Completion Report

Ruesink, Jennifer

Period: 2/1/2012 - 1/31/2013
Project: R/LME-3 - *Spatial and temporal factors influencing recruitment variability in estuarine bivalves*

:: STUDENTS SUPPORTED

Freshley, Nicole, nfreshley@gmail.com, Western Washington University, *no department*, status:cont, *no field of study*, *no advisor*, degree type:BA, degree date:2011-12-01, degree completed this period:No
Student Project Title: *none*

Involvement with Sea Grant This Period: *none*

Post-Graduation Plans: *none*

:: CONFERENCES / PRESENTATIONS

How Ocean Acidification Works, Columbia River Maritime Museum, public/profession presentation, 20 attendees, 2013-02-23
How ocean acidification works, Willapa Bay Interpretive Center, public/profession presentation, 10 attendees, 2012-07-09
National Shellfisheries Association, “Temperature and pH as drivers of oyster larval survival over 50 years of field data”, public/profession presentation, 70 attendees, 2012-03-25
Ecological Society of America, “Climate variability influences recruitment in a multi-decadal time series” (poster), public/profession presentation, 3000 attendees, 2012-08-06
Pacific Coast Shellfish Growers Association annual meeting “Substrate effects on manila clam settlement and survival", public/profession presentation, 150 attendees, 2012-09-25

:: ADDITIONAL METRICS

K-12 Students Reached:0

Acres of degraded ecosystems restored as a result of Sea Grant activities:0

Curricula Developed:0

Resource Managers who use Ecosystem-Based Approaches to Management:0

Volunteer Hours:50
Sorting sieved samples for clam recruits - UW undergraduate for research credit; retiree citizen scientist

HACCP - Number of people with new certifications:0

Cumulative Clean Marina Program - certifications:0

:: PATENTS AND ECONOMIC BENEFITS

Description	Patents	Economic	Businesses	Businesses	Jobs	Jobs

			Benefit ($)	Created	Retained	Created	Retained
Larval samples in Willapa Bay, Wash. in August 2011 led to predictions of a small settlement of Pacific oysters around August 20. Economic benefit estimate based on number of shell placed on tideflat (500,000) x 1 oyster/shell at maturity x $0.10 per oyster. R/LME-3	**Actual** (2/1/2012 - 1/31/2013) :	0	50000	0	0	0	0
	Anticipated (2/1/2013 - 1/31/2014) :	0	50000	0	0	0	0
Large August 2012 set identified in oyster bulletins, immediate benefit of reduced cost of needing to purchase 60,000 bags of hatchery seed ($5 each) for remote setting. R/LME-3	**Actual** (2/1/2012 - 1/31/2013) :	0	300000	0	0	0	0
	Anticipated (2/1/2013 - 1/31/2014) :	0	0	0	0	0	0

:: TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
Long-term dataset of hourly water temperature at Nahcotta, Willapa Bay, WA, provided to shellfish growers to inform operations. R/LME-3	**Actual** (2/1/2012 - 1/31/2013) :	1	1	Shellfish growers have "rule of thumb" activities associated with different water temperatures. This water temperature data set and oyster recruitment data set will be publicly available as archived data associated with planned publication.	10
	Anticipated (2/1/2013 - 1/31/2014) :	0	0		
Oyster bulletins for Willapa Bay shellfish growers detailing location, abundance, growth of different bivalve larvae for use in deciding when to deploy settlement substrate. R/LME-3	**Actual** (2/1/2012 - 1/31/2013) :	1	1	Shellfish growers, state shellfish manager for the Willapa Bay Oyster Reserves (WDFW). 40 reported only count 30 here, 10 captured above	30
	Anticipated (2/1/2013 - 1/31/2014) :	0	0		

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

:: ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2012 - 1/31/2013) : 20
Anticipated (2/1/2013 - 1/31/2014) : 0
Shell placed for oyster set on August 18, 2012 by approximately half the companies in Willapa Bay and by WDFW on the Oyster Reserves. This is a one-time modification because larval sampling is not funded in 2013.

Number of fishers using new techniques
Actual (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0

Sustainable Coastal Development
Actual (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0

Coastal Ecosystems
Actual (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0

:: PARTNERS

Partner Name: Washington State Department of Fish and Wildlife
Partner Name: Washington State University Extension, Pacific County, type: academic, scale: state

:: IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant-funded research reveals the effects of invasive eelgrass and warming waters on clams and oysters in Washington's Willapa Bay**

Type: accomplishment
Description:
Relevance: Washington's Willapa Bay is one of the most productive shellfish-growing areas in the United States. But several environmental factors, including rising water temperatures and the growth of nonnative eelgrass, may affect future harvests. The Pacific County and Washington State Noxious Weed Boards sought information about the impacts of nonnative Zostera japonica to help determine whether to approve control measures.
Response: Washington Sea Grant-supported researchers studied clam recruitment in various Willapa Bay habitats and compared settlement and post-settlement mortality in nonnative eelgrass and in unaffected habitats, and with and without sediment enhancements such as gravel and shell. They also compiled data on water temperatures and oyster recruitment from 1942 to 2012 and used structural equation modeling to evaluate the relationship between them.
Results: The researchers found that over the long term Manila clams were more abundant on graveled substrate than in areas colonized by Zostera japonica. They also concluded that Pacific oyster production benefitted from warmer waters. On average, warming water has advanced Pacific oyster recruitment by one day per decade, and warmer years result in both earlier and larger recruitment. Temperature changes had no discernible effect on the recruitment of native Olympia oysters.

Recap:
Washington Sea Grant-supported research shows that Manila clams fare better on graveled substrate than amid nonnative eelgrass, and finds evidence that climate-related warming aids the recruitment of Pacific oysters.

Comments:
Primary Focus Area – COCC (SCD),
Secondary Focus Area – OCEH (SSSS)

Associated Goals: Improve capacity to manage ocean and coastal ecosystems and resources for societal benefit

under changing climatic and demographic conditions (SCD, Efficiency).
Improve understanding and management of emerging and cumulative threats to ocean and coastal health (SSSS, Supply).

Related Partners:

Agriculture Research Service (USDA, ARS)
Lummi Nation
Oregon State University (OSU)
Pacific Shellfish Institute
Puget Sound Partnership
Taylor Shellfish Company
University of Washington, Department of Biology, College of Arts and Sciences (UW)
US Department of Agriculture (USDA)
US Environmental Protection Agency (US EPA)
Washington State Department of Fish and Wildlife
Whiskey Creek Shellfish Hatchery
Title:

Type:
Title: **Washington Sea Grant helps oyster growers get a bigger oyster crop by scattering shells just when larvae need them**

Type: impact
Relevance, Response, Results:
Relevance: Natural larval settlement represents a variable but important source of seed oysters for shellfish growers and state reserves on Washington's Willapa Bay, which produces 10 percent of all U.S. oysters. Growers deploy post-harvest shells at times and places where larval settlement will soon occur, to take advantage of the larvae's preference for clean shell. They have only a brief window of time to obtain maximum settlement. The sooner they learn when larvae are ready to settle, the more shell they can distribute.
Response: A Washington Sea Grant-funded research team sampled bivalve larvae every three to five days and issued bulletins to a listserv of oyster growers, advising them of the best times to deploy shells.
Results: As larvae approached setting size in August 2012, their abundance was about an order of magnitude larger than it was in 2011, based on standardized spat collectors with 11 spat per shellface. This resulted in a commercially viable set, an estimated $50,000 benefit to Willapa Bay oyster growers.

Recap:
A larval sampling program supported by Washington Sea Grant enabled oyster growers in Washington's Willapa Bay to place clean shells at the optimal time for Pacific oyster settlement.

Comments:
Primary Focus Area – LME (SSSS)
Secondary Focus Area – COCC (SCD)

Associated Goals: Support conservation and sustainable use of living marine resources through effective and responsible approaches, tools, models and information for harvesting wild and cultured stocks and preserving protected species (SSSS Industry).
Assist coastal communities and marine-dependent businesses in planning and making decisions that provide local and regional economic benefits, increase resilience and foster stewardship of social, economic and natural

resources (SCD Economies).

Related Partners: *none*

Title: **Washington Sea Grant-funded research reveals the secrets of Manila clam settlement and survival for use by growers**

Type: impact

Relevance, Response, Results:

Relevance: Manila clam harvests have increased tremendously in Washington's Willapa Bay over the past two decades, and production has expanded into new parts of the bay. But the existing literature provides no reliable guidance about the timing and location of larval settlement or its contribution to commercial yields.

Response: Researchers supported by Washington Sea Grant developed standardized, mesh-enclosed gravel spat collectors to detect newly settled microscopic clams.

Results: The project revised previous thinking about the Manila clam recruitment season and found evidence that the peak season in Willapa Bay is June to October. This information was extremely valuable to clam growers planning culture activities and is anticipated to support future economic benefits. The spat collectors also detected substantial movements of larger one- to two-month-old clams, providing new insight into of the survival rate of young clams.

Recap:

A Washington Sea Grant-funded study provided clam growers extremely valuable information about the recruitment timing and post-settlement survival.

Comments:

Primary Focus Area – COCC (SCD)

Secondary Focus Area – LME (SSSS)

Associated Goals: Assist coastal communities and marine-dependent businesses in planning and making decisions that provide local and regional economic benefits, increase resilience and foster stewardship of social, economic and natural resources (SCD Efficiency).

Support conservation and sustainable use of living marine resources through effective and responsible approaches, tools, models and information for harvesting wild and cultured stocks and preserving protected species (SSSS, Industry).

Related Partners: *none*

:: PUBLICATIONS

Title: **Rising water temperature shifts recruitment patterns in a non-native but not native oyster: evidence over seven decades**

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2013

Uploaded File: *none*

URL: *none*

Abstract:

Climate warming may disproportionately benefit non-native species because of enhanced reproductive rates, phenological shifts, and climate matching. To evaluate this interaction of climate change and biological invasion, we applied structural equation modeling to time series (1942-2012) of native and non-native oyster recruitment in Willapa Bay, Washington, USA. Summer water temperatures in the bay warmed on average 0.1°C per decade and had interannual variability related to broader regional climate indices (El Niño-Southern Oscillation, Pacific

Decadal Oscillation), but not coastal upwelling. Recruitment in the non-native Pacific oyster (Crassostrea gigas) tracked this temperature variation, but not in the native Olympia oyster (Ostrea lurida). Temperature-tracking resulted in a phenological shift of 1 day earlier and 10% more recruitment per decade in C. gigas. In contrast, O. lurida had constant timing and magnitude of recruitment throughout the time series, although overall earlier and higher recruitment than for C. gigas. A climate change fingerprint was evident only in the non-native oyster at the recruitment life stage, which projects enhanced invasion under continued warming as well as more reproductive overlap between the native and non-native species.

Citation:
Ruesink J.L., and A.C. Trimble. In review. Rising water temperature shifts recruitment patterns in a non-native but not native oyster evidence over seven decades. Global Change Biology

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **Influence of substratum on non-native clam recruitment in Willapa Bay, Washington, USA**

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2013
Uploaded File: *none*
URL: *none*
Abstract:
Two non-native clam species were studied for habitat-related performance in Willapa Bay, Washington, USA. Across 86 quadrats sampled for adult clams throughout the bay, excluding areas directly seeded with clams, Manila clams (Ruditapes philippinarum) and Eastern soft-shell clams (Mya arenaria) had higher densities on graveled and (for R. philippinarum) shelled tideflats relative to finer sediments, and densities increased with intertidal elevation to the highest elevation sampled (+1.5 m relative to mean lower low water). Substrate type as a driver of clam recruitment was tested in four separate experiments. These experiments demonstrated little effect of substrate type on densities of newly-settled clams, but large differences developed by the following spring due to differential post-settlement mortality. Final densities did not differ between any treatments with added gravel, round rock, or shell (tested in two experiments), but were significantly higher in these coarse sediments than in background tideflat. The non-native seagrass Zostera japonica appeared not to affect settlement but reduced the survival benefit from adding gravel or shell. These patterns are consistent with small predators reducing wild-set clams by an order of magnitude within 1 year and prior to shell lengths of 1 cm.

Citation:
Ruesink J. L., N. Freshley, S. Herrold, A.C. Trimble, and K. Patten. In review. Influence of substratum on non-native clam recruitment in Willapa Bay, Washington, USA. Aquaculture Environment Interactions.

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **Spatio-temporal recruitment variability of naturalized Manila clams (Ruditapes philippinarum) in Willapa Bay, Washington, USA**

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2013
Uploaded File: *none*
URL: *none*
Abstract:
Manila clam (Ruditapes philippinarum) harvests from Willapa Bay, Washington, USA have increased by an order of magnitude over two decades. This increased yield of a non-native species has been supported by natural

recruitment as well as some planting of small clams. Manila clam settlement was recorded over two years in clam spat collectors (mesh bags containing gravel) and occurred in June to October at water temperatures >13°C. At 16 sites in the bay, clam abundance was measured at multiple life stages (larvae, settlers, recruits <20 mm, adults >20 mm), and correlations examined among life stages to determine influences on spatial pattern. A major spawning event occurred on 8 Jul 2010 in association with high temperatures. Larval density did not adequately predict immediate settlement in spat collectors ($r2=0.15$, $P=0.08$) but was a successful predictor of spatial variation in recruit density the following summer ($r2=0.49$, $P=0.002$). In addition, recruits and adults occurred at approximately equal densities and with strong spatial coherence ($r2=0.81$, $P<0.0001$). Despite larval collection on a single day out of an extended reproductive period, larval delivery appeared to be a major driver of large-scale pattern in natural yield of clams. At the same time, post-settlement processes contributed to severe losses of settlers, even though recruits and adults were sampled in sediments with large grain size expected to reduce predation. Post-settlement factors reducing yields, especially compared across intertidal habitats, remain to be determined.

Citation:
Ruesink J.L., K. van Raay, A. Witt, S. Herrold, N. Freshley, A. Sarich, and A.C. Trimble. Spatio-temporal recruitment variability of naturalized Manila clams (Ruditapes philippinarum) in Willapa Bay, Washington, USA. Submitted. Fisheries Research.

Copyright Restrictions + Other Notes:

Journal Title: *none*

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

No Leveraged Funds Reported This Period

WASHINGTON SEA GRANT COMPLETION NARRATIVE

WSG Project Number: R/LME-3
Project title: Spatial and temporal factors influencing recruitment variability in estuarine bivalves

PI: Jennifer Ruesink, Biology, University of Washington

SECTION I: Progress report for the period 2/1/2012-1/31/2013

Activities: We continued to monitor water temperatures at the Port of Peninsula through iButtons programmed to collect hourly data. This contribution is important because WDFW ended their temperature collection in 2009, and a thermometer associated with a multi-probe sensor established by the Pacific Shellfish Institute has large gaps due to probes malfunctioning. Without our temperature loggers, a record stretching back to 1942 would not be continuing.

We continued monthly monitoring clam recruitment at monthly intervals. This continued monitoring helped clarify seasonal patterns of settlement and also alerted us to a new source of clam mortality for individuals <1 mm in shell dimension – some may have been killed by a predator that makes irregularly-shaped holes in the shell. Otherwise, however, few dead clams are seen with intact shells.

We found another five years of data on oyster recruitment from the earliest years of the extended time series. These data were in files that we obtained from Washington Department of Fish and Wildlife. As part of the manuscript on long-term oyster recruitment, quantitative data will be archived.

Finally, our major focus was data analysis and manuscript preparation.

Participants: Jennifer Ruesink, Alan Trimble, Nicole Freshley

Results: This summer in Willapa Bay appears to have been the first commercial Pacific oyster set that was successfully captured by shellfish growers since 2004.

One manuscript has been submitted on the spatial and seasonal patterns of Manila clam recruitment. A manuscript will soon be submitted, with partners at Washington State University, regarding substrate effects on settlement and post-settlement densities of two non-native clams, Manila clams and eastern soft-shell clams. A third manuscript is in preparation that documents how non-native and native oyster recruitment have changed over 70 years.

Challenges: We were misled by the appearance of small clams in spat collectors during the winter and initially interpreted this as winter reproduction. However, a more logical explanation is that clams recruited earlier but migrated into the spat collectors.

SECTION II: Summary report

1. PROJECT OBJECTIVES (from the original proposal)

How and why does bivalve recruitment vary? This is the central topic of the proposal, which we will address through a powerful combination of analysis of remarkable historic data, and intensive modern spatial sampling. Answering this question is essential to (although undoubtedly not sufficient for) sustainable natural resource use and evaluation of consequences of anthropogenic environmental change. Specific objectives are: 1) Evaluate long-term records of environmental conditions (daily water temperature, meteorological observations, water chemistry) for evidence of spatio-temporal patterns, and their predictive power for the magnitude and timing of oyster recruitment. 1A. Summarize temporal trends in environmental variables, and in timing and magnitude of recruitment; 1B. Assess amount of interannual variation in recruitment timing and magnitude explained by water temperature; 1C. Make available online hundreds of historic documents about oyster recruitment and performance. 2) Compare present-day spatial and temporal patterns of larval abundance and recruitment among 3 estuarine bivalves. 2A. Determine spatial patterns of spawning and recruitment in 3 bivalve species; 2B. Relate changes in larval abundance of 3 bivalve species to environmental correlates; 2C. Prepare and distribute weekly Oyster Bulletins during summer.

2. METHODOLOGY

2.1 Spatial and seasonal pattern of clam recruitment

<u>2.1.1 Study site and species</u>

Willapa Bay, Washington, USA (N46.5° W124.0°) is a mesotidal estuary of 35,700 ha area (Ruesink et al. 2006). Extensive tideflats emerge at low tide, representing more than half of the bay's total area (Borde et al. 2003). Oyster aquaculture occurs at tidal elevations around mean lower low water (MLLW; Feldman et al. 2000), and Manila clam aquaculture generally occurs at higher intertidal elevations (1-2 m above MLLW). Water residence time increases from 1 wk in the northern region of the bay close to the estuary mouth to >1 mo in the southern region during summer periods of low river flow (Banas et al. 2007).

The first eastern Pacific populations of *R. philippinarum* were recorded from British Columbia in 1936 (Carlton 1992). In Willapa Bay, it arrived as a hitchhiking species with oysters introduced from Japan, certainly by the mid-1940s. This introduction date emerges from a statement in a 1950 report: "Several years ago it was noted that the Japanese clam *Paphia* [*Ruditapes* = *Venerupis* = *Tapes*] *philippinarum* closely related to our native little neck, *Paphia* [*Leukoma* = *Protothaca*] *staminea*, was present in the bay and was tending to form beds in favorable conditions. This species has now spread widely over the bay and has assumed commercial importance, especially on the west side of Long Island where it is unusually abundant" (Kincaid 1950). This region where Manila clams were first conspicuous in Willapa Bay corresponds to "SW" in figures. Over the past 20 years, yields of Manila clams reported from Willapa Bay have increased by an order of magnitude, beginning in the "SW" region and expanding into the northern regions of the bay closer to the estuary mouth (Fig. 1). Currently, clam production in

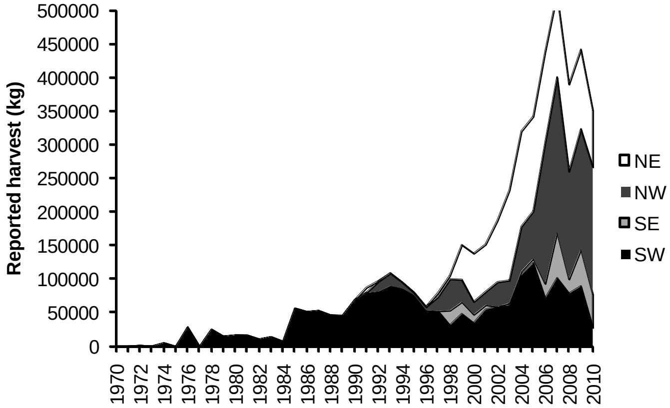

Figure 1. Fresh weight of Manila clams (*Ruditapes philippinarum*) harvested from 1970-2010 in Willapa Bay, Washington, USA, based on grower reports to Washington Department of Fish and Wildlife, which are not mandatory. Prior to 1986, harvests were aggregated across management units but are assumed to derive primarily from the SW region.

2.1.2 Settlement time series

We implemented clam spat collectors that were mesh bags (15 x 10 cm, 1 mm mesh size, n=5 replicates) filled with pea gravel placed on the sediment surface at +1 m MLLW within a commercial clam bed (site W3; Fig. 2). Bags were replaced at weekly (Jun to Sep 2010 and 2011) to monthly (Sep 2010 to Dec 2012) intervals. Upon collection, each bag was washed thoroughly through a sieve series (1.7 mm, 0.5 mm, 0.147 mm). In weekly samples, most clams were collected on the 0.147 mm sieve; these samples were dyed with Rose Bengal (at least 20 min), rinsed, and examined under a dissecting microscope (20x) for clams. Monthly samples contained clams both on the 0.5 mm sieve, which were counted directly under a dissecting microscope, and on the 0.147 mm sieve. Those on the 0.147 mm sieve were frequently overwhelmed by accumulated sediment, in which sand grains were about the same size as clams. To aid counting, we took advantage of small density differences between sand and clams and collected material that settled slowest through a saturated sucrose solution (Munroe et al. 2004), or from multiple decants after agitating the sample in a 100-ml graduated cylinder and waiting 10-20 seconds for the sand to collect at the bottom (Strasser et al. 1999). This lighter fraction was then dyed with Rose Bengal, rinsed, and examined under a dissecting microscope for clams. *R. philippinarum* comprised about half of the clams collected on 0.147 mm sieves and about 20% of those collected on the 0.5 mm sieves, with the remainder primarily a second introduced species (Eastern soft-shell clam, *Mya arenaria*) and a small fraction (<5%) of native *Macoma* spp. We compared this settlement of *R. philippinarum* to water temperatures logged at 1-hr intervals within 1 km of the site (iButton, Dallas Semiconductor).

2.1.3 Spatial patterns of larvae, settlers, recruits, and adults

We selected 16 sites throughout Willapa Bay to examine clam abundance at multiple life stages. Larval density was determined on 8-9 July 2010, immediately following a water temperature spike that induced widespread spawning. Clam larvae were counted in duplicate 20-gal (44 L) samples pumped at high tide from 6.5 m depth at each of 16 sites (see Fig. 2). Water samples were filtered in the field on a small boat through a pre-filter (300 microns) and collected on a 55-micron mesh. This material was washed into a bottle with ~20 ml seawater, and at least one 1-ml sample was examined in the laboratory for larvae on a Sedgewick-Rafter slide under a compound microscope. Clam larvae were distinguished from other bivalves by shape (Loosanoff et al. 1966) and by color under polarized light. On 8-9 July 2010, and again on 18 August 2010, also coinciding with a water temperature peak, clam larvae in mid-Willapa Bay exceeded 8600 per 44 L, nearly an order of magnitude higher than in other larval collections from June-August (mean =

900 per 44 L, SE = 165, n=20). Given characteristic larval development rates, clam spat collectors (mesh bags as described above) were placed at each site beginning on 18-19 July 2010. At each site, we placed 3 bags (across 10 m) at two sub-sites (>100 m apart) at a tidal elevation of +0.7-1.3 m MLLW. Spat collectors were picked up 6 days later and frozen until processed, as described above.

One year later, from 23 Jun-7 Jul 2011, we surveyed the same 16 sites for Manila clams. We surveyed a broad range of tidal elevations and substrates at each site but for this cross-site comparison focus on gravel or shell substrates at +0.7-2 m MLLW. When these were actively used for aquaculture, we made sure that they had never been planted and had not been harvested within the past year. We determined recruit density in cores (10 cm diameter, 5 cm deep) sieved to 1 mm, and we determined adult density in 0.25 m^2 quadrats raked and examined by hand (n = 1-8 per site, average 3.5). We set a size cut-off between recruits and adults of 20 mm, which reflects both a biological change associated with reproductive capacity (Holland and Chew 1974) and an upper size reached by clams after one year of growth (Toba et al. 2007). We analyzed these data by regression across life stages, with log-transformed site averages as samples.

2.2 70-year pattern of oyster recruitment

Two oyster species naturally reproduce in Willapa Bay, Washington, USA (N46.5° W124.0°): the native Olympia oyster (*Ostrea lurida*) and the Pacific oyster (*Crassostrea gigas*) first introduced in 1928 from Japan (White et al. 2009). Pacific oysters are widely introduced for aquaculture, with an establishment rate near 20% (Ruesink et al. 2005), and range limits typically align with temperature (Carrasco and Baron 2010). Temperature-sensitivity is obvious in the species' reproductive biology, given gametogenesis at temperatures above 17°C, spawning at 20°C, accelerated larval development at higher temperatures, and larval mortality below 16°C. Olympia oysters reproduce at lower water temperatures than Pacific oysters, with spawning possible above 13°C. Olympia oysters are ovoviviparous, with larvae brooded inside the mantle cavity of their mother before release as planktotrophic larvae, whereas Pacific oysters are broadcast-spawners and develop as larvae entirely in the water column. Both recruit to hard substrates to initiate the benthic phase of their life cycle.

Oyster recruitment in Willapa Bay was measured on consistent shell substrate, called shellstrings. Each week in summer, 20-25 oyster shells, strung face down on a wire, were placed at mean lower low water at 2-8 established stations. Stations were arrayed in the southern part of Willapa Bay that has relatively high water residence time (Banas et al. 2007); oysters rarely recruit in the northern part of the bay closer to the ocean (Chapman and Esveldt 1943). Weekly shellstrings were collected a week after deployment, and the number of new recruits counted under a dissecting microscope. Seasonal shellstrings were deployed each week and removed after recruitment had finished, usually September. Counts of newly-settled oysters were made on shell faces (smooth internal side), separating Pacific and Olympia oysters by morphology (Trimble et al. 2009). These data were reported weekly during summer months for Willapa Bay (1942-1983) in Oyster Bulletins, and station-by-station data were available from original paper data sheets. After 1983, shellstrings were discontinued due to funding constraints at Washington Department of Fish and Wildlife. In 2002, we reinitiated weekly shellstrings.

Recruitment magnitude was based on the sum of weekly recruits per shellface at each station, averaged across stations. For Pacific oysters, this sum across weekly shellstrings was strongly related to maximum seasonal settlement during years when both weekly and seasonal shellstrings were deployed (n=36 years r^2=0.9). Further, Pacific oyster recruitment has been given a categorical score by commercial growers every year since 1936 (Kincaid 1968). These categories map well onto observed quantitative variation in recruits per shellface: non-commercial (1.74+0.75 SE, n=25), commercial (10.8+5.3 SE, n=10), good (35.6+18.4 SE, n=7), and excellent (45.3+19.8 SE, n=10). Timing ("set day") was the date when shells were outplanted and received at least 1 recruit per shellface during that week at any station. No set day was possible to calculate in years of poor recruitment.

A few problems arose in the time series. Slight variations in methods occurred through the years, for instance, bags of shell were placed initially, and dowels with 11 shells were used recently. To address this discrepancy, all data were standardized to recruits per shellface. Because of the commercial focus on Pacific oysters, shellstrings were delayed until just prior to Pacific oyster settlement in some years. This issue affected only native oyster data, since this species typically recruits earlier than the non-native. To address the fact that early settlers may not have been measured, we included date of first shellstring deployment as a covariate in analyses of native oyster recruitment. From 1942 to 1944, native and non-native oyster recruits were not distinguished. However, spawning dates for non-native oysters were reported, so we assumed that all recruits prior to three weeks post-spawning were native oysters, and all those afterwards were Pacific oysters.

Water temperature has been measured near the marine edge of typical oyster recruitment in Willapa Bay (N46.5007° W124.0304°; Chapman and Esveldt 1943) at 1-3 hr intervals throughout the day since 1942. Daily average water temperatures were further averaged to produce monthly values. Pacific Decadal Oscillation values, reflecting North Pacific sea surface temperature anomalies, were used directly as monthly values beginning in 1942 (jisao.washington.edu/pdo). Coastal upwelling was incorporated as monthly averages from the NOAA NCEP-NCAR reanalysis, based on the northerly component of 150-mb winds measured at nearby coastal gridpoints (e.g. 45°N 125°W), a time series extending from 1949 to present (http://iridl.ldeo.columbia.edu/SOURCES/.NOAA/.NCEP-NCAR/.CDAS-1/.MONTHLY/.Diagnostic/.above_ground/.v/).

Structural equation modeling (SEM) was carried out separately for each oyster species and for response variables of recruitment timing and magnitude (log-transformed). Environmental predictors were included as monthly values, with June applied to native oysters and July and August (averaged) applied to introduced Pacific oysters, reflecting typical patterns of reproduction. Each SEM included a direct link from water temperature to recruitment, and from other environmental variables (PDO, upwelling) to water temperature. To test for temperature trends, a link was included from year to temperature. Covariance in observed exogenous variables was included between PDO and upwelling and between year and upwelling, but not between year and PDO, because PDO is already detrended. For *O. lurida*, date of first shellstring deployment was included as a further potential predictor of recruitment measured each year, given that shellstrings in some years were not initiated until larval samples indicated that Pacific oysters were close to settling. SEMs were evaluated for significant path coefficients in AMOS (http://amosdevelopment.com). Models that did not adequately fit data ($P<0.05$) were re-evaluated after adding links from each exogenous variable (year, PDO, upwelling) directly to recruitment. Because of missing data, means and intercepts were both estimated; maximum likelihood was used to assess model fit.

3. RATIONALE

3.1 Clam recruitment

Clam recruitment studies are the first to have been carried out in Willapa Bay, Washington. Therefore, all the data collected during this Sea Grant-funded project provide novel quantitative insight into the seasonality of clam recruitment and the location throughout the bay and across intertidal habitats.

Recruitment of marine organisms can be highly variable in space and time, and the coincidence of factors resulting in particularly strong year-classes remains unknown or case-specific. Because mortality rates for planktonic larvae are very high (15-25% loss d-1 in bivalves; Strathmann 1985), small changes in these rates, compounded over the developmental period, can lead to dramatic recruitment variability. Variability may also emerge due to larval transport, for instance offshore water movement at particular times or in particular places reduces the number of benthic settlers (Broitman et al. 2008, Barshis et al. 2011). Post-settlement mortality, which can exceed 90% in juvenile benthic marine invertebrates (Gosselin and Qian 1997), may or may not decouple the initial arrival of benthic individuals from their densities measured at a later age or size (Hunt and Scheibling 1997). Recruitment underlies numerous ecologically- and economically-important phenomena, such as spread and impact of invasive species (Grosholz 1996), yield of commercially-exploited stocks (Lehodey et al. 2006), and community structure and function (Connolly and Roughgarden 1999). In this paper, we document spatial and temporal variation in the Manila clam *Ruditapes philippinarum* (Adams & Reeve, 1850) across multiple life stages to determine constraints on production in an eastern Pacific estuary.

Worldwide Manila clam culture increased from 1,846,436 t in 2001 to 3,248,013 t in 2009, when *R. philippinarum* ranked third among aquaculture species in total production (UN FAO). Although much of the production derives from its native range (western Pacific from the Sea of Okhotsk to Singapore), established populations occur in western North America (Wonham and Carlton 2005), Poole Harbor, England (Humphreys et al. 2007), the Atlantic coast of Europe (Rodriguez-Moscoso et al. 1992, Dang et al. 2010), and in the Mediterranean Sea (Adriatic; Sbrenna and Campioni 1994). Aquaculture of *R. philippinarum* relies on either hatchery-raised larvae or natural recruitment to replenish stocks. In general, production suffers where natural recruitment is low or variable (Iishi et al. 2001, Toba et al. 2007, Dang et al. 2010). Reproductive periods in *R. philippinarum* are constrained by temperature, with food supply further determining gonad development (Delgado and Pérez-Camacho 2007b). Clams conditioned at 14°C are able to spawn (Delgado and Pérez-Camacho 2007a), but at 12°C cannot (Mann 1979). After broadcast spawning, larval development in *R. philippinarum* lasts 1.5-2 wk, and metamorphosis occurs at a size of ~200 microns (Zhang and Yan 2006). Larval duration up to 3 wk has occurred in some studies (Robinson and Breese 1984). Larval survival is poor below 10°C and above 30°C, as well as at salinities below 10 (Robinson and Breese 1984, Numaguchi 1998). Newly-settled clams grew to 2.8 mm in two months after settling in July but only to 0.5 mm in two months after settling in September and ceased growth altogether from November to

January (Williams 1980). Early mortality, possibly due to meiofaunal predators, can result in 99% losses of settlers within a year (Williams 1980). Clam spat can move 10s of cm in a month (Williams 1980).

We hypothesized that, given consistent benthic substrate, the estuarine distribution of Manila clams across all life stages would be a function of larval supply. We tested this hypothesis through a spatial analysis of larvae, settlers, juvenile and adult clams across 16 sites spanning 30 km in a coastal estuary. Further, we examined the temporal pattern of settlement across two years at a single site for comparison with reproductive dynamics of populations worldwide. Here we invoke typical terminology, with settlement referring to the transition between planktonic larval and benthic stages and measured within 1 wk, whereas recruitment is defined operationally and generally refers to larger individuals that have experienced post-settlement mortality. This study tests the critical role of larval supply in structuring commercial populations for harvest. At the same time, it informs how recruitment processes determine the distribution and abundance of *R. philippinarum* as a non-native species capable of changing benthic community structure and function when grown at high densities in aquaculture (Nizzoli et al. 2006, 2007, Whiteley and Bendell-Young 2007).

3.2 Oyster recruitment

In contrast to clam recruitment, which does not have a long-term record in Willapa Bay, oyster recruitment studies reach back many decades. The first quantitative studies coincide with oyster seed embargoes from Japan during World War II (Chapman and Esveldt 1943). The larval sampling and spat collection stations that we used during this Sea Grant-funded project were consistent with historical stations and therefore enabled evaluation of long-term recruitment variability.

Rising global temperatures have clear biological fingerprints in phenological shifts, generally towards earlier life history events, and in poleward range expansions from altered reproductive output at range edges (Parmesan 2006). Warming conditions are expected to aid non-native species (Stachowicz et al. 2002, Huang et al. 2011) because of improved climate matching or temperature-enhanced reproductive rates allowing escape from small population size. Additionally, shifts to earlier phenologies in non-native species may increase invasibility in accordance with vacant niches or priority effects (Wolkovich and Cleland 2010). In contrast, environmental changes represent departures from long-term conditions to which native species have adapted. Therefore, native species may be less responsive to or less aided by environmental change than non-natives, although some evidence indicates similar climate-related phenological shifts for plants in their native and non-native ranges (Hulme 2011). Here we examine unique long-term estuarine datasets to evaluate a climate change fingerprint for the timing and magnitude of recruitment in a native and non-native oyster.

Although warming trends have been documented in many marine environments over the past century, research has clarified that the pace of warming varies spatially (Burrows et al. 2011), and for intertidal species in particular, environmental temperature may reflect the timing of exposure to air, not simply ambient water conditions (Helmuth et al. 2002). Sea surface temperatures in the north Pacific vary at decadal scales, with periods of relatively warm water offshore from the continental U.S. alternating with relatively warm periods offshore from

Alaska, as indexed by the Pacific Decadal Oscillation (PDO). The PDO has biological correlates in ocean productivity and salmon returns (Mantua et al. 1997). Along the west coast of North America, summer upwelling brings cold, nutrient-rich water to the surface. Upwelling is projected to increase off northern California due to temperature differentials between land and ocean driving increased summer wind strength (Snyder et al. 2003). Increased upwelling could locally ameliorate rising water temperatures, although it also drives chemical changes such as reduced oxygen and higher dissolved inorganic carbon known to influence survival of coastal organisms (Grantham et al. 2004, Barton et al. 2012). Through tidal advection, ocean conditions can influence water properties in coastal estuaries such as Willapa Bay, Washington, USA (Roegner et al. 2002). In sum, environmental variability that occurs at scales of tides to decades could underlie temperatures experienced by estuarine organisms, and this variability may obscure climate warming.

Through long-term estuarine water temperature records, it may be possible to evaluate mechanistic coupling between climate variability and local biological responses of oysters, which are ecological foundation species, as well as economically valuable (Ruesink et al. 2005). Temperature-sensitivity of oyster reproduction leads to the prediction that both the native and non-native oyster in Willapa Bay will have some variation in the timing and magnitude of reproduction that is related to temperature. However, because of higher optimal temperatures for the non-native than native oyster, non-natives are expected to be disproportionately enhanced under conditions of rising coastal water temperatures.

4. SIGNIFICANCE OF RESULTS

4.1 Clam recruitment

In Willapa Bay, *R. philippinarum* showed seasonal reproductive patterns. In the monthly spat collectors, *R. philippinarum* larger than 0.5 mm first appeared in August samples, were more abundant in October samples, and had an extended tail lasting through winter (Fig. 2B). However, newly-settled clams were collected on the 0.147 mm screen primarily in June through October (Fig. 2B). Water temperatures generally exceed 13°C starting in mid-May and continuing through mid-October (Fig. 2A). The temporal pattern of settlement, based on the smallest clams in spat collectors, would be expected for minimal reproductive temperatures near 13°C.

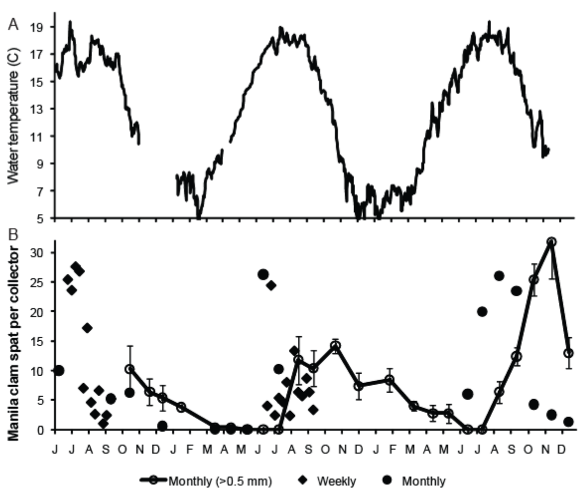

Figure 2. A) Daily average water temperature in Willapa Bay near site W3. B) Manila clams (*Ruditapes philippinarum*) settling into spat collectors (15x10 cm mesh bags of gravel) replaced at weekly-to-monthly intervals at site W3, +1 m MLLW. Points show mean of 3-5 bags, error bars are standard errors. Line connects values for >0.5 mm clams in monthly samples, and filled points show clams <0.5 mm in weekly and monthly samples (error bars removed for clarity).

Willapa Bay has lower water temperatures (annual temperature range ~5-19°C) than many other locations where *R. philippinarum* reproduction has been studied; however, it matches the common pattern of extended reproductive activity during summer months. Examples of locations with reproduction from May/June to September/October include: Incheon Bay, Korea (~3-26°C; Uddin et al. 2012); Jinju Bay, Korea (~8-25°C; Kang et al. 2007); Brittany, France (~5-22°C; Beninger and Lucas 1984); Po River, Italy (~5-28°C; Sbrenna and Campioni 1994); and Hood Canal, Washington, USA (~4-21°C, Holland and Chew 1974). The reproductive season is longer where winter temperatures are warmer, e.g. Ria de Vigo, Spain (~11-22°C, spawning April-November; Rodriguez-Moscoso et al. 1992). Similarly, Manila clam reproductive season was longer in a warmer year in Arcachon Bay, France (Dang et al. 2010). Where temperatures are even warmer (e.g. 12-28°C in Ariake Sound, Japan), two spawning periods – April to August and October to January – may coincide with intermediate temperatures (Iishi et al. 2001). Multiple spawning peaks have been reported for other locations as well (see excellent compilations by Ponurovsky and Yakovlev (1992) and Dang et al. (2010)), although in most cases they occur within an extended period of suitable conditions and may reflect reconditioning between bouts of spawning.

Spat collectors placed at 16 sites around Willapa Bay for 6 days in July 2010 indicated substantial spatial variation in settlement of *R. philippinarum* (Fig. 3). However, this variation was not adequately explained by peak larval densities immediately prior to settlement (Fig. 4). Curiously, recruitment of clams the following year was much better predicted by larval spatial pattern (Fig. 4). This relationship was strong despite two issues that would be expected to break down any relationship: we measured larval densities at all 16 sites over a 2-day period out of an extended reproductive season; and the 11-month gap between larval and recruit measurements could allow both build-up of recruits due to ongoing settlement and loss of recruits due to post-settlement mortality. Regardless of the degree of site-by-site matching, all stages tended to be higher in the southern than northern region, which aligns with retention under extended water residence time in the south (Banas et al. 2007). Four of our sites were north of the line corresponding to performance differences in Pacific oysters in Willapa Bay (Chapman and Esveldt 1943); this line also divides the NW and NE from SW and SE regions in Figure 2. Extrapolating from settlement into spat collectors, the four sites north of this line had settlement rates of 108 m^{-2} (SE=46, n=4), and those in the higher-retention region had settlement rates of 746 m^{-2} (SE=212, n=12).

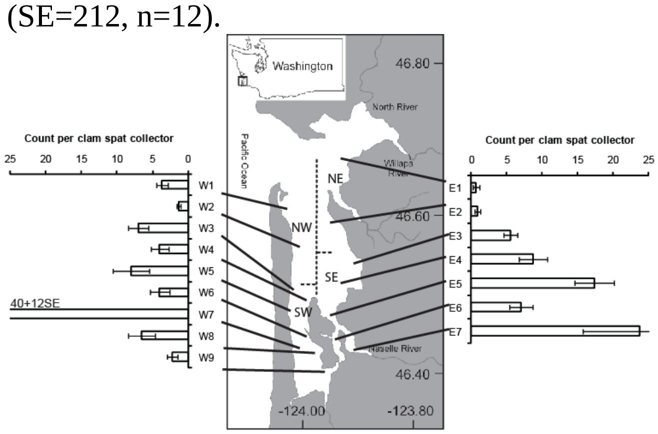

Figure 3. Settlement of Manila clams (*Ruditapes philippinarum*) in Willapa Bay, Washington, from 18-26 July 2010 in spat collectors (15x10 cm mesh bags of pea gravel). Sites are divided into east (E) and west (W) sides, and regions (NW, NE, SW, SE) refer to clam management areas for reporting harvests. Error bars show standard errors of 6 replicate spat collectors.

Although settlers and recruits were measured with different techniques (spat collectors vs. cores), both were in standardized substrates with large sediment grain size, rather than only mud or sand. Recruits had many months to build up but were not any higher in density than arrived in 6 days of settlement (northern recruits: 47 m^{-2} + 25 SE, n=4; southern recruits: 240 m^{-2} + 44 SE, n=12; compare to settlement above). This discrepancy suggests settlers suffer high mortality. Many causes of small clam mortality have been documented in previous studies. Among biotic sources, predators can disproportionately influence small *R. philippinarum* (Cigarria and Fernandez 2000). Consequently, addition of gravel and shell can dramatically increase clam survival, as documented in Oakland Bay, Washington (Thompson 1995). Where predators are rare, Manila clams may suffer density-dependent mortality (Melía et al. 2004). Abiotic conditions such as low temperatures or salinity also kill Manila clams, for instance, several studies suggest salinities of 10 or below cause metabolic and feeding problems in adults and juveniles (Kim et al. 2001, Nakamura et al. 2005). One study site in Willapa Bay had second-highest settlement into spat collectors but no detectable juveniles in the following year (compare site E7 in Fig. 4A and 4B), consistent with salinities below 10 due to winter-season rainfall and local river flow. However, the sites were not arranged in a way that provides a general test of whether proximity to rivers influences post-settlement survival. Thus, we can simply conclude that, although post-settlement mortality of Manila clams appears high and perhaps spatially variable, it does not break down the large-scale spatial pattern of population distribution established by larval delivery.

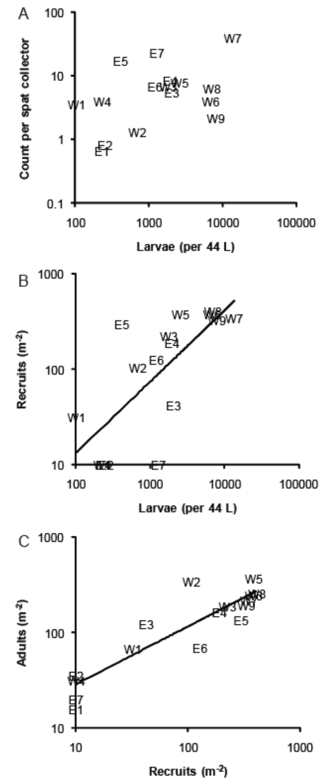

Figure 4. Manila clam (*Ruditapes philippinarum*) life stages across 16 sites in Willapa Bay. A) Settlement vs. larval densities. B) Recruitment vs. larval densities. C) Adult vs. recruit densities. Cores with no recruits are graphed at 10 m^{-2} to accommodate log-scale axes. Each point is shown as a site code label, as in Figure 2. Larvae were measured on 8/9 Jul 2010; settlers on 18-26 Jul 2010; recruits (<20 mm) and adults (≥20 mm) in June-July 2011.

The final life-stage correlation between adults and recruits was strong (Fig. 4), with both stages occurring at approximately equal densities. Including both recruits and adults, Manila clam densities reached a maximum of about 500 m^{-2} in Willapa Bay, which aligns with a moderate density for aquaculture (e.g. Nizzoli et al. 2007) but appears higher than many other locations where clams are not seeded (Humphreys et al. 2007, Whiteley and Bendell-Young 2007, Dang et al. 2010). Nevertheless, all of these locations show similar ratios in spring, with juveniles (<20 mm) about as dense as adults (>20 mm).

4.2 Oyster recruitment

Summarizing the entire recruitment time series (Fig. 5), native Olympia oysters recruited a month earlier than non-native Pacific oysters, with median set day of 11 July (n=39) and 5 August (n=36), respectively. Recruitment magnitude was higher for native than non-native oysters: 8.1 vs. 2.1 recruits per shellface (*O. lurida* log-transformed = 0.91+0.12 SE, n=43; *C. gigas* log-transformed = 0.32+0.15 SE, n=52). Because shellstrings were first deployed as late as August in some years, *O. lurida* estimates are biased towards later and lower total recruitment, and the interspecific differences may actually be larger. On the other hand, the lapse in shellstring deployment between 1983 and 2002 coincided with a series of excellent Pacific oyster sets according to categorical assessments (Fig. 5), which would mean that average settlement magnitude could be larger than calculated.

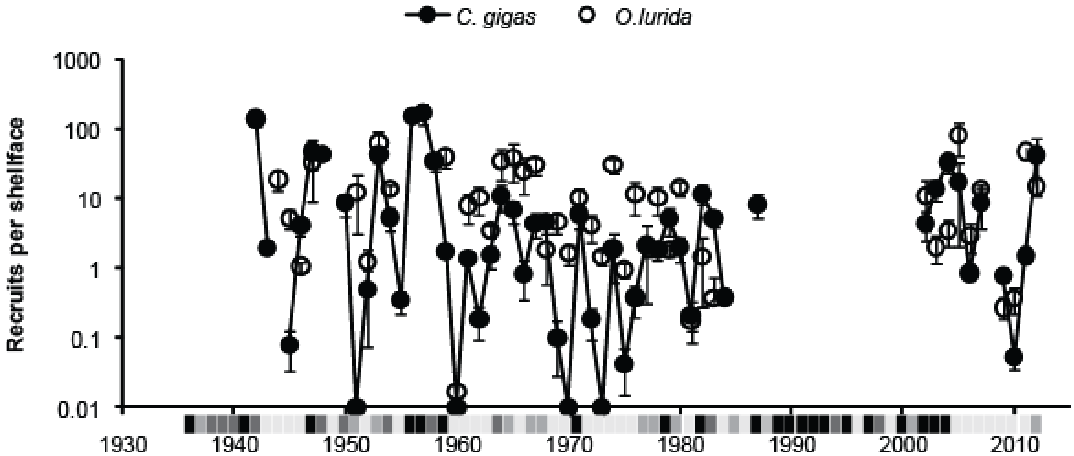

Figure 5. Settlement of non-native (*Crassostrea gigas*) and native oysters (*Ostrea lurida*) in Willapa Bay, based on the sum across weekly shellstrings. Error bars show standard errors across 2-8 sites in southern Willapa Bay. Non-native oyster set is coded as a categorical variable along the x-axis, with 4 categories of increasing grayscale.

In structural equation models, recruitment of non-native *C. gigas* was related to summer water temperature, but that of native *O. lurida* was not. With higher water temperature, *C. gigas* recruitment occurred earlier and at higher magnitude (Fig. 6).

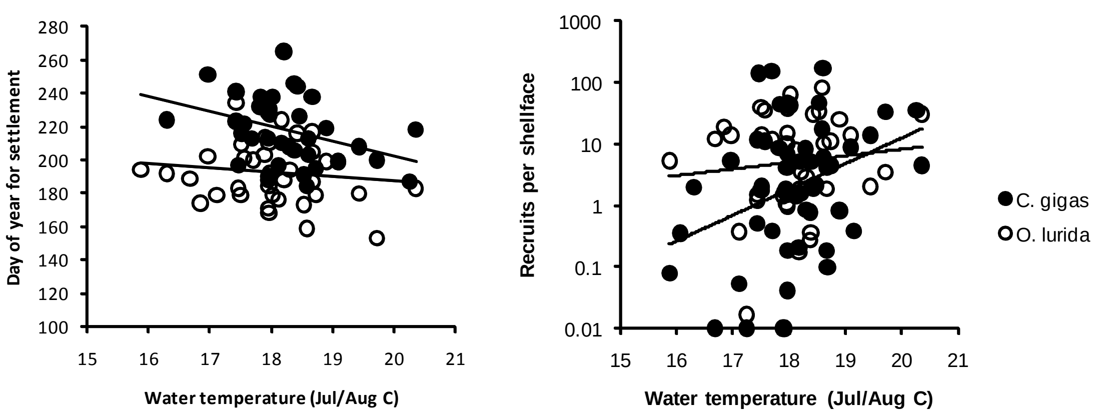

Figure 6. (A) Set day for oysters in relation to Jul/Aug temperature. For *C. gigas* r^2=0.14, for *O. lurida*, r^2=0.01. (B) Magnitude of oyster recruitment in relation to Jul/Aug temperature. For *C. gigas*, r^2=0.11, for *O. lurida* r^2=0.01. Both oyster species are plotted with respect to the same environmental variable to provide a visual contrast, but statistical analyses for *O. lurida* were carried out with June water temperature because of its lower temperature requirements for reproduction and earlier settlement relative to *C. gigas*.

Summer water temperatures were highly variable from 1942 to 2012 but showed similar warming trends in both June and July/August averages (Fig. 7a). The overall trend was about 0.1°C per decade, while recruitment advanced for *C. gigas* about 9 days with each 1°C; overall this results in a phenological advance of about one day per decade, in line with averages for marine organisms (Thackeray et al. 2010). Because summer temperatures in Willapa Bay typically range around lower thermal limits for reproduction in *C. gigas*, it is not surprising that warmer years result in earlier and more recruitment. At the same time, the relationship between local water temperature and broader climate, as indicated by PDO (Fig. 7b), provides an indirect driver of decadal-scale variability in recruitment. Thus estuarine Pacific oyster recruitment adds to the growing list of biological phenomena showing decadal-scale variation aligned with the PDO. Upwelling-related temperature changes typically occur at much shorter time scales (days to weeks) than the PDO (decades), and upwelling was unrelated to bay temperatures at longer scales, that is, month or 2-month averages applied in these structural equation models.

Native Olympia oysters appear much better adapted to local environmental conditions than their non-native counterparts, based on their ability to reproduce earlier, at water temperatures reached reliably in summer in Willapa Bay, and their higher average magnitude of recruitment (Fig. 5). However, native oysters, which were overexploited by early 1900s, remain rare, whereas non-natives form large areas of intertidal reefs (Dumbauld et al. 2011). Overall, warming trends favor non-native oyster reproduction at the same time that temperatures still appear better for native than non-native reproduction, but clearly other factors influence their relative abundance at other phases of the life cycle, with native oysters particularly sensitive to post-recruitment factors (Trimble et al. 2009).

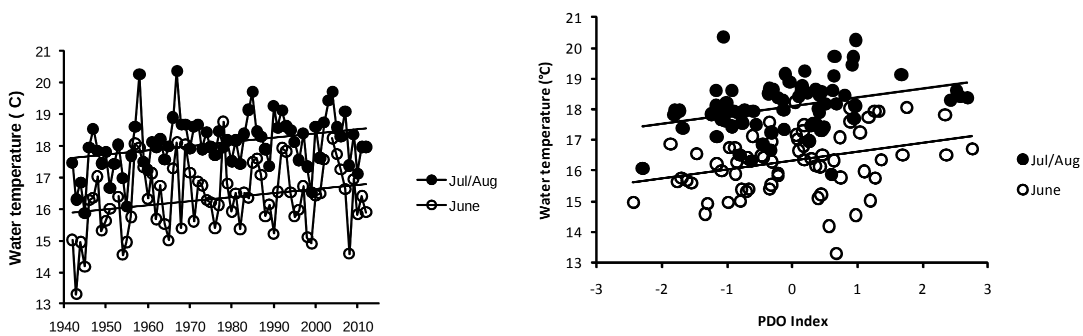

Figure 7. (A) Average summer water temperature in Willapa Bay from 1942 to 2012. Trend lines show best-fit regression for each series. (B) Average summer water temperature in Willapa Bay in relation to PDO index during the same interval; each point represents one June or July/August average from 1942 to 2012.

Despite recent concerns regarding commercial failures of non-native Pacific oyster recruitment in Willapa Bay, the pattern is consistent with relatively cool summers over the past five years, also coinciding with a likely shift from positive to negative PDO regime. Warming will provide an economic advantage in reliable oyster seed production, while it also risks increase or expansion of this non-native species, as has been documented anecdotally in northern Europe (Diederich 2005, Wrange et al. 2010).

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