

DISTRIBUTION AND TRANSPORT OF OLYMPIA OYSTER *OSTREA LURIDA* LARVAE IN NORTHERN PUGET SOUND, WASHINGTON

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ABSTRACT As efforts for restoring Olympia oyster *Ostrea lurida* populations have expanded, there is an increased need to understand local factors that could influence the long-term success of these projects. To address concerns over potential limitations to recruitment at a restoration site in northern Puget Sound, WA, a study was developed to characterize physical processes governing larval transport in conjunction with larval abundance and environmental factors. Larval presence was not associated with tide cycle, season, or a combination of tide cycle and season. In terms of location, larvae were more likely to be present at offshore and intertidal sites versus the estuarine lagoon, where the adult population resides. Larval density was higher during late summer ebbs versus early summer floods. Across sampling dates and locations, larval sizes ranged from 184 to 263 μm , indicating that larvae were released into the water column throughout the reproductive season and retained in the embayment for at least ~ 16 days. Throughout different tidal cycles in Skagit Bay, acoustic Doppler current profilers were used to measure current direction and velocities, concurrent with plankton sampling. Surface currents in the study area alternated between a clockwise and a counterclockwise gyre during initial ebb and flood tides, respectively. Larvae exported from the source population during initial to midebbs are swept into a northward gyre and potentially retained at intertidal sites alongshore. These results will provide resource managers attempting to restore native bivalves with the ability to expand populations by identifying optimal areas for habitat enhancement through natural recruitment.

KEY WORDS: *Ostrea lurida*, nearshore circulation, Olympia oyster, larval transport, Puget Sound, recruitment, restoration

INTRODUCTION

Successful restoration of marine invertebrate species with complex life cycles is often highly dependent on larval dispersal and the recruitment dynamics of the species. Sessile invertebrate populations are particularly affected by larval dispersal because environmental factors that drive larval transport can determine adult population locations, densities, and stability (e.g., Roughgarden et al. 1988, Shanks 2013). Further complicating recruitment success of marine invertebrates is the availability of suitable habitat within a system. Many invertebrate populations are known to be affected by habitat bottlenecks, where life-stage-specific population reductions can occur because of lack of suitable habitat (e.g., Wahle & Steneck 1991, Beck 1995, Trimble et al. 2009). Among other stressors, modification or removal of specific habitat types by human activities can lead to the collapse of populations (e.g., Jackson et al. 2001, Kirby 2004, Lotze & Milewski 2004), presumably due in part to recruitment failure. Consequently, restoration efforts of sessile marine invertebrates, particularly species with long-lived planktonic larvae, can be improved with knowledge of their larval transport pathways, connectivity among populations, and habitat limitations.

Oyster populations have declined globally because of a multitude of factors including overharvesting and habitat destruction (Kirby 2004, Beck et al. 2011, zu Ermgassen et al. 2012). Understanding larval dispersal patterns can be paramount in improving scientists' ability to develop spatially explicit oyster habitat restoration plans (Sponaugle et al. 2002, Kim et al. 2013). For example, if information existed on

particular restoration sites in relation to larval dispersal and population connectivity characteristics, managers could better target locations for habitat restoration efforts. These site-specific data characterizing larval dispersal typically are not incorporated into shellfish restoration plans, and more importantly, managers rarely have the means available to collect baseline data with which to evaluate the long-term success of the restoration project. Thus, while significant resources have been devoted to the recovery and restoration of oyster habitats (Beck et al. 2011, Baggett et al. 2015), scientists and managers can further improve the likelihood of restoration success by collecting site-specific data to determine what environmental conditions affect larval retention, recruitment, and survival in areas of interest (Fitzsimons et al. 2020).

The Olympia oyster *Ostrea lurida* (Carpenter, 1864) was once found in coastal estuaries from British Columbia, Canada to Baja California, Mexico (Polson & Zacherl 2009). Despite the broad geographic distribution of this species, populations are less than 1% of historical numbers and they are absent in some of their historical locations (zu Ermgassen et al. 2012, Blake & zu Ermgassen 2015, Hatch & Wyllie-Echeverria 2016). Restoration and enhancement efforts to protect Olympia oysters began in earnest around 1999 and are continuing to become more widespread throughout their range (Peter-Contesse & Peabody 2005, White et al. 2009, Wasson et al. 2015). As the number of restoration projects have increased along the coast, so has interest in quantifying factors that affect recruitment (Carson 2010, Wasson et al. 2016). The lack of recruitment synchrony along much of the range of *O. lurida* highlights the need for studying local population dynamics of Olympia oysters within particular estuarine systems (Wasson et al. 2016). This information could be especially important in complex fjord systems such as Puget Sound, WA, where subbasins within the

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Sound are largely defined by complex oceanographic features (Moore et al. 2008). Although knowledge of larval distribution, retention, and recruitment success in Puget Sound is expanding (e.g., Hatch et al. 2018, Hintz 2018, Becker et al. 2020, McIntyre et al. 2020), larval recruitment dynamics are still not well understood in many parts of Puget Sound, including northern Whidbey basin where *Olympia* oyster beds were historically located.

Because the life cycle of *Ostrea lurida* is relatively well described, scientists can apply their understanding of larval development and timing to research focused on improving restoration efforts for this species. *Olympia* oysters brood their larvae for a period of ~2 wk before the release of D-stage veligers (163–187 μm) into the water column (Stafford 1913, Pritchard et al. 2015). Most populations exhibit two annual larval release pulses, with one prominent peak early in the season (late May to early June) and a minor peak in the late season (mid-July) (Hopkins 1937, Pritchard et al. 2015). Currently, the literature suggests that planktonic larval duration (LD) ranges broadly from 1 to 8 wk following release. These differences are likely due to location as well as temperature, food availability, and salinity (Peteiro & Shanks 2015, Pritchard et al. 2015, Lawlor & Arellano 2020). Upon release, *Olympia* oyster larvae are actively mobile and able to regulate their position within the water column, thus controlling their ability to take advantage of, or resist, transport by prevailing currents (Peteiro & Shanks 2015, McIntyre 2018). Importantly, results describing the presence of tidally timed vertical migration behavior in *Olympia* oyster larvae differ on a geographic basis (Peteiro & Shanks 2015, McIntyre 2018). Describing the horizontal and/or vertical distribution of *Olympia* oyster larvae at different developmental stages could help predict when and where these larvae might settle in a particular estuary.

From 2012 to 2017, approximately 500,000 *Olympia* oyster seed were spread across two restoration sites on the Swinomish Indian Tribal Community (SITC) reservation, with the intent of establishing self-sustaining populations that could act as larval sources to additional sites in northern Whidbey basin [one of the 19 target native oyster restoration sites in Puget Sound (Blake & Bradbury 2012) and an area that historically supported *Olympia* oyster populations (Hatch & Wyllie-Echeverria 2016)]. These two restoration sites, located in northern Skagit and Similk bays (Fig. 1), are tidal lagoons perched on the upper beach where water flow from the surrounding bay is restricted by a tidal channel (Beamer et al. 2003). These sites were initially chosen for restoration because of the low flow conditions and the presence of lagoon channels that would allow the oysters to remain inundated over all tide cycles (Barber et al. 2015). Restoration efforts also included the addition of unseeded UV-treated *Crassostrea gigas* (Thunberg, 1793) shells to ~0.04 ha for *Olympia* oyster larvae to settle on within the lagoons. *Olympia* oyster beds with a large adult population should export larvae within their local estuaries and potentially regionally. Although it is generally thought that *Olympia* oyster larvae remain relatively close to their source population, larvae have been shown to travel upward of 75 km in southern California (Carson 2010). Given that there are no other known populations of *Olympia* oysters in Skagit and Similk bays, the estimated population of 129,000 individuals (circa 2017, 82,000 in Skagit Bay (SB) and 47,000 in Similk Bay, SITC Fisheries Department, unpublished data) in these two restoration sites

establishes the potential for native oysters to spread to other suitable habitat in northern Whidbey basin. Notably, *Olympia* oyster larvae in this region are habitat-limited (i.e., there are no existing beds of oyster shells outside these restoration sites) and determining where larvae are moving and when they will reach settlement size would allow SITC to more appropriately locate and enhance areas with preferred oyster habitat (Pritchard et al. 2015, Wasson et al. 2015, Zacherl et al. 2015).

In 2015, both restoration sites and the surrounding near-shore areas were monitored for signs of reproduction and recruitment by checking the brooding status of oysters and examining deployed *Crassostrea gigas* shells for signs of recruitment (Barber et al. 2015, 2016, Greiner et al. 2015). It was determined that oysters in the lagoons were successfully brooding (Barber et al. 2016), but no evidence of recruitment was found at any of these monitoring sites over a 3-y survey period (Barber et al. 2015, SITC Fisheries Department, unpublished data). Possible explanations for the lack of recruitment include insufficient adult populations (low larval supply), ineffective monitoring methodology for the habitat, suboptimal conditions in the lagoons for larval survival, and/or tidal currents and circulation transporting larvae out of the monitoring area. The lack of suitable habitat (i.e., oyster shell) outside the lagoons is of particular concern. Specifically, larvae may have been transported out of the lagoon but retained within Skagit and Similk bays, where there is currently little suitable habitat for settlement.

Based on these uncertainties, this study was designed to improve the understanding of potential limitations to native oyster recruitment in northern Puget Sound. This particular research focused solely on the restoration site in SB, within and around an area referred to as Lone Tree Lagoon (LTL). Because of the observed lack of recruitment at the restoration site within the lagoon habitat, this study primarily focused monitoring on the tidelands and offshore sites adjacent to LTL. If larvae are exported out of the lagoon shortly after release, densities of small larvae would be expected to be highest at sites directly adjacent to the lagoon channel and offshore. As the season progresses, this study hypothesized that larval sizes would become more mixed and densities at locations within the embayment would follow patterns dictated by the predominant circulation. A thorough understanding of the physical processes governing larval transport within this region is necessary for making informed decisions regarding future expansion of native oyster restoration projects on Swinomish tidelands. This research addressed the following broad goals: (1) collect plankton samples to investigate associations between larvae and hydrodynamic processes and to describe spatial and temporal changes in larval abundance, size, and density; (2) determine the effectiveness of recruitment samplers; and (3) describe tidal currents and circulation around LTL and northern SB.

Specifically, the spatial distribution of *Ostrea lurida* larvae was examined along with the nearshore transport processes that may affect dispersal during larval export from LTL. This work was carried out both to characterize potential transport pathways and to evaluate recruitment potential along the shoreline north of the adult LTL oyster population. Using plankton samples, the following questions were also investigated: Is there a relationship between the presence and absence of larvae by location, tide cycle, season, or a combination of these factors? Is there a difference in the density of larvae by tide and

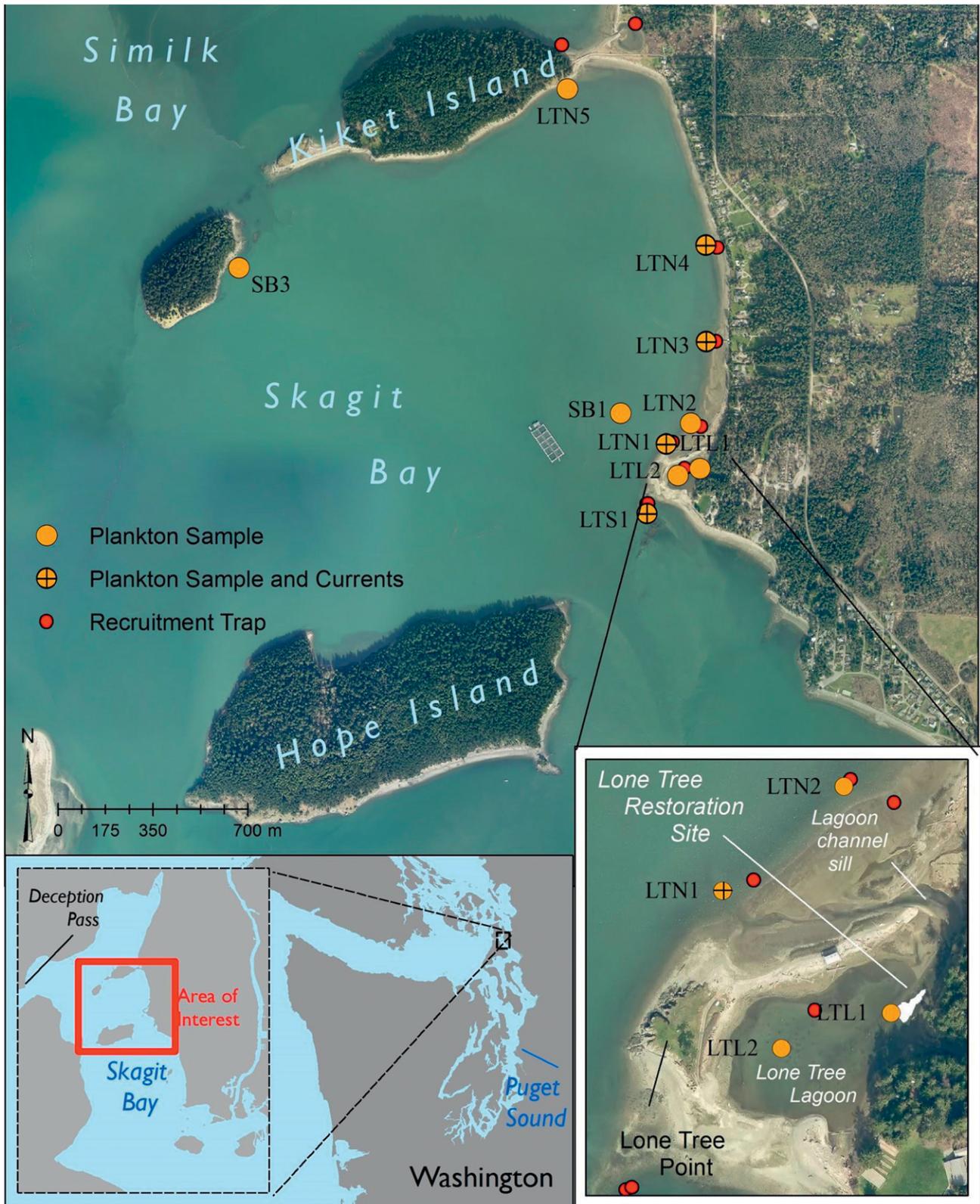


Figure 1. Locations of larval sampling, larval recruitment sampler sites, and current profile transects (transect starting point shown). Note that although recruitment samplers were deployed in Similk Bay, no plankton sampling was conducted there. Skagit Bay 3 was a pilot site and was only sampled twice during the study.

season? And can change in larval size classes be qualitatively described across space and through time? Finally, recognizing that other resource managers may be experiencing similar problems with recruitment, SITC wanted to develop reproducible methods that could be applied to other bivalve restoration projects in the hopes of improving the ability to properly locate restoration sites that target natural recruitment.

MATERIALS AND METHODS

Study Site

Skagit Bay is located in the northern Puget Sound region of Washington state in the northwest of the United States. Lone Tree Lagoon, the target restoration site for this study, is located within northern SB and is geomorphically considered a pocket estuary or tidal channel lagoon (Beamer et al. 2003). The lagoon is approximately 1.6 ha in size and is perched on the tidelands with a barrier beach along the western edge. Marine waters from SB are able to enter the lagoon through a channel during flood tides greater than +1.2 m relative to the North American Vertical Datum 1988 (NAVD88), and water exits the lagoon as the tide ebbs. During the summer months, winds in the area are generally calm (<10–20 km/h), predominantly from the west, pushing surface waters northward toward Kiket Island and a tombolo at the northern end of the bay (Fig. 1).

As part of this native oyster restoration work, a small bed (roughly 0.02 ha) of *Olympia* oysters was established at the head of the channel inside LTL (Fig. 1). Based on shoreline surveys and discussions with other *Olympia* oyster experts in the state, this region is believed to be the only source of *Olympia* oyster larvae in SB; however, it is important to remember that a second *Olympia* oyster restoration site is located in nearby Similk Bay (Barber et al. 2015). The next nearest *Olympia* oyster restoration site is located in Fidalgo Bay, roughly 35 km away by water from SB in a different oceanographic subbasin of Puget Sound. Because this species tends to exhibit local larval retention (e.g., Becker et al. 2020), it is highly unlikely, though not impossible (Carson 2010), that Fidalgo Bay serves a source population for SB. Nevertheless, when taking regional circulation patterns into account, this study hypothesized that the larvae recorded during this research were likely to have originated from one of the two restoration sites in either Skagit or Similk Bay.

Sampling Strategy

Three independent methods were used both in sequence and concurrently to investigate the distribution of *Ostrea lurida* larvae and evaluate potential larval transport pathways and retention in SB: (1) surface water plankton sampling, (2) deployment of larval recruitment samplers, and (3) current profiling. The surface water plankton sampling took place at monitoring stations that were established within and outside of the restoration site in LTL, whereas the current profiling and deployment of larval recruitment samplers occurred at a subset of the surface water plankton sampling sites (Fig. 1). Two sites were established within LTL for the plankton sampling: one over the oyster bed (LTL1) and the other farthest from the outlet channel at the southwest portion of the lagoon (LTL2). Five intertidal sites for plankton monitoring and recruitment samplers were established north of the lagoon channel

outlet along the eastern shore of SB at 0.6 m NAVD88 tidal elevation [from the lagoon channel north to the head of SB: (Lone Tree North = LTN) LTN1, LTN2, LTN3, LTN4, and LTN5]. Finally, two plankton sampling stations were established offshore in deeper waters within SB (SB1 and SB3) (Fig. 1).

Surface Water Plankton Sampling

The relative abundance of *Olympia* oyster larvae was examined at sites in SB and LTL by collecting surface water plankton pump samples weekly from June 13 to August 31, 2017 (Fig. 1). Each week, sampling efforts would alternate between ebb and flood tides (i.e., 1 wk sampling on the ebb and the following week sampling on the flood), and sites were sampled at +1.2 to +1.8 m NAVD88 tides (Hopkins 1937, Peteiro & Shanks 2015). It should be noted that tide height in LTL does not correspond with tide height at the intertidal and offshore sites because of the sill in the lagoon. Initially, six sites were sampled per week: the two lagoon sites (LTL1 and LTL2), one offshore site (SB1), and three intertidal sites (LTN1, LTN2, and LTN3). As sampling became more efficient, the number of sites sampled per week was increased to eight, with sampling at all five of the intertidal sites each week plus the two lagoon sites and the offshore site (Table 1). Site SB3 Pilot was only sampled on two dates in an exploratory attempt to determine the western boundary of larval distribution within northern SB (Table 1).

For each sample, 150 L of water was pumped through a 125- μ m plankton net using a modified battery-powered bilge pump moved vertically through the top 1.5 m of the water column (McIntyre 2018). Samples were transferred from the detachable cod end of the net to a jar with filtered seawater and stored in a portable cooler. Following the same procedure each time, samples were further filtered through a 180- μ m sieve and transferred into a container, obtaining concentrated samples that were \sim 30 mL (ranging from \sim 25 to 35 mL). The live concentrated samples were subsampled in three 1-mL replicates and observed under an Olympus CKX1 compound microscope and/or a Motic DM143 dissecting microscope, both set at 40 \times magnification. Specimens were visually identified based on size and morphology, photographed, and measured (Loosanoff et al. 1966, Shanks 2001). Although *Olympia* oyster larvae can be released at sizes as small as 150 μ m, this project used 180 μ m as size at release based on other studies (Pritchard et al. 2015, McIntyre 2018). Following release and entrance to the water column, *Olympia* oysters can grow to \sim 300–320 μ m before settling (Hori 1933, Hopkins 1936). Thus, length was measured at the widest point parallel to the hinge using Motic Image Plus 2.0 (Motic China Group Co. Ltd. 2007) software to determine the approximate age of the larvae.

As the original intent of this research was to document only the presence or absence of larvae at these specific sites, the number of *Olympia* oyster larvae by sample volume had to be retroactively calculated. It is necessary to emphasize that the process of standardizing larval count by volume magnified larval counts in this report, making them appear higher than what was actually captured; this is not ideal, but the process allows for comparisons with other published research (e.g., McIntyre 2018, McIntyre et al. 2020). To standardize these data by volume, the count of *Olympia* oyster larvae per subsample was summed (e.g., 1 + 1 + 2 = 4) and divided by three (the

TABLE 1.
Summary of *Olympia* oyster larvae found at each site by date, tidal phase, and larval size range.

Date	Tide stage	Size range (µm)	Lagoon		Intertidal					Offshore		Weekly mean	SE
			LTL1	LTL2	LTN1	LTN2	LTN3	LTN4	LTN5	SB1	SB3*		
June 13, 2017	Ebb	223–240	0	0	6.7	0	0	–	–	13.3	–	3.3	2.3
June 19, 2017	Flood	225–226	0	0	0	0	0	–	–	13.3	–	2.2	2.2
June 27, 2017	Ebb	NA	0	0	0	0	0	–	–	0	–	0	0
July 5, 2017	Flood	NA	0	0	0	0	0	–	–	0	0	0	0
July 12, 2017	Ebb	182–251	6.7	0	0	13.3	6.7	–	–	6.7	–	5.6	2
July 18, 2017	Flood	211–233	0	6.7	0	13.3	0	–	–	0	–	3.3	2.3
July 27, 2017	Ebb	205–245	0	0	0	0	0	0	0	0	73.3	8.1	8
August 2, 2017	Flood	184–247	0	0	6.7	0	0	0	20.0	13.3	–	5.0	2.7
August 9, 2017	Ebb	202–259	0	20.0	6.7	26.7	26.7	20.0	66.7	26.7	–	24.2	7.0
August 15, 2017	Flood	202–263	0	0	13.3	0.0	26.7	0	6.7	6.7	–	6.7	3.3
August 32, 2017	Ebb	199–238	6.7	0	6.7	33.3	0	0	13.3	6.7	–	8.3	3.9
August 31, 2017	Flood	240	0	0	0	0	0	0	0	6.7	–	0.8	0.8
Site mean	–	–	1.1	2.2	3.3	7.2	5.0	3.0	17.8	7.8	36.7	–	–
SE	–	–	0.7	1.7	1.3	3.4	3.0	3.3	10.3	2.3	36.7	–	–

Counts are estimated from smaller sampled volumes and multiplied to a standard 100 L.

* Pilot site.

number of 1 mL subsamples taken per 150 L) to obtain the mean number of larvae per milliliter (e.g., $4/3 = 1.3$ larvae/mL). Because the entire 150 L sample was represented in the ~30 mL concentrated solution, the mean number of larvae/mL was multiplied by 30 to estimate the total number of larvae/150 L (e.g., $1.3 \text{ larva/mL} \times 30 = 40 \text{ larvae/150 L}$). From this information, the number of larvae estimated to be present in a 100-L sample was calculated. Unless otherwise noted, larval counts presented in this article are counts standardized to 100 L.

For an initial qualitative analysis, cumulative larval density by sampling site was mapped to gain a better understanding of where the majority of larvae were located throughout the sampling season. Next, larval counts were converted to presence/absence data, and Pearson's chi-square tests were used to determine if there was an association between the presence or absence of larvae and the following variables: tide cycle, early or late summer, and TideSeason (R Core Team 2017). The term "TideSeason" refers to the combination of larval count data in the following four categories: early summer ebb (ESE), early summer flood (ESF), late summer ebb (LSE), and late summer flood. Early summer dates ranged from June 13 through July 18, 2017, whereas late summer dates ranged from July 27 through August 31, 2017; these dates divided the entire summer sampling season in half, with each half containing six sampling dates (e.g., ESE: $n = 3$, ESF: $n = 3$) (Table 1). A Fisher's exact test was used to investigate possible associations between location (lagoon, intertidal, or offshore) and larval presence because the contingency table values were unacceptably low for the chi-square function in R (R Core Team 2017). When appropriate, *post hoc* tests were run using Fisher's exact tests and a Bonferroni adjusted P value of 0.0167 to account for multiple pairwise comparisons (Sokal & Rohlf 2012). Kruskal–Wallis and *post hoc* Conover–Iman tests were used to investigate differences in the distribution of larval count by TideSeason (SYSTAT version 13; Sokal & Rohlf 2012).

The range of larval shell lengths was graphed by location and week to qualitatively describe change in the distribution of

larval sizes across space and through time. Larvae were binned into three size classes (180–200, 201–240, and >240 µm) based on observations in the SITC laboratory and changes in morphology generally found at different developmental stages (Hori 1933, Loosanoff et al. 1966, Shanks 2001). Although the literature on size at various development stages varies widely, size classes for this study were selected by considering numerous sources of published data and from observations on when the larvae in this geographic region were entering larger, late-stage development (Loosanoff et al. 1966, McIntyre 2018). Of particular importance, although McIntyre (Western Washington University, unpublished data) documented that size does vary with age, a correlation between size range and age is still evident, allowing researchers to estimate general age. Furthermore, the largest size class was selected based on work that described some larvae from northern Puget Sound with developed eye spots at 240 µm or larger (Lawlor & Arellano 2020). It is important to note that most larvae from this region appear ready to settle at shell lengths ranging from ~260 to 300 µm (Hori 1933, Loosanoff et al. 1966). This study opted to focus on the size at which larvae developed eye spots, which means the larvae were nearing metamorphic competency. From these larval size measurements, temporal developmental progression was predicted by calculating an estimated date for when the larvae were released from the parent oyster using a growth rate of ~4.7 µm/day. The estimated larval growth rate was calculated based on assumptions that larvae are 180 µm when released from the parent and that they reach a maximum size of 320 µm over a period of 30 days (Hopkins 1937). This calculated duration is referred to as "planktonic LD."

Quantifying Recruitment

Three recruitment sampler methods were used in this study: (1) "shell strings" (Allen et al. 2015, Becker et al. 2020), (2) shell bags (Dinnel 2016), and (3) modified "egg crates" (Hopkins 1937). Shell strings are currently the most widely used

recruitment sampler used in Puget Sound Olympia oyster restoration efforts (Allen et al. 2015, Dinnel 2016). To date, the use of shell strings to verify recruitment in SB has been unsuccessful, likely the result of the small scale of the SITC restoration project (2015–2017, SITC Fisheries Department, unpublished data). As a result, this current study used different recruitment samplers (shell bags and modified egg crates) in addition to shell strings to compare method effectiveness.

Shell strings were constructed by drilling a hole in 11 clean, UV-treated, Pacific oyster shells and stringing them onto a 60-cm wooden dowel, nacre-side down. The top 10 oyster shells were the settlement substrate, and the bottom-most shell was considered sacrificial as it was usually buried by fine sediment throughout the deployment period. Shell bags consisted of nylon mesh bags containing approximately 70 UV-treated unseeded Pacific oyster shells. Modified egg crates were built to take advantage of the Olympia oyster preference to settle on the underside of suitable substrate (shell, rocks, etc.) (Hopkins 1937). These structures were constructed of molded fiberglass dock grating consisting of 3.8×3.8 cm cells stacked two high and six across and deployed horizontally on the substrate surface.

All three recruitment sampler designs were deployed at each designated intertidal location (Fig. 1) on May 30, 2017 and collected on September 14, 2017. At each site, the recruitment samplers were deployed at +0.6 and +1.8 m NAVD88 to test for recruitment variability by elevation and location. After the deployment period, the shell strings, shell bags, and modified egg crates were examined for the presence of Olympia oyster recruitment under a magnifying glass and/or dissecting microscope.

Currents, Circulation, and Larval Transport

Measurements of nearshore circulation and transport were made through paired moving-boat acoustic Doppler current profiler (ADCP) and drifter studies. This effort aimed to gather information characterizing surface water transport processes and flow paths and did not have the capacity to evaluate all of the potential variability because of winds, waves, and runoff that affect estuarine circulation. To focus on processes likely affecting larval transport when larvae are exported from the lagoon, sampling emphasized periods of high tide, ebb, and initial flood tide relative to the elevation of the LTL sill.

Profiles of current speeds, directions, and backscatter intensity (a proxy for suspended particulate concentration) were collected using an ADCP along cross-shore transects of SB. One transect was located immediately south of the channel outlet at station LTN1 and two to the north at stations LTN3 and LTN4 (Fig. 1). A fourth transect was also sampled farther south between Lone Tree Point and Hope Island (LTS1) to characterize the dominant circulation entering and exiting the study area along its southern boundary. Measurements were made on May 26, 2017, 1 day before the maximum May spring tide, and June 27, 2017, 2 days after the maximum June spring tide. These sampling dates were selected to capture conditions influencing the maximum potential transport of Olympia oyster larvae during the peak periods in which the adults are known to be brooding late-stage larvae (presumably ready for release) (Barber et al. 2016). Weather conditions during circulation measurements were characteristic of summer, with calm winds

on May 26 and low west winds (<8 km/h) on June 27. Skagit River flow was normal for the season.

Data were collected using a SonTek RiverRay ADCP and differential GPS mounted to a portable catamaran boat with Bluetooth communications to a computer following standard U.S. Geological Survey methods to examine circulation and discharge (Grossman et al. 2007, 2018, Mueller et al. 2013). The ADCP sampled at a rate of 1 Hz, which translated to an average data point spacing of 1.25 m along a track at maintained boat speed (average of 4.5 km/h). The data were processed using SonTek WinRiver, U.S. Geological Survey Velocity Mapping Tools, and MathWorks MatLab software. Current speeds and directions were calculated after removing boat motion recorded by the GPS. All data were averaged by 0.5 m depth intervals and plotted along-track as 3D plots, with summary calculations of averaged speed and direction of the upper 2 m of the surface waters pertinent to the used larval sampling method.

Surface current data were also collected using GPS-tracking drifters on May 26, June 27, and during five of the weekly surface water plankton sampling sessions described earlier. Drifters were deployed at the confluence of the lagoon outlet (LTN1) to map the movement of water near the lagoon channel outlet. The 30-cm tall drifters were constructed out of PVC tubing and designed to float in the top 20 cm of water, tracking the surface currents with minimal air resistance (Austin & Atkinson 2004). On ADCP sampling dates, drifters were deployed and retrieved roughly hourly, coinciding with the duration of ADCP sampling periods. Drifters deployed during plankton sampling events were released before conducting the first plankton sample and collected roughly 45–60 min later. The Garmin 64s GPS units in the drifters were set to collect waypoints at 10-sec intervals for the duration of each deployment. Acoustic Doppler current profiler and drifter results were mapped in groups by time representing ~ 1 h, with a common scale to examine variability in flow among transects and in relation to circulation into and out of the bay across the southern boundary of the study area.

RESULTS

Surface Water Plankton Sampling

Sampling was conducted across sites in northern SB once a week for 12 wk, and *Ostrea lurida* larvae were recorded during each of the sampling weeks except June 27 and July 5, 2017. Larvae of *O. lurida* were found in the water column at all 10 of the sites sampled in this study at least once. Of the 86 total samples collected, 56 (or 65%) contained no larvae. Across all samples and dates, a total of 78 larvae (not standardized to larvae per 100 L) were recorded, reflecting the low relative abundance of larvae in the study area; succeeding numbers in this report are standardized to larvae/100 L. The exploratory site, SB3, which was only checked twice during the field season, had the highest larval density across all survey sites and dates (73.3 larvae/100 L) on July 27, when no other larvae were found at other sites (Table 1). When considering all other sites, the highest density of *O. lurida* occurred on August 9 at LTN5 (66.7 larvae/100 L; Table 1). The highest weekly mean density of larvae across all sites was recorded on August 9 (24.2 ± 7.0 SE larvae/100 L; Table 1). Excluding SB3 Pilot, the lowest cumulative mean density of *O. lurida* larvae by site was found over the

oyster bed in LTL at site LTL1 (1.1 ± 0.7 SE larvae/100 L), and the highest cumulative mean density was found at intertidal site LTN5 (17.8 ± 10.3 SE larvae/100 L; Table 1, Fig. 2). Lone Tree North 2 had the second highest cumulative mean density of larvae of all the intertidal sites (7.2 ± 3.4 SE larvae/100 L). The lowest mean densities at the intertidal sites were observed at the mouth of the lagoon outflow (LTN1; 3.3 ± 1.3 SE larvae/100 L)

and midway up the bay at LTN4 (3.3 ± 3.3 SE larvae/100 L). Within the lagoon, LTL2 mean densities across all dates were twice as high as those of LTL1 at 2.2 ± 1.7 SE (Table 1, Fig. 2).

Pearson's chi-square tests ($n = 86$ for all tests) showed that the presence of larvae was not associated with tide cycle (Chi-square = 2.51, $df = 1$, $P = 0.11$), early or late summer (Chi-square = 2.42, $df = 1$, $P = 0.12$), or TideSeason

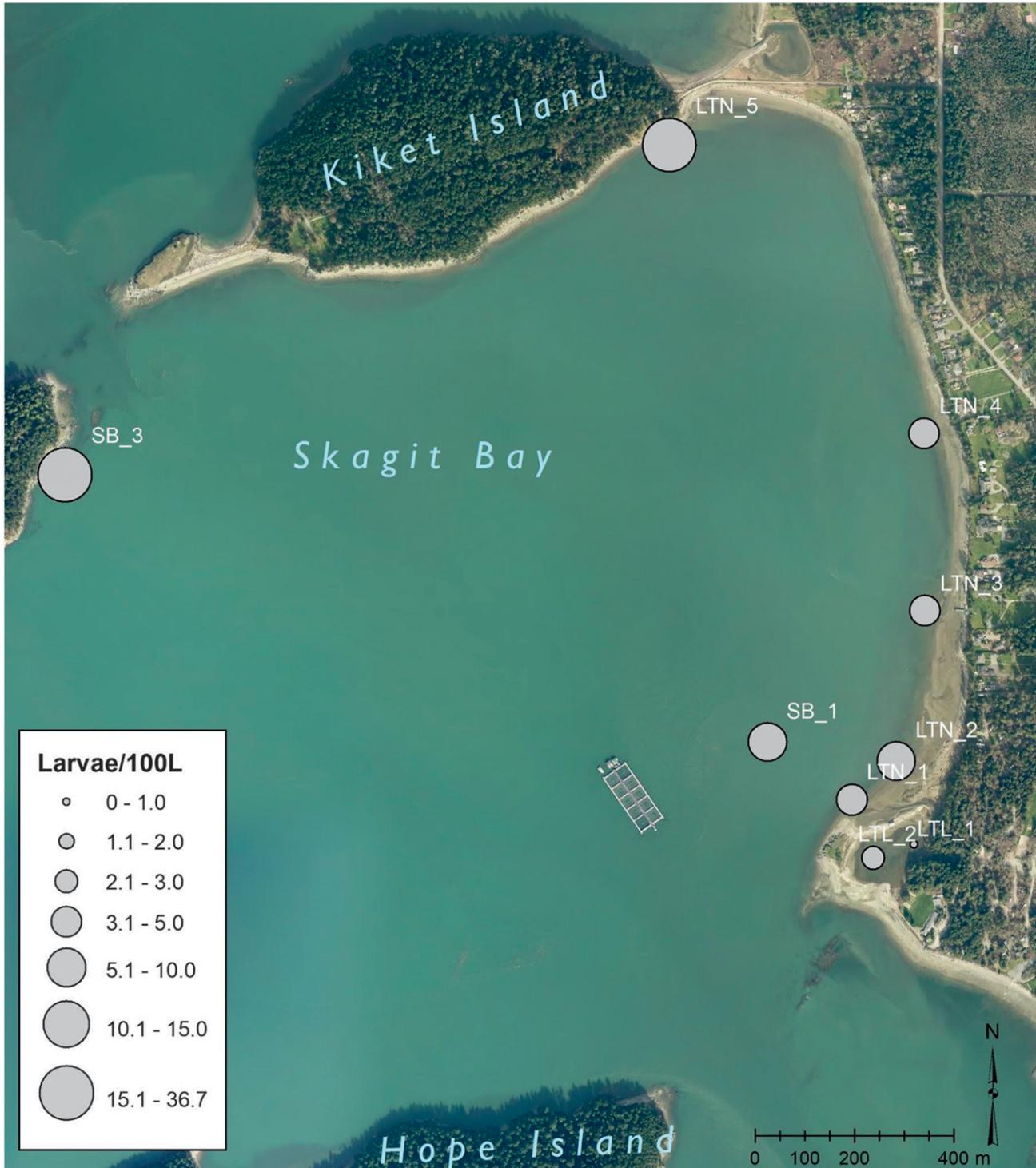


Figure 2. Cumulative mean density of Olympia oyster larvae/100 L at nine sampling sites throughout the 12-wk study period.

(Chi-square = 6.32, $df = 3$, $P = 0.09$). There was an association, however, between the presence of larvae and the sampling location ($P = 0.0137$). *Post hoc* pairwise comparisons indicate that the primary difference was between the lagoon and offshore sites, where larvae were more likely to be present at the offshore sites versus lagoon sites ($P = 0.005$). No difference was found between intertidal versus lagoon sites ($P = 0.168$) and intertidal versus offshore sites ($P = 0.07$). The Kruskal–Wallis results found a significant difference in larval count by Tide-Season (Chi-square = 8.295, $df = 3$, $P = 0.04$, Fig. 3). Conover–Iman *post hoc* tests found that more larvae were counted during the LSE versus the ESF ($P = 0.005$, Fig. 3). No other significant differences between TideSeason were detected.

Olympia oyster larvae represented a range of sizes and developmental stages at the lagoon, intertidal, and offshore sites throughout the study period (Figs. 4 and 5, Table 1). Sizes of *Ostrea lurida* larvae ranged from 182 to 263 μm ($n = 78$), with the majority of specimens between 200 and 240 μm (Fig. 5). Although no qualitative correlation was observed between size and sampling date (Fig. 4), overall the smallest larval specimen was found in mid-July and the largest was found in late August (Fig. 5, Table 1). Although larvae greater than 240 μm were found on the very first day of sampling, larger larvae appeared to be more prevalent in August (Figs. 4 and 5). Across all dates, the mean estimated planktonic LD at the time of sampling (based on the size of the larvae) was 9.5 ± 0.4 SE days and ranged from 0 to 18 days post-release (Fig. 6).

Quantifying Recruitment

No recruitment was observed on any of the three styles of recruitment samplers. Because of the lack of recruitment on all

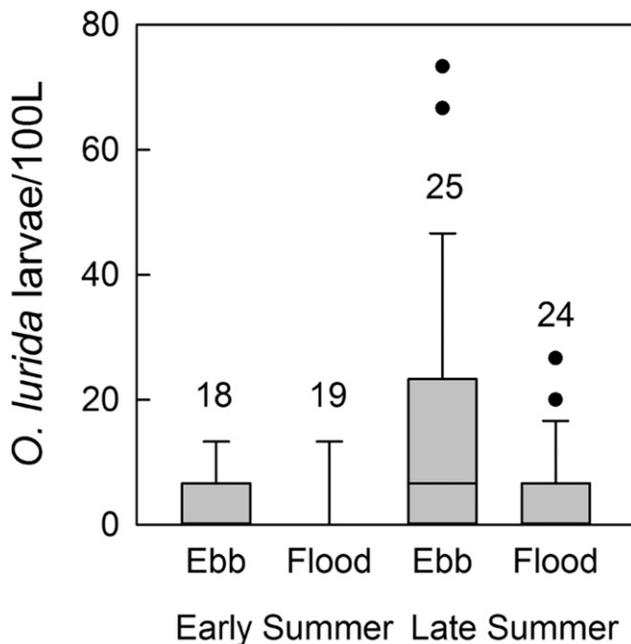


Figure 3. Distribution of the estimated number of *Olympia* oyster larvae/100 L by tidal cycle and season in 2017. Early summer = June 13–July 18, 2017; late summer = July 27–August 31, 2017. Numbers above box plots indicate sample size.

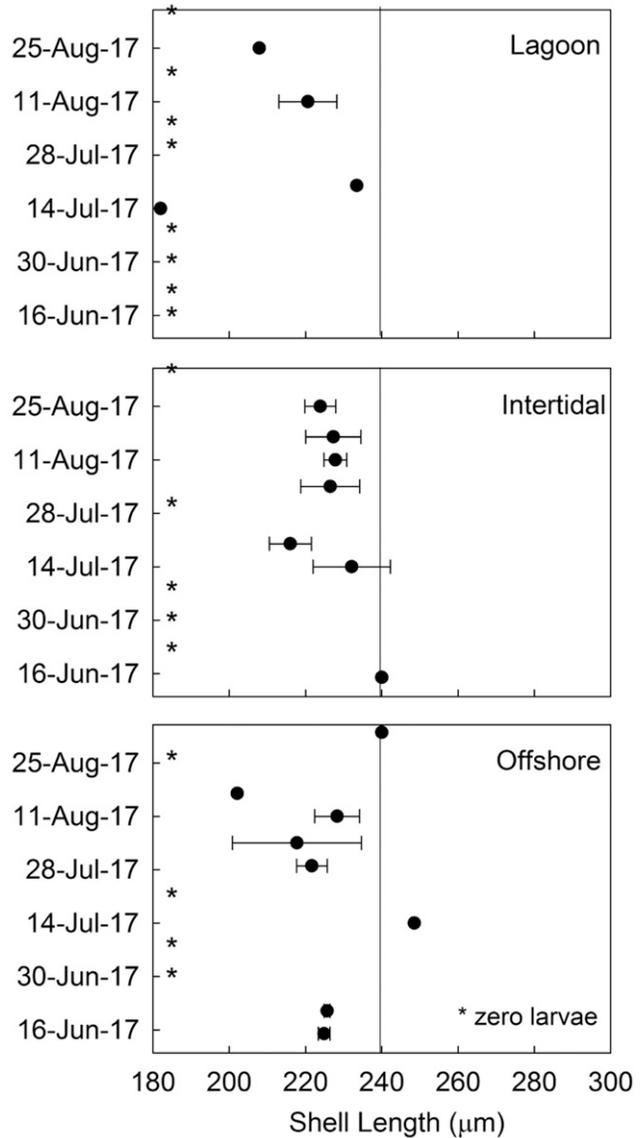


Figure 4. Range of shell lengths from *Olympia* oysters collected over the 12-wk period from June 13 to August 31, 2017 in lagoon, intertidal, and offshore sites in northern SB, WA. The line at 240 μm represents the size at which oysters in this region are likely nearing metamorphic competency.

shell strings and modified egg crates and the first 40% of shell bags, the remaining 60% of shell bags were not processed.

Currents, Circulation, and Larval Transport

Thirty-five ADCP transects covering ~25 km were made along four principal cross-shore transects on May 26, 2017 and June 27, 2017 over ebb and flood tides. Near-surface currents were estimated by averaging the upper 2.0 m of ADCP observations. These surface water velocities were mapped alongside observed GPS drifter tracks, illustrating circulation patterns for surface waters on two separate days (Figs. 7 and 8). Surface current speeds were generally higher offshore and across the southern boundary of the study area between Hope Island and Lone Tree Point (≥ 100 cm/sec) than nearshore (10–30 cm/sec). The degree of variability in current speeds and directions

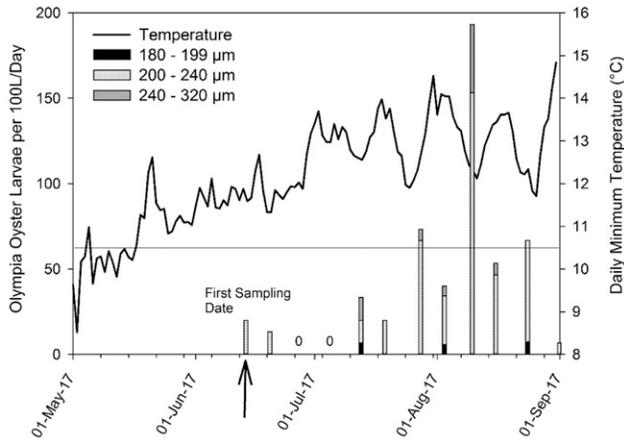


Figure 5. Total number of *Olympia* oyster larvae/100 L by sampling date binned into three size classes: 180–200 µm, 201–240 µm, and greater than 240 µm with daily minimum temperature in LTL. The horizontal line depicts 10.5°C, the water temperature above this represents the critical temperature at which spawning occurs in LTL. Arrow indicates the date larval sampling began.

depended on tide state and location, with evidence of higher speeds at depth than at the surface during mid-flood (e.g., Fig. 9A) and higher speeds at the surface during high tide to initial ebb (e.g., Fig. 10A). Pronounced vertical and horizontal shear and flow reversals were found at many tide states. For example, southerly flow was recorded within 100–200 m of the shore during mid- to late-flood and high tides (Figs. 7–9, green and yellow colors in Fig. 10B) and initial ebb (Fig. 8-2, green and yellow colors in Fig. 10B). During both of these tide states, offshore flow was directed north (blue and red colors in Figs. 9B and 10B). Despite relatively low current speeds in the shallow

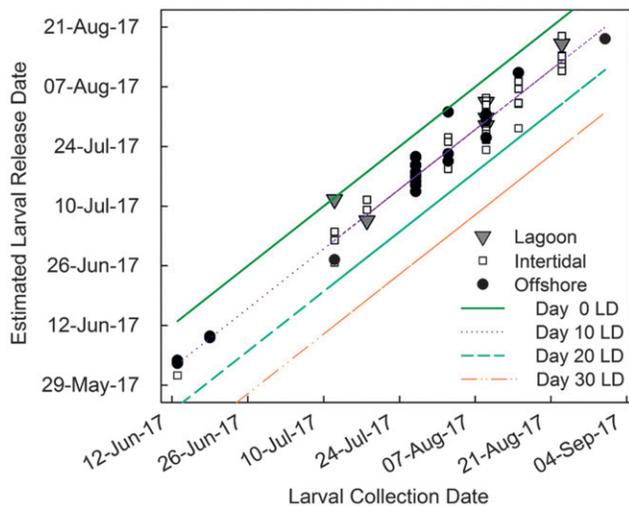


Figure 6. Plot of estimated larvae release date versus collection date by site. Duration milestones from 0 (upper most) to 30 days in 10-day increments are depicted with lines. This plot predicts the amount of time the collected larvae ($n = 78$) had been in the water based on larval size and if those larvae were near or at metamorphic competence (~240–260 µm for this study). A planktonic LD of 0 days represents larvae at ~180 µm, 10 days ~227 µm, 20 days ~274 µm, and 30 days ~320 µm.

nearshore (ranging from 5 to 20 cm/sec; Figs. 9A and 10A), these areas were consistently characterized by high acoustic backscatter (reflecting high turbidity) and, presumably, the resuspension of fine sediment (Figs. 9C and 10C).

From mid-flood tide through high tide and to midebb, northerly surface flow into the study area from SB was associated with a general clockwise pattern of flow in northern SB and along the shoreline north of LTL (Figs. 7-9, 8-1, 8-5, and 11). The zone of southerly flow along the LTL shore appeared to expand farther offshore as the clockwise gyre developed (Fig. 8-2, 8-4). During strong northward flow into the study area from the south, a small eddy was observed in the vicinity of Lone Tree Point that directed flow alongshore toward and sometimes north of the lagoon outlet (Figs. 7-1, 8-4, 8-6, and 11). Conversely, from midebb to the early flood, a strong southern flow at the boundary between Hope Island and Lone Tree Point was associated with a general counterclockwise pattern of flow (Figs. 7-4, 7-8 and 11). During this period of the tide cycle, circulation offshore of the LTL channel was directed northward, with what appears to be an eddy offshore of Lone Tree Point directing flow southward close to the shore (Fig. 11). Between these periods, there are incidents close to shore when flow in the north was oriented in the opposite direction from flow in the middle or southern portions of the study area (Figs. 7-2, 7-5 and 8-6), perhaps reflecting shear and mixing as the larger scale eddies evolved.

Drifter results were consistent with circulation patterns observed in the ADCP data, illustrating possible surface water trajectories for oyster larvae. In the vicinity of the LTL channel, drifter data showed a relatively strong offshore flow toward the west-northwest during the initial flood and through the high tide into early ebb (Figs. 7-7 and 8-1, 8-4). Immediately offshore of the LTL shorelines, a relatively strong northward alongshore pattern was observed during early to midebb (Fig. 8-3, 8-6). As flow out of the bay to the south and a counterclockwise gyre strengthened, the offshore component of flow from the LTL channel mouth also appeared to increase (Fig. 7-2, 7-8). When shear zones and divergent flow were observed, drifters displayed more alongshore trajectories (e.g., Fig. 7-1, 7-2, 7-5). These transitional periods likely contribute to subtle differences in alongshore and across-shore transport patterns.

DISCUSSION

These results represent important advancements in the understanding of *Ostrea lurida* recruitment limitations in SB. Specifically, *Olympia* oyster larval density was found to be extremely low in SB [maximum 66.7 larvae/100 L in this study versus ~1,570 larvae/100 L in nearby Fidalgo Bay (Hatch et al. 2018)] likely because of the relatively small scale of the SITC restoration efforts. Regardless of the low density of larvae, this study is the first to quantify the presence of *Olympia* oyster larvae in SB. In addition to confirming the presence of larvae at one of the 19 target restoration sites in Washington state (Blake & Bradbury 2012), these results demonstrate that larval density varies spatially and temporally in complex ways within the study area. By describing the transport pathways in the vicinity of the restoration site, this project is now better able to determine recruitment potential within the region and target habitat enhancement efforts in specific areas that appear to have a higher probability of late-stage larval delivery.

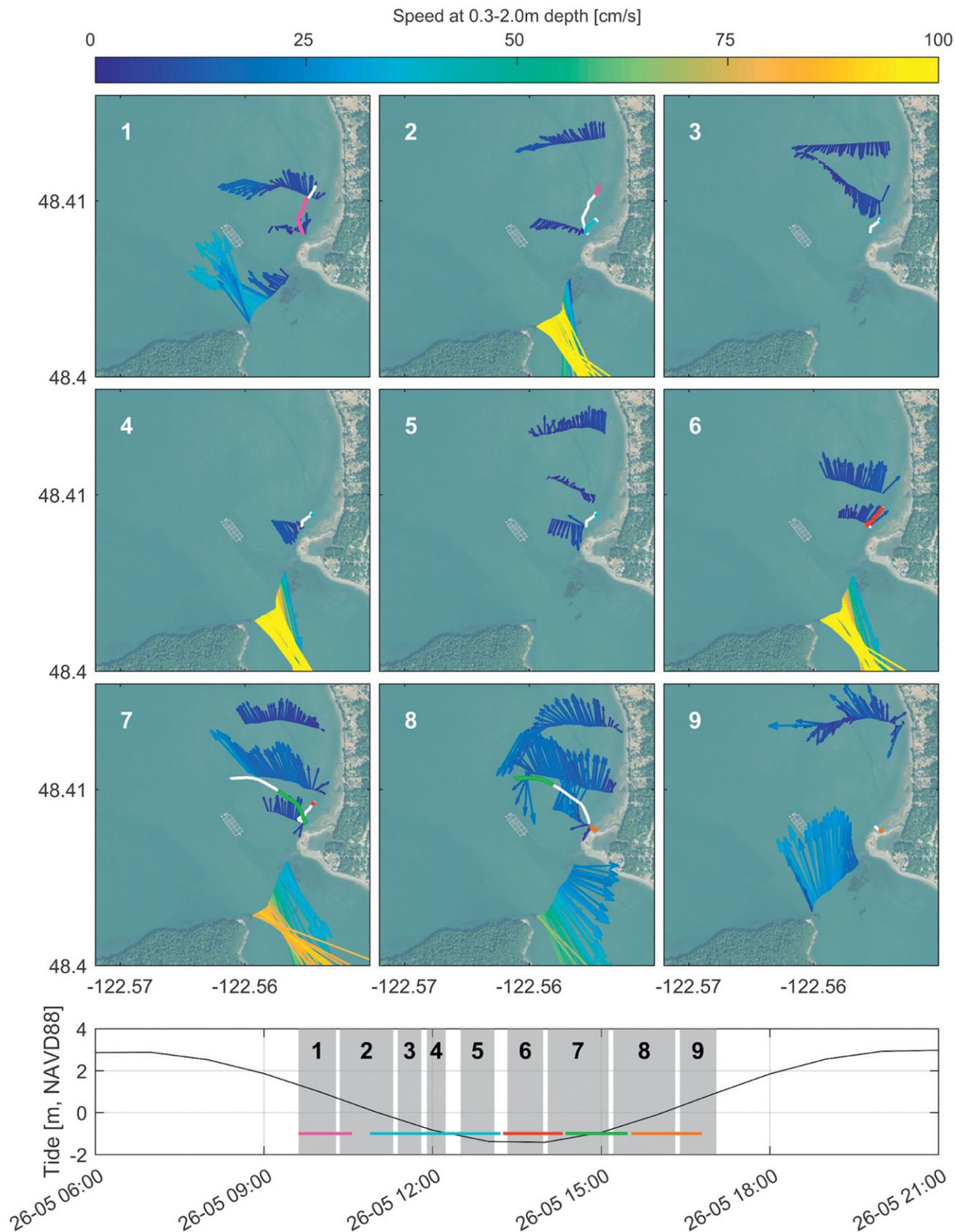


Figure 7. Map showing surface (0–2 m depth) averaged flow directions and speed from moving ADCP measurements and drifter tracks in northern SB for the nine time periods sampled during spring tide conditions on May 26, 2017. Corresponding time periods are denoted as numbers on the tide locator plot. Note that drifter results are colored by time span in the tide locator plot, with white lines showing cumulative track path when spanning multiple ADCP deployments. Latitude and longitude are shown as the y and x axes, respectively.

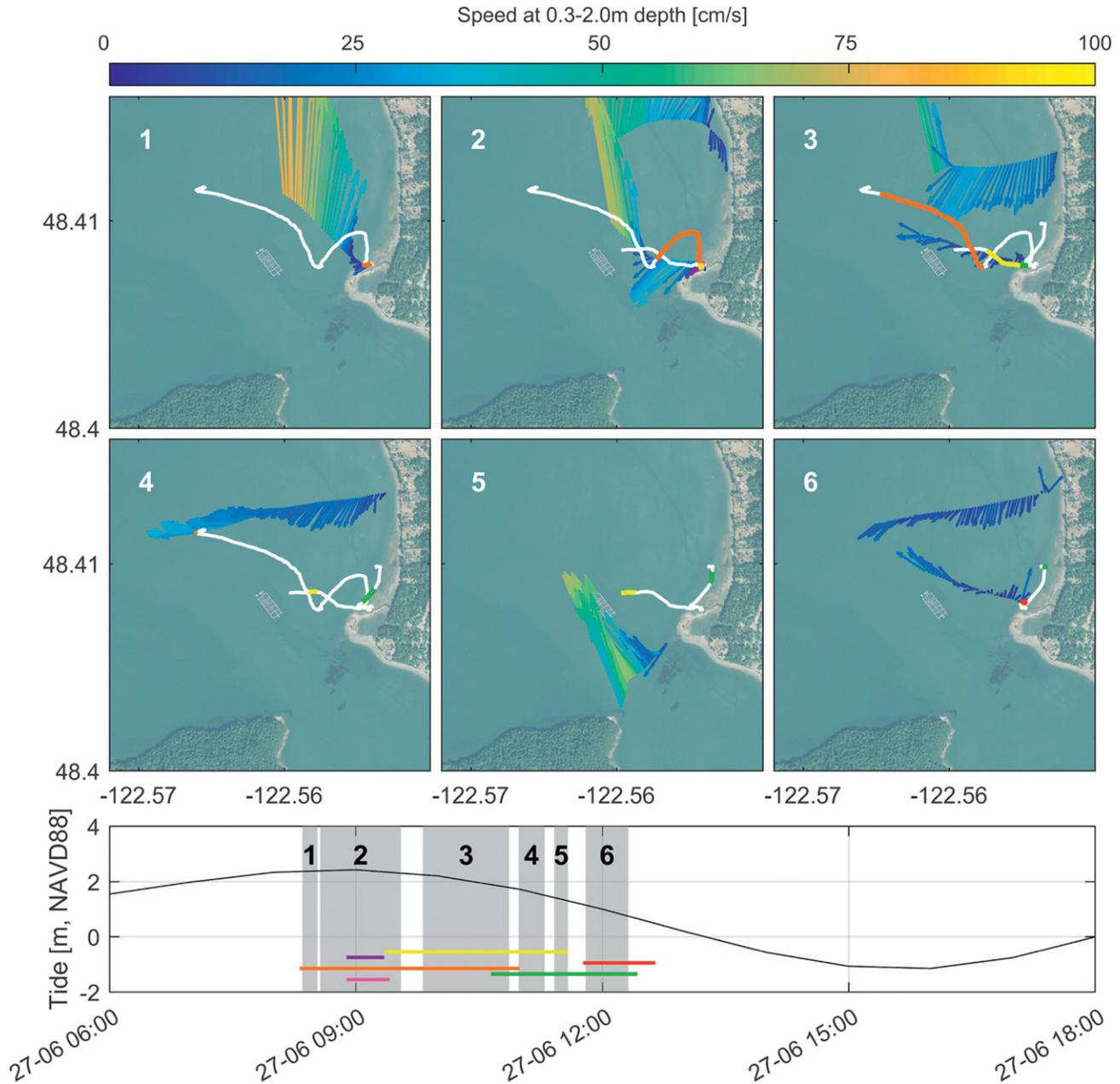


Figure 8. Map showing surface (0–2 m depth) averaged flow directions and speed from moving ADCP measurements and drifter tracks in northern SB for the six time periods sampled during spring tide conditions on June 27, 2017. Corresponding time periods are denoted as numbers on the tide locator plot. Note that drifter results are colored by time span in the tide locator plot, with white lines showing the cumulative track path when spanning multiple ADCP deployments. Latitude and longitude are shown as the Y and X axes, respectively.

Surface Water Plankton Sampling

Results suggest that summer 2017 was characterized by an initial wave of larval release that produced relatively lower densities than a second, stronger larval pulse from mid- to late summer. After June 19, 2017, a 2-wk period was observed with zero sampled larvae until July 12 when the beginning of the second pulse of larvae was recorded. This pulse lasted 8 wk, peaking in mid-August before tapering off. The timing and duration of these two larval pulses were not unexpected, given that Barber et al. (2016) found that LTL Olympia oysters

brooded from early May to early August, peaking in late May to early June. This timing coincides with records of larval presence in SB in this study. Furthermore, Olympia oysters in cooler northern waters, such as those in Puget Sound, have been found to spawn one to two times over a period of approximately 6 wk (Hopkins 1936, Couch & Hassler 1989). Indeed, newly released larvae (<200 μm) were found during three distinct time periods in this study, indicating that the oysters were still releasing larvae as late as August 2, 2017.

Larval presence was not associated with tide cycle, early versus late summer, or TideSeason, but a relationship was

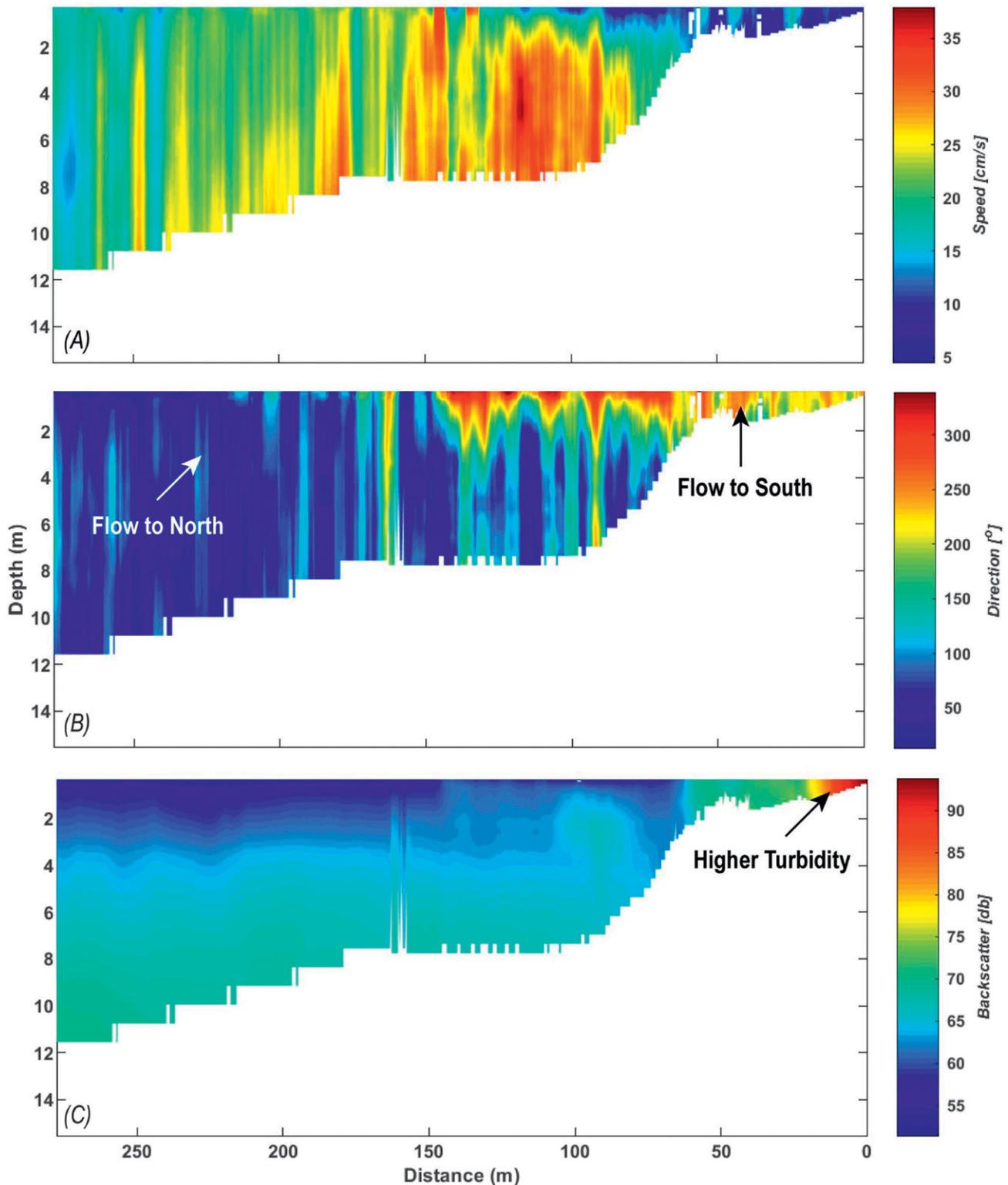


Figure 9. Cross-shore vertical profile of (A) current speed, (B) direction, and (C) backscatter intensity along Transect LTN1 offshore of the Lone Tree channel mouth during mid-flood of May 26, 2017. Results show flow reversal with southerly flow nearshore (green and yellow colors in B) and northerly flow offshore (blue and red colors in B). Note high backscatter nearshore (C) occurring with relatively low current speeds ranging 5–20 cm/sec (A).

described between larval presence and location (i.e., lagoon, intertidal, and offshore). Specifically, larvae were more likely to be present offshore versus in the lagoon. Although it was surprising to find very few larvae in the water column directly over

the established oyster beds inside LTL, there are plausible explanations for the observed higher offshore densities. First, the majority of newly released larvae could have been overwhelmed by current speeds in the lagoon channel and exported from the

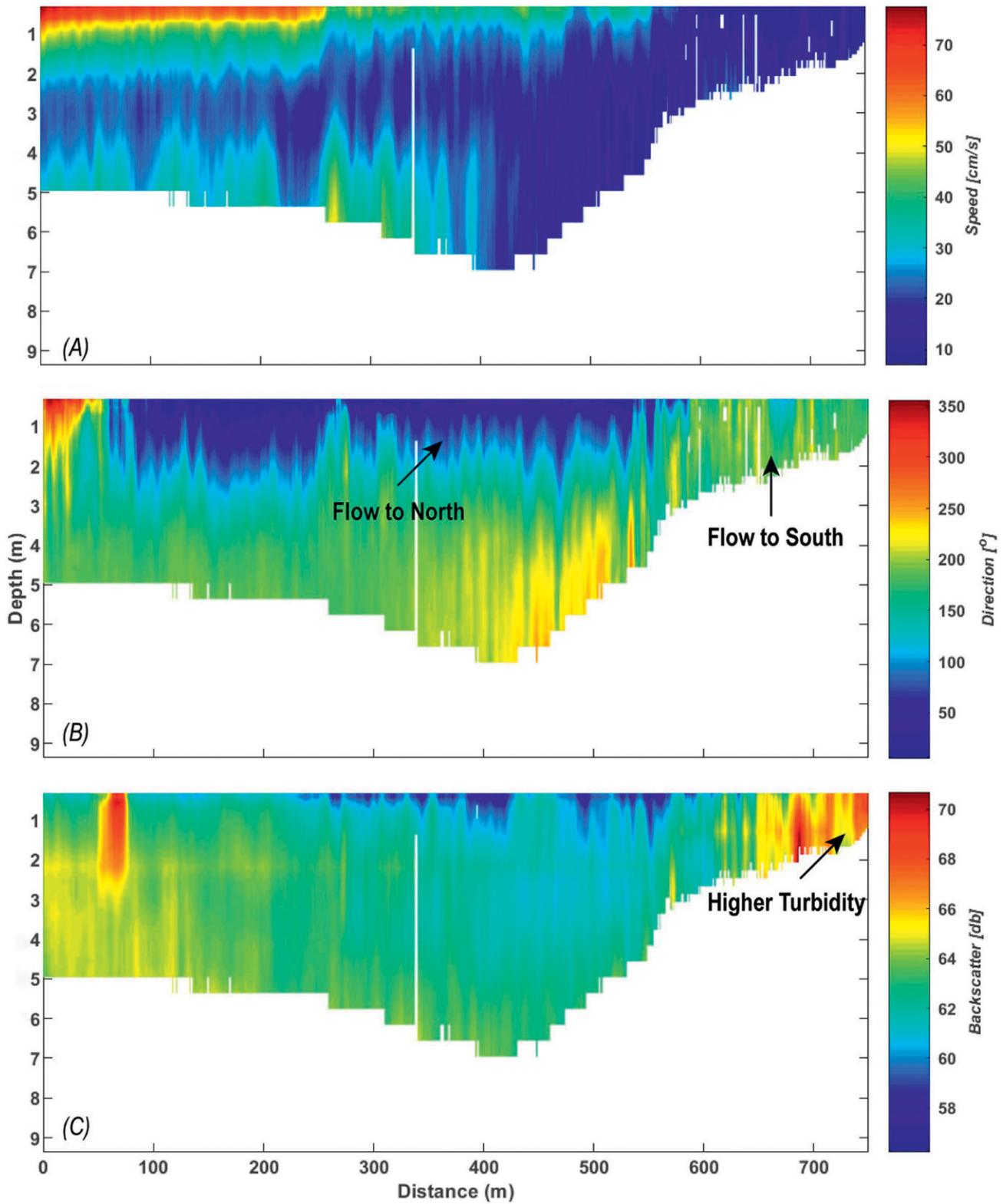


Figure 10. Cross-shore vertical profile of (A) current speed, (B) direction, and (C) backscatter intensity along Transect LTN4 during high tide and initial ebb of June 27, 2017. Results show flow reversal with southerly flow nearshore (green and yellow colors in B) and northerly flow offshore (blue colors in B). Note high backscatter nearshore (C) occurring with relatively low current speeds ranging 5–20 cm/sec (A).

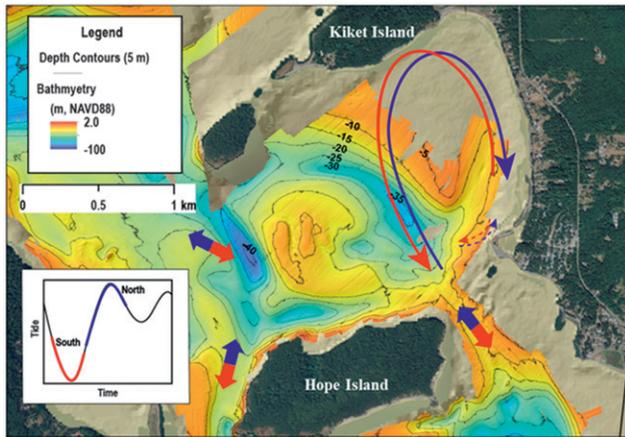


Figure 11. Map showing bathymetry, tidal exchange pathways into and out of the study area (double-headed blue–red arrows), and observed surface flow patterns (solid lines) and eddies (dashed) with respect to tide state (inset). Red and blue colors in the tide status inset correspond with the red and blue lines in the main figure.

lagoon to surrounding waters (e.g., swept northward during initial ebbs to the offshore sites). But larvae, including developing to late-stage larvae with an estimated planktonic LD of 11 days, were still recorded within the lagoon. It remains unclear whether these developing to late-stage larvae were retained in the lagoon or if they were transported back in from the bay during a flood tide. Second, because of the sill elevation in LTL, the water level was typically much lower and slower moving when sampled for plankton during floods than during ebbs. Because McIntyre (2018) found that *Olympia* oyster larvae are distributed deeper in slower moving water (the McIntyre study sampled up to 4 m depth), it is possible that larvae were entrained within the oyster bed during these low water events and the plankton pump used in this study could not sample them properly. Interestingly, of the four times larvae were found in LTL, three were found during early ebbs (i.e., higher water level).

Larval counts were also found to be higher during LSEs versus ESFs (Fig. 3). Regardless of the tide stage, this is not surprising simply because the density of larvae increased dramatically as the summer progressed (Fig. 5). Although there was no statistical difference between ESE versus ESF or between LSE versus flood, the pattern of slightly higher larval densities on ebbs versus floods (during both early or late summer) is intriguing and merits further investigation (Fig. 3). Future studies, with larger sample sizes, could examine possible relationships between larval count and water flow/direction to determine if there are stronger predictors of larval abundance not considered in this research. This study was not designed to research vertical migrations in *Ostrea lurida* larvae in SB; therefore, it is important to remember that these results focus on only the presence of larvae in the top 1.5 m of the water column. Nevertheless, it is interesting that higher concentrations of larvae were found in surface waters during the ebb, whereas Peteiro and Shanks (2015) describe tidally timed vertical migrations in Coos Bay, OR, with larvae higher in the water column during flood tides. Thus, this study hypothesizes that SB larvae located in the first 1.5 m of water were either (1) entrained

in the bay by the clockwise current that develops during initial to mid ebbs or (2) caught in a counterclockwise current during the late-ebb and initial flood, transported south out of the study area, and unable to reenter the northern area of the bay.

In addition to improving the understanding of factors that influence the presence and/or density of larvae, SITC was interested in describing qualitative change in larval size across space and through time. Because the population of oysters in LTL is the only known population of *Olympia* oysters in SB, larvae in the smallest size class were assumed to have been recently released from adults in LTL. Therefore, it was not surprising that the smallest larva was found in LTL. But these results also demonstrate that small larvae can be easily exported from LTL to the offshore site (SB1), where the second smallest larva was collected. No qualitative relationship was detected between size range and site (e.g., there was no obvious size class at the offshore site compared with a different size class at the intertidal sites) or size range and time (e.g., there was no obvious early-season cohort in the early summer followed by a different late-season cohort in the late summer). This observation makes sense because larvae were probably released throughout the summer and younger larvae likely mixed with older larvae in the water column.

The absence of larvae greater than 263 μm in this study is not entirely surprising, given that smaller larvae are known to concentrate on the surface more often than larger larvae, which are more likely to be recorded near the bottom (Becker et al. 2020). Nonetheless, it is interesting that McIntyre (2018) found larger larvae in near-surface waters in Fidalgo Bay, whereas no larvae greater than 263 μm were found in this study. *Olympia* oyster larvae greater than 260 μm from this region have been recorded probing the surface of laboratory petri dishes with their feet and appear ready to begin the process of settling (McIntyre, Western Washington University, 11/2019, personal communication). Thus, in SB, it is possible these competent larvae were located closer to bottom substrates and out of reach of the larval pump. Bivalve larvae, particularly *Olympia* oyster larvae, are also known to close their valves and sink in the water column when disturbed (Stafford 1913, Arellano & McIntyre, Western Washington University, 11/2019, personal communication). Because the plankton pump sampling technique used in this study was limited to 1.5 m depth, it is possible that these larger larvae were disturbed and initiated movement to depths deeper than the pump. Of course, larger larvae may not have been observed if those greater than 260 μm larvae had already settled or if the overall population density was too low to detect those larger larvae (because larval population would be lower at larger sizes because of increased mortality). Furthermore, if larvae are moved largely passively, perhaps large late-stage larvae are simply heavier and end up distributed relatively deeper than the smaller larvae. Any hypothesis that suggests larger larvae may be located closer to the bottom can be supported by the fact that McIntyre (2018) found very few larvae greater than 260 μm in nearby Fidalgo Bay, where it is known that a large portion of the larvae are retained and successfully recruit to the substrate (Dinnel 2016). Finally, the strong circulation conditions observed in the study area likely contributed to a gradual export of larvae out of the region during each tide cycle. This potential winnowing of the larval pool would likely explain why the majority of the larvae found were small to medium sized and the larger size class was virtually absent.

Olympia oyster planktonic larval development rates can vary depending on post-release environmental conditions and food resources (Stafford 1913, Becker et al. 2020, Lawlor & Arellano 2020). The mean estimated planktonic LD of 9.5 ± 0.4 SE days (Fig. 6) from this study suggests that most of the larvae ranged from newly released to late-stage larvae and roughly corroborates with an estimated LD of 1 wk in Fidalgo Bay (Becker et al. 2020). But time to complete development from D-stage veligers to viable settlers can differ from 1 to 8 wk (depending on the study), a wide time range that can result in variability in morphological development (e.g., larval size and/or age when gill and foot development occurs) (as reviewed in Pritchard et al. 2015). Recently, brooding Olympia oysters from Fidalgo Bay were transferred from the field to the laboratory, and their newly emerged larvae were cultured for 28 days for tracking their growth (McIntyre, Western Washington University, unpublished data). In this study, larvae showed considerable variation in size among individuals, even from the same brood, with larval lengths ranging from 205 to 232 μm at 7 days and 219–300 μm at 21 days following release. Although these data suggest that some correlation between size and age is evident, variability in larval growth rates was still high, suggesting it is difficult to estimate LD based on size alone. Alternatively, there are numerous possible latent effects of suboptimal environmental conditions occurring over brooding and LD that could prohibit a successful transition from larval stage to settlement (e.g., Lawlor & Arellano 2020). As noted previously, the oysters in LTL spawn at cooler temperatures than oysters from previously published studies (Barber et al. 2016). Olympia oysters spawning at lower temperatures can take up to 8 wk to develop larvae after the detection of gametogenesis; this increased time in the mantle could be energetically taxing to both the parent and the larvae (Santos & Chew 1992). Also, low food and reduced pH conditions have been shown to reduce larval growth and delay metamorphosis, increasing larval time in the water column and/or potentially increasing mortality rates (Hettinger et al. 2013, but see Lawlor & Arellano 2020). The LTL restoration site is consistently inundated with reduced pH and salinity conditions (SITC Fisheries Department, unpublished data), especially during the early spawning season when the Skagit River exhibits peak discharge (Babson et al. 2006). Improved understanding of the variability of environmental conditions might help determine whether pocket estuaries (lagoons) are conducive to the long-term survival of the Olympia oyster beds. Perhaps the lagoons exhibit conditions that are too energetically expensive for successful larval development and recruitment at sufficient numbers to maintain adult populations.

Quantifying Recruitment

The complete lack of settlement observed on all three styles of recruitment samplers was not entirely surprising, given the current size of the SB Olympia oyster population and the actual number of larvae recorded in this experiment. Other Olympia oyster restoration projects in Puget Sound have been successful in capturing recruits using shell strings and shell bags (Dinnel 2016, Hatch et al. 2018, Becker et al. 2020), as well as modified egg crates (Hopkins 1937). In Fidalgo Bay, Dinnel (2016) noted a relationship between years of failed settlement and the lack of recruitment to collection devices. It is also not

uncommon for sites across the range of Olympia oysters to experience periodic recruitment failures (Wasson et al. 2016).

Currently, it is unknown whether Olympia oyster larvae are responding to settlement cues (e.g., hard substrates or existing populations) or if circulation patterns in estuaries with successful recruitment are more favorable for local larval retention. Olympia oysters have been shown to preferentially settle on hard substrates (e.g., shell, rocks, wood, and anthropogenic debris) at lower intertidal/upper subtidal elevations where water properties are relatively stable through tidal cycles (as reviewed in Pritchard et al. 2015). Becker et al. (2020) provide evidence that conspecific presence and water flow serve as important settlement cues in Fidalgo Bay. Also, larval dispersal distance in Puget Sound Olympia oyster populations is generally thought to be low, as recruitment densities have been shown to decrease with increasing distance from Olympia oyster beds (Dinnel 2018). Although larvae greater than 240 μm were present in the water column in areas where recruitment samplers were located in this study, larval presence does not guarantee successful settlement and metamorphosis if suitable habitat cannot be located (as reviewed in Pritchard et al. 2015). These results indicate that larvae are exported from the lagoon habitats (where the restored beds are located) into SB where, at the time of this study, there was little to no naturally occurring preferential settling habitat.

It is also plausible that significantly higher larval densities are needed to observe recruitment. As of 2017, the SB Olympia oyster population was estimated at $\sim 82,000$ individuals, of which 61,000 were outplanted as hatchery seed during the winter of 2016 (SITC Fisheries Department, unpublished data). Recruitment was observed to be higher at the nearby Fidalgo Bay restoration site after the population size exceeded 100,000 individuals (Dinnel 2018). If it is hypothesized that larval density is related to adult population size, it is interesting that extremely small Olympia oyster populations such as those in Elkhorn Slough, CA ($\sim 5,000$ individuals), show many years with little to zero recruitment (Wasson et al. 2015), whereas a larger population in Port Gamble Bay, WA (~ 1.47 million individuals), has exhibited regular recruitment since 2014 (PSRF 2017). Also, as the Fidalgo Bay adult population increased (>2.9 million individuals, circa 2018), recruitment was observed at locations both farther from the restoration site and in lower quality habitats. Dinnel (2018) also noted that the addition of new shell plots in intertidal areas rapidly attracted a significant number of settlers, demonstrating how Olympia oysters are likely habitat-limited in Fidalgo Bay. Results from Dinnel (2018) highlight the need for increasing both the size of the Olympia oyster population and the area of available habitat in target restoration sites to increase larval density and, hopefully, chances of successful sustained recruitment to the nearshore environment. Thus, it is hypothesized that the combination of low larval density, strong currents, inadequate settlement cues, and/or lack of suitable habitat was likely responsible for the lack of settlement on the recruitment samplers during this study.

Currents, Circulation, and Larval Transport

Olympia oyster larvae are slow swimming but capable of propelling themselves in the water (~ 0.1 cm/sec swimming speed) and exhibit evidence of tidally timed vertical migrations in an Oregon estuary (Peteiro & Shanks 2015). Specifically,

Peteiro and Shanks (2015) found that *Olympia* oyster larvae were located deeper on ebb versus flood tides in an estuary with vertical variability in current velocities (similar to SB). The same study also described how these weak swimmers could easily be overcome by strong currents. Interestingly, a study conducted in Fidalgo Bay found different results in that larvae were more likely to be located shallow at higher current velocities and deeper at lower velocities, with no evidence of tidally timed vertical migrations; it remains unknown if the larvae were recorded at these depths because of active or passive behaviors (McIntyre et al. 2020). Despite contrasting results between these studies in Washington and Oregon, it seems unlikely that *Ostrea lurida* larvae would behave like passive particles in the water column unless their swimming abilities were overcome by strong currents. Thus, because (1) larvae appear to behave dissimilarly across regions and (2) data do not exist on the vertical distribution of larvae in SB, water transportation processes had to be considered from a passive particle perspective for this study, recognizing that *Olympia* oysters are relatively weak swimmers.

Current and drifter data revealed a complex alternating pattern of circulation across northern SB. Proximal to Lone Tree Point (Fig. 1), the larger scale alternating clockwise and counterclockwise patterns of flow and small eddies are consistent with the interaction of strong currents driven by tides and winds entering the study area from southern SB and Deception Pass. These results are also likely consistent with the complex bathymetry and shoreline morphology in the study area (Fig. 11) (Grossman et al. 2018). Specifically, the deep circular troughs around Hope and Kiket Islands would be expected to direct flow in a clockwise pattern as northward flood initially enters the study area from the southeast and is progressively influenced by flow from the west around Hope Island and from Deception Pass. This pattern is consistent with previous circulation studies in the region that showed an asymmetric surface flow directed northward during flood, high tide, and early ebb that lasted ~60%–70% of the time (Grossman et al. 2007). The associated clockwise surface flow observed during this study period is also consistent with the clockwise flow noted by Stober and Salo (1973). Adding to the complexity of these results are the intermittent jets of flow and shear zones offshore of the beaches north of the lagoon; these are likely to influence and contribute to vertical mixing. Subtle variability in the onshore and offshore components of alongshore flow was noted and would likely be strongly affected by winds and waves that were not sampled in this study.

The alternating pattern of clockwise and counterclockwise flow that, respectively, set up northerly and southerly flow immediately alongshore the outlet of LTL could have important implications for larval transport, residence time, and, ultimately, locations of highest potential for larval recruitment to the nearshore. Although these results were insufficient for estimating the full range of potential residence time and transport patterns of water in the study area, surface transport distances were calculated for the observed current velocities and directionality. The alongshore current velocities observed indicate that water parcels can be transported 0.5–8.0 km during each flood and ebb cycle. This suggests that passive particles may be transported north of the study area beyond Kiket Island and into Similk Bay during northerly flow, and south into greater SB during southerly flows.

As the sill into LTL is perched at +1.2 m NAVD88, larval export from the lagoon is more likely during the ebb when water flows out of the lagoon, than when waters flow into the narrow lagoon channel during the late flood. Depending on when larvae are exported from LTL during the ebb, there are two potential trajectories that could occur (in combination or singularly): (1) during the initial to midebb, conditions seemingly favor entrainment and longer larval residence time within the study area (Figs. 7-1, 7-2 and 8-2, 8-6) and/or (2) during the late-ebb, conditions may entrain larvae in the strong counterclockwise southerly flows leaving SB (Fig. 7-4, 7-8). Regarding the first potential trajectory, larvae exiting LTL could either be swept into the small alongshore northward gyre or the larvae could be exported to the west and then entrained in the larger clockwise gyre; either of these outcomes would be favorable for larval retention. In terms of the second potential trajectory, larvae transported from LTL are likely to either be swept west into the alongshore gyre and moved south into the stronger currents, or the larvae could be exported to the north in the counterclockwise gyre and eventually exported to the south; either of these outcomes may not be favorable for larval retention in the study area. Future studies could investigate when in the tidal cycle the larval export from LTL is the greatest to determine which of these trajectories are more likely to contribute to the results described in this study.

The presence of a range of larval sizes (182–263 μm) suggests that larvae were retained within SB (or they were exported and then reentered) for at least 18 days. Larval presence, particularly at the north end of the bay and the north side of Lone Tree Point (at the southern boundary of the shoreward eddy at LTL2, Fig. 2), further supports the hypothesis that hydrodynamic conditions are conducive for larval retention in SB. Furthermore, larvae retained in SB are more likely to reenter LTL (the restoration site) during mid-flood once the tide reaches +1.2 m NAVD88, when flow into the lagoon is highest. Given that observed densities of larvae were lower during flood tides at sampling sites, however, it is unlikely that many mature larvae were transported back to the restoration site once they were exported from the lagoon. Thus, future habitat enhancement should instead focus on the beaches to the north of the LTL channel where larvae may be entrained during initial to midebbs. Notably, the sampling method in this study could not reach deeper waters, and, therefore, it remains unknown if larvae were concentrated at depth during specific tidal cycles. Second, the larval data were collected only during mid-flood and midebb tidal stages. Given the observed circulation patterns, it is plausible larval densities were higher at sites during slack tides; further studies could encompass multiple tidal stages.

Synthesis

The presence of larvae, even at low densities, in northern SB is encouraging for restoration purposes. This result is one of the most valuable outcomes of this study, proving that despite the relatively small size of the *Olympia* oyster population in this target restoration area, adults are successfully releasing larvae that are capable of developing to at least the size at which they become competent. Furthermore, combining these larval data with results on currents and circulation has allowed for the development of a description of potential larval transport

pathways in the surface waters of northern SB. Importantly, larval retention within the study area may be bolstered by the northward alongshore transport associated with the initial tideebb, when larvae are most likely to be exported from LTL. Whereas these results provide insight into some aspects of northern SB hydrodynamics, the study shows the importance of understanding fine scale processes affecting retention, dispersal, and settlement as well as large-scale circulation to inform local population enhancement. It is expected that complex current structures and mixing dynamics, outside of the realm of this study, affect flow and larval dispersal. A hydrodynamic model, able to resolve both spatial scales, could help estimate residence times within the domain important to evaluating potential spatial scales of restoration application and the extent that external factors influence restoration outcomes. This type of model, paired with recruitment sampling, could improve the ability of managers to assess the likelihood and fraction of larvae that are dispersed to potential recruitment sites across the area of interest. Future work would also benefit from an improved understanding of larval vertical migrations in SB and the extent to which larvae can physically overcome the relatively strong regional currents.

Finally, because Olympia oysters exhibit local adaptation (e.g., Bible & Sanford 2016, Heare et al. 2017, Silliman et al. 2018), SITC aims to increase the local native oyster population by habitat enhancement and natural recruitment within the embayment. These results will help identify future habitat

enhancement sites where later-stage larvae are more likely to concentrate in surface waters. For example, because transport processes described in this article are conducive for larval delivery from LTL to nearby northern beaches, SITC will target placing unseeded oyster shells at locations such as LTN2 and LTN5 (Figs. 1 and 2). The use of locally collected data to guide restoration efforts will, ideally, lead to a higher likelihood of individual project success.

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