Using multiple natural tags provides evidence for extensive larval dispersal across space and through time in summer flounder

Running title: High spatiotemporal dispersal in summer flounder

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Abstract

Dispersal sets the fundamental scales of ecological and evolutionary dynamics and has important implications for population persistence. Patterns of marine dispersal remain poorly understood, partly because dispersal may vary through time and often homogenizes allele frequencies. However, combining multiple types of natural tags can provide more precise dispersal estimates, and biological collections can help reconstruct dispersal patterns through time. We used SNP genotypes and otolith core microchemistry from archived collections of larval summer flounder (*Paralichthys dentatus*, n = 411) captured between 1989-2012 at five locations along the U.S. East coast to reconstruct dispersal patterns through time. Neither genotypes nor otolith microchemistry alone were sufficient to identify the source of larval fish. However, microchemistry identified clusters of larvae (n = 3-33 larvae/cluster) that originated in the same location, and genetic assignment of clusters could be made with substantially more confidence. We found that most larvae likely originated near a biogeographic break (Cape Hatteras) and that larvae were transported in both directions across this break. Larval sources did not shift north through time, despite the northward shift of adult populations in recent decades. Our novel approach demonstrates that summer flounder dispersal is widespread throughout their range, both on intra- and inter-generational timescales, and may be a particularly important process for synchronizing population dynamics and maintaining genetic diversity during an era of rapid environmental change. Broadly, our results reveal the value of archived collections and of combining multiple natural tags to understand the magnitude and directionality of dispersal in species with extensive gene flow.

Introduction

Dispersal sets the fundamental scales over which ecological and evolutionary dynamics of populations occur. Dispersal drives connectivity, or the exchange of individuals among populations (Cowen & Sponaugle 2009), and the degree of connectivity influences population dynamics (Huffaker 1958; Gotelli 1991; Hanski & Gilpin 1997; Runge *et al.* 2006), community composition (Connolly *et al.* 2001), evolution (Wright 1931; Slatkin 1987), persistence (Botsford *et al.* 2001; Hastings & Botsford 2006), and management strategies (Fogarty & Botsford 2007). Yet, understanding dispersal in the marine realm is challenging, especially since dispersal may vary over time (Reis-Santos *et al.* 2013; Nanninga & Berumen 2014) and may homogenize allele frequencies (Gleason & Burton 2016; Sandoval-Castillo *et al.* 2018). Our understanding of dispersal through time is often limited by our ability to sample across relevant seasonal, interannual, or decadal scales. Fortunately, natural history collections provide powerful, underappreciated, and often underutilized opportunities to retrospectively study biological diversity in populations of interest (Schwartz *et al.* 2007; Johnson *et al.* 2011; Pimm *et al.* 2015). As unique repositories of life on Earth, natural history collections preserve individuals and their natural markers across space and time (Watanabe 2019). These specimens are particularly useful for investigating a wide range of ecological and evolutionary processes (Holmes *et al.* 2016; Webster 2018), especially during an era of rapid environmental change (Meineke *et al.* 2019).

A variety of natural markers have been used to study the extent and rate of exchange between populations (Thorrold *et al.* 2002). Assignment methods using genetic markers have been developed to determine the most likely population an individual or a group of individuals belongs to, or to exclude individuals of interest from potential populations of origin (see review by Manel, Gaggiotti, & Waples, 2005). Genetic assignment methods (Paetkau *et al.* 1995, 2004;

84 Rannala & Mountain 1997; Cornuet *et al.* 1999; Pritchard *et al.* 2000) have been used to ascertain
85 population membership or infer dispersal between populations of fishes (Shaklee *et al.* 1999;
86 Primmer *et al.* 2000; Nielsen *et al.* 2001, 2012; Glover *et al.* 2008), birds (Claramunt & Wright
87 2018; Townsend & Navarro-Siguenza 2018), reptiles (Berry *et al.* 2004), polar bears (Paetkau *et*
88 *al.* 1995), deer (Frantz *et al.* 2006), and humans (Rannala & Mountain 1997). While genetic
89 assignment has most often been used with putatively neutral loci, using non-neutral (candidate)
90 loci that are more spatially diverged can be particularly useful in species with high rates of gene
91 flow (Nielsen *et al.* 2009, 2012; Freamo *et al.* 2011).
92 Connectivity and dispersal studies using genetic markers have clearly been informative,
93 but recently, approaches that utilize multiple types of markers have highlighted complementary
94 results on different timescales or illuminated otherwise hidden patterns (Bradbury *et al.* 2008;
95 Papetti *et al.* 2013; Tanner *et al.* 2014; Barton *et al.* 2018; Reis-Santos *et al.* 2018). A number of
96 marine dispersal studies in particular have started to combine genetics with microchemistry,
97 another type of natural marker. Otoliths (fish ear stones), statoliths (related structures in
98 invertebrates), and shells are structures that grow over an individual's lifetime, are metabolically
99 inert once deposited, and incorporate trace elements into their inorganic ($CaCO_3$) and organic
100 matrices (Thorrold *et al.* 2007). Thus, microchemistry reflects the site-specific environmental
101 characteristics of ambient waters in which each individual resided, starting with the natal core
102 that is formed upon fertilization at the spawning and hatching site (Thorrold *et al.* 1997).
103 Microchemistry can be used to retroactively detect residency and movement within or between
104 estuarine and marine systems provided that spatial gradients in temperature, salinity, or water
105 chemistry exist (Gillanders & Kingsford 1996; Thorrold *et al.* 2001; Vasconcelos *et al.* 2008;
106 Schaffler *et al.* 2009). However, unlike genetic markers that can integrate over multiple

107 generations to offer a deeper historical perspective (Lowe & Allendorf 2010), microchemistry
108 data within otoliths are limited to individual lifespans (Gillanders 2002). Combined approaches
109 using genetics and microchemistry promise to improve our understanding of dispersal, but studies
110 have generally analyzed these datasets in parallel rather than in a truly integrated framework
111 (Bradbury *et al.* 2008; Papetti *et al.* 2013; Barton *et al.* 2018; but see Tanner et al. 2014 & Reis-
112 Santos *et al.* 2018).
113 Many recent studies of marine larvae have demonstrated that dispersal is more
114 constrained than previously imagined (Jones *et al.* 2005; Almany *et al.* 2017; Baetscher *et al.*
115 2019). Larval dispersal may be particularly limited around biogeographic breaks, such as Cape
116 Hatteras in North Carolina. Cape Hatteras has been found to be an important barrier to larval
117 dispersal for a variety of invertebrates and fish (Baker *et al.* 2008; Roy *et al.* 2012) because the
118 Gulf Stream transports larvae offshore and because its divergence results in a steep thermal
119 gradient (Briggs 1974).
120 One species with a distribution straddling Cape Hatteras and that is thought to experience
121 limited dispersal across Cape Hatteras (Wilk *et al.* 1980; Kraus & Musick 2001) is summer
122 flounder (*Paralichthys dentatus*). The directionality and magnitude of larval summer flounder
123 dispersal remains unknown. Summer flounder inhabit waters from Nova Scotia, Canada to
124 Florida, USA (Packer *et al.* 1999). Relatively homogenous allele frequencies at most loci suggest
125 substantial dispersal throughout this range, though candidate loci under spatially divergent
126 selection have also been identified (Jones & Quattro 1999; Hoey & Pinsky 2018). Larval summer
127 flounder are spawned over the continental shelf, with the majority occurring between Cape Cod,
128 Massachusetts and Cape Lookout, North Carolina (Smith 1973) during the fall and early winter
129 when adults move offshore (Able & Fahay 2010). It is unknown whether more specific spawning

grounds exist. Larval summer flounder develop in the coastal ocean, but ingress to estuaries soon before settling down to their juvenile habitat; a process that is thought to take ~30 days in ambient spring temperatures (Keefe & Able 1993). Ingressing larvae have been collected and archived at sites throughout the species range since the 1980s. With archived specimens and allele frequency differences along the coast at candidate loci, summer flounder offer an ideal opportunity to test the use of multiple natural markers to assign larvae back to their natal origins over 24 years.

In this study, we combine double-digest restriction-site associated DNA sequencing (ddRADseq) and otolith core microchemistry data on collections of larval summer flounder from 1989-2012 to investigate natal origins and dispersal over time. We ask: (1) Do larval summer flounder exhibit regional genetic population structure and has it remained stable over a quarter century? (2) How do elemental signatures from the natal core of larval otoliths differ across space and time? and (3) Does combining genetic and otolith markers improve our understanding of the extent to which larval dispersal has varied across space and through time?

Materials and Methods

Larval collections & curation

To explore regional patterns of larval population structure and connectivity throughout the majority of the species' range, we leveraged several ongoing larval ingress survey and collection programs along the U.S. East coast (Figure 1 & Table 1). We primarily obtained larvae collected at the Rutgers University Marine Field Station (RUMFS; Little Egg Inlet, New Jersey) and the National Oceanic & Atmospheric Administration's Beaufort (North Carolina) Laboratory starting in 1989. At both ingress locations, ichthyoplankton were collected from a bridge during night-

153 time flood tides on a weekly basis and sorted to species (see Sullivan *et al.* 2006; Able *et al.* 2011
154 for sampling protocol). To ensure adequate sample size for this study, larvae were pooled by
155 month and 50 larvae were assembled from each of three time periods: 1989-1993, 1998-2002 and
156 2008-2012. These time periods are hereafter referred to as early, middle and late, respectively. To
157 sample from the peak ingress periods for New Jersey (NJ) and North Carolina (NC),
158 approximately five larvae were selected from winter (January-March) and five from fall
159 (October-December) for each year. Additional ingressing summer flounder larvae were obtained
160 from collections taken at Roosevelt Inlet, Delaware (DE; 2008-2010), York River, Virginia (VA;
161 2008-2010), and North Inlet, South Carolina (SC; 2008) to extend the spatial sampling of this
162 species' range. Larvae from these additional sites were taken from both winter and fall periods,
163 when possible, to match the collection periods in New Jersey and North Carolina; Virginia and
164 Delaware specimens were among those reported in Ribeiro *et al.* (2015). Virginia specimens are
165 cataloged in the Nunnally Ichthyology Collection at the Virginia Institute of Marine Science
166 (VIMS Catalog Numbers 19445-19494). All larval summer flounder (n = 411) had been stored in
167 95% ethanol at their respective institutions.
168
169 *Population structure analyses*
170 Methods for obtaining genotypes at 1,904 loci across 293 larval individuals are detailed in
171 the Supporting Information (Appendix S1). We performed principal component analysis (PCA)
172 using the *adegenet* v.2.0.1 (Jombart 2008) and *ade4* v.1.7-10 (Chessel *et al.* 2004) packages in R
173 v.3.3.3 (R Core Team 2017). We then sorted individuals into regions (collected north or south of
174 Cape Hatteras, NC) and time periods (early, middle & late) and performed two hierarchical
175 analyses of molecular variance (AMOVA) using the *ade4* method of the *poppr.amova* function in

176 the *poppr* v.2.4.1 package (Kamvar *et al.* 2014) in R with 1,000 permutations each. The first
177 AMOVA tested for differences in genetic variance among time periods (nested within regions)
178 and differences between regions. The second AMOVA examined differences between regions
179 (nested within time periods) and differences between time periods (Excoffier *et al.* 1992). Weir
180 and Cockerham's pairwise F_{ST} was also calculated using the *pairwise.WCfst* function in the
181 *heirfstat* v.0.04-22 package (Goudet 2005) in R for each pair of unique ingress site and time
182 period groups. These analyses tested how genome-wide allele frequencies differed on average
183 across space and time.
184 Next, we used STRUCTURE v.2.3.4 (Pritchard *et al.* 2000) to determine the number of
185 putative populations. We ran STRUCTURE using all 1,904 loci and a burn-in of 10,000 iterations
186 followed by 200,000 Markov chain Monte Carlo (MCMC) steps assuming admixture and
187 correlated allele frequency models with prior information on sampling location and time period.
188 We ran 10 replicates of K from 1-5, where K is the number of population clusters, and we
189 checked for parameter stabilization. We also ran STRUCTURE with the 1,646 remaining loci
190 after removing 258 loci not in Hardy-Weinberg proportions (HWP; $p < 0.01$, exact test, *pegas*
191 v.0.10 package), but the results were effectively identical and are not further discussed.
192 To determine the optimal number of clusters, the 10 replicates for each K were input into
193 STRUCTURE HARVESTER (Earl & VonHoldt 2012) and visualized with CLUMPP (Jakobsson
194 & Rosenberg 2007) and Distruct (Rosenberg 2004). Based on previous work (Hoey & Pinsky
195 2018), we expected difficulty determining the optimal K and so we used both the mean likelihood
196 value (L(K)) and ΔK (Evanno *et al.* 2005).
197
198 *Genetic assignment of individual larvae*

As previously reported in Hoey & Pinsky (2018), fifteen of 1,137 loci in adult summer flounder were found to be associated with distance along the coast, depth, bottom temperature and/or bottom salinity, and exhibited allele frequency differences along the coast. Of the 15 candidate loci previously identified in adults, 10 passed our filtering criteria in larval fish as described in 'Bioinformatics & genotyping' (Appendix S1). Generalized additive models (GAMs) with a binomial error structure were fit for each of these 10 candidate loci to relate individual allele counts of adults to distance along the coast. We used the *predict.gam* function in the *mgcv* package (Wood 2011) in R to predict allele frequencies of our candidate loci at 10 equidistant reference locations across the adult summer flounder sampling range (Figures 1 & S1). These GAM-determined allele frequencies formed the 'genetic map' to which larval summer flounder were assigned.

We used these 10 loci to determine assignment accuracy of different sized larval clusters. We simulated groups of one, five, 10, 20 or 30 diploid individuals from each of the 10 potential spawning reference locations based on allele frequencies at the 10 candidate loci (Table S2) using a custom R script. We did this 1,000 times and assigned (Paetkau *et al.* 1995) each simulated individual or group of individuals to the most likely reference location using a custom R script employing equation 10 of Rannala & Mountain (1997). We then examined the percentage of correct assignments.

To calculate individual genetic assignment, we calculated the genotype likelihood for each observed individual using the GAM-determined allele frequencies at 10 loci and a custom R script employing equation 10 of Rannala & Mountain (1997) at 10 distances along the coast (Table S2). We then assigned each individual larva to the spawning location with the maximum genotype likelihood.

222

223 *Otolith microchemistry analyses*

224 Detailed methods for obtaining microchemistry data from larval otolith cores may be
225 found in the Supporting Information (Appendix S2). To test the null hypothesis of no difference
226 in natal core microchemistry among larvae ingressing to different estuaries or during different
227 time periods, the effects of ingress site and time period for each elemental ratio (Sr:Ca, Mg:Ca,
228 Mn:Ca, Fe:Ca, Cu:Ca, Cd:Ca, Ba:Ca, Sn:Ca, Pb:Ca & U:Ca) were analyzed using a two-way
229 analysis of variance (ANOVA) following $\log_{10}$-transformation. Multivariate analysis of variance
230 (MANOVA) was also used to test for differences in combined larval otolith core trace elements
231 among ingress sites and time periods. Data were scaled and then nonmetric multidimensional
232 scaling (nMDS) was performed using the *nmds* function in the *ecodist* v.2.0.1 package (Goslee &
233 Urban 2007) in R for each time period.

234 As an additional test of the extent to which larvae ingressing to the same site also shared
235 similar natal core signatures, we performed linear discriminant function analysis (LDA) despite
236 the likely incorrect assumption of a single larval source per ingress location. The typical use of
237 LDA classifies individuals based on the microchemistry of known locations. This was not our
238 goal. Instead, we tested whether ingress site could be predicted from natal core signatures. If
239 ingress site could be accurately predicted, it would suggest that larvae ingressing to the same
240 estuary had been born near each other at similar natal sites, even though such natal sites were
241 geographically far from the ingress site. If ingress site could not be predicted, it would suggest
242 substantially more mixing between natal sites and ingress sites. We used all 10 scaled elemental
243 ratios and the leave-one-out jackknife procedure in the *MASS* v.7.3-47 package (Venables &
244 Ripley 2002) in R. We calculated 68% confidence ellipses for individuals captured from each

245 ingress site using the *ellipse* v.0.3-8 package (Murdoch & Chow 1996) in R, and used these 141
246 individuals as a training dataset for LDA to define otolith natal core signatures for larvae
247 ingressing at each site. We then predicted the ingress site of the remaining 56 individuals using
248 the elemental signature at the natal core. Posterior group membership probabilities were also
249 determined.

250 To avoid making *a priori* assumptions about group membership, we also performed
251 clustering for each time period separately using all 10 elements. The optimal number of clusters
252 was determined using the *fviz_nbclust* and *NbClust* functions in the *factoextra* v.1.0.5
253 (Kassambara & Mundt 2017) and *NbClust* v.3.0 (Charrad *et al.* 2014) packages, respectively, in
254 R. The optimal number of clusters then informed k-means clustering using the *kmeans* function in
255 the *stats* package.

256

257 *Assignment and exclusion of larval groups using otolith microchemistry & genetics*

258 For larvae with both otolith microchemistry and genetic data, we utilized both kinds of
259 data to further investigate larval origins, rather than relying on either data type alone. Since larvae
260 dispersed from the continental shelf to the ingress estuary in which they were captured, we used
261 otolith microchemistry at the natal core to cluster individuals that were likely spawned together in
262 the same offshore water mass. We then assigned natal origins to the clusters of larvae using
263 genetic assignment and exclusion tests. We utilized groups of larvae and pooled genotype data
264 for increased assignment accuracy (Baudouin *et al.* 2004).

265 First, we subset the data by time period (early, middle & late) and performed clustering in
266 the same fashion as for the larvae with only otolith microchemistry data.

Second, we used genetic assignment and exclusion tests to determine the natal origins of clustered larvae. For assignment, we calculated the observed likelihood that each cluster originated from each of the 10 potential spawning reference locations using the GAM-determined allele frequencies at the 10 candidate loci (Rannala & Mountain 1997). We assigned each cluster to the spawning location with the maximum likelihood.

For the exclusion method, we used a Monte Carlo resampling method that employs allele frequencies from reference locations (Rannala & Mountain 1997; Cornuet *et al.* 1999) to generate distributions of the likelihood criteria that each larval cluster originated from a given spawning location. For comparison against a given cluster composed of Z individuals, we randomly constructed Z genotypes from the allele frequencies in each of the 10 reference locations at each of the 10 loci in adult summer flounder (Table S2). We repeated this 10,000 times to produce the expected distributions of likelihood values for Z individuals that originated in each of the 10 potential locations of origin. For each cluster, the resampled distribution of likelihood criteria was compared to the corresponding observed genotype likelihood for each reference location, and the probability that the cluster of individuals originated from the reference location was calculated as the proportion of resamples with genotype likelihoods less than the observed value (Cornuet *et al.* 1999). Unlike the assignment method, this method allowed us to calculate a measure of confidence that a cluster of larval individuals originated from a potential spawning location. Similar to Berry *et al.* (2004), each cluster of larval individuals was assigned to or excluded from potential source locations in three ways. Individuals were (i) assigned to the most likely location, (ii) excluded from all locations with $\geq 80\%$ confidence of exclusion ($P \leq 0.20$), and (iii) excluded from all locations with $\geq 95\%$ confidence of exclusion ($P \leq 0.05$).

289 To examine whether our dataset had evidence of siblings dispersing together, we
290 conducted an exploratory sibship analysis with Colony (Jones & Wang 2010). No siblings were
291 detected and the analysis was not pursued further.
292
293 **Results**
294 *Genotyping results*
295 The average number of quality-filtered reads per individual was 868,180 ± 811,927 (mean
296 ± SD). Mapping to our reference assembly resulted in average coverage of 13x. Variant calling
297 across larvae and adults identified 422,767 putative SNPs, and of these, 1,904 loci with an
298 average read depth of 71x across 293 larvae passed filtering.
299
300 *Population structure*
301 A PCA suggested that larval summer flounder were genetically similar across space and
302 time at a genome-wide scale (Figure S2), and these results were confirmed using AMOVA,
303 regardless of hierarchical level (Tables S3 & S4). Pairwise F_{ST} values between ingress site and
304 time period groups were generally quite small (-0.0016 to 0.0019), except for those including the
305 one larva from North Inlet, SC (Table S5).
306 After testing $K = 1$ to $K = 5$ in STRUCTURE, the mean likelihood value (L(K)) and the
307 Evanno method (ΔK) indicated $K = 1$ and $K = 2$ clusters for the full dataset containing 1,904 loci,
308 respectively. We interpret these results as a lack of population structure in larvae because all
309 individuals were admixed at approximately the same proportions, regardless of the K value
310 (Figure S3).
311

312 *Individual assignment using genetics*

313 Simulated individual larval genetic assignment using the 10 candidate outlier loci
314 revealed weak resolution for assigning individuals back to location of origin, though with greatest
315 confidence for larvae originating in the extreme northern (A) and extreme southern (J) locations
316 (Figure 2A; ~47% accuracy in both cases). Our ability to assign individual larvae was limited
317 because genotype likelihoods were quite similar between potential source locations (Figure S4).
318 However, simulated individuals drawn from north (A-E) or south (F-J) of Cape Hatteras, NC
319 were usually assigned back to the correct north (~57-78% accuracy) or south side of Cape
320 Hatteras (~53-79% accuracy; Figure 2A).

321 Across all time periods, observed larvae ingressing to Little Egg Inlet, NJ; Roosevelt
322 Inlet, DE; and York River, VA were equally likely to be assigned back to sources north and south
323 of Cape Hatteras (52% vs. 48% for NJ; 56% vs. 44% for DE; and 46% vs. 56% for VA,
324 respectively). The majority of larvae ingressing to Beaufort, NC (69%) were assigned to sources
325 south of Cape Hatteras and the individual from North Inlet, SC likely originated from the
326 southern-most (J) reference location (Figure S5).

327

328 *Otolith microchemistry*

329 Otolith microchemistry resulted in high-quality data for 197 larval individuals. Otolith
330 core microchemistry varied significantly among larvae ingressing to different estuaries for Mg,
331 Mn, Fe, Ba and Sn (two-way ANOVA across ingress site and time period, $p < 0.01$; Table S6 &
332 Figure S6) and over time for Mg and Pb (two-way ANOVA, $p < 0.01$; Table S6 & Figure S7).
333 The combined elemental signatures at the natal otolith core significantly differed among ingress
334 sites (MANOVA: Pillai's trace = 0.929, $F_{3,193} = 8.35$, $p < 0.0001$) and time periods (MANOVA:

335 Pillai's trace = 0.368, $F_{2,194} = 4.199$, $p < 0.0001$). Segregation between ingress sites located north
336 (NJ and DE) and south (VA and NC) of Cape Hatteras was evident in the nMDS based on otolith
337 core microchemistry within each time period (Figure 3).

338 When no *a priori* assumptions about group membership were made, clustering of larvae
339 based on otolith microchemistry data revealed that many clusters were composed of larvae that
340 ingressed either to the same estuary or to adjacent estuaries (Figure S8). Even with the likely
341 incorrect assumption of a single larval source per ingress location, LDA analysis also showed that
342 individuals captured at an ingress site had natal signatures characteristic of other larvae captured
343 at the same ingress site, suggesting that they were spawned in roughly similar locations (Figures
344 S9 & S10), even when LDA was performed for each time period separately (Figure S11). In
345 reality, ingress sites likely include larvae from multiple natal sources. Mg, Mn, Fe, and Sn drove
346 many of the patterns observed in LDA classification.

347

348 *Cluster assignment and exclusion using otolith microchemistry & genetics*

349 In contrast to individual larval assignments, we found greatly improved accuracy when
350 assigning clusters of larvae identified through shared elemental signatures, especially as the size
351 of the larval clusters increased (Figures 2 & S4). Multiple k-means clustering techniques
352 determined that the optimal number of larval clusters were six, two, and three for the early,
353 middle and late time periods, respectively (Figure S12).

354 When clusters were assigned to the most likely reference location, eight of the clusters
355 (73%) were assigned to the reference locations nearest Cape Hatteras, NC (Locations E & F). The
356 remaining three clusters were assigned to Location C or Location G (Figure 1 & Table 2). In

particular, the method had confidence in the assigned origins of the larger clusters from the middle and late time periods (Figure 2D-E).

Larvae ingressed to estuaries both close to and far from their most likely location of origin. For example, individuals in clusters E1, E5 and E6 all ingressed at Beaufort, NC and were most likely to originate from the reference locations closest to Beaufort, NC (Locations F & G). In contrast, some individuals in cluster M2 ingressed to Little Egg Inlet, NJ, but likely originated off southern Virginia (Location E; Figure 4).

The assignment results revealed substantial dispersal across the putative biogeographic break at Cape Hatteras. For example, the majority of individuals in cluster M2 ingressed at Beaufort, NC, but were most likely to originate from the reference location just north of Cape Hatteras, NC (Location E; Figure 4). In addition, the majority of individuals in cluster L1 ingressed at Roosevelt Inlet, DE and Little Egg Inlet, NJ, but were most likely to originate south of Cape Hatteras, NC (Location F; Figure 4). The assignment results suggest that exchange of larval summer flounder throughout the species range is common, frequently extends across Cape Hatteras, and sometimes occurs in the direction opposite the dominant Gulf Stream.

The exclusion method suggested that clusters E3 and L2 had low probabilities ($p < 0.20$) of originating from southern reference locations and could therefore be excluded with high confidence (Table 2). These results supported and were consistent with the assignment results. Other clusters could not be confidently excluded from particular reference locations.

Discussion

Natural history collections, and natural tags intrinsic to preserved specimens, are useful for investigating a wide range of ecological and evolutionary processes (Webster 2018). We

utilized intra-generational otolith microchemistry and inter-generational genetic markers, separately and in combination, from archived larval summer flounder captured across a quarter century to reveal that contemporary dispersal during the larval stage is sufficiently widespread to result in extensive population mixing along the U.S. East coast. Neither genetics nor otolith microchemistry alone were adequate for identifying the origins of larval fish because allele frequencies were homogeneous among source locations and because elemental signatures at potential spawning locations could not be validated. However, natal origins could be identified with greater accuracy when data from candidate loci and otolith microchemistry were combined in an integrated approach. We found that many larvae were most likely to originate in the vicinity of Cape Hatteras, larvae dispersed both near and far from their site of origin, and dispersal sometimes occurred in the opposite direction to the northward-flowing Gulf Stream.

Single- and multi-marker approaches to infer dispersal

Genetic assignment tests are widely used to determine the population of origin for an individual or a group of individuals in order to infer dispersal rates, identify immigrants, recognize hybridization events or classify the proportion that each source population contributed to a mixture of individuals (Manel *et al.* 2005). However, the utility of these methods may be reduced when effective population size is large and genetic differentiation is weak (Berry *et al.* 2004; Allendorf *et al.* 2010), as is often the case for many species (Ward *et al.* 1994; Waples 1998), including summer flounder (Hoey & Pinsky 2018). Low genetic differentiation at neutral markers has limited efficacy for population assignment because of ongoing gene flow. However, candidate outlier loci, or gene-associated markers, that arise due to divergent environmental selection, are promising for population assignment because of their elevated differentiation

403 compared to neutral markers (Nielsen *et al.* 2009, 2012; Freamo *et al.* 2011; Benestan *et al.* 2015;
404 DeSaix *et al.* 2019). Genetically diverged candidate loci contain higher information content,
405 allowing greater assignment accuracy than would be possible using an equal or greater number of
406 neutral loci. For example, Freamo *et al.* (2011) achieved 85% assignment accuracy when using
407 14 candidate outlier loci compared to 75% assignment accuracy when using 67 neutral loci in
408 salmon, and studies in other systems have achieved equal success using many more markers
409 (Rannala & Mountain 1997; Benestan *et al.* 2015; DeSaix *et al.* 2019). The 10 candidate outlier
410 loci available for summer flounder were differentiated across geography, but only weakly so,
411 which resulted in low power to assign individuals to populations of origin. Identification of
412 additional candidate outlier loci would likely improve our power to distinguish the origins of
413 individual larval summer flounder when using only genetic assignment.
414 Otoliths are useful for studying dispersal and population connectivity within individual
415 lifespans of fishes (Thorrold *et al.* 2001). The conventional use of otolith microchemistry for
416 population assignment requires that a chemical atlas can be accurately created, typically by
417 capturing individuals at a location that corresponds to the otolith section being studied (i.e.,
418 captured at the natal site when analyzing the otolith core). By capturing individuals from all
419 known natal locations, a reference elemental atlas is developed to which other individuals can be
420 assigned (Gillanders 2002; Shima & Swearer 2009). As a result, these studies are often limited to
421 species spawned in shallow bays and estuaries where water chemistry differences are greatest.
422 However, many marine species are spawned in more open, coastal environments, often resulting
423 in a temporal disconnect between the natal core and the individual's capture location. Chemical
424 atlases are difficult to recreate for such species, meaning that for summer flounder, otolith
425 microchemistry alone could not be used to explicitly define the specific natal location(s). Rather,

426 otolith microchemistry indicated that ingressing summer flounder larvae spanned a range of natal
427 environmental signatures. Some groups of larvae were spawned in the same offshore location and
428 dispersed together, but mixing along the coast was also evident. We note that traditional
429 assignment of summer flounder natal sources using otolith microchemistry could potentially be
430 achieved using several long-term collections of ichthyoplankton on the continental shelf,
431 including the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) and the
432 Ecosystem Monitoring (EcoMon) programs (Richardson *et al.* 2010; Walsh *et al.* 2015), which
433 would be useful to corroborate our results.

434 In many cases, neither microchemistry nor genetics alone are particularly helpful for
435 assigning individuals to source populations (Cornuet *et al.* 1999; Gillanders *et al.* 2001; Manel *et*
436 *al.* 2005). In this paper, we instead combined both natural tags together to resolve natal sources in
437 a more spatially explicit context and to achieve greater power for assignment. We first clustered
438 individuals from each time period using elemental microchemistry (Tanner *et al.* 2012), and then
439 used allele frequencies at spatially differentiated candidate loci for genetic population
440 assignment. In doing so, we were able to account for baseline differences in elemental chemistry
441 within each time period while concurrently taking advantage of increased power for genetic
442 assignment of groups (Baudouin *et al.* 2004) despite relatively few genetic markers. Assignment
443 validation indicated particularly robust results when clusters were composed of ~20 individuals
444 or more, which was true of all our clusters except those from the earliest time period. Our
445 combined multi-marker approach allowed for a higher resolution understanding of larval
446 dispersal along the U.S. East coast over a quarter century than would otherwise be possible using
447 a single-marker. Similar approaches are likely to be useful for future connectivity studies of
448 coastally spawned species.

449

450 *Dispersal across biogeographic breaks*

451 Due to the positive correlation between pelagic duration and dispersal distance (Shanks
452 2009), marine populations were once thought to be highly connected and well-mixed, but recent
453 investigations of realized population connectivity in the sea have documented much more limited
454 dispersal (Palumbi 2003; Jones *et al.* 2005; Almany *et al.* 2017; Baetscher *et al.* 2019).
455 Biogeographic breaks are areas where physical processes create sharp physical and biochemical
456 discontinuities. Such discontinuities occur in all oceans and are thought to limit larval exchange
457 (Galarza *et al.* 2009). Thus, biogeographic breaks provide interesting and important opportunities
458 to understand dispersal scales in the ocean (Cowen *et al.* 2006). We tested for larval summer
459 flounder dispersal across Cape Hatteras, a known biogeographic break for a variety of other
460 invertebrates and fish (Briggs 1974; Baker *et al.* 2008; Roy *et al.* 2012), and a purported barrier
461 for summer flounder (Wilk *et al.* 1980; Kraus & Musick 2001). However, we found larval
462 dispersal to be bidirectional across Cape Hatteras, suggesting that Cape Hatteras does not
463 function as a strong biogeographic barrier to movement for summer flounder larvae. We also
464 found that larval summer flounder ingressed to locations both near and far from their most likely
465 origin (~100-500 km), providing empirical evidence for substantial connectivity across space in
466 the sea.

467 Both biological characteristics of summer flounder and oceanographic processes likely
468 influenced the high population connectivity we observed. Summer flounder are highly fecund and
469 exhibit serial spawning across an extended season, though peak spawning coincides with the
470 autumn breakdown of the thermocline and the resulting plankton bloom (Morse 1981).
471 Northward movement across Cape Hatteras could be achieved if larvae were entrained in the

472 Gulf Stream and subsequently concentrated and transported back west across the continental
473 shelf via warm core rings (a type of mesoscale eddy; Hare & Cowen 1996; Hare *et al.* 2002) or
474 filaments between mesoscale eddies (Harrison *et al.* 2013) prior to ingress. The southwest
475 propagation of warm core rings (Auer 1987) and the southerly flow of shelf and slope waters due
476 to the Labrador Current (Bumpus 1973) could facilitate southward dispersal of larvae spawned in
477 the Mid-Atlantic Bight, with flux across Cape Hatteras occurring via wind or buoyancy-driven
478 intrusions (Stegmann & Yoder 1996; Grothues *et al.* 2002). Biological characteristics, such as
479 iteroparity and considerable larval production, may also interact with physical oceanographic
480 features to increase retention and upstream spread of larval summer flounder in the Mid- and
481 South Atlantic Bights (Byers & Pringle 2006).
482
483 *Dispersal over time: implications, assumptions & opportunities*
484 Empirical estimates of dispersal over time are crucial for validating our theoretical
485 understanding of the variable nature of larval dispersal and its consequences (Siegel *et al.* 2008)
486 and for improving fisheries management (Fogarty & Botsford 2007). An increasing number of
487 studies have examined dispersal over intraseasonal (Cook 2011) and interannual (Kraus & Secor
488 2005; Hogan *et al.* 2012; Reis-Santos *et al.* 2013) scales, but dispersal estimates for more than
489 two cohorts and over decadal scales are rare. Similar to our theoretical understanding, these
490 empirical studies indicate that dispersal distances, local retention and patterns of larval
491 connectivity can be quite variable over time. In summer flounder, we also found variation in
492 dispersal patterns, with some estuaries receiving more larvae from particular spawning locations
493 in certain time periods. However, we found instances of larval summer flounder originating both
494 near and far from their ingress site, and this phenomenon appeared regularly in each of three time

periods examined over a 24-year timeframe. Our finding of a high degree of larval connectivity over decadal timescales in summer flounder suggests that larval dispersal is frequent and extensive enough to result in genetic near-homogeneity.

The utility of spatially divergent loci for genetic population assignment with temporally spaced samples is dependent on allele frequency differences being stable over time. Allele frequencies at candidate loci may be difficult to detect or may fluctuate over time due to the transient nature of small-effect loci (Yeaman 2015) or spatially varying environmental selection acting in each generation (Hedrick *et al.* 1976; Bernatchez 2016). However, the majority of our candidate outlier loci in adult summer flounder were associated with bottom temperature, and a strong thermal gradient due to the Gulf Stream exists along the U.S. East coast (Briggs 1974). Recent evidence points to a weakening of the Atlantic meridional overturning circulation (AMOC), of which the Gulf Stream is an important element, as well as a northward shift of the Gulf Stream, and that these changes are likely due to climate change (Caesar *et al.* 2018; Thornalley *et al.* 2018). This is reflected in the increased occurrence of southern species at the Little Egg Inlet, NJ site (Morson *et al.* 2019). Despite these changes, the persistence of a thermal gradient suggests that selection for temperature-associated genetic markers has existed over the last few decades as well.

Dispersal may promote or constrain adaptive divergence across an environmental landscape (Lenormand 2002; Garant *et al.* 2007). Within the context of rapid environmental change, high connectivity over time may be particularly advantageous because it increases adaptive potential through beneficial gene flow. With a baseline understanding of dispersal in summer flounder, ongoing and future dispersal will be easier to evaluate. We took advantage of existing natural history collections – invaluable, long-term datasets of life's diversity (Lister &

518 Climate Change Research Group, 2011) – to retroactively investigate dispersal patterns over
519 time. Such collections have also been key to investigating phenotypic (Cross *et al.* 2018),
520 phenological (Primack *et al.* 2004), and distributional shifts (Moritz *et al.* 2008), further
521 highlighting the importance of archived collections for studying ecological and evolutionary
522 patterns and processes. Furthermore, these studies illustrate the need for long-term sampling
523 programs to make connections with governmental, academic, or private (e.g., open non-profit)
524 research collections so that specimens resulting from their sampling can be cataloged, curated,
525 and made available (and known) to the broader research community (Singer *et al.* 2018).
526 Appropriately preserved specimens not only serve as historical baselines by documenting
527 biogeography and morphology in space and time, but also harbor a wealth of information in the
528 form of genetic, biochemical, and geochemical natural tags, which themselves can be time
529 capsules of information. However, these collections are most useful when accessible and
530 available to the research community and properly cared for in perpetuity. The nature of our
531 summer flounder specimens allowed for a combined otolith-genetic approach, identifying
532 instances of bidirectional dispersal both near and far from sites of origin across an environmental
533 gradient and over time. Future studies combining contemporary specimens and those available in
534 natural history collections have great potential to reveal how historical and ongoing
535 environmental changes have and will continue to impact the ecological and evolutionary
536 trajectory of organisms (Johnson *et al.* 2011; Webster 2018; Meineke *et al.* 2019), if we can use
537 collections in innovative and synergistic ways and revisit them as new approaches are developed
538 to extract information.
539
540 *Consequences for summer flounder management*

Summer flounder support economically important commercial and recreational fisheries, especially in the Mid-Atlantic where biomass is highest (Packer *et al.* 1999), and our findings have important implications for summer flounder biology and conservation. The results from our multi-marker approach indicate that the majority of larvae most likely originated in the vicinity of Cape Hatteras. The consistency with which larval clusters were assigned back to the Cape Hatteras region through time was striking, despite the northward shift of summer flounder populations in recent decades (Nye *et al.* 2009; Bell *et al.* 2014). Even though the majority of summer flounder are thought to spawn from Cape Cod, MA to Cape Lookout, NC along the continental shelf (Smith 1973; Able & Fahay 1998), our data suggest that spawning adults in the vicinity of Cape Hatteras comprise a particularly important source of production and contribute disproportionately to coastwide annual recruitment. The Cape Hatteras region could be targeted for spawning stock protection if management needs warranted. In addition, shared dispersal trajectories may have demographic consequences by affecting the distribution of phenotypes in the subsequent life stages (Shima & Swearer 2016). Further research using samples captured as soon as possible after spawning (i.e. ichthyoplankton samples captured offshore), additional adult samples, larvae that ingressed to estuaries not sampled in this study and/or other natural and artificial tags would be useful to further ground-truth our findings and confirm if individuals spawned in the vicinity of Cape Hatteras contribute disproportionally to the next generation. Additionally, the use of biological-oceanographic (biophysical) models would be helpful for understanding how larvae spawned throughout the species' range disperse along the coast.

Conclusions

563 We demonstrated how an integrated multi-marker approach can improve estimates of
564 contemporary dispersal, and how natural history collections can greatly extend the temporal scale
565 of investigations examining ecological and evolutionary processes. By using natural tags that
566 span intra- and inter-generational timescales, our combined approach enabled a higher resolution
567 understanding of the magnitude and directionality of dispersal over time. Our results provide
568 direct evidence of high connectivity in a marine species, and contrasts with recent evidence of
569 high self-recruitment in the sea. High dispersal over space and time appears to be quite common
570 in some marine species and may be a particularly important mechanism maintaining genetic
571 diversity and evolutionary potential in populations during an era of rapid environmental change.
572

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598 **Data Accessibility**

599 Raw sequencing reads are archived in the NCBI Sequence Read Archive (SRA) database (Acc.
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602

603 **Author Contributions**

604 JAH, MLP, KWA and FJF designed the study; KWA, EJH, GTK, TET, JCT, JAH and FJF
605 obtained and organized the samples; JAH prepared the ddRADseq libraries, performed the
606 bioinformatics and analyzed the cleaned genetic dataset; FJF and QAW dissected and performed
607 laser ablation inductively coupled plasma mass spectrometry on cleaned larval otoliths; JAH and

608 FJF analyzed elemental ratios; JAH, MLP, and FJF designed analysis methods; all authors
609 discussed results; JAH wrote the manuscript; all authors edited the manuscript.

610 **References**

611 Able KW, Fahay M. (1998) *The First Year in the Life of Estuarine Fishes in the Middle Atlantic*
612 *Bight*. Rutgers University Press.
613 Able KW, Fahay MP (2010) *Ecology of Estuarine Fishes: Temperate Waters of the Western*
614 *North Atlantic*. Johns Hopkins University Press, Baltimore, MD.
615 Able KW, Sullivan MC, Hare JA *et al.* (2011) Larval abundance of summer flounder
616 (*Paralichthys dentatus*) as a measure of recruitment and stock status. *Fishery Bulletin*, **109**,
617 68–78.
618 Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation
619 genetics. *Nature Reviews Genetics*, **11**, 697–709.
620 Almany GR, Planes S, Thorrold SR *et al.* (2017) Larval fish dispersal in a coral-reef seascape.
621 *Nature Ecology & Evolution*, **1**, 1–7.
622 Auer SJ (1987) Five-year climatological survey of the Gulf Stream system and its associated
623 rings. *Journal of Geophysical Research*, **92**, 11709–11726.
624 Baetscher DS, Anderson EC, Gilbert-Horvath EA *et al.* (2019) Dispersal of a nearshore marine
625 fish connects marine reserves and adjacent fished areas along an open coast. *Molecular*
626 *Ecology*, **28**, 1611–1623.
627 Baker P, Austin JD, Bowen BW, Baker SM (2008) Range-wide population structure and history
628 of the northern quahog (*Merceneria merceneria*) inferred from mitochondrial DNA
629 sequence data. *ICES Journal of Marine Science*, **65**, 155–163.
630 Barton DP, Taillebois L, Taylor J *et al.* (2018) Stock structure of *Lethrinus laticaudis*
631 (Lethrinidae) across northern Australia determined using genetics, otolith microchemistry
632 and parasite assemblage composition. *Marine and Freshwater Research*, **69**, 487–501.
633 Baudouin L, Piry S, Cornuet JM (2004) Analytical Bayesian approach for assigning individuals
634 to populations. *Journal of Heredity*, **95**, 217–224.
635 Bell RJ, Richardson DE, Hare JA, Lynch PD, Fratantoni PS (2014) Disentangling the effects of
636 climate, abundance, and size on the distribution of marine fish: an example based on four
637 stocks from the Northeast US shelf. *ICES Journal of Marine Science*, **72**, 1–12.
638 Benestan L, Gosselin T, Perrier C *et al.* (2015) RAD-genotyping reveals fine-scale genetic
639 structuring and provides powerful population assignment in a widely distributed marine
640 species, the American lobster (*Homarus americanus*). *Molecular Ecology*, **24**, 3299–3315.
641 Bernatchez L (2016) On the maintenance of genetic variation and adaptation to environmental
642 change: considerations from population genomics in fishes. *Journal of Fish Biology*, **89**,
643 2519–2556.
644 Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular*
645 *Ecology*, **13**, 551–561.
646 Botsford LW, Hastings A, Gaines SD (2001) Dependence of sustainability on the configuration
647 of marine reserves and larval dispersal distance. *Ecology Letters*, **4**, 144–150.
648 Bradbury IR, Campana SE, Bentzen P (2008) Estimating contemporary early life-history
649 dispersal in an estuarine fish: Integrating molecular and otolith elemental approaches.
650 *Molecular Ecology*, **17**, 1438–1450.
651 Briggs JC (1974) *Marine Zoogeography*. McGraw-Hill Book Company, New York.
652 Bumpus DF (1973) A description of the circulation on the continental shelf of the East coast of
653 the United States. *Progress in Oceanography*, **6**, 111–157.
654 Byers JE, Pringle JM (2006) Going against the flow: Retention, range limits and invasions in

advective environments. *Marine Ecology Progress Series*, **313**, 27–41.
Caesar L, Rahmstorf S, Robinson A, Feulner G, Saba V (2018) Observed fingerprint of a weakening Atlantic Ocean overturning circulation. *Nature*, **556**, 191–196.
Charrad M, Ghazzali N, Boiteau V, Niknafs A (2014) NbClust : An R Package for determining the relevant number of clusters in a data set. *Journal of Statistical Software*, **61**, 1–36.
Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package - I : One-table methods. *R News*, **4**, 5–10.
Claramunt S, Wright NA (2018) Using museum specimens to study flight and dispersal. In: *The Extended Specimen: Emerging Frontiers in Collections-Based Ornithological Research* (ed Webster MS), pp. 127–141. CRC Press, Boca Raton, FL.
Connolly SR, Menge BA, Roughgarden J (2001) A latitudinal gradient in recruitment of intertidal invertebrates in the Northeast Pacific Ocean. *Ecology*, **82**, 1799–1813.
Cook GS (2011) Changes in otolith microchemistry over a protracted spawning season influence assignment of natal origin. *Marine Ecology Progress Series*, **423**, 197–209.
Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.
Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. *Science*, **311**, 522–527.
Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, **1**, 443–466.
Cross EL, Harper EM, Peck LS (2018) A 120-year record of resilience to environmental change in brachiopods. *Global Change Biology*, **24**, 2262–2271.
DeSaix MG, Bulluck LP, Eckert AJ *et al.* (2019) Population assignment reveals low migratory connectivity in a weakly structured songbird. *Molecular Ecology*, **28**, 2122–2135.
Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, **14**, 2611–2620.
Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, **491**, 479–491.
Fogarty MJ, Botsford LW (2007) Population connectivity and spatial management of marine fisheries. *Oceanography*, **20**, 112–123.
Frantz A., Tigel Pourtois J, Heuertz M *et al.* (2006) Genetic structure and assignment tests demonstrate illegal translocation of red deer (*Cervus elaphus*) into a contiguous population. *Molecular Ecology*, **15**, 3191–3203.
Freamo H, O'Reilly P, Berg PR, Lien S, Boulding EG (2011) Outlier SNPs show more genetic structure between two Bay of Fundy metapopulations of Atlantic salmon than do neutral SNPs. *Molecular Ecology Resources*, **11**, 254–267.
Galarza JA, Carreras-Carbonell J, Macpherson E *et al.* (2009) The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. *Proceedings of the National Academy of Sciences*, **106**, 1473–1478.
Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on

700 contemporary adaptation. *Functional Ecology*, **21**, 434–443.
701 Gillanders BM (2002) Connectivity between juvenile and adult fish populations: Do adults
702 remain near their recruitment estuaries? *Marine Ecology Progress Series*, **240**, 215–223.
703 Gillanders BM, Kingsford MJ (1996) Elements in otoliths may elucidate the contribution of
704 estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Marine*
705 *Ecology Progress Series*, **141**, 13–20.
706 Gillanders B, Sanchez-Jerez P, Bayle-Sempere J, Ramos-Espla A (2001) Trace elements in
707 otoliths of the two-banded bream from a coastal region in the south-west Mediterranean: are
708 there differences among locations? *Journal of Fish Biology*, **59**, 350–363.
709 Gleason LU, Burton RS (2016) Genomic evidence for ecological divergence against a
710 background of population homogeneity in the marine snail *Chlorostoma funebralis*.
711 *Molecular Ecology*, **25**, 3557–3573.
712 Glover KA, Skilbrei OT, Skaala Ø (2008) Genetic assignment identifies farm of origin for
713 Atlantic salmon *Salmo salar* escapees in a Norwegian fjord. *ICES Journal of Marine*
714 *Science*, **65**, 1–9.
715 Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological
716 data. *Journal of Statistical Software*, **22**, 1–19.
717 Gotelli NJ (1991) Metapopulation models: The rescue effect, the propagule rain, and the core-
718 satellite hypothesis. *The American Naturalist*, **138**, 768–776.
719 Goudet J (2005) HIERFSTAT, a package for R to compute and test hierarchical F-statistics.
720 *Molecular Ecology Notes*, **5**, 184–186.
721 Grothues TM, Cowen RK, Pietrafesa LJ *et al.* (2002) Flux of larval fish around Cape Hatteras.
722 *Limnology and Oceanography*, **47**, 165–175.
723 Hanski I, Gilpin ME (Eds.) (1997) *Metapopulation Biology: Ecology, Genetics, and Evolution*.
724 Academic Press, San Diego, CA.
725 Hare JA, Churchill JH, Cowen RK *et al.* (2002) Routes and rates of larval fish transport from the
726 southeast to the northeast United States continental shelf. *Limnology and Oceanography*, **47**,
727 1774–1789.
728 Hare JA, Cowen RK (1996) Transport mechanisms of larval and pelagic juvenile bluefish
729 (*Pomatomus saltatrix*) from South Atlantic Bight spawning grounds to Middle Atlantic
730 Bight nursery habitats. *Limnology and Oceanography*, **41**, 1264–1280.
731 Harrison CS, Siegel DA, Mitarai S (2013) Filamentation and eddy-eddy interactions in marine
732 larval accumulation and transport. *Marine Ecology Progress Series*, **472**, 27–44.
733 Hastings A, Botsford LW (2006) Persistence of spatial populations depends on returning home.
734 *Proceedings of the National Academy of Sciences*, **103**, 6067–6072.
735 Hedrick PW, Ginevan ME, Ewing EP (1976) Genetic polymorphism in heterogeneous
736 environments. *Annual Review of Ecology and Systematics*, **7**, 1–32.
737 Hoey JA, Pinsky ML (2018) Genomic signatures of environmental selection despite near-
738 panmixia in summer flounder. *Evolutionary Applications*, **11**, 1732–1747.
739 [dataset]Hoey, JA, Fodrie FJ, Walker QA *et al.* (2020) Data from: Using multiple natural tags
740 provides evidence for extensive larval dispersal across space and through time in summer
741 flounder. https://doi.org/10.5281/zenodo.3670955.
742 Hogan JD, Thiessen RJ, Sale PF, Heath DD (2012) Local retention, dispersal and fluctuating
743 connectivity among populations of a coral reef fish. *Oecologia*, **168**, 61–71.
744 Holmes MW, Hammond TT, Wogan GOU *et al.* (2016) Natural history collections as windows

745 on evolutionary processes. *Molecular Ecology*, **25**, 864–881.
746 Huffaker CB (1958) Experimental studies on predation: Dispersion factors and predator-prey
747 oscillations. *Hilgardia*, **27**, 795–835.
748 Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for
749 dealing with label switching and multimodality in analysis of population structure.
750 *Bioinformatics*, **23**, 1801–1806.
751 Johnson KG, Brooks SJ, Fenberg PB *et al.* (2011) Climate change and biosphere response:
752 Unlocking the collections vault. *BioScience*, **61**, 147–153.
753 Jombart T (2008) Adegenet: A R package for the multivariate analysis of genetic markers.
754 *Bioinformatics*, **24**, 1403–1405.
755 Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. *Current*
756 *Biology*, **15**, 1314–1318.
757 Jones WJ, Quattro JM (1999) Genetic structure of summer flounder (*Paralichthys dentatus*)
758 populations north and south of Cape Hatteras. *Marine Biology*, **133**, 129–135.
759 Jones OR, Wang J (2010) COLONY: A program for parentage and sibship inference from
760 multilocus genotype data. *Molecular Ecology Resources*, **10**, 551–555.
761 Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of
762 populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, **2**, e281.
763 Kassambara A, Mundt F (2017) factoextra: Extract and Visualize the Results of Multivariate
764 Data Analyses.
765 Keefe M, Able KW (1993) Patterns of metamorphosis in summer flounder, *Paralichthys*
766 *dentatus*. *Journal of Fish Biology*, **42**, 713–728.
767 Kraus RT, Musick JA (2001) A brief interpretation of summer flounder, *Paralichthys dentatus*,
768 movements and stock structure with new tagging data on juveniles. *Marine Fisheries*
769 *Review*, **63**, 1–6.
770 Kraus RT, Secor DH (2005) Application of the nursery-role hypothesis to an estuarine fish.
771 *Marine Ecology Progress Series*, **291**, 301–305.
772 Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology and*
773 *Evolution*, **17**, 183–189.
774 Lister A, Group CCR (2011) Natural history collections as sources of long-term datasets. *Trends*
775 *in Ecology and Evolution*, **26**, 153–154.
776 Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity?
777 *Molecular Ecology*, **19**, 3038–3051.
778 Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: Matching biological questions
779 with appropriate techniques. *Trends in Ecology and Evolution*, **20**, 136–142.
780 Meineke EK, Davies TJ, Daru BH, Davis CC (2019) Biological collections for understanding
781 biodiversity in the Anthropocene. *Philosophical Transactions of the Royal Society B:*
782 *Biological Sciences*, **374**, 20170386.
783 Moritz C, Patton JL, Conroy CJ *et al.* (2008) Impact of a century of climate change on small-
784 mammal communities in Yosemite National Park, USA. *Science*, **322**, 261–264.
785 Morse WW (1981) Reproduction of the summer flounder, *Paralichthys dentatus* (L.). *Journal of*
786 *Fish Biology*, **19**, 189–203.
787 Morson JM, Grothues T, Able KW (2019) Change in larval fish assemblage in a USA east coast
788 estuary estimated from twenty-six years of fixed weekly sampling. *PLoS ONE*, **14**,
789 e0224157.

790 Murdoch D, Chow ED (1996) A graphical display of large correlation matrices. *The American*
791 *Statistician*, 50, 178-180.
792 Nanninga GB, Berumen ML (2014) The role of individual variation in marine larval dispersal.
793 *Frontiers in Marine Science*, **1**, 1–17.
794 Nielsen EE, Cariani A, Mac Aoidh E *et al.* (2012) Gene-associated markers provide tools for
795 tackling illegal fishing and false eco-certification. *Nature Communications*, **3**, 1–6.
796 Nielsen EE, Hansen MM, Schmidt C, Meldrup D, Grønkjaer P (2001) Population of origin of
797 Atlantic cod. *Nature*, **413**, 272.
798 Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine
799 fishes: Identifying adaptive variation in space and time. *Molecular Ecology*, **18**, 3128–3150.
800 Nye JA, Link JS, Hare JA, Overholtz WJ (2009) Changing spatial distribution of fish stocks in
801 relation to climate and population size on the Northeast United States continental shelf.
802 *Marine Ecology Progress Series*, **393**, 111–129.
803 Packer DB, Griesbach SJ, Berrien PL *et al.* (1999) Essential Fish Habitat Source Document: Life
804 History and Habitat Characteristics. *NOAA Technical Memorandum NMFS-NE Series*, **151**,
805 1–88.
806 Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population
807 structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.
808 Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-
809 time estimation of migration rate: a simulation-based exploration of accuracy and power.
810 *Molecular Ecology*, **13**, 55–65.
811 Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine
812 reserves. *Ecological Applications*, **13**, 146–158.
813 Papetti C, Di Franco A, Zane L *et al.* (2013) Single population and common natal origin for
814 Adriatic *Scomber scombrus* stocks: evidence from an integrated approach. *ICES Journal of*
815 *Marine Science*, **70**, 387–398.
816 Pimm SL, Alibhai S, Bergl R *et al.* (2015) Emerging technologies to conserve biodiversity.
817 *Trends in Ecology & Evolution*, **30**, 685–696.
818 Primack D, Imbres C, Primack RB, Miller-Rushing AJ, Del Tredici P (2004) Herbarium
819 specimens demonstrate earlier flowering times in response to warming in Boston. *American*
820 *Journal of Botany*, **91**, 1260–1264.
821 Primmer CR, Koskinen MT, Piironen J (2000) The one that did not get away: individual
822 assignment using microsatellite data detects a case of fishing competition fraud.
823 *Proceedings of Biological Sciences*, **267**, 1699–1704.
824 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus
825 genotype data. *Genetics*, **155**, 945–959.
826 R Core Team (2017) R: A language and environment for statistical computing. R Foundation for
827 Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
828 Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes.
829 *Proceedings of the National Academy of Sciences*, **94**, 9197–9201.
830 Reis-Santos P, Tanner SE, Aboim MA *et al.* (2018) Reconciling differences in natural tags to
831 infer demographic and genetic connectivity in marine fish populations. *Scientific Reports*, **8**,
832 10343.
833 Reis-Santos P, Tanner SE, Vasconcelos RP *et al.* (2013) Connectivity between estuarine and
834 coastal fish populations: Contributions of estuaries are not consistent over time. *Marine*

Ecology Progress Series, **491**, 177–186.
Ribeiro F, Hale E, Hilton EJ *et al.* (2015) Composition and temporal patterns of larval fish communities in Chesapeake and Delaware Bays, USA. *Marine Ecology Progress Series*, **527**, 167–180.
Richardson DE, Hare JA, Overholtz WJ, Johnson DL (2010) Development of long-term larval indices for Atlantic herring (*Clupea harengus*) on the northeast US continental shelf. *ICES Journal of Marine Science*, **67**, 617–627.
Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.
Roy EM, Quattro JM, Greig TW (2012) Genetic management of black sea bass: Influence of biogeographic barriers on population structure. *Marine and Coastal Fisheries*, **4**, 391–402.
Runge JP, Runge MC, Nichols JD (2006) The role of local populations within a landscape context: Defining and classifying sources and sinks. *The American Naturalist*, **167**, 925–938.
Sandoval-Castillo J, Robinson NA, Hart AM, Strain LWS, Beheregaray LB (2018) Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (*Haliotis laevigata*), along a longitudinal environmental gradient. *Molecular Ecology*, **27**, 1603–1620.
Schaffler JJ, Reiss CS, Jones CM (2009) Spatial variation in otolith chemistry of Atlantic croaker larvae in the Mid-Atlantic Bight. *Marine Ecology Progress Series*, **382**, 185–195.
Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution*, **22**, 25–33.
Shaklee JB, Beacham TD, Seeb L, White BA (1999) Managing fisheries using genetic data: case studies from four specis of Pacific salmon. *Fisheries Research*, **43**, 45–78.
Shanks AL (2009) Pelagic larval duration and dispersal distance revisited. *Biological Bulletin*, **216**, 373–385.
Shima JS, Swearer SE (2009) Larval quality is shaped by matrix effects: Implications for connectivity in a marine metapopulation. *Ecology*, **90**, 1255–1267.
Shima JS, Swearer SE (2016) Evidence and population consequences of shared larval dispersal histories in a marine fish. *Ecology*, **97**, 25–31.
Siegel DA, Mitarai S, Costello CJ *et al.* (2008) The stochastic nature of larval connectivity among nearshore marine populations. *Proceedings of the National Academy of Science*, **105**, 8974–8979.
Singer RA, Love KJ, Page LM (2018) A survey of digitized data from U.S. fish collections in the iDigBio data aggregator. *PLoS ONE*, **13**, 1–20.
Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.
Smith WG (1973) The distribution of summer flounder, *Paralichthys dentatus*, eggs and larvae on the continental shelf between Cape Cod and Cape Lookout, 1956-1966. *Fishery Bulletin*, **71**, 527–548.
Stegmann PM, Yoder JA (1996) Variability of sea-surface temperature in the South Atlantic Bight as observed from satellite: Implications for offshore-spawning fish. *Continental Shelf Research*, **16**, 843–849.
Sullivan MC, Able KW, Hare JA, Walsh HJ (2006) *Anguilla rostrata* glass eel ingress into two, U.S. east coast estuaries: Patterns, processes and implications for adult abundance. *Journal*

880 *of Fish Biology*, **69**, 1081–1101.
881 Tanner SE, Pérez M, Presa P, Thorrold SR, Cabral HN (2014) Integrating microsatellite DNA
882 markers and otolith geochemistry to assess population structure of European hake
883 (*Merluccius merluccius*). *Estuarine, Coastal and Shelf Science*, **142**, 68–75.
884 Tanner SE, Vasconcelos RP, Cabral HN, Thorrold SR (2012) Testing an otolith geochemistry
885 approach to determine population structure and movements of European hake in the
886 northeast Atlantic Ocean and Mediterranean Sea. *Fisheries Research*, **125**–**126**, 198–205.
887 Thornalley DJR, Oppo DW, Ortega P *et al.* (2018) Anomalously weak Labrador Sea convection
888 and Atlantic overturning during the past 150 years. *Nature*, **556**, 227–230.
889 Thorrold SR, Jones CM, Campana SE (1997) Response of otolith microchemistry to
890 environmental variations experienced by larval and juvenile Atlantic croaker
891 (*Micropogonias undulatus*). *Limnology and Oceanography*, **42**, 102–111.
892 Thorrold SR, Jones GP, Hellberg ME *et al.* (2002) Quantifying larval retention and connectivity
893 in marine populations with artificial and natural markers. *Bulletin of Marine Science*, **70**,
894 291–308.
895 Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal homing in a marine fish
896 metapopulation. *Science*, **291**, 297–299.
897 Thorrold S, Zacherl D, Levin L (2007) Population connectivity and larval dispersal using
898 geochemical signatures in calcified structures. *Oceanography*, **20**, 80–89.
899 Townsend PA, Navarro-Siguenza AG (2018) What bird specimens can reveal about species-level
900 distributional ecology. In: *The Extended Specimen: Emerging Frontiers in Collections-*
901 *Based Ornithological Research* (ed Webster MS), pp. 111–125. CRC Press, Boca Raton,
902 FL.
903 Vasconcelos RP, Reis-Santos P, Tanner S *et al.* (2008) Evidence of estuarine nursery origin of
904 five coastal fish species along the Portuguese coast through otolith elemental fingerprints.
905 *Estuarine, Coastal and Shelf Science*, **79**, 317–327.
906 Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*. Springer, New York.
907 Walsh HJ, Richardson DE, Marancik KE, Hare JA (2015) Long-term changes in the distributions
908 of larval and adult fish in the northeast U.S. shelf ecosystem. *PLoS ONE*, **10**, 1–31.
909 Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high
910 gene flow species. *Journal of Heredity*, **89**, 438–450.
911 Ward RD, Woodwark M, Skibinski DOF (1994) A comparison of genetic diversity levels in
912 marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, **44**, 213–232.
913 Watanabe ME (2019) The evolution of natural history collections. *BioScience*, **69**, 163–169.
914 Webster MS (2018) The Extended Specimen. In: *The Extended Specimen: Emerging Frontiers in*
915 *Collections-Based Ornithological Research* (ed Webster MS), p. 240. CRC Press, Boca
916 Raton, FL.
917 Wilk SJ, Smith WG, Ralph DE, Sibunka J (1980) Population structure of summer flounder
918 between New York and Florida based on linear discriminant analysis. *Transactions of the*
919 *American Fisheries Society*, **109**, 265–271.
920 Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation
921 of semiparametric generalized linear models. *Journal of the Royal Statistical Society, Series*
922 *B (Statistical Methodology)*, **73**, 3–36.
923 Wright S (1931) Evolution in Mendelian Populations. *Genetics*, **16**, 97–159.
924 Yeaman S (2015) Local adaptation by alleles of small effect. *The American Naturalist*, **186**, S74–

925 S89.
926

927 Tables & Figures

928 Table 1. Sampling years, source and sample size for each sampled collection or dataset of larval
929 summer flounder from north to south. See Figure 1 for locations.

Year(s)	Source	$n_{sampled}$	$n_{otolith}$	$n_{genetic}$	$n_{otolith\ \&\ genetic}$
1989-1993	Little Egg Inlet, NJ	51	7	8	4
1998-2002	Little Egg Inlet, NJ	50	32	38	26
2008-2012	Little Egg Inlet, NJ	50	34	6	4
2008-2010	Roosevelt Inlet, DE	50	41	50	41
2008-2010	York River, VA	50	25	44	23
1989-1993	Beaufort, NC	55	17	52	17
1998-2002	Beaufort, NC	54	25	49	21
2008-2012	Beaufort, NC	50	16	45	15
2008	North Inlet, SC	1	0	1	0
	Total	411	197	293	151

930

931 Table 2. Larval summer flounder cluster assignment and exclusion results from each time period. Clusters were assigned to the
932 most likely (ML) spawning reference location along the coast (see Figure 1). Clusters were also excluded from potential
933 reference locations at two significance levels: 0.20 and 0.05.

	Size (n = 151)	**Location A (north)**	**Location B**	**Location C**	**Location D**	**Location E**	**Location F**	**Location G**	**Location H**	**Location I**	**Location J (south)**
Early (1989-1993)											
E1	n = 4						ML				
E2	n = 3					ML					
E3	n = 4					ML			$p < 0.20$	$p < 0.20$	$p < 0.20$
E4	n = 4			ML							
E5	n = 3							ML			
E6	n = 3							ML			
Middle (1998-2002)											
M1	n = 21					ML					
M2	n = 26					ML					
Late (2008-2012)											
L1	n = 33						ML				
L2	n = 22					ML	$p < 0.20$	$p < 0.20$	$p < 0.20$	$p < 0.05$	$p < 0.05$
L3	n = 28						ML				

934

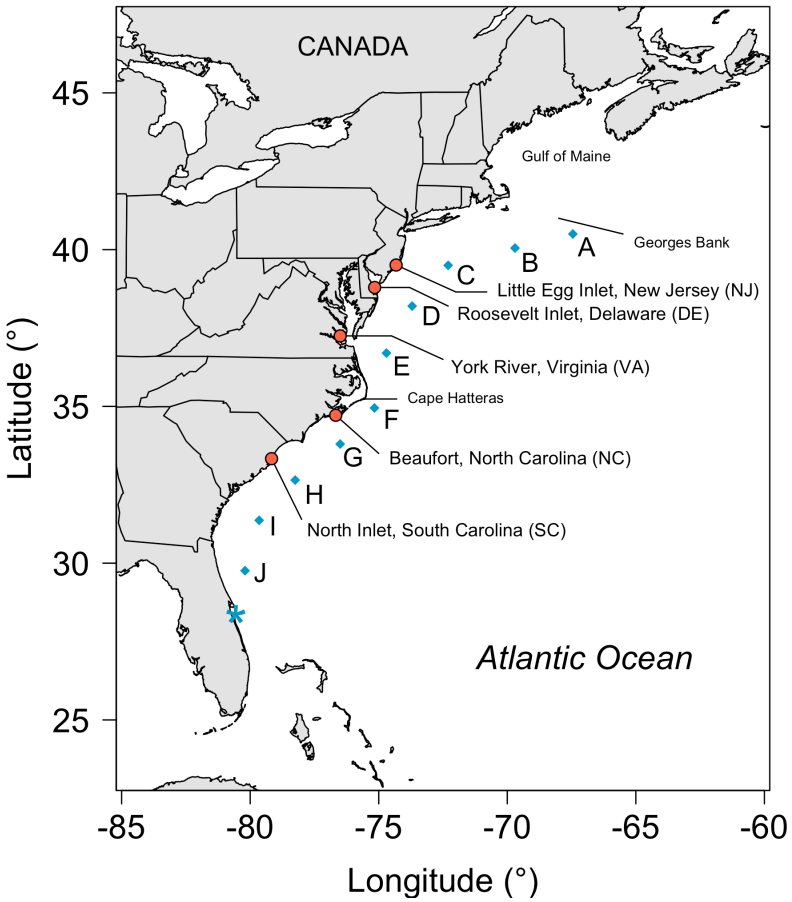

Figure 1. Locations of larval summer flounder (n = 411; circles) sampled from ingress sites along the U.S. East coast between 1989-2012. Locations A through J denote spawning reference locations to which larvae were assigned. These locations represent distances along the coast calculated from a southern point (*).

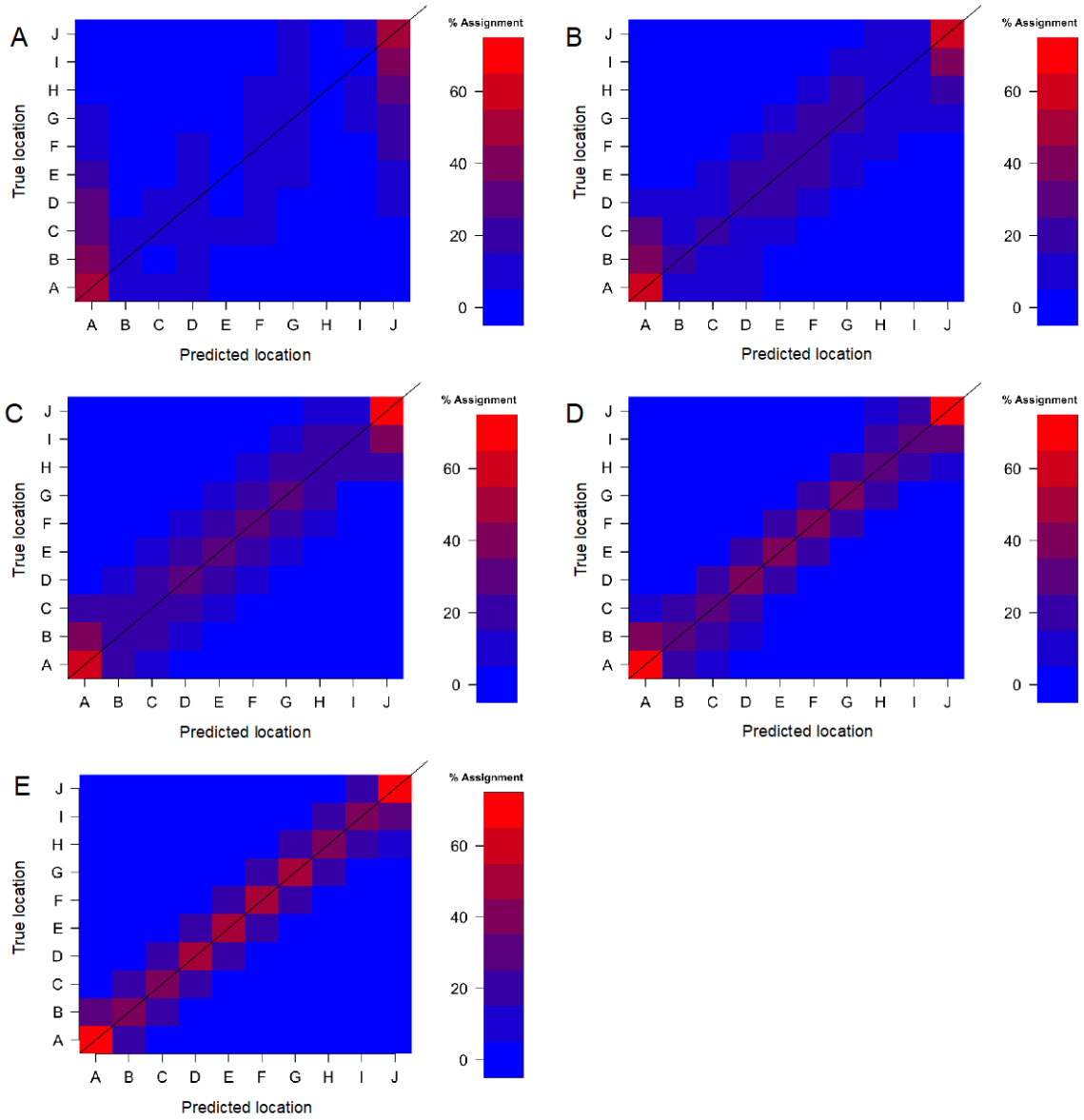

Figure 2. Increased assignment accuracy as the number of individuals used for assignment increased. Alleles of A) 1 individual, B) 5 individuals, C) 10 individuals, D) 20 individuals, and E) 30 individuals were simulated from each of 10 locations (A – J) using the 10 allele frequencies in Table S2. Genotype likelihoods were calculated for individuals or groups of individuals for each spawning location, and these were then assigned to the most likely location. The percentage of correct assignments increased and coalesced around the 1:1 line as the number of individuals increased.

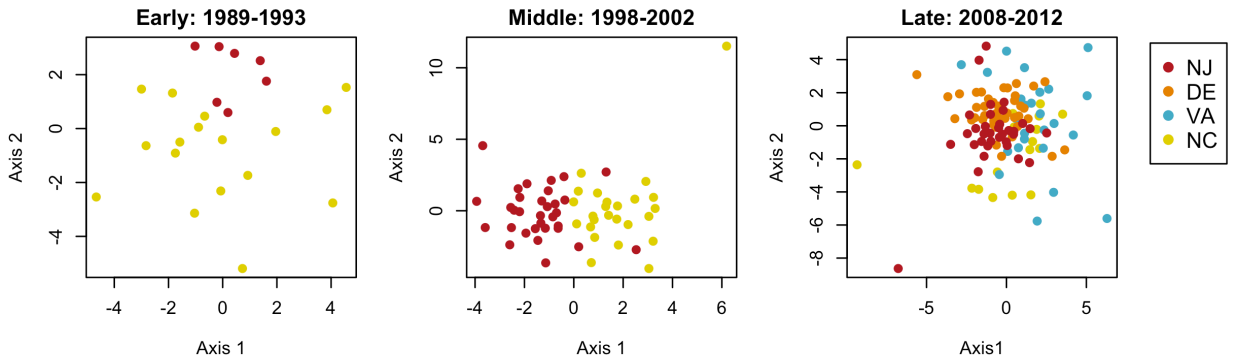

Figure 3. Nonmetric multidimensional scaling (nMDS) plots for summer flounder larvae caught within each time period using 10 elemental otolith microchemistry ratios (Sr, Mg, Mn, Fe, Cu, Cd, Ba, Sn, Pb & U) relative to Ca. Segregation between ingress sites located north (NJ: New Jersey and DE: Delaware) and south (VA: Virginia and NC: North Carolina) of Cape Hatteras was visible through time.

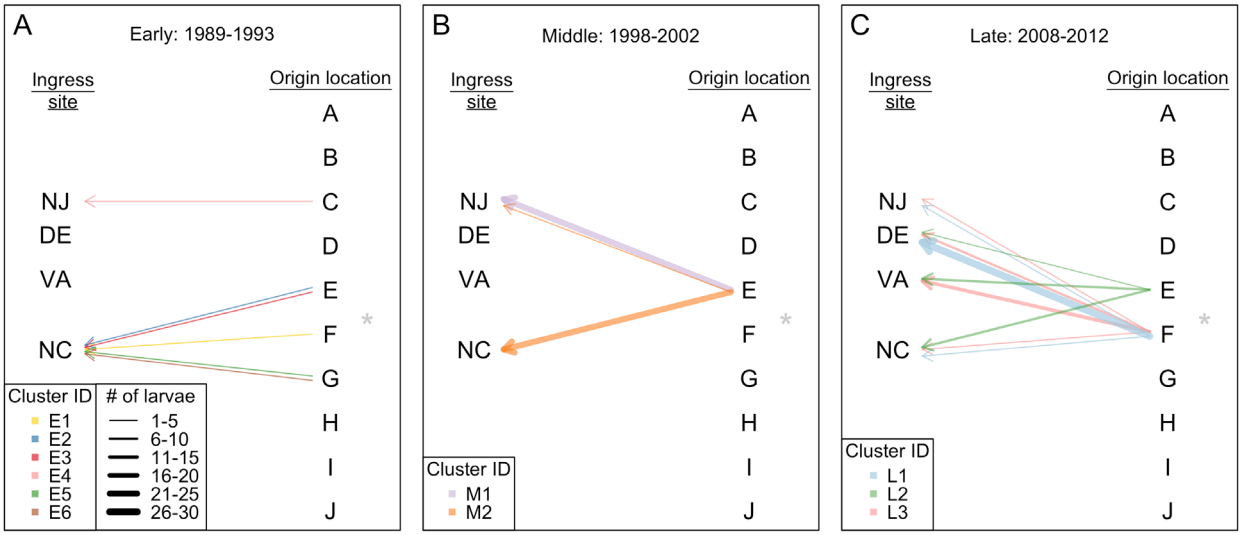

Figure 4. Schematic depicting the likely origin on the continental shelf (A-J; ordered from north to south, see Figure 1) and estuarine destination of dispersing summer flounder larvae. The width of the arrow indicates how many larvae ingressed to sites (NJ: New Jersey, DE: Delaware, VA: Virginia, NC: North Carolina) in the A) early (1989-1993), B) middle (1998-2002), and C) late (2008-2012) time periods. Each cluster of larvae is represented by a unique color. The location of Cape Hatteras, a biogeographic break for many marine species, is indicated by a gray *.