1	Macrofaunal assemblages on oyster aquaculture and rock reef habitat
2	in Long Island Sound
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12	RUNNING PAGE HEAD: macrofauna within oyster aquaculture and reef habitats
13	Abstract
14	Trap sampling was used to survey relative abundance of juvenile fish and invertebrates
15	near three habitats including an off-bottom oyster cage farm (cage farm), a traditional on-
16	bottom oyster culture area (shell bottom), and a natural cobble and boulder reef (rock reef)
17	in a coastal embayment in Long Island Sound near Milford, CT. Ten traps, deployed on
18	two subsites within each habitat (20/habitat), were allowed to soak for ~24 hours, 2-3 times
19	per week, from June through September 2016. Juvenile finfish assemblages appeared
20	similar on the cage farm and rock reef while composition of the invertebrate community
21	was more variable among the three habitats. These preliminary observations suggest that
22	oyster cage farms may provide functional habitat populated by structure-oriented finfish
23	communities similar to those found on natural rock reefs in Long Island Sound.

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24 Aquaculture of the Eastern Oyster Crassostrea virginica in coastal Connecticut is conducted primarily using traditional on-bottom culture on leased shellfish beds, where live shellfish and 25 shell cultch are distributed on sediments for grow-out and spat collection. Oysters and shell on 26 substrate add complexity to low relief areas and create a hard bottom irregular surface on 27 otherwise featureless seafloor (Dumbauld et al. 2009). Many species of juvenile fish associate 28 29 with the cover provided by shell bottom (e.g., Auster et al. 1991; Able et al. 1995), where spaces among and between oysters and empty shell valves may provide respite from current and wave 30 action (Meyer and Townsend 2000). Shellfish that serve as attachment surfaces for sessile 31 32 invertebrates may enhance prey resources for ecologically important fish and crustacean predators (Arve 1960; Ozbay et al. 2014; Lehnert and Allen 2002). Some adult fish that mature 33 at smaller sizes are known to spawn on subtidal shell bottom (Crabtree and Middaugh 1982). 34 Habitat afforded by oysters and shell may provide critical refuge and resources for small 35 organisms and young fish in areas where biogenic features are otherwise limited. 36 More recently, use of three-dimensional off-bottom aquaculture cages has increased in 37 popularity, allowing growers to raise more oysters on a smaller footprint. Composed of a wire 38 framework and containing mesh bags or trays of oysters, these cages add vertical structure and 39 40 surface area to low relief seafloor. Anecdotal observations by shellfish growers have reported fish interacting with the structure provided by submerged oyster aquaculture cages. Over time, 41 the surface of multi-tiered cages become colonized by epibenthic, emergent, and encrusting 42 43 organisms, which may provide fish with camouflage and shelter, refuge from predation, respite from high current flow, and a food source (Shumway et al. 2003; DeAlteris et al. 2004; 44 Dumbauld et al. 2009; Ozbay et al. 2014). Microenvironments created by cage farms may 45 46 concentrate prey resources for foraging fish (Ozbay et al. 2014) and serve as nurseries for new

recruits (Erbland and Ozbay 2008). Aquaculture gear, which remains on the seafloor for
extended periods, may increase carrying capacity within an ecosystem (DeAlteris et al. 2004).
Single oyster cages represent discrete and highly localized sources of structure (Erbland and
Ozbay 2008) that may be important in areas with little natural seafloor relief. Clusters of cages
on shellfish farms augment existing habitat and may boost abundance and diversity of
commercially and recreationally important fish species (e.g., DeAlteris et al. 2004; Erbland and
Ozbay 2008; Marenghi et al. 2010).

Studies suggest that oyster cages may offer attractive habitat to fish species associated 54 55 with hard bottom environments (e.g., Tallman and Forrester 2007; Erbland and Ozbay 2008; Ozbay et al. 2014). Two studies in New England estuaries have compared aquaculture gear to 56 natural seafloor as habitat for fish and other organisms. In Point Judith Bay, Rhode Island, 57 DeAlteris et al. (2004) used a combination of lift-nets, quadrat sampling of sessile invertebrates, 58 drop-nets, and a suction dredge to assess organisms associated with oyster cages, submerged 59 aquatic vegetation, and non-vegetated seabed. They reported species richness and abundance 60 near oyster cages similar to that of submerged aquatic vegetation and greater than that of non-61 vegetated seabed. Another Rhode Island study by Tallman and Forrester (2007) used trapping to 62 63 evaluate relative fish density among oyster cages, natural rock reefs and an artificial reef in Narragansett Bay. These authors found that oyster cages, while providing suitable habitat for reef 64 fish, contained fish assemblages that differed in composition from rock reef communities. 65 66 In Connecticut, the value of oyster aquaculture as habitat for fish and invertebrate assemblages, relative to natural structured habitat, is not well studied. This is of particular 67 interest where structured habitat is relatively sparse, such as leased bottom in the central basin of 68 Long Island Sound (LIS). We used trapping to collect preliminary data on composition and 69

relative abundance of juvenile finfish and invertebrate assemblages associated with habitat

reated by off-bottom oyster cage culture (cage farm) and traditional on-bottom oyster culture

72 (shell bottom) and a naturally occurring rock and cobble reef (rock reef).

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74 METHOD

Study sites.—This field study was conducted within a single embayment west of Charles Island
near Milford, CT in the central basin of Long Island Sound and was located around a midpoint of
41°11.249 N and -73° 04.185 W (Figure 1). The three study habitats included an off-bottom
oyster cage farm (cage farm), traditional on-bottom oyster culture area (shell bottom) and a
natural cobble and boulder reef (rock reef). Each habitat was divided into 2 subsites (A and B)
for sampling.

Distances between
habitat sampling areas measured from 745 to 1537 meters. Topography on inshore shellfish beds
in this embayment (in the absence of shellfish or cages) is known to be generally uniform with
low seafloor relief (e.g., Goldberg et al. 2012, 2014; Meseck et al. 2014; Mercaldo-Allen et al.
2016) and little benthoscape variation (Zajac et al. 2000).

In Connecticut, much of the seafloor in the inshore coastal zone is leased to shellfish growers and shellfish beds and periodically cultivated for hydraulic harvest of the Northern Quahog *Mercenaria mercenaria* and/or collection of eastern oysters using dragged metal cages. Two active shellfish lease areas, one containing bottom cages and the other supporting traditional on-bottom oyster culture, were chosen as study sites based on permission by leaseholders to access shellfish areas while creating minimal disruption to ongoing aquaculture operations.

92	The cage farm was located on a 0.11 km ² shellfish lease (12M), approved for up to 200
93	cages (each 91 long x 122 wide x 61 cm high) within a 0.014 km^2 gear area. We focused our
94	sampling effort adjacent to four large novel cages situated within the northeast quadrant (Figure
95	2). These cages measured 132 cm high, 183 cm deep, and 121.9 cm wide and were composed of
96	twenty 91.4 x 121 cm trays holding a total of 300,000 oysters (Figure 2). The large cages were
97	located in close proximity to a second shellfish farm (L385), approved for up to 250 cages (each
98	38 cm high and 61 cm wide) within a 0.25 km ² area. Sampling subsites (A and B) were placed
99	134.4 meters apart at opposite sides of the large cage array and adjacent to the second farm.
100	Cages were not disturbed for maintenance during the study.
101	The mixed shell bottom habitat was located within an active traditional on-bottom culture
102	area, where live oysters and empty shell valves are found together on the seafloor. Subsites (A
103	and B) were placed 332.8 meters apart along the delineation between two shellfish leases L305
104	and L392. These leases, which measured 0.20 km^2 in size, were bordered by additional shellfish
105	leases where on-bottom oyster culture was practiced.
106	The rock reef is horseshoe-shaped, covers approximately 0.25 km ² , and varies in rock
107	density and size, with boulders up to 1 meter high. The two subsites (A & B) were situated 198.4
108	meters apart, in areas where dive surveys indicated 70% or greater boulder coverage (Mercaldo-
109	Allen et al. 2011). Although located on shellfish lease L386, the presence of hard substrate on
110	the reef precludes active shellfish harvesting here.
111	For the purposes of our study, the oyster cage farm, on-bottom oyster culture area and
112	rock reef were considered discrete habitats, potentially fulfilling different functional roles for
113	benthic fish and mobile invertebrates. Proximity of cages to one another and location of shellfish
114	farms at shallow water depths limited biological sampling options. Irregular topography on the

reef precluded fish collection using nets. For this reason, traps were selected as sampling devices since they could be placed on the reef and near shellfish aquaculture sites without interfering with oyster grow-out or commercial harvesting operations.

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Field sampling.—Traps sit on the seafloor and primarily target demersal and epibenthic fish and 119 mobile invertebrates while trap openings and mesh size select for animal size. This method of 120 assessing relative abundance and diversity of juvenile fish has proven useful in describing 121 macrofaunal assemblages in complex environments, where sampling options are limited (e.g. 122 123 Grabowski et al. 2005; Tallman and Forrester 2007). Our traps were sized to target the juvenile life stage, when fish rely most on structured habitat. Traps were constructed in-house, measured 124 46 x 23 x 23 cm and consisted of a wire frame lined with 3-mm mesh (Figure 3). Each trap was 125 126 configured with a single central chamber, double entry, and was fitted with a flexible 25-mmdiameter ring opening at both entrances. A 5-kg steel plate along the base provided ballast and 127 stability. Structure-oriented fish and invertebrates may have little incentive to leave shelter, 128 therefore traps were baited with whole fresh Northern Quahogs, placed in perforated plastic 129 cups, to provide an attractant. 130

Ten traps were deployed at each subsite for a total of 20 traps per habitat and were spaced 7.6 meters apart. Gear was arranged in two strings of three traps and one string of four traps. Strings were placed in line across the rock reef and shell bottom subsites and clustered near the large cages, reflective of differences in spatial scale of the three habitat types. High cobble and boulder densities at the reef ensured that traps were distributed on or adjacent to natural structure. Traps were placed as close as possible to cages without becoming tangled, < 30 meters. Traps were deployed between 8 and 11 am, soaked for 24-hours and retrieved on the subsequent

day. Trapping was conducted 2-3 days per week. During sampling trips, temperature, salinity
and dissolved oxygen were measured just above the sediments in bottom waters at each habitat
and site in the vicinity of the traps using a handheld Yellow Springs Instrument Co. ProDO
optical dissolved oxygen and temperature meter and a Yellow Springs Instrument Co. salinity,
conductivity and temperature meter.

A total of 23 sampling trips were conducted from June through early September 2016. 143 Upon retrieval, traps were inspected and all organisms identified, enumerated, and released. 144 Black Sea Bass were measured for standard length (SL) to the nearest 0.1 mm. The number of 145 146 fish and invertebrates caught per trap hour was used as an index of relative abundance. Catch per unit effort (CPUE) for fish and invertebrates was calculated as number per trap divided by soak 147 time (24 hours). This study presents preliminary and descriptive data on fish and invertebrate 148 149 abundance on 3 habitat types; access to a single oyster cage farm and rock reef for trap sampling within the study embayment precluded adequate replication for statistical comparison among 150 habitats. 151

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153 RESULTS AND DISCUSSION

154 *Environmental Data.*—Environmental parameters were similar across habitats. Mean seawater 155 temperature (\pm SD) measured 21.2 \pm 3.2 °C, mean salinity was 27.7 \pm 1.4 and mean dissolved 156 oxygen concentrations measured 5.7 \pm 1.2 ppm.

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Fish Assemblages on Cage Farm, Shell Bottom and Rock Reef.—Fish community composition
and abundance data are shown in Figure 4. The eight fish species observed, in order of overall
abundance, included Black Sea Bass *Centropristis striata*, Scup *Steotomus chrysops*, Oyster

161 Toadfish Opsanus tau, Cunner Tautoga adspersus, Tautog Tautoga onitus, Conger Eel Conger oceanicus, Bluefish Pomatomus saltatrix and Smallmouth Flounder Etropus microstomus. A 162 single Smallmouth Flounder, collected on the shell bottom habitat, was not shown in the graph. 163 Juvenile finfish assemblages on the cage farm and rock reef were similar in composition 164 with comparable numbers of Black Sea Bass, Cunner, Oyster Toadfish and Tautog. These 165 166 species are generally structure-oriented, demonstrate site fidelity and show strong affinity for 167 shelter (e.g. Able et al. 2005). Shelter is a functional requirement influencing abundance of temperate reef fishes, such as the wrasses Cunner and Tautog (Auster 1989; Tupper and Boutilier 168 169 1997) and is closely tied to successful recruitment in Black Sea Bass (Drohan et al. 2007). In a Rhode Island study, Tallman & Forrester (2007) found no discernable difference in Black Sea 170 Bass density between reef and cage sites, consistent with the results reported here. However, 171 172 they report higher numbers of Cunner near rock and artificial reefs while Scup (age 1+) and Tautog were more prevalent near oyster cages. In Delaware, four species of southern reef fish, 173 including Tautog, were observed near shellfish cages but absent from adjacent oyster reef areas 174 (Erbland and Ozbay 2008; Marenghi et al. 2010). Habitats with greater surface area and elevated 175 176 relief generally support higher numbers of species and individual organisms (Garacía Charton 177 and Ruzafa 1998). Boulder and cobble reefs, while sparse in the central basin of LIS, provide islands of topographically diverse cryptic habitat known to support a variety of fish and 178 invertebrates. The study reef is characterized by a dense growth of hydroids, bryozoans, sponges 179 180 and seaweeds inhabiting cobble and boulder surfaces (Mercaldo-Allen et al. 2011) providing camouflage and cover to young fish. The presence of sponges has been associated with reduced 181 predation among young Black Sea Bass (Sharf et al. 2006). Although the three-dimensional 182 183 relief created by aquaculture gear is structurally and functionally different from that of boulders

and cobble on natural reefs, oyster cages also support diverse assemblages of fish (Ozbay et al.
2014).

In our study, Black Sea Bass and Scup were more abundant on shell bottom, relative to 186 the other habitats, while relatively fewer Cunner and oyster toadfish were found on shell bottom, 187 and no Tautog were found. The importance of shell bottom as habitat for young Black Sea Bass 188 is well established (Lehnert and Allen 2002; Sharf et al. 2006) and a study in Chincoteague Bay, 189 Maryland found this species to be three times more abundant on shelled bottom versus unplanted 190 seafloor (Arve 1960). In a South Carolina estuary, Lehnert and Allen (2002) noted high numbers 191 192 of Black Sea Bass on shell bottom during the summer months. Although structure-oriented, Black Sea Bass are known to leave reef areas in pursuit of prey in nearby low relief 193 environments (Lindquest et al. 1994; Steimle and Figley 1996). 194 Elevated numbers of Black Sea Bass and Scup on shell substrate could represent an 195 artifact of our sampling method. Trapping provides a useful index of relative fish and 196 invertebrate abundance across difficult to sample benthic habitats, but may fish more efficiently 197 in one habitat versus others (Tallman and Forrester 2007), potentially introducing bias into 198 density estimates. Traps placed on low vertical relief seafloor may attract fish in search of shelter 199 200 as well as food. Higher numbers of fish collected on the shell bottom habitat may also reflect the somewhat larger spatial area covered by this habitat type. Despite these caveats, the high 201 numbers of juvenile Black Sea Bass and Scup we observed substantiate the value of oyster shell 202 203 bottom as habitat for these species. Higher abundances of bottom feeding fish and associated prey (Plunkett and LaPeyre 2005) and more diverse fish communities (Lehnert and Allen 2002) 204 have been previously documented in shell substrate relative to adjacent featureless bottom. 205 206 Although cultch and live oysters provide less vertical relief than cages and boulders, our study

and others (e.g., Grabowski et al. 2005; Plunkett and LaPeyre 2005; Dumbauld et al. 2009) show
that shell bottom provides habitat for a variety of fish and crustacean species.

209 Microhabitats created by live oysters and shell, rock reefs and oysters in cages may 210 confer a variety of benefits to newly settled and juvenile fish. We noted two size classes of juvenile Black Sea Bass present on all 3 habitats in mid-August, when young-of-the-year first 211 212 appeared in traps, and were easily distinguished by size from the one-year+ population (Figure 213 5). Interactions between fish and structured habitat are most profound at early life stages (Diaz et al. 2003), when vulnerability to predation make fish reliant on cryptic habitat that contains a 214 215 ready food source (Scharf et al. 2006). Similar to high relief cobble and boulders, interstitial spaces supplied by wire mesh cages and oyster bags may exclude large-bodied predators, 216 protecting small fish (DeAlteris et al. 2004; Scharf et al. 2006). Higher topographic complexity 217 218 has been linked to successful recruitment in temperate reef fish, such as Cunner (Tupper and Boutilier 1997). Structure provided by oyster aquaculture may boost fish and invertebrate 219 survival during early life stages, by enhancing availability of nursery habitat, and reducing 220 potential bottlenecks to settlement and recruitment (DeAlteris et al. 2004; Marenghi et al. 2010). 221 222

Invertebrate Assemblages on Cage Farm, Shell Bottom and Rock Reef.— Invertebrate
community composition and species abundances are shown in Figure 6. Dominant species in
order of overall abundance included the Portly Spider Crab *Libinia emarginata*, Atlantic Mud
Crab *Panopeus herbstii*, Channeled Whelk *Busycotypus canaliculatus*, Flatclaw Hermit Crab *Pagurus pollicaris*, and American Lobster *Homarus americanus*. Invertebrates present in low
abundance (N<3) (not shown in figure) included Purple Sea Urchin *Arbacia punctulata*,

229 Common Sea Star *Asterias forbesi*, Blue Crab *Callinectes sapidus*, Atlantic Rock Crab *Cancer*230 *irroratus*, and Green Crab *Carcinus maenas*.

Channeled Whelk, which graze on hard substrate, occurred in high abundance on the reef 231 relative to the other habitats. Interestingly the DeAlteris et al. (2004) study found American 232 Lobsters sheltering inside oyster cages, but we observed them on the reef only. Despite the 233 generally low abundance of American Lobsters in LIS, rock reef environments remain key 234 nursery areas for juveniles (Mercaldo-Allen et al. 2011). Portly Spider Crabs were highly 235 associated with the cage farm and shell bottom and occurred in low numbers on the reef. Long 236 237 limbs allow these crabs to ascend cages to feed on organisms encrusting surfaces. Flatclaw Hermit Crab occurred in similar numbers on the aquaculture sites and in less abundance on the 238 reef while Atlantic Mud Crabs occurred on all habitats but were most numerous on the cage 239 240 farm. These two species of predatory crabs are important members of the oyster community where they find refuge and forage among shellfish and empty shell valves (Williams 1983; Day 241 & Lawton 1988; Grabowski and Kimbro 2005). Invertebrate and crustacean species associated 242 with live oysters and shell cultch are prey for resident and transitory predatory fishes (e.g., 243 Lehnert and Allen 2002; Plunket and LaPeyre 2005; Ozbay et al. 2014). Aquaculture of oysters, 244 245 whether in cages or grown on the seafloor, may represent valuable estuarine habitat (Dumbauld et al. 2009), and a foundation for growth of productive sessile and mobile invertebrate 246 communities (Ozbay et al. 2014). 247

Conclusion.—Our study found that similar assemblages and abundances of juvenile temperate
 reef fish on oyster cage farm and rock reef habitats. Relative abundance of species within
 invertebrate communities were more variable among habitats. Off-bottom oyster cages, rock

251	reef and on-bottom traditional shellfish culture areas provide varying levels of habitat complexity
252	and seafloor relief that each support ecologically valuable finfish and invertebrate communities.
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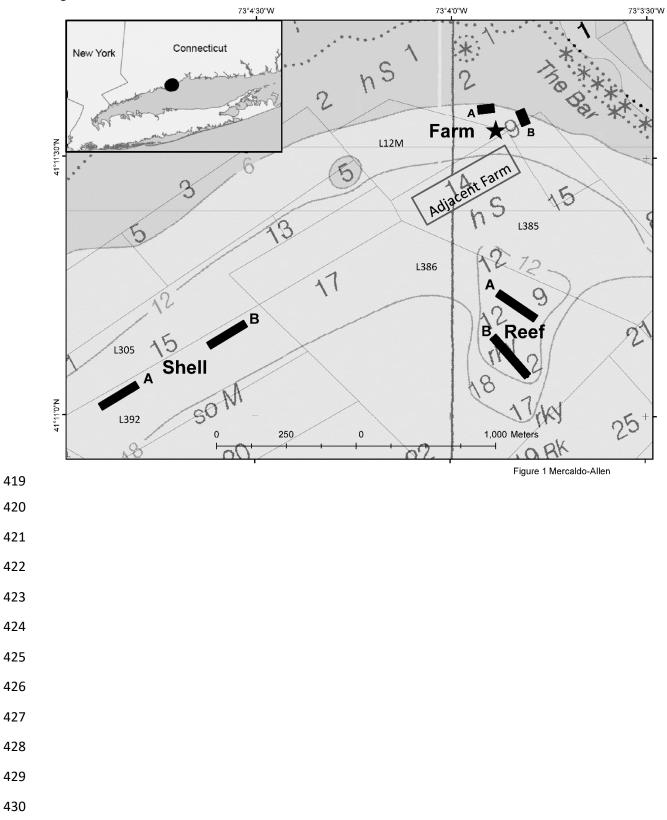
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393	Figure Captions:
394 395 396 397 398 399 400	Figure 1: Map shows study sites on shellfish leases located near Milford, CT in Long Island Sound. Habitats include an oyster cage farm (farm), shell bottom (shell) and a rock reef (reef). Box shows location of an adjacent shellfish farm also containing oyster cages. Depths at mean low water indicated in feet (1 ft = 0.31m). Black boxes indicate locations of fish trap sampling on subsites (A and B). Black lines represent lease boundaries. Star represents center of large 4 cage array.
401 402	Figure 2: Large off-bottom oyster cage style used by the grower at the shellfish farm.
403 404	Figure 3: Fish trap style used in field study.
405 406 407 408 409 410	Figure 4: Dominant finfish species at the oyster cage farm (farm), shell bottom (shell) and rock reef (reef) habitats during summer 2016. Numbers represent catch per unit effort (±standard error) over the course of the season for each species. The left y-axis indicates total number of individuals collected for all species except Black Sea Bass. The right y-axis indicates abundance of Black Sea Bass.
411 412 413 414	Figure 5. Time series of Black Sea Bass standard length measurements. Points connected by lines represent mean standard length of all 1+ individuals averaged across each habitat on a single date. Points not connected by lines represent individual YOY Black Sea Bass.
415 416 417	Figure 6: Dominant invertebrate species at the oyster cage farm (farm), shell bottom (shell) and rock reef (reef) habitats during summer 2016. Numbers represent catch per unit effort (±standard error) over the course of the season for each species.



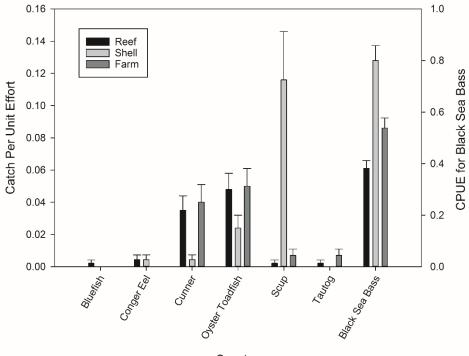




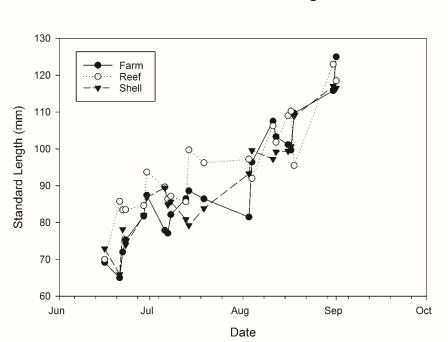
- 435 Figure 3







Species



Black Sea Bass Standard Length 2016

Invertebrate Abundance 2016

