

1 **Reproductive phenology of the eastern oyster, *Crassostrea virginica* (Gmelin, 1791), along a**
2 **temperate estuarine salinity gradient**

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4 Kaili M. Gregory ^{1,2}

5 Dr. Katherine McFarland^{1,3}

6 Dr. Matthew P. Hare¹

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8 ¹*Department of Natural Resources and the Environment, Cornell University, Ithaca, NY;*

9 ²*Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL;*

10 ³*NOAA Fisheries NEFSC, Milford Laboratory, Milford, CT*

11

12 **ABSTRACT**

13 Low salinity can negatively affect reproduction in estuarine bivalves. The spatial and
14 temporal extent of these effects are important to inform models of population dynamics,
15 environmental risk assessments, restoration efforts, and predictions of climate change effects.
16 We hypothesized that oysters at low salinity sites would have delayed gametogenesis compared
17 to their higher salinity counterparts in downstream experimental cages. The timing of
18 gametogenesis and spawning was observed June – August for 2-year-old oysters from three
19 distinct ancestries (Native, Hatchery, Aquaculture), outplanted at age 1 month along the salinity
20 gradient (3–30 psu) of a temperate estuary. A second season of data was collected from 3-year-
21 old Aquaculture oysters (comparable to year 1 data) and Native adult oysters transplanted one
22 year prior. Dermo was very low both years. A delay in gametogenesis and spawning was
23 observed for all ancestries at low salinity relative to higher salinity sites during July and August
24 of the first year but not the second year. In contrast, June showed the reverse pattern with
25 northern low salinity sites having more advanced gonad index (2.65) than a high salinity site
26 (1.46). This difference in average gonad index was 2.65 vs 1.46, respectively, for the Native line
27 and 2.62 vs 2.08 for Aquaculture. Low salinity seemed to not only induce earlier gametogenesis
28 in June, but also extended the reproductive season relative to higher salinity sites. Among oyster
29 ancestries, the Aquaculture line stood out as having 30 – 48% lower gametogenic synchrony
30 within sites, but only in 2018. Despite some dependence of reproductive phenology on salinity
31 variation, the Native low salinity population demonstrates notable reproductive plasticity in the
32 completion of a reproductive cycle across a wide range of salinities, an encouraging result for
33 potential future restoration strategies.

34 Keywords: gametogenesis, salinity, ancestry, histology, reproduction, *Perkinsus marinus*

35 INTRODUCTION

36 Estuaries are ecologically vital ecotones characterized by a salinity gradient (Telesh and
37 Khlebovich 2010). Average salinity levels fluctuate with tide, weather, river discharge, and
38 season, with a general rise in salinity as proximity to the ocean increases (Warner et al. 2005).
39 Many species are adapted to the variable salinity conditions of estuaries, whereas others are
40 interlopers that take advantage of estuarine habitats during one or more life stages (Able 2005;
41 Lellis-Dibble 2008). Among estuarine adapted species, physical niche is often described using a
42 habitat suitability index constructed from viability or growth rate across different combinations
43 of estuarine environments (Barnes et al. 2007; Swannack et al. 2014; Linhoss et al. 2016). These
44 indices help guide habitat protection priorities and restoration planning by modelling how
45 species would fare in specified areas. There are two aspects of population variation that are
46 rarely accounted for with these indices: (1) phenotypic plasticity and (2) genetic differences
47 among local or supplemented populations. Phenotypic plasticity can dramatically broaden the
48 realized niche of a population, so experiments that inform performance indices should ideally
49 include a full range of acclimation effects to accurately predict performance at the margin.
50 Genetic differences among local populations, originating from within-generation selection rather
51 than multigenerational adaptation (Marshall et al. 2010; Sanford and Kelly 2011), have potential
52 to affect population resilience through variation in life history traits, including the degree of
53 phenotypic plasticity (Eierman and Hare 2015).

54 With metapopulation structure as our within-estuary model, and given the added
55 productivity and resilience expected from portfolio effects when component populations have
56 somewhat independent life history or population dynamics (Lipcius et al. 2008; Schindler et al.
57 2010), it is important to document performance variation among the diverse habitats within an

58 estuary. Here, we focus on a trait that is poorly known for many estuarine organisms -
59 gametogenic and spawning phenology relative to the salinity gradient. Spawning phenology
60 differences across an estuarine metapopulation could have a large impact on recruitment and
61 population dynamics because the timing of larval production, relative to hydrodynamic,
62 microalgal, and community ecology dynamics, can be expected to constrain or promote larval
63 survivorship (Starr et al. 1990; Morgan 1995).

64 Our study organism is the eastern oyster (*Crassostrea virginica*, Gmelin, 1791) because
65 of its keystone role, contributing valuable ecosystem services to estuarine communities such as
66 water filtration, benthic pelagic coupling, and a structurally complex benthic reef habitat used by
67 many commercial fish species (Coen et al. 2007; Beck et al. 2011; Bricker et al. 2020; Rose et al.
68 2021). The synergistic mix of coastal degradation, overharvesting, disease, eutrophication, and
69 climate change threaten to degrade oyster populations to functional extinction in some regions, if
70 it has not done so already (Beck et al. 2011). While salinity is considered to be a weak or
71 negligible environmental factor for triggering spawning in eastern oysters relative to temperature
72 and chemical cues (Thompson et al. 1996), its effect on pre-spawning gametogenic development
73 and overall reproductive timing is much less studied. More fully understanding the effect of
74 salinity variation on reproductive phenology has the potential to inform eastern oyster population
75 dynamic models, environmental risk assessments, restoration efforts, and improve predictions of
76 the effects of climate change (Barnes et al. 2007; Marshall et al. 2010; Levinton et al. 2011). For
77 these reasons, this study primarily examines the effect of natural estuarine salinity variation on
78 the reproductive phenology of the eastern oyster.

79 Unfortunately, many estuaries no longer have extant populations of eastern oysters along
80 the entire salinity gradient. In fact, the desire to restore full metapopulation structure within

81 estuaries provides an important motivation for understanding how salinity variation affects
82 phenology. The effects of salinity variation on overall reproductive timing has received little
83 attention outside of aquaculture settings. It is well-established that the timing of spawning for
84 eastern oysters is closely correlated with rising temperatures, particularly in temperate estuaries
85 where reproduction has a more pronounced seasonality (Loosanof and Davis 1952; Davis and
86 Calabrese 1964; Bourlès et al. 2009). The interactive effects of salinity and temperature
87 influence multiple aspects of oyster life history traits such as mortality, growth, and survival at
88 all life stages (Davis and Calabrese 1964; Rybovich et al. 2016; McFarland et al. 2022). Multiple
89 studies have shown that low salinity environments (< 6 psu) can inhibit or depress
90 gametogenesis in oysters (Butler 1949; Loosanof 1953; Shafee and Daoudi 1991; Shumway
91 1996; Honig et al. 2014; Volety et al. 2017), but the effect of salinity variation specifically on
92 reproductive phenology has been much less studied. Butler (1949) addressed how natural salinity
93 variation affects gametogenic progression using histology, yet that case involved unusually
94 protracted and extreme low salinity conditions in the upper Chesapeake Bay rather than the
95 average salinity gradient experienced by an estuarine oyster metapopulation. The strongest data
96 relating salinity to reproductive phenology that we are aware of come from a 15-year time series
97 of monthly oyster collections at five sites along the Caloosahatchee River Estuary, Florida,
98 ranging from 12 to 28 average salinity in September (the annual salinity low point). Based on
99 gonad index averages over 15 years for wet, moderate, and dry subsets, McFarland et al. (2022)
100 found that the upper-most site had the earliest gametogenic development and the longest overall
101 reproductive season. Dermo disease was negatively correlated with salinity and average gonad
102 index, making it difficult to infer the separate phenology effects of these two factors (McFarland
103 et al. 2022).

104 Dermo disease is caused by the pathogen *Perkinsus marinus*. Moderate to heavy infection
105 reduces an oyster's investment in reproduction and shell growth, with the potential to cause
106 mortality and devastate oyster populations (Chu and La Peyre 1993; Powell et al. 1996). This
107 disease poses a pervasive risk to populations across the eastern seaboard and is thus of great
108 concern to management and restoration planning (Andrews 1988; Dittman et al. 2001;
109 Mackenzie 2007). Non-lethal infection intensities can have significant negative effects on gonad
110 size and gametogenic development (Dittman et al. 2001). Dermo disease risk is largely limited to
111 high and moderate salinity environments (≥ 12 psu) because of the pathogen's environmental
112 limitations (Powell et al. 1996; Levinton et al. 2011). Thus, while oyster gametogenesis is
113 inhibited in extreme low salinity environments, such areas can also serve as a refuge from
114 disease (La Peyre et al. 2003; La Peyre et al. 2016). Documenting Dermo prevalence can not
115 only help to interpret potential mechanisms limiting gametogenesis between high and low
116 salinity sites, but it also provides a critical baseline metric to inform restoration planning and
117 monitoring the health of restored populations.

118 The Hudson River Estuary (HRE) provides a particularly interesting case study. This
119 estuary includes multiple named water bodies in the brackish zone above and below New York
120 City (Fig. 1). The HRE was once home to a highly abundant, economically valuable population
121 of eastern oysters (Franz 1982; Kurlansky 2006). Overharvesting, water pollution, habitat
122 destruction, and urbanization led to a precipitous decline of the population in the early 20th
123 century (Kurlansky 2006; Mackenzie 2007). Today, a self-sustaining eastern oyster population is
124 largely absent from the HRE except for a small remnant population in the Tappan Zee-
125 Haverstraw Bay (TZHB) portion of the estuary near Irvington, NY (Fig. 1), where average
126 salinity is low (3-12 psu) (Medley 2010; Starke et al. 2011). Recent unpublished data (Hare)

127 suggest that the TZHB population, while reproducing annually and self-recruiting consistently
128 (Levinton et al. 2011; McFarland and Hare 2018; AKRF Inc. et al. 2021), is barely contributing
129 any spat recruitment downstream that could help recover a self-sustaining population in lower
130 parts of the estuary where water quality has improved and restoration efforts abound (Stinnette et
131 al. 2018; McCann 2019). Based on published habitat suitability indices for *C. virginica*, the
132 persistence and reproductive output of the TZHB population at very low salinities is enigmatic
133 but provides hope that when restoration broodstock are needed, local native oysters will be
134 considered a viable option. Our goal was to test the spawning capacity of TZHB oysters when
135 transplanted to other parts of the estuary, and measure salinity effects on gametogenic timing.

136 To increase the generality of this study we compared gametogenic phenology of oysters
137 from three population sources. Choosing an oyster source for restoration typically requires
138 evaluation of potential trade-offs with respect to restoration efficacy, involving logistics,
139 availability, cost, genetic diversity and relative fitness in the specific restoration environment
140 (Hornick and Plough 2019; Hornick and Plough 2022). There currently are no relative
141 performance data available on gametogenesis and phenology for oysters with distinct ancestries,
142 even though these phenotypes can vary due to either local adaptations (genetic factors) or
143 acclimation to distinct environments (epigenetic factors). Local native transplants (source 1:
144 “Native”), recommended to maximize genetic diversity while minimizing maladaptive gene flow
145 (Camara and Vadopalas 2009), involve transplants from naturally recruiting populations within
146 the same estuary or region. For simplicity we will refer to naturally recruited oysters as “Native”,
147 acknowledging that they are not necessarily pristine or genetically non-admixed with aquaculture
148 lines at present or in the past. Native transplants can potentially be done at any post-settlement
149 life stage, and do not involve hatchery propagation. Hatchery propagated stocks for restoration or

150 supplementation can derive from wild broodstock spawned in the hatchery (source 2:
151 “Hatchery”) or from artificially selected lines used in aquaculture (source 3: “Aquaculture”).
152 Most U.S. eastern seaboard hatcheries producing oysters sell primarily to aquaculture growers
153 and do not typically work with wild broodstock, whereas restoration hatcheries and some
154 research hatcheries spawn and propagate wild oysters for restoration (Hornick and Plough 2019).
155 Thus, there can be many practical trade-offs affecting the decision to use one oyster source or
156 another for restoration. A recent survey of New York/New Jersey projects in which oysters were
157 seeded on restored benthic habitat revealed that 19 out of 20 had obtained seed from an out of
158 state hatchery, with at least partial use of local broodstock in only 3 cases (McCann 2019).

159 Here, we compare the timing of gametogenic development and among-oyster synchrony
160 for these three oyster ancestries along the salinity gradient in a temperate estuary. By quantifying
161 gonad development at locations ranging from 3 to 30 psu, we test for the effects of salinity and
162 ancestry on reproductive phenology. Our first hypothesis is that gametogenesis is delayed in the
163 early-reproductive season (June) at sites with lower salinity relative to the higher salinity sites
164 (Butler 1949; Shafee and Daoudi 1991; Shumway 1996; Honig et al. 2014; Volety et al. 2017).
165 Comparing oyster ancestries, our hypothesis is that the Native line, from the local TZHB
166 population, will demonstrate the most distinct phenology out of the three lines. More
167 specifically, the long history of the TZHB population at an isolated, low salinity location
168 (McFarland and Hare 2018), leads us to hypothesize that local adaptation or acclimation has
169 reduced gametogenic suppression at low salinity, compared to the Aquaculture and Hatchery
170 lines, both of which involved hatchery-produced seed (juveniles) from moderate salinity
171 broodstock. Accordingly, the gametogenic delay predicted by hypothesis 1 is only expected in
172 these latter two lines.

173

174 **METHODS**

175 **Study Species**

176 Eastern oysters (hereafter, oysters) are protandrous hermaphrodites, maturing first as
177 males and later becoming females, and spending some period of the winter, depending on
178 temperatures, with undifferentiated sex (Kennedy and Battle 1964). After sex differentiation,
179 oysters begin gametogenesis, building and developing gonad tissue and gamete cells (eggs or
180 sperm). The oyster is a synchronous spawner, triggered by temperatures greater than 20°C to
181 release gametes into the water column as nearby individuals do the same (Loosanof and Davis
182 1952; Kennedy and Battle 1964; Barber et al. 1991; Barber 1996; Mann et al. 2014). In
183 temperate waters like the HRE, the reproductive period (gametogenesis and spawning) begins in
184 the Spring and ends in the Fall. As it gets colder, oysters sometimes achieve a smaller fall spawn
185 (Hayes and Menzel 1981) and then enter a quiescent stage in the winter during which gonads are
186 dormant and mostly undifferentiated until the next spring (Kennedy and Battle 1964; Hayes and
187 Menzel 1981; Mann et al. 2014).

188

189 **Outplant locations**

190 During a previous study initiated in 2016, juvenile oysters were deployed in cages along
191 the estuarine salinity gradient to measure and compare survivorship and growth over 3 years. For
192 this study we sampled lines from six of these outplant locations in both 2018 and 2019 (Fig. 1).
193 The locations at Irvington Boat Club (IRV), Hastings on the Hudson (HH), and Yonkers Science
194 Barge (SB) represent the low salinity region of the HRE (3-13 psu). The Redhook (RED)
195 location represents an intermediate salinity (15-25 psu) in New York Harbor. Paerdegat Basin

196 (PGB) and Kingsborough Community College (KCC) are both in Jamaica Bay (connected to the
197 lower HRE) and have the highest salinities (20-30 psu) (Fig. 1). Temperature and salinity were
198 recorded as point data during each sampling trip at each location, along with semi-continuous
199 measurements collected using YSI 600 OMS (YSI Inc. Yellow Springs, Ohio) sondes deployed
200 at KCC and RED. Environmental data for HH, IRV and SB were obtained from Riverkeeper
201 Incorporated's Water Quality Program in the Hudson River Estuary
202 (<https://www.riverkeeper.org/water-quality/udson-river/>), the Hudson River Environmental
203 Conditions Operating System (HRECOS) location at Piermont Pier (across the river from IRV),
204 and the Center for the Urban River at Beczak (sonde deployed at SB) ([http://hudson.dl.stevens-](http://hudson.dl.stevens-tech.edu/hrecos/d/index.shtml)
205 [tech.edu/hrecos/d/index.shtml](http://hudson.dl.stevens-tech.edu/hrecos/d/index.shtml)). Water quality data for PGB were obtained from a Harbor Water
206 Quality dataset provided by the New York City Department of Environmental Protection
207 (<https://data.cityofnewyork.us/Environment/Harbor-Water-Quality/5uug-f49n>).

208

209 **Oyster ancestry**

210 At each location, three distinct oyster lines were compared: Native, Hatchery, and
211 Aquaculture. The 2018 Native line was obtained in August/September 2016 as natural spat
212 recruits on bivalve shell deployed in the vicinity of the TZHB remnant population, then
213 transplanted as 1 month old spat to various cage grow-out locations (Fig. 1). The Hatchery line
214 represents wild genetic diversity from a moderate-salinity population with standard hatchery
215 procedures imposing some genetic bottlenecking (Hornick and Plough 2019). The Hatchery line
216 was produced by strip-spawning 12 males and 12 females collected from a native population in
217 Edgartown Great Pond, a moderate-salinity (15-25 psu) lagoon in Martha's Vineyard,
218 Massachusetts. The larvae were then cultured by the Martha's Vineyard Shellfish Group and sent

219 to the Cornell Cooperative Extension hatchery in Southold, New York, to set on micro-clutch
220 and grow out in a nursery upwelling system for three weeks during August 2016 before
221 deploying to sites across the HRE (Fig. 1). Larval culture was at 27-28 psu and the nursery was
222 28 psu. Finally, a moderate-salinity aquaculture line was acquired as seed oysters (clutch-less
223 spat) in August 2016 for outplant in the same cages. We estimated that all three lines were within
224 approximately 2-3 months of the same age and were thus approximately two years old when
225 sampled in 2018.

226 The 2019 samples consisted of the same Aquaculture line (now 3 years old) and TZHB
227 native oysters dredged as adults (mean length = 74.57 ± 1.13 mm) in June 2018. The dredged
228 samples were immediately transplanted to experimental cages at the study sites (Fig. 1). Unlike
229 the 2018 TZHB native oysters that developed from spat in the experimental cages at each study
230 site (nearly full post-settlement opportunity for developmental plasticity), 2019 TZHB native
231 oysters had acclimated as adults for 1 year at each study site before summer sampling in 2019.
232 The 2019 Native cohort is recognized and considered a fourth treatment group because of their
233 alternate collection and deployment strategy. Due to common ancestry, the 2019 cohort is still
234 included in the discussion of ancestry effects on salinity.

235

236 **Oyster sampling and histology preparation**

237 Oysters were collected and sampled for histology three times during the summer of 2018
238 (June 15-21, July 8-13 and August 8-13). Logistical and financial constraints limited collections
239 to two dates in 2019 (July 28-August 1 and September 1-4), and Aquaculture individuals were
240 not available at every location in 2019. The number of individuals that could be histologically
241 analyzed was limited by oyster survivorship as well as funding. At each sampling site visit, shell

242 height was measured to the nearest millimeter using Mitutoyo Calipers (Mitutoyo America Co.,
243 Aurora, IL, USA) from the hinge to the farthest edge of the shell prior to dissections.

244 In 2018, oysters were transported live in coolers with ambient HRE water to the Fort
245 Totten Urban Field Station Laboratory in Queens, New York, (US Forest Service) where
246 dissection and fixation of tissue sections was performed. In 2019, oysters were transported back
247 to Cornell University live in coolers with ice packs, location specific HRE water, and aeration
248 for processing the next day. After cleaning and shucking the oysters, a standardized 4mm cross
249 section was taken to include reproductive tissue at the intersection of the labial palps and gills
250 (Morales-Alamo and Mann 1989; Howard et al. 2004). The tissue cross section was placed in a
251 labeled plastic cassette and preserved in Davidson's Fixative for 7 days and then 70% ethanol
252 (Fisher et al. 1996). Samples were then sent to the Cornell University Animal Health Diagnostic
253 Center for histology slide preparation with hematoxylin and eosin staining.

254

255 **Categorical gametogenesis analysis**

256 A common approach to measuring oyster reproductive status is with qualitative
257 categorical scoring of gametogenesis progression from histological sections (Fisher et al. 1996).
258 We scored the 5 stages defined by Lango-Reynoso et al. (2000), Barber (1996), and Kennedy &
259 Battle (1964) (Table 1). Histology slides were examined with a compound microscope under
260 100x total magnification to determine sex and gonad developmental stage. Gametogenesis was
261 scored by KMG. A subset of oysters with borderline category assignments were also scored
262 independently by MPH until consistent agreement was obtained.

263

264 **Dermo disease testing**

265 To test for the presence of *P. marinus* and measure intensity of infection, a small section
266 of rectum tissue and approximately 0.5 cm² from the posterior gill tissue were removed from the
267 same individuals on which histology was performed and placed in a culture tube containing 9.5
268 mL of Ray's Thioglycollate Medium (RFTM; (Ray 1952; Ray 1954). Then, 0.5mL antibiotic
269 solution (equivalent to 500 units of penicillin G and 500 units of streptomycin per mL of
270 medium) and 50 µL of nystatin solution were added to the culture tube. The cultures were
271 incubated at 25-26 °C in the dark for 5-7 days. After the incubation period, all tissues were
272 removed from each tube and placed on a clean glass microscope slide. The tissue was macerated
273 using a razor blade and 3-4 drops of Lugol's iodine was added before applying a cover slip. Each
274 slide was immediately examined at 100-400x magnification for Dermo cells using the Mackin
275 scale of 0 to 5, with 0 indicating no infection and 5 heavily infected (Mackin 1962; Dittman et al.
276 2001). Because infections were so sparse, several slides were sent for confirmatory readings by
277 Iris Burt at Rutgers Haskin Shellfish Research Lab, New Jersey. All individuals sampled in 2019
278 for histology (n = 240) were tested for levels of *P. marinus* infection.

279

280 **Statistical Analyses**

281 The proportions of each gametogenic stage were calculated for each unique combination
282 of location, month, and line. A set of ordinal logistic models were created to test the relationship
283 between proportions of each gonad development stage and location, month, sex, and line for the
284 2018 sampling year (Table 2). The moderate-salinity site (RED) had extremely low cumulative
285 survivorship (by August 2018: Native- 3%, Hatchery- 0%, Aquaculture- 9%), resulting in a small
286 sample size, so it was not included in the model. Given that complete or nearly complete
287 gametogenic data were available for sites representing two environmental extremes in the

288 estuary, northern low salinity and southern high salinity, location was used as a proxy for salinity
289 and included in all models. Month was included in all models because observations were made at
290 this time interval during the typical main gametogenic season for oysters at this latitude
291 (Kennedy and Battle 1964). All resulting possible interaction effects were included for 2018
292 models. The 2019 model did not include a line variable because of limited data across ancestries,
293 and because the Native line was based on adult transplants, not juvenile transplants like in 2018.
294 Instead, 2019 Aquaculture data are described qualitatively, and only Native data were included
295 in a location + month + location*month model to best mirror the top ranked model in the 2018
296 model selection (Table 2). Models were ranked in terms of AIC values with the criterion that
297 well supported models have a delta AIC value < 2 (Burnham and Anderson 2002). Pairwise *post*
298 *hoc* contrasts were performed using the ‘emmeans’ package (Lenth 2022). All statistical analyses
299 were performed using R and RStudio (R Core Team 2018).

300 To quantify the synchrony of oysters, we calculated the Shannon-Wiener Diversity Index
301 (hereafter, diversity index) for each unique combination of month, location, and line (Shannon
302 and Weaver 1949; Tuomisto 2012). The formula, where p_i is the proportion of the total sample
303 found to have each of the five gametogenic categories, captures evenness of gametogenic stages
304 at a given time and place (Eqn. 1). Used this way, the index has an inverse relationship with
305 synchrony such that as the index increases, the level of synchrony decreases. The diversity index
306 was compared across locations and lines using generalized linear models (Table 3), following the
307 formulas created for models 1-7 of the 2018 ordinal logistic modeling (Table 2). Models were
308 ranked in terms of AIC values, but because this diversity metric lacks replication within sites and
309 lines, *post hoc* analyses were not possible. These analyses were performed using R and RStudio
310 (R Core Team 2018).

311 **Eqn 1.** $H = -\sum p_i * \ln(p_i)$

312

313 **RESULTS**

314 **Environmental Conditions**

315 Across locations, temperature varied from 0.9 - 30.1°C throughout the year (Fig. 2).

316 There was a seasonal flux in temperature, with only slight variation between years and among

317 locations. Treated as two regions, the northern sites (IRV, HH, SB) and southern sites (PGB,

318 KCC) saw little difference in average temperature during any sampling month (Fig. 2). Salinity

319 variation (Fig. 2) was non-overlapping for northern (0.9-15.7 psu) and southern sites (18.4-30.1

320 psu), supporting our use of these locations as proxy variables for low and high salinity,

321 respectively. For each site, there was little variation in salinity between years, except at KCC and

322 SB. In 2018, there was a gradual decrease in salinity at KCC (29.9 to 19.0 psu) between April

323 and October, but in 2019 the trend was reversed, with salinity increasing from 23.8 to 30.1 psu in

324 those months. The SB site had much lower spring salinity in 2019 than in 2018, but

325 unfortunately insufficient oyster numbers remained at SB to allow an analysis for 2019.

326

327 **Gametogenic Development Stage**

328 Based on model selection criteria using AIC values, the top ranked model for 2018 was

329 model 7: Location + month + line + location*month + location*line + month*line (Table 2).

330 Model 11 was also supported, differing from model 7 only in the inclusion of sex as a parameter.

331 Sex was determined to be an uninformative parameter based on the criteria in (Arnold 2010), so

332 the *post hoc* analysis was thus performed only for model 7. All sampling occasions in 2018 and

333 2019 demonstrated either a roughly even or male-biased sex ratio (Table 4, Table S1). In August

334 of 2018 and in all 2019 samples, the Aquaculture line at PGB became 100% male. For the linear
335 models of the diversity index, the top model was model 4: location + month + line + month*line,
336 indicating that location effects on synchrony were not interacting with line or month (Table 3).

337 In June 2018, only Native and Aquaculture could be compared between low and high
338 salinity sites and in both cases gametogenic development had advanced more at low salinity. A
339 greater proportion of individuals were mature or spawning at the low salinity site SB (75-80%)
340 compared to the high salinity site PGB (0-20%; Native $p < 0.0001$, $n = 26$; Aquaculture $p =$
341 0.0001 $n = 26$; Hatchery could not be tested; Tables 4, 5). For both lines, early and late active
342 stages were found in no more than 20% of individuals at low salinity site SB, yet at high salinity
343 these stages were still 100% and 70% of Native and Aquaculture samples, respectively (Fig. 3a,
344 g). The low salinity site HH showed the same trend in both oyster lines, with a near absence of
345 the early active stage, but was less extreme (post hoc contrast significant only for the Native line,
346 Table 5). For the Native line at high salinity, the complete lack of mature or later stage
347 individuals contrasted with the diverse gametogenic stages observed in Aquaculture at high
348 salinity, while patterns were similar among all three lines at low salinity (Fig. 3). One exception
349 is that Native and Hatchery lines achieved some June spawning at low salinity, but Aquaculture
350 only had spawners at higher salinity sites in June. The diversity index was highest, and thus
351 synchrony was lowest, for the Aquaculture line in the South (Table 6).

352 In July, gametogenesis had not advanced much in any line at low salinity sites whereas at
353 the moderate and higher salinity sites the proportion of spawning and reabsorbing stages had
354 increased, where comparisons could be made to June. Thus, the June pattern of relatively
355 advanced phenology at low salinity had reversed by July. Phenology again differed between low
356 salinity SB+HH (average 0.9% spawning or reabsorbing individuals across the three lines and

357 two sites) and high salinity PGB (21%), with 4 out of 6 post hoc contrasts $p < 0.05$ (table 5). An
358 even greater proportion of spawning and/or reabsorbing stages was observed at the moderate
359 salinity RED site than at high salinity PGB. In contrast, the two low salinity sites, SB and HH,
360 both were dominated by late active and mature stages with almost no spawners (Table 5, Fig. 3b,
361 e, h). Comparing the ancestry lines, the most striking difference was a slightly slower phenology
362 in Aquaculture as indicated by 18.6% early active oysters averaged across all sites compared
363 with 4.5% in Hatchery and Native combined (Fig. 3b, e, h). In fact, Aquaculture phenology went
364 slightly backwards from June to July (more early active stage at all sites except PGB), even
365 while spawning increased (Fig. 3h). All ancestry lines contained all 5 stages when tallied over all
366 locations in July, but the among-location average diversity index was 1.2 for the Aquaculture
367 line, indicating lower synchrony than Native and Hatchery with 0.84 and 0.62 diversity indices,
368 respectively (Table 6). The July Hatchery line within-locale synchrony was higher (diversity
369 index lower) than in any other month and line (Table 6).

370 In August, gametogenic progression to a later mix of stages was observed in all lines, at
371 all sites. All three lines continued to show a higher proportion of later stages at higher salinity
372 sites, dominated by spawning and reabsorption, relative to low salinity sites where late-active
373 and mature stages were abundant (all $p < 0.0026$, Table 5, $n = 51, 60, 58$, for HH, SB, PGB,
374 respectively all $n = 20$; Table 4). Relative to July, low salinity sites in August progressed less than
375 higher salinity sites except for the Aquaculture line. The only other line distinction of note was
376 the Native oysters having 44% pre-spawning stages at high salinity PGB, whereas Hatchery and
377 Aquaculture lines were $> 85\%$ spawning and reabsorbing stages (Fig. 3 c, f, i). The diversity
378 index was again overall highest for the Aquaculture line.

379 The 2019 data only included two months, and data collection was shifted 2 weeks later
380 relative to 2018. Two sites were included for both the high salinity and low salinity regions, but
381 complete data were only obtained for the Native line (adult transplants that had acclimated for a
382 year in this case, as opposed to 2 years growth from spat on site for 2018 data). No Hatchery line
383 oysters were available in 2019. At all sites oysters demonstrated gametogenic progression
384 between the two sampling dates (Fig. 4). The *post hoc* contrasts between locations found little
385 difference in stage proportions for either month (Table 7, Fig. 4a, b). This spatial gametogenic
386 synchrony was coupled with temporal synchrony within sites based on low average diversity
387 index values of 0.38 and 0.44 for July/August and early September, respectively (Table 8). At
388 the high salinity sites where the Aquaculture and Native lines could be compared, the former had
389 substantially more advanced gametogenic stages at the end of July (90+% spawning and
390 reabsorption stage for Aquaculture, but only 15-45% for Native), but only modest differences
391 with the same trend at the beginning of September.

392

393 **Dermo Disease**

394 Only 3 of 240 individuals had *P. marinus* spores detected (1.3%). All detected infections
395 were in the Native line at PGB during the September sampling period. Two samples had Mackin
396 intensity levels of 0.5, and another had a level of 1.

397

398

399 **DISCUSSION**

400 In one of the first studies to measure salinity effects on gametogenic phenology in a
401 bivalve, and compare oyster lines with different ancestries, we found that in July and August of

402 2018 all three oyster lines had delayed gametogenic development at low salinity sites relative to
403 high salinity sites, confirming our first hypothesis that low salinity would cause a delay.
404 Surprisingly, the reverse was true in June 2018 for the two lines that could be compared in
405 different salinities up and down river. June spawners only occurred at low salinity for Native
406 oysters and contributed to a weighted average gonad index of 2.38-2.92 at low salinity sites,
407 compared to only 1.46 at high salinity. The Aquaculture line showed this trend less dramatically,
408 with weighted averages of 2.62 vs. 2.08 at low and high salinity, respectively, partly because the
409 few June spawners were observed only at high and moderate salinity sites. The June pattern of
410 more advanced oyster gonad index proportions at low salinity relative to high salinity was
411 reversed in July due to gonad index stasis at low salinity versus progression toward spawning
412 and reabsorption at high salinity.

413 Our second hypothesis, that the Native line would have the most distinct phenology
414 characterized by earlier (less delayed) gametogenesis at low salinity, was rejected. All three lines
415 had similar gonad index distributions at low salinity in June. Instead, the most striking distinction
416 observed in the Native line was the extent of gonad index delay at high salinity in June, as
417 indicated by zero oysters with a 'maturing' or later stage (weighted average gonad index 1.46),
418 whereas at the same site the Aquaculture line had 25% maturing or spawning oysters (2.08). By
419 July the Native oysters had compensated and had gonad index proportions similar to the other
420 lines. The other line distinction of note in 2018 was the consistently higher diversity of gonad
421 index stages in the Aquaculture line throughout the summer, indicating 30-48% less synchrony at
422 any particular time or place.

423 Low variation in temperature among sites and minimal Dermo infections detected
424 overall, highlight regional salinity differences as likely key to phenology variation among sites in

425 2018, but causal inferences are tentative because oyster cage outplant sites differed in other
426 unmeasured ways. The 2019 sampling provided fewer spatial and line comparisons. Despite
427 similar environmental conditions in the two years, 2019 patterns indicated greater synchrony and
428 only slight salinity effects relative to 2018. We discuss these findings with reference to
429 environmental variation and future restoration goals.

430

431 **Phenology variation**

432 *Month*

433 The model that best explains 2018 gametogenic stage proportions includes month,
434 location, line, and their interactions. Given the summer temperature variation (Fig. 2), month is
435 the most obvious factor of expected importance since the species is considered to have locally
436 synchronous spawning triggered by rising temperatures and conspecific spawning (Thompson et
437 al. 1996; Bernard et al. 2011; Aranda et al. 2014). While some spawning occurred in each month
438 (average proportion of spawners and reabsorbing individuals equal to 3.5%, 7.5%, and 46.8% in
439 June, July, and August 2018, and 58.63% and 97.5% in July/August and September 2019), the
440 spawning period in the HRE peaked in August and continued into September for these
441 outplanted, caged oysters. An August peak for native TZHB oysters is supported by spat
442 monitoring in the HRE, where the most abundant recruitment of spat (after a 2-3 week larval
443 stage) has been observed in September (McFarland and Hare 2018). Our results suggest an
444 extension of the spawning season for the HRE relative to nearby Long Island Sound where
445 spawning peaks in July with spent oysters by August (Ford et al. 1990; Barber et al. 1991). Cold
446 Adirondack mountain Spring melt waters in the Hudson River, or differences in phytoplankton

447 bloom timing and concentration, could be responsible for this geographic difference (Hofmann et
448 al. 1992; Bernard et al. 2011).

449

450 *Salinity*

451 Our hypothesis of delayed gametogenic progression at lower salinities was only observed
452 after the month of June, suggesting that the initiation of gametogenesis is less constrained by
453 salinity variation, but that low summer salinity at northern sites relative to southern sites could be
454 causing a delay at later gonad maturation stages. By the August 8-13 sampling period in 2018,
455 northern low salinity sites (HH, SB) had fallen to near 5 psu and a majority of oysters were still
456 in pre-spawning stages, a trend we expected based on literature suggesting reproductive
457 depression caused by low salinity (Butler 1949; Shafee and Daoudi 1991; Shumway 1996; Honig
458 et al. 2014; Volety et al. 2017). In contrast, the majority of oysters at the high salinity PGB site
459 (approximately 25 psu) were spawned or reabsorbing for all three lines in August. These June
460 and later Summer findings in Hudson River oysters are similar to those reported by McFarland et
461 al. (2022) for eastern oysters in the Caloosahatchee River Estuary in Florida. They found over 15
462 years of monthly sampling, across 5 sites ranging from 12 to 28 average salinity, that average
463 gonad index at sites had a negative relationship with salinity. The lowest salinity site had the
464 earliest gametogenic development; it had a 15-year average March gonad index of 2 while all
465 other sites had averages below 1.5 (Fig. 7 in McFarland et al. 2022). We did not measure Spring
466 gametogenesis in Hudson River oysters, but early initiation in the northern TZHB population
467 seems unlikely given that it often experiences extended periods below salinity 5 during Spring
468 (Levinton et al. 2011). Instead, we speculate that the advanced June gametogenesis observed
469 here results from a rapid compensation for stressful Spring conditions. Whatever the mechanism,

470 the early gametogenic progression that produced some spawning in northern low salinity sites,
471 coupled with a small proportion of spawners in August relative to high salinity sites, indicates
472 that oysters at low salinity sites in the Hudson River also had relatively extended reproductive
473 seasons similar to what was reported in the Caloosahatchee River (McFarland et al. 2022).

474 Contrary to salinity effects observed in 2018, oysters analyzed in 2019 showed no
475 significant differences in gonad index among sites. We don't know what interannual differences
476 caused this change in phenology pattern along the salinity gradient, but the summer variation in
477 salinity at northern sites was less in 2018, lacking a 5 psu drop in August or September (Fig. 2).
478 Phenology comparisons between years are tenuous with respect to salinity contrasts because of
479 the varied sampling dates, uneven oyster line availability, and altered Native outplant method.
480 Factors such as food availability (Luna et al. 2000; Bernard et al. 2011) or local freshwater inputs
481 (i.e. river discharge) (Baba et al. 1999; Wilson et al. 2005) could drive interannual variation, but
482 such factors were outside the scope of this study. We recommend further study to determine the
483 key interannual factors that can change reproductive phenology patterns so dramatically.

484 In terms of outplant method, it is notable that the 2019 Native dredged oysters were able
485 to develop gametes at a variety of salinities only one year after transplant. Furthermore, the fact
486 that both outplant strategies (outplanted as spat, outplanted dredged adults) resulted in oysters
487 that were able to undergo a full reproductive cycle is encouraging for future restoration efforts
488 considering the potential advantages associated with restoration using locally-adapted species
489 (Hofmann et al. 1992; Camara and Vadopalas 2009; Flanagan et al. 2018; Hornick and Plough
490 2019; Hornick and Plough 2022). This phenotypic plasticity is characteristic of oysters, but
491 presumably has limits that need to be accounted for in restoration planning (Li et al. 2017; Li et
492 al. 2018; Li et al. 2021).

493

494 *Ancestry*

495 The prediction that the Native line is adapted to low salinity and therefore would
496 uniquely demonstrate a lack of reproductive depression at low salinity was only consistent with
497 patterns observed in June 2018. Unfortunately, only comparisons between Native and
498 Aquaculture strains were possible in June. The spatial contrast in gonad index between low and
499 high salinity sites was much stronger in the Native line. The average proportion of early active
500 and mature individuals for Natives in the north was 0.08 and 0.58 respectively, compared to 0.54
501 and 0.0 at high salinity PGB. The overall gonad index average for Natives was higher at low
502 salinity vs high salinity sites, 2.65 vs 1.46. This trend also was detectable in Aquaculture
503 individuals but was less extreme (gonad index 2.62 in the north vs 2.08 at high salinity PGB).
504 This suggests that TZHB Native oysters may have local adaptations that allow them to rapidly
505 compensate for gametogenic delays experienced during very low Spring salinity levels, perhaps
506 in response to overall metabolic depression (Gurr et al. 2020).

507 In August there was no single oyster line that stood out in terms of phenology or salinity
508 effects, but there were differences in the gonad index progression with Native slowest (3.37
509 average gonad index), Hatchery intermediate (3.54) and Aquaculture most advanced (3.82).
510 These differences and trend are similar in the northern low salinity sites vs high salinity PGB,
511 just shifted lower and higher than the overall average, respectively (3.03, 3.17, 3.50 in north;
512 4.06, 4.30, 4.45 at PGB). This trend for 2018 indicates that under some conditions the Native line
513 has a more extended reproductive season than the other oyster lines. A lengthening of the
514 gametogenic period can be interpreted as a depression of gametogenesis that has been observed

515 by previous studies. (Butler 1949; Shafee and Daoudi 1991; Shumway 1996; Honig et al. 2014;
516 Volety et al. 2017).

517 The 2019 samples allowed line comparisons only at high and low salinity sites. At these
518 sites the gonad index differences between lines were not large, but the 2018 trend of a more
519 extended reproductive season for the Native line was repeated in 2019. At the end of July gonad
520 index averages for low and high salinity were 3.05 and 3.85 for Native, and 4.66 at high salinity
521 sites for Aquaculture. In early September the difference was similar, 4.56 and 4.70 for Native,
522 and 4.93 at high salinity sites for Aquaculture. In September, the transplanted Natives were
523 majority post-spawning at low salinity sites, suggesting that the Natives had completed a cycle of
524 gametogenesis and spawning.

525 One of the biggest differences among lines was in synchrony as measured by the
526 diversity index. When averaged across all sites and over the entire 2018 season, the stage
527 diversity in Native, Hatchery, and Aquaculture was 0.78, 0.81, and 1.05, respectively. Thus,
528 Aquaculture was the outlier with less synchrony. We are not aware of other comparable data and
529 can only speculate that long term culture and domestication led to relaxed selection (i.e., more
530 random change under genetic drift) on sensory systems that contribute to synchrony under
531 natural conditions. However, the same strain difference in synchrony was not observed in 2019,
532 so this difference among strains probably relates to distinct expressions of genotype by
533 environment plasticity (Eierman and Hare 2015).

534

535 **Conclusions and context for future oyster restoration**

536 This study demonstrated differences in reproductive phenology along the HRE salinity
537 gradient that were associated with locality (low vs. high salinity regions) and oyster ancestry

538 line. These novel results in a temperate estuary suggest that reproductive phenology within an
539 eastern oyster metapopulation is not simply a function of temperature, but also varies along the
540 salinity gradient. The 2018 patterns were consistent with salinity effects on phenology
541 documented elsewhere, but our interannual results caution against generalizing from the 2018
542 patterns because genotype by environment interactions likely have strong effects. Additional
543 replication is necessary to further understand genotype by environment effects. Future studies
544 would be valuable that can experimentally link aspects of phenology with specific environmental
545 drivers. In particular, future studies should sample in Spring as well as Summer because salinity
546 effects seem to vary at different stages, and experimental designs to test for reproductive
547 compensation would help evaluate the consequences of stressors.

548 Similar to previous reports in the HRE (Levinton et al. 2011; McFarland and Hare 2018),
549 the near absence of Dermo infections in this study makes it reasonable to infer salinity effects
550 separate from disease interactions. Further, the lack of Dermo presence across all sites supports
551 the conclusion that disease is not the reason for the Native TZHB population's current isolation
552 at low salinities, but further study would be necessary to confirm this.

553 The moderate salinity Aquaculture line studied here succeeded in spawning across
554 diverse environments, attesting to its plasticity with respect to salinity. However, it also showed
555 lower gametogenic within-site synchrony compared to the other lines, perhaps indicating
556 domestication effects lowering a fitness-related trait. Selective breeding for commercially
557 valuable traits can cause reduced genetic diversity and potentially reduced lifetime fitness in a
558 natural environment, making aquaculture lines often the least desirable option for restoring
559 oyster populations (Baggett et al. 2014). Enhancing or restoring a population with stocks that do
560 not possess adequate genetic variation, or that have maladaptive variation, could be detrimental

561 to overall population fitness and diversity (Camara and Vadopalas 2009; Morvezen et al. 2016;
562 Hornick and Plough 2019; Hughes et al. 2019; Hornick and Plough 2022).

563 With the growing threat of climate change, species inhabiting estuaries will likely be
564 exposed to more frequent and extreme storms that can rapidly shift salinity to the edge of their
565 tolerances. Investigating the effect of salinity on reproductive phenology is only one aspect of
566 research that is essential to understand and possibly mitigate the potential effects of climate
567 change on estuarine oyster populations. The Native TZHB population is at the edge of habitable
568 salinity variation with little quality habitat to expand to upstream (Starke et al. 2011), and it is
569 not known to occur in any habitat further south except as sparse spat (new recruits; Medley
570 2010) or adults on pilings in Hudson River Park, Manhattan (Fitzgerald et al. 2020). The ability
571 of TZHB oysters to survive and reproduce at moderate salinities, as evidenced in this study,
572 indicates that its continued isolation north of New York City is likely a function of
573 hydrodynamics and/or stressful downstream water quality for the mobile larval stage. If poor
574 water quality in the lower HRE amplifies larval mortality above typical levels, then further water
575 quality improvements will be needed before restoration with any oyster line has a chance of
576 establishing a self-sustaining metapopulation (i.e., promoting the full life cycle) in the lower
577 estuary. Assuming adequate water quality, transplantation of adults or spat to seed hard
578 substrates in moderate salinity habitats could help protect the population from climate change
579 effects by reestablishing metapopulation dynamics (Lipcius et al. 2008). Here, results
580 demonstrate that the TZHB population provides a valuable resource for transplant restoration
581 strategies that could leverage and expand local genetic variation.

582 **TABLES**

583 **Table 1.** Descriptions of gametogenic development stages for *Crassostrea virginica* (Kennedy
584 and Battle 1964; Barber 1996; Lango-Reynoso et al. 2000)

Stage Number	Stage Title	Description
0	Inactive	Follicles are nonexistent or elongated, with walls consisting or undifferentiated germinal epithelium. Sex cannot be determined
1	Early-active	Follicles contain oogonia or spermatogonia and primary oocytes or spermatocytes (no free oocytes or spermatozoa)
2	Late-active	Secondary (free) oocytes and spermatocytes predominate in the follicles; there are some spermatozoa
3	Mature	Mature gametes (ova or spermatozoa) totally filling the follicles; presence of ova with distinct nucleus and nucleolus, spermatozoa oriented with tails toward the follicle lumen
4	Spawned	Follicles have gaps devoid of gametes, although numerous gametes may still remain, follicle walls may be broken. Redevelopment as evidenced by increased number of primary oocytes or spermatocytes
5	Reabsorbing	Follicles have a shrunken appearance and contain numerous phagocytes and products of reabsorption; gametes are refractory, and development is not evident

585

586 **Table 2.** Results of 2018 model selection of ordinal logistic models for gametogenic

587 development stages. Models are ranked in terms of AIC values

Model #	Description	Df	Log Likelihood	AIC	Delta AIC	Weight
7	Location + month + line + location*month + location*line + month*line	22	-496.620	1037.2	0.00	0.504
11	Location + month + line + location*month + location*line + month*line + sex	23	-496.217	1038.4	1.19	0.277
2	Location + month + location*month	12	-508.137	1040.3	3.03	0.110
5	Location + month + line + location*month	14	-506.842	1041.7	4.45	0.055
9	Location + month + location*month + sex	23	-507.850	1041.7	4.46	0.054
4	Location + month + line + month*line	14	-533.466	1094.9	57.69	0.000
6	Location + month + line + location*line	12	-534.097	1096.2	58.95	0.000
3	Location + month + line	10	-538.836	1097.7	60.43	0.000
10	Location + month + line + sex	11	-538.836	1099.7	62.43	0.000

1	location + month	8	-541.883	1099.8	62.53	0.000
8	location + month + sex	9	-541.883	1101.8	64.53	0.000

588

589 **Table 3.** Results of 2018 model selection of generalized linear models of Shannon Diversity

590 Index. Models are ranked in terms of AIC values

Model #	Description	Df	Log Likelihood	AIC	Delta AIC	Weight
4	Location + month + line + month*line	12	6.422	11.2	0.0	0.745
1	location + month	6	-1.699	15.4	4.24	0.089
3	Location + month + line	8	0.199	15.6	4.44	0.081
7	Location + month + line + location*month + location*line + month*line	20	12.032	15.9	4.78	0.086
2	Location + month + location*month	10	-0.444	20.9	9.73	0.006
5	Location + month + line + location*month	12	1.555	20.9	9.73	0.006
6	Location + month + line + location*line	12	1.358	21.3	10.13	0.005

591

592

593 **Table 4.** Sex ratios of oysters sampled for histology (# females: # males). Locations are listed by

594 latitude, from north to south. Locality abbreviations explained in Fig. 1 legend and in the

595 text. Native oysters in 2019 were dredged and outplanted as adults

Location	Line	June 2018	July 2018	August 2018	July/August 2019	September 2019
IRV	Native	-	-	-	11:9	11:9
HH	Aquaculture	3:10	7:13	7:13	-	-
	Hatchery	3:10	5:7	4:8	-	-
	Native	3:10	10:10	6:13	9:11	5:15
SB	Aquaculture	4:9	5:15	5:15	-	-
	Hatchery	3:10	8:12	6:14	-	-
	Native	2:11	7:13	7:13	-	-
RED	Aquaculture	2:8	1:19	2:18	-	-
	Hatchery	-	-	-	-	-
	Native	-	-	1:5	-	-
PGB	Aquaculture	2:11	5:15	0:20	0:20	0:20
	Hatchery	-	6:12	4:16	-	-

	Native	5:8	4:16	5:13	7:13	4:16
KCC	Aquaculture	-	-	-	4:16	7:13
	Native	-	-	-	9:11	4:16

596

597

598 **Table 5.** Results of pairwise *post hoc* test (p-values) for the 2018 model comparing locations

599 within each month, for each line. Locality abbreviations explained in Fig. 1 legend and in

600 the text

a.) June	Native	Hatchery	Aquaculture
HH-SB	0.3761	0.0076	0.0347
HH-PGB	0.0002	N/A	0.1102
SB-PGB	<.0001	N/A	0.0001

601

b.) July	Native	Hatchery	Aquaculture
HH-SB	0.4015	0.7865	0.9991
HH-PGB	0.4366	0.0581	0.0010
SB-PGB	0.0420	0.1531	0.0018

602

c.) August	Native	Hatchery	Aquaculture
HH-SB	0.9410	0.3073	0.6345
HH-PGB	0.0010	<.0001	<.0001
SB-PGB	0.0002	0.0026	<.0001

603

604 **Table 6.** Shannon-Wiener Diversity Index values for each unique combination of month,

605 location, and line for 2018. Locality abbreviations explained in Fig. 1 legend and in the

606 text

	Location	Native	Hatchery	Aquaculture
June	HH	0.911	1.073	0.690
	SB	0.937	0.790	0.687
	RED	-	-	1.280
	PBG	0.690	-	1.199
July	HH	0.562	0.888	0.999
	SB	0.999	0.423	1.106
	RED	0.673	-	1.476

	PBG	1.141	0.557	1.234
August	HH	0.874	0.824	1.345
	SB	0.708	0.824	1.345
	RED	-	-	0.441
	PBG	0.349	1.07	0.824

607

608

609 **Table 7.** Results of pairwise *post hoc* test (p-values) for the 2019 model comparing locations

610 within each month for the Native line. Locality abbreviations explained in Fig. 1 legend

611 and in the text

	July/Aug.	Sept.
IRV-HH	0.9998	1.00
IRV-PGB	0.9928	0.9998
IRV-KCC	0.1817	1.00
HH-PGB	1.00	0.9976
HH-KCC	0.3711	0.9992
PGB-KCC	0.5431	1.00

612

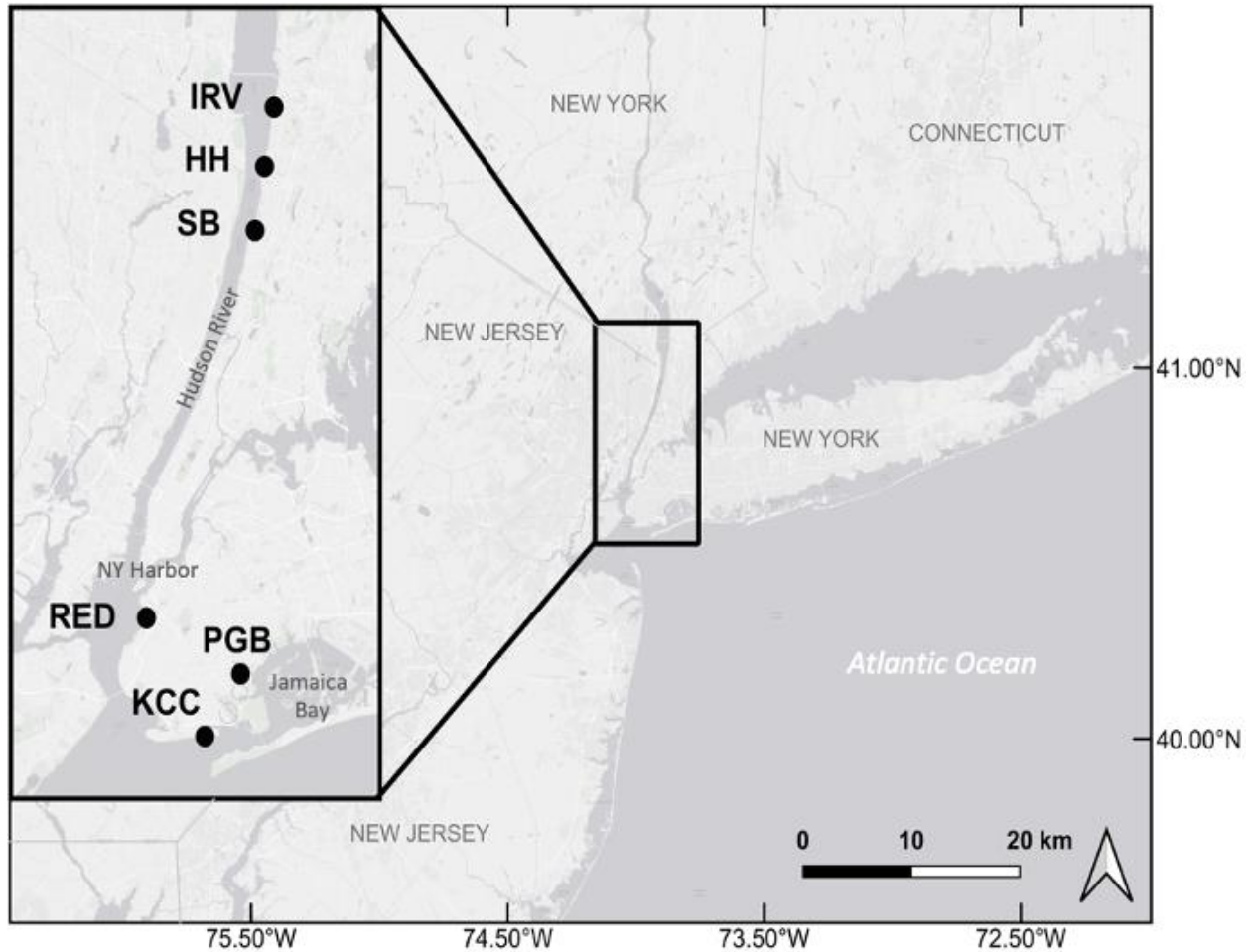
613 **Table 8.** Shannon-Wiener Diversity Index values for each unique combination of month,

614 location, and line for 2019. Locality abbreviations explained in Fig. 1 legend and in the

615 text. *Native oysters dredged and outplanted as adults

	Location	Native*	Aquaculture
July/Aug	IRV	0.199	-
	HH	0.381	-
	PGB	0.263	0.525
	KCC	0.680	0.276
Sept	IRV	0.491	-
	HH	0.514	-
	PGB	0.376	0.263
	KCC	0.387	0

616 **FIGURES**



617

618 **Fig. 1** Map of sampling locations on the Hudson River Estuary in New York, United States.

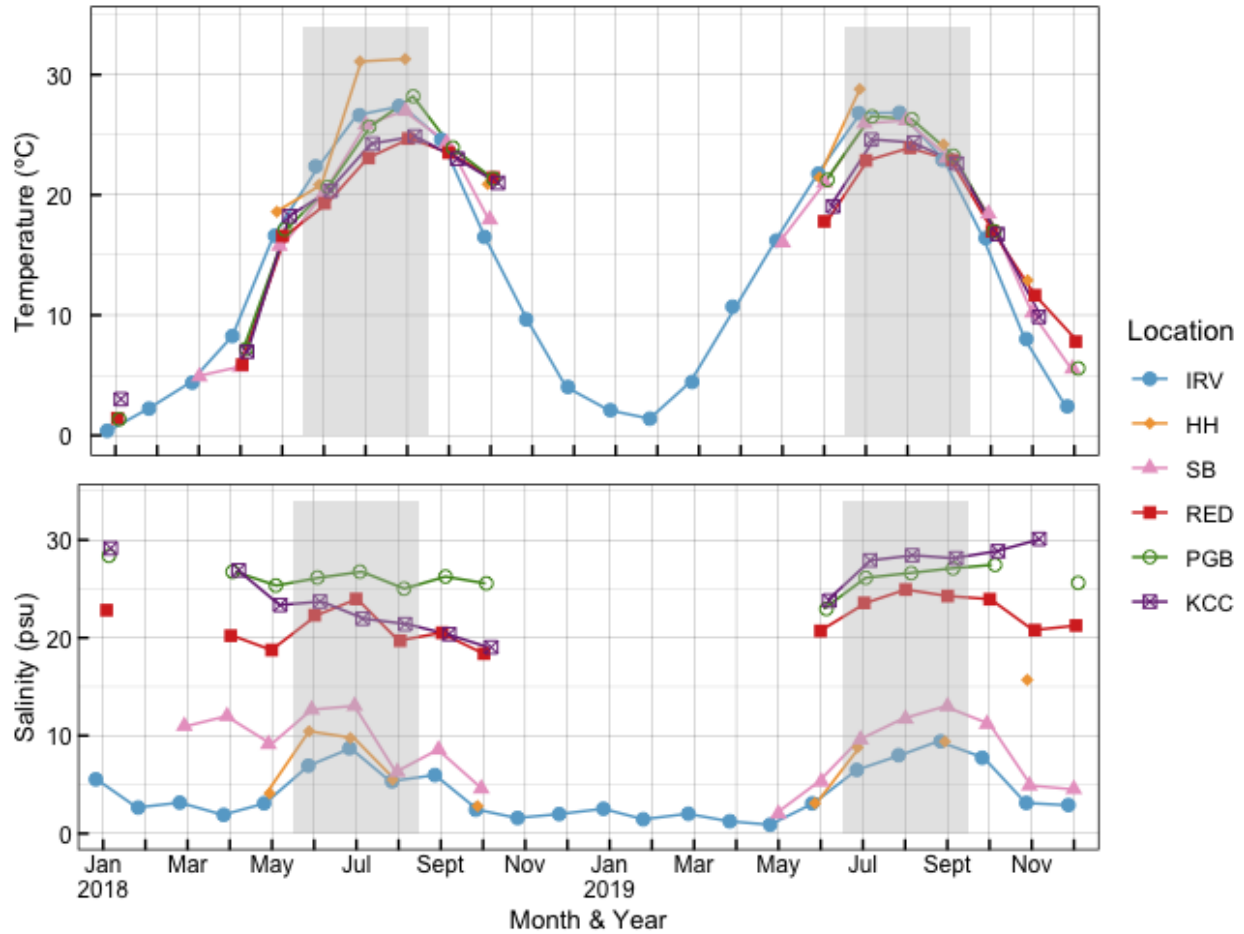
619 Sampling locations are in three named regions of the estuary: Irvington Boat Club (IRV),

620 Hastings on the Hudson (HH), and Yonkers Science Barge (SB) are north of New York

621 City in the “Lower Hudson River”, also known as the North River; Redhook (RED) is in

622 New York Harbor; Paerdegat Basin (PGB), and Kingsborough Community College

623 (KCC) are both in Jamaica Bay.



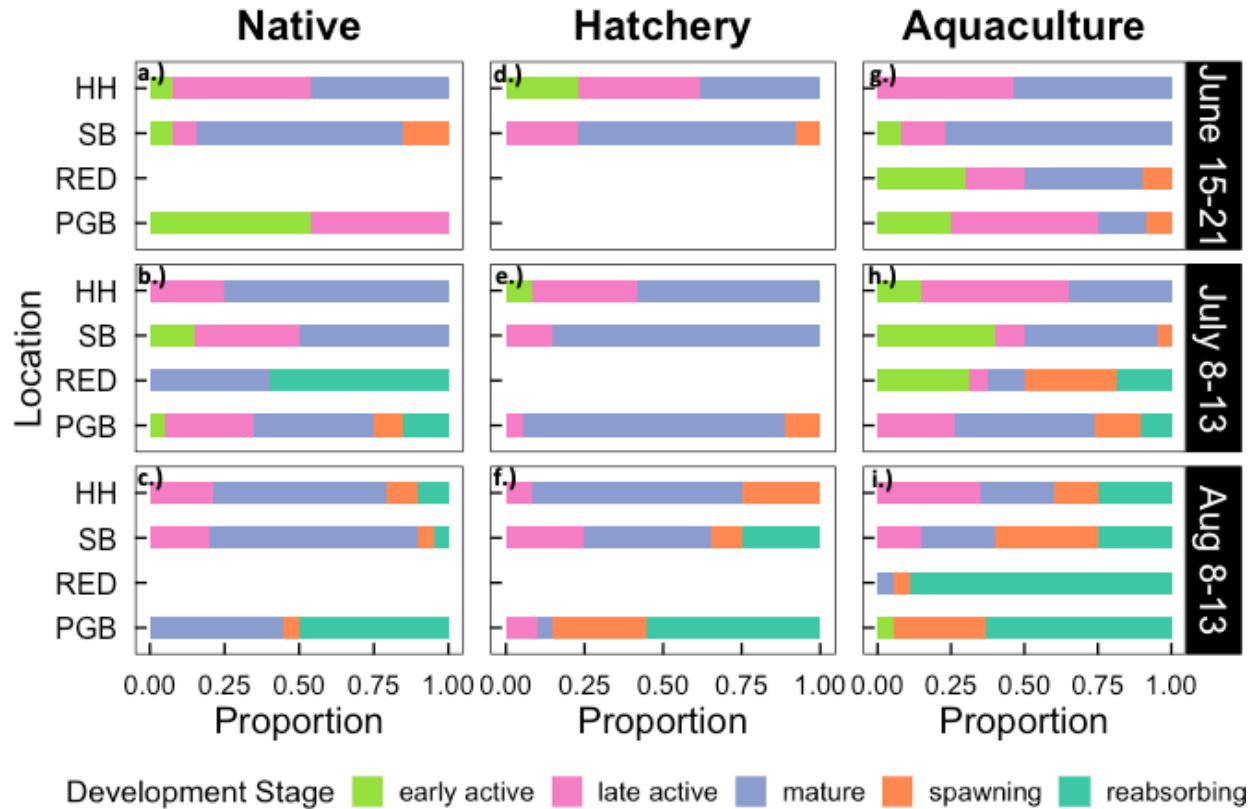
624

625 **Fig. 2** Temperature and salinity patterns in the Hudson River Estuary from January 2018 -

626 December 2019. Shaded areas indicate sampling periods. Locations are listed in order by

627 latitude, from north to south. Locality abbreviations explained in Fig. 1 legend and in the

628 text



629

630

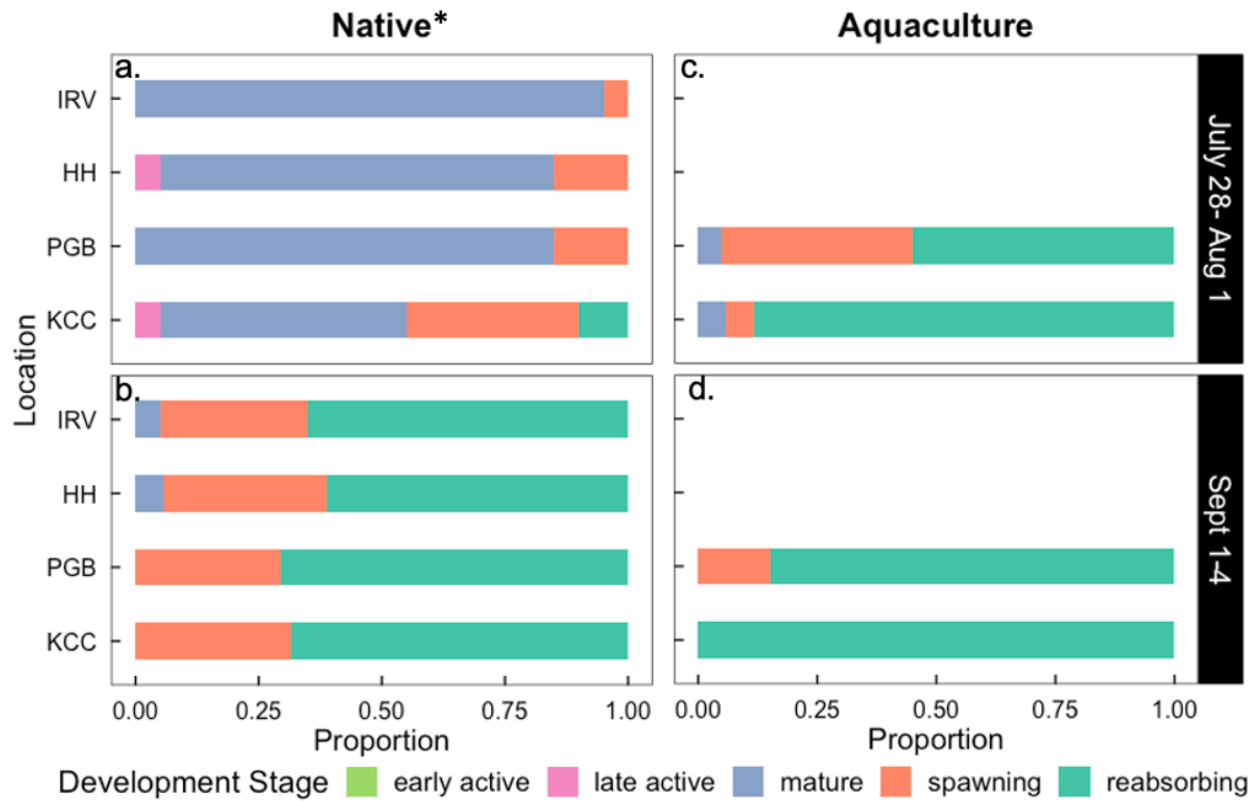
631

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633

634

Fig 3. Gametogenic development stage proportions from 2018 oyster samples by month and location. a-c.) Native line. d-f.) Hatchery line. g-i.) Aquaculture line. Sample sizes were n=13 in June and n=20 in July and August with some exceptions (see Table 2). Locations are listed in order by latitude, from north to south. Locality abbreviations explained in Fig. 1 legend and in the text



635

636 **Fig 4.** Gametogenic development stage proportions for 2019 oyster samples by month and line,

637 including Native line (a-b.) and Aquaculture line (c-d.). N=20 in all cases. Locations are

638 listed in order by latitude, from north to south. Locality abbreviations explained in Fig. 1

639 legend and in the text. *Native oysters dredged and outplanted as adults

640

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921 **ELECTRONIC SUPPLEMENTARY INFORMATION**

922 **Table S1.** Lengths of oysters sampled for histology by sex (female, male, average length (mm) ±
 923 S.E). Locations are listed in order by latitude, from north to south. Locality abbreviations
 924 explained in Fig. 1 legend and in the text

Location	Line	June 2018	July 2018	August 2018	July/August 2019	September 2019
IRV	Native	-	-	-	69.0 ± 4.5: 66.4 ± 4.9	71.2 ± 4.4, 64.6 ± 4.8
HH	Aquaculture	36.7 ± 1.8, 32.9 ± 2.2	45.2 ± 3.8, 41.0 ± 2.3	51.4 ± 4.2, 49.0 ± 2.5	-	-
	Hatchery	53.3 ± 3.8, 46.5 ± 1.8	35.6 ± 2.7, 34.6 ± 2.4	N/A	-	-
	Native	34.7 ± 4.5, 36.6 ± 1.3	42.8 ± 3.8, 38.7 ± 3.1	N/A	63.9 ± 3.4, 62.6 ± 5.1	71.2 ± 4.4, 64.6 ± 4.8
SB	Aquaculture	63.0 ± 7.4, 55.9 ± 4.8	45.8 ± 3.0, 45.5 ± 1.6	53.7 ± 5.6, 51.6 ± 4.1	-	-
	Hatchery	52.7 ± 3.4, 49.1 ± 2.7	56.0 ± 6.4, 59.0 ± 5.6	74.0 ± 3.6, 61.5 ± 2.1	-	-
	Native	42.0 ± 0.0, 38.2 ± 2.3	49.7 ± 3.2, 42.0 ± 2.1	47.1 ± 3.1, 52.0 ± 2.4	-	-
RED	Aquaculture	70.5 ± 17.5: 60.5 ± 6.2	64.0 ± 0, 45.6 ± 4.2	72.0 ± 8.0, 57.8 ± 3.6	-	-
	Hatchery	-	-	-	-	-
	Native	-	-	45.0 ± 0, 48.2 ± 2.6	-	-
PGB	Aquaculture	94.5 ± 1.5, 68.5 ± 4.3	55.2 ± 3.0, 59.4 ± 2.9	0, 52.7 ± 2.7	0, 95.0 ± 2.4	0, 91.3 ± 2.7
	Hatchery	-	65.3 ± 3.1, 57.8 ± 3.0	48.2 ± 5.0, 58.3 ± 3.2	-	-
	Native	49.2 ± 3.0, 44.6 ± 1.7	45.5 ± 3.5, 43.7 ± 1.7	45.8 ± 3.1, 52.8 ± 3.0	67.1 ± 4.7, 68.6 ± 3.2	81.5 ± 8.2, 68.3 ± 2.6
KCC	Aquaculture	-	-	-	91.5 ± 8.0, 85.7 ± 3.4	91.0 ± 5.8, 82.5 ± 3.7
	Native	-	-	-	73.1 ± 5.6, 70.6 ± 4.6	86.5 ± 4.4, 76.9 ± 2.7

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