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Experiments in conservation aquaculture to optimize restoration for Olympia oysters *Ostrea lurida* in Elkhorn Slough, CA, USA

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ABSTRACT: Foundation species are emerging as a focus in restoration to enhance long-term ecosystem recovery. Conservation aquaculture can support population recovery for depleted and low-density coastal foundation species, including oysters. Long-term restoration success may be constrained by conditions that reduce oyster survival and growth rates during the first months after transferring from the hatchery to the natural habitat. We conducted a series of experiments with aquaculture-raised, juvenile Olympia oysters *Ostrea lurida* in central California (USA) to inform adaptive management and develop best restoration practices. Oysters were outplanted to 2 tidal elevations. Low-elevation oysters initially had higher growth and survival, but after 1 yr, there was no difference in size or survival between elevations. The effect of age on survival in the estuary was tested by delaying outplanting for groups of oysters from the same cohort. Oysters that spent more time in the hatchery survived better than those outplanted earlier. In a separate experiment comparing 3 age groups outplanted at the same time, older juveniles had markedly higher survival rates than younger groups. Oysters settled on various substrate types had different survival rates. Juveniles on shell substrates generated cluster structures that are more typical in natural habitats. Cages did not inhibit growth and supported higher survival rates than uncaged substrates. This study demonstrates how conservation aquaculture provides an opportunity to conduct restoration experimentally for recovering foundation species.

KEY WORDS: Restoration · Olympia oyster · Aquaculture · Estuary · Outplant · Salt marsh

1. INTRODUCTION

Foundation species generate living habitats and play a disproportionate role in maintaining community structure and ecosystem stability (Dayton 1972). They modulate critical ecosystem processes, and their growth provides biogenic structure, hosting diverse communities that span multiple trophic levels (Ellison 2019). In coastal ecosystems, foundation species improve water quality, cycle nutrients, support nurseries for commercially valuable fisheries, protect shorelines from wave energy attenuation, and provide important food sources for diverse consumers (Angelini et al. 2015, Christianen et al. 2017, Borst et al. 2018). Native foundation species are declining rapidly in coastal habitats world-wide. Corals, mangroves, seagrasses, marsh plants, and oysters are severely reduced in abundance and distribution relative to pre-industrialization baselines (Lotze et al. 2006, Beck et al. 2011, Li et al. 2018). This habitat loss can have a strong effect on local communities and economies. Restoration and management of founda-

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tion species is thus a growing priority for coastal zone management. Re-establishing these habitat-forming populations actively strengthens long-term, ecosystem-level processes (Douvere 2008, Scyphers 2012, Rullens et al. 2019).

In near-shore and aquatic environments, restoration can work in tandem with one of the coastal zone's fastest growing industries: aquaculture. 'Conservation aquaculture' is the cultivation of an aquatic organism for the planned management and protection of a natural resource (Froehlich et al. 2017). Conservation aquaculture techniques are modified from commercial practices to enhance fishery stocks and recover ecosystem functions in marine and estuarine populations (Overton et al. 2024). This strategy has been used to successfully recover depleted populations and enhance the abundance of many species globally, in some cases for decades (e.g. corals: Omori 2019, Boström-Einarsson et al. 2020; oysters: Bersoza-Hernández et al. 2018, Fitzsimons et al. 2020). Current successful conservation aquaculture programs include fishes, vegetation, bivalves, corals, and other invertebrates (Drawbridge 2002, Froehlich et al. 2017, Carranza & zu Ermgassen 2020, Gentry et al. 2020, Alleway et al. 2021, Ridlon et al. 2023).

Conservation aquaculture is a powerful restoration tool for low-density populations whose growth is limited by on-going reproduction failures (Anders 1998, Ridlon et al. 2021a). Hatchery-raised juveniles grow in aquaculture conditions to a size that is less vulnerable to predation and environmental stressors. They are then transferred, or outplanted, into the natural habitat without further husbandry intervention. For example, commercial aquaculture was successfully adapted for an endangered white abalone recovery program to produce juveniles that were released into the wild (Rogers-Bennett et al. 2016). This strategy requires substantial planning and coordination as well as adequate knowledge of the population's ecology, organismal life history, husbandry, and dedicated resources to enhance species recovery (Zhang et al. 2018, Diefenderfer et al. 2021, Waltham et al. 2021). When the target species is not common in aquaculture settings, careful experimentation is required to identify best practices and/or key bottlenecks for stimulating long-term restoration.

The Olympia oyster *Ostrea lurida* is the only native oyster along the Northeast Pacific's temperate coast, from central Baja California (USA) to British Columbia (Canada) (Polson & Zacherl 2009, Raith et al. 2015). Oysters are foundation species that engineer 3-dimensional habitat structures, filter the water transferring nutrients and nitrogen to the benthos, facilitate recovery from disturbance, support biodiversity, and prevent shoreline erosion (Kimbro & Grosholz 2006, Grabowski et al. 2012, Gray & Langdon 2019). Olympia oysters are also a traditional food resource for humans and other predators (Erlandson 1988, Moss 1993, Baker 1995). Like many oyster species, Olympia oysters have declined in abundance and distribution; several populations are functionally extinct (White et al. 2009, Kornbluth et al. 2022). Their diminished populations are a consequence of historical overfishing, impaired water quality, and coastal development (Pritchard et al. 2015). Restoration efforts for Olympia oysters are underway along the Pacific coast (Ridlon et al. 2021b), and there is growing recognition of the important role for conservation aquaculture in the recovery of this species (Ridlon et al. 2021a).

Olympia oysters are not a common aquaculture species, and few hatcheries produce them for either commercial or conservation objectives. Therefore, only few experimental tests of best conservation aquaculture practices are published for Olympia oysters (Greiner et al. 2015, Zabin et al. 2016, Silliman et al. 2018, Wasson et al. 2020). Conjunct with the origins of both commercial and conservation aquaculture for this species, evaluations and recommendations for Olympia oyster aquaculture predominantly come from Washington state (Townsend 1896, Peter-Contesse & Peabody 2005, Blake & Bradbury 2012, Barber et al. 2016, Heare et al. 2017). Aquaculture for shellfish typically comprises 2 phases: (1) the hatchery for reproduction, and (2) outplanting for transferring juveniles into the habitat (Fitzsimons et al. 2020). Hatchery methods can be modified from related commercial species, but outplanting strategies differ for each new species and location. Unlike hatchery methods, outplanting techniques for conservation cannot be adapted from commercial practices because the fundamental target outcomes are very different. Commercial aquaculture places oysters in coastal waters only temporarily for later harvest and focuses on maximizing short-term growth. In contrast, conservation aquaculture outplants remain in the estuary permanently with the goal of providing a source of larvae and biogenic habitat (Brumbaugh & Coen 2009, Araki & Schmid 2010, McDonald et al. 2023).

One challenge is to determine where along the intertidal gradient to outplant hatchery-raised oysters. The balance between submersion below and emersion above the water can affect the success of oyster restoration efforts in estuaries (Fodrie et al. 2014, Smith & Castorani 2023). At higher elevations, exposure to air reduces feeding time and raises temperature and desiccation stress for oysters (Bishop & Peterson 2006, Kimbro et al. 2009). Lower elevations have greater risk from sedimentation, hypoxia, predation, and competition for space with invasive fouling species (Johnson & Smee 2014, Zabin et al. 2016, Valdez et al. 2017). Another important consideration is to determine the optimal length of time to retain juveniles in the hatchery before outplanting. Hatcheries may aim to minimize time in the tanks and outplant early in order to reduce material, labor, and economic costs to the conservation activity. However, delaying outplanting to an optimum size could confer higher survival rates if larger or thicker-shelled oysters are more resistant to habitat-related stressors such as desiccation, hypoxia, predation, disease, parasitism, or competition (Bricelj et al. 1992, Davis & Barber 1994, Bible et al. 2017). Substrate type is another important consideration in restoration design and outcomes for oysters (Fitzsimons et al. 2020). Natural or artificial surfaces that oysters attach to can have varied effects on recruitment (White et al. 2009), growth rates, and the 3-dimensional complexity that clusters of oysters create (Goelz et al. 2020). Additionally, certain substrates can prove more efficient for settling oysters in a hatchery setting (Rodriguez-Perez et al. 2019, Colsoul et al. 2021). Finally, aquaculture outplant success will be affected by predation. Cages can protect oysters from large predators (Grason & Buhle 2016, Cheng et al. 2022), but may also affect oyster growth and survival by disrupting water flow, increasing sedimentation, and increasing competition by providing hard substrate for biofilms, algae, or other sessile organisms (Lenihan 1999, Fodrie et al. 2014, Johnson & Smee 2014).

We conducted aquaculture-based Olympia oyster restoration experimentally to evaluate our methods and optimize restoration success. Our first objective was to compare oyster growth and survival between 2 intertidal elevations to determine where to outplant juvenile oysters. Second, we determined how long to keep juveniles in the hatchery before outplanting them into the natural habitat. The third objective was to compare outcomes on 3 different settlement substrates. The fourth objective was to determine whether predator-exclusion cages affected growth or survival. We conducted these experiments by outplanting hatchery-raised oysters in Elkhorn Slough in central California, an estuary that has a very small oyster population, low oyster densities (Wasson 2010), and frequent estuary-wide recruitment failure (Wasson et al. 2016), and therefore ranks high for conservation aquaculture implementation (Ridlon et al. 2021a). Our study was designed to inform restoration for this species. It serves as a model for using experimentation to guide conservation aquaculture practices and adaptive management for recovery of coastal foundation species.

2. MATERIALS AND METHODS

2.1. Study site

Elkhorn Slough (Fig. 1) is a small, relatively isolated estuary in central California (USA), located south of San Francisco Bay, about 170 km by sea, along the coastline as a larva would travel. The average daily difference between the lowest and highest tide is 1.6 m; the maximum difference on king tides is approximately 2.5 m. Most rainfall occurs between October and May. The estuary has been highly altered by changing land uses, extensive diking, and draining converted wetlands to agricultural areas (Caffrey et al. 2002). Agricultural run-off has resulted in eutrophic conditions (Hughes et al. 2011).

The Elkhorn Slough National Estuarine Research Reserve and partners conduct coastal habitat restoration in the region. Restoring native oysters to self-sustaining populations is part of the Reserve's broader restoration goals. Oysters are found at tidal elevations ranging from about 0.4 m below to 0.9 m above mean lower low water (MLLW) at Elkhorn Slough. At the lower end of this range, hard substrates are typically covered by non-native sessile species (bryozoans, sponges, and tunicates in particular) and sediment, while at the upper end of this range, oysters comprise the dominant cover on hard substrates (Zabin et al. 2016). Much of the estuary is dominated by deep mud as a result of anthropogenic eutrophication, and oysters only survive on substrates that avoid burial in the mudflats (Wasson 2010).

Initial oyster restoration efforts in this estuary focused on providing suitable hard substrate for wild recruitment. High natural recruitment was recorded in 2012. Thousands of juvenile oysters recruited onto clam shells that were deployed as restoration substrates. These oysters had high survivorship and growth rates. The next year, no oysters recruited to newly deployed clam shells (Zabin et al. 2016). Estuary-wide recruitment failure was subsequently found to be common at Elkhorn Slough (Wasson et al. 2016). A coastwide assessment highlighted Elkhorn Slough as 1 of 3 restoration priority estuaries in California where conservation aquaculture should be expanded (Ridlon et al. 2021a). The use of conservation aquaculture was piloted in 2018 specifically to supplement the small, isolated, recruitment-limited populations

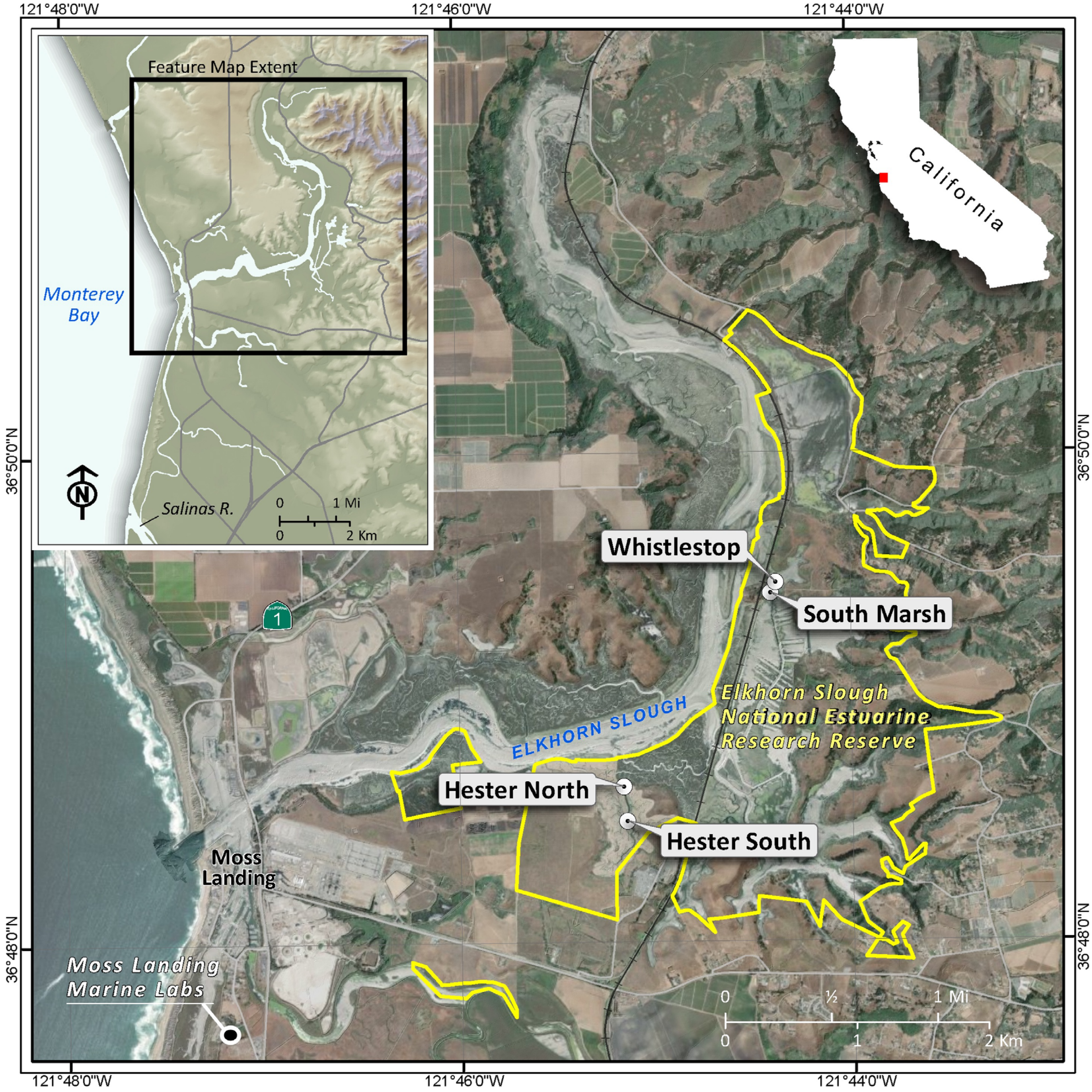

Fig. 1. Geographic setting of Elkhorn Slough National Estuarine Research Reserve boundaries (yellow outline), the hatchery facility at Moss Landing Marine Labs, and the 4 outplant sites

there (Wasson et al. 2020). The following work described here comprised the next phase of expanding conservation aquaculture at Elkhorn Slough. These experiments were designed to inform further efforts to increase oyster density and distribution in the estuary.

Oyster restoration at the Elkhorn Slough Reserve previously focused on the South Marsh and Whistlestop areas (Fig. 1). These sites are accessible for monitoring and community engagement. Archeological data from these sites suggest oysters were present and harvested by Native American people for centuries (King 1982). Water residence times are relatively long (>1 wk) and may facilitate larval retention, unlike the more marine-influenced areas with short residence time where recruitment failure is common (Wasson et al. 2016). In this study, oysters were again outplanted at South Marsh and Whistlestop. In addition, oysters were outplanted to a new site, Hester Creek, within a large-scale tidal marsh restoration project, Hester Marsh. Here, sediment was added in 2017 to restore elevation to the formerly diked and subsided marsh habitat (Haskins et al. 2021). For this study, we outplanted oysters at a northern and a southern area of Hester Creek, referred to below as Hester North and Hester South; the latter is a different location than South Marsh, despite the similarity in names (Fig. 1).

2.2. Hatchery production

Adult oysters were collected from the South Marsh and Whistlestop sites and transported to the hatchery facility at Moss Landing Marine Laboratories in May 2021. This designated broodstock group was kept in static-water tanks with ambient seawater for 3 wk and fed live microalgae mixes of *Tisochrysis lutea*, *Diacronema lutheria*, *Chaetoceros calcitrans*, *C. muelleri*, and *Tetraselmis suecica*.

Following this conditioning period, we increased the water temperature 1°C d^{-1} to a maximum 20°C to induce spawning (Wasson et al. 2020). The broodstock produced 3 distinct, sequential cohorts of oyster larvae between July and September 2021, with approximately 30 d separating each cohort's age. Each cohort was also monitored separately in the estuary for the following experiments (Table 1).

Larvae were stocked in tanks at 1 oyster ml^{-1} and maintained using static-water culturing methods. Larvae were fed daily using the live microalgae mixes described above. Once competent, larvae were settled using 'spat-on-shell' techniques which included gaper clam *Tresus nuttallii* shell, red abalone *Haliotis rufescens* shell, or ceramic tile substrates hung throughout the tanks' water column. Empty clam shells with a minimum 15 cm length were collected from Elkhorn Slough and abalone shells 10–20 cm were donated by the Monterey Abalone Company in Monterey, CA, USA. All shells were scrubbed clean using seawater and sun-dried for a minimum of 72 h. Tiles used were 15 × 15 cm ceramic composition with 1 glazed and 1 unglazed face (Daltile brand ceramics). All substrates were pre-conditioned by hanging in seawater tanks for 14 d with the same microalgal concentrations described above. Settled juvenile oysters grew on these substrates in aerated, static-water tanks. Water was changed daily, and oysters were fed live microalgae with each water change until they were outplanted into the Slough 9–22 wk later.

2.3. Monitoring juvenile growth and survival

Oyster size was measured as the longest edge-to-edge length to the closest mm on the largest oyster for each substrate. This method facilitated rapid, repeatable measurements collected by teams of volunteers on hundreds of substrates during the brief exposed low tide period. By focusing on the largest individual, we assessed the potential for growth under each experimental condition, rather than average size per substrate. Average size per substrate can result in measurements that underestimate growth potential because some oysters' growth is constrained due to crowding at high densities. For the tidal elevation experiment, size was calculated by averaging the largest 2 oysters per substrate, while only the single largest was measured in all other experiments. Growth rate was quantified as size divided by time since outplant (days). Survival was quantified as the

Table 1. Larval Olympia oyster cohorts used for restoration experiments. Spawn: last release of swimming larvae from brood; Settlement: first date that all juveniles settled on provided substrates; Age: weeks after settlement; Count: total number of live oysters on monitoring date; Size: calculated from the largest shell length measured on each substrate (mean ± SE). The final column shows which cohorts were used for which restoration experiments. Cohort 3 Outplant Count and Size show the total number of oysters alive and average size for each group on their respective outplant dates

Cohort information			Outplant				Restoration monitoring July 2022				Cohort placement
Cohort	Spawn	Settlement	Outplant date	Age (wk)	Count	Size (mm)	Weeks in field	Age (wk)	Count	Size (mm)	Restoration experiment
1	10 July 2021	24 July 2021	2 Dec 2021	22	4927	18±1	32	52	2768	45 ± 1	Outplant age[a]; substrate; caging
2	15 Aug 2021	29 Aug 2021	2 Dec 2021	14	2372	11±1	32	46	881	44 ± 1	Tidal elevation; outplant age[a]
3	16 Sept 2021	30 Sept 2021	2 Dec 2021	9	581	4±1	32	41	49	38 ± 1	Outplant age[a,b]
			14 Jan 2022	15	510	7±1	26	41	99	40 ± 1	
			27 Feb 2022	21	563	11±1	20	41	183	36 ± 1	
Total					8953				3980		

[a] Outplant age/size experiment comparing 3 cohorts outplanted at the same time
[b] Outplant age experiment comparing 1 cohort outplanted at 3 different times

number of live oysters on the substrate unit, divided by the original number of live oysters at outplant. For tiles, only the rough, downward facing side was assessed; virtually no oysters settled on the smooth upper side. For the shells, all surfaces were assessed, as oysters settled everywhere.

2.4. Tidal elevation

To determine whether tidal elevation affects juvenile oyster growth and survival, we outplanted juveniles from Cohort 2 in December 2021 after 15 wk of growth in the hatchery. While Elkhorn Slough Reserve preferentially uses natural substrates such as clam shells for oyster settlement, tiles were used for this study because the exact position of the oysters in the tidal frame could be more tightly constrained using flat horizontal surfaces. Tiles were transferred to 3 sites in the estuary and deployed hanging horizontally from PVC stakes with zip ties (Fig. 2C,I), with the rough, oyster-covered side down, facing the seafloor. Juvenile oysters were outplanted at 2 elevations: MLLW (referred to from here on as 'low') and MLLW+0.4 m ('high'). Over the year, MLLW is exposed (out of water) 3% of the time, while +0.4 is exposed 14% of the time (Elkhorn Slough Reserve unpublished water level monitoring data). Previous work in this estuary had found differences among wild oyster recruitment at −0.3 vs. +0.3 m MLLW (Zabin et al. 2016); higher elevations are logistically easier for restoration and monitoring, so here we focused on comparing 2 higher elevations. Tidal elevations were obtained by laser leveling from a nearby permanent benchmark. We hung 2 high and 2 low tiles from the same stake on 4 separate zip ties with 40 cm between the 2 elevations. In this experiment, each tile substrate was a replicate. We conducted the experiment at 3 sites: 10 tiles were outplanted to each elevation at Hester North and Hester South, and 17 tiles were outplanted to each elevation at Whistlestop.

Across all sites and treatments, the initial size of the largest juvenile oyster per tile was 11 ± 0.25 mm (mean ± SE), and the density was 21 ± 4 oysters per tile at the time of outplanting. Tiles were randomly selected from hatchery tanks, and initially, there were no significant differences detected in size or density among tiles assigned to the 2 elevations or the 3 sites. We counted the total number of live oysters and measured the lengths of the 2 largest oysters on each substrate the day they were outplanted and again 15, 32, 52, and 83 wk after outplanting. We used visual estimates to quantify percent cover by macroscopic sessile invertebrates (hereafter referred to as fouling cover) on the oysters at Week 83. Fouling cover was divided into barnacles and non-barnacles, since it appeared that barnacles were common at high elevations, and other, mostly soft-bodied, fouling species at low elevations. We compared oyster growth rate, survival, and fouling cover at 83 wk between tidal elevations and among sites using a 2-way ANOVA, with tidal elevation, site, and the interaction between tidal elevation and site as factors and tile as replicate.

2.5. Age at outplant

2.5.1. Comparing the same cohort outplanted at different times

To test whether outplanting age affects juvenile oyster growth and survival, we staggered the outplant date for juvenile oysters from Cohort 3 (settled on clam shells 24–31 September 2021). One-third were outplanted after growing for 9 wk in the hatchery (on 2 December 2021, when individuals from other cohorts were also outplanted); another third after 15 wk (14 Jan 2022); and the final third after 21 wk (27 Feb 2022). For all treatments ('9 week', '15 week', and '21 week'), oysters settled on clam shell substrates were hung on zip ties from vertical PVC stakes and outplanted to the South Marsh restoration site. Each stake contained 6 clam shell substrates deployed at MLLW+0.2 m, about 1 m above the mudflat (Fig. 2A,B). Each stake was a replicate, with size averaged and count summed across the 6 clam shell substrates per stake (n = 9 for each treatment).

All oysters from this cohort, whether still located in the hatchery or already deployed in the field, were measured and counted at each outplanting date (2 December 2021; 14 January 2022; and 27 February 2022), plus 1 additional 6 wk interval (13 April 2022), then again on 18 July and 2 December 2022. ANOVAs were used to compare (1) initial size at outplanting, (2) survival in the first 6 wk period in the estuary immediately following outplanting, and (3) growth and survival after 12 mo among the 3 groups (n = 9). To compare growth and survival in hatchery vs. field conditions, we conducted a separate analysis for the first 12 wk of the experiment (2 December 2021 to 27 February 2022). Hatchery growth and survival were determined for the last outplanted '21 week' group, which spent all 12 wk in the hatchery. This was compared to field growth and survival determined for the first outplanted '9 week' group in the Slough for that entire period.

Fig. 2. Photos of experimental design and oyster growth on different substrates. (A) Restoration stake design for hanging juvenile oysters settled on clam shells. (B) Array of replicate stakes placed parallel to the shoreline, set to a standard elevation and distance from the substrate. (C) Elevation experiment replicates with tiles at 2 height treatments. (D) Clusters of oysters growing on shells inside the cage treatment. (E) Clam shell with juvenile settled oysters. (F) Ceramic tile illustrating the variability in size and density when oysters were outplanted. Also shown are 1 yr aggregate growth morphologies on (G) a single abalone shell, (H) several clam shells, and (I) tiles in high- and low-elevation treatments

2.5.2. Comparing different cohorts outplanted at the same time

We also assessed the effect of age on growth and survival by comparing oysters spawned at different times throughout the summer, then outplanted at the same time (over 3 d between 30 November and 2 December 2021) on substrates hanging from PVC stakes in the same design and tidal elevation as previously described. Stakes contained either 6 clam shells or 4 tiles with settled oysters attached, all from the same cohort. Size measurements were taken at outplanting and 32 wk later, on 18 July 2022. Each stake was a replicate with average size and total count for all substrates measured per stake. To avoid variation due to site or substrate differences, we only statistically compared cohorts outplanted on the same substrate type (tile or clam shell) at the same sites. Cohort 1 was settled on both clam shells and tiles, while Cohort 2 was settled only on tiles and Cohort 3 only on clam shells. Specifically, we compared Cohort 1 on tiles (n = 13) to Cohort 2 (n = 74) at South Marsh, Whistlestop, and Hester Marsh, and Cohort 1 on clam shells (n = 11) to Cohort 3 (n = 9) at South Marsh only, but could not directly compare Cohorts 2 and 3 since they were on different substrates. ANOVAs were used to examine differences in size, growth rate, live oyster counts, and survival using the replication and comparisons described above (only comparing cohorts at the same sites on the same substrates). To visualize overall trajectories, growth and survival for the 3 cohorts was plotted across all sites and substrates.

2.6. Substrates

We tested the effect of substrate on juvenile growth and survival by comparing oysters from Cohort 1 settled onto 3 substrate types: clam shells, abalone shells, and tiles at Hester Marsh North and Hester Marsh South. Size was measured, and all oysters were counted on all substrates on the outplanting day (30 November 2021) and 32 wk later (18 July 2022). Stakes either contained 6 clam shells, 6 abalone shells, or 4 tiles with settled oysters attached, deployed at about 0.2 m above MLLW, about 1 m above the mudflat. For each stake, we averaged the size of the largest oyster per substrate and summed the total number alive on all substrates (clams shells: n = 6, abalone shells: n = 4, tiles: n = 11). ANOVAs were used to compare size at outplant, and size and survival after 32 wk, among substrates.

2.7. Cages

To determine the effect predator-exclusion cages had on juvenile growth and survival, we outplanted some oysters from Cohort 1 inside cages designed for commercial oyster aquaculture at Hester Marsh. The 15 l cages (Fig. 2D) had 12 mm mesh made of polyethylene (SEAPA Pty). Two cages were suspended from separate horizontal lines at MLLW+0.2 m, about 1 m above the mudflat, approximately 500 m apart. Cages contained oysters attached to both clam shells and abalone shells. With just 2 cages, low sample size precluded this being a robust experiment like those previously described. We included an assessment of the caged vs. adjacent uncaged oyster clusters as an initial proof-of-concept exploration of the viability of cages for outplanting hatchery-raised clusters. Average size and number were quantified for each substrate type; we treated these as 4 replicates (2 substrate types, 2 cages), although the 2 substrate types within the same cage may not have been independent. These were compared to averages from stakes with Cohort 1 oysters attached to clam shells and abalone shells (n = 10) at the same site. Measurements were taken at outplant on 30 November 2021 and after 32 wk on 18 July 2022. ANOVA was used to compare size and survival between these caged and uncaged oysters after 32 wk.

All statistical analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021). For all ANOVAs, the assumption of normality was confirmed using Shapiro-Wilk tests, with p-values greater than 0.05 indicating normally distributed data. Bartlett's test for homogeneity of variances was used to confirm that variances were equal between groups for each comparison. The data used in the parametric tests met these assumptions, and no data transformations were used.

3. RESULTS

3.1. Hatchery production and overall outplanting outcomes

A total of 8953 juvenile oysters were outplanted to 4 sites in Elkhorn Slough between 30 November 2021 and 27 February 2022. In July 2022, 3980 (45%) were still alive. Overall, Cohort 1 had the highest survival through July (56%) while Cohort 3 had the lowest (20%) and Cohort 2 was in between (40%). Oysters across all cohorts and treatments had grown to an average of 41 ± 2 mm in July 2022, with some differences across cohorts (Table 1). Only tidal elevation and outplanting size experiments (Cohorts 2 and 3, respectively) were monitored in December 2022, 1 yr post-outplanting. After 1 yr, total survival of Cohort 2 was 35%, with an average size of 55 ± 1 mm, and covering a median 75%, mean 62 ± 4% of the substrate. Total survival of Cohort 3 was 18%, and average size after 1 yr in the estuary was 52 ± 1 mm. Cohort 2 was again monitored in July 2023, by which time survival was 22% of the originally outplanted count.

3.2. Tidal elevation

Growth was rapid for both elevation treatments and all sites in this experiment. However, the temporal trajectory of growth during this time differed between elevations (Fig. 3A). Low-elevation oysters grew more than oysters at the high elevation during the first 32 wk, but their growth slowed dramatically after that. High-elevation oysters grew more slowly during the first 32 wk, but continued to increase in size substantially between 32 and 52 wk. Growth at both elevations slowed significantly after the 52 wk measurement. After 83 wk, oyster size was not different between tidal elevations, but was different among sites (Table 2, Fig. 4A).

Survival was high for all treatments and sites; mean survival was 51 ± 3% (SE) at 52 wk, and 42 ± 3% at 83 wk. The temporal trajectory of survival clearly differed among tidal elevations during the first period after outplanting (Fig. 3B). Ultimately, the proportion of oysters at low and high tidal elevations that survived at 83 wk since outplant was not different, and there was no difference among sites (Table 2, Fig. 4B).

At 83 wk, fouling on the oysters covered an average of 41 ± 4% of their shells' surface area. Barnacles (likely mainly *Balanus* sp.), were the only fouling organism growing directly on oysters at high elevation, but were minimal on low-elevation oysters where fouling was dominated by other organisms, including bryozoans (especially *Bugula* sp.) and sponges (mostly *Hymeniacidon permolis*). Barnacle cover was greater at high elevation, while non-barnacle cover was greater at low elevation; there were also strong site differences and site × elevation interactions (Table 2, Fig. 4C,D).

3.3. Age at outplant

3.3.1. Comparing the same cohort outplanted at different times

Growth trajectories among 3 different outplanting ages differed slightly in the first 18 wk, but these differences did not persist (Fig. 5A). Size at outplant was distinctly different among all 3 treatments (Fig. 6A); oysters outplanted after 9 wk in the hatchery averaged 4 ± 1 mm when outplanted, those outplanted after 15 wk averaged 7 ± 1 mm, and those outplanted after 21 wk averaged 11 ± 1 mm. After 1 yr, there was no difference, and all oysters in all 3 treatments together averaged 52 ± 1 mm (Fig. 6C), with no difference among treatments (ANOVA $F_{2,25} = 0.038$, $p = 0.962$).

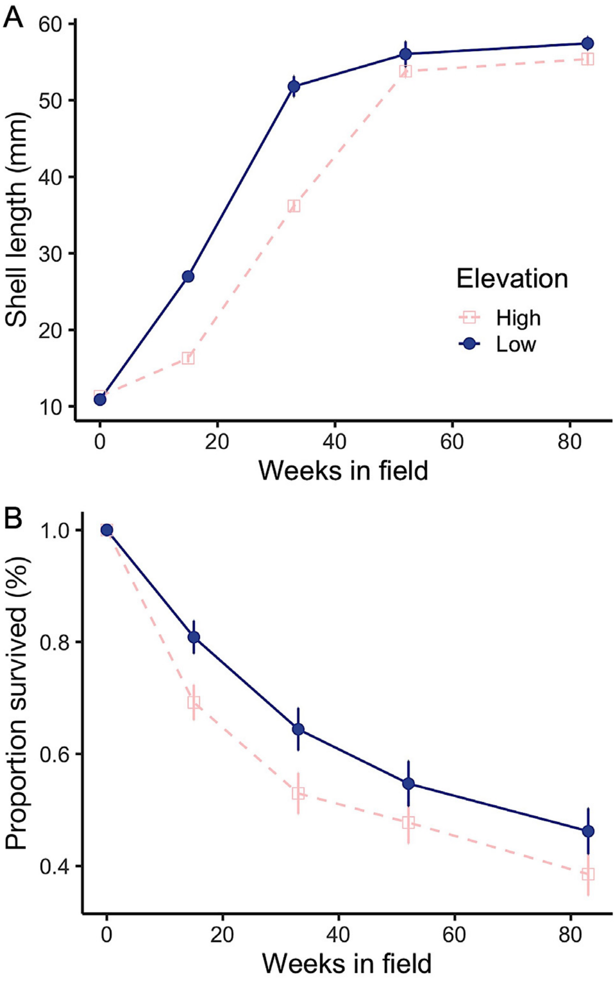

Fig. 3. Oyster trajectories at 2 tidal elevations. Oyster (A) size and (B) survival monitored at 0, 15, 32, 52, and 83 wk after outplanting. Standard error is shown

Table 2. Effects of tidal elevation and site. Two-way ANOVA results for juvenile oyster growth and survival, and for fouling cover by other sessile animals on the restoration substrates. Significant ($p < 0.05$) treatments are highlighted in **bold**

Variable	Treatment	df	*F*	p
Growth	Elevation	1, 68	2.855	0.096
	Site	**2, 68**	**12.070**	**<0.0001**
	Interaction	2, 68	0.364	0.696
Survival	Elevation	1, 68	1.975	0.165
	Site	**2, 68**	**3.201**	**0.047**
	Interaction	2, 68	0.556	0.576
Fouling by barnacles	**Elevation**	**1, 68**	**53.250**	**<0.0001**
	Site	**2, 68**	**49.630**	**<0.0001**
	Interaction	**2, 68**	**35.510**	**<0.0001**
Fouling by non-barnacle spp.	**Elevation**	**1, 68**	**117.950**	**<0.0001**
	Site	**2, 68**	**15.110**	**<0.0001**
	Interaction	**2, 68**	**15.120**	**<0.0001**

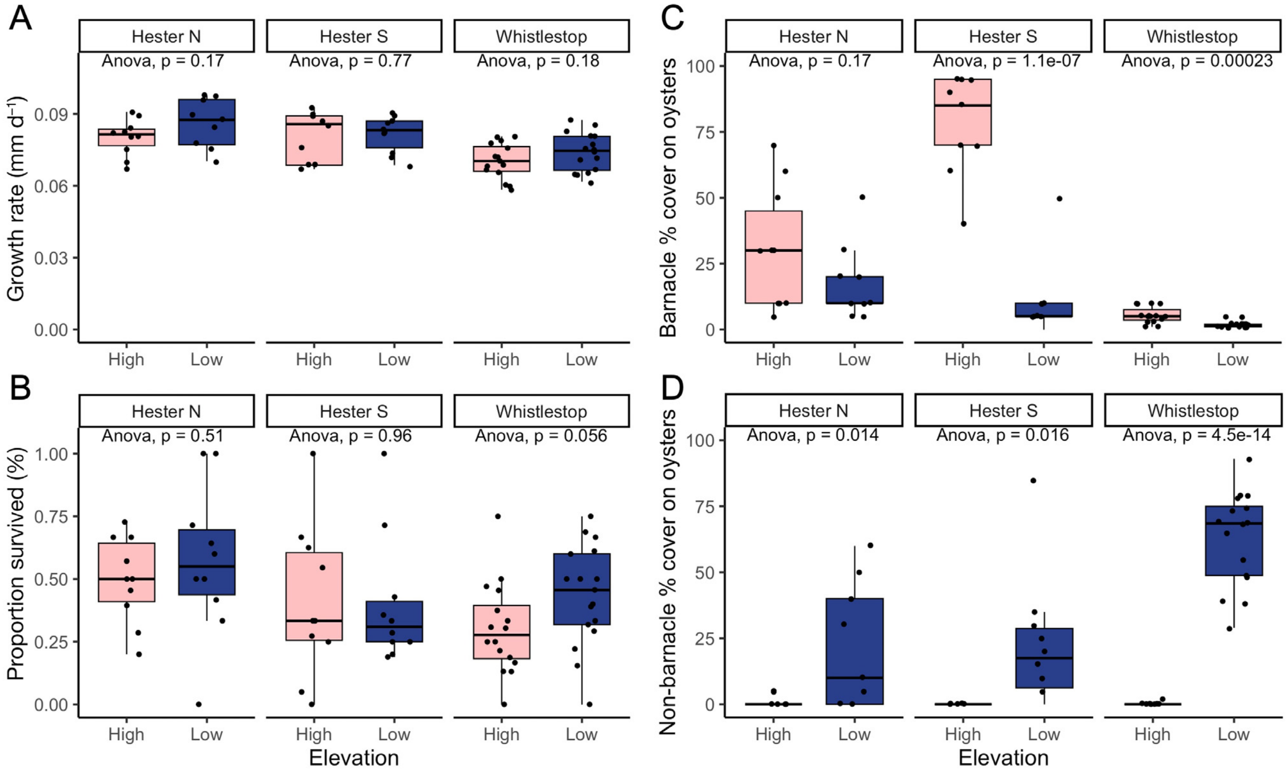

Fig. 4. Comparison of 2 tidal elevations for oyster (A) growth rate and (B) survival, and percent of oyster shells covered by (C) barnacles and (D) all non-barnacle organisms at 3 sites after 83 wk in the field. Box plots show median, 25th and 75th percentiles, minimum, maximum, and observed data values. Minimum and maximum lines do not include outliers (1.5 times the interquartile range less/greater than the first/third quartile)

Survival trajectories also differed among the 3 outplanting ages (Fig. 5B). Initially when the experiment started on 2 December 2021, all treatment groups contained similar numbers of oysters (ANOVA $F_{2,25}$ = 1.174, p = 0.326). Survival during the first 6 wk in the field was similar for each group (Fig. 6B); however, because the groups differed in length of time spent in the hatchery, and survival was higher in the hatchery, differences in their early survival trajectory (Fig. 5B) persisted and re-

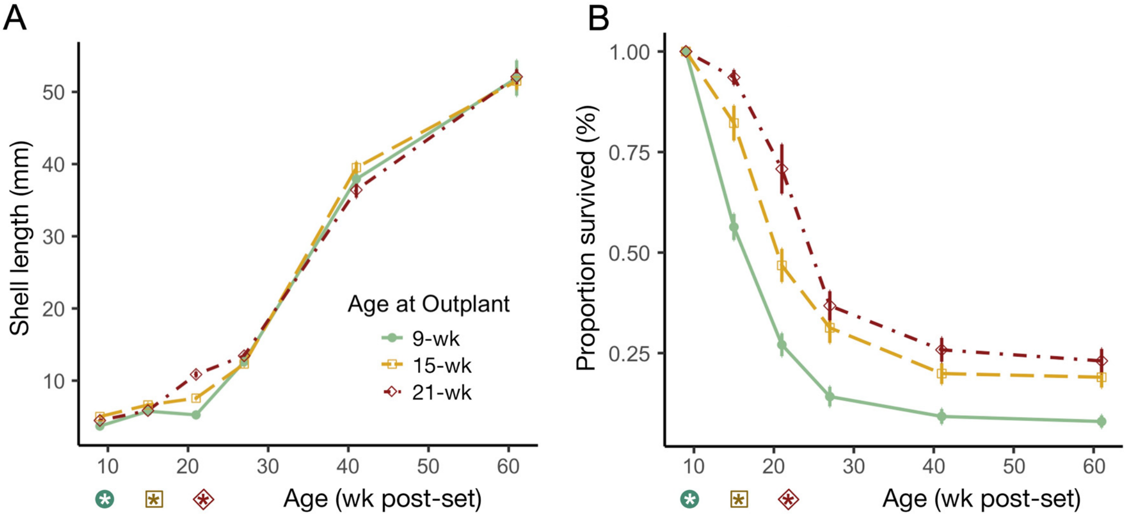

Fig. 5. Trajectories for oysters from Cohort 3 deployed at different ages. Oyster (A) size and (B) survival monitored at 9, 15, 21, 27, 41, and 61 wk after settlement. Standard error is shown. Asterisks on *x*-axes indicate when outplant occurred for each treatment group

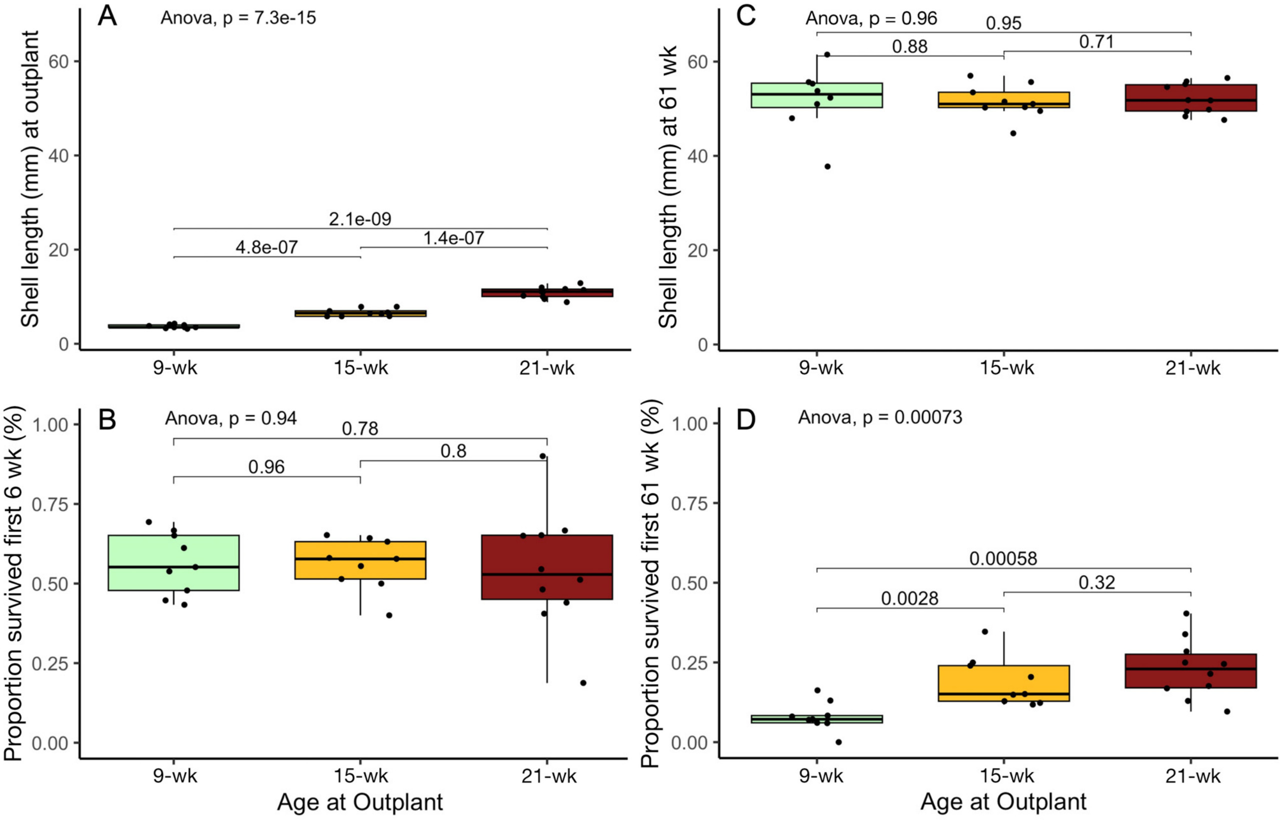

Fig. 6. Comparison of oysters from Cohort 3 outplanted at different ages: (A) size at outplant (which was staggered at 6 wk intervals among groups), (B) survival in the first 6 wk after outplanting, (C) size and (D) survival at 61 wk post-settlement. Overall p-values for ANOVAs testing effect of age at outplant are shown; p-values from *t*-tests shown for pairwise comparisons among groups. Box plot elements as in Fig. 4

sulted in differences in final survival at the end of a year (Fig. 6D).

Comparison of the first and the last outplanted group for the first 12 wk, i.e. same-aged oysters that spent these 12 winter weeks entirely in the field vs. in the hatchery, reveals strong differences in these environments. Growth (Fig. 7A) and survival (Fig. 7B) were higher in the hatchery than the field. While the growth differences were no longer evident by the end of the year (the smaller ones had caught up, Fig. 6C), the survival differences persisted (Fig. 6D).

3.3.2. Comparing different cohorts outplanted at the same time

Sizes differed among cohorts. Each cohort was 4 wk older than the next and therefore, juveniles from Cohort 1 were larger than those from Cohort 2 (Fig. 8A) and Cohort 3 (Fig. 8D) when outplanted. After 32 wk, there was no difference in size between Cohort 1 and 2 (Fig. 8B), but Cohort 3 remained smaller than Cohort 2 (Fig. 8E).

Survival also differed among cohorts. Cohort 1 had significantly higher survival than Cohorts 2 (Fig. 8C) and 3 (Fig. 8F) after 32 wk. Growth and survival of Cohort 2 were not compared directly with Cohort 3 in this analysis because they were settled on different substrate types.

3.4. Substrates

Growth patterns differed among substrates. Juvenile oysters settled on tiles were smaller when outplanted than those on clam or abalone shells, despite being the same age (Fig. 9A). Oysters were then similarly sized after 32 wk due to more rapid growth by oysters on tiles (Fig. 9C). Survival also differed among substrates. Oyster larvae in the hatchery settled on abalone shells at a much higher rate than on the clam shells or tiles (despite similar size of the substrate types), and therefore had higher numbers per unit when outplanted compared to the other 2 substrates (Fig. 9B). After 32 wk, survival was lowest on the abalone shells, and oysters

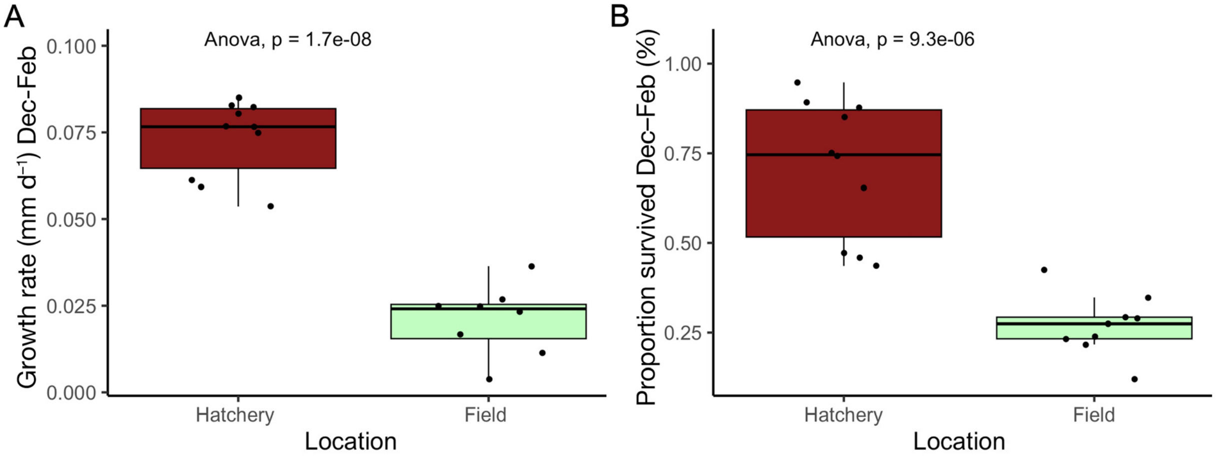

Fig. 7. Comparison of juvenile oysters in the hatchery vs. in the field: (A) growth rate and (B) survival for Cohort 3 oysters that spent a 12 wk period in the hatchery vs. in the field. p-value for ANOVA is shown for each comparison. Box plot elements as in Fig. 4

on tiles had somewhat greater survival than those on clam shells (Fig. 9D).

Morphology of individual oysters and structure of aggregations also differed among substrates. On 2-dimensional tile surfaces, oysters mostly spread horizontally in the same plane as the substrate, generating generally flat shell morphologies (Fig. 2F). On both shell types with convex shapes (Fig. 2E,G), they grew horizontally and vertically (perpendicular to the plane of the substrate). High settlement density also contributed to 3-dimensional growth when shells pushed up against each other. The resulting oyster aggregations thus formed more complex 3-dimensional cluster structures on shells than on tiles (Fig. 2H, I).

3.5. Cages

Oysters in cages were slightly larger than uncaged oysters after 32 wk (Fig. 10A). Survival was markedly higher for caged than uncaged oysters after 32 wk (Fig. 10B). Cages effectively retained oysters that detached from their substrates. Cages remained generally unfouled at Hester Marsh sites, with few to no sessile organisms growing on the cages throughout the study.

4. DISCUSSION

4.1. Aquaculture as a vital tool for oyster restoration

Aquaculture is increasingly recognized for its potential as a conservation tool for restoring imperiled coastal species (Froehlich et al. 2017, Ridlon et al. 2023), including oysters (Carranza & zu Ermgassen 2020, Fitzsimons et al. 2020, Colsoul et al. 2021). We found aquaculture-based restoration to be highly successful in Elkhorn Slough, with overall survival of outplanted oysters approximately 45% at 1 yr post-settlement. While estuary-wide wild recruitment failure has occurred in most years (Wasson et al. 2020), all stages of reproduction from gamete production through settlement were viable in the hatchery. Approximately 4000 reproductively mature 1 yr old oysters resulted from this single, small-scale effort. The estuary's population, estimated below 1000 individuals in 2018 (Wasson et al. 2020), thus increased 4-fold. Growth and survival were relatively high compared to hatchery-raised Olympia oysters outplanted in other estuaries (Barsh et al. 2004, Trimble et al. 2009, Valdez et al. 2017, Lowe et al. 2019). Overall, these results support conservation aquaculture expansion to recover depleted Olympia oyster populations (Ridlon et al. 2021a).

Using aquaculture to enhance oyster restoration requires improved understanding of the conditions that maximize success, defined here as maximizing survival and reproductive output of cultured oysters both within the hatchery and after outplanting into the wild. We focused on 3 main questions central to conservation aquaculture: (1) where to outplant, based on intertidal height and estuary site; (2) when to outplant, specifically, how long to keep oysters in the hatchery; and (3) how to outplant, to determine whether substrates or caging impact oyster performance or morphology. The answers to these questions support long-term restoration expansion by informing site selection and outplanting methodologies, decreasing hatchery costs, and matching management plans to defined restoration outcomes.

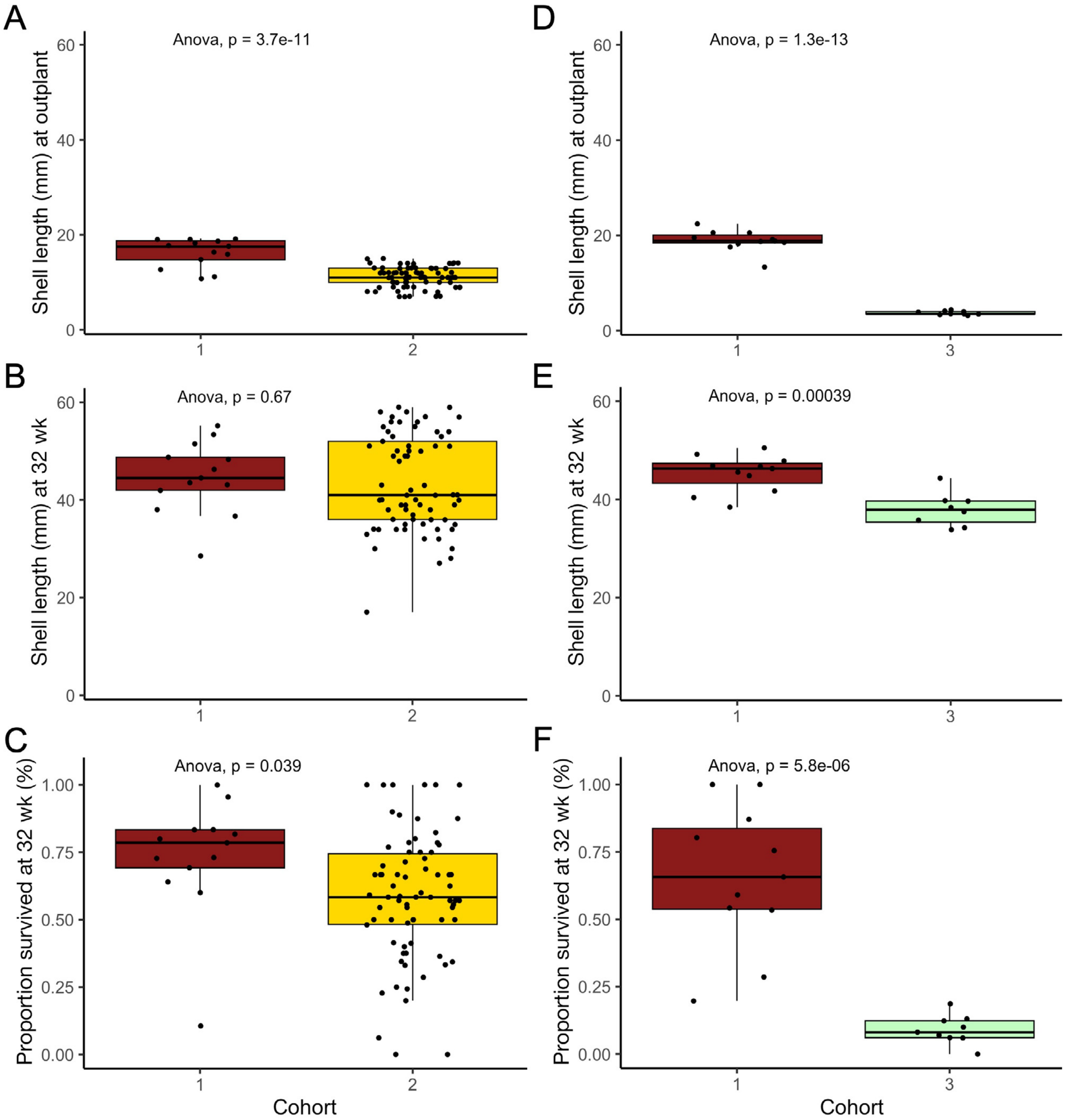

Fig. 8. Comparison of cohorts. Cohort 1, age 22 wk post-settlement compared to Cohort 2, age 14 wk, in (A) size at outplanting, (B) size after 32 wk in the field, (C) survival after 32 wk in the field. This comparison was all on tile substrates. Cohort 1, age 22 wk, compared to Cohort 3, age 9 wk, in corresponding graphs (D–F); these were all on clam shells. Box plot elements as in Fig. 4

4.2. Where to outplant: tidal elevation

Where to place hatchery-raised juveniles is a crucial decision in conservation aquaculture and restoration efforts in general. For sessile, intertidal species like oysters, the optimal elevation for outplanting involves potential tradeoffs that affect the organism differently throughout its life history (Townsend 1896, Crosby et al. 1991, Fodrie et al. 2014). These tradeoffs influence long-term success in oyster restoration (Smith & Castorani 2023). At higher elevations, oysters are exposed to air more frequently and for longer periods, resulting in reduced feeding time and increased thermal and desiccation stress. At lower

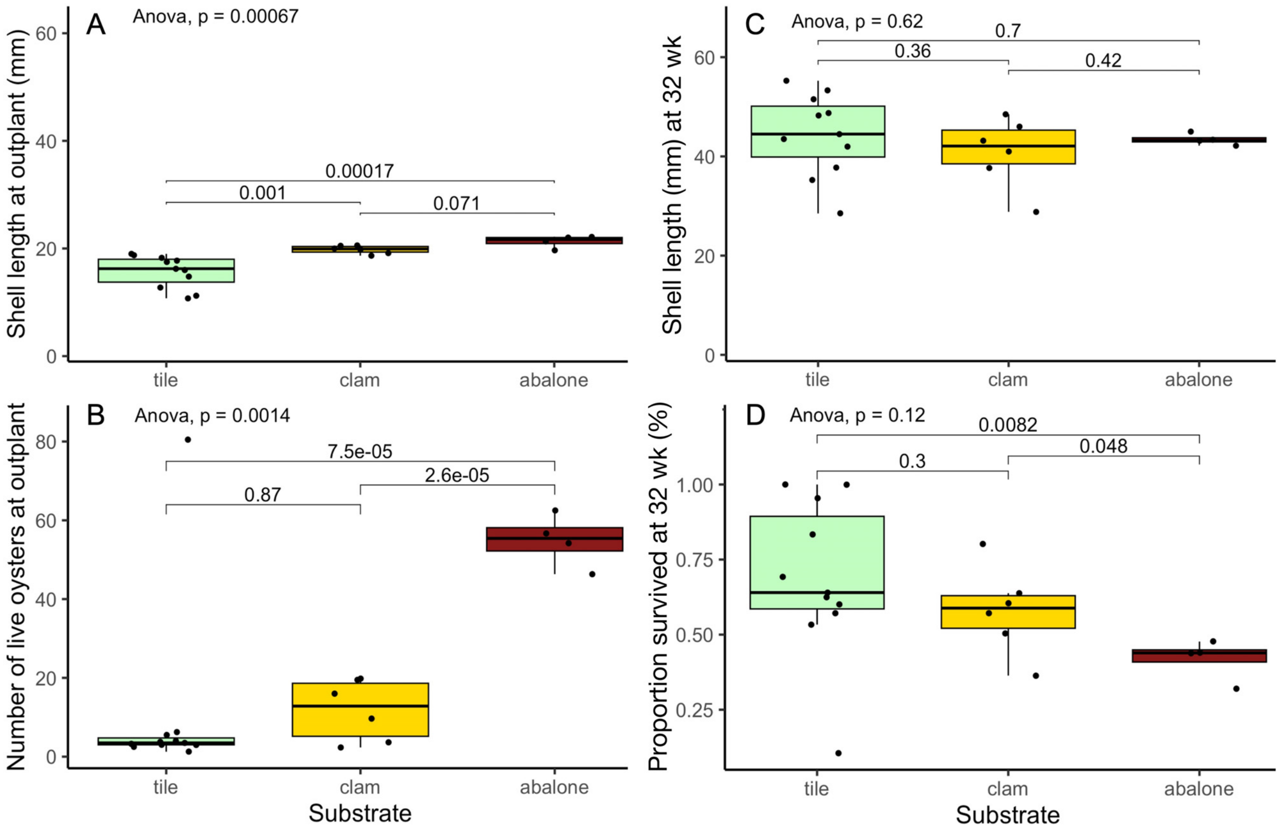

Fig. 9. Comparison of settlement substrates: (A) size at outplant, (B) live oyster count at outplant, (C) size and (D) survival 32 wk after outplant. Overall p-values for ANOVAs testing effect of substrate are shown, and p-values from *t*-tests shown for pairwise comparisons among groups. Box plot elements as in Fig. 4

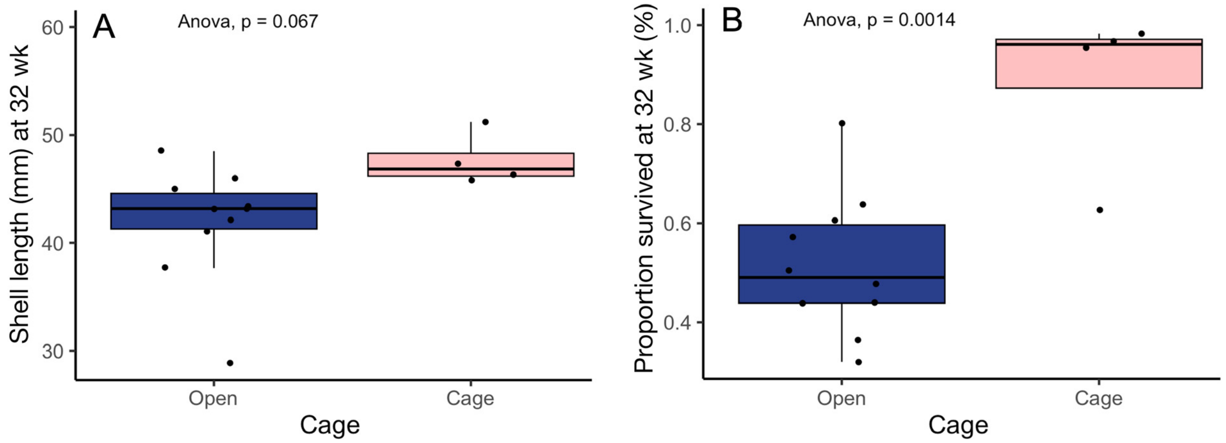

Fig. 10. Comparison of caged vs. uncaged oysters: (A) size and (B) survival for Cohort 1 oysters after 32 wk in the field at Hester Marsh. p-value for ANOVA is shown for each comparison. Box plot elements as in Fig. 4

elevations, oysters remain submerged, with increased food availability and reduced exposure stress (Bishop & Peterson 2006, Kimbro et al. 2009, Deck 2011). However, release from these physical constraints at lower elevations can increase competition with other non-native sessile species (Johnson & Smee 2014, Zabin et al. 2016) and predation by subtidal predators (Trimble et al. 2009, Valdez et al. 2017). Oysters at lower elevations are also more susceptible to detrimental effects from sedimentation in soft-bottom habitats (Wasson 2010, Dinnel 2018, Fuentes et al. 2020, McArdle et al. 2022).

In our study, increased submersion at lower elevations improved growth rates for oysters in the first 6 mo after outplanting, likely due to increased feeding time. Survival rates were also better at low elevations, perhaps due to reduced stress from temperature fluctuations, greater dissolved oxygen availability along with reduced desiccation. This experiment also demonstrated that oysters at MLLW grew at a consistent rate from December through July and reached reproductive size, nearly their maximum size, within 1 yr post-settlement. At the same time, initially slow growth at the high elevation markedly increased after March compared to the winter months. These findings are consistent with expected growth and survival trends across a tidal gradient for both wild recruits (White et al. 2009) and hatchery-raised Olympia oysters (Barsh et al. 2004, Trimble et al. 2009, Valdez et al. 2017, Lowe et al. 2019).

The initial size and survival differences detected between elevations equalized a year after outplanting. Growth rate of Olympia oysters is shown to slow as oysters reach adult sizes (Peter-Contesse & Peabody 2005, Barber et al. 2015). This size threshold occurred earlier for the faster-growing oysters at low elevations and later for the high-elevation treatment. The lower-elevation treatment had greater survival rates in the first 6 mo, but lower survival rates in the second 6 mo. This was perhaps due to increased predation by subtidal predators that select larger Olympia oysters (Barsh et al. 2004), which was indicated by observations of dead juveniles with shell damage consistent with crab predation. Faster growth, as seen at the low elevation, can additionally lead to thinner shells in juvenile oysters (Paynter & Dimichele 1990, Bible et al. 2017, McAfee et al. 2017). We did not measure shell thickness, but thinner shells at lower elevations may incur further vulnerability to predators that must break shells to access their prey.

Across an intertidal gradient, oyster performance can also be affected by positive as well as negative species interactions (Reeves et al. 2020, McAfee et al. 2021). Many fouling organisms, for example, provide shading that reduces desiccation and thermal stress on oysters at higher elevations (Neeb Wade 2016, Zabin et al. 2021), but others can compete with oysters for space and food at lower elevations (Buhle & Ruesink 2009, Deck 2011, Wasson et al. 2014). In our study, oysters outplanted at high elevation were almost exclusively fouled by barnacles, while low-elevation oysters were covered by several other organisms (bryozoans, sponges, tunicates). To avoid potential competition among these fouling species and oysters, restoration substrates can be moved to higher intertidal elevations after the initial period of rapid oyster growth is completed at lower elevation, an adaptive management approach piloted by Zabin et al. (2016).

Site selection is a primary consideration for long-term persistence in restored oyster habitats (Ziegler et al. 2018). Restoration success in this study was not very sensitive to site selection in Elkhorn Slough. Hatchery-raised oysters had similar size and survival across the 4 sites over their first year, although long-term differences were observed between sites after 83 wk. Success at the 2 Hester Marsh sites was important because it demonstrated that outplanting can initiate population expansion into newly created marsh habitat without a present oyster population (Haskins et al. 2021).

4.3. When to outplant: size

Timing for releasing cultured organisms is critical for all conservation aquaculture efforts (Sarrazin & Legendre 2000, Zeug et al. 2020, Crossman et al. 2023). There are both benefits and costs to longer hatchery times. More time spent in the hatchery allows organisms to grow larger before outplanting. Larger organisms typically have increased protection from predation and physical stressors than their smaller conspecifics and are expected to survive better after release. Hatchery selection or acclimation to tank conditions over time in the hatchery, however, may reduce resistance to environmental stresses and decrease overall fitness (Taris et al. 2006, Camara & Vadopalas 2009, Spencer et al. 2020). Hatchery costs associated with feeding and husbandry increase with time in hatcheries as well and can limit the scalability of restoration efforts. We additionally hypothesized that our hatchery conditions may slow oyster growth compared to the field due to limited feeding time and less diverse microalgae diet (Pit & Southgate 2000). Faster growth in the estuary would support earlier outplanting if it would counteract size-related mortality.

Surprisingly, oysters grew faster in the hatchery than the field for small (~5 mm) oysters grown in these differential conditions between December and February. While this was unexpected, there are relatively low chlorophyll levels in the estuary (2–4 $\mu g\ l^{-1}$) during this season (Elkhorn Slough Reserve monitoring data, https://cdmo.baruch.sc.edu/dges). This hatchery advantage could be seasonal, and juveniles outplanted in warmer seasons, when Elkhorn Slough chlorophyll levels are higher (10–15 µg in summer), can still be predicted to grow faster in the field than in the hatchery. For this reason, hatcheries for many filter-feeding bivalves may strategically spawn and settle juveniles outside the natural reproduction season to align outplanting and juvenile growth with maximum feed availability in their respective estuary system (Loosanoff & Davis 1963, Mann 1983, Nascimento-Schulze et al. 2021).

Survival was also affected by time in the hatchery. For oysters from the same cohort outplanted at different times through the winter, survival was higher in the hatchery than in the field. Those held back longer had higher year-long survival simply from this early effect. Survival was not higher following outplant to the field at different sizes/ages in this experiment. All of these oysters were very small when outplanted and perhaps below a threshold where increased size provides a benefit. In contrast, size differences in the experiment comparing different oyster cohorts outplanted at the same time were greater between treatment groups. Here we did find that larger juveniles from the oldest cohort survived in the estuary better than smaller oysters from the 2 younger cohorts.

Future studies can determine minimum oyster size thresholds with refined sensitivity and investigate how the relationship between size and survival may change seasonally. Overall, increased time in the hatchery improved survival for this species in these local conditions. Determining optimal outplant size can improve conservation aquaculture efficiency by restricting time in the hatchery to this size threshold. This will likely vary based on outplanting season and local adaptations. Restoration programs will need to balance size-dependent survival with their hatchery methods, outplanting design, and habitat conditions.

4.4. How to outplant: substrates and caging

Conservation aquaculture approaches must be tailored to local restoration goals and management values. The estuary and wetland habitats in Elkhorn Slough are located within a marine protected area and are managed for sustaining natural ecosystem functions. A variety of substrates have been used successfully to restore oyster reefs worldwide, including Olympia oysters (Fitzsimons et al. 2020, Goelz et al. 2020). The Elkhorn Slough Reserve specifically aims to restore biogenic, low-profile oyster beds that were natural in this system for millennia (Wasson et al. 2020). Accordingly, the substrates and aquaculture methods in this study were tested to generate clusters of oysters aggregated on a single substrate. We demonstrated differential performance based on substrate type. Gaper clam shells fit Elkhorn Slough's restoration goals best because they yielded better survival than abalone shells and improved structural complexity from clustering compared to tiles (Fig. 2H). The clam shell substrates were locally available within the estuary and represent a naturally occurring substrate in this habitat. Clam shells also proved more durable, remaining sturdy and supporting long-term biogenic oyster clusters. In our study, abalone shells were obtained from a neighboring aquaculture facility, not the estuary. Abalone shells had the highest initial oyster settlement densities, but competition for space as oysters grew seemed to result in density-dependent mortality. The abalone shells were only effective as short-term substrates as most become brittle, cracking or breaking after 1 yr in the Slough.

Innovative designs are needed for restoring Olympia oysters to eutrophic mudflats. Sedimentation is the biggest obstacle to restoration success for Olympia oysters throughout the entire range of the species (Ridlon et al. 2021b). Outplanting oysters in Elkhorn Slough is particularly challenging due to a deep, highly organic, largely anoxic mud layer resulting from eutrophication (Hughes et al. 2011). Oysters are easily buried, and heavy substrates sink quickly in many parts of the Slough. The stake design for hanging substrates was developed to match Reserve restoration goals such as adaptive management, long-term monitoring, accessibility, and community engagement. They are quickly assembled in the field and facilitate easy transportation between the hatchery and outplanting sites. Community events were organized around assembly and outplanting efforts that connected stakeholders, students, and volunteers with oyster conservation. The design is useful for its mobility around the sites, accessibility for monitoring, adaptability to experimental treatments, and for facilitating easy return to the hatchery as broodstock adults.

Cages suspended from stakes also contributed to site-specific restoration goals by mitigating burial in the mud and supporting high growth and survival for dense oyster clusters. Although cages do not generate naturalistic habitat, they can be valuable for conservation aquaculture especially in the juveniles' first year. Higher growth and survival inside cages demonstrated that cages did not restrict water flow nor increase competition from settling organisms. Sea otters sometimes forage on hatchery-raised oysters in Elkhorn Slough (Wasson et al. 2020), so caging is an option to allow oyster recovery and a keystone predator population to coexist.

4.5. Conclusions: lessons from an experimental approach to restoration

This study demonstrated the value of incorporating an experimental approach to restoration. Conservation aquaculture facilitated restoration experiments because many juveniles from the same cohort could be settled on replicate substrates. The results of the field experiments supported practical decision making and directly informed future management. For Elkhorn Slough, we learned that hatchery-raised oysters do best when outplanted around MLLW, on clam shells, at a size greater than 10 mm. Success was high at multiple sites, including a marsh restoration area with no adult oysters. In addition, caging can effectively support oysters without compromising survival or growth if sea otter predation becomes important. Few experimental publications rigorously quantify conservation aquaculture methods or results for Olympia oysters (Greiner et al. 2015, Barber et al. 2016, Zabin et al. 2016, Heare et al. 2017, Silliman et al. 2018). Thus, these results support a growing knowledge base for Olympia oyster conservation. More broadly, the experimental approach to restoration described here can serve as a model for conservation aquaculture projects with other foundation species.

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