1 Species-specific abundance of bivalve larvae in relation to biological and physical conditions in
2 a Cape Cod estuary: Waquoit Bay, Massachusetts (USA)
3
4 Christine M. Thompson[1,*], Richard H. York[2], Scott M. Gallager[1]
5
6 [1]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543
7
8 [2]Shellfish Constable, Town of Mashpee, Mashpee, MA 02649
9
10 KEYWORDS: bivalves, larval supply, transport, spawning, estuaries, shellfish, time series
11
12 RUNNING HEAD: Species-specific abundance of bivalve larvae
13
14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29   *email cthompson@umces.edu
30   Present address: University of Maryland Center for Environmental Science, Horn Point
31   Laboratory, Cambridge, MD 21613

ABSTRACT

32
33
34   Physical and biological conditions impact recruitment and adult population structure of

35 marine invertebrates by affecting early life history processes from spawning to post-settlement.

36 We investigated how temperature, salinity and phytoplankton influenced larval abundance and

37 larval size structure for three species of bivalves over two non-consecutive years in Waquoit

38 Bay, MA. Abundance and size of *Mercenaria mercenaria* (quahog), *Anomia simplex* (jingle

39 clam), and *Geukensia demissa* (ribbed mussel) larvae were compared between locations in the

40 bay and with environmental conditions. Shell birefringence patterns using polarized light

41 microscopy were used to distinguish species. Larval abundances for all three species were higher

42 in 2009 than in 2007 and were positively correlated with temperature in both years. Differences

43 in larval abundance and size structure between bay sites were attributed to salinity tolerances and

44 potential source locations. Higher survival in 2009 than in 2007, as determined by number of

45 pediveligers, was likely due to higher temperatures and greater food availability during the peak

46 abundance months of July and August in 2009. Yearly differences in larval growth and survival

47 can have a large impact on recruitment. Knowing the optimal periods and locations for larval

48 abundance and survival can be useful for isolating species-specific patterns in larval dispersal

49 and to aid resource managers in enhancing or restoring depleted populations.

50

INTRODUCTION

The dispersal and supply of planktonic invertebrate larvae has important consequences for benthic adult population structure (Roughgarden 1988). The strength of larval flux to a given habitat or area can vary from year to year because of environmental conditions (Thorson 1950, Shirley and Shirley 1989, Gaines and Bertness 1992). Larval supply can be influenced by many factors including the timing of larval release or spawning, local hydrographic effects, larval behavior, and quality of larvae (Scheltema 1986, Todd 1998). Mean densities of bivalve larvae can vary greatly been consecutive years (Carriker 1961, Fegley 2001). Particularly for areas where commercial adult populations are managed, larval supply can be a link in the relationship between reproductive output and population growth (Botsford et al. 1998).

Understanding bivalve larval supply is essential to understanding the relationship between larval abundance and the population structure at later stages (Pineda et al. 2007, Cowen and Sponaugle 2009). Many bivalve species are harvested commercially, and natural population stocks are typically managed by studying adult survivorship and fecundity without accounting for the larval period (Orensanz et al. 1991). When measurements of adult reproductive effort do not support their subsequent recruitment, it could be due to larval success which is rarely measured. It is difficult to study pelagic larvae because of their microscopic size, short larval period compared to the adult lifespan, high mortality, and ability to disperse long distances (Levin 2006). A need for more in-depth studies of bivalve larvae has been expressed for years (Carriker 1988, Mann 1988), but progress has lagged behind that of other invertebrate larvae because of a lack of usable techniques to identify bivalve larvae at the species level (Garland and Zimmer 2002).

73   Many of the biological and physical controls on bivalve larval abundance are subject to

74   seasonal and annual variation that can affect yearly recruitment dynamics (Botsford et al. 1994).

75   Wind speed and direction can affect estuarine retention time (Geyer 1997), leading to

76   fluctuations in larval import and export from an estuary (Boicourt 1988, Gaines and Bertness

77   1992, Belgrano et al. 1995). Adult spawning can be affected by water temperature and adult

78   fecundity (Keck et al. 1975, Kassner and Malouf 1982), which can affect larval survival, growth

79   and recruitment (Loosanoff et al. 1951, Davis and Calabrese 1964, Brousseau 1977, Gallager et

80   al. 1986, Pechenik et al. 1990, Dekshenieks et al. 1993). Areas of low salinity can be intolerable

81   to certain species of bivalves (Loosanoff and Davis 1963). Furthermore, environmental factors

82   such as food availability and water temperature will affect the length of the larval period in the

83   plankton (Bayne 1965, Hodgson and Bourne 1988) which can affect survival and dispersal

84   distance (Loosanoff et al. 1951, Jorgensen 1981, Raby et al. 1994, Wilson and Meekan 2001).

85   Timing of phytoplankton blooms has been shown to affect timing of larval abundance in

86   barnacles, mussels and urchins (Starr et al. 1991), fish larvae (Townsend and Cammen 1988) and

87   crab larvae (Shirley and Shirely 1989), but under estuarine conditions typical bloom patterns do

88   not always occur (Litaker et al. 1987, Tomasky-Holmes 2008). These factors can vary spatially

89   with certain areas being more favorable for survival or retention than others. Although it is

90   challenging to isolate the effects of one particular environmental variable on larval abundance

91   and survival in the field, by concentrating on a few environmental variables over a long time

92   series we may be able to discern which factors have a greater effect on larval abundance on a

93   seasonal and bay-wide scale.

94   A majority of studies on larval bivalve growth and feeding have been performed in

95   laboratories (e.g. Loosanoff and Davis 1963), demonstrating growth and survival responses to

4

96   temperature, salinity, and food availability (Loosanoff et al. 1951, Davis and Calabrese 1964,

97   Bayne 1965, Gallager et al. 1986, Dekshenieks et al. 1993). Effects of these factors on larval

98   growth rates in the field is not well documented due to challenges with larval sampling, as well

99   as confounding factors such as advection and mortality due to predation. A few studies have

100   attempted to follow growth and survival of larval cohorts from estimates of their size frequency

101   distributions (Jorgensen 1981, Chicharo and Chicaro 2001, Rigal et al. 2010), but this is most

102   applicable for closed systems with high retention.

103   The purpose of our study was to investigate the biological and physical factors affecting

104   larval abundance and survival of three bivalve species for 2007 and 2009 in Waquoit Bay, an

105   embayment on Cape Cod, MA. *Mercenaria mercenaria* (quahog), is a commercially important

106   shellfish resource for the bay and is found in open waters with sandy bottoms; *Guekensia*

107   *demissa* (ribbed mussel) grows along the banks in marsh channels and plays an ecologically

108   important role by filtering particles and removing nitrogen (Jordan and Valiela 1982) and

109   stabilizing shorelines and fertilizing marsh plants (Bertness 1984). *Anomia simplex* is a

110   widespread fouling organism around Cape Cod often found attached to rocks and shells (Eckman

111   1987). We compared time series of abundance and size of these three species of bivalve larvae

112   from four sites in Waquoit Bay from May through mid-October (when water temperatures

113   exceeded 15ºC) and applied a state-of-the-art image-analysis method using shell birefringence

114   patterns to distinguish larval species (Twiari and Gallager 2003a,b, Thompson et al. *in press*).

115   Environmental conditions prevailing during two non-consecutive years of data collection

116   allowed us to compare a warm, dry year (2007) to an initially cooler, wet year (2009). We

117   hypothesized that better food quality in 2009 would result in more growth and show better

118   survival of larvae. We used abundance of pediveliger larvae as a proxy for larval survival to

5

119 competency as we were unable to estimate growth rates. We addressed the following questions:

120 (1) Does larval abundance and survival differ spatially and temporally? and (2) How do

121 environmental variables at each site and between years influence larval supply? This study

122 presents a novel effort to address species-specific questions in bivalve larval supply in Waquoit

123 Bay and relate them to population dynamics and management.

124 **METHODS**

125   **Study Site and Sampling Locations.** Waquoit Bay is a 16 km^2 estuary on the south

126 shore of Cape Cod, Massachusetts. The average depth in the bay is 2.5 m with an average tidal

127 range of about 0.5 m (Howes et al. 2004). Waquoit Bay exchanges water with outer Nantucket

128 Sound through two inlets with a residence time of 2-3 days and is subject to occasional

129 enhancement or reduction of exchange via wind forcing (Geyer 1997). The main freshwater

130 input to Waquoit Bay is through groundwater, but several sub-embayments exchange water with

131 the main bay and vary with freshwater and nutrient inputs (Howes et al. 2004). Residence times

132 in the sub-embayments are longer than the main bay on the order of several days to weeks

133 (Howes et al. 2004, Tomasky-Holmes 2008). We sampled at four sites representing different

134 areas of the bay (Fig. 1). The Menauhant site (MN) is the western inlet to the bay, Little River

135 (LR) is a well-mixed sub-embayment on the eastern side, Waquoit Bay – Metoxit Point site

136 (WB) is located in the middle of the bay proper, and the Childs River site (CR) is a sub-

137 embayment upstream of the western inlet and has the lowest salinities and high nitrogen

138 concentrations. In 2007 weekly samples were taken from 23 May – 26 October and in 2009

139 samples were taken weekly from 7 May – 14 October. These periods correspond to temperatures

140 exceeding 15ºC, which cause spawning of most local bivalves.

141   **Larval Sampling Procedure.** On each sampling date, all four sites were sampled within

142   3-5 hours. The Waquoit Bay – Metoxit Point samples were taken from a boat both years. At each

143   other site, 2007 samples were taken from a boat and 2009 samples were taken from the same

144   dock the boat samples were taken from to save time. A bilge pump (West Marine BilgePro 2200)

145   attached to a hose and powered either from the boat console or a portable 12V battery was used

146   to collect samples with a flow rate of at 18-24 L/min. Samples were taken by slowly moving the

147   pump through the surface to 20 cm above the bottom in order to get a depth-integrated sample of

148   100-200 L. Water was filtered through a 53 μm nylon mesh PVC screen with a pre-screen of 333

149   μm mesh which was discared. The pre-screen may have removed some large pediveliger larvae

150   but none were ever found in select 333 μm fractions that were collected. All filtered samples

151   from the 53 μm fraction were immediately preserved in 4% buffered formalin.

152   **Sample Processing and Larval Identification.** Plankton samples were first counted in

153   full or by volumetric sub-sampling for denser samples (to ensure at least 300 individuals were

154   counted per sample) under a dissection microscope. Volumes were standardized to one cubic

155   meter. One-hundred larvae were then subsampled from the total sample (or the total sample was

156   used if it contained fewer than 100 individuals) and individually imaged using a Zeiss IM35

157   microscope fitted with Moticam 1000 digital camera, polarization filter, and full wave

158   compensation plate. Motic Images Plus (version 2.0; Motic China Group, Ltd.) was used to

159   capture each polarized image.

160   Several criteria for identification were used to ensure accuracy. Field identification

161   guides of Chanley and Andrews (1971) and Loosanoff et al. (1966) were used for morphology

162   and size criteria. A polarized image library of confirmed hatchery reared and molecularly

163   identified field collected larvae was used to confirm birefringence patterns for each species.

164   These patterns have been shown to be species-specific and can aid in larval identification (Tiwari

165   and Gallager 2003a,b, Thompson et al. *in press*). Based on these criteria, we visually sorted

166   images into fourteen species categories. Only the larval images that were identified as *Anomia*

167   *simplex*, *Geukensia demissa*, or *Mercenaria mercenaria*, composing about one-third of the total

168   images, were used in further analysis as library images of these species were identified using

169   PCR methods (Hare et al. 2001). These sorted larvae were used to test an automated image-

170   analysis method. Accuracies from control tests using these criteria to visually sort four species of

171   known larvae ranged from 85-100% and were not significant between size classes (Thompson et

172   al. *in press*), however, accuracies for both visual and automated methods are expected to

173   decrease with increasing numbers of species categories. Agreements with manually classified

174   larvae (this study) and computer classified larvae using a six-species training set ranged from 72-

175   82% for the three species studied here, which provides some estimate of the accuracies of the

176   classifications in this study.

177   Measurements of each larval shell were made by masking each larval image from its

178   background and cropping it to only the region of the larval shell. An edge-detection image

179   analysis routine in MATLAB (version R2009a; Mathworks, Inc.) was used to obtain major and

180   minor axes in pixels, which were converted to microns by calibration with a stage micrometer.

181   To assess abundance of pediveliger larvae, we used larvae greater than 200 µm as a cutoff for

182   *Mercenaria mercenaria* and *Geukensia demissa*, and greater than 175 µm for *Anomia simplex*

183   based on literature estimates (Chanley and Andrews 1971).

184   **Phytoplankton Counts.** Alongside each larval sample, 100 mL of unfiltered water was

185   sampled from the water column. Phytoplankton were identified and counted on a hemacytometer

186   slide with a light microscope a few hours after collection. Cell sizes were measured with an

187   optical micrometer. Subsamples of 10^{-3} to 10^{-1} ml were counted depending on phytoplankton

188   density. The larger volumes were examined by counting multiple chambers per sample.

189   **Environmental Data.** Measurements of temperature, salinity, pressure (depth),

190   chlorophyll a fluorescence, and other parameters were recorded in 15 minute intervals from

191   moored units (YSI 6600 sonde, YSI Inc.) at each sampling location (Fig. 1). Three sites (MN,

192   WB, and CR) were maintained by the Waquoit Bay National Estuarine Research Reserve's

193   (WBNERR) seawater quality monitoring program (SWMP), and the Little River instrument was

194   maintained by the Mashpee Shellfish Constable. Wind speed and direction were recorded from a

195   weather station at the WBNERR facility on the north end of Waquoit Bay. Data from these

196   instruments were averaged daily during the sampling period.

197   A handheld instrument (YSI 650 MDS, YSI Inc.) recorded instantaneous temperature,

198   and salinity at the time and location of plankton collection. Measurements were taken at the

199   surface, middle, and bottom of the water column. Values at these depths were averaged for each

200   sample. If salinity and/or temperature differed between by one unit or more between the surface

201   and bottom the water column was considered stratified.

202   **Time series Analysis.** Autocorrelation analysis on time series of each species'

203   concentration and mean size at each site was performed to determine the scale of independence

204   for the samples and if there was periodicity. Series means were initially subtracted from each

205   value to detrend the data prior to analysis. Only autocorrelations at lags of 1-2 weeks were

206   considered meaningful based on the total length of our time series (less than 20%, Emery and

207   Thomson 1997). To determine if larval abundance and size structure were coherent between

208   sites, cross-correlation analyses were performed between pairs of sites for species concentrations

209   and mean sizes. Data were lagged in both directions by weekly time steps. In the few cases of

210 missing data points due to a lost sample or instrument failure (no more than two per series), the

211 missing data point was interpolated using a quadratic spline to ensure continuity of the time

212 series for analysis. The time scale for independent samples for each time series was determined

213 by the time point where the autocorrelation was no longer significantly different from zero (alpha

214 = 0.05). Degrees of freedom were calculated by dividing the total length of the time series by the

215 time scale for independent realizations. Although this is a less conservative approach than using

216 decorrelation times (the time point when the autocorrelation function crosses the x-axis), we

217 chose this method because the time series was only 24 points and most of the time series' were

218 not autocorrelated.

219   We explored possible associations between larval concentrations and physical

220 measurements of temperature, salinity and chlorophyll using the autocorrelation and cross-

221 correlation methods described above. For these cross-correlations, the log of larval concentration

222 was used to normalize the variance of larval time series with respect to the physical variables.

223 Temperature and salinity values were recorded simultaneously with each larval sample, and

224 chlorophyll fluorescence from the continuous records were averaged for the tidal period when

225 the sample was taken. We only regarded correlations with salinities at no time lags to be

226 biologically relevant, as the salinity time series oscillates predominately at a tidal frequency,

227 much shorter than our weekly sampling interval. All statistical tests were performed using

228 MATLAB and SYSTAT (version 12.0; SPSS, Inc) software.

229 **RESULTS**

230   **Environmental Setting.** Records from water quality monitoring instruments from the

231 main bay site (WB) indicate that bay water temperatures in spring and early summer in 2007

232 were warmer and had lower chlorophyll concentrations than in 2009 (Fig. 2a, c). Initial spawning

10

233   temperatures over 16°C occurred in May at all sites, ideal spawning temperatures over 20°C

234   were reached by late May in 2007, but not until late June in 2009. Temperatures warmed earlier

235   at the Little River site (Thompson 2011). During August, water temperatures and chlorophyll

236   concentrations were higher in 2009 than in 2007. Salinities were similar between the two years.

237   Salinity oscillated around 27-32 PSU with slightly fresher bay water in 2009 (Fig. 2b).

238   We grouped phytoplankton into flagellate and diatom categories to represent available

239   food sources for larvae (Fig. 3). Flagellates included dinoflagellates and represent the

240   background available food source for larvae in the bay, and diatoms found were both pennate

241   (such as *Nitzschia sp.*) and centric forms (*Chaetocerous sp.*, *Thalassiosira sp.*, *Skeletonema sp.*)

242   and represent a more nutritious source when available. Food was extremely low in Little River,

243   Menauhant, and the main bay (Fig. 3a,b,d) in May and June 2007. The higher chlorophyll

244   observed in 2009 was a result of diatom blooms starting in mid-June.

245   The typical summer prevalent southwest wind pattern for southern Cape Cod was

246   observed for both years (Fig. 4), with average onshore wind speeds around 0.5-1 m/s. This would

247   lead to increased freshwater buildup and stratification within the bay (Geyer 1997). In spring and

248   fall of 2009 there were sporadic storm events changing wind speed and direction, leading to

249   more mixing and flushing of bay water during these periods.

250   **Species-specific Concentration and Size Distribution.** Concentrations of all three

251   species were higher in 2009 than in 2007 (Fig. 5-7). For both years, peak abundance occurred in

252   July and August. For *Anomia simplex*, larvae were present from late June through August in

253   2007 and from June through September with a few individuals into October in 2009 (Fig. 5).

254   This species appeared to spawn weekly throughout its period of abundance with large portions of

255   smaller larvae and pediveligers appearing simultaneously. *A. simplex* was observed first in the

256   inlet site (MN) in 2007, but in 2009 the inner bay sites, Waquoit Bay and Little River, had the

257   highest early concentrations. Few *A. simplex* were observed at Childs River. Pediveliger larvae

258   were present throughout July and August of 2009 for all sites except Childs River, indicating

259   more survival. Pediveligers only appeared 1-2 times any site in 2007.

260   Larvae of the ribbed mussel, *Geukensia demissa*, were mostly observed July through

261   October both years, with some spawning in late June in 2007 (Fig. 6). Two distinct and equal

262   spawning events were observed in 2007, while in 2009 there was a mass spawn in July and a

263   smaller spawn afterwards (with the exception of Childs River which had three large peaks).

264   Abundances were greatest at Little River and Childs River where marshes are prevalent. In 2009,

265   *G. demissa* larvae were first observed from the Menauhant inlet site, far from the upper marsh

266   regions where adults are more abundant. Pediveligers were present in August in 2007 and July

267   through September in 2009, but in lower abundance compared to the other species.

268   *Mercenaria mercenaria* larvae appeared the earliest (May-June) of all the species

269   suggesting a lower spawning temperature threshold (Fig. 7). Little River and Waquoit Bay had

270   the highest concentrations of larvae in 2009, but in 2007 the highest concentrations were

271   observed at the inlet and less from the bay sites. *M. mercenaria* concentrations had bi-weekly

272   peaks in 2007 and 2009 with modes in July and August. Pediveliger larvae were prevalent in

273   2009 at all sites with highest concentrations in August and lasting through October, but only

274   occurred at the bay site in 2007.

275   **Spatial Patterns in Concentration and Mean Size.** Correlations were performed as a

276   way to assess the consistency, or coherence, of larval abundance and size with space and time.

277   There was no significant autocorrelation beyond a one week lag for either year for both the

278   abundance and mean size time series for all species with the exception of *Geukensia demissa*

279 abundance at Childs River in 2009, which was correlated for three weeks. We subtracted degrees

280 of freedom by two or three for the autocorrelated time series in the cross-correlations. Significant

281 correlations between sites (no lags) are depicted in Fig. 8. All correlation coefficients and

282 significant lag times are reported in supplementary material (Figs. S1-S2).

283   Comparing locations, there were significant correlations in abundance between the sites

284 on the eastern sides of the bay (Little River and Waquoit Bay site) and the western channels

285 (Menauhant inlet and Childs River), though these relationships were not consistent for all species

286 or for both years. *Anomia simplex* was the least well-mixed species in the bay showing consistent

287 separation between sites. In 2007, *A. simplex* concentrations at both Menauhant and Waquoit

288 Bay lagged behind Little River for 1-2 weeks ($r = 0.73$ and 0.52, respectively) and no sites were

289 correlated with Childs River. Mean sizes were significantly correlated for the three lower sites

290 (Fig. 8a), and also lagged two weeks behind Little River ($r = 0.73$ for MN and $r = 0.91$ for WB).

291 In 2009, Little River and Waquoit Bay were significantly cross-correlated for *A. simplex*

292 abundance ($r = 0.79$), as were Menauhant and Childs River ($r = 0.66$), and there was a two week

293 lag in cross-correlations between the two sides of the bay (Fig. 8b).

294   *Geukensia demissa* was more well-mixed between sites in 2009, but in 2007 there was

295 some spatial separation between east and west sites. *G. demissa* concentrations at Waquoit Bay

296 were correlated with both Little River and Menauhant (Fig. 8c, $r = 0.62$ and $r = 0.73$,

297 respectively), and Menauhant was correlated to Childs River ($r = 0.76$). Waquoit Bay and Little

298 River concentrations had significant one-week lags behind Menauhant and Childs River

299 (WB:MN $r = 0.67$, LR:MN $r = 0.71$, LR:CR $r = 0.51$). Only Menauhant and Childs River had

300 correlations with sizes in 2007 ($r = 0.65$), and all sites were lagged to Little River. In 2009,

301 positive and significant correlations were observed between Little River, Waquoit Bay, and

13

302   Menauhant (LR:WB r = 0.94, LR:MN r = 0.75, MN:WB r = 0.74) and between Childs River and

303   Waquoit Bay (r = 0.58) in 2009 (Fig. 8d). All mean sizes were strongly correlated in 2009

304   indicating strong coherence in population structure throughout the bay (LR:MN r = 0.59, LR:CR

305   r = 0.66, LR:WB r = 0.66, MN:CR r = 0.71, MN:WB r = 0.80).

306   *Mercenaria mercenaria* had little population coherence in 2007, but high coherence

307   among the more downstream sites in 2009. In 2007, abundances at Menauhant and Waquoit Bay

308   had a significant correlation (p = 0.69) and Little River abundances lagged all other sites by 1-2

309   weeks (Fig. 8e). All sites lagged Childs River. In 2009, Little River abundances and sizes were

310   significantly correlated with the Menauhant and Waquoit Bay sites (LR:MN r = 0.71/0.52,

311   LR:WB r = 0.76/0.79) and Childs River lagged Little River by one week (r = 0.63). Similar to

312   the other species, *M. mercenaria* time series' at Childs River were not correlated with many

313   other sites.

314   **Relationship to Environmental Factors.** Cross-correlations between environmental

315   conditions and individual species suggested that temperature was most influential at predicting

316   larval abundance for each species on a temporal scale, but salinity was more important on a

317   spatial scale. We compared the abundance and distributions of each species in relation to average

318   water column temperature and salinity by plotting larval concentration (on a log scale) as a

319   function of both these variables (Fig. 9). Childs River was almost always stratified when samples

320   were taken, while Menauhant and Wauquoit Bay - Metoxit Point were occasionally stratified.

321   Bivalve larvae were commonly found in samples taken during high temperature and

322   salinity conditions. Temperature and salinity trends were similar for both years, though 2009 had

323   a wider range of salinities, due to lower Childs River salinities (Fig. 9, squares). Little River

324   (Fig. 9, circles) had the highest ranges of observed temperatures in 2007, and Little River and

325 Menauhant (Fig. 9, triangles) had the highest ranges of temperatures in 2009. Results from both

326 years show that larval concentrations peak above 20°C indicating that these three species are

327 warm-water spawners. Salinity ranges were broader for *Geukensia demissa* with abundant

328 samples through mid-salinity ranges, but *Anomia simplex* and *Mercenaria mercenaria* favored

329 higher salinities (Fig. 9).

330   Temperature was the environmental variable that had the most significant correlations

331 with larval concentration time series (Fig. 10). Cross-correlations were significant between

332 temperature and *Anomia simplex* and *Geukensia demissa* concentration both years with the

333 exception of *A. simplex* at Childs River in 2007. In 2009, all species and sites were positively and

334 significantly correlated with temperature with the exception of *Mercenaria mercenaria* at Little

335 River. The only significant correlation with salinity was negative for *A. simplex* at Menauhant in

336 2007 ($r = -0.615$).

337   There were a few significant correlations with chlorophyll in 2007, but they were low and

338 possibly not biologically relevant. Waquoit Bay chlorophyll was negatively cross-correlated with

339 *Geukensia demissa* larvae ($r = -0.44$), and Childs River chlorophyll was positively cross-

340 correlated with *Anomia simplex* (0.47). We used phytoplankton counts for a better estimate of

341 larval food abundance. Comparing larval abundances to the presence of phytoplankton from the

342 counts (Fig. 3), higher survival in the later months of 2009 may be attributed to the diatom

343 bloom at many of the sites, which was absent in 2007. See supplementary material for all

344 correlation coefficients (Fig. S3-S4).

345   **Synthesis.** Based on the information in this study, the proposed mechanism for higher

346 concentrations and sizes in 2009 compared to 2007 is that initially cooler temperatures delayed

347 mass spawning but wetter conditions encouraged phytoplankton growth. Once species spawned

348 temperatures increased and diatoms were abundant encouraging rapid growth and better survival

349 of larvae. Higher wind speeds let to more circulation in the bay causing larvae to be transported

350 further upstream and retained.

351 **DISCUSSION**

352   The purpose of this study was to investigate environmental factors that could influence

353 larval abundances on a weekly scale. We were able to document two very different years in

354 terms of biological and physical characteristics, and we propose this had a pronounced affect on

355 larval concentrations and survival. Larval growth depends mainly on nutritional factors and

356 larval survival depends on predation, advection, and initial density (Fegley 2001). We used

357 density of veliger and pediveliger larvae to interpret larval survival which also may imply

358 successful growth, however, growth rates were not calculated as we would have to successfully

359 isolate cohorts and assume no larval loss. This study suggests annual patterns in water

360 temperature and food availability can lead to yearly fluctuations in larval abundance. These

361 differences can affect potential settling of adults, emphasizing the importance of recognizing

362 larval stages to explain population dynamics in management applications. Because enhancement

363 efforts can rely heavily on spat collection to measure recruitment, knowing optimal times and

364 locations to place collectors is valuable to maximizing returns.

365   **Species-Specific Concentration and Size.** We compared species-specific yearly

366 differences in larval abundance and survival, which is rare for studies of bivalve larvae. The few

367 field studies that have been performed on *Mercenaria mercenaria* reported large differences in

368 abundance between years (Carriker 1961, Fegley 2001). Despite its commercial importance to

369 the area, there is little documentation of *M. mercenaria* spawning for Cape Cod. Spawning

370 temperatures for *M. mercenaria* near New Bedford, Massachusetts have been reported at 21°C

16

371 and can occur as low as 18-19°C in more northern areas (Eversole 2001). Spawning of *M.*

372 *mercenaria* has been documented for areas south of Long Island from June – November

373 (Loosanoff et al. 1951, Keck et al. 1975). Our study showed that *M. mercenaria* larvae were

374 present in low concentrations as early as mid-May in both 2007 and 2009 at water temperatures

375 above 16°C. A Rhode Island study performed in 1954 found larvae appearing by the end of May

376 with highest abundances of *M. mercenaria* larvae in mid to late June, however late July and

377 August had the highest concentrations in our study (Fegley 2001). Carriker (1961) found larvae

378 appearing in late May with highest densities in July when sampling from 1948-1951 in Little

379 Egg Harbor, NJ. The differences between our study and older studies of *M. mercenaria* could

380 reflect differences in regional populations as well as possible adaptations to climate change.

381   *Mercenaria mercenaria* had a coherent population structure within Waquoit Bay in 2009

382 as both abundance and sizes were correlated between sites indicating the population was well-

383 dispersed and uniform throughout the bay. There was less coherence in 2007. Results indicated

384 that Little River and Waquoit Bay could be source or spawning locations as larvae were more

385 abundant and initially appeared there. A major biomass and high density source of spawning

386 quahogs was located in a ten hectare shellfish aquaculture farm in the Seapit River that flows

387 tidally between Waquoit Bay and Childs River. The tidal direction during a major spawning

388 period would determine the concentration of larvae from the spawn transported to the main bay.

389 Tides flowing in the Childs River direction during a big spawn in 2007 might explain the high

390 concentrations observed there and at Menauhant with other sites lagging behind, with larvae

391 ultimately succumbing to low salinities or export. In 2009, a tide flowing in the opposite

392 direction might explain the high concentrations and retention of *M. mercenaria* in the main bay.

393 The highest abundance of *M. mercenaria* pediveligers was in August 2009 at all sites, with

394 Childs River in particular having a high proportion of pediveliger larvae. This may be due to

395 lower salinities in Childs River discouraging pediveliger larvae from settling and causing them to

396 remain in the water column. *M. mercenaria* spawned again later in the season and may have

397 settled at Little River and the main bay, despite not growing as fast as in mid-summer. In 2011,

398 two-year old *M. mercenaria* made up a large portion of adults and most of the seed in the

399 Waquoit Bay population (R. York, unpublished result). This can now be traced to the favorable

400 conditions and high larval supply in mid-summer 2009.

401   For the jingle clam, *Anomia simplex*, different conclusions can be made. Abundance and

402 population structure were separated between sides of the bay, and no correlations between

403 abundances and sites were observed in 2007 when overall concentrations were low. Little River

404 was a likely source population, and early 2007 data indicated a possible source of *A. simplex*

405 larvae from the inlet. However, *A. simplex* did not seem to stay in Little River, as more

406 pediveligers were found in Menauhant and the main bay. There was some evidence for this

407 species to be found in bay waters on ebb tides, supporting an estuarine source and export for *A.*

408 *simplex*. More upstream transport from the inlet may have caused *A. simplex* abundance in

409 Childs River to be correlated with Menauhant in 2009. As the adult lifespan for *A. simplex* is

410 only 1-2 years (Chanley and Andrews 1971), year-to-year differences in larval abundance would

411 depend on the previous year's recruitment and might explain the patchiness and inconsistencies

412 observed between years. The multitude of peaks in the time series suggest *A. simplex* spawns

413 continuously, and our data suggest these spawns may come from different locations. Larval

414 periods of 3-4 weeks are typical for this species (Chanely and Andrews 1971), so retention in the

415 bay system may be difficult.

416   Despite both *Mercenaria mercenaria* and *Anomia simplex* achieving sizes that could lead

417   to settlement in Waquoit Bay, *Geukensia demissa* larvae showed little evidence of survival to

418   pediveligers or retention in 2009 despite maintaining stable concentrations in the bay with well-

419   mixed populations. It is likely that marsh areas in the bay and channels like Little River were

420   sources of larvae for *G. demissa*, though some larvae were found in the inlet site early in 2009.

421   *G. demissa* spawned with 2-3 distinct cohorts, but the middle cohort that had the highest

422   concentrations did not result in many pediveligers observed later. It remains uncertain why *G.*

423   *demissa* had reduced survival compared to the other species when all were present concurrently.

424   Food availability was better in the early and later periods, and *G. demissa* may have been more

425   vulnerable to export. Rigal et al. (2010) found that tidal efflux resulted in a lack of settlement-

426   stage gastropod larvae in an embayment. For instance, larvae spawned in marsh channels on an

427   outgoing tide could be instantly flushed out of the bay and not retained as well as *M. mercenaria*

428   larvae spawned in the middle of the bay and caught in a gyre or transported to a channel with

429   higher retention times.

430   **Spatial Patterns in Concentration.** Time series of abundances at different sites can

431   allow for predictions of dispersal. Larval abundance at most sites was autocorrelated for a

432   maximum of two weeks, which is on the order of water residence times and the larval

433   development period. Each species had different trends: *Anomia simplex* was the least dispersed,

434   but *Geukensia demissa* and *Mercenaria mercenaria* were well dispersed at times, particularly

435   during 2009. This suggests that there are both periods of limited dispersal and homogeneity

436   throughout a spawning season for different species. Other studies have failed to show temporal

437   correlations between sites with younger *M. mercenaria* larvae, but older larvae were found to be

438   more dispersed, although these studies were performed over a greater spatial range (Fegley

439   2001). Childs River often showed distinct abundance patterns from the other sites, which could

440   be a result of its upstream location, lower salinities, higher nutrients, and longer retention times

441   (Tomasky-Holmes 2008).

442   There are several physical factors that may have contributed to the observed differences

443   in larval abundance. All sites were separated by only 1-2 km, thus the sometimes large

444   differences in concentrations between sites emphasize the patchiness within the system. Flow

445   dynamics through an inlet are different than flow through estuarine channels and open water. In

446   Waquoit Bay, the inlets have the strongest flows and exchange water rapidly with the main bay.

447   Flows through sub-embayments vary, exchanging waters over 1-3 days with the bay proper, but

448   have much longer residence times within the whole bay system due to marsh storage areas

449   (Howes et al. 2004). In addition, sporadic wind forcing from the north can disrupt stratification

450   and lead to increased flushing of the bay (Geyer 1997), which may be responsible for occasional

451   decreases in concentration. Increased wind speeds in 2009 could have led to increased mixing

452   and transport in the bay and could account for the larger presence of larvae, particularly *Anomia*

453   *simplex* and large *Mercenaria mercenaria* larvae, upstream at Childs River.

454   **Relationships with Environmental Conditions.** We looked at larval abundance over

455   two years when environmental conditions within the bay differed with respect to temperature,

456   chlorophyll and food quality. We chose these conditions based on environmental influences that

457   may operate on our weekly sampling scale. Other factors, such as tidal flow and wind stress need

458   to be investigated at higher frequencies (i.e. Roegner et al. 2007). If we use total abundance as a

459   proxy for spawning activity, spawning was less in 2007 despite higher temperatures. In 2009,

460   bivalve larval concentrations for the Waquoit Bay site were as large as 90/L with individual

461   species' concentrations ranging from 8.5-22/L. This is extremely high, even for an estuary, and

462   may indicate multiple mass spawnings. Typical reported peaks range from a few hundred to

463   thousand bivalves per m^3 (Wood and Hargis 1971, Andrews 1983, Garland 2000), although

464   reported densities from Carriker (1961) were up to 70/L. This suggests Waquoit Bay is an

465   abundant pool of bivalve larvae when conditions are right.

466   Temperature was revealed to be important in predicting larval abundance. Abundances of

467   each species indicated that 20ºC and above are optimal temperatures for larval abundance, most

468   likely due to peak spawning. In 2009, spawning for *Geukensia demissa* occurred later than in

469   2007 because temperatures warmed up later in 2009. This delayed spawn could have led to the

470   higher larval concentrations observed if adult gonads were allowed to ripen longer. Correlations

471   with temperature were seen at all sites both years, but they were highest in 2009. *Mercenaria*

472   *mercenaria* was the only species found consistently present below 20ºC. Temperature has been

473   shown to have significant correlations with other bivalve and reef fish larval abundance in other

474   studies (Chicharo and Chicharo 2001, Wilson and Meekan 2001), most likely due to the role it

475   plays in increasing metabolic and growth rates as well as the growth of algae, which can be good

476   for survivorship if enough food is present.

477   Salinity and chlorophyll showed weaker correlations with larval abundance. Salinity did

478   not vary much temporally, but showed variations between sites. Negative cross-correlations with

479   *Anomia simplex* and salinity at Menauhant in 2007 suggest that there could have been tidal effect

480   with more larvae present on outgoing tides, but this pattern is not necessarily causation and

481   merits further investigation on a tidal scale (Thompson 2011). Site-specific differences in

482   salinities highlight potential larval tolerances for each species, and low salinities can reduce

483   survivorship of sensitive species. *G. demissa* had the highest abundance during low salinity

484   periods in Childs River, and *Anomia simplex* was the least tolerant of low salinity conditions.

485   However, this relationship could also be explained by proximity to sources or different patterns

486   in dispersal between species. Although we did see some significant correlations to chlorophyll,

487   these were weak and suggest that larvae are not necessarily associated with areas of high or low

488   chlorophyll. It is possible that the extremely high concentrations of larvae associated with these

489   samples were effective at grazing the phytoplankton down to the lower levels observed,

490   particularly for 2009. In 2007, blooms of large dinoflagellates at Childs River created a lot of

491   turbidity and could be a deterrent to larvae. Overall, relationships to chlorophyll were

492   inconsistent, and it may be necessary to investigate this question with higher frequency sampling

493   to see any patterns (Domingues et al. 2011). Other field studies of bivalve larvae have also failed

494   to find associations with chlorophyll (Tremblay and Sinclair 1990, Raby et al. 1994).

495   **Food Quality.** We compared the abundance of diatoms, a quality larval food source, and

496   flagellates, usually a mediocre food source, for both years. There was very little available

497   phytoplankton that would be suitable for larval ingestion and growth in May and June of 2007.

498   Together with higher temperatures, this would have led to more starvation for these cohorts.

499   Diatom blooms coinciding with higher temperatures in July and August 2009 could have led to

500   their increased survival.

501   In 2007, lower numbers of pediveligers could suggest that low food quality may have

502   limited larval survival and growth, although other food sources may have been present. Bivalve

503   larvae typically consume food particles in the pico- to nano- plankton size range of 0.5–12 μm,

504   occasionally ingesting large particles up to 30 μm if abundant (Baldwin and Newell 1995).

505   However, it is well documented that bivalve larvae are capable of ingesting bacteria (Douillet

506   1993, Gallager et al. 1994, Tomaru et al. 2000), although it mostly supplements growth on a

507   phytoplankton-based diet (Baldwin and Newell 1991). Larvae can grow in estuarine conditions

22

508   with low natural phytoplankton abundances, although growth patterns are species-specific (Crisp

509   et al. 1985), and diets are likely supplemented with bacteria or detritus (Fritz et al. 1984). Larvae

510   can continue shell growth without food by depleting tissue for energy (Crisp et al. 1985).

511   More predation could explain the observed low abundance of pediveliger larvae in 2007

512   if food was not limiting. Although predation may be relatively low for larvae overall, certain

513   predators, if abundant, are capable of reducing a bivalve larval population by upwards of 80%

514   (Johnson and Shanks 2003). In 2007 the ctenophore *Mnemiopsis leidyi* was more abundant than

515   in 2009 (C.M. Thompson, pers. obs.) and could have reduced larval abundance through top-

516   down control.

517   **Conclusions.** By performing a species-specific study using a new method to identify

518   bivalve larvae, we were able to depict spatial and temporal trends and uncover environmental

519   factors that may regulate larval supply for each species. We observed general patterns affecting

520   larval abundance, such as seasonal temperature and site-specific salinity differences, which are

521   likely to have the greatest effect on survivability. Species-specific patterns suggested that for a

522   commercial species like *Mercenaira mercenaria*, higher recruitment based on larval supply alone

523   would likely be achieved in a year where high temperatures are coupled with abundant quality

524   food. For an ecologically important species like *Geukensia demissa*, our study suggests that

525   larval survival was low, and that larval supply may be subsidized from other marshes or limited

526   to the few individuals retained in high-retention areas of the bay. We used cross-correlation

527   analyses to determine whether such characteristics had a relationship with larval abundance,

528   however these results are correlative but not necessarily causation. There could be many other

529   mechanisms leading to the observed patterns in larval abundance and survival that were not

530   investigated in this study, such as tidal flow, wind-driven transport, or environmental conditions

531 and spawning rates from nearby populations. Furthermore, we did not test correlations separately

532 for size classes which can sometimes lead to different patterns (Fegely 2001). Because Waquoit

533 Bay has abundant shellfish resources for both recreational and commercial fisheries and such a

534 high abundance of larvae, a study such as ours is necessary to understand the factors regulating

535 these valuable resources and to managing future populations and biodiversity.

536 **ACKNOWLEDGEMENTS**
537
549
550 **LITERATURE CITED**
551
552 Andrews JD (1983) Transport of bivalve larvae in James River, Virginia. J Shellfish Res 3(1):29-
553   40
554 Baldwin BS, Newell RIE (1991) Omnivorous feeding by planktotrophic larvae of the eastern
555   oyster *Crassostrea virginica*. Mar Ecol Prog Ser 78:285-301
556 Baldwin BS, Newell RIE (1995) Relative importance of different size food particles in the
557   natural diet of oyster larvae (*Crassostrea virginica*). Mar Ecol Prog Ser 120:135-145
558 Bayne BL (1965) Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.).
559   Ophelia 2(1): 1-47
560 Belgrano A, Legendre P, Dewarumex JM, Frontier S (1995) Spatial structure and ecological
561   variation of meroplankton on the Belgian-Dutch coast of the North Sea. Mar Ecol Prog
562   Ser 128:51-59
563 Bertness MD (1984) Ribbed mussels and *Spartina alterniflora* production in a New England salt
564   marsh. Ecology 65(6): 1794-1807.
565 Boicourt WC (1988) Recruitment dependence on planktonic transport in coastal waters. In:
566   Rothschild, B.J. (ed.), *Toward a Theory on Biological-Physical Interactions in the World*
567   *Ocean*. Kluwer Academic Publishers, p 183-202
568 Botsford LW, Moloney CL, Hastings A, Largier JL, Powell TM, Higgins K, Quinn JF (1994)
569   The influence of spatially and temporally varying oceanographic conditions on
570   meroplanktonic metapopulations. Deep-Sea Res II 41:107-145

Botsford LW, Wing SR, Largier JL (1998) Population dynamics and management implications of larval dispersal. S Afr J of Mar Sci 19:131-142

Brousseau DJ (1977) Spawning cycle, fecundity, and recruitment in a population of soft-shell clam, *Mya arenaria*, from Cape Ann, Massachusetts. Fish Bull 76(1):155-166

Carriker MR (1961) Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve, *Mercenaria mercenaria*. J Elisha Mitchell Sci Soc 77: 168-241

Carriker MR (1988) Bivalve larval research, in transition: a commentary. J Shellfish Res 7(1):1-6

Chanley P, Andrews JD (1971) Aids for identification of bivalve larvae of Virginia. Malacologia 11:45-119

Chicharo L, Chicharo MA (2001) Effects of environmental conditions on planktonic abundances, benthic recruitment and growth rates of the bivalve mollusc *Ruditapes decussates* in a Portuguese coastal lagoon. Fish Res 53:235-250

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Annu Rev Mar Sci 1:443-466

Crisp DJ, Yule AB, White KN (1985) Feeding by oyster larvae: the functional response, energy budget and a comparison with mussel larvae. Mar Biol Ass UK 65:759-783

Davis HC, Calabrese A (1964) Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica.* Fish Bull 63(3):643-655

Dekshenieks MM, Hofmann EE, Powell EN (1993) Environmental effects on the growth and development of eastern oyster, *Crassostrea virginica* (Gmelin, 1971), larvae: a modeling study. J Shellfish Res 12(2):241-254

Domingues CP, Almeida MJ, Dubert J, Nolasco R, Cordeiro N, Waap S, Sequeira A, Tavares S, Queiroga H. 2011. Supply of crab larvae to an estuary in the eastern Atlantic upwelling system exhibits predictable and haphazard variation at different temporal scales. Mar. Ecol. Prog. Ser. 425: 113-124.

Douillet P (1993) Bacterivory in Pacific oyster *Crassostrea gigas* larvae. Mar Ecol Prog Ser 98:123-134

Eckman JE (1987) The role of hydrodynamics in recruitment, growth, and survival of *Argopecten irradians* (L.) and *Anomia simplex* (D'Orbingy) within eelgrass meadows. J Exp Mar Biol Ecol 106:165-191

Emery WJ, Thomson RE (1997) Data Analysis Methods in Physical Oceanography. Pergamon Press, New York

Eversole AG (2001) Reproduction in *Mercenaria mercenaria*. IN: Kraeuter JN, Castagna M (Eds.) Biology of the Hard Clam. Elsevier, pp 221-260

Fegley SR (2001) Demography and dynamics of hard clam populations. IN: Kraeuter JN, Castagna M (Eds.) Biology of the Hard Clam. Elsevier, pp 383-422

Fritz LW, Lutz RA, Foote MA, Van Dover CL, Ewart JW (1984) Selective feeding and grazing rates of oyster (*Crassostrea virginica*) larvae on natural phytoplankton assemblages. Estuaries 7:513-518

Gaines SD and Bertness MD (1992) Dispersal of juveniles and variable recruitment in sessile marine species. Nature 360:579-580

Gallager SM, Mann F, Sasaki G (1986) Lipid as an index of growth and viability in three species of bivalve larvae. Aquaculture 56:81-103

Gallager SM, Waterbury JB, Stoecker DK (1994) Efficient grazing and utilization of the marine cyanobacterium *Synechococcus sp*. by larvae of the bivalve *Mercenaria mercenaria*. Mar Biol 119:251-259

Garland ED (2000) Temporal variability and vertical structure in larval abundance: the potential roles of biological and physical processes. PhD dissertation, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution, Woods Hole, MA

Garland ED, Zimmer CA (2002) Techniques for the identification of bivalve larvae. Mar Ecol Prog Ser 225:299-310

Geyer WR (1997) Influence of wind on dynamics and flushing of shallow estuaries. Est Coast Shelf Sci 44:713-722

Hare, M.P., S.R. Palumbi, and C.A. Butman. 2000. Single-step species identification of bivalve larvae using multiplex polymerase chain reaction. Mar. Biol. 137: 953-961.

Howes B, Kelley WW, Ramsey JS, Samimy R, Schlezinger D, Ruthven T, Eichner E (2004) Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for the Quashnet River, Hamblin Pond, and Jehu Pond, in the Waquoit Bay System in the Towns of Mashpee and Falmouth, Massachusetts. Massachusetts Estuaries Project, Massachusetts Department of Environmental Protection. Boston, MA

Hodgson CA, Bourne N (1988) Effect of temperature on larval development of the spiny scallop, *Chlamys hastata* Sowerby, with a note on metamorphosis. J Shellfish Res 7: 349-357.

Johnson KB, Shanks AL (2003) Low rates of predation on planktonic marine invertebrate larvae. Mar Ecol Prog Ser 248:125-139

Jordan TE, Valiela I (1982) A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a New England salt marsh. Limnol Oceanogr 27(1):75-90

Jorgensen CB (1981) Mortality, growth, and grazing impact of a cohort of bivalve larvae, *Mytilus edulis* L. Ophelia 20(2):185-192

Kassner J, Malouf RE (1982) An evaluation of "spawner transplants" as a management tool in Long Island's hard clam fishery. J Shellfish Res 2(2):165-172

Keck RT, Maurer D, Lind H (1975) A comparative study of the hard clam gonad developmental cycle. Biol Bull 148:243-258

Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. Integr Comp Biol 46(3):282-297

Litaker W, Duke CS, Kenney BE, Ramus J (1987) Short-term environmental variability and phytoplankton abundance in a shallow tidal estuary. Mar Biol 96:115-121

Loosanoff VL, Miller WS, Smith PB (1951) Growth and setting of larvae of *Venus mercenaria* in relation to temperature. J Mar Res 10:59-81

Loosanoff VL, Davis HC (1963) Rearing of bivalve mollusks. Adv Mar Biol 1:1-136

Loosanoff VL, Davis HC, Chanley PE. (1966) Dimensions and shapes of larvae of some marine bivalve mollusks. Malacologia 4:351-435

Mann R (1988) Field studies of bivalve larvae and their recruitment to the benthos: a commentary. J of Shellfish Res 7(1):7-10

Orensanz JM, Parma AM, Iribarne OO. (1991) Population dynamics and management of natural stocks. In: Shumway SE (ed.) Scallops: biology, ecology, and aquaculture. Elsevier, NY, p 625-713

Pechenik JA, Eyster LS, Widdows J, and Bayne BL (1990) The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* L. larvae. J Exp Mar Biol Ecol 136: 47-64

661   Pineda J, Hare JA, and Sponaugle S (2007) Larval transport and dispersal in the coastal ocean
662   and consequences for population connectivity. Oceanography 20(3): 22-39.
663   Raby D, Lagadeuc Y, Doson JJ, Mingelbier M (1994) Relationship between feeding and vertical
664   distribution of bivalve larvae in stratified and mixed waters. Mar Ecol Prog Ser 103:275-
665   284
666   Rigal F, Viard F, Ayata S, Comtet T (2010) Does larval supply explain the low proliferation of
667   the invasive gastropod *Crepidula fornicata* in a tidal estuary? Biol Invasions Pub Online
668   4 Feb. 2010
669   Roegner GC, Armstrong DA, Shanks AL. 2007. Wind and tidal influences on larval crab
670   recruitment to an Oregon estuary. Mar. Ecol. Prog. Ser. 351: 177-188.
671   Roughgarden JS, Gaines SD, Possingham H (1988) Recruitment dynamics in complex life
672   cycles. Science 241:1460-1466
673   Scheltema RS (1986) On dispersal and planktonic larvae of benthic invertebrates: an eclectic
674   overview and summary of problems. Bull Mar Sci 39(2):290-322
675   Shirley SM, Shirley TC (1989) Interannual variability in density, timing, and survival of Alaskan
676   red king crab *Paralithodes camtschatica* larvae. Mar Ecol Prog Ser 54:51-59
677   Starr M, Himmelman JH, Therriault JC (1991) Coupling of nauplii release in barnacles with
678   phytoplankton blooms: a parallel strategy to that of spawning in urchins and mussels. J
679   Plank Res 13(3): 561-571
680   Thompson CM (2011) Species-specific patterns in bivalve larval supply to a coastal embayment.
681   PhD dissertation, Massachusetts Institute of Technology/Woods Hole Oceanographic
682   Institution, Woods Hole, MA
683   Thompson CM, Hare MP, and Gallager SM. In press. Semi-automated image analysis for the
684   identification of bivalve larvae from a Cape Cod estuary. Limnol. Oceanogr.-Meth.
685   Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Bio Rev
686   25:1-45
687   Tiwari S, Gallager SM (2003a) Optimizing multiscale invariants for the identification of bivalve
688   larvae. Proceedings of the 2003 IEEE International Conference on Image Processing,
689   Barcelona, Spain, September 14-17, 2003
690   Tiwari, S, Gallager SM (2003b) Machine learning and multiscale methods in the identification of
691   bivalve larvae. Proceedings of the Ninth IEEE International Conference on Computer
692   Vision, Nice, France, October 14-17, 2003
693   Todd CD (1998) Larval supply and recruitment of benthic invertebrates: do larvae always
694   disperse as much as we believe? Hydrobiologia 375/376:1-21
695   Townsend DW Cammen LM (1988) Potential importance of the timing of spring plankton
696   blooms to benthic-pelagic coupling and recruitment of juvenile demersal fishes. Biol
697   Oceanogr 5:215-229
698   Tomaru Y, Kawabata S, Nakano S (2000) Consumption of picoplankton by the bivalve larvae of
699   Japanese pearl oyster *Pinctada fucata martensii*. Mar Ecol Prog Ser 192:195-202
700   Tomasky-Holmes G (2008) Nutrient supply, water residence time, temperature, and grazing as
701   controls of size-fractionated phytoplankton biomass in shallow temperate estuarine
702   ecosystems. PhD dissertation, Boston University, Boston, MA
703   Tremblay MJ, Sinclair M. (1990) Sea scallop larvae *Placopecten magellanicus* on Georges
704   Bank: vertical distribution in relation to water column stratification and food. 61:1-15.

705   Wood L, Hargis WJ (1971) Transport of bivalve larvae in a tidal estuary. IN: Crisp DJ (Ed.)
706   Proceedings of the Fourth European Marine Biology Symposium. Bangor, 1969.
707   Cambridge University Press, Cambridge, p 29-44
708   Wilson DT, and Meekan. 2001. Environmental influences on patterns of larval replenishment in
709   coral reef fishes. Mar. Ecol. Prog. Ser. 222: 197-208.
710

711

712
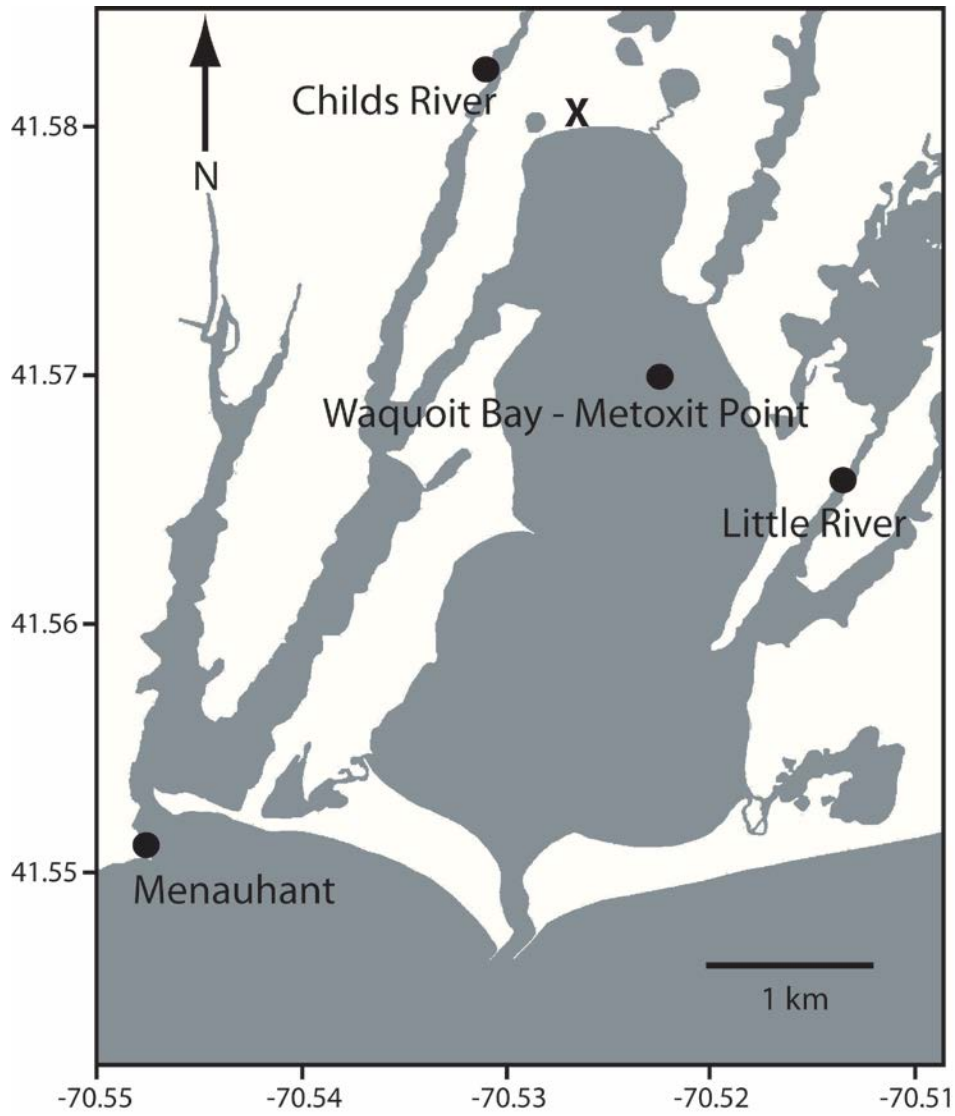
Figure 1.   Map of Waquoit Bay showing the four larval sampling sites (black dots). The 'X'
marks the location of the Waquoit Bay National Estuarine Research Reserve and weather station
where wind data was collected.
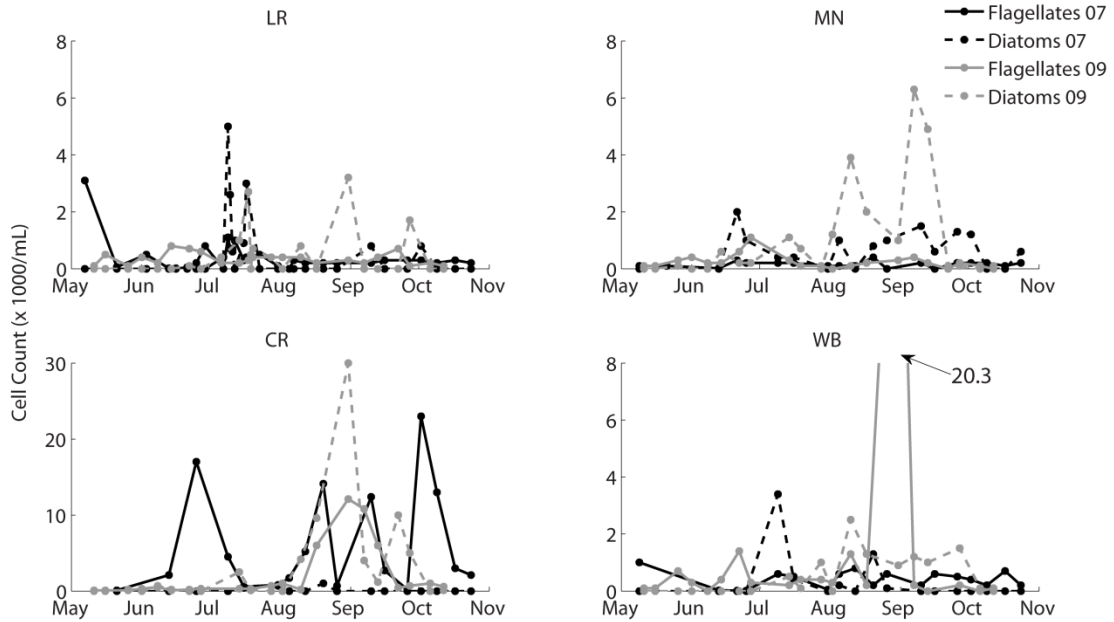
716
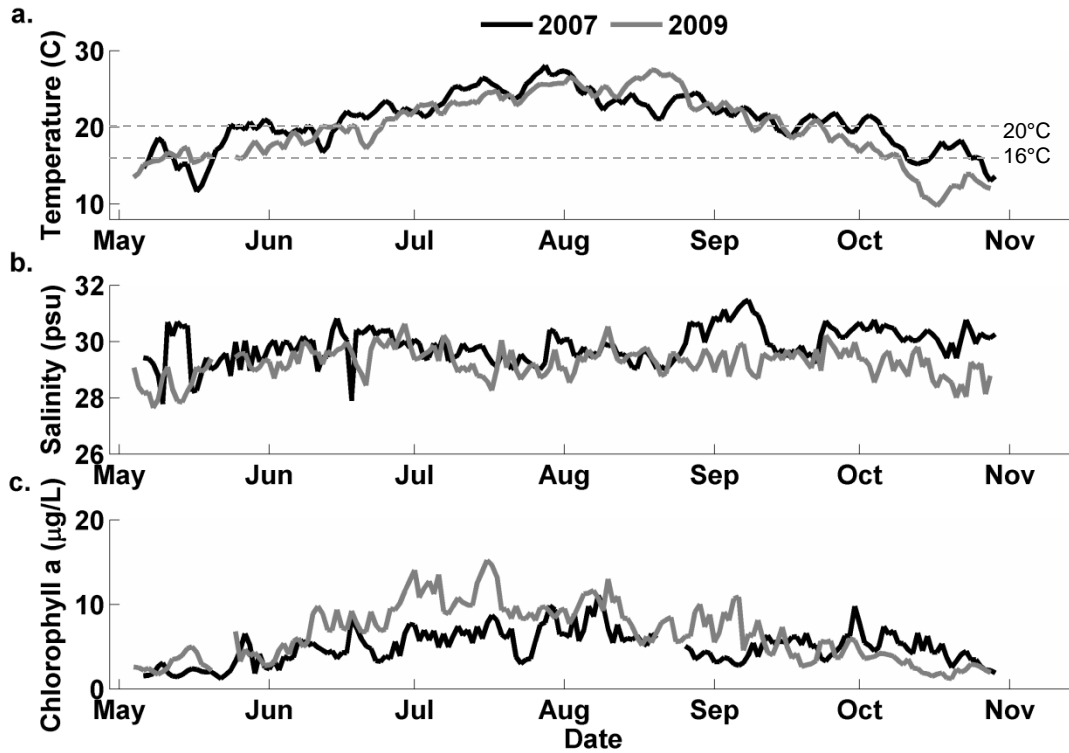
Figure 2. Daily averaged (a) temperature (b) salinity and (c) chlorophyll a for Waquoit Bay –
Metoxit Point. All recorded data were averaged daily from moored loggers for the sampling
periods of May through October in 2007 and 2009. Dashed lines in (a) represent lower and
higher threshold spawning times of 16°C and 20°C, respectively.

721

722

723

Figure 3.   Phytoplankton counts for all sites in 2007 (black) and 2009 (gray). Total counts of
flagellates (solid line) and diatoms (dashed lines) from water samples on each sampling date are
plotted for each site. Flagellates included counts for flagellates and dinoflagellates and diatoms
consisted of centric, pennate, *Chaetoceros sp.*, *Skeletonema sp.*, *Thalassiora sp.*, and *Nitzchia sp.*
Note the different axis scale for (c) and outlier value for (d). Site abbreviations: LR = Little
River; CR = Childs River; MN = Menauhant; WB = Waquoit Bay – Metoxit Point.
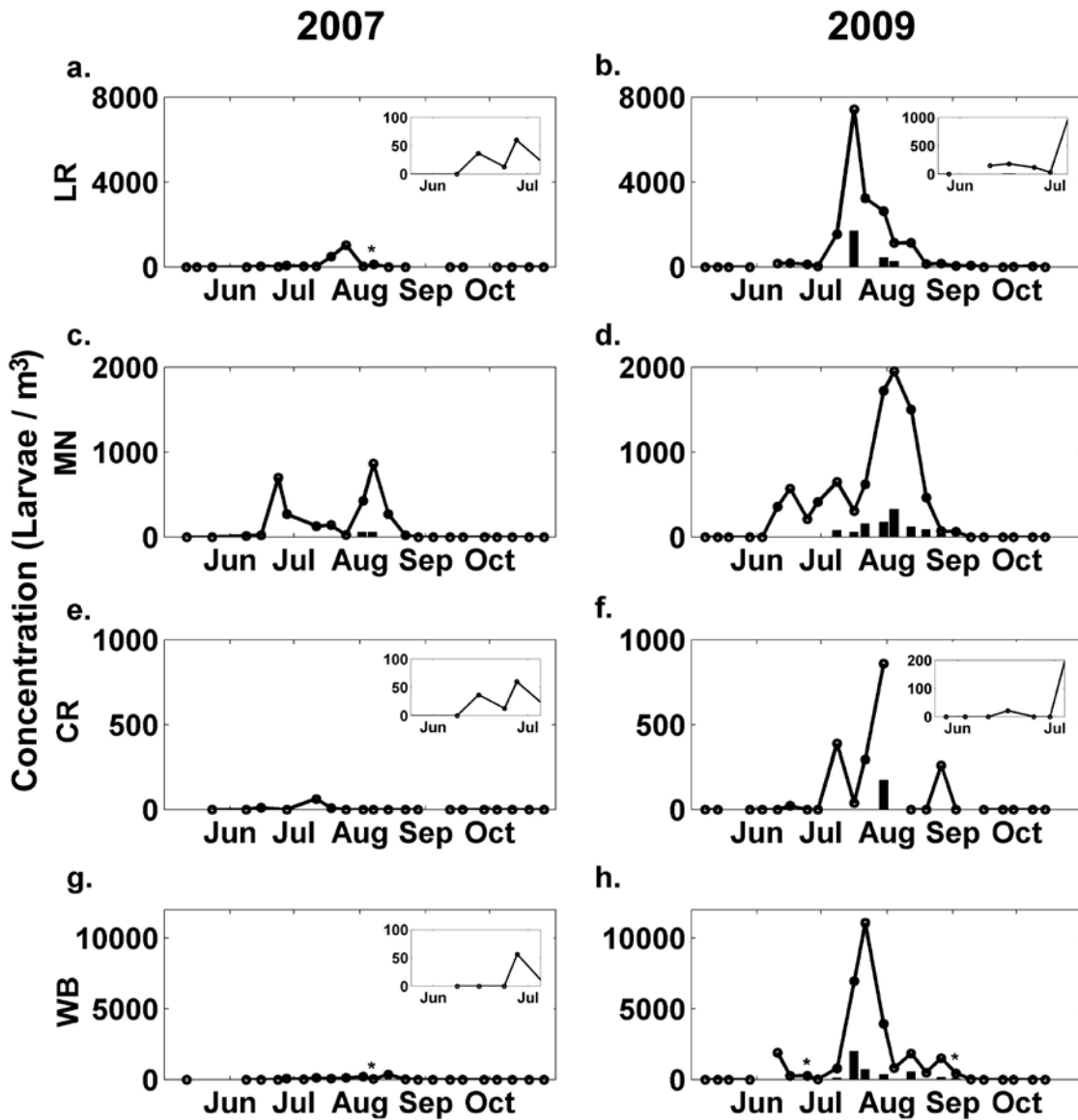
730
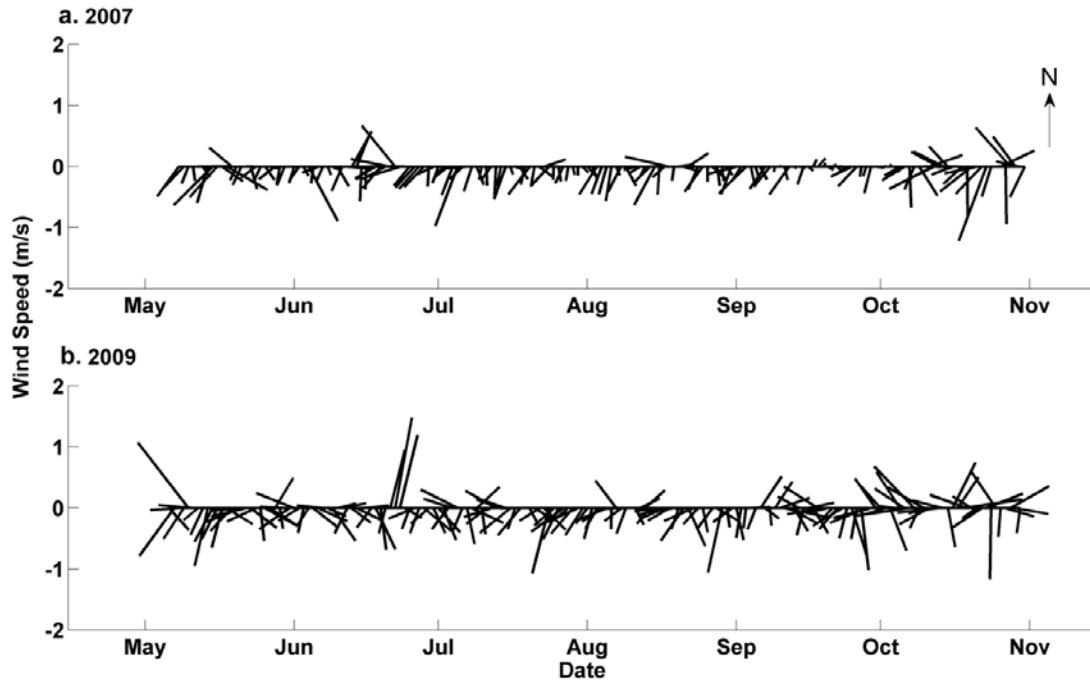
731

732   Figure 4. Time series of wind vectors for Waquoit Bay for (a) 2007 and (b) 2009. Wind speed
733   (m/s) and direction were averaged daily from May through October for both years. The weather
734   station was located at the north tip of the bay at the Waquoit Bay National Estuarine Research
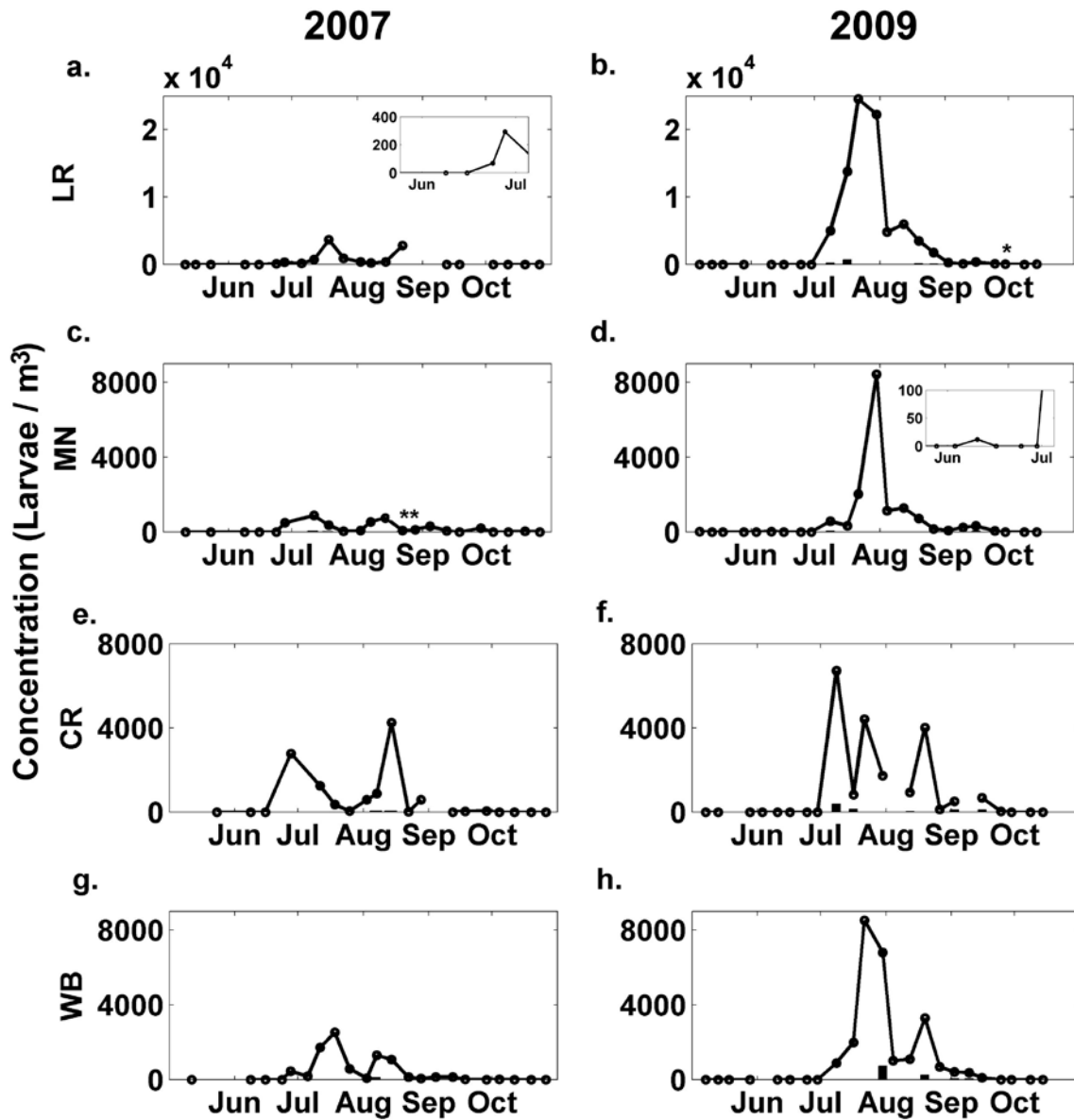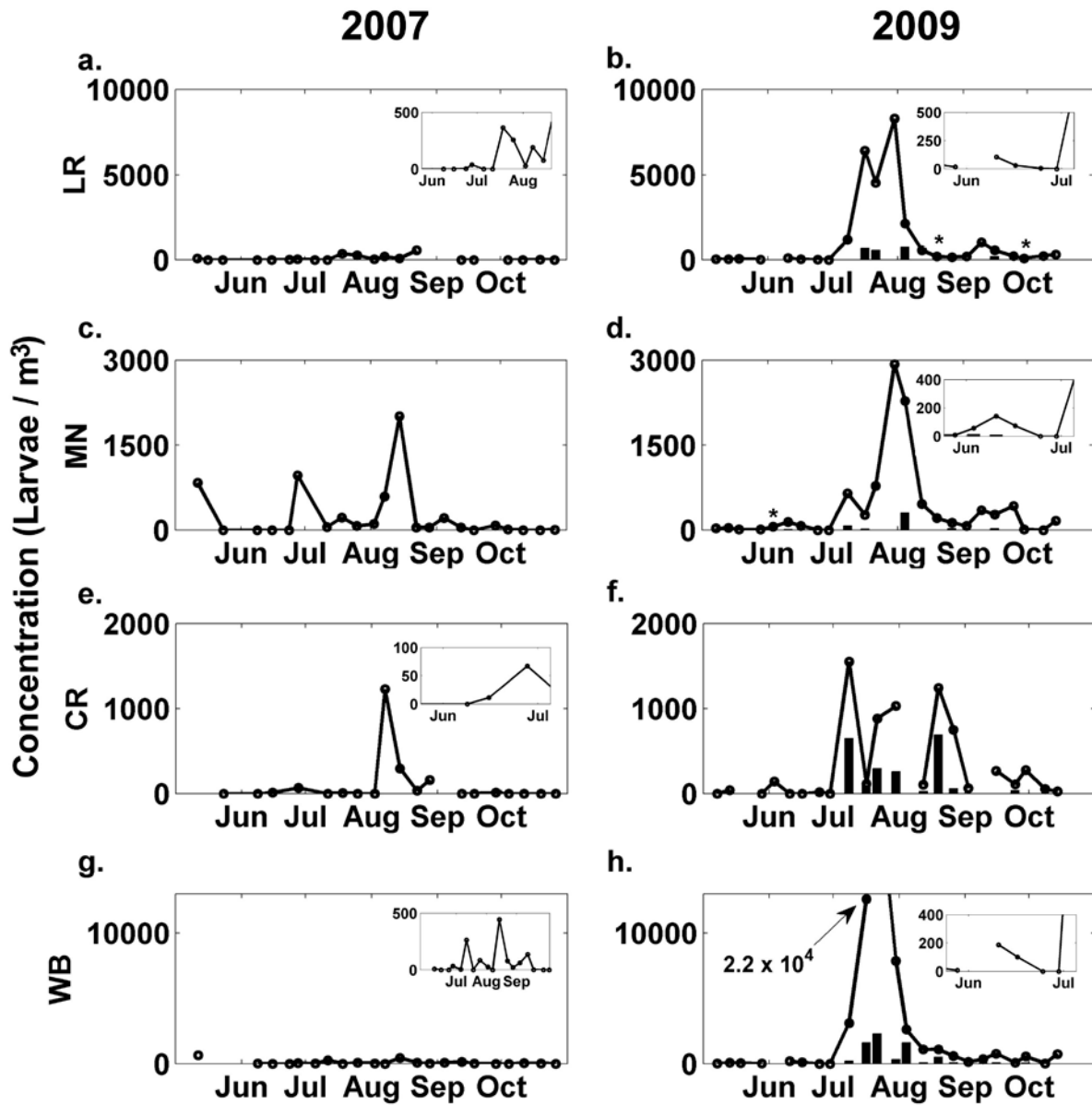735   Reserve.

736

737

Figure 5. Time series of *Anomia simplex* larvae at each sampling site for 2007 and 2009. Black lines show total concentration and bars underneath represent concentration of pediveliger (> 175 µm) larvae. Inserts show a zoomed in area to depict trends if not visible on full graph. Asterisks represent samples where pediveliger larvae were present but concentrations were too small to appear on figure. Site abbreviations: LR = Little River; CR = Childs River; MN = Menauhant; WB = Waquoit Bay – Metoxit Point

738

739
740
741
742
743
744
745

Figure 6. Time series of *Geukensia demissa* larvae at each sampling site for 2007 and 2009. Black lines show total concentration and bars underneath represent concentration of pediveliger (> 200 µm) larvae. Inserts show a zoomed in area to depict trends if not visible on full graph. Asterisks represent samples where pediveliger larvae were present but concentrations were too small to appear on figure. Site abbreviations: LR = Little River; CR = Childs River; MN = Menauhant; WB = Waquoit Bay – Metoxit Point

Figure 7. Time series of *Mercenaria mercenaria* larvae at each sampling site for 2007 and 2009.
Black lines show total concentration and bars underneath represent concentration of pediveliger
(> 200 µm) larvae. Inserts show a zoomed in area to depict trends if not visible on full graph.
Asterisks represent samples where pediveliger larvae were present but concentrations were too
small to appear on figure. Site abbreviations: LR = Little River; CR = Childs River; MN =
Menauhant; WB = Waquoit Bay – Metoxit Point
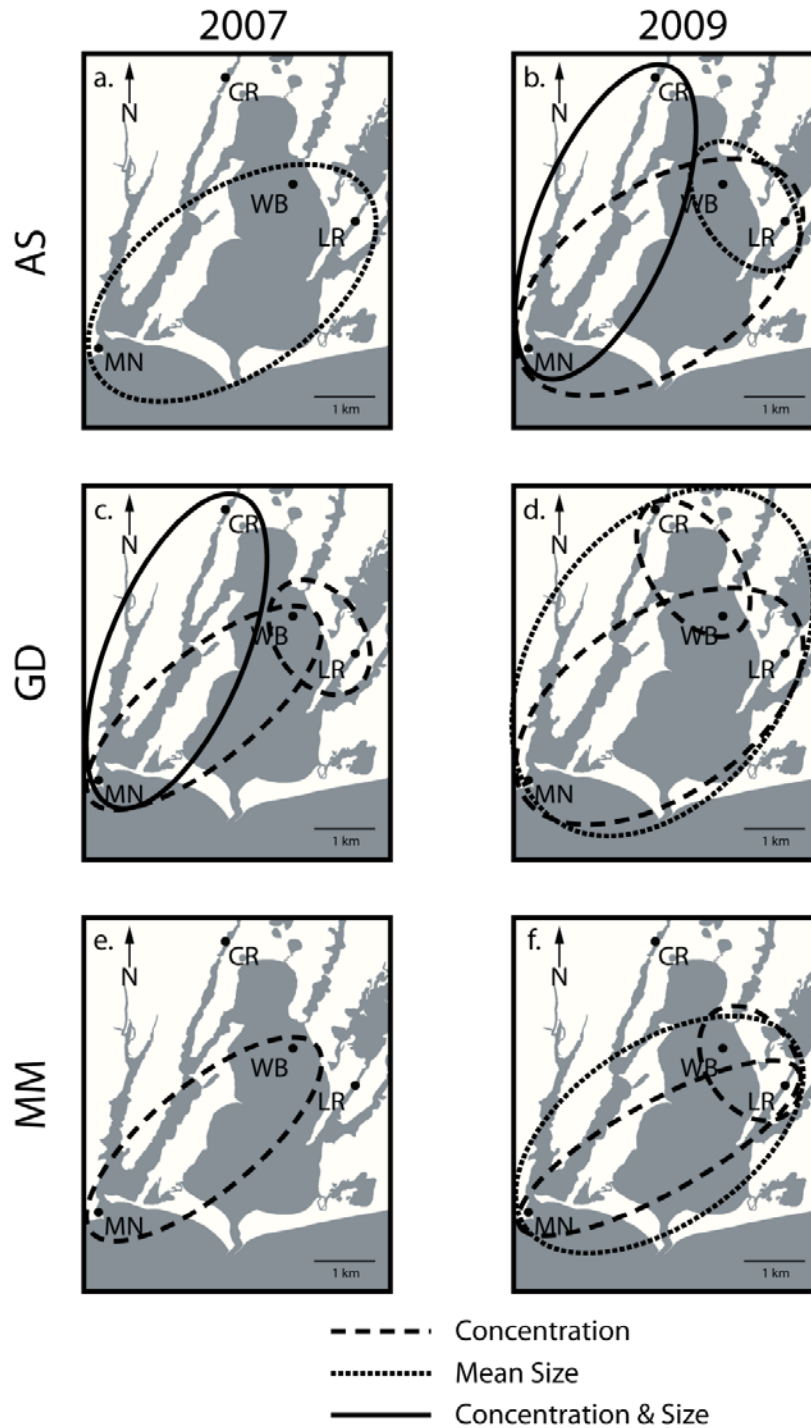
Figure 8. Correlations between time series of concentrations and mean size of bivalve larvae.
Ellipses represent positive and significant cross-correlations (p > 0.05) between sampling sites.
Solid lines represent significant correlations for both concentration and mean size. Species
abbreviations: AS = *Anomia simplex;* GD = *Geukensia demissa*; MM = *Mercenaria mercenaria*.
Site abbreviations: LR = Little River; CR = Childs River; MN = Menauhant; WB = Waquoit Bay
– Metoxit Point
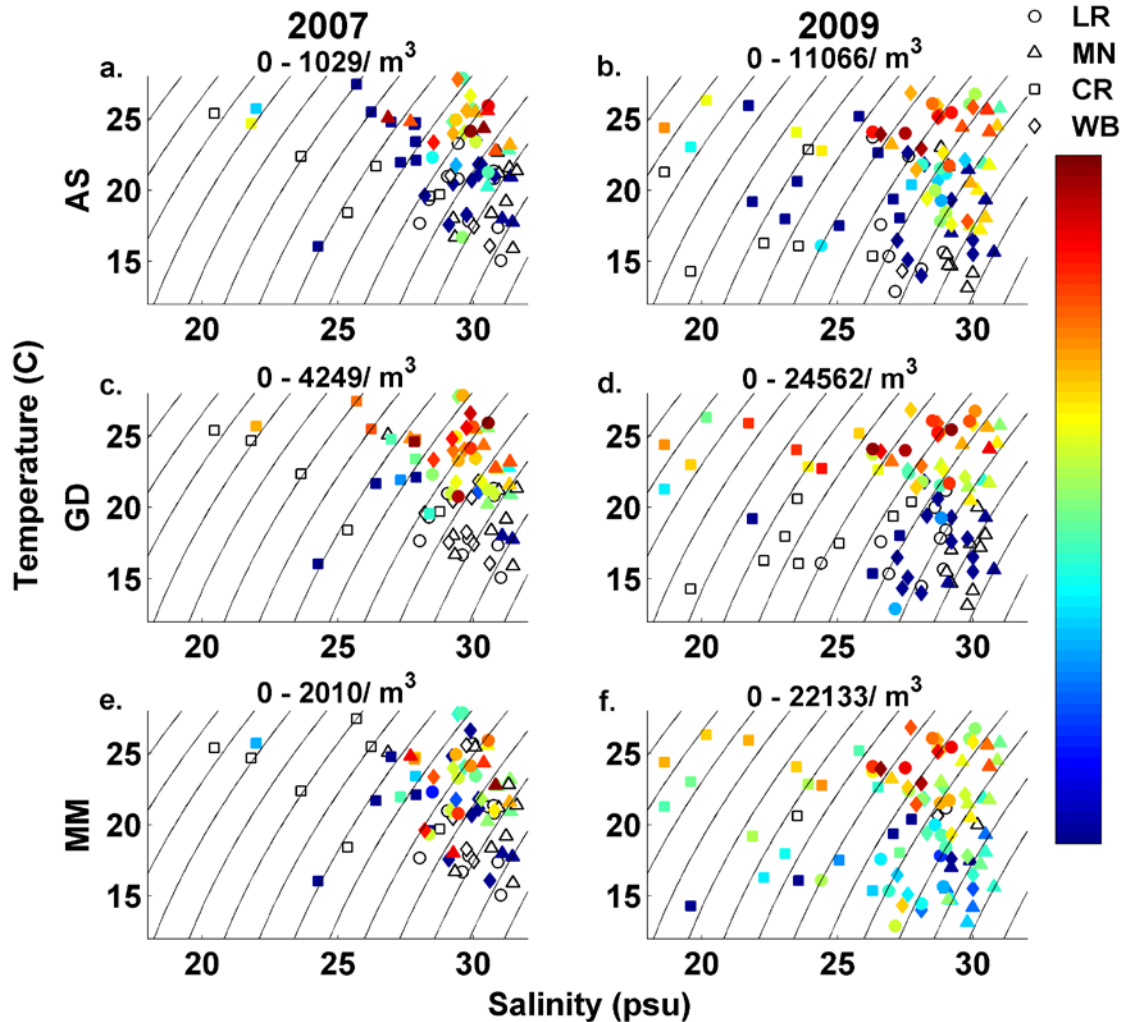
Figure 9.   Temperature-salinity-plankton plots of three species of bivalve larvae at all four sites in 2007 (a,c,e) and 2009 (b,d,f). The location of each data point represents the temperature and salinity as recorded during each sample. The color of each point represents the concentration of each species as determined from the percentage observed in each subsample and total concentration. Numbers at the top of each figure are the range of concentrations for each species that correspond to the colorbar. Concentrations are on a log scale. Each site is depicted with its own symbol. Unfilled symbols represent zero larvae. Black lines represent constant density at one sigma-t unit. Species abbreviations: AS = *Anomia simplex;* GD = *Geukensia demissa*; MM = *Mercenaria mercenaria*. Site abbreviations: LR = Little River; CR = Childs River; MN = Menauhant; WB = Waquoit Bay – Metoxit Point.
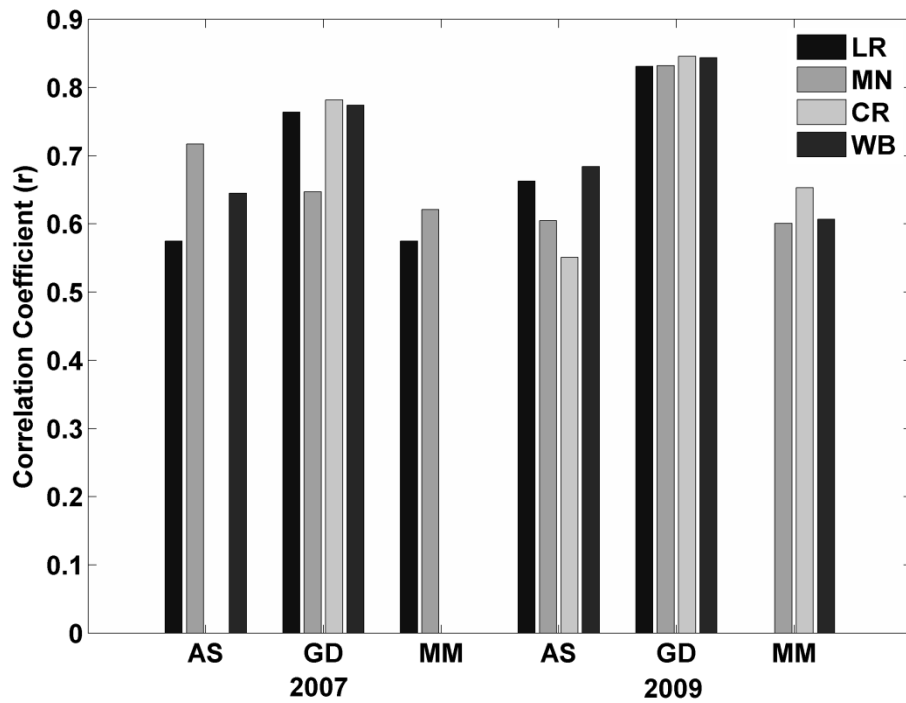
Figure 10   Correlation coefficients for each larval concentration time series and water
temperature during sample collection. Correlation coefficients are Pearson's r and significant for
alpha = 0.05. Species abbreviations: AS = *Anomia simplex*, GD = *Geukensia demissa*, MM =
Mercenaria mercenaria. Site abbreviations: LR = Little River; CR = Childs River; MN =
Menauhant; WB = Waquoit Bay – Metoxit Point

Supplementary Figures

Table S1. Matrix of cross-correlation coefficients (r) for concentration and shell length data between sites for each species in 2007. All reported correlation coefficients were significant ($p < 0.05$) and adjusted for autocorrelation of the lowest frequency. Bold values were significant for the decorrelation time of the series. Integers in parentheses represent if there was a significant lag between the sites in the columns and the rows. A positive lag means the sites in the columns lagged the sites in the rows by the factor, and a negative lag means the sites in the rows lagged behind the sites in the columns. ns = not significant

	---- Little River ----		---- Menauhant ----		---- Childs River ----	
	Conc.	Size	Conc.	Size	Conc.	Size
Anomia simplex						
Menauhant	0.73 (-3)	0.73 (-2)				
		0.67 (-1)				
		0.61 (0)				
Childs River	ns	ns	ns	ns		
Waquoit Bay	0.52 (-1)	0.65 (-3)	0.58 (-1)	0.63 (-1)	ns	ns
		0.91 (-2)		0.69 (0)		
		0.78 (-1)				
		0.58 (0)				
Geukensia demissa						
Menauhant	0.71 (1)	0.58 (-3)				
Childs River	0.51 (1)	0.68 (-1)	0.53 (-1)	0.65 (0)		
			0.76 (-1)			
Waquoit Bay	0.62 (0)	0.63 (-1)	0.68 (-1)	ns	ns	ns
			0.73 (-1)			
Mercenaria mercenaria						
Menauhant	0.54 (1)	ns				
Childs River	0.66 (2)	ns	**0.81 (1)**	ns		
Waquoit Bay	0.73 (1)	ns	**0.69 (0)**	ns	**0.83 (-1)**	ns

805

Table S2. Matrix of cross-correlation coefficients (r) for concentration and shell length data between sites for each species in 2009. All reported correlation coefficients were significant (p < 0.05) and adjusted for autocorrelation of the lowest frequency. Bold values were significant for the decorrelation time of the series. Integers represent if there was a significant lag between the sites in the columns and the rows. See Table S1 caption for description of lags. ns = not significant

| | ---- Little River ---- | | ---- Menauhant ---- | | ---- Childs River ---- | |
	Conc.	Size	Conc.	Size	Conc.	Size
Anomia simplex						
Menauhant	**0.84 (-2)**	0.65 (-1)				
		0.81 (0)				
		0.75 (1)				
		0.54 (2)				
Childs River	**0.83 (-2)**	ns	0.66 (0)	0.59 (0)		
			0.67 (1)			
Waquoit Bay	**0.79 (0)**	0.67 (-1)	0.67 (1)	0.67 (-2)	0.55 (1)	ns
		0.88 (0)	0.84 (2)	**0.85 (-1)**	**0.89 (2)**	
		0.63 (1)		**0.88 (0)**		
				0.73 (1)		
Geukensia demissa						
Menauhant	**0.79 (-1)**	0.59 (0)				
	0.75 (0)	0.61 (1)				
Childs River	0.56 (1)	0.67 (0)	0.47 (-1)	0.71 (0)		
		0.71 (1)	0.45 (1)	0.54 (1)		
Waquoit Bay	**0.73 (-1)**	0.66 (0)	**0.74 (0)**	0.57 (-1)	0.62 (-2)	0.71 (0)
	0.94 (0)	0.64 (1)	**0.80 (1)**	0.80 (0)	0.56 (0)	0.69 (1)
	0.60 (1)	0.68 (2)		0.81 (1)		
				0.61 (2)		
Mercenaria mercenaria						
Menauhant	**0.67 (-2)**	**0.52 (0)**				
	0.79 (-1)	**0.49 (1)**				
	0.71 (0)					
Childs River	0.63 (-1)	ns	ns	ns		
Waquoit Bay	**0.61 (-1)**	**0.79 (0)**	**0.87 (1)**	**0.41 (-1)**	ns	ns
	0.76 (0)		**0.85 (2)**	**0.56 (0)**		
	0.85 (1)					

812

813

814   Table S3   Significant Pearson correlation coefficients (r) from cross-correlations of larval
815   concentrations to temperature, salinity and chlorophyll time series for each species in 2007.
816   Correlations were performed between full time series as well as independent samples only when
817   larvae were present. All reported correlations were significant at $p < 0.05$. Numbers in front of
818   cross-correlations represent the lag of the larval time series with respect to the physical series
819   (i.e., a lag of -1 would mean larval concentration lagged the physical time series by one week).
820   No correlations were significant when accounting for full decorrelation time of each time series.

	Anomia simplex	*Geukensia demissa*	*Mercenaria mercenaria*
Temperature			
Little River	0.58 (0)	0.76 (0)	0.48 (-1)
		0.60 (1)	0.58 (0)
		0.60 (2)	
Menauhant	0.72 (0)	0.65 (0)	0.62 (0)
Childs River		0.78 (0)	
Waquoit Bay	0.63 (-1)	0.58 (-1)	
	0.65 (0)	0.77 (0)	
Salinity			
Menauhant	-0.62 (0)		
Chlorophyll			
Childs River	0.47 (0)		
Waquoit Bay		-0.44 (0)	

821

822

823

824

825

826

827

828

829

830

831

832   Table S4   Significant Pearson correlation coefficients (r) from cross-correlations of larval
833   concentrations to temperature and chlorophyll for each species in 2009. No significant
834   correlations were observed with salinity. Correlations were performed between full time series as
835   well as independent samples only when larvae were present. All reported correlations were
836   significant at p < 0.05. See table S3 for description of lags. Time-series were adjusted for
837   autocorrelation of the lowest frequency for both time-series. Bold values indicate significant
838   correlations accounting for full decorrelation time.

	Anomia simplex	*Geukensia demissa*	*Mercenaria mercenaria*
Temperature			
Little River	0.66 (0)	0.54 (-2)	
		0.72 (-1)	
		0.83 (0)	
		0.79 (1)	
		0.62 (2)	
Menauhant	0.61 (0)	0.73 (-1)	0.60 (0)
	0.64 (1)	**0.83 (0)**	0.62 (1)
	0.68 (2)	0.80 (1)	
	0.69 (3)	0.64 (2)	
	0.63 (4)		
Childs River	0.55 (0)	0.57 (-2)	0.55 (-3)
		0.74 (-1)	0.54 (-2)
		0.85 (0)	0.57 (-1)
		0.77 (1)	0.65 (0)
		0.61 (2)	0.54 (1)
Waquoit Bay	0.68 (0)	0.58 (-2)	0.61 (0)
	0.71 (1)	0.73 (-1)	0.59 (1)
	0.73 (2)	**0.84 (0)**	
	0.69 (3)	**0.83 (1)**	
		0.73 (2)	
Chlorophyll			
Little River			-0.66 (1)
Childs River			-0.44 (-2)
			-0.51 (-1)

839

840