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RESEARCH ARTICLE

Experimental test of oyster restoration within eelgrass

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Abstract

1. Both seagrasses and oysters are foundation species valued for their wide range of ecosystem services, but their space competition sets a constraint on joint benefits. A reserve for native Olympia oysters (*Ostrea lurida*) was established in lower Hood Canal (Washington State, USA) more than a century ago but is now devoid of that species and dominated by native eelgrass (*Zostera marina*). This situation sets up a conservation conflict because management activities for one species are at odds with the protection of another.
2. In experimental enhancement plots, Olympia oysters were outplanted at low density, which successfully maintained eelgrass density and production. One method was used in 2013 (seeded cultch, 8% cover) and two additional methods in 2015 (anchored cultch and single oysters, the latter at 4% cover).
3. For all outplant methods, oysters experienced 99% annual mortality, associated with the attraction of non-native and native predators. Shell cover remained steady for a year and then declined rapidly, as shell accumulation did not exceed sedimentation rates.
4. Eelgrass *per se* does not preclude Olympia oysters, given that the two species were observed to co-occur at a coastal estuarine site (Willapa Bay, Washington). However, even when socio-political constraints on restoration activities were overcome, ecological constraints remained from predation. Competition between these two protected species was avoided, but it may be the case that top-down control on oysters was particularly acute owing to low oyster density and/or the environmental conditions of eelgrass beds.

KEYWORDS

alien species, ecosystem approach, intertidal, invertebrates, macrophytes, restoration, seagrass meadow, sedimentation

1 | INTRODUCTION

A major challenge in conservation ecology arises when management activities for one species are at odds with the protection of another. When protected species occupy two trophic levels, then interventions that aid a higher trophic level may come at a cost to the adjacent lower trophic level. Such is the case for anadromous salmon, of which several populations are listed as threatened or endangered. Birds and marine mammals consuming these salmon have their own protection, making it challenging to work out ways to maintain both trophic levels (Keefer, Stansell, & Tackley, 2012). A similar conundrum arises when non-native plants provide habitat for endangered birds, leading to biodiversity risks from rapid eradication of invasives (Lampert, Hastings, Grosholz, Jardine, & Sanchirico, 2014; Zavaleta, Hobbs, & Mooney, 2001). These two examples identify predator–prey interactions and facilitation (habitat provision) as challenges in ecosystem-based management. This study reports on a third category of interaction in which two protected species interact via competition.

Distinct efforts in Washington State, USA, are directed towards increasing native Olympia oysters (*Ostrea lurida*) and native eelgrass (*Zostera marina*). Olympia oysters suffered from overexploitation soon after the arrival of Euro-American settlers to the west coast. Outer coast estuaries were depleted by 1900 (Kirby, 2004), Puget Sound populations dropped within a few decades thereafter, and efforts at cultivation ceased to provide measurable harvest by the mid-20th century (Baker, 1995; White, Trimble, & Ruesink, 2009), though a handful of growers have continued to cultivate Olympia oysters as a boutique product through to the present. Restoration efforts guided by the state's Department of Fish and Wildlife have emerged in the past decade and focus on 19 priority sites overlapping former natural oyster beds (Blake & Bradbury, 2012; Cook, Shaffer, Dumbauld, & Kauffman, 2000). Eelgrass has no net-loss protection at Federal, State, and local levels (Clean Water Act, 1972 section 404, WAC 173-26-221, WAC 220-110-250, Washington State Environmental Policy Act (RCW 43.21C.010)) and is considered essential fish habitat under the Endangered Species Act, Magnuson-Stevens Act, and Washington's Growth Management Act (RCW 36.70A.060). Increasing the area of eelgrass (20% by 2020) is a goal of the Puget Sound Partnership (Hamel et al., 2015).

Space conflicts between *O. lurida* and *Z. marina* arise in part because they have overlapping distributions in the low intertidal and shallow subtidal zones. Olympia oysters are sensitive to temperature extremes (Baker, 1995; White, Buhle, Ruesink, & Trimble, 2009), and *Z. marina* to desiccation (Boese, Alayan, Gooch, & Robbins, 2003). Consequently, this overlap may have led to limitation of eelgrass by Olympia oyster beds in the past, as suggested for Willapa Bay, Washington (Blake & ZuErmgassen, 2015), but an intentional transition from eelgrass back to oysters would not be allowed under current policies. A third foundation species in some areas is the Pacific oyster (*Crassostrea gigas*), introduced as a commercial replacement for Olympia oysters about a century ago. Its tolerance of low-tide conditions exceeds that of *Z. marina* and *O. lurida*, so *C. gigas* forms conspicuous reefs on both soft and rocky substrata, generally above mean lower low water (MLLW). Pacific oysters are popular for recreational harvest, support tribal and non-tribal commercial fisheries on wild and enhanced stocks, and are cultivated by a shellfish aquaculture industry in Washington state that leads national production (Goldburg, Elliott, & Naylor, 2001). In addition to intertidal reefs, Pacific oysters can overlap with eelgrass meadows and reduce shoot density, especially above 5–20% cover of shell (Wagner et al., 2012), and can compete with Olympia oysters when they co-occur (Buhle & Ruesink, 2009).

In Hood Canal, Washington (a fjord connected to Puget Sound), historical populations of Olympia oysters occurred in two large beds, which became part of the State's network of Oyster Reserves in the late 1800s. Several decades later, when Kincaid (1920, p. 51) surveyed these reserves, he reported that Quilcene Bay had 'extensive growths of eelgrass which will require considerable efforts to overcome'. In contrast, the Clifton Reserve at the head of Hood Canal had been outfitted with intertidal dykes to hold water and protect Olympia oysters from environmental extremes, where 'the freedom of these beds from eelgrass and the usual animal enemies is striking' (Kincaid, 1920, p. 53). At the same time, oyster resources in British Columbia, 150 km to the north, were surveyed at an earlier stage in their exploitation:

> *Along the sides of the channel open at intervals the mouths of narrow or broad sloughs, which begin shallow towards the margins of the bay and deepen as they approach the channel or body of bay-water at low tide. Down these flow currents during falling tide and up them at rising tide. At the lowest spring-tides they may be largely empty or reduced to narrow, shallow strips of drainage-water. At low neap tides they are broad and overflow considerable areas of grass-covered tide-flats. These are the best areas for the native oyster. It is along them or in patches here and there that the native oyster was originally and is yet chiefly to be found. The oysters are either covered with shallow water at low tide or exposed for only short intervals, which the eel-grass acts as a strainer in keeping the water back, and preventing complete drainage, or falls over and protects the oysters from direct heat of the sun. (Stafford, 1916, p. 150)*

The perspectives from a century ago illuminate competing hypotheses regarding the suitability of co-locating Olympia oysters in native eelgrass. At the present time, the 'Guide to Olympia Oyster Restoration and Conservation' emphasizes other factors altogether, particularly sediment conditions and top-down species interactions (Wasson et al., 2015). Olympia oysters require hard substratum for settlement, and their post-settlement performance can be reduced by smothering with fine sediments or by predators. Further down the list of concern are factors such as competition and low salinity that kill oysters at some sites. Temperature constraints, both in air and water, appear in this secondary category as well, and their most obvious influence may be to limit intertidal extent rather than site-level occurrence (Wasson et al., 2015). Although native eelgrass per se is not documented as a constraint to Olympia oysters on the US west coast (Wasson et al., 2015), it is possible that native eelgrass could trap fine sediments (de Boer, 2007) or provide oysters with structural protection from some types of predators (Heck & Orth, 2006).

Earlier studies relevant to Olympia oysters in *Z. marina* suggest limiting outplants to <10% cover to maintain eelgrass (Archer, 2008; Wagner et al., 2012), a guideline that was incorporated in outplanting Olympia oysters to a priority restoration site in Washington state in the present study. The focal questions were: (1) How well do outplanted Olympia oysters survive and grow at the site using different outplant methods? (2) What are responses of *Z. marina* in terms of density and productivity? Also, surveys of oysters at the study site and two other sites with persistent Olympia oyster populations were done to: (3) describe the intertidal zonation of Olympia oysters in comparison to *Z. marina* and introduced Pacific oysters.

2 | METHODS

2.1 | Outplant site

The Mission Creek study site (47.4238°N, 122.8748°W, WGS 84) is at the head of Hood Canal in Lynch Cove, coincident with the former Clifton Oyster Reserve. This reserve was designated at more than 500 acres (200 ha) in 1895, as part of a network of locations where oyster settlement was reliable and young oysters could be sold for grow-out on private tidelands. Excessive removal of shell probably degraded this reserve for on-going recruitment, since Kincaid (1920, p. 53) looked back on a time when 'originally a great natural oyster bed spread out upon the flats developed as part of the delta of the Union river'. (Mission Creek and Union River enter the head of Hood Canal within 2 km, spanning the former reserve area.) Sales of Olympia oysters from the reserve occurred until 1929, even as the acreage was reduced, but the state discontinued the reserve in 1933. The tideflat was then predominantly leased to Belfair Oyster for Pacific oyster cultivation from 1934 to 1962. Presently eelgrass occurs in a meadow largely below -0.4 m MLLW at the site (Yang, Wheat, Horwith, & Ruesink, 2013) and 50 m from the closest oyster dyke, which marks the lower edge of Pacific oyster reefs. Sediment within eelgrass at Mission Creek averages 2.87% organic content (SE=0.12%, N=3), with the inorganic portion made up of 5.74% silt and clay (<0.063 mm grain size, SE=0.85%, N=3) and the remainder as sand.

2.1.1 | Survey of oyster populations

Oysters (both *Ostrea lurida* and introduced *Crassostrea gigas*) were surveyed for size and abundance across intertidal zones at the Mission Creek study site in July 2015. For comparative purposes, two other locations in Washington state were selected for a similar survey where Olympia oysters were known to occur (Figure 1). Twanoh (47.378093°N, 122.974870°W) is located within 10 km of Mission Creek in lower Hood Canal. However, the sites differ in substratum, with Mission Creek consisting of muddy sand and Twanoh having pebbles. Nahcotta (46.49585°N, 124.02610°W), which is a site in the coastal plain estuary of Willapa Bay, is in a different water body from Mission Creek and receives regular recruitment of Olympia oysters (Trimble, Ruesink, & Dumbauld, 2009). All three sites share habitat characteristics in terms of Pacific oyster reefs at a mid-tide elevation, with eelgrass below mean lower low water, although this eelgrass is patchy at Twanoh. For each survey, 10 quadrats were placed along 100 m transects (50 m at Nahcotta) at five tidal elevations: +0.6 m, +0.3 m, 0 m, –0.3 m, and –0.6 m relative to MLLW. These elevations were determined during a falling tide based on the predicted times for particular water levels. At Nahcotta, no transect was done at -0.6 m, and at Mission Creek, a transect was added at +0.15 m. Quadrat size was selected for each transect based on oyster density, such that on average at least 10 oysters were present per quadrat. Consequently, quadrats were 0.0625 m^2 when oysters were dense and up to 1 m^2 in sparse oysters. Pacific and Olympia oysters were counted within the quadrat and the first 10 of each species were measured from hinge to edge (shell height) to the nearest cm.

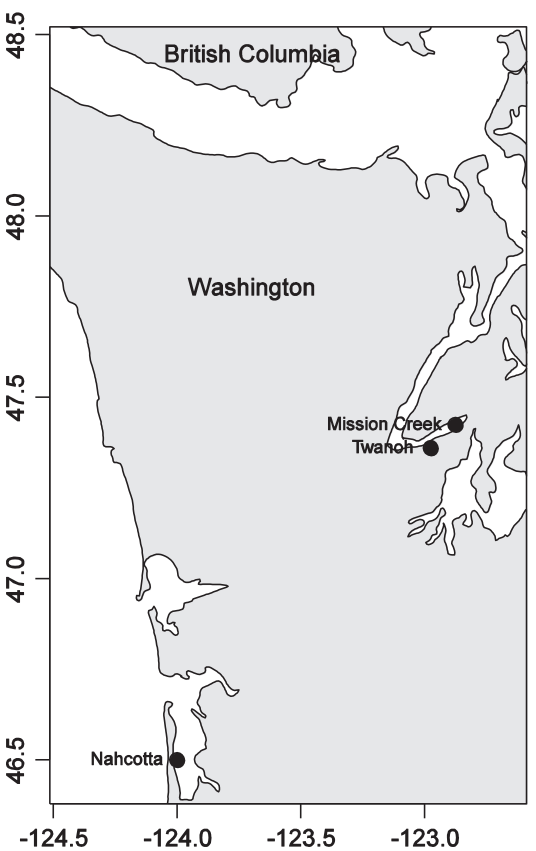

FIGURE 1 Map of Washington State, USA, showing the site for Olympia oyster outplants (Mission Creek) and two other sites that were surveyed and shown to have persistent Olympia oysters

For analysis of oyster surveys, Pacific and Olympia oyster densities (log(x+1)-transformed) were compared across tidal elevations at each site where both were present, with elevation, species, and their interaction as fixed effects. A statistically-significant interaction (elevation x species) would be evidence of different distributions for the two species.

2.1.2 | 2013 Oyster outplant

Adult Olympia oysters serving as broodstock were collected from mid-Hood Canal sites and conditioned to produce larvae that were settled in a hatchery in April 2013. Larvae settled both on Pacific oyster shell in mesh bags (cultch) and on small shell fragments to make single oysters (microcultch). On 20 Aug 2013, seeded cultch was outplanted into 10x10 m plots at the Mission Creek study site. Seven plots within eelgrass were established randomly between –0.3 and –0.6 m MLLW. Within each plot, five bags of seeded cultch (85 cm long x 20 cm diameter) were distributed evenly by hand. The contents of one bag cover 1.5 m^2 with shell; therefore, the initial target cover was 8% (5 bags x 1.5 m^2 per 100 m^2). Each cultch plot had an adjacent reference plot, also 10x10 m. The reference plots adjacent to the cultch plots allowed for detection of any drifting seeded cultch, but seeded cultch never appeared in these adjacent samples. The seven cultch and reference plots were examined seasonally between 2013 and 2015, each plot sampled with five subsamples of 0.25 m^2, which were done on a diagonal across the plot. Data were recorded for oysters (number of pieces of cultch, percentage cover of cultch, number of oysters on cultch, shell height in mm), eelgrass (density, size, growth), and predators (number of non-native oyster drills *Ocenebra inornata*). Overall, these measurements include structural (number of oysters) and functional aspects of the site (survival of oysters, productivity of eelgrass), in keeping with recent guidelines for post-restoration monitoring of oysters (Baggett et al., 2014, 2015).

For eelgrass biometrics, counts of shoots were made in the five sub-plots of 0.25 m^2 within each plot. The first five shoots encountered in each sub-plot were measured for sheath length. At least three shoots were marked for growth within each plot (not sub-plot) by poking small holes with sharpened wire near the top of the leaf sheath (Zieman, 1974). After 2–3 days, growth was determined non-destructively by the extension of the fastest-growing leaf (usually leaf 2) relative to the size of the shoot as measured by sheath length.

To test the response of *Z. marina* to oyster outplants, density, size, and growth of *Z. marina* on reference and cultch plots were compared. Because shell was present on the cultch plots from August 2013 through summer 2014, four seasonal samples of density and sheath length during this period (five for growth) were used in evaluating *Z. marina* response. Each eelgrass biometric was examined separately as a response variable in linear mixed effects analyses. Fixed effect was treatment, and random effects were sample date and paired plots within sample date. The significance of treatment was based on comparing the calculated F-value to a critical value at an a priori α-level of 0.05, based on the Satterthwaite approximation for denominator degrees of freedom (necessary for mixed-effects models). Error was well described by a Gaussian assumption for *Z. marina* biometrics. To test the response of oyster drills to outplants, a similar model structure was applied with the following exceptions. First, it was necessary to populate five of 22 reference samples with 1s rather than 0s to avoid singularities during analysis, but this modification reduced any differences between reference and cultch plots, and therefore represented a conservative change. Second, error for predator counts was described as poisson-distributed. Analyses were carried out with the 'nlme' (Pinheiro, Bates, DebRoy, Sarkar & R Core Team, 2016) and 'lme4' (Bates, Maechler, Bolker, & Walker, 2015) packages in R (R Development Core Team, 2016). Because outplants of oysters occurred only on one treatment, no comparisons across treatments were possible, and patterns of cultch and oysters were described over 2 years following outplant.

2.1.3 | 2015 Oyster outplant

On 30 Jul 2015, two additional outplant methods were implemented within 2x2 m plots, using cultch and single oysters from the original hatchery production in 2013, which had been held in northern Hood Canal at Thorndyke Bay. Sample size was limited by the availability of restoration-grade oysters. Between –0.3 m and –0.6 m MLLW, four blocks of three treatments were established: seeded cultch anchored in place, single oysters, and reference plots without oysters. Plots were separated by 3 m within blocks. Seeded cultch were culled for shells with at least one native oyster, and sets of 10 cultch were drilled and strung on galvanized wire to surround a wooden garden stake (Trimble et al., 2009; Figure S1. Supplementary material). Anchored cultch were pushed into the sediment until cultch was just at the sediment surface, with 14 stakes in each 2x2 m plot for a target cover of shell of <10%. Oysters on the central stake in each plot were measured for shell height at outplant (mean= 21.1 mm, SD= 6.0, N=45). Single oysters were added to the appropriate plots at total numbers of 260 to 290 in the 2x2 m plots. The mean shell height of these oysters was 26.5 mm (SD= 4.4, N=20), and cover was 4%.

Oysters, eelgrass, and predators were examined in the plots 3 and 9 months after they were set up (30 Oct 2015, 9 May 2016). Counts of *Z. marina*, *O. inornata* and *Pisaster ochraceus* (seastars) were made in the central 1 m^2 of all plots, and single oysters were counted in the single-oyster treatment. Anchored oysters were counted on the central stake, on which oysters had been measured initially. Oysters were scored for whether they were alive or dead, and if dead, whether the shell had a round hole indicating drilling by *O. inornata*. Linear mixed effects analyses were applied to all response variables, with treatment a fixed effect and block a random effect. Error structure was Gaussian for *Z. marina* density, and Poisson-distributed for predator counts. Oysters were compared between anchored and single treatments for three response variables, all evaluated at 3 and 9 months with respect to the initial oysters outplanted in July: fraction remaining (includes live and dead when re-sampled), fraction live, and fraction drilled. Analyses on oysters followed the same general structure as for other response variables in 2015, specifically fixed effect of treatment and random effect of block.

3 | RESULTS

3.1 | Oyster surveys

Pacific and Olympia oysters were found in surveys at Twanoh and Nahcotta, but only Pacific oysters at Mission Creek. Where both species were present, Pacific oysters were distributed at higher elevations and achieved 10-fold higher density than Olympia oysters (Figure 2, Table 1). The elevation of highest density for Olympia oysters was not consistent among sites or even between the east- and west-facing sides of Twanoh. On the east-facing side of Twanoh, Olympia oysters were most dense (8 m^{-2}) at 0 m MLLW, but on the west-facing side were most dense (6 m^{-2}) at –0.6 m MLLW. At Nahcotta, Olympia oysters exceeded 60 m^{-2} within Pacific oyster reefs at +0.3 m MLLW, and were less dense (but still abundant relative to Twanoh) at lower elevations, i.e. 10–20 m^{-2}, overlapping with *Z. marina*. The size distribution of Olympia oysters at Twanoh and Nahcotta was dominated by oysters around 3 cm in shell height, much smaller than Pacific oysters with median sizes >10 cm.

3.2 | 2013 Oyster outplants

After the 2013 outplant of Olympia oysters, cultch persisted for a year at around 7%, but in the second year declined to <1% (Figure 3a). Olympia oyster mortality occurred before decline in cultch. Given one oyster on each piece of cultch initially, starting densities were around 13 m^{-2}, but only 1.4 m^{-2} remained 3 months later, and less than 0.4 m^{-2} after 5 months and more (Figure 3b). Oysters that were measured over the period of monitoring were generally larger than at outplant, but few reached the median size of 3 cm found in surveys (Figure 3c). The density of oyster drills increased in cultch relative to reference plots (Figure 4a, Table 2).

None of the metrics of eelgrass density, size, or growth responded to the addition of seeded cultch in 2013 (Figure 5, Table 2). Because of the paired design of cultch and reference plots, potential space competition between oysters and eelgrass was effectively tested despite substantial spatio-temporal variability in eelgrass biometrics, which varied seasonally and with tidal elevation (Figure S2).

3.3 | 2015 Oyster outplants

Three months following the 2015 outplants of anchored and single oysters, less than half of the oysters in any plot remained alive (mean 20%, Figure 6), and 99% were dead or missing from plots after 9 months. A substantial fraction (15% at 3 months, 15% of those remaining at 9 months) showed evidence of drilling. Those gaping but without drill holes may have been consumed by *P. ochraceus*, as two seastars were observed consuming oysters on both re-sampling dates.

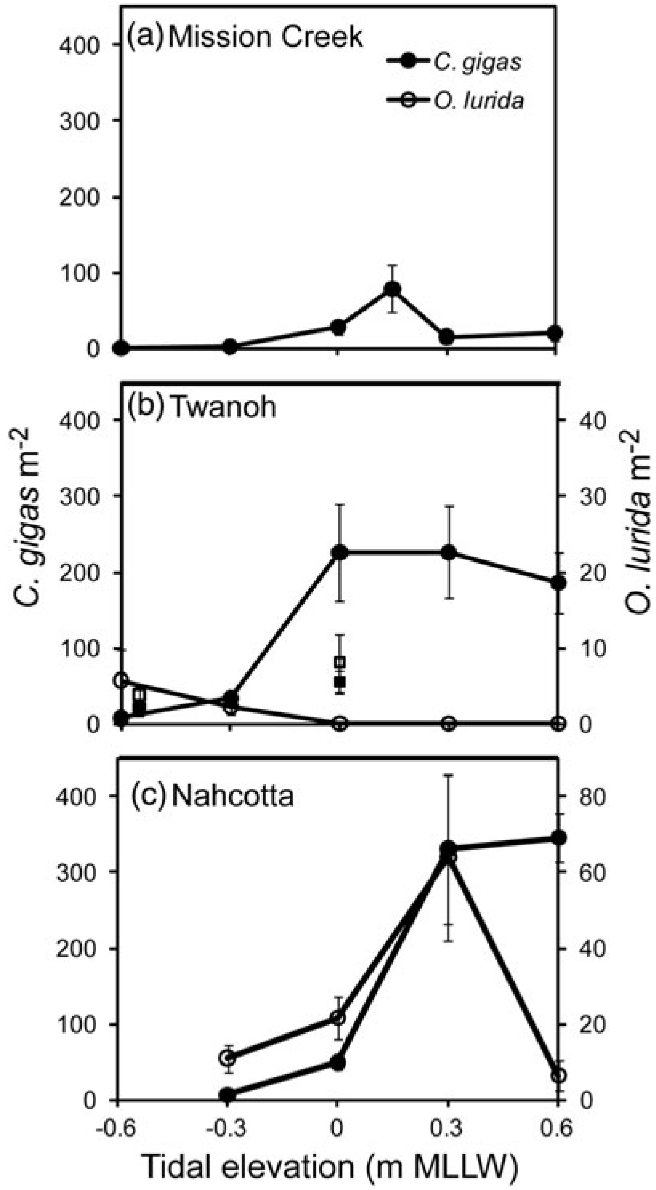

FIGURE 2 Density of Olympia oysters (*Ostrea lurida*) and Pacific oysters (*Crassostrea gigas*) across tidal elevations at (a) Mission Creek, (b) Twanoh in lower Hood Canal, and (c) Nahcotta in Willapa Bay. Surveys at Twanoh were primarily on the west side of the tideflat, but square symbols (Solid = *C. gigas*. Open = *O. lurida*) show east side samples. Error bars show standard errors from 10 quadrats

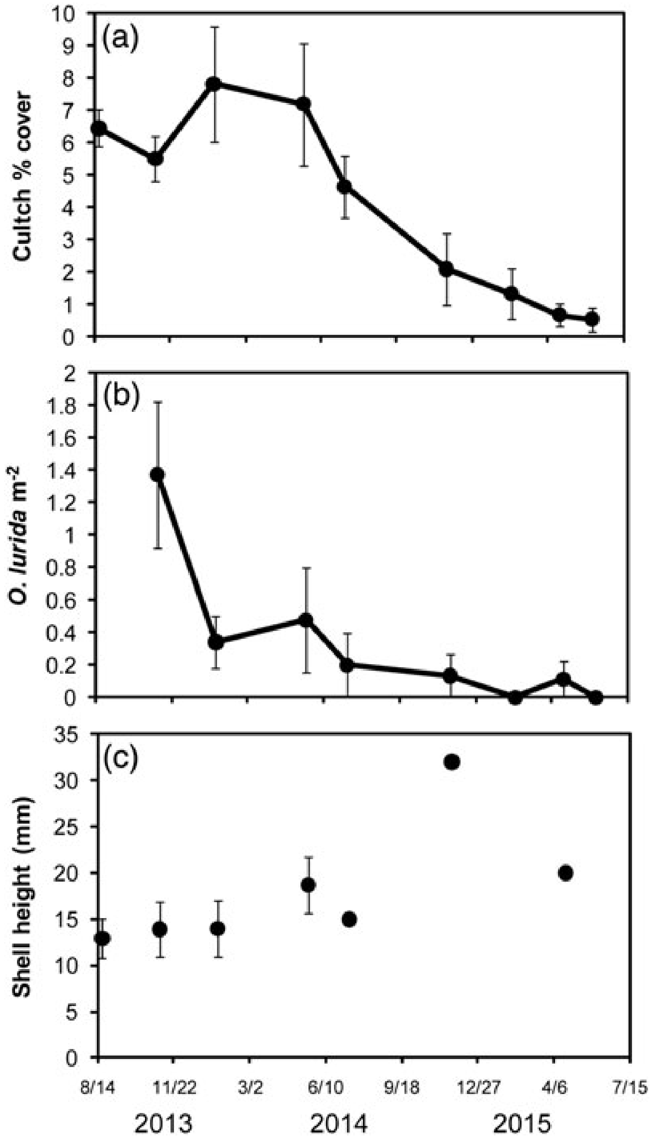

FIGURE 3 Olympia oysters (*Ostrea lurida*) outplanted in 2013 as seeded cultch at the Mission Creek study site. (a) Cover of shell (cultch); (b) density of live Olympia oysters; (c) shell height. Error bars in panels A and B represent standard errors from up to seven cultch plots, and in panel C represent standard deviation of any oysters found live during sampling

TABLE 1 Results of analyses comparing intertidal distribution of *Ostrea lurida* and *Crassostrea gigas* at two sites where both were surveyed in July 2015

	Density at Twanoh	Density at Nahcotta
Oyster species	$F_{1,86}$=398, P<0.0001	$F_{1,44}$=20.8, P<0.0001
Tidal elevation	$F_{4,86}$=6.3, P=0.0002	$F_{3,44}$=15.7, P<0.0001
Species x Elevation	$F_{4,86}$=25.2, P<0.0001	$F_{3,44}$=10.9, P<0.0001

Treatment differences occurred only in the fraction of oysters remaining (whether live, dead, or drilled), which was higher for anchored than for single oysters after 9 months (Table 3). Although treatments showed a tendency towards higher predator densities on plots with added oysters, the differences relative to reference plots were not statistically significant in this case (Figure 4b, c, Table 4). Eelgrass density did not change significantly in plots with anchored or single oysters relative to reference plots (Figure 5e , Table 4).

4 | DISCUSSION

Ecosystem-based management emerged from the recognition that connections among species and other parts of the system, both ecological and social, challenge any effort to manage a single species on its own (Leslie & McLeod, 2007). This over-arching perspective informed the Olympia oyster project from its inception, due to the value of maintaining submerged aquatic vegetation while restoring native shellfish beds. Nevertheless, post-treatment monitoring revealed additional constraints on Olympia oyster survival and

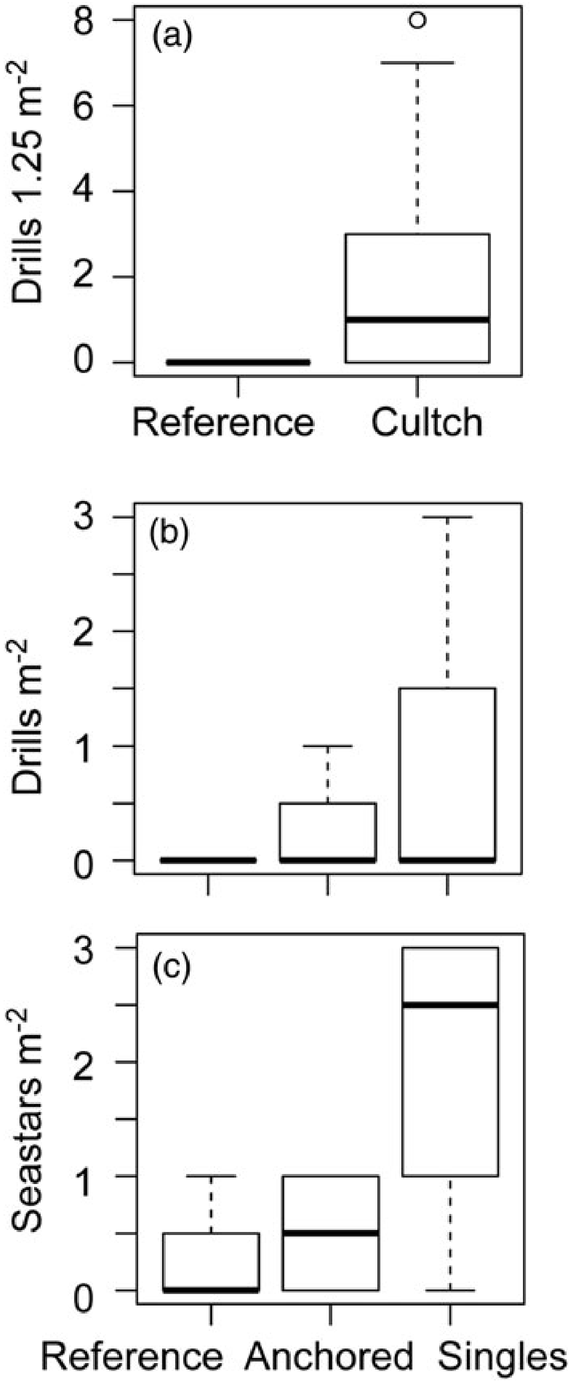

FIGURE 4 Predator density in cultch and reference plots at Mission Creek. (a) *Ocenebra inornata* sampled four times up to one year after 2013 outplants of seeded cultch; (b) *Ocenebra inornata* 3 months after 2015 outplants of anchored and single oysters; (c) *Pisaster ochraceus* 3 months after 2015 outplants. Boxes show median and interquartile range, whiskers show range, with points as outliers

therefore the build-up of self-sustaining populations. Survival rates of outplanted oysters were ~1% after a year following outplants in 2013 of seeded cultch and in 2015 of anchored cultch and single oysters. These low-density outplants were successful, however, in managing two protected species simultaneously, in that efforts to improve one (Olympia oysters) were carried out without harm to the other (eelgrass; Figure 5).

The poor performance of Olympia oysters in this study was unexpected, but the persistence of native eelgrass was consistent with other research regarding their interaction. In Netarts Bay, Oregon, Pacific oyster shell with Olympia oysters attached was put in plots at three densities, 4%, 11%, and 19% cover, and monitored from March to September 2007 (Archer, 2008). Relative to controls, density and cover of *Z. marina* were significantly lower with 19% cultch cover, and had a trend of lower cover without statistical significance with 11% cultch cover, but eelgrass persisted with 4% cultch cover (Archer, 2008). Olympia oysters grew during the study period, but no long-term measurements of oyster bed persistence were available. Olympia oysters were also outplanted in eelgrass in Willapa Bay, Washington, showing that after 1 year, about one-quarter of Olympia oysters survived in treatments where shell substratum was stabilized, but frequently disappeared when shells were not anchored in place (Trimble et al., 2009). The survey of Olympia oysters at Nahcotta, in Willapa Bay, reinforced the possibility of co-location, with Olympia oysters at densities of 10–20 m^{-2} in small clusters on top of the soft sediment within the eelgrass zone (Figure 2c). Of oysters found a year after outplant in Fidalgo Bay, Washington, 70–95% were alive, but outplants occurred only without eelgrass (Dinnel, Peabody, & Peter-Contesse, 2009). In summary, the survival of Olympia oysters in the present study was much lower than in other outplants with published monitoring.

To make sense of the outcome of this study requires expanding an ecosystem-based perspective beyond Olympia oysters and native eelgrass to include other native and non-native species. The most likely limiting factors at Mission Creek map logically on to the suite summarized in the 'Guide to Olympia Oyster Restoration and Conservation' (Wasson et al., 2015). Soft sediments provide little opportunity for settlement, and the loss of cultch from the outplant site after a year is consistent with smothering (Figure 3). Still, particularly with anchored cultch, experiments rapidly revealed that it was not sediment alone that limited oyster survival, given top-down control as an early constraint (Figure 6).

4.1 | Predation

Two species of introduced oyster drills are known to feed on Olympia oysters and have been shown in feeding trials, mesocosms, and across-site surveys to account for low survival (Buhle & Ruesink, 2009; Kimbro, Grosholz, & Baukus, 2009; Sanford et al., 2014). On

TABLE 2 Results of linear mixed effects analyses of predators (*Ocenebra inornata*) and eelgrass (*Zostera marina*), comparing treatments for 1 year after outplants of cultch in August 2013. All analyses include sample date and plot pairs as random effects

	Oyster drills	Eelgrass density	Sheath length	Growth extension per day	Growth standardized to sheath length
Cultch vs. Reference treatment	$F_{1,21}$=20.1, P<0.01	$F_{1,21}$=0.50, P=0.49	$F_{1,21}$=1.23, P=0.28	$F_{1,23}$=0.13, P=0.72	$F_{1,23}$=1.13, P=0.30

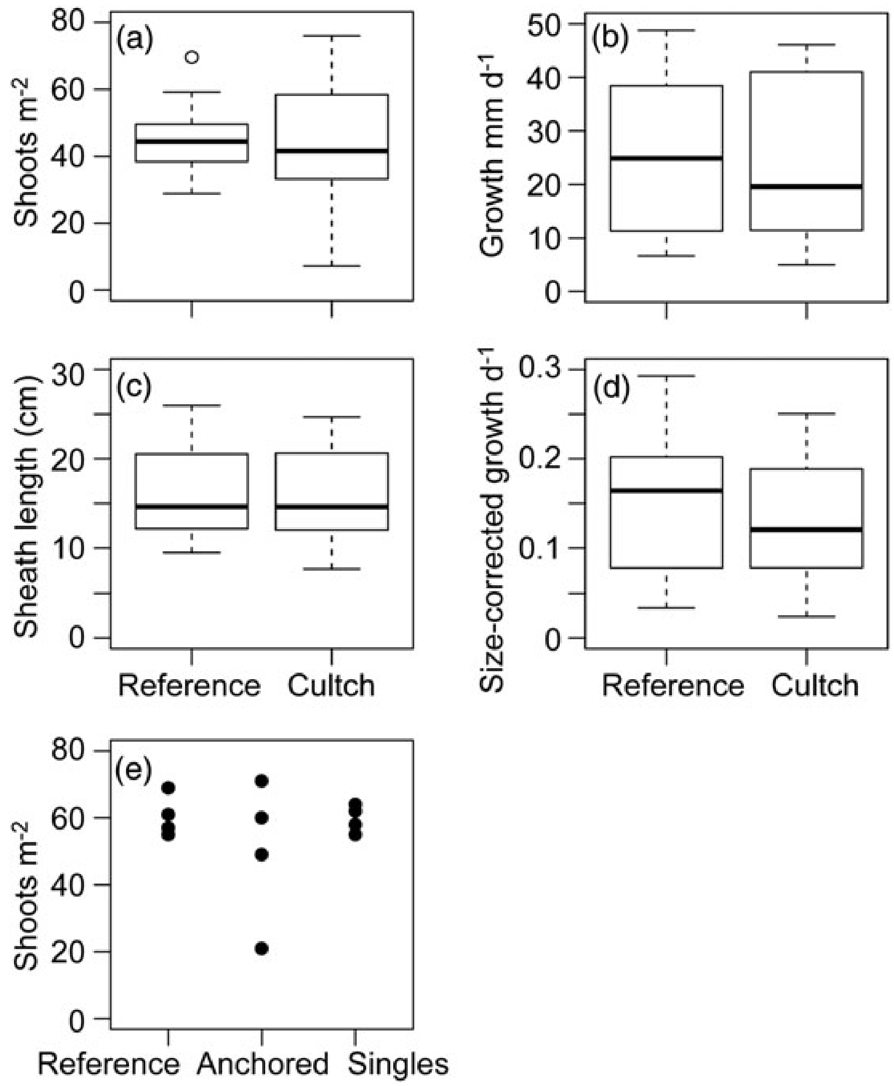

FIGURE 5 Biometrics of eelgrass (*Zostera marina*) following Olympia oyster outplant in comparison with reference plots. Between November 2013 and August 2014, eelgrass was measured for (a) density, (b) growth as linear extension of the fastest-growing leaf, (c) size, and (d) growth standardized by sheath length. (e) In October 2015, eelgrass was measured for density. Boxplots are described in Figure 4

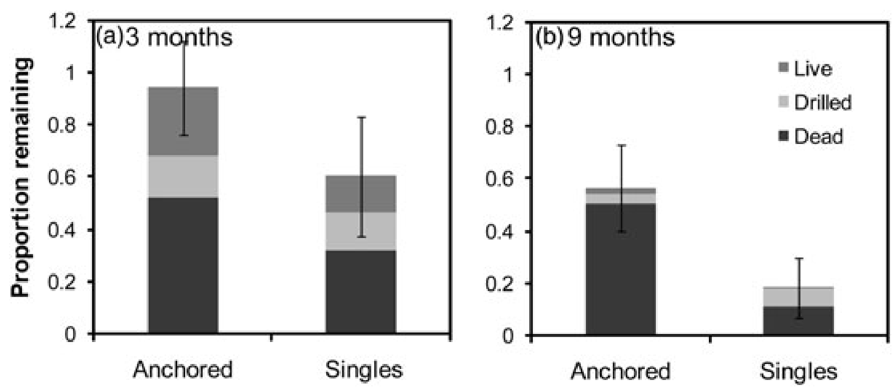

FIGURE 6 Olympia oysters (*Ostrea lurida*) outplanted on 30 July 2015 as anchored or single oysters at Mission Creek, re-sampled (a) 30 October 2015, and (b) 9 May 2016. Each bar represents results from four plots, dividing oysters into three categories (dead, drilled, or live). Error bars (SE) are for total oysters remaining, relative to initial outplants

TABLE 3 Results of linear mixed effects analyses of Olympia oysters (*Ostrea lurida*) when outplanted as anchored and single oysters, with statistical significance implying performance differed between these treatments. Oysters were re-sampled at 3 and 9 months. Analyses include block as a random effect

	Fraction remaining	Fraction live	Fraction drilled
3 months	$F_{1,3}$=5.45, *P*=0.1	$F_{1,3}$=0.63, *P*=0.48	$F_{1,3}$=0.16, *P*=0.71
9 months	$F_{1,3}$=14.4, *P*=0.03	N/A, only 2 live oysters sampled	$F_{1,3}$=1.27, *P*=0.34

TABLE 4 Results of linear mixed effects analyses of predators (*Ocenebra inornata* and *Pisaster ochraceus*) and eelgrass (*Zostera marina*), comparing treatments 3 months after outplants in 2015. Analyses include block as a random effect

	Oyster drills and egg clusters	Seastars	Eelgrass density
Anchored, singles and reference treatment	$F_{2,6}$=0.73, *P*=0.3	$F_{2,6}$=3.0, *P*>0.05	$F_{2,6}$=0.87, *P*=0.46

the other hand, self-sustaining populations of *O. lurida* co-occur with oyster drills in several areas of Washington state, including Willapa Bay, North Bay (N47.401°, W122.822°), and Liberty Bay (N47.722°, W122.655°; B. Blake, pers. obs.). Small (5 cm) seastars were observed feeding on *O. lurida* in 2015–2016, even though an epizootic disease had recently reduced seastar densities regionally (Eisenlord et al., 2016). In the event of further recovery of seastars, restoration could become more constrained by these native predators. Seastars have long been implicated as setting the lower limit of Olympia oysters in Hood Canal (Kincaid, 1920), and Olympia oysters may now be pinched in their distribution by oyster drills attracted from Pacific oyster reefs at higher elevations. Efforts to reduce oyster drills as part of Olympia oyster restoration could have practical problems, even though oyster drills are regulated as a pest species (WAC 220-72-011). These regulations primarily address shellfish transfers, not pest control, and no methods have been developed for effective drill control other than removal by hand (Buhle, Margolis, & Ruesink, 2005). The attraction of drills to outplanted oysters (Figure 4) provides a possible technique for 'baiting' drills to improve removal rates, but could also result in drills supplemented by this additional food source, and a larger drill problem in the long term. This possibility of bottom-up enhancement of a pest through oyster restoration needs further examination.

4.2 | Competition

Pacific oysters can reduce the performance of co-occurring Olympia oysters (Buhle & Ruesink, 2009) and may serve as a recruitment sink, attracting larvae to settle at inappropriately high elevations (Trimble et al., 2009). In this restoration project, however, Pacific oysters were spatially distinct, and the mechanism of impact through a recruitment sink should not be a factor for outplanted oysters. In addition, the surveys found successful co-occurrence of Olympia oysters within and below Pacific oysters at the other sites (Figure 2), reflecting more general observations in Hood Canal over the past several decades (B. Blake, pers. obs.). Looking forward, better understanding of factors that generate the lower limit of Pacific oysters would be valuable, in order to understand if they are likely to settle on Olympia oysters restored into soft sediment. As in Trimble et al. (2009), anchored oysters at Mission Creek in 2015 were colonized by fouling organisms such as tunicates. However, at present, despite moderate exposure to competitors throughout their native range, Olympia oysters are not considered particularly sensitive to this stressor (Wasson et al., 2015).

4.3 | Restoration methods

Olympia oysters naturally occur in a variety of habitat types (Stafford, 1916). These habitats include beds where hard substrate is provided by the build-up of their own shells, and densities of reproductive-size oysters can exceed 100 m^{-2} (White, Buhle et al., 2009, referring to North Bay, but in 2016, densities were substantially lower, J. Ruesink, pers. obs.). In addition, populations occur at lower density as sparse singles or clusters in habitats such as pebble beaches (e.g. Twanoh, also including intertidal seeps), on cobble (Kimbro et al., 2009), or attached to scattered shell of other species. The occurrence of Olympia oysters within the lower parts of non-native Pacific oyster reefs at Nahcotta adds to this list (Figure 2). Olympia oysters also recruit to anthropogenic surfaces such as rip-rap, sunken trash (Baker, 1995), and floating docks (Groth & Rumrill, 2009). Ecological functions of water filtration and habitat provision for other species likely increase with density and area of Olympia oysters, making high-density beds a priority for conservation and restoration (Blake & Bradbury, 2012). Yet these high-density beds were also targeted by Euro-American fisheries and harmed through the concurrent removal of the shell base along with live oysters. So, among nearly 40 high-density beds known to have existed in Puget Sound, only a handful have sufficient oysters that they do not require restoration (but other considerations also influence the identification of priority restoration sites; Blake & Bradbury, 2012).

For shellfish more generally, low-density restoration may be hampered by sedimentation that exceeds shell accretion rates (Harding, Southworth, & Mann, 2012) or by low relief that keeps oysters in hypoxic bottom water (Schulte, Burke, & Lipcius, 2009). Shellfish may also be recruitment-limited (Wasson et al., 2015), although at Mission Creek, larvae regularly settle when hard substratum is provided at a suitable tidal elevation (Valdez & Ruesink, in review). In retrospect, comparative outplants outside eelgrass and at higher oyster density would have given substantial additional insight into limiting factors, but at the outset of the project, attention was focused primarily on how oysters would influence eelgrass.

Methods for enhancement of Olympia oysters span a wide range, tuned to local environmental history, conditions, and limiting factors (Wasson et al., 2015). Efforts in Newport Bay, CA, have included placement of Pacific oyster shell as habitat for naturally-recruiting oysters, including layers that range from 4" to 12" thick, with the latter persisting longer in the face of sedimentation (Zacherl, Moreno, & Crossen, 2015). In San Francisco Bay, still taller structures of shell or concrete have been tested as recruitment substrates maintained above the sediment and potentially protecting shorelines as part of living shorelines design (Latta & Boyer, 2015). In Washington State, substrate enrichment of loose Pacific oyster shell is used to expand the footprint of developing populations with reliable recruitment, and areas with low densities of adult oysters have been enhanced with shell and seeded cultch (Dinnel et al., 2009). The present study confirms that eelgrass persists through low-density outplants of Olympia oysters, avoiding a potential management conflict from space competition of protected species. Poor performance of Olympia oysters leaves open whether top-down control was particularly acute due to low oyster density and/or the environmental conditions of eelgrass beds. Given that Olympia oyster populations are widely recognized to be constrained by sedimentation (Wasson et al., 2015), low-density outplants may also be insufficient to provide the density or structure needed to rebuild Olympia oyster populations within eelgrass.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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