Biological Conservation 250 (2020) 108745

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Policy analysis

A scientific framework for conservation aquaculture: A case study of oyster restoration in central California

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ARTICLE INFO

Keywords:
Conservation aquaculture
Crassostrea
Ostrea
Hatchery
Recruitment

ABSTRACT

The emerging field of conservation aquaculture focuses on the potential for incorporating aquaculture techniques into restoration. Extensive loss of oyster reefs worldwide has led to restoration initiatives that sometimes incorporate aquaculture, but few scientific studies of this approach have been published. We developed a scientific framework to determine whether aquaculture is an appropriate conservation tool, and applied it to Olympia oysters (*Ostrea lurida*) in Elkhorn Slough, an estuary in central California, USA. Over 12 years of monitoring, we documented precipitous declines in density, highlighting the need for restoration. We tracked settled oysters and found that growth and survivorship is high, showing that hatchery-raised juveniles have the potential to survive to reproductive age. No natural recruitment has occurred in the estuary in seven years, suggesting that this population is recruitment limited. Thus, we determined a need for conservation aquaculture. We produced juvenile oysters from local broodstock in a hatchery and settled them on native clam shells, which we attached to stakes to form small clusters that mimic natural biogenic habitat created by this species. We deployed these near the upper limit of the intertidal range of oysters, where oyster cover dominates over non-native fouling species. The outplanted oysters grew to adult, reproductive size within months of outplanting, and survivorship was generally high, providing the first new generation of oysters in this estuary in seven years. The science-based approach we implemented and our incorporation of traditional restoration principles of natural habitat structure and dominance by native species can serve as a model for conservation aquaculture for oysters and other species.

1. Introduction

Conservation aquaculture is defined as human cultivation of an aquatic organism for the planned management and protection of a natural resource (Froehlich et al., 2017). Aquaculture generates about half of globally consumed seafood (Edwards et al., 2019), but has earned a negative reputation among conservationists, due to habitat loss, pollution, and the spread of invasive species often associated with these operations (Islam, 2005; De Silva, 2012). However, aquaculture can be implemented sustainably, and bivalve aquaculture in particular has been identified as one of the lowest-impact forms of marine aquaculture and sources of animal-based food (Hilborn et al., 2018), capable of providing some ecological benefits including water filtration and structural habitat (Naylor et al., 2000; Costa-Pierce, 2010). Aquaculture techniques have also long been used to enhance wild populations of fished species (Taranger et al., 2014) and as a management tool for harvested populations, including oysters (Breitburg et al., 2000; Lorenzen et al., 2013; Couvray et al., 2015).

The emerging interest in conservation aquaculture (Froehlich et al., 2017) emphasizes the importance of ecologically responsible methods

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https://doi.org/10.1016/j.biocon.2020.108745
Received 22 October 2019; Received in revised form 20 July 2020; Accepted 24 July 2020
0006-3207/

of implementing and scientifically rigorous methods of evaluating the use of aquaculture techniques for conservation goals. In contrast to conventional aquaculture, conservation aquaculture purposefully aligns with the conservation goals for a species, which can include production not only for consumption, but to enhance or restore wild populations (Froehlich et al., 2017). Its techniques also seek to minimize the risks associated with conventional aquaculture. For example, conservation hatchery protocols address risks related to the release of hatchery-reared organisms, including preserving the genetic diversity of the wild population and minimizing the propagation of invasive species (Flagg et al., 1999; Crim et al., 2015).

Oysters can act as foundation species through habitat creation and amelioration of environmental stressors, such as dampening of storm surges (Beck et al., 2011). Oysters have also provided food for people around the world for millennia, including 2000 years of aquaculture in some regions (e.g. in China: Guo et al., 1999). Aquaculture of oysters has increased over the past century, and it has become common in many regions to switch from culturing native species to culturing predominantly fast-growing, hardy introduced species. For example, the Pacific oyster (*Crassostrea gigas*), native to east Asia, is currently the dominant aquaculture species grown on the west coast of North America (Conte and Moore, 2001), where it was introduced after the collapse of the native oyster fishery (Kirby, 2004). This species has likewise been introduced to many other coasts around the world, where it has often spread beyond aquaculture areas and established self-sustaining introduced populations (Brandt et al., 2008, Troost, 2010, Kochmann et al., 2012, Wörner et al., 2019). Meanwhile native oyster beds, beleaguered by overharvest, habitat loss, and introduced diseases and predators, have decreased dramatically (White et al., 2009). In the United States, there has been an 88% loss in oyster biomass (Zu Ermgassen et al., 2012) and worldwide, an estimated 85% of oyster reefs have been lost, a figure exceeding the estimated loss of coral reefs (Beck et al., 2011).

Globally, efforts are underway to restore lost oyster populations, both for consumption, and for the ecosystem services they provide (Coen et al., 2007; Grabowski and Peterson, 2007; Beck et al., 2011). Aquaculture has been identified as vital to rebuilding native populations that have severely declined (e.g. *Ostrea edulis*: Pogoda et al., 2019), especially where recruitment is a limitation to maintaining viable populations (Steppe et al., 2016). Aquaculture techniques have successfully supported the restoration of various wild populations of native oysters (most notably *Crassostrea virginica*: Brumbaugh et al., 2000, Schulte et al., 2009), and may be particularly useful when integrated into other habitat restoration efforts and/or fisheries management techniques (Breitburg et al., 2000, Powers et al., 2009). However, widespread, systematic application of aquaculture techniques to achieve conservation goals for oysters are hindered by a lack of scientific evaluation and regular monitoring, among other challenges (Kennedy et al., 2011).

Here, we develop and apply a scientific framework for determining whether aquaculture may be an effective component of conservation efforts for a marine foundation species (Fig. 1). The first step is to determine whether intervention is needed at all – populations that are stable or recovering may not need restoration action. The second step is to evaluate environmental conditions – if these are not suitable, then restoring the degraded ecosystem processes or health may be the first priority. The third step is to examine post-recruitment survival: if it is low, then hatchery-produced juveniles are likely to die. The final step is to assess whether failed reproduction is likely to be limiting the population, since aquaculture is primarily a tool for addressing recruitment limitation.

The Olympia oyster (*Ostrea lurida*) is well-suited to the application of this conservation framework. It is the only oyster native from Baja California to British Columbia, on the temperate west coast of North America (Polson and Zacherl, 2009). Populations were historically abundant enough to be harvested, and native oysters were commercially cultured (Conte and Moore, 2001), but populations have declined precipitously across the range — to the point of functional extinction in some estuaries (i.e. ≤1% of historical abundances; Zu Ermgassen et al., 2012). Restoration of Olympia oyster populations has gained momentum in recent years, and aquaculture has been incorporated into a small subset of restoration projects. In particular, the state of Washington in partnership with the Puget Sound Restoration Fund has pioneered the use of aquaculture for this species (Blake and Bradbury, 2012). However, integrating these techniques into restoration efforts for Olympia oysters is comparatively new, and very few scientific studies of conservation aquaculture of this species have been published (Archer, 2008; Dinnel et al., 2009; Barber et al., 2015; Valdez et al., 2017). Aquaculture techniques could be used to enhance populations across its range, especially where adult numbers are low or where recruitment is a limitation to maintaining viable populations. Rigorous scientific evaluation of the efficacy of this conservation tool is now critical.

We applied the conceptual framework (Fig. 1) to Olympia oysters in Elkhorn Slough, an estuary in central California, USA. We assessed restoration need through long-term monitoring of densities of adult populations in the estuary. We determined whether conditions are suitable and post-settlement survival is robust by quantifying survival and growth rates of settled oysters. We evaluated whether recruitment is likely a critical bottleneck for population recovery by tracking recruitment for over a decade. Since application of this framework revealed that conservation aquaculture might be a potentially valuable tool, we conducted a small-scale proof-of-concept test of aquaculture techniques and evaluated success of the outplanted, hatchery-raised juveniles. To align with stakeholder values, we developed and tested a naturalistic design for the outplanted oysters that mimics the natural biogenic-structured habitat that clusters of Olympia oysters create, and provides dominance of the oyster over non-native species. The approach we used, with scientific data informing restoration planning and evaluating outcomes, can serve as a model for new conservation aquaculture projects for oysters and other aquatic species.

2. Methods

2.1. Study system

Elkhorn Slough (Fig. 2) is a small estuary (1200 ha) in central California (latitude 36.8, longitude −121.7), where all significant rainfall occurs between October and May. The average daily difference between the lowest and highest tide is 1.6 m; the maximum difference on king tides is about 2.5 m. The estuary has been highly altered by human land uses. Extensive diking and draining converted wetlands to agricultural areas. The Salinas River, which once connected to the Slough, was diverted, and an artificial mouth was created to support Moss Landing Harbor, increasing tidal energy in the Slough (Caffrey et al., 2002). Agricultural run-off has resulted in eutrophic conditions, including extensive algal mats (Hughes et al., 2011; Wasson et al., 2017).

Since the 1970s, various conservation organizations and resource management agencies have worked together to restore healthier ecosystems in Elkhorn Slough (Caffrey et al., 2002). The Elkhorn Slough National Estuarine Research Reserve aims to return processes and habitats to conditions similar to what naturally occurred in the region over the past hundreds to thousands of years, prior to the dramatic alterations resulting from European colonization. As a part of this, the Reserve set a goal of doubling the native oyster population within its boundaries (Fig. 2) over the course of a decade. In much of the estuary, oysters only survive if they can avoid burial in the deep organic mud that prevails; the size of the smallest substrate containing live oysters correlates with the depth of the mud (Wasson, 2010). Restoration efforts thus have provided hard substrate above the mud. Substrates deployed in 2012, a year with high recruitment, supported thousands of new oysters with high survivorship and growth, but ones deployed in

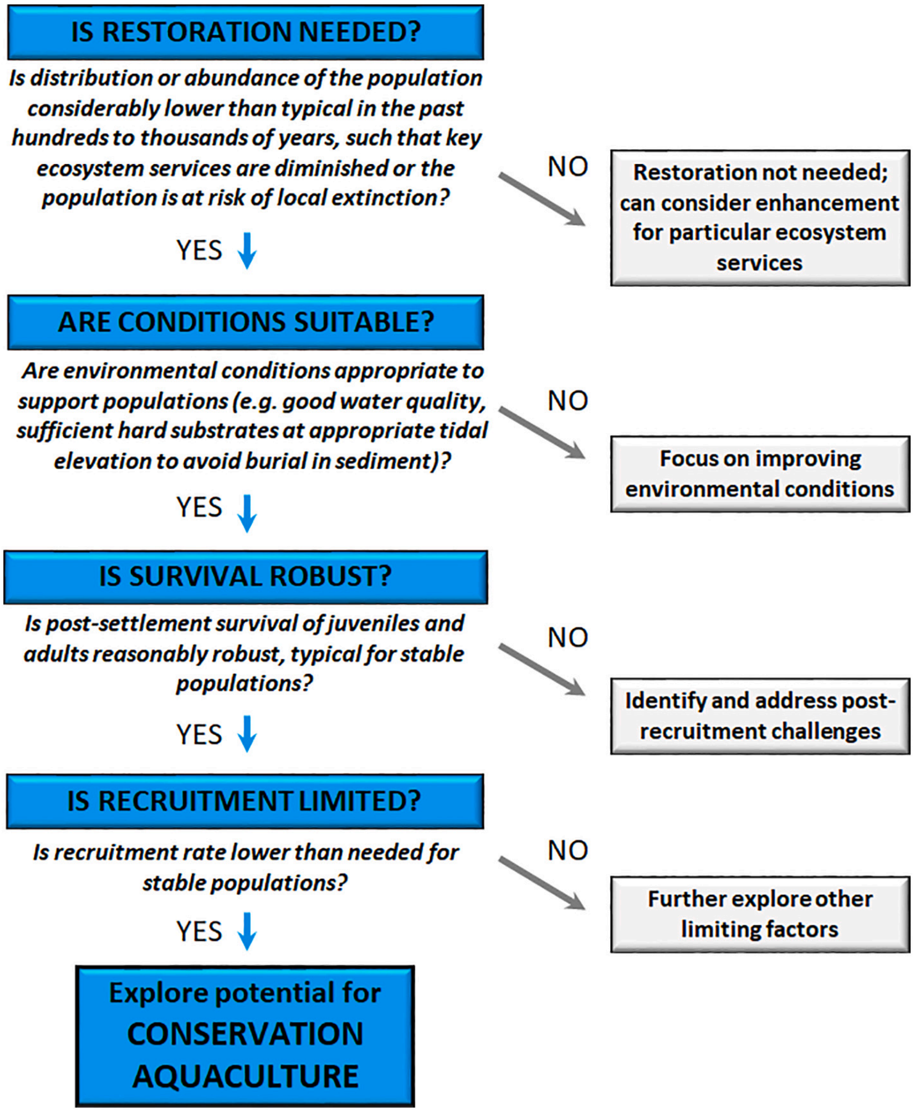

Fig. 1. Conceptual framework for incorporating conservation aquaculture in restoration. The purpose of this framework is to determine whether conservation aquaculture is an appropriate tool for a particular estuary or region. While this was designed for oysters, the general framework is applicable to other aquatic species. Note that options are shown as either/or for simplicity, but in reality, practitioners may need to invest in multiple components (e.g. habitat restoration and conservation aquaculture).

2013, a year with little to no recruitment, had none (Zabin et al., 2016). Oysters are found at tidal elevations ranging from about 0.4 m below to 0.6 m above Mean Lower Low Water (MLLW) at Elkhorn Slough. At the lower end of this range, there is high cover by non-native sessile species (bryozoans, sponges, and tunicates in particular), while at the upper end of this range, oysters comprise the dominant cover on hard substrates (Zabin et al., 2016).

2.2. Long-term demographic trends in wild population

To assess changes in oyster density over time and determine whether the population needs restoration intervention to prevent local extinction (first step in Fig. 1), we conducted long-term monitoring of adult populations. Permanent fixed transects were established in Fall 2007 at the four sites with the largest adult populations in the estuary (Wasson, 2010) and were assessed every five years (Fall 2007, 2012, 2017). An additional survey was conducted before five years had passed, in Summer 2019, to provide information on population status concurrent with restoration efforts. At all sites, transects were located parallel to shore in the areas with the highest densities of oysters, which occurred on rip-rap and gravel at elevations approximately 0.2–0.3 m above MLLW. At three of the sites the transects were 20 m long, as that was approximately the extent of rip-rap and thus dense oysters; at Kirby Park, which has more extensive rip-rap, the transect was 30 m. Transect endpoints were permanently marked with rebar stakes. A 0.5 × 0.5 m quadrat was placed every 2 m along the transect, and all live oysters within it counted and measured.

To more extensively assess adult populations at restoration sites (two locations in South Marsh and one in Whistlestop, Fig. 2), a complete area search was conducted in the 100 m stretch of shoreline centered on the three restoration sites (e.g. 50 m to either side) prior to outplanting. The entire intertidal zone was thoroughly searched and all live oysters counted.

2.3. Short-term juvenile survival and growth in wild population

To determine whether conditions are suitable for oysters and whether post-settlement survival is robust (steps 2 and 3 in Fig. 1), we tracked growth and survival of a cohort of oysters that recruited in the wild in July–August 2012 at the same four focal sites in Elkhorn Slough

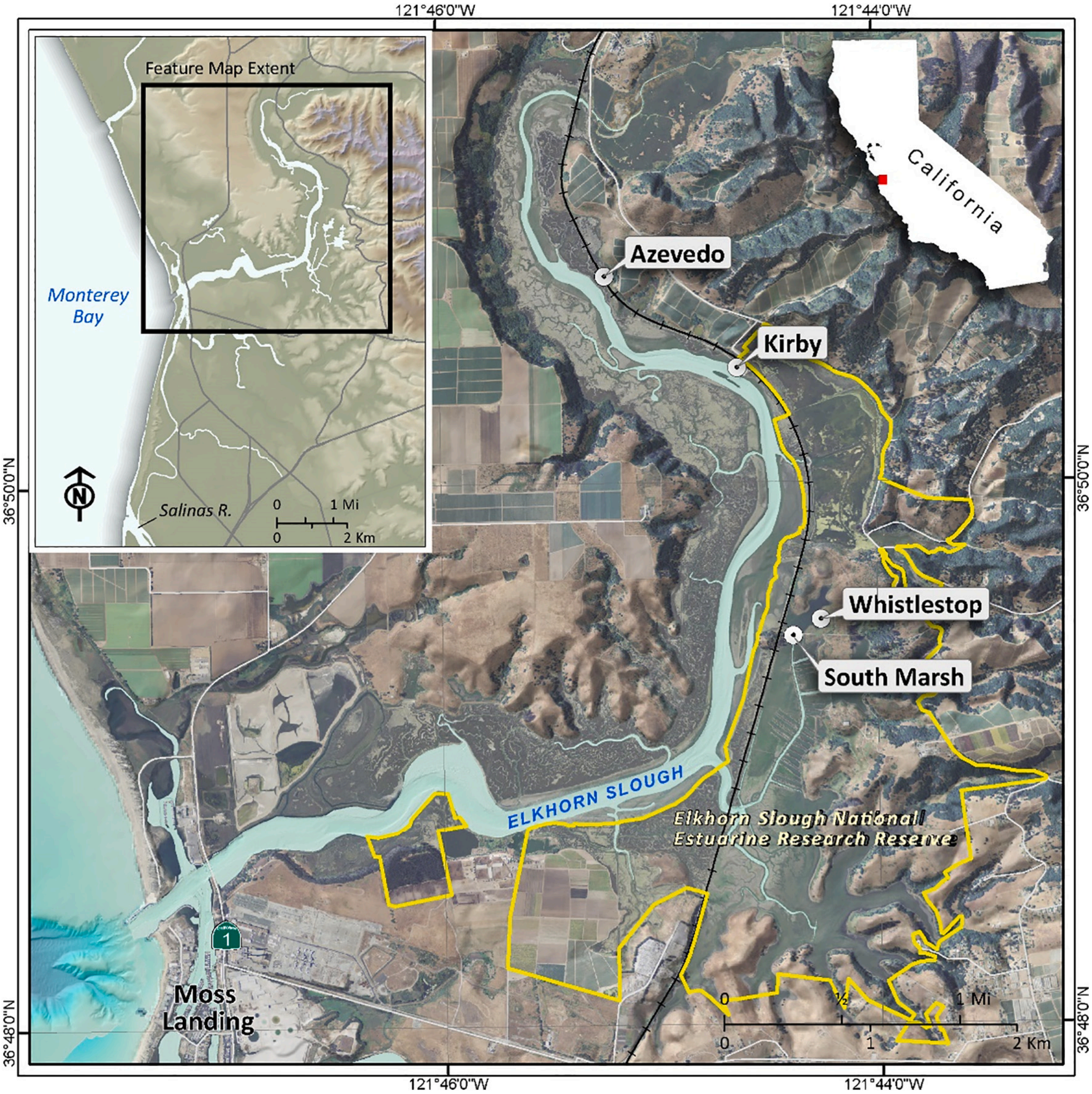

Fig. 2. Focal sites for monitoring and restoration. The boundaries of the Elkhorn Slough National Estuarine Research Reserve are shown in yellow. Full site names are North Azevedo Pond, Kirby Park, Whistlestop Lagoon, and South Marsh; shorter names are used on the map and in the text for convenience. The hatchery was located in Moss Landing, shown at the bottom left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from October 2012 through November 2013. (Additional sites and tidal elevations were also monitored; see Supplemental Information.) At each site, nine ceramic tiles (10 × 10 cm) were deployed at approximately MLLW. Tiles were cleaned and photographed quarterly. Photographs were analyzed using the program ImageJ, with individual oysters tracked over time. Survival was calculated as a monthly rate for Fall 2012 (October 2012–January 2013), Winter 2013 (January–April 2013), Spring 2013 (April–July 2013), and Summer 2013 (July–November 2013). To determine monthly survival, we calculated how many 'months' (defined as exactly 30 day periods for consistency among periods) had passed since the previous assessment (by dividing the number of days by 30), and calculated the proportion that had survived (number at most recent assessment/number at previous assessment). We then raised this proportion to the exponent 1/'months'. At one site, Whistlestop, very little natural recruitment occurred on the tiles, so tiles from Kirby and South Marsh with a cohort of settlers were moved there in December 2012 and tracked subsequently as at the other sites.

2.4. Long-term recruitment monitoring of wild population

In order to quantify spatial and temporal variability in recruitment and determine whether recruitment is a critical bottleneck to oyster populations in the estuary (step 4 in Fig. 1), we established a long-term monitoring program in 2007. We monitored recruitment annually 2007–2019 at the four focal sites. In some years recruitment was monitored at other sites throughout the estuary as well, but virtually no recruitment was ever detected at the other sites so they are not included here.

To assess recruitment, we suspended 10 × 10 cm ceramic tiles from PVC stakes with cable ties, with the elevation of the tile estimated to be near MLLW (we visited the sites multiple times in advance at tides predicted to be at MLLW and set flags to estimate this tidal elevation). Five tiles were deployed per site. They were checked approximately one year after deployment; the underside of each tile (where most recruits preferentially settle) was carefully examined and all oysters were counted and measured. Our assessment of recruitment thus focuses on juveniles entering the adult population, not on the early stages of settlement and growth. In some years, we had checked the tiles monthly

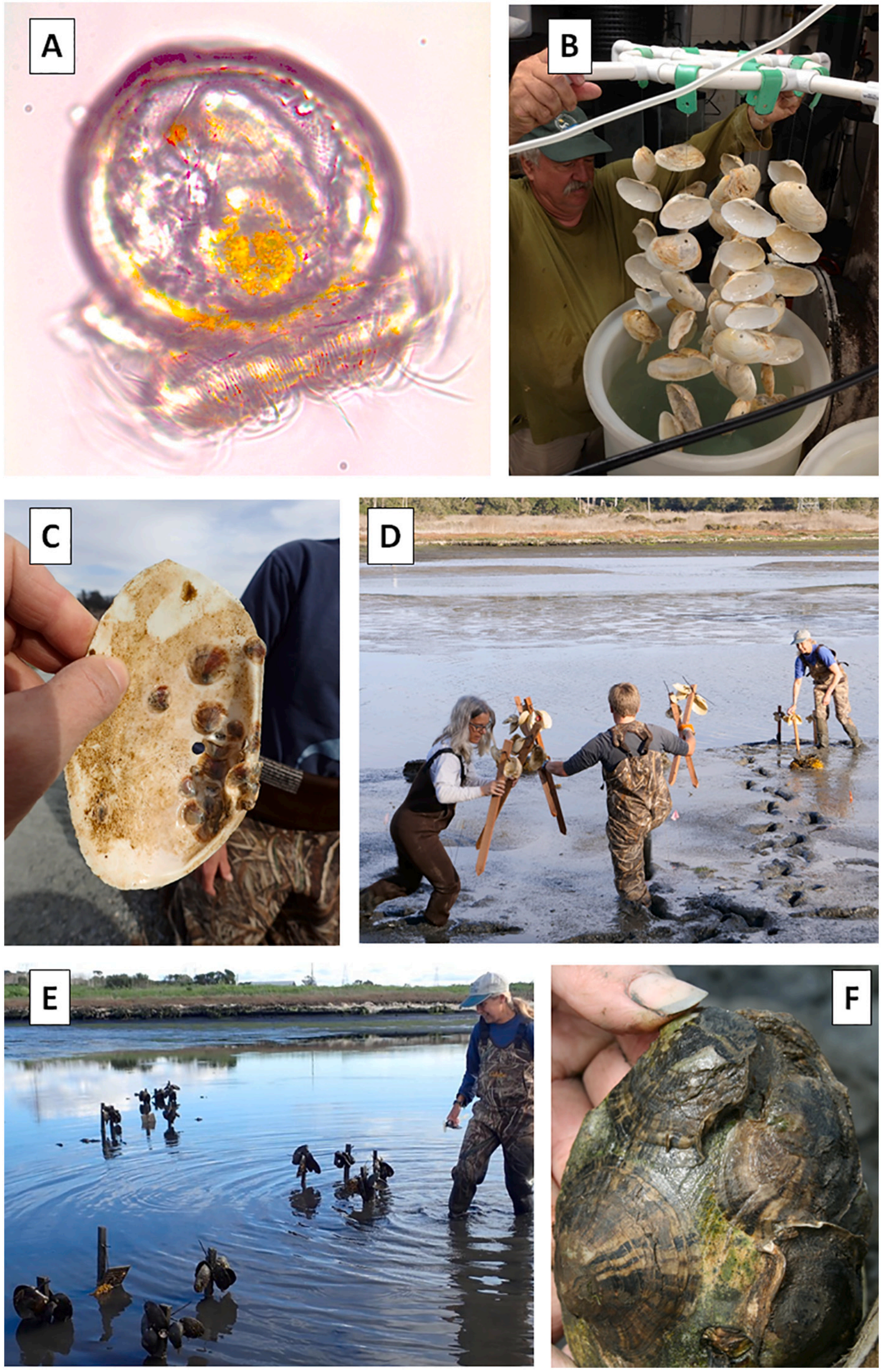

Fig. 3. Hatchery production and outplanting. A: Olympia oyster larva grown in hatchery (photo D. Gossard). B: Strings of clams shells provide larval settlement substrate at hatchery (photo D. Gossard). C: Small juveniles on a clam shell at time of outplant (photo C. Zabin). D: Deployment of stakes with outplanted oysters on clam shells (photo E. Garcia). E: Groups of stakes being monitored (photo K. Beheshti). F: Juvenile oysters at reproductive size eight months after deployment (photo A. Frisbee).

and found early survival was high. However, many years had no recruitment, making monthly checks unhelpful, so we chose annual monitoring for long-term recruitment assessment.

2.5. Hatchery methods

Adult *Ostrea lurida* (85 oysters) were collected from Elkhorn Slough in May 2018 to serve as broodstock. They were transferred to Moss Landing Marine Laboratory and initially fed dead microalgal shellfish diet, then switched to live algal cultures (see Supplemental Information for more details on hatchery methods). Only male *O. lurida* release their gametes into the water column which are in turn taken into the mantle cavity of the female oysters where the eggs are stored, fertilized, and held until they are released as larvae (Fig. 3A). This series of events was induced after about a month of conditioning the broodstock by gradually increasing the temperature in holding tanks, simulating seasonal temperature increases observed in the field (Seale and Zacherl, 2009). Following larval release, larvae were separated from adults, fed live algal cultures, and examined regularly for developmental stage.

When a larval eyespot and foot were evident, strings of gaper clam shells were suspended in the larval tanks as a settlement substrate (Fig. 3B), and food levels were increased. About one month post-settlement, all clam shells with visible live spat were consolidated and hung within a large tank with flow-through seawater (flow-through ceased for a daily 8 hour feeding period). Throughout the larval culture period, larval cohorts and the spat resulting from them were kept in separate containers according to their period of release. Clam shells were transported to the restoration sites for outplanting.

2.6. Outplant methods and monitoring

We chose three nearby sites to receive the hatchery-raised oysters. A rip-rap berm in South Marsh was selected because it had the greatest total number of adults on the Reserve, but had undergone strong declines over time. Our goal was to place hatchery-raised oysters close enough to the adults remaining at this site to allow for fertilization (sperm is readily diluted on fast currents, and so we suspect breeding distance is only a few meters). Our earlier restoration work (Zabin et al., 2016) established that crab predation on juveniles is higher in rap-rap than mud, so we also selected a second nearby site in a mudflat without hard substrate or adult oysters, 60 m away. Finally, we selected a location in nearby Whistlestop Lagoon, 225 m from the other sites. This area previously supported many oysters, but they all died in 2012–2013 when the culvert through the berm connecting this area to South Marsh collapsed, resulting in stagnant water. Tidal exchange was restored through a large box culvert in 2014, but oysters remained absent due to lack of recruitment. This site also has piles of oyster shells from past Native American harvests, thus making it a priority for restoration.

Because all three sites are close together and subject to strong tidal exchange, we assume water quality conditions were very similar (see www.elkhornslough.org/water for monthly water quality samples at South Marsh vs. Whistlestop monitoring stations). Over the monitoring period (22 October 2018–9 March 2020), data from a nearby sonde collecting water quality data every 15 min (available form http://cdmo.baruch.sc.edu/dges/) revealed the following averages and standard deviations: temperature 15.8 ± 3.2 °C, salinity 31.9 ± 2.2, dissolved oxygen 7.4 ± 1.5 mg/L, pH 7.9 ± 0.19. Chlorophyll, collected monthly from this station over the same period was 5.75 ± 4.6 μg/L.

To engage the community in oyster restoration, we organized a large public outreach event on 22–23 October 2018 for outplanting the oysters. Volunteers assisted the project team by attaching clam shells holding the hatchery-raised oysters to wooden stakes. The clam shells had been drilled (Fig. 3C) prior to deployment in hatchery tanks as a settlement substrate, and the wooden stakes were drilled as well, to allow for clam shells to be attached with plastic zip ties (30 cm, UV resistant). The stakes were redwood, 60–90 cm long, pointed at one end, about 3 cm wide and 2 cm thick. The longer stakes were used for the muddier sites.

Between 3 and 7 shells were attached to each stake, along with an identifying numbered tag. All shells on a single stake came from the same larval cohort (A-D). Following assembly, volunteer teams counted all the oysters per shell and measured the largest oyster per shell, recording this for each stake. For the most recently released larval cohort (D), where oysters were barely visible, mostly < 1 mm in size, numbers and sizes represent estimates.

Stakes were deployed in clusters of 3–4, inserted into the mud so that the shells attached to the top were situated about 0.4 m above MLLW, along an approximately 20 m stretch of shoreline at each site (Fig. 3D, E). This relatively high tidal elevation was chosen because an earlier study had shown that native oyster dominance over non-native fouling species is optimized at higher elevations (Zabin et al., 2016). A total of 67 stakes were deployed, 23 at the South Marsh berm, 20 in the nearby South Marsh mudflat, and 24 in the Whistlestop mudflat. All four larval cohorts (A–D) were represented at each site.

Oysters were monitored approximately 6 weeks after deployment (3 December 2018), 32 weeks after deployment (7 June 2019), and 72 weeks after deployment (9 March 2020). Since it is difficult to see small oysters inside the clam shells near the umbo, some of the small oysters were not observed in the initial counts but detected in later counts, so for some substrate shells, total oyster number per shell appeared to increase over time (providing survivorship numbers > 100%). We converted survival to a monthly rate as described above in Section 2.3. For each stake, we summed all the live oyster counts from all shells, and calculated the average size of the largest live oyster measured per shell. All analyses used individual stakes as replicates. For the initial period, all 67 stakes were included in the analyses. Stakes with no live oysters were dropped from the analysis for determining growth or survival over the previous period (though all were included in calculating survival over the whole period). In March 2020, 19 stakes had no live oysters; the remaining 48 stakes were used as replicates for analyses of growth and survival over the last period. We examined average sizes and counts separated by larval cohort and outplant site, and plotted a regression of growth over time. At the last monitoring date, we estimated percent cover on each clam shell by oysters (for those shells with live oysters remaining) and by any species other than oysters.

2.7. Statistical analyses

We used the ggpubr package in R version 3.5.2 for all analyses (R Core Team, 2018). For comparisons by site, year, or other groupings, we created box plots where the midline is the median, the upper and lower limits of the box represent the 75th and 25th percentile, and the “whiskers” extend up/down to the largest/smallest point within 1.5 times the interquartile range; any outliers beyond that range are shown individually as points. In order to determine whether outplant sites or larval cohorts had significant effects on juvenile size or survival, we used Kruskal Wallis tests.

3. Results

3.1. Long-term demographic trends in wild population

Monitoring of permanent transects revealed a clear decline in oyster density over time (Fig. 4A). By coincidence, this long-term monitoring program with five year sampling intervals happened to result in transects being assessed in Fall of 2007 and 2012, in both cases following a summer with high recruitment. In both of those surveys, oyster densities were moderately high at most sites, with the exception of Whistlestop in 2012, where failed culverts had led to water quality impairment. In 2017, following five years with no recruitment, densities had declined markedly; by 2019, densities had dropped to zero or near zero at all sites. Indeed, in a search of 100 m stretches of coastline encompassing our 2018 outplant sites, we found zero live oysters at two of the sites, and a total of about 100 live oysters at the South Marsh Berm site.

Size class distribution makes clear that these trends are related to aging of the 2012 cohort and the absence of recruitment. Average size per quadrat in these permanent transects increased over time (Fig. 4B). Similar trends were reflected in minimum sizes. The smallest measured oyster per quadrat was < 10 mm at all sites in 2007 and 2012, reflecting recruitment in the preceding summer months. In 2017, the smallest measured oysters were 30–40 mm, and these were typically ones prevented from growing larger due to crowding with other oysters. By 2019, the smallest measured oysters in transects were 47–50 mm.

3.2. Short-term juvenile survival and growth in wild population

Survivorship of the cohort of oysters that settled in July–August

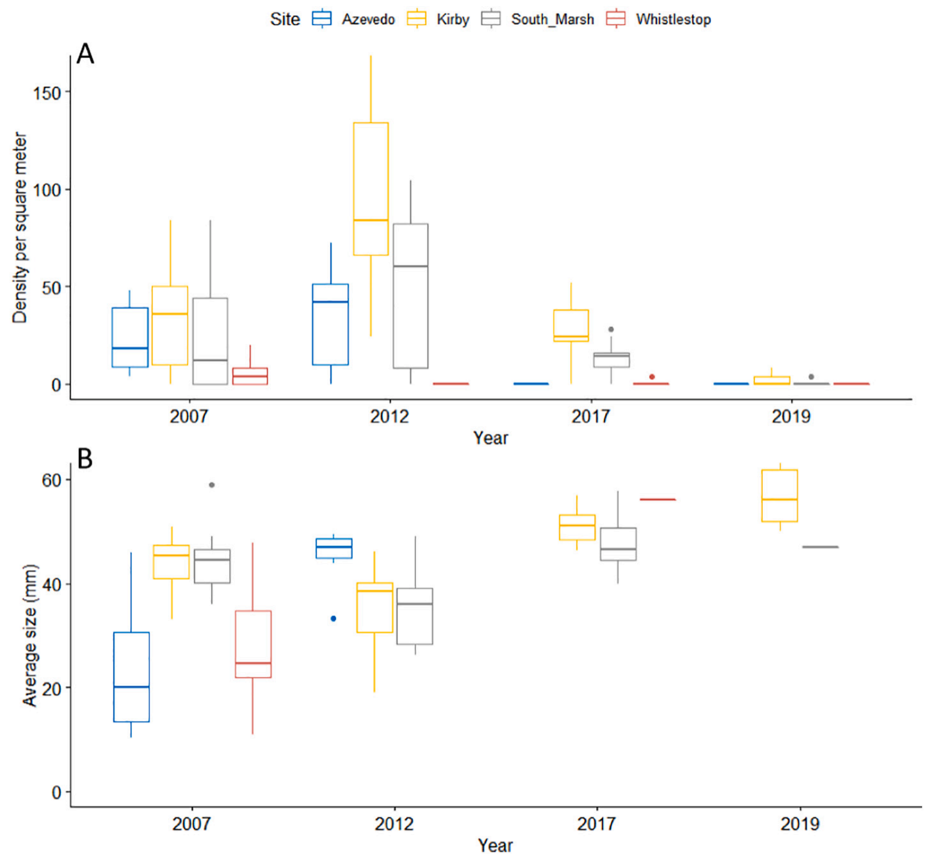

Fig. 4. Long term demographic changes over time in permanent transects at four focal sites. A: Oyster density. B: Oyster size.

2012 varied by site and period (Fig. 5A; Supplemental Figs. S1–2). Survival across the year was lowest at Azevedo (a site with tidal restriction and impaired water quality). Overall, survivorship (proportion of oysters per tile that survived) averaged across the four focal sites for all quarters was 0.79 ± 0.30 per month.

Growth rates of this cohort were high; one year after settlement oysters were 41 ± 10.3 mm in size, averaged across the four focal sites. Growth varied across sites and time periods (Fig. 5B, Supplemental Figs. S3–4). Size after one year was lowest at Azevedo, and highest at Whistlestop, where no wild recruitment had occurred on tiles (so the measured oysters had been relocated to Whistlestop from Kirby and South Marsh).

3.3. Long-term recruitment monitoring in wild population

Monitoring showed strong variation in recruitment over time (Fig. 6). In 2007 and 2012, all four sites had recruitment, with high densities of juveniles recorded at three of four of these. Between 2008 and 2011 there was very limited recruitment at some sites. From 2013 to 2018, there was virtually zero recruitment: a single oyster was found on one tile, of the 20 tiles per year examined at these four sites for 6 years.

3.4. Hatchery results

The wild collected adults were readily induced to spawn in the laboratory. Sperm release occurred 2 and 10 days following initial temperature increase; larval release occurred over multiple days or in distinct batches 11–48 days after water temperature increase (see Table S1).

Four distinct batches of larvae released in different periods were outplanted at the same time, Cohort A (about 122 days since larvae were released), Cohort B (97 days), Cohort C (about 87 days), and Cohort D (about 30 days). This final batch resulted from an unexpected late spawning event; since the hatchery efforts were concluding, the spat from Cohort D were outplanted even though they were at a size known to be at high risk.

3.5. Outplant monitoring

In October 2018, initial measurements and counts of hatchery-raised oysters on shells just prior to outplanting yielded an estimate of about 2300 juveniles in the larger size classes (larval cohorts A-C) and about 17,000 juveniles in the tiny size class from the late spawning event (larval cohort D). The former were an order of magnitude larger than the latter (Table 1).

In December 2018, six weeks following outplanting, the tiny juveniles from larval cohort D had experienced high mortality, but the other cohorts displayed reasonably high survival (Fig. 7A). All juveniles had grown substantially, with cohorts C and D still lagging behind A and B (Fig. 7B).

In June 2019, 32 weeks after outplanting, and just under a year following initial larval release, all cohorts were found to have had high survival since the last monitoring check, with cohort D showing much improved survival over the early period, but still lower than the other cohorts (Fig. 7A, Table 1). All cohorts had grown substantially (Fig. 3F), with cohorts C and D still lagging behind A and B (Fig. 7B).

In March 2020, 72 weeks after outplanting, survival rate was found to be high since the last monitoring 40 weeks earlier. During this period, survival was similar across all larval cohorts – larval cohort D no longer had higher mortality than the others (Fig. 7A). All cohorts had grown since the last monitoring, but at a lower rate than in previous periods. Juveniles from larval cohort D had caught up in size to the other cohorts, but those from cohort C still lagged behind (Figs. 7B,

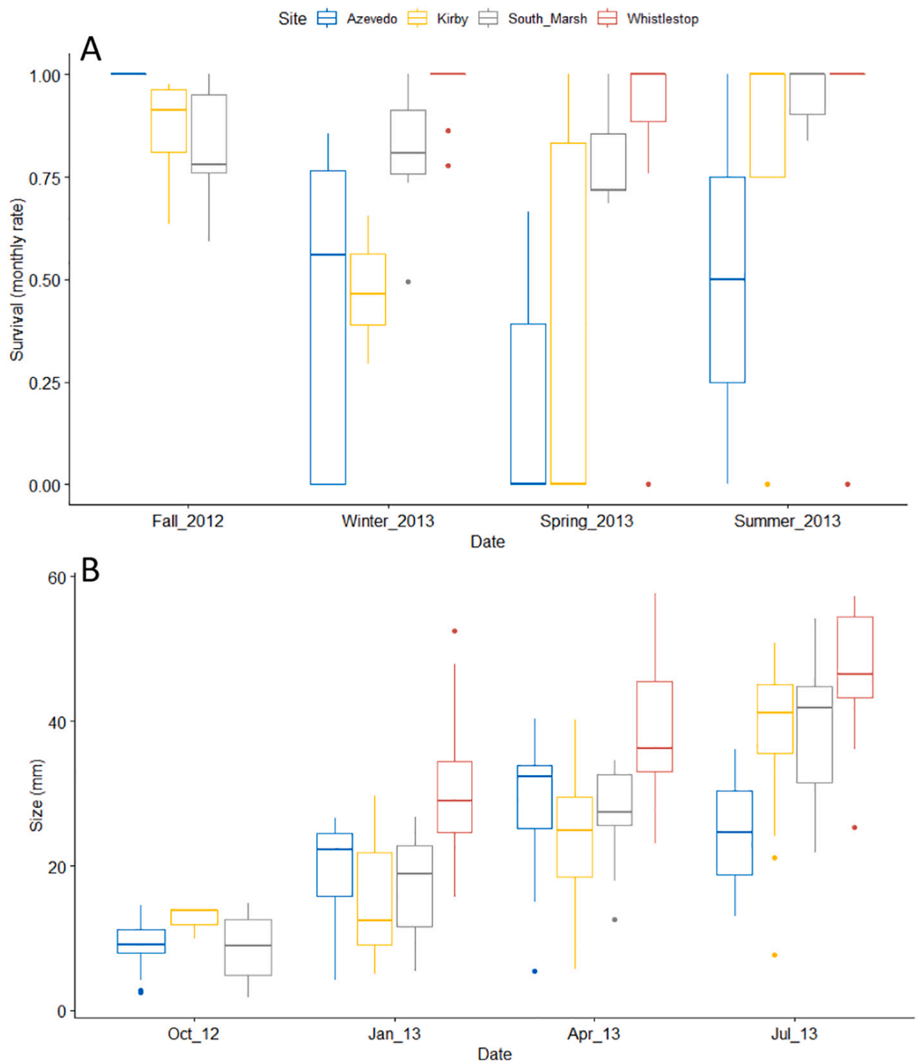

Fig. 5. Short-term demography of a wild recruitment cohort settled on tiles in July–August 2012. A: Survival (proportion of oysters per tile that survived each quarter expressed as a monthly rate). B: Average size (per tile) over time by site.

S7–S8). Overall survival across the whole period was not significantly different for larval cohorts A-C, but was lower for larval cohort D (Fig. S7), as a result of the high mortality in early periods.

Performance of outplanted oysters was generally similar across the three nearby sites. Survival was not different among sites in the first monitoring period and somewhat lower at the rocky South Marsh site in the second monitoring period. However, in the final monitoring period, survival was significantly lower at the muddy South Marsh site than the

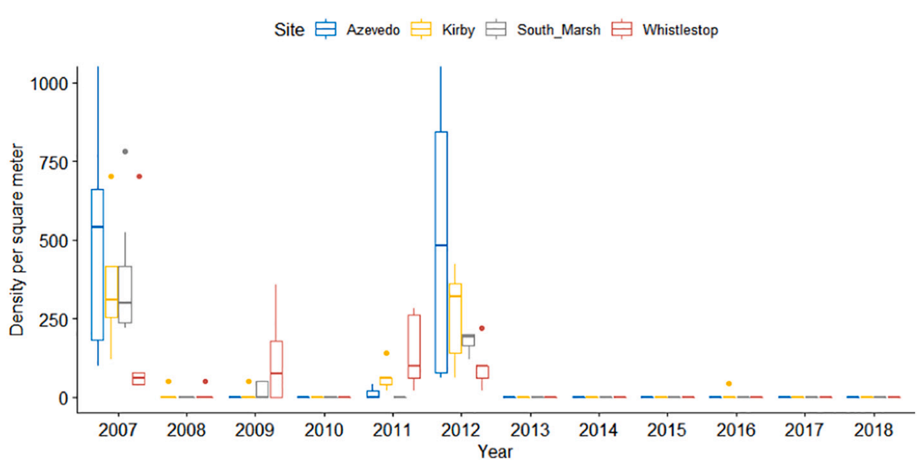

Fig. 6. Long-term recruitment patterns at four focal sites. Density of recruits per m^2 is shown over time. Virtually no recruitment has occurred in the estuary since 2012.

Table 1
Summary of outplanted oyster survival and growth across all sites and stakes. Results are presented separately for juveniles from larval cohorts A–C (87–122 days old when outplanted) vs. larval cohort D (30 days old). Number of oysters is the sum across all stakes for four monitoring dates: the date of deployment (week 0) and three times after that (6, 32, and 72 weeks after deployment). Survival rate was calculated as the proportion that survived between each set of consecutive monitoring dates, as well as across the whole period. The rate was standardized to a monthly (30 d) survival rate to allow for comparison across periods of different length. Size is the average (in mm) across all stakes per larval cohort and period. Note that the numbers in this master summary differ somewhat from those in figures, where stake was used as replicate.

	Number of oysters				Survival rate (per month)				Size (mm)			
weeks since outplant	*0*	*6*	*32*	*72*	*first 6*	*6 to 32*	*32 to 72*	*entire 72*	*0*	*6*	*32*	*72*
Larval cohorts A-C	2326	1995	1601	782	0.90	0.97	0.93	0.94	13.8	20.2	38.0	47.2
Larval cohorts D	16791	477	342	164	0.08	0.95	0.92	0.76	1.2	7.9	27.7	46.6

other two sites (Fig. S9A). At this site, the mortality of oysters was largely due to the clam shells they were on being partly broken or entirely removed from the stakes. In the past, camera trapping has revealed both raccoons and sea otters interacting with our oyster restoration projects. In this case, the broken/missing clam shells almost entirely occurred in stakes facing a deeper channel (on the seaward not landward side), so we infer that damage was caused by foraging sea otters (Fig. S10). Size of outplants did not differ significantly among sites at any monitoring date (Fig. S9B).

Qualitative assessments revealed that oysters comprised the dominant cover on the clam shells at the first two monitoring dates after outplanting. At the third date, a quantitative survey confirmed this was the case; in March 2020, percent cover on the clam shells was 32 ± 28% for oysters vs. 4.2 ± 3.8% for all other sessile species

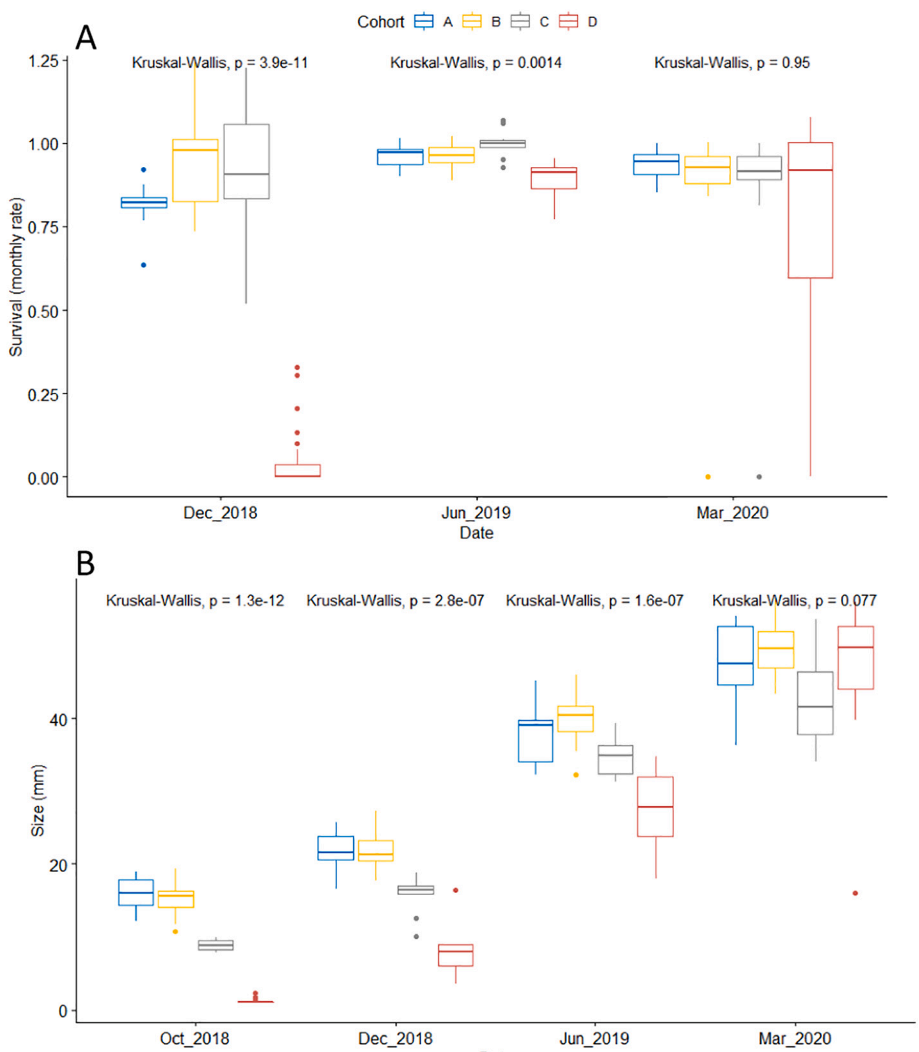

Fig. 7. Success of outplanted oysters. A: Survival between monitoring dates expressed as monthly rate. B: Size of oysters at outplant and at subsequent monitoring dates. All calculations used stake as replicate. Number of stakes per larval cohort was initially 9, 19, 10 and 29 for cohorts A–D respectively and this is what was used for initial calculations. By March 2020, various stakes had no live oysters, especially for cohort D that had suffered high mortality, so number of stakes per larval cohort was 9, 18, 9 and 12, respectively.

combined (mostly non-native bryozoans and sponges).

4. Discussion

4.1. Conceptual framework for pursuing conservation aquaculture

Aquaculture is one of many tools in the conservation practitioner's toolbox. To guide decision-making for when this tool may be appropriate, we developed a flowchart that illustrates a pathway for determining whether aquaculture may be an effective part of conservation efforts (Fig. 1). This model cannot do justice to the nuances in such decision-making, nor the variations among focal species, but serves to highlight, in a simplified manner, the logical flow for the process. Below, we briefly discuss each step in the process, and then use Olympia oysters at Elkhorn Slough as a case study for application of this approach.

4.1.1. Restoration need

The first step is to determine whether restoration efforts are needed at all, by comparing current population distributions to intact reference sites or to past reference conditions, over whatever temporal and spatial scale is considered appropriate by the stakeholders (Gann et al., 2019). For oysters at Elkhorn Slough, there are no nearby intact reference sites. Oysters were present in Native American middens at many sites around the estuary for seven thousand years (Caffrey et al., 2002). While around 80,000 native oysters were harvested from the estuary in just a few days in the 1920s, by 2007 the entire estuary's population was estimated at 5000 individuals (Wasson, 2010), and it has declined since then to less than 1000. Our long-term monitoring data presented here show a dramatic decline in densities. There is thus clearly a need for restoration to increase distribution and abundance, and to prevent local extinction, as happened in the nearest population to the south, Morro Bay (Polson and Zacherl, 2009). The closest populations of Olympia oysters to Elkhorn Slough are approximately 150 km along the coast to the north (San Francisco Bay) and 450 km to the south (Carpinteria Slough). The estuary's isolation makes it particularly vulnerable to local extinction, and preventing this is critical not only for the Elkhorn Slough ecosystem, but for some coast-wide population connectivity to remain.

4.1.2. Suitability of environmental conditions

If the appropriate habitat or environmental conditions that allow the focal species to survive are absent or greatly diminished, then restoration efforts should probably focus there. For instance, if water quality is not suitable, there is no use in pursuing aquaculture or other efforts until it is improved. For oysters in the Elkhorn Slough area, the extent of appropriate habitat has been dramatically decreased due to diking and tidal restriction (Wasson, 2010). Some efforts are underway to restore diked habitats, but progress is slow. The demographic data presented here reveals issues with mortality and growth at two tidally restricted sites that had impaired water quality. However, there are hundreds of hectares of appropriate habitat in the undiked portions of the estuary, and we documented high survival and growth at sites with natural tidal exchange. Some of these are too muddy to allow for natural, low profile biogenic oyster beds, but there are extensive areas with rip rap that host ample habitat for oysters, yet are mostly bare. Thus, providing more habitat alone is unlikely to restore the population.

4.1.3. Post-recruitment challenges

If there is higher mortality following recruitment than typical for stable populations, then it is unwise to consider conservation aquaculture until the factors causing the mortality have been addressed. At Elkhorn Slough, we demonstrated in this study that survivorship and growth of Olympia oysters post-recruitment appear to be robust. The relatively gradual decline of adult densities in permanent transects also supported this finding.

4.1.4. Recruitment limitation

After considering all of the above, the final step (Fig. 1) is to consider the role of reproduction in limiting the population. If recruitment is significantly lower than typical in stable populations, there is a potential role for aquaculture to assist in overcoming this limitation (Brumbaugh and Coen, 2009). For Elkhorn Slough oysters, our current investigation demonstrated that recruitment is a critical factor limiting the population. Substantial recruitment only occurred in two of the twelve years of monitoring. Moreover, in the most recent seven consecutive years, there was virtually no recruitment anywhere in the estuary: one single juvenile was observed on hundreds of tiles that were deployed. In an earlier study comparing recruitment at 37 sites across the range of the Olympia oyster, the longest documented period with zero or near zero recruitment at any site was three years (Wasson et al., 2016). So this seven-year absence of recruitment is unusual across the range of the species. The lack of recruitment at Elkhorn Slough is clearly visible in demography of adult populations, with increase in average size and decrease in density of live oysters in the seven years since recruitment occurred.

4.2. Restoration success with aquaculture

Our pilot test of conservation aquaculture was promising. While our project was designed to be a small-scale proof-of-concept, it is likely to have a very strong local effect. We estimate that the estuary-wide population of Olympia oysters consisted of less than 1000 individuals in the year before our project; we have doubled this number, with about 1000 hatchery-raised adults now living in Elkhorn Slough and augmenting the previous population. In the wetlands of the Elkhorn Slough Reserve where we conducted the project, we estimate there were 100 adults in the year prior to the project; we have increased this by an order of magnitude. This initial proof-of-concept can now be scaled up. The causes of recruitment failure in the estuary are unknown, but an earlier comparative study revealed that strong marine influence (likely leading to difficulties with larval retention) and limited adult populations were key correlates of recruitment failure (Wasson et al., 2016). Increasing the adult population size at Elkhorn Slough, ideally from thousands to millions of oysters, should decrease the likelihood of recruitment failure. We thus intend to rely on conservation aquaculture to increase the estuary's oyster population size to achieve our goal for the estuary: a self-sustaining population, with regular natural recruitment of new cohorts. Since retention of larvae is critical for isolated populations, the adult population should be centered away from strong marine influence, in the portions of the estuary with longer residence time and slower currents (Peteiro and Shanks, 2015; Pritchard et al., 2016).

Our pilot study suggests hatchery-raised juveniles are likely to thrive in the estuary. We documented higher survival and growth rates for the hatchery-raised outplants than for a wild cohort that had settled in 2013. Since these cohorts were monitored in different years and by different methods, we cannot conclude that hatchery-raised juveniles generally perform better than wild ones in this estuary, but we can rule out any major disadvantage to hatchery-raised oysters. All three nearby sites had good performance of outplants, though one had breakage of the clam shell clusters in the final period, likely due to sea otter predation. Larvae released at different times in the hatchery had differential success: the most recent, youngest cohort suffered high initial mortality, but the survivors had similar size and survival rates by the end of the monitoring period. The next youngest larval cohort had consistently lower sizes throughout the study; this variability highlights the value of using multiple larval release cohorts in outplanting.

The survival rates we documented for hatchery-raised outplants were at the higher end of what has been reported for Olympia oysters from field experiments. A recent study found high spatial variation in survivorship of hatchery-raised outplants in two Washington estuaries, ranging from < 0.2 to > 0.9 over 3.5 months (Lowe et al., 2019). Sites near the mouth of estuaries and in eelgrass beds had lower survivorship than ones farther inside the estuary or in mudflats. Valdez et al. (2017) also documented extremely low survivorship of hatchery-raised Olympia oysters in eelgrass beds, of about 0.01 over 9 months, with loss attributed largely to predation. In an experiment in Humboldt Bay, survivorship of hatchery-raised outplants that were caged was much higher (around 0.47) than uncaged (around 0.18) with mortality attributed to a non-native oyster drill (Koeppel, 2011). These data come from experimental studies conducted as academic research; there are few published data available on survivorship of Olympia oyster outplants in restoration projects. One restoration project in Fidalgo Bay, Washington, reported the ratio of live to dead oysters on cultch as a proxy for survivorship, and documented it to be very high (0.74–0.97; Dinnel et al., 2009). The paucity of published data on restoration aquaculture survivorship highlights the need for future studies ideally including coordinated restoration experiments with Olympia oysters.

The growth rates we documented at Elkhorn Slough were among the highest documented for hatchery-raised, outplanted Olympia oysters. Lowe et al. (2019) reported a range of growth rates from 0.3–3.1 mm/month across sites during the summer season, when warm temperatures and high phytoplankton concentrations should result in maximum growth; the outplants at Elkhorn Slough grew about 3 mm/month in the cooler winter-spring months (October–June). In a restoration project in Netarts Bay, Oregon, growth appeared slower than at Elkhorn, with hatchery-raised Olympia oysters reaching 30–40 mm after 1–2 years (Archer, 2008) rather than in < 1 year at Elkhorn Slough. It is possible that warmer summer temperatures at lower latitudes in this California project led to higher growth rates, or that these were due to the relatively high chlorophyll concentrations in this eutrophic estuary (Hughes et al., 2011).

While we did not directly measure reproduction to avoid damage to individuals in the only new cohort of native oysters in the estuary since 2012, a recent study of Olympia oysters in San Francisco Bay (Moore et al., 2016) found mature sperm and oocytes were present in less than 3 months following larval settlement, at sizes below 30 mm. Thus, our hatchery-raised cohort reached potential reproductive size within months of outplant.

4.3. Naturalistic restoration design

Commercial oyster aquaculture typically employs off-bottom culture on racks or lines, and aims to grow individual oysters suitable to be eaten off the half shell (Conte and Moore, 2001; Forrest et al., 2009). However, our work was conducted on a nature reserve that follows a restoration philosophy of attempting to return habitats and biodiversity to conditions representative of what was present before the dramatic anthropogenic alterations of the past 150 years. Since Olympia oysters naturally form small biogenic clusters on mudflats, our goal was to mimic this habitat structure. We accomplished this with clusters of native clam shells providing settlement substrate to the hatchery-raised oysters. We did this at a very small scale, with only about 70 clusters, but this approach could easily be scaled up by orders of magnitude. Our project thus served as an example of the marriage of some elements from commercial aquaculture with others from classic ecological restoration aiming for historic conditions.

There are other naturalistic approaches to oyster restoration. In estuaries where the slope is gentle and mud is firm, loose shells containing hatchery-raised oysters can be spread on the mudflat (Peter-Contesse and Peabody, 2005; Kennedy et al., 2011; Barber et al., 2015; Dinnel, 2016). Where mud is deeper, shell can also be bagged, and bags can be stacked. Another alternative is to culture oysters under ideal conditions of temperature and food for reproduction, and to simply allow the larvae that are generated to colonize the estuary. In Humboldt Bay, adult oysters were simply held in a tank preventing predator access but allowing flushing with local water, and this supplied larvae and subsequently adults to an area that previously had very few oysters (David Couch, pers. com.). In many estuaries where Olympia oysters occur, there is ample bare space on substrates at the appropriate elevation, so deployment of new hard substrates may not be necessary to increase estuary-wide population size. Simply providing more larvae may elegantly increase population sizes, in systems where recruitment is limited and larval and juvenile survival is high, especially in small estuaries where such supplementation would be most likely to have a measurable impact.

Another consideration in deploying hard substrate in Elkhorn Slough, as in other West Coast estuaries, is the prevalence of non-native species on these substrates (Wasson et al., 2001). At the nature reserve where we conducted this project, the goal is to restore native-dominated habitats. An earlier study found that native oyster recruitment and growth is higher near MLLW, but the ratio of native oysters/non-native species is greater at higher elevations (Zabin et al., 2016). Informed by this work, we chose a relatively high elevation near the upper range of this oyster for outplanting our hatchery-raised juveniles. It is possible that the high mortality of the tiny size class of outplants was intensified by longer exposure to warm air temperatures and desiccation stress at this elevation. However, survivorship and growth of larger outplants was excellent at this elevation. Oysters comprise virtually the only cover on the substrates after 8 months (Fig. 3F), so we accomplished the goal of native dominance. Optimal tidal elevations for oyster restoration likely differ by region, affected by differing thermal stress from exposure to excessively low or high air temperatures during low tide and by distribution of competitors and predators. Selecting the appropriate tidal elevation to achieve restoration goals, which may include oyster attributes and cover by non-native species, is an important consideration for restoration planning.

5. Conclusions

We have developed a conceptual framework for determining the need for, and likely success of, conservation aquaculture. The investigations we undertook, that demonstrated that recruitment is limiting but survivorship and growth of juveniles and adults are robust, are transferable to any other system and can help guide decisions about whether restoration aquaculture is the right conservation tool. Next, small-scale restoration projects incorporating aquaculture can be used as a proof-of-concept before investing more heavily. Our initial success has served this purpose and will help to garner funding and support for larger future projects aimed at generating a hatchery-grown adult population of sufficient size to become self-sustaining. In the future, additional data on spatial variation in Olympia oyster survival and growth could be combined with the conceptual framework we developed to create a geospatial tool for identifying priority locations where conservation aquaculture is likely to be successful, or particularly valuable in providing ecosystem services, such as has been developed for *Crassostrea* on the US Atlantic coast (Theuerkauf et al., 2019).

We also demonstrated that a naturalistic restoration design mimicking typical Olympia oyster clusters could be coupled with aquaculture. Large-scale commercial oyster aquaculture in California and increasingly elsewhere involves off-bottom culturing, and raising single individuals rather than clusters. But where natural, biogenic structured habitat is important due to the restoration philosophy of regional stakeholders and/or the key ecosystem services such habitat provides, it is possible to develop substrates for hatchery-raised juveniles that mimic historic conditions. Likewise, where dominance by native species is important, restoration can be conducted under conditions where the target species are favored over non-natives, as we achieved by locating our restoration substrates relatively high in the intertidal zone, where oysters survive much better than non-native fouling species.

We have provided a case study integrating techniques from commercial aquaculture with principles of traditional restoration ecology. There are many opportunities for expansion of science-based restoration aquaculture, with clear conservation objectives, data informing strategic planning and used to evaluate success, and with publications reporting on the findings.

CRediT authorship contribution statement

K Wasson: Conceptualization, Investigation, Formal analysis, Writing – original draft; **D Gossard:** Methodology, Investigation, Formal analysis (aquaculture); **L Gardner**, **P Hain**, **J Bible:** Methodology (aquaculture); **C Zabin**, **S Fork**, **A Deck:** Investigation (field monitoring); **A Ridlon:** Writing – original draft; **B. Hughes:** Conceptualization, Funding acquisition; **all authors:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding for the monitoring of recruitment, demography, and outplants was provided by a grant to the Elkhorn Slough National Estuarine Research Reserve (ESNERR) by NOAA's Office for Coastal Management. California Department of Fish and Wildlife provided facilities and other key support at ESNERR. The NERR Science Collaborative supported the collection of data on juvenile survival and growth on tiles in 2012–2013, and we are grateful to project leaders M. Ferner, E. Grosholz, A. Chang, and M. Latta for allowing us to use data from this collaboration. Hatchery funding was provided by the Anthropocene Institute and California Sea Grant. M. Graham, S. Hamilton and J. Harvey provided critical resources to support the work at Moss Landing Marine Laboratories. M. Elliot, J. Inovejas, M. Rintoul, and J. Newman were instrumental in the aquaculture work. B. Lyon developed the calculation tool for converting survival to monthly rates. R. Jeppesen supplied the chlorophyll data. D. Brumbaugh, P. Dayton, J. Ruesink, and two anonymous reviewers provided suggestions that improved the clarity and conservation framework of this paper. Too many ESNERR staff and volunteers to name assisted with the oyster monitoring and outplant, but we are grateful to them all.

Supplementary Information

Supplementary information to this article can be found online at https://doi.org/10.1016/j.biocon.2020.108745.

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A scientific framework for conservation aquaculture: a case study of oyster restoration in central California

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SUPPLEMENTAL INFORMATION

Juvenile survival and growth from natural recruitment

We monitored growth and survival of naturally recruited oysters at nine sites in Elkhorn Slough from October 2012 through November 2013. The nine sites included the four focal sites (South Marsh, Whistlestop, Azevedo, and Kirby), as well as five additional sites that had few to no adult oysters present as determined by site surveys (West Bennett Slough just north of Jetty Road bridge; Hudson Landing at Elkhorn Road pullout, Moss Landing Harbor just north of Moss Landing Road, North Marsh near culverts; Vierra near the old power plant outflow in the lower main channel). At these five sites and one of the focal sites (Whistlestop), where there was little to no natural recruitment, tiles bearing recruits were moved from Kirby and South Marsh in December 2012, so survival and growth could be tracked. In all cases, recruits had settled in July-August 2012, so the same cohort was being tracked at all sites. At South Marsh, Kirby and Moss Landing Harbor, we monitored at two tidal elevations, MLLW and approximately 0.3 m below MLLW.

Survival was high at most sites during Fall 2012 (October 2012-January 2013), then decreased in Winter 2013 (January-April 2013) and Spring 2013 (April-July 2013), particularly at Azevedo, and then again was high during Summer 2013 (July-November 2013) (Fig. S1). During the Winter and Spring periods of higher mortality, survival was greater at MLLW than 0.3 m below MLLW (Fig. S2).

Growth was highest at Whistlestop and lowest at Azevedo, but otherwise fairly consistent among sites, with size one year after recruitment averaging around 40 mm at most sites (Fig. S3). Elevation did not have a strong effect on growth across the three sites where elevations at both MLLW and 0.3 m below MLLW were monitored (Fig. S4).

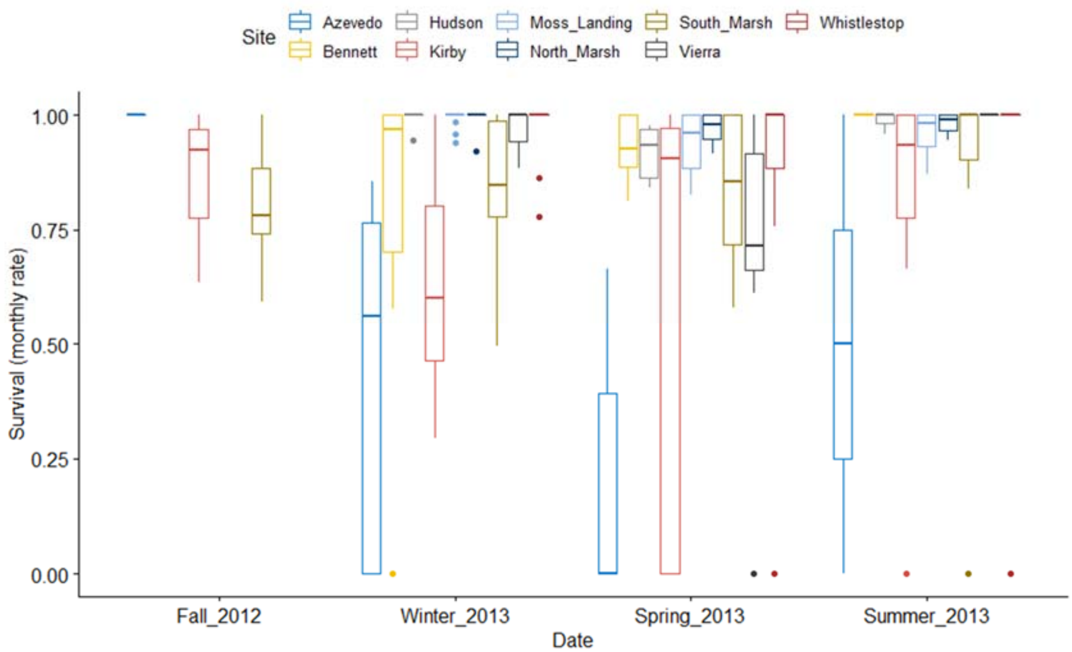


Figure S1: Wild oyster survival rates across nine sites. Oyster numbers were assessed on tiles quarterly at nine Elkhorn Slough sites, at tidal elevations of MLLW and 0.3 below MLLW, tracking a cohort that recruited in July-August 2012. For each date, the survival rate since the last monitoring survey is reported (as a standardized monthly rate).

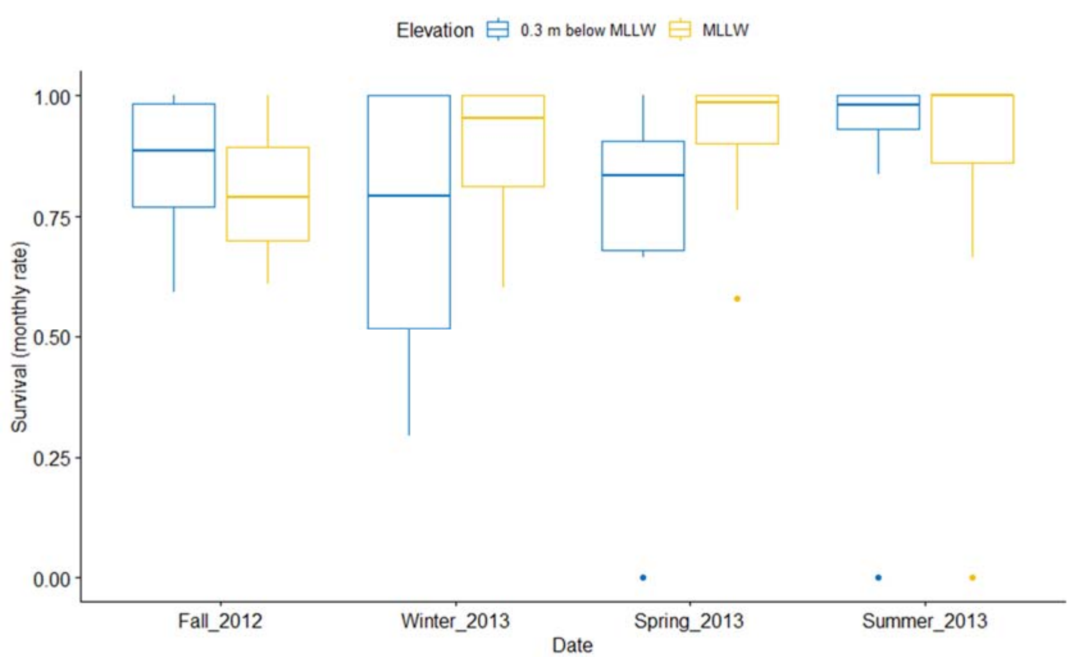


Figure S2: Wild oyster survival rates by elevation. Oyster numbers were assessed on tiles quarterly at two elevations (MLLW and 0.3 m below MLLW) at three Elkhorn Slough sites, tracking a cohort that recruited in July-August 2012.

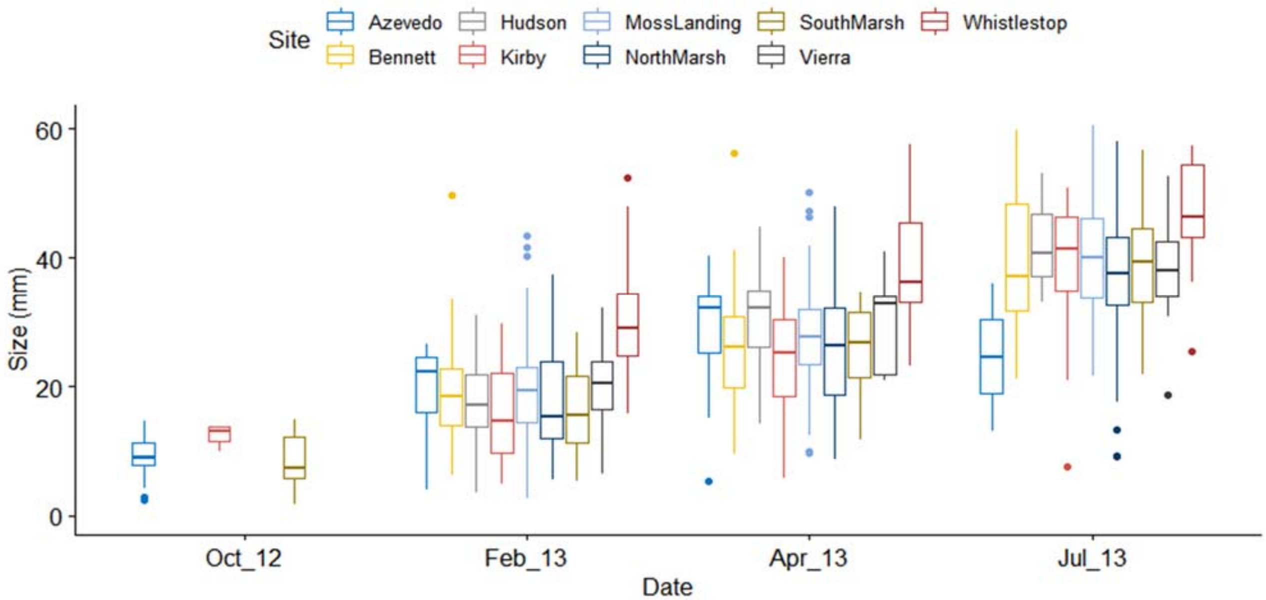


Figure S3: Wild oyster size over time across nine sites. Oyster sizes were measured quarterly at nine Elkhorn Slough sites, at tidal elevations of MLLW and 0.3 m below MLLW, tracking a cohort that recruited in July-August 2012. At most sites, oysters were about 40 mm in size one year after settlement.

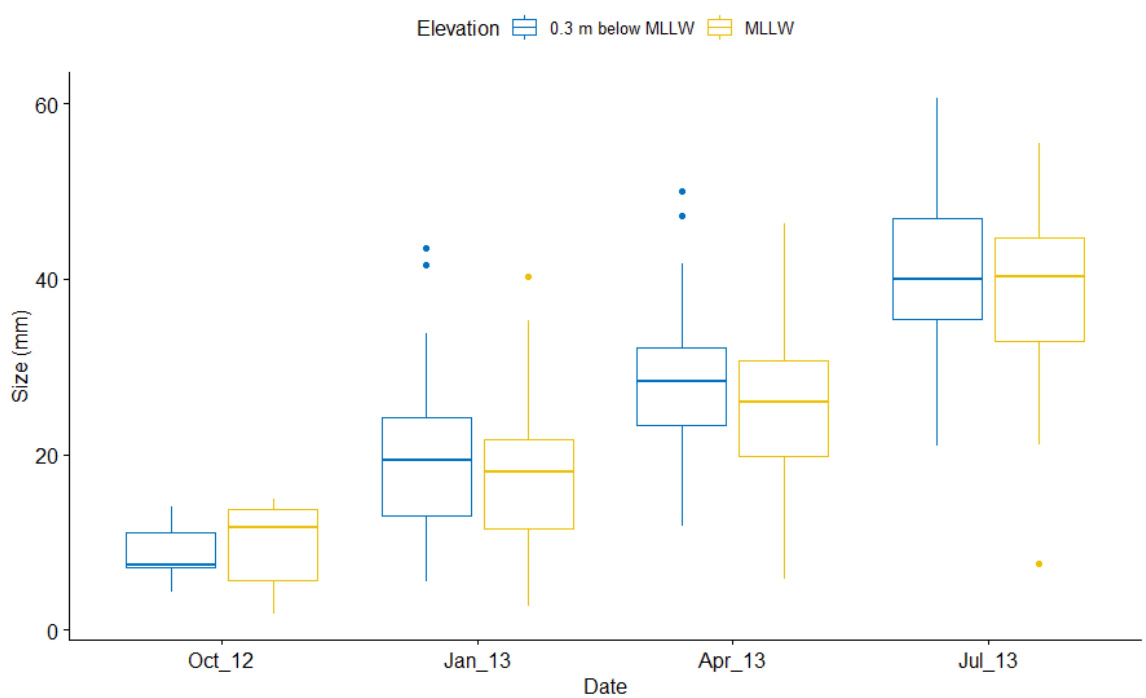


Figure S4: Wild oyster size over time by elevation. At three sites, oysters were tracked at two elevations (MLLW and 0.3 m below MLLW). Sizes did not differ by elevation.

Aquaculture Methodology

Adult *Ostrea lurida* (85 individuals) were collected in May 2018 from two Elkhorn Slough sites near the restoration sites; they were not collected from the restoration sites because so few adults were present there (less than 100 at South Marsh, zero at Whistlestop). One source site (Kirby Park), about 1.7 km north of the restoration area, has the greatest remaining oyster numbers in the estuary. Oysters were gently pried from rip-rap at this site. The other site (North Azevedo channel), about 2.2 km north of the restoration area, was selected because oysters grow on a gravel bar and are thus easily collected as separate individuals.

The oysters were brought to Moss Landing Marine Laboratories and cleaned with a brush and sponge to remove fouling organisms and sediment. They were held initially within a 60 L tank for broodstock conditioning and were fed approximately 300,000 cells/ml of Shellfish Diet 1800 (Reed Mariculture) per day in a static system. Following an 8 hour feeding period the tank was reverted back to 4 L/min flow through systems for water exchange to occur until the next feeding. The tank was cleaned weekly.

TABLE S1: Broodstock fecundity and spawn timing. Approximate quantities of *O. lurida* larvae released within broodstock tanks and associated broodstock origin.*Sperm releases prior to larval releases. Sperm release for Azevedo was not recorded. **Larval release on 7/13/18 experienced 100% mortality. Larval cohort D's temperature stress timing was relative to adding the remaining 30 Kirby Park broodstock to the water bath on 8/24/18.

Release date	Days since initial temperature stress ramping	Larval Cohort	Broodstock origin	Approximate larval quantity	Larvae per captia
22 June 2018	10		Kirby Park	*	
23 June 2018	11	A	Kirby Park	100,000	4,000
8 July 2018	26	B	Kirby Park	200,000	8,000
13 July 2018	31	**	Kirby Park	100,000	4,000
27-30 July 2018	45-48	C	Azevedo	400,000	16,000
26 August 2018	2		Kirby Park	*	
21-24 September 2018	28-31	D	Kirby Park	300,000	8,571

On 12 June 2018, 25 oysters from each collection site were isolated in a 15 L vessel each and placed within an external water bath to control water temperature. Bath temperature was increased from 15 C° to 25 C° (± 2 C°) by 1 C° daily to incrementally increase temperature in the oyster vessels to 21 C°. Feeding regimen was changed to 300,000 cells/ml of mixed live

Chaetocerous calcitrans and CCMP463 *Tisochrysis lutea*, grown on site. Water exchange occurred once per day, prior to feeding, using 5 µm particle size filtered and UV-sterilized water. Vessels were cleaned before each water replacement. On 24 August 2018, the initial 50 broodstock were returned to flow through systems and remaining 35 broodstock (all from Kirby) were placed in vessels in the water bath.

Broodstock sperm releases were observed 2 days and 10 days after initial temperature increase, and pediveliger larval releases occurred between 11 and 48 days after initial temperature increase (Table S1). Larvae were isolated by siphoning culture water through a 100 µm mesh screen into a 10L bucket. After thorough homogenization via mixing, larvae were counted by taking five 1mL samples and multiplying by 10,000 after (rounded to the nearest 100,000 larvae). Larvae were then placed into conical bottom tanks with mild aeration. Larvae were consolidated if releases occurred within a week. The larval feeding regimen consisted of approximately 50,000 cells/ml of live *T. lutea*. Water was fully exchanged twice a week after isolating larvae using a 100 µm mesh screen and cleaning the inside of each tank. After each water exchange, a sample of larvae was examined microscopically for eyespot and foot development on individual pediveligers. Additionally, for larval cohort D, maximum length of pediveligers in these samples taken pre-water exchange were measured using photoimaging software. Marginal elongation of pediveliger mean length was observed with an increase of 1.5µm * day^{-1} (Simple linear regression; $n = 5$; $p = 0.032$).

Upon confirmation of larval eyespot and foot development, strings of clam shells were suspended within larval tanks as a substrate to encourage settlement**.** Water replacements and feeding regimens continued as previously described until no swimming pediveliger larvae remained in the tank. The post-settlement daily feeding regimen included an additional 50,000 cells/ml of *C. calcitrans*. Visual inspection of tank water clarity at the start of the day (prior to feeding) was used as an indicator to determine daily live microalgae feeding regimens. As microalgae were consumed more readily by settled oysters, feeding was increased incrementally by 25,000 cells/ml of each species.

After one month post-settlement, clam shell strings were consolidated and hung within a 450L conical bottom tank. Spat were fed 150,000 cells/ml of each *C. calcitrans* and *T. lutea*. Following an 8 hour static feeding period the tank was placed on a flow-through system permitting 200% water exchange daily.

Spat growth was monitored using a subset of spat maximum shell length collected 1-2 times per week on a variable dissecting microscope and Image-pro Plus 7.0 software (Fig. S5; Table S2). Maximum length growth rates for spat varied between 0.167mm * day^{-1} and 0.268mm * day^{-1} and had a mean growth rate of 0.197mm * day^{-1} ± 0.011mm * day^{-1}.

Survival was tracked for a subset of newly settled juveniles from larval cohorts A, B and D (Fig. S6). Survival was high for A and B, and low for D, but this may simply be the result of different observation periods: A and B were tracked starting 50-60 days post-release, presumably after initial mortality had occurred, while D was tracked starting about 15 days post-release, likely during the period of highest mortality.

Our experience with raising Olympia oysters in a hatchery provided some lessons learned that can inform future work, and some suggestions for additional research. The most resource consuming part of any oyster hatchery is cultivating microalgae and this was certainly the case for this study and a major limitation on production effort. In order to minimize the limitations that live microalgae culture can have on Olympia oyster restoration aquaculture, future efforts need to address a number of aspects that could improve the species aquaculture production efficiencies. These include developing a spawning induction protocol that more rapidly and reliably predicts larvae release. This would reduce the need to produce microalgae for long periods before and after expected larval releases. We demonstrated some success with this by utilizing a cold water (9°C - 13°C) flow-through tank to store “back-up” oysters in temporary reproductive stasis (Ryan Crim, pers. com.) Subsequent temperature ramping with these oysters produced rapid spawns and larvae releases. Secondly, rigorous assessment of survival rates of juveniles out-planted to the field at different sizes would aid in the identification of the minimal practical outplanting size for acceptable survival rates, thus reducing microalgae culture effort. Finally, investigating when or if juveniles can be transitioned to commercially available dead algal cell concentrations would also be advantageous in that it would reduce the costs and resources required with live microalgae culture and offer another inexpensive redundancy against microalgae culture crashes. Research on the above mentioned aspects would help to generally increase the efficiency of Oylmpia oyster hatchery production and reduce the cost of restoration significantly.

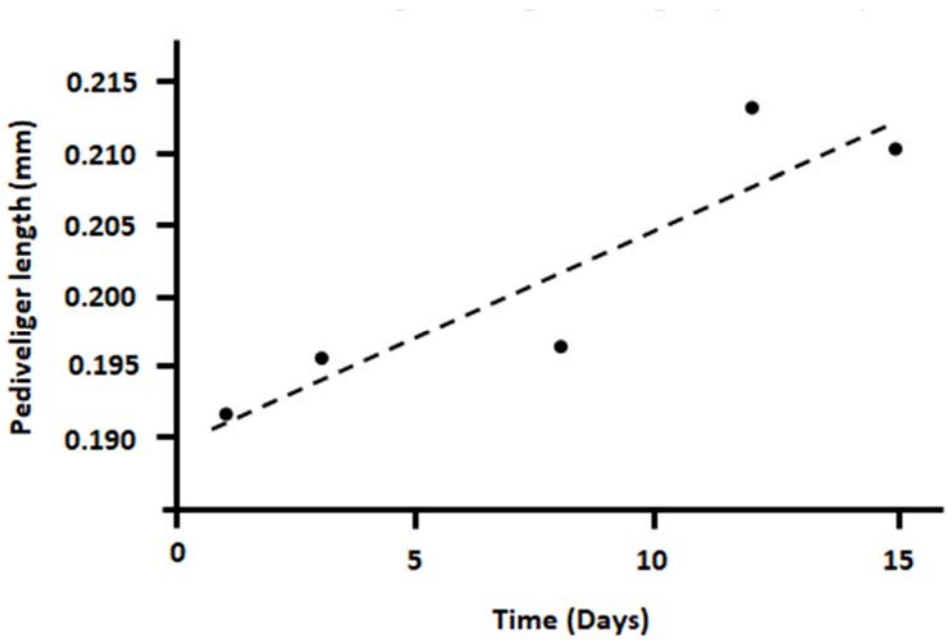


Figure S5: Pediveliger Growth. Growth rates measured for subsampled *O. lurida* pediveligers from Larval Cohort D showed a marginal increase in size (1.5µm per day) over 15 days post-larval release.

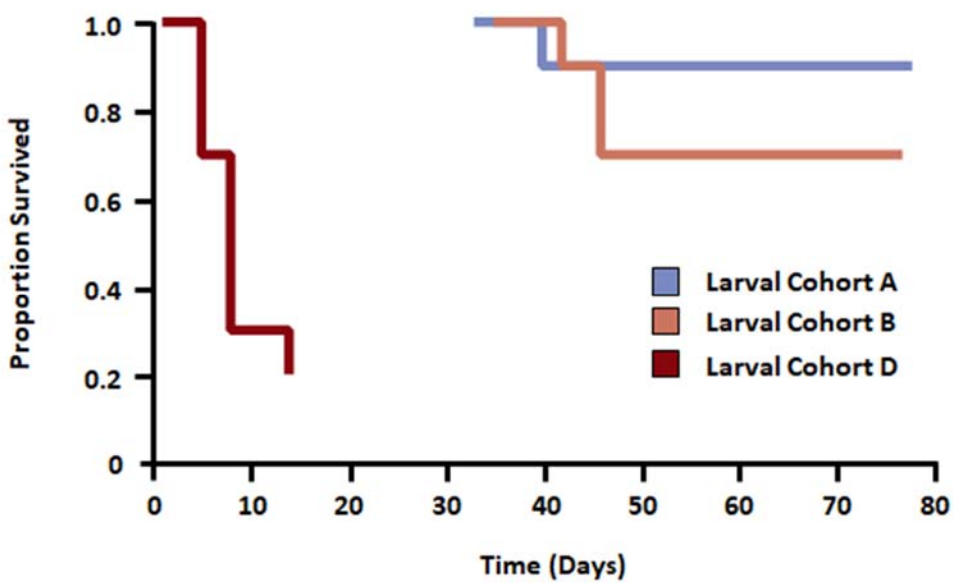


Figure S6. Post-settlement survival in the hatchery. Survival of oysters measured at different times for Larval Cohort A (90% after 45 days), B (70% after 42 days), and C (20% after 13 days) (n = 10 for each larval cohort) measured at different starting times post-settlement.

Table S2. Post-settlement growth in the hatchery. Growth rates were calculated using a simple linear regression for change in maximum length over time for 15 individuals (measured for up to 45 days) within the hatchery setting. These growth rates were obtained from settlers on two shells from each of Larval Cohorts A and B, and were calculated between 62-107 days post-release for Cohort A and 50-92 days post-release for Cohort B. n=number of days.

Larval Cohort	Shell	Oyster Number	Growth Rate ($\frac{mm}{day}$)	n (# of days)	p-value
A	A1	1	0.259	16	<0.0001
A	A1	2	0.179	16	<0.0001
A	A1	3	0.182	16	<0.0001
A	A1	4	0.179	16	<0.0001
A	A1	5	0.229	16	<0.0001
A	A2	1	0.159	16	<0.0001
A	A2	2	0.238	16	<0.0001
A	A2	3	0.239	16	<0.0001
A	A2	4	0.206	16	<0.0001
B	B1	2	0.167	15	<0.0001
B	B1	3	0.186	15	<0.0001
B	B1	4	0.076	15	<0.0001
B	B2	1	0.204	15	<0.0001
B	B2	2	0.192	15	<0.0001
B	B2	4	0.215	15	<0.0001
B	B2	5	0.268	15	<0.0001

Monitoring of outplanted oysters

Effects of larval release cohort

We examined how different larval cohorts fared in terms of survival and growth. Across the entire monitoring period (October 2018-March 2020), survival rate was similar across larval cohorts A-C, but significantly lower for larval cohort D (Fig. S7A).

The average size of juveniles after 72 weeks was significantly lower for larval cohort C, but similar for the other three cohorts (Fig. S7B). The growth rate for cohort D was faster than for the other cohorts, allowing these initially smallest juveniles to catch up in size by the end of the monitoring period (Fig. S8).

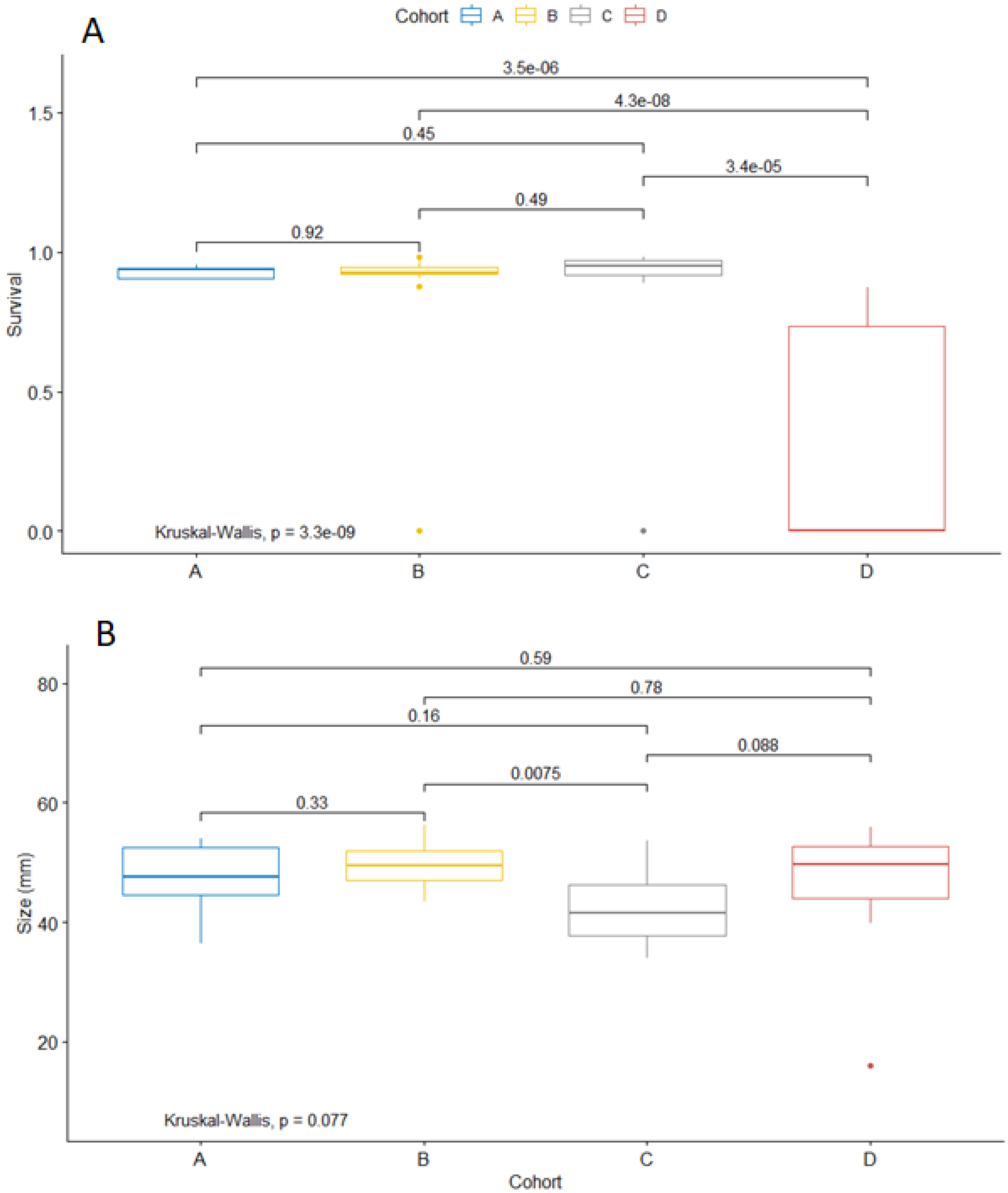


Figure S7. Survival and size of outplanted oysters. A: Survival (expressed as monthly rate) from October 2018 to March 2020. **B**: size in March 2020,72 weeks after outplant. The stake is the replicate for these analyses. Number of stakes per larval cohort was initially 9, 19, 10, and 29 for cohorts A-D respectively, and this is what was used for survival calculations. By March 2020, various stakes had no live oysters, especially for cohort D that had suffered high mortality, so number of stakes per larval cohort was 9, 18, 9, and 12, respectively.

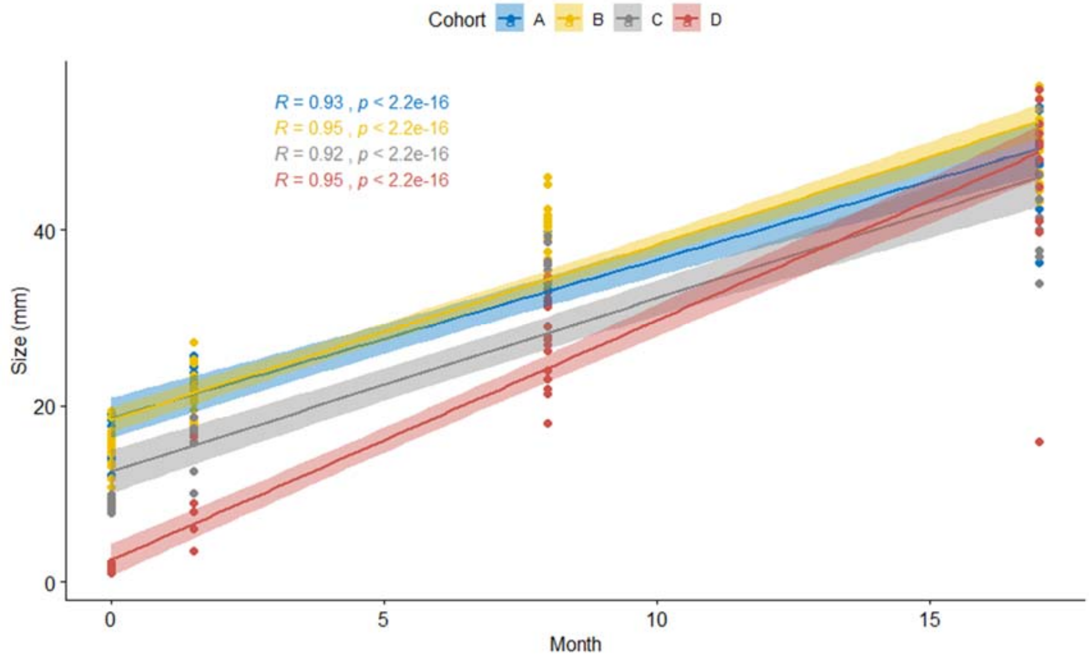
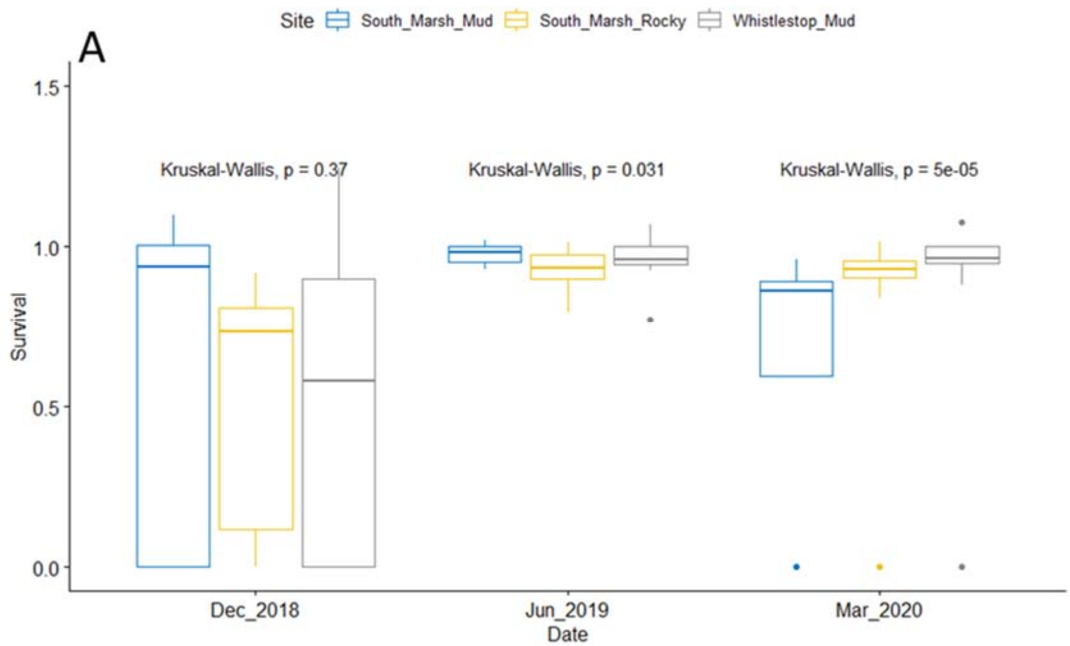


Figure S8. Oyster size over time. Maximum size of oysters per clam shell substrate averaged by stake is shown for the four monitoring dates.

Effects of outplant site

Overall, the three nearby outplant sites had similar performance of outplanted juveniles. In the first period after outplant, with the highest mortality of all periods, similar survival rates were observed at the three sites. In the second period, survival was significantly (but only slightly) lower at the rocky South Marsh site. In the final period, survival was significantly lower at the muddy South Marsh site (Fig. S9A). Sizes were similar across sites at all time periods (Fig. S9B).

At the muddy South Marsh site, evidence suggested that sea otters had damaged some of the stakes. Sea otters forage regularly on the Elkhorn Slough Reserve, but generally in subtidal areas. The muddy South Marsh site is closer to a subtidal channel than the other two sites. At this site, on the March 2020 sampling date, there were broken clam shells on various stakes, and clam shells were entirely missing from some stakes. Raccoons also can cause such damage (we observed them interacting with clam shell reefs in camera trapping during our 2012 restoration efforts). However, raccoons approach from land, while sea otters approach from the subtidal. In this case, the pattern of stake damage was consistent with sea otter predation, as most damaged stakes were on the seaward side closest to the subtidal channel (Fig. S10).



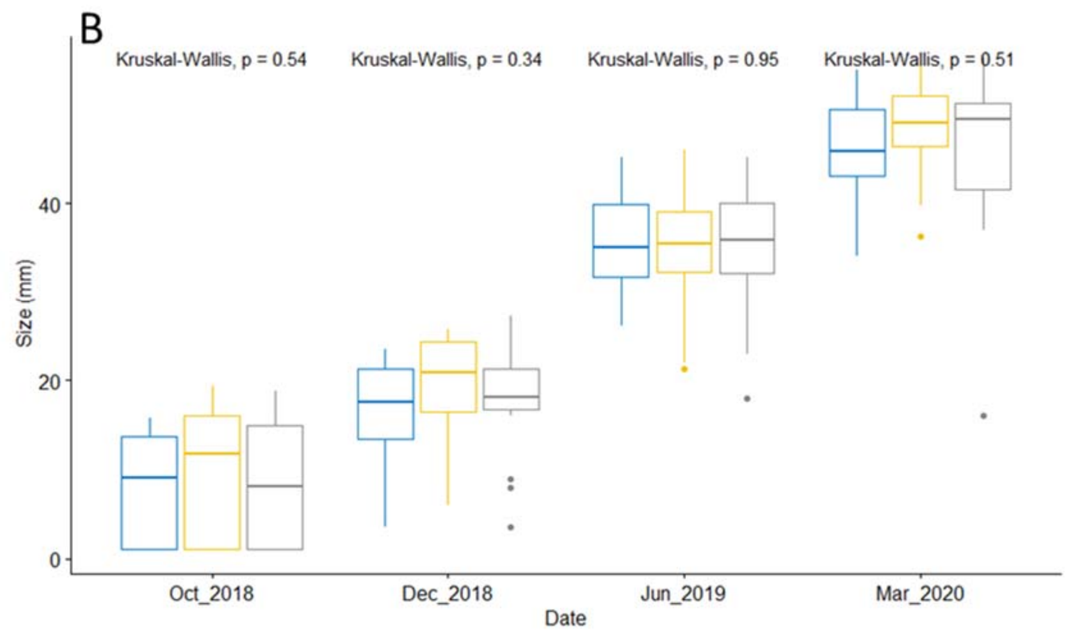


Figure S9. Comparison of outplant sites. **A:** Survival (expressed as monthly rate) shown for the period preceding each monitoring date. **B**: Size at each monitoring date. All analyses are conducted with stake as replicate. Number of stakes per site was initially 20, 23, and 24, South Marsh Mud, South Marsh Rocky, and Whistlestop Mud, respectively. By March 2020, various stakes had no live oysters, so number of stakes for these sites had decreased to 12, 19, and 17, respectively.

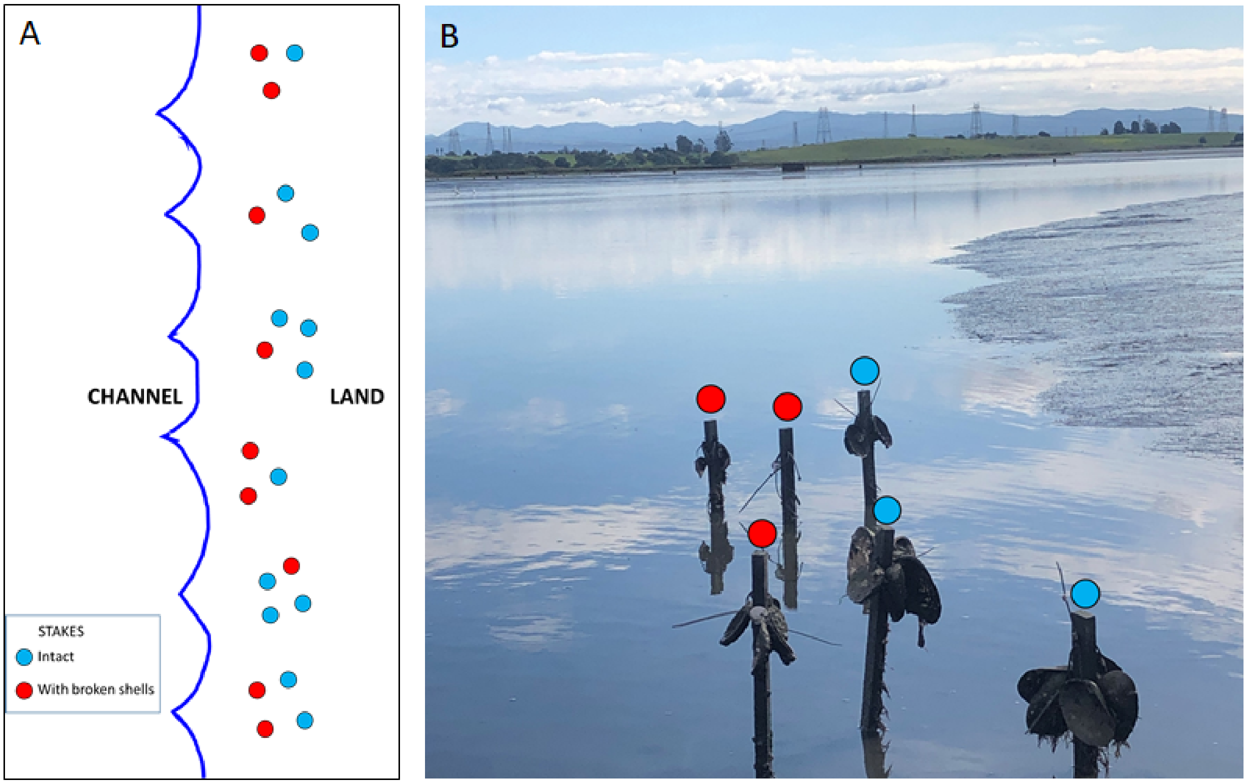


Figure S10. Shell damage attributed to sea otters. A: Schematic of South Marsh mud site, showing stakes with shell damage in red, and those without damage in blue. **B**: Photo of the six stakes at the top of the schematic in A, coded similarly to show damage vs. intact status. The farthest right of the damaged (red) stakes has no clam shells remaining at all.