximate reason for
442 both the NRE-wide shifts in community structure between 1955-1956 and 2013-2015, as well as
443 the compositional squeezing of reef-associated communities across the NRE over time (i.e.,
444 decrease in across-NRE beta and entire-NRE alpha diversity). During the 1950s, most intertidal
445 oyster reefs in the euhaline NRE also supported subtidal reef skirts that extended to depths ~0.5
446 m below mean low water (Wells 1961). While these fringe subtidal reef habitats in the lower
447 NRE may have been sourced primarily from dislodged oyster clumps shed from the intertidal
448 sections of the reefs (combined with some *in situ* oyster recruitment/growth), these living
449 biogenic structures persisted consistently enough to support subtidal reef-associated fauna, such
450 as bryozoans and anemones. As salinity regimes in the lower-to-middle NRE have trended
451 higher over time, however, we speculate that marine-adapted predators and pests, such as stone
452 crabs (*Menippe mercenaria*), oyster drills, and *Clionid* boring sponges, exerted strong predation
453 (Chestnut and Fahy 1953) and bioerosion (Lunz 1943) pressures that extirpated subtidal reef
454 structure, and subsequently, associated subtidal fauna. Even though the WRR station sampled
455 during 2013-2015 was ~0.5 km farther up-estuary relative to the WR reef sampled in the 1950s,
456 we did not observe any significant subtidal fringe reef during our repeated, year-round visits to

457 that site. By comparison, Wells (1961) specified that he was able to locate and sample from both
458 intertidal and subtidal reef zones at WR.

459 Salinity-related shifts in faunal distributions also contributed to the decline in species turnover
460 across the NRE over time. In 1955-1956, 10 families had up-estuary limits at WR; but by 2013-
461 2015, five of those families had expanded their ranges up-estuary to CR. Two of those families,
462 Caprellids and Leptochellids, are tightly associated with mossy bryozoans, such as *Bugula*
463 *neritina*, which themselves are excluded by low salinity (Kitamura and Hirayama 1985).
464 Similarly, Eunicid polychaetes (Garcês and Pereira 2011) and Venerid clams (Davis and
465 Calabrese 1964) both moved up-estuary between the 1950s and 2010s, and generally exhibit low
466 fitness at salinities <15 ppt. Thus, the shift in mean salinities at CR from ~16 ppt in 1955-1956 to
467 ~21 ppt in 2013-2015 would almost certainly reflect a decrease in abiotic stress for those taxa.
468 Atlantic oyster drill distribution also shifted up-estuary over the last 60+ years. In 1955-1956,
469 drills were not documented farther up-estuary than GP, but during 2013-2015 this species was
470 routinely collected at WRR (~7 km up-estuary from GP). Due to the nature of our stratified-
471 haphazard sampling across reef zones, and since drills typically avoid aerial exposure (Johnson
472 and Smee 2014), we suspect drills were collected from the low perimeter of the WRR reef. Still,
473 their up-estuary expansion is doubly notable: drills are important low-intertidal and subtidal
474 predators and they are limited by salinities <18 ppt (Federighi 1931, Manzi 1970). While our
475 statistical analyses operated at the family level, it was also significant that Wells (1961) collected
476 the Balanid barnacle *Amphibalanus improvisus* at CR, while this species was absent during
477 2013-2015 throughout the NRE. *A. improvisus* populations are generally limited to areas with
478 salinities <15 ppt (Gordon 1969), which makes this species another potentially important “canary
479 in the coal mine” reflecting saltwater intrusion into the upper NRE.

480 As in many human-dominated coastal systems, the drivers of NRE environmental change (e.g.
481 salinity) are multifaceted – including channelization, sea-level rise, rainfall, and storm-water
482 drainage infrastructure. Mechanical dredging of navigational channels alters coastline
483 bathymetry, and in the case of the NRE contributed to an increase in tidal range and tidal prism,
484 as well as decreased estuarine water residence time, potentially leading to saltwater intrusion
485 (Johnston 1981). To serve the state port in Morehead City, NC (in the lower NRE), Beaufort
486 Inlet has been dredged to new depths at least five times since 1911 (Years and depths: 1911, 6.0
487 m; 1936, 9.1 m; 1961, 10.7 m; 1978, 12.2 m; and 1994, 13.7 m;), a cumulative inlet depth
488 increase >100% over time (Zervas 2003). Zervas (2004) explored long-term trends in water
489 levels across NC, and noted that three locations defined by inlet/channel dredging (Oregon Inlet,
490 Beaufort Inlet, Wilmington) saw large and statistically significant increases in tidal ranges, while
491 other locations had no such increases. For the Beaufort Inlet - NRE system, this increase in tidal
492 range approaches 10 cm since the early 1970s (Zervas 2003), increasing ocean-estuary exchange
493 during each tidal cycle. Additionally, to create the port and adjacent ship-turning basin, extensive
494 deltaic salt marshes that formerly dampened connectivity between the NRE and ocean have been
495 converted in relatively deep open water. Despite important differences in embayment
496 size/orientation and tidal regime between systems, dredge/fill activities in Tampa Bay were
497 projected to account for a 4-ppt increase in estuarine salinity (Zhu et al. 2015), akin to the long-
498 term shifts in the NRE (>5 ppt). Exacerbating these changes, RSLR may change whole-estuary
499 volume, with increased oceanic influence (i.e. potential saltwater intrusion if bottom bathymetry
500 does not keep pace with SLR). Immediately adjacent to PI reefs, the National Oceanic and
501 Atmospheric Administration tide station 8656483 revealed an 18-cm increase in mean sea level
502 across 1973-2015 (~3.5-mm-yr⁻¹ RSLR; NOAA 2021). Finally, Beaufort Inlet dynamics may

503 have also contributed to the salinity-driven faunal patterns we observed in 2013-2015 (but not
504 long-term salinity patterns *per se*), as Beaufort Inlet widened from 1.15 km in 2010 to 1.75 km in
505 2015 (Seymour et al. 2019), furthering the coastal ocean influence in the NRE.

506 Alterations to the physical and environmental structure of estuaries also has implications for
507 the magnitude and duration of freshwater pulses, called freshets, that are critical in limiting
508 predators and pests that attack subtidal oysters (while oysters exhibit greater environmental
509 tolerance for these low-salinity events; Bahr and Lanier 1981; *sensu* Bender et al. 1984). In this
510 respect, the long-term increases in mean and minimum salinities in the NRE run counter to
511 projected higher annual rainfall (Polsky et al. 2000) and the occurrence of wetter tropical
512 cyclones (Paerl et al. 2019) in this region. This disconnect may be explained by the more-rapid
513 flushing of major pulse freshets in the NRE over time due to storm-water infrastructure,
514 channelization, and RSLR. Following the passage of hurricanes Connie and Diane in August,
515 1955 (combined, >400-mm rainfall), salinities throughout the entire NRE were ≤ 10 ppt for at
516 least 14 consecutive days (Wells 1961). This period of reduced salinity was highlighted by Wells
517 (1961) to cause some oyster mortality throughout the NRE, but more notably, significant
518 reductions in the abundance of oyster drills – including within subtidal fringing reefs near
519 Beaufort Inlet (i.e., SS, PI, GP). By comparison, hurricane events during the 2013-2015 sampling
520 effort, Dorian (2013, 200 mm), Arthur (2014, 100 mm), Ana (2015, 100 mm) lowered NRE
521 salinities by <3 ppt, while those modest effects were completely absent within 2-3 tidal cycles of
522 storm passage (Tice-lewis 2018). We conclude that physical modifications of the NRE (e.g.,
523 channelization/ditching, higher sea levels), combined with the relatively small size of this
524 watershed, outweigh the long-term changes in precipitation patterns in regulating local salinity
525 regimes.

526 Following these shifts in estuarine environmental envelopes, the net movement of the subtidal-
527 intertidal transition line appears to be up estuary in the NRE. We estimate that the transition line
528 between subtidal (up estuary) and intertidal (down estuary) reefs has shifted from 12 km to 13.5
529 km away from Beaufort Inlet. This estimate is based on several lines of evidence, including: the
530 magnitude of community shifts at WR/WRR and CR; NRE-wide salinity patterns available from
531 the NCDMFSS database (i.e. former and present-day locations of ~ 15 ppt within the estuary);
532 current extent of subtidal and intertidal reefs in the NRE; and recent targeted experiments on the
533 settlement, growth, and survival of subtidal oysters across the NRE (Sorg 2017). In 2012, Sorg
534 (2017) deployed oyster settlement substrates (e.g., oyster shell, marl) at five sites across the
535 salinity gradient of the NRE running from near the PI site to up-estuary of the CR site. By 2016,
536 Sorg (2017) found that constructed reefs ~1 km up-estuary of the WR/WRR sites exhibited
537 substantial oyster mortality and reef degradation after five years due to extensive *Cliona celata*
538 impacts, while two sites near CR demonstrated only modest bioerosion impacts. Additionally,
539 constructed reef down-estuary from WR/WRR failed to develop due largely to intense drill
540 predation, bioerosion, and other mortalities of juvenile oysters (Sorg 2017).

541 Present-day subtidal and intertidal reef distributions in the NRE are likely also impacted by the
542 legacy of destructive shellfish harvest (zu Ermgassen et al. 2013). In this context, however, the
543 patterns reported in Sorg (2017) are critical in highlighting the first-order importance of
544 environmental drivers of oyster reef distributions, as those constructed reefs were exempted from
545 harvest pressure. Similarly, Powers et al. (2009) conducted surveys in the waters immediately
546 around the NRE, and found that oyster sanctuaries only met conservation success thresholds in
547 euhaline regions when reefs were sited intertidally. Even in the absence of destructive harvest,
548 subtidal eastern oyster reefs did not, and do not, flourish in NRE regions defined by relatively

549 high salinities. Thus, it seems unlikely that destructive harvest is the primary causal agent for the
550 multidecadal patterns of reef distributions we have documented.

551 Long-term physicochemical alterations to the NRE have led to a form of coastal “narrowing”
552 for subtidal reefs (Pontee 2013), as the envelope of suitable environmental conditions for these
553 reefs has migrated into the more constricted “neck” of the NRE. Given the strong preference for
554 oysters with a subtidal morphology for human harvest (thicker, more rounded shells, with less
555 cementing among oysters), this shift has important economic consequences. Moreover, water-
556 quality degradation and resultant shellfish harvest closures - encroaching on oysters from the up-
557 estuary end - have exacerbated the impacts of saltwater intrusion in the NRE regarding the
558 benefits these reefs confer for humans. As such, subtidal, harvestable oyster reefs are not just
559 experiencing narrowing driven by shifts in their downstream margin; but rather, a “coastal
560 squeeze” along both upstream (water quality) and downstream (salinity) margins. While water-
561 quality related closures do confer some *de facto* reserve protection for shellfish in the upper
562 regions of systems like the NRE, these closures also reduce seafood supply and economic
563 opportunities for fishermen. Moreover, there is often reduced incentive for shellfish habitat
564 enhancement in closed waters, given the absence of potential direct economic returns related to
565 future harvest (Fodrie et al. 2018). Furthermore, water-quality closures in the upper NRE are
566 correlated with high sedimentation rates (Mattheus et al. 2009) that can further degrade oyster
567 fitness under any conservation or restoration initiatives (Rothschild et al. 1994, Thomsen and
568 McGlathery 2006, Beck et al. 2011).

569 A century ago, the upper 4.5 km of the NRE was suitable for subtidal reef growth/persistence
570 and harvest (Grave 1905). Today, the combination of saltwater intrusion and water-quality
571 closures has truncated this zone to a ~0.75-km remnant (along the main axis of estuary, Fig. 1).

572 Extrapolating these shifts forward suggests that the transition line for subtidal-intertidal reefs
573 may someday occur up-estuary of the shellfish-closure line, completely eliminating the area of
574 the NRE suitable for subtidal reef persistence and harvest. While salinity records and reef
575 presence/absence data suggest the up-estuary movement of this transition line over decadal
576 scales, we consider it likely that the location of the break between subtidal and intertidal reefs
577 may ‘wobble’ in the NRE based on conditions during any few select years. For instance, the
578 North Atlantic Oscillation (NAO) is correlated with winter rainfall patterns that should increase
579 (positive NAO) or decrease (negative NAO) freshets in the NRE during 3-5-year horizons
580 (Ottersen et al. 2001). However, we do not think NAO phase had a major effect on the
581 community-level shifts we observed, as both the late 1950s and mid 2010s were defined by
582 moderately negative NAO conditions (National Centers for Environmental Information
583 [noaa.gov]). Even more sporadically, strong wet storms, such as Hurricane Florence in 2018,
584 could temporarily relocate the NRE transition line farther down-estuary if oyster pests are
585 extirpated following these major pulse events (*sensu* Wells 1961).

586 As the causes for NRE shifts are multifaceted in nature, so must be the actions to abate or
587 reverse “squeeze” on oysters. Limiting saltwater intrusion will likely require increased efforts to
588 halt global climate change and associated RSLR (Douglas 1991). We also encourage managers
589 to balance the economic drivers of channel/inlet dredging with the suite of environmental costs
590 or benefits that these estuarine modifications stimulate – in particular, changes in the amount and
591 quality of natural capital or infrastructure. We perceive that during the next decade, improving
592 up-estuary water quality may be the most effective means of combatting squeeze on subtidal,
593 harvestable oysters. This may be achieved through continued or expanded efforts to reduce non-
594 point-source pollutants, improve wastewater/stormwater management, and implement low-

595 impact development (Kennish 2002, Fodrie et al. 2018). Finally, an important caveat is that
596 subtidal reef enhancement projects in the middle and lower NRE may still support oyster
597 harvests for a few years before predators, bioeroders, and pests degrade reef productivity, given
598 lags between when oysters colonize available substrate and when their enemies subsequently
599 recruit to young reefs (Fodrie et al. 2014). In this context, the use of novel, mobile substrates to
600 support oyster settlement, survival, and growth could be explored. These mobile substrates could
601 be periodically transferred upstream of the subtidal-intertidal transition (or just into the intertidal)
602 to enhance subtidal reef structure, function, and persistence in the middle and lower NRE.

603 As subtidal reefs retreat up-estuary, intertidal reefs are becoming a relatively more dominant
604 component of this temperate estuary. Importantly, intertidal reefs are distinct from subtidal reefs
605 in terms of the suite and magnitudes of ecosystem services they provide. In addition to being less
606 marketable than their subtidal analogues, and owing primarily to their time out of water,
607 intertidal reefs can be expected to provide relatively lower water filtration/purification capacity
608 (Bahr and Lanier 1981), reduced provision of habitat for mobile nekton (Byers et al. 2015), and
609 decreased organic carbon burial (Fodrie et al. 2017). Yet, intertidal reefs may be efficient
610 nitrogen sinks (Piehler and Smyth 2011) and be significantly more important in shoreline
611 stabilization (Grabowski et al. 2012) relative to subtidal counterparts. While intertidal reefs
612 provide their own form of resilience to climate change via their ability to outpace RSLR
613 (Rodriguez et al. 2014), it is also notable that reduced diversity of reef types and reef-associated
614 fauna at the estuary scale may have complex, poorly understood impacts on ecological resilience
615 in estuaries like the NRE (Elmqvist et al. 2003, Stachowicz et al. 2007). Using oyster reefs as a
616 model, but applicable to all biogenic habitats that exist along strong environmental gradients,
617 these dynamics exemplify how human-driven shifts in key abiotic properties such a salinity,

618 temperature, or water level can have profound effects on coastal ecosystem structure and
619 functions.

620

621 **Acknowledgements**

622 We are grateful to field and laboratory hands, as well as J. Plumlee who created Figure 1.
623 Funding was provided by the North Carolina Marine Resources Fund (supported by the Division
624 of Marine Fisheries Coastal Recreational Fishing License program) and the National Science
625 Foundation (#1926395). This work was conducted under protocol 16-123.0 of the University of
626 North Carolina at Chapel Hill's Office of Animal Care and Use. M. Tice-Lewis, N. Lindquist, A.
627 Rodriguez, C. Fieseler, and J. Fodrie conceived the study. M. Tice-Lewis, S. Zhang, G. Redding,
628 and Q. Walker conducted field and laboratory work. M. Tice-Lewis and J. Fodrie analyzed the
629 data. M. Tice-Lewis and J. Fodrie drafted the manuscript and all coauthors provided editorial
630 input. Comments from two anonymous reviewers helped strengthen and clarify the manuscript.

631

632 **Literature Cited**

633 Able, K. W. 2016. Natural history: an approach whose time has come, passed, and needs to be
634 resurrected. *ICES Journal of Marine Science* 73:2150–2155.

635 Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure
636 of beta diversity. *Ecology Letters* 9:683–693.

637 Asch, R. G., and B. Erisman. 2018. Spawning aggregations act as a bottleneck influencing
638 climate change impacts on a critically endangered reef fish. *Diversity and Distributions*
639 24:1712–1728.

640 Baggett, L. P., S. P. Powers, R. D. Brumbaugh, L. D. Coen, B. M. Deangelis, J. K. Greene, B. T.

641 Hancock, S. M. Morlock, B. L. Allen, D. L. Breitburg, D. Bushek, J. H. Grabowski, R. E.
642 Grizzle, E. D. Grosholz, M. K. La Peyre, M. W. Luckenbach, K. A. McGraw, M. F. Piehler, S.
643 R. Westby, and P. S. E. zu Ermgassen. 2015. Guidelines for evaluating performance of oyster
644 habitat restoration. *Restoration Ecology* 23:737–745.

645 Bahr, L. M., and W. P. Lanier. 1981. The ecology of intertidal oyster reefs of the South Atlantic
646 coast: a community profile. United States Fish and Wildlife Service, Washington D.C.

647 Barry, J. P., C. H. Baxter, R. D. Sagarin, and S. E. Gilman. 1995. Climate-related, long-term
648 faunal changes in a California rocky intertidal community. *Science* 220:671–680.

649 Beck, M. W., R. D. Brumbaugh, L. Airoidi, A. Carranza, L. D. Coen, C. Crawford, O. Defeo, G.
650 J. Edgar, B. Hancock, M. C. Kay, H. S. Lenihan, M. W. Luckenbach, C. L. Toropova, G.
651 Zhang, and X. Guo. 2011. Oyster reefs at risk and recommendations for conservation,
652 restoration, and management. *BioScience* 61:107–116.

653 Bender E. A., Ted J. C., and Michael E. G. 1984. Perturbation experiments in community
654 ecology: theory and practice. *Ecology* 65:1–13.

655 Byers, J. E., J. H. Grabowski, M. F. Piehler, A. R. Hughes, H. W. Weiskel, J. C. Malek, and D.
656 L. Kimbro. 2015. Geographic variation in intertidal oyster reef properties and the influence of
657 tidal prism. *Limnology and Oceanography* 60:1051–1063.

658 Chase, J. M., and J. A. Myers. 2011. Disentangling the importance of ecological niches from
659 stochastic processes across scales. *Philosophical Transaction of the Royal Society B: Biological*
660 *Sciences* 366:2351–2363.

661 Chestnut, A. F., and W. E. Fahy. 1953. Studies on the vertical distribution of setting of oysters in
662 North Carolina. *Proceedings of the Gulf and Caribbean Fisheries Institute* 5:106–112.

663 Cloern J. E., and A. D. Jassby. 2012. Drivers of change in estuarine–coastal ecosystems:

664 discoveries from four decades of study in San Francisco Bay. *Reviews in Geophysics*
665 50:RG4001.

666 Davis, H. C., and A. Calabrese. 1964. Combined effects of temperature and salinity on
667 development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *Fishery Bulletin*
668 63:643–655.

669 Dayton, P. K. 1985. Ecology of kelp communities. *Annual Review of Ecology and Systematics*.
670 16:215–245.

671 Doody, J. P. 2004. ‘Coastal squeeze’ – an historical perspective. *Journal of Coastal Conservation*
672 10:129–138.

673 Douglas, B.C. 1991. Global sea-level rise. *Journal of Geophysical Research* 96:6981–6985.

674 Elmqvist, T., C. Folke, M. Nyström, G. Peterson, J. Bengtsson, B. Walker, and J. Norberg. 2003.
675 Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the*
676 *Environment* 1:488–494.

677 Ensign, S. H., M. W. Doyle, and M. F. Piehler. 2013. The effect of tide on the hydrology and
678 morphology of a freshwater river. *Earth Surface Processes and Landforms* 38:655–660.

679 Fabricius, K. E., G. De’ath, M. L. Puotinen, T. Done, T. F. Cooper, and S. C. Burgess. 2008.
680 Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone.
681 *Limnology and Oceanography* 53:690–704.

682 Federighi, H. 1931. Salinity death-points of the oyster drill snail, *Urosalpinx Cinerea* Say.
683 *Ecology* 12:346–353.

684 Fodrie, F. J., C. H. Peterson, C. Voss, and C. Baillie. 2018. North Carolina strategic plan for
685 shellfish mariculture: a vision to 2030. Raleigh, North Carolina, USA. 177 pgs.

686 Fodrie, F. J., A. B. Rodriguez, C. J. Baillie, M. C. Brodeur, S. E. Coleman, R. K. Gittman, D. A.

687 Keller, M. D. Kenworthy, A. K. Poray, J. T. Ridge, E. J. Theuerkauf, and N. L. Lindquist. 2014.
688 Classic paradigms in a novel environment : inserting food web and productivity lessons from
689 rocky shores and saltmarshes into biogenic reef restoration. *Journal of Applied Ecology*:1314–
690 1325.

691 Fodrie, F. J., A. B. Rodriguez, R. K. Gittman, J. H. Grabowski, N. L. Lindquist, C. H. Peterson,
692 M. F. Piehler, and J. T. Ridge. 2017. Oyster reefs as carbon sources and sinks. *Proceedings of*
693 *the Royal Society B* 284:20170891.

694 Garcês, J. P., and J. Pereira. 2011. Effect of salinity on survival and growth of *Marphysa*
695 *sanguinea* Montagu (1813) juveniles. *Aquaculture International* 19:523–530.

696 Goff, J. A., L. Lugin, , S.P. Gulick, K. Thirumalai, and Y. Okumura. 2015. Oyster reef die-offs
697 in stratigraphic record of Corpus Christi Bay, Texas, possibly caused by drought-driven extreme
698 salinity changes. *The Holocene* 26:511-519.

699 Gordon, C. M. 1969. The apparent influence of salinity on the distribution of barnacle species in
700 Chesapeake Bay (Cirripedia). *Crustaceana* 16:139–142.

701 Grabowski, J. H., R. D. Brumbaugh, R. F. Conrad, A. G. Keeler, J. Opaluch, C. H. Peterson, M.
702 F. Piehler, S. P. Powers, and A. R. Smyth. 2012. Economic valuation of ecosystem services
703 provided by oyster reefs. *Bioscience* 62:900–909.

704 Grave, C. 1901. The oyster reefs of North Carolina: a geological and economic study. *The Johns*
705 *Hopkins Universtiy Circulars* 151:1-9.

706 Grave, C. 1905. Investigations for the promotion of the oyster industry of North Carolina. *Report*
707 *of Commissioner of Fish and Fisheries* 1903:247-341.

708 Gunter, G. 1956. Some relations of faunal distributions to salinity in estuarine waters. *Ecology*
709 37:616-619.

710 Hoyt, W. D. 1920. Marine algae of Beaufort, N. C., and adjacent regions. Bulletin of the Bureau
711 of Fisheries 36:367-556.

712 Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments.
713 Ecological Monographs 54:187–211.

714 Johnson, K. D., and D. L. Smee. 2014. Predators influence the tidal distribution of oysters
715 (*Crassostrea virginica*). Marine Biology 161:1557–1564.

716 Kennish, M.J. 2002. Environmental threats and environmental future of estuaries. Environmental
717 Conservation 29:78-107.

718 Kirby-Smith, W. W., and J. D. Costlow. 1989. The Newport River Estuarine System. UNC Sea
719 Grant College Publication. Raleigh, North Carolina, USA. 58 pgs.

720 Kitamura, H., and K. Hirayama. 1985. The effect of low salinity on the early growth of a
721 bryozoan *Bugula neritina* in the sea and laboratory. Bulletin of the Japanese Society of
722 Scientific Fisheries 51:1401–1405.

723 La Peyre, M. K., B. S. Eberline, T. M. Soniat, J. F. La Peyre. 2013. Differences in extreme low
724 salinity timing and duration differentially affect eastern oyster (*Crassostrea virginica*) size class
725 growth and mortality in Breton Sound, LA. Estuarine, Coastal and Shelf Science 135:146-157.

726 Lenihan, H. S., and C. H. Peterson. 1998. How habitat degradation through fishery disturbance
727 enhances impacts of hypoxia on oyster reefs. Ecological Applications 8:128–140.

728 Lunz, G. R. 1943. The yield of certain oyster lands in South Carolina. American Midland
729 Naturalist 30:806–808.

730 Manzi, J. J. 1970. Combined effects of salinity and temperature on the feeding, reproductive, and
731 survival rates of *Eupleura caudata* (Say) and *Urosalpinx cinerea* (Say)(Prosobranchia:
732 Muricidae). The Biological Bulletin 138:35–46.

733 Mattheus, C. R., A. B. Rodriguez, and B. A. McKee. 2009. Direct connectivity between
734 upstream and downstream promotes rapid response of lower coastal-plain rivers to land-use
735 change. *Geophysical Research Letters* 36:L20401.

736 McDougall, K. D. 1943. Sessile marine invertebrates of Beaufort, North Carolina: a study of
737 settlement, growth, and seasonal fluctuations among pile-dwelling organisms. *Ecological*
738 *Monographs* 13:321–374.

739 Morley, J. W., R. L. Selden, R. J. Latour, T. L. Frölicher, R. J. Seagraves, and M. L. Pinsky.
740 2018. Projecting shifts in thermal habitat for 686 species on the North American continental
741 shelf. *PLoS ONE* 13:e0196127.

742 Morris, J. T., P. V. Sundareshwar, C. T. Nietch, B. Kjerfve, and D. R. Cahoon. 2002. Responses
743 of coastal wetlands to rising sea level. *Ecology* 83:2869–2877.

744 NOAA. 2021. Sea Levels. 11/10/2021: <https://tidesandcurrents.noaa.gov/sltrends/sltrends.html>

745 National Shellfish Sanitation Program. 2017. Guide for the Control of Molluscan Shellfish 2017
746 Revision.

747 NCDEQ (North Carolina Department of Environmental Quality) 2021. North Carolina Coastal
748 Habitat Protection Plan 2021. Department of Environmental Quality, Raleigh, NC. 266 p

749 Ochieng, C. A., F. T. Short, and D. I. Walker. 2010. Photosynthetic and morphological responses
750 of eelgrass (*Zostera marina* L.) to a gradient of light conditions. *Journal of Experimental*
751 *Marine Biology and Ecology* 382:117–124.

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

754 Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P. R. Minchin, R.
755 B. O'hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, and H. W. Maintainer.

2019. Package “vegan”. A Community Ecology Package. Community ecology package.

Ottersen, G., B. Planque, A. Belgrano, E. Post, P. C. Reid, and N. C. Stenseth. 2001. Ecological effects of the North Atlantic Oscillation. *Oecologia* 128:1-14.

Park, K., J. F. Valentine, S. Sklenar, K. R. Weis, and M. R. Dardeau. 2007. The effects of Hurricane Ivan in the inner part of Mobile Bay, Alabama. *Journal of Coastal Research* 23:1332-1336.

Paerl, H. W., N. S. Hall, A. G. Hounshell, R. A. Luettich, K. L. Rossignol, C. L. Osburn, and J. Bales. 2019. Recent increase in catastrophic tropical cyclone flooding in coastal North Carolina, USA: Long-term observations suggest a regime shift. *Scientific Reports* 9:1–9.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42.

Piehler, M. F., and A. R. Smyth. 2011. Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. *Ecosphere* 2:1–17.

Polsky, C., J. Allard, N. Currit, R. Crane, and B. Yarnal. 2000. The Mid-Atlantic region and its climate: past, present, and future. *Climate Research* 14:161–173.

Pontee, N. 2013. Defining coastal squeeze: a discussion. *Ocean and Coastal Management* 84:1–4.

Powers, S. P., C. H. Peterson, J. H. Grabowski, and H. S. Lenihan. 2009. Success of constructed oyster reefs in no-harvest sanctuaries: implications for restoration. *Marine Ecology Progress Series* 389:159–170.

R Core Team (2020). 2020. R: A language and environment for statistical computing.

Ralston, D. K., and W. R. Geyer. 2019. Response to channel deepening of the salinity intrusion, estuarine circulation, and stratification in an urbanized estuary. *Journal of Geophysical*

779 Research: *Oceans* 124:4784–4802.

780 Rodriguez, A. B., F. J. Fodrie, J. T. Ridge, N. L. Lindquist, E. J. Theuerkauf, S. E. Coleman, J.
781 H. Grabowski, M. C. Brodeur, R. K. Gittman, D. A. Keller, and M. D. Kenworthy. 2014. Oyster
782 reefs can outpace sea-level rise 4:493–497.

783 Rothschild, B. J., J. S. Ault, P. Gouletquer, and M. Heral. 1994. Decline of the Chesapeake Bay
784 oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress*
785 *Series* 111:29–39.

786 Ruppert, E. E., and R. S. Fox. 1989. *Seashore animals of the Southeast: a guide to common*
787 *shallow-water invertebrates of the southeastern Atlantic Coast*. University of South Carolina
788 Press, Columbia, South Carolina, USA.

789 Sagarin, R. D., J. P. Barry, S. E. Gilman, and C. H. Baxter. 1999. Climate-related change in an
790 intertidal community over short and long time scales. *Ecological Monographs* 69:465–490.

791 Seymour, A. C., J. T. Ridge, E. Newton, A. B. Rodriguez, and D. W. Johnston. 2019.
792 Geomorphic response of inlet barrier islands to storms. *Geomorphology* 339:127–140.

793 Snedaker, S. C. 1995. Mangroves and climate change in the Florida and Caribbean region:
794 scenarios and hypotheses. *Hydrobiologia* 295:43–49.

795 Sorg, G. 2017. Identifying sustainable substrates for subtidal oyster reef construction. Masters
796 Thesis. University of North Carolina at Chapel Hill. 56 pgs.

797 Stachowicz, J. J., J. F. Bruno, and J. E. Duffy. 2007. Understanding the effects of marine
798 biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and*
799 *Systematics* 38:739–766.

800 Thomsen, M. S., and K. McGlathery. 2006. Effects of accumulations of sediments and drift algae
801 on recruitment of sessile organisms associated with oyster reefs. *Journal of Experimental*

802 Marine Biology and Ecology 328:22–34.

803 Tice-lewis, M. L. 2018. Natural and Anthropogenic Drivers of Oyster Reef Community
804 Dynamics. Masters Thesis. University of North Carolina at Chapel Hill. 103 pgs.

805 Walles, B., F. J. Fodrie, S. Nieuwhof, O. J. D. Jewell, P. M. J. Herman, and T. Ysebaert. 2016.
806 Guidelines for evaluating performance of oyster habitat restoration should include tidal
807 emersion: Reply to Baggett et al. Restoration Ecology 24:4–7.

808 Wells, H. W. 1961. The fauna of oyster beds, with special reference to the salinity factor.
809 Ecological Monographs 31:239–266.

810 Winslow, F. 1889. Report on the Sounds and Estuaries of North Carolina: With Reference to
811 Oyster Culture. United States Coast and Geodetic Survey. Washington D.C.

812 Zervas, C. E. 2003. Long term changes in tidal response associated with the deepening of
813 navigational channels. Proceedings of the 13th Biennial Coastal Zone Conference Baltimore,
814 Maryland, USA.

815 Zervas, C. E. 2004. North Carolina bathymetry/topography sea level rise project: determination
816 of sea level trends. NOAA Technical Report NOS CO-OPS 041. Silver Spring, Maryland. 39
817 pgs.

818 Zhu, J., R. H. Weisberg, L. Zheng, and S. Han. 2015. Influences of channel deepening and
819 widening on the tidal and nontidal circulations of Tampa Bay. Estuaries and Coasts 38:132–
820 150.

821 zu Ermgassen, P. S. E., M. D. Spalding, R. E. Grizzle, and R. D. Brumbaugh. 2013. Quantifying
822 the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. Estuaries
823 and Coasts 36:36–43.

824

825 Table 1. Summary of Newport River Estuary oyster reef site characteristics. Insights from Grave (1901) are provided to give historical
 826 context to oyster reef sites relevant to the multidecadal analysis.

Reef	Grave 1901	Wells 1955-1956	This study 2013-2015	Distance to Inlet (km)	Reef Type	Aerial exposure regime	Wells (1961) Months sampled	Current study Months sampled
Shark Shoal	Not noted	Extant	Nonextant	1.8	Groin	Intertidal, subtidal fringe	1955: 7, 8, 9, 10, 11 1956: 1, 2, 3, 4, 5, 6, 7, 9	Not sampled
Pivers Island	Not noted	Extant	Extant	3.5	Fringing	Intertidal, subtidal fringe	1955: 7, 8, 9, 10, 11 1956: 1, 2, 3, 4, 5, 6, 7, 9, 10	2013: 7, 8, 10, 12 2014: 2, 4, 6, 8, 10 2015: 1, 3, 5, 7, 10, 10, 12
Gallants Point	Not noted	Extant	Nonextant	6.6	Bar	Intertidal, subtidal fringe	1955: 7, 8, 9 1956: 7, 8, 9	Not sampled
Green	Extant	Not noted	Nonextant	9.9	Patch	Not noted	Not sampled	Not sampled
White Rock	Extant	Extant	Nonextant	12.5	Patch	Intertidal, subtidal	1955: 7, 8, 8, 9 1956: 8, 9	Not sampled
White Rock Replacement	Extant	Not noted	Extant	13.1	Patch	Intertidal	Not sampled	2013: 7, 8, 10, 12 2014: 2, 4, 6, 8, 10 2015: 1, 3, 5, 7, 12
Turtle Rock	Extant	Extant	Nonextant	13.4	Not noted	Not noted	Not sampled	Not sampled
Lime Kiln	Extant	Not noted	Extant	14.5	Flats connected to bar	Subtidal, few intertidal crests	Not sampled	Not sampled
Cross Rock	Extant	Extant	Extant	14.7	Flats connected to bar	Subtidal, few intertidal crests	1955: 5, 7, 8, 8, 9, 10, 11 1956: 1, 2, 4, 5, 6, 7, 9, 10	2013: 7, 8, 10, 12 2014: 2, 4, 6, 8, 10 2015: 1, 3, 5, 7, 10, 10, 12

828 Table 2. Oyster-reef associated fauna and their frequencies of occurrence (0-100% scale) in the Newport River Estuary (NRE) during
829 1955-1956 (Wells 1961) and 2013-2015 surveys. Oyster reefs included Pivers Island (PI), White Rock (WR) or White Rock
830 Replacement (WRR), and Cross Rock (CR). Taxa reported by Wells (1961) include those in >20% of all NRE samples (five sites),
831 although 1955-1956 frequency of occurrence may be <20% across the three sites included here.

Taxonomic group	1955-1956			2013-2015			Taxonomic group	1955-1956			2013-2015		
	PI	WR	CR	PI	WRR	CR		PI	WR	CR	PI	WRR	CR
ARTHROPODA							BRYOZOA						
Amphipoda							Cheilostomatida						
Caprellidae	86	33	0	0	0	13	Bugulidae	64	67	20	6	7	0
Corophiidae	57	67	87	0	14	94	Electridae	29	100	93	0	0	0
Gammaridae	64	33	20	6	7	6	Membraniporidae	86	17	0	0	0	0
Melitidae	100	100	93	38	79	88	Schizoporellidae	86	17	0	0	0	0
Tanaidacea							Ctenostomatida	0	0	0	0	0	0
Leptocheliidae	36	33	0	0	0	13	Alcyonidiidae	21	67	7	0	0	0
Isopoda							Nolellidae	0	33	0	0	0	0
Sphaeromatidae	21	33	93	13	100	94	Victorellidae	29	33	47	0	0	0
Decapoda							CILIOPHORA						
Diogenidae	36	17	0	6	0	0	Heterotrichea						
Panopeidae	100	67	80	100	100	100	Folliculinidae	93	67	53	0	0	0
Diptera							CLITELLATA						
Tabanidae	0	17	20	6	0	0	Oligochaeta						
Maxillopoda							Tubificidae	0	0	0	6	21	44
Balanidae	93	100	87	81	79	88	CNIDARIA						
Chtamalidae	36	0	0	0	0	0	Anthozoa						
Sacculinidae	0	0	0	44	79	56	Actiniaria spp.	100	33	13	38	50	38
Pycnogonida							Gorgoniidae	57	17	0	0	0	0
Nymphonidae	29	0	0	0	0	0	Hydrozoa						
							Campanulariidae	50	67	47	0	0	0

832

Taxonomic group	1955-1956			2013-2015			Taxonomic group	1955-1956			2013-2015		
	PI	WR	CR	PI	WRR	CR		PI	WR	CR	PI	WRR	CR
<i>Table 2. Continued</i>							POLYCHAETA						
MOLLUSCA							Errantia						
Bivalvia							Eunicidae						
Anomiidae	36	33	7	6	0	0	93	17	0	94	79	6	
Arcidae	21	0	0	0	0	0	Nereididae						
Mytilidae	100	50	100	100	100	100	100	100	100	100	100	100	
Pholadidae	43	0	0	0	0	0	Phyllodocidae						
Tellinidae	0	0	0	0	7	19	100	33	13	44	79	19	
Veneridae	57	33	0	38	57	75	Syllidae						
Gastropoda							64	33	7	6	0	0	
Buccinidae	7	0	0	0	0	0	Sedentaria						
Calyptraeidae	36	50	13	0	43	25	Capitellidae						
Cerithiidae	57	0	0	0	0	0	71	33	20	6	0	19	
Columbellidae	93	50	7	0	7	0	Sabellariidae						
Fascioliariidae	50	0	0	0	0	0	93	50	13	0	0	0	
Fissurellidae	71	0	0	6	0	0	Sabellidae						
Muricidae	86	0	0	6	21	0	86	50	13	13	0	6	
Pyramidellidae	100	33	33	75	79	38	Serpulidae						
NEMERTEA							100	50	27	25	36	13	
Nemertea spp.	86	50	13	0	21	25	Spionidae						
PLATYHELMINTHES							79	50	80	25	64	69	
Polycladida							Terebellidae						
Stylochidae	43	17	20	0	0	0	79	17	0	13	21	6	
PORIFERA							PORIFERA						
Demospongia							Demospongia						
Chalinidae							Chalinidae						
Clionidae							Clionidae						
Halichondriidae							Halichondriidae						
TUNICATA							TUNICATA						
Ascidiacea							Ascidiacea						
Molgulidae							Molgulidae						
Styelidae							Styelidae						
VERTEBRATA							VERTEBRATA						
Actinopterygii							Actinopterygii						
Gobiidae							Gobiidae						

835 Figure 1. (A) Eastern oyster (*Crassostrea virginica*) reefs in the Newport River Estuary (NRE)
836 sampled for reef-associated fauna during 1955-1956 (Wells 1961) and/or 2013-2015 to assess
837 multidecadal stability or change in this temperate coastal system. Reef sites included Shark
838 Shoal (SS), Pivers Island (PI), Gallants Point (GP), White Rock (WR) or White Rock
839 Replacement (WRR), and Cross Rock (CR). Distinct symbols represent reefs sampled during
840 the 1950s only, 2010s only, or 1950s and 2010s. (B-G) Area within the NRE, 1904-present,
841 where subtidal oysters can persist and be harvested for human consumption (black shaded
842 regions). This area, which has experienced “coastal squeeze” over time, is defined by the
843 subtidal-intertidal oyster transition zone (downstream margin) and shellfish harvest closure
844 boundaries (upstream margin), and is shown at the time of: (B) Grave’s (1905) and (C) Well’s
845 (1961) reef surveys; following (D) water-quality related permanent shellfish harvest closures in
846 1972; with downstream extensions of permanent harvest closures in (E) 1998 and (F) 2005. In
847 (G) 2016, this area appeared to have truncated further in accordance with reef surveys during
848 2013-2015, as well as an additional downstream extension of permanent harvest closures.

849 Figure 2. Community composition patterns of reef-associated fauna across the NRE during 1955-
850 1956 and 2013-2015 based on nonmetric multidimensional scaling (nMDS, stress =0.15). Each
851 datum represents a single reef sample, with symbol shapes and colors (open/closed) delineating
852 reef identity and sampling period. Environmental factors that correlated with faunal
853 community patterns are included as vectors (moving in the direction of increasing “year” and
854 “salinity” across nMDS space). Ellipses represent 95% confidence intervals for each of the six
855 site*period groups. Oyster reefs included Pivers Island (PI), White Rock (WR) or White Rock
856 Replacement (WRR), and Cross Rock (CR).

857 Figure 3. Jaccard distance as a measure of between-reef species turnover (beta-diversity) in
858 1955-1956 (Wells 1961) and 2013-2015. Data are presented as means +/- one standard
859 deviation. Oyster reefs included Pivers Island (PI), White Rock (WR) or White Rock
860 Replacement (WRR), and Cross Rock (CR). Statistically significant ($\alpha=0.05$) differences in
861 species turnover across PI-WR/WRR, PI-CR, and WR/WRR-CR in the 1950s versus 2010s,
862 based on Mann-Whitney U analyses, are indicated by asterisks. Sample sizes used for between-
863 reef comparisons are also provided.

864 Figure 4. Multidecadal trends in annual mean, maximum, and minimum salinity across the
865 Newport River Estuary, in the vicinity of: Pivers Island (PI); White Rock and White Rock
866 Replacement (WR/WRR); and Cross Rock (CR) oyster reefs. For PI, sources included Hoyt
867 (1920), Wells (1961), the North Carolina Division of Marine Fisheries Shellfish Sanitation
868 (NCDMFSS) program, and the present study. For WR/WRR and CR, salinity data were
869 sourced from Wells (1961), NCDMFSS, and the present study. In each panel, data are depicted
870 as a scatterplot for annual means, with bars extending above and below mean values to
871 represent annual maximum and minimum salinity records, respectively. For each reef, solid
872 and dashed lines show the least-squares regressions for annual maximum and minimum values,
873 respectively.

874

875

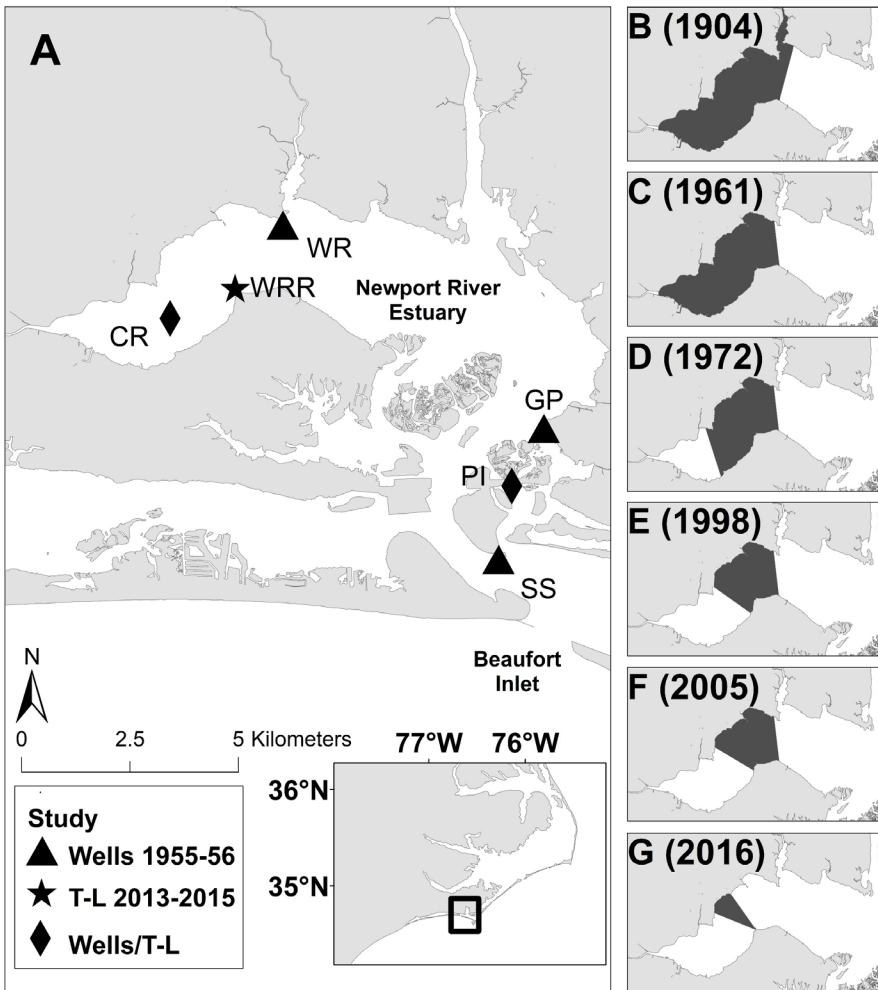
876

877

878

879

880 Figure 1.



881

882

883

884

885

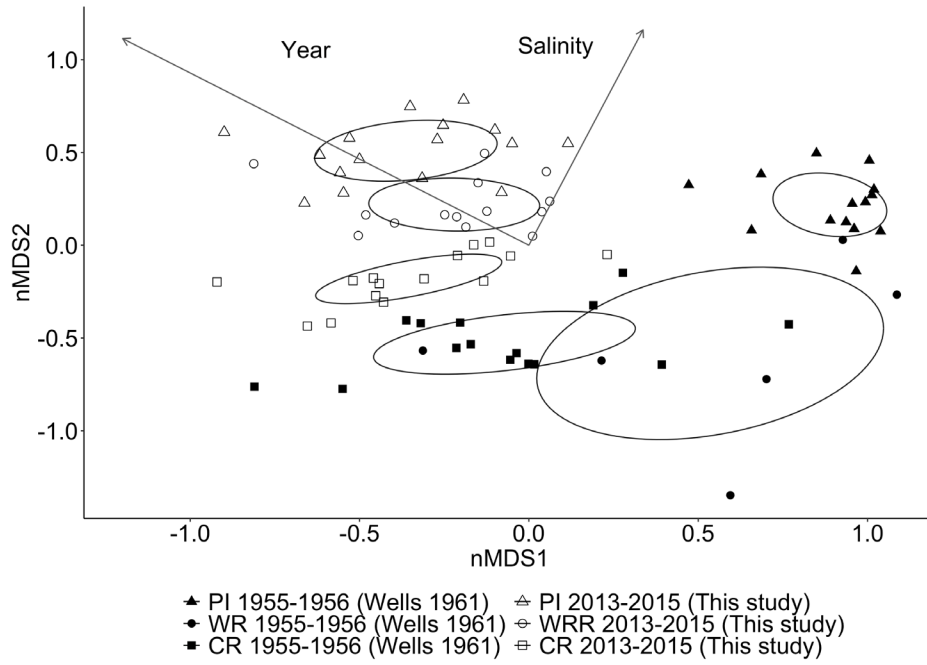
886

887

888

889

890 Figure 2.



891

892

893

894

895

896

897

898

899

900

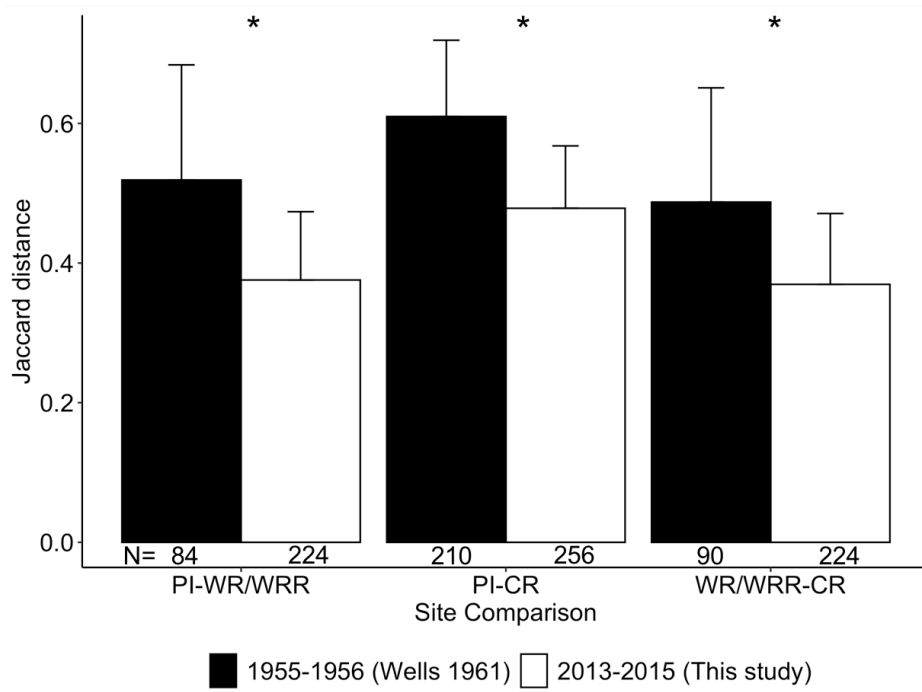
901

902

903

904

905 Figure 3.



906

907

908

909

910

911

912

913

914

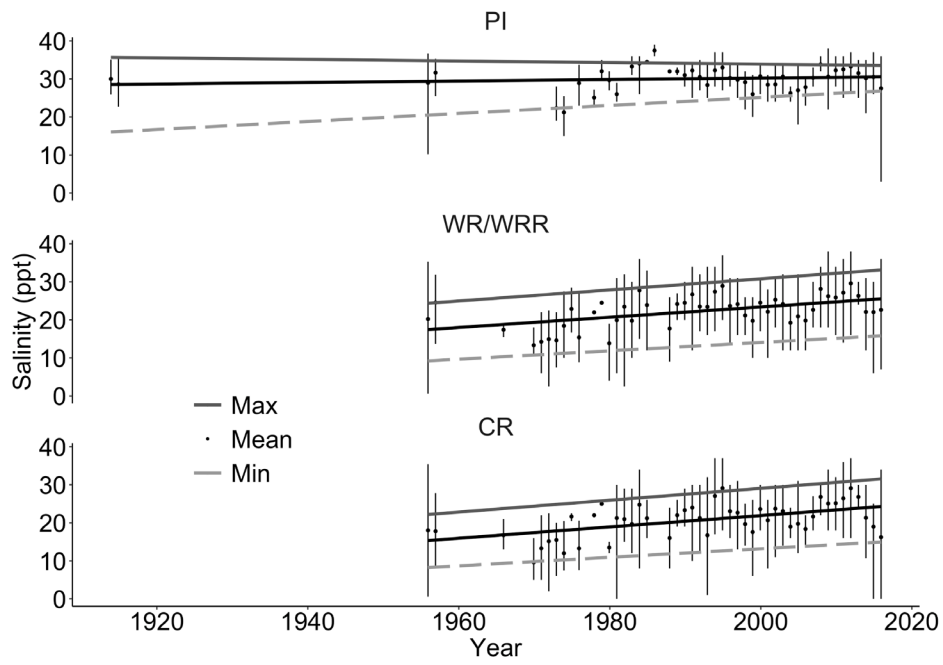
915

916

917

918

919 Figure 4.



920