Sexual development and symbionts of native Olympia oysters *Ostrea lurida* naturally settled on cultch deployed in San Francisco Bay, California

James D. Moore*, Blythe C. Marshman, Rena Obernolte, and Robert Abbott

California Department of Fish and Wildlife, Bodega Marine Laboratory, P. O. Box 247, Bodega Bay, CA 94923 USA (JDM, BCM)

Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, CA 95616, USA (JDM)

Isla Arena Consulting, Emeryville, CA 94608 USA (RO, deceased)

6 Captain Drive #429E, Emeryville, CA 94608 USA (RA)

Correspondent: jim.moore@wildlife.ca.gov

Attempts to restore depleted oyster populations are taking place worldwide. The design of effective restoration programs can benefit significantly from knowledge of basic biological processes such as ontogenetic and seasonal reproductive patterns and the presence of potential agents of disease. In June 2007 we deployed oyster shell cultch in a series of mounds in San Francisco Bay, California, USA. In monthly sampling, sixty of the largest oysters recruited onto the cultch were examined histologically to track the reproductive development of the initial settlers. Symbiont presence was also recorded. Sexual development was already occurring in August 2007, 56 days after cultch deployment, with immature males comprising 18% of the sample. Mature sperm and oocytes were observed in September 2007, 92 days following cultch deployment. Brooded larvae were observed in October-November 2007 and April-June 2008, indicating a relatively long reproductive season and confirming that oysters that settle in late spring can reproduce as females by autumn. These results suggest the capacity for rapid population expansion when suitable habitat is available. The oysters were minimally affected by disease, in contrast to the native oyster (*Crassostrea virginica*) of the US Atlantic and Gulf coasts.

Key Words: *Ostrea lurida*, restoration, cultch, reproduction, disease

The native Olympia oyster (*Ostrea lurida)* was historically important in the ecology of intertidal estuarine communities along much of the west coast of North America. For thousands of years it was an important food source for humans (Baker 1995). The mid-nineteenth century California Gold Rush spurred intensive harvest of oysters in San Francisco Bay and subsequently in numerous other embayments in California, Oregon, and Washington (Baker 1995, Kirby 2004). Since the shells of their predecessors provided solid substrate that is often limited in such bays, the harvest of oysters resulted in a significant reduction of habitat. In San Francisco Bay, this harvest, along with siltation from hydraulic mining in the Sierra Nevada mountain range, the filling of tidal flatlands, and increasing numbers of competing non-native species, drastically reduced traditional oyster habitat while seawalls and armored shorelines provide potential new habitat. Despite no significant harvest for more than 100 years, oyster populations have failed to recover (Kirby 2004). The decline of *O. lurida* is far from unique, since oyster populations and oyster reefs have diminished or disappeared in many regions of the world (Kirby 2004, Beck et al. 2011, zu Ermgassen et al. 2012).

Over the past decade, interest in restoring native oyster populations has increased throughout the United States (McGraw 2009, Trimble et al. 2009, White et al. 2009, Beck et al. 2011, Kennedy et al. 2011, State Coastal Conservancy 2010, Wasson 2010, Wasson et al. 2015). Relative to the native eastern oyster (*Crassostrea virginica)*, the Olympia oyster is vastly understudied and active recovery efforts have just recently begun. Recruitment dynamics, habitat requirements, genetics, reproduction, disease, and the utility of deploying artificial reef structures are recognized as important information sets for implementing effective restoration strategies (McGraw 2009, State Coastal Conservancy 2010, Wasson 2010,Wasson et al. 2015). A number of recent studies examined *O. lurida* populations in Washington (Trimble et al. 2009, White et al. 2009), Oregon (Groth and Rumrill 2009, Pritchard et al. 2015), and Southern California (Polson and Zacherl 2009, Seale and Zacherl 2009). Populations are diminished from historical levels at all locations, and both reduced recruitment and limited habitat are commonly cited as key impediments to recovery.

Critical factors in recruitment success include reproductive output and larval survival. Members of the genus *Ostrea* employ a reproductive strategy that includes protandric hermaphroditism and larval brooding before release as veligers. The reproductive biology of *O. lurida* was first reported by Stafford (1913) in British Columbia, Canada, who observed that sperm develops in aggregates (later commonly referred to as sperm balls, morulae, or spermatozeugmata), larvae are brooded before release, and that "each individual is bisexual, hermaphroditic, monoecious." He also noted that younger individuals had sperm but no ova, i.e., protandry. The continuous cycling between male and female sexes was later described as rhythmical consecutive hermaphroditism (Mackie 1984). In contrast, members of the genus *Crassostrea* have monoecious gonads displaying alternative hermaphroditism in which adults develop a single sex that may or may not change during the subsequent season, with protandry being typical, e.g., younger animals tend to be male and older tend to be female (Mackie 1984).

Wesley Coe produced a series of reports (1930, 1931a, 1931b, 1932a, 1932b, 1934) after studying *O. lurida* of known approximate age that settled onto wooden or concrete blocks submerged for various lengths of time off a pier in La Jolla, California, during 1926-1931. The pier is located on the open coast, an unusual environment to encounter settling *O. lurida*. Coe stated that the initial male stage is followed by a female phase, another male phase, and a period of recuperation, but this cycle is suspended when temperatures

fall below 16 °C in fall and resumes the following spring when temperatures again reach 16 °C. The dramatic effects of elevated temperature on the rate of reproductive maturation in *O. lurida* were briefly reported by Santos et al. (1993). Adult oysters, presumably from a wild population in Washington State, were collected in January when water temperatures were 8 °C and the oysters were rapidly acclimated to 12, 18, or 21 °C. Those held at 21 °C produced large numbers of larvae after 2-3 weeks, followed by those at 18 °C after 3-4 weeks, while for the population at 12 °C a small number of brooders were noted at 8-9 weeks, at which time the experiment was terminated. More recently, Oates (2013) examined reproductive patterns in large (>30 mm shell length) *O. lurida* from two locations in Coos Bay, Oregon, conducting histology on 30 animals monthly from January to December 2012. Gonads were categorized as female, male, or hermaphroditic and predominantly female, predominantly male, or with equal representation of both sexes. Animals were also assigned a gonad maturity stage and oocyte diameter was measured. Gametogenesis was observed from May to September with brooded larvae seen from July to September. Differences in timing between the two sites was attributed to salinity stress (<15 ppt) at the site farther from the mouth of the bay. To our knowledge no other research on Olympia oyster reproduction has been published during the past seventy years.

By periodically examining a single set of the European flat oyster (*Ostrea edulis)* in England, Cole (1942) provided a detailed description of sequential sexual development in that species over time. His descriptions are quite similar to those of Coe in California, but with clearly defined animal ages and more detailed information on the sizes and numbers of oysters examined. Cole categorized the sequential stages of development summarized herein. In the indifferent or undeveloped stage the distal wall of each gonoduct is lined with ciliated epithelium and the wall closest to the digestive gland was lined with gonadal precursors, which even at this earliest stage could be identified as spermatogonia and oogonia. This is followed by a young male stage in which spermatogenesis commences, immature sperm balls develop, and oocytes along the follicle wall begin to expand, followed by a first male stage containing ripe and maturing sperm balls while oocytes along the follicle wall expand in size and number. The next stage is a male-to-female transition in which mature sperm balls are still present in follicles and ducts while developing oocytes completely line the follicle walls. The subsequent first female stage is characterized by follicles filled with mature oocytes interspersed with residual spermatocytes and occasional oogonia and spermatogonia lining follicle walls. Even at this stage residual mature sperm balls may still be present. Release of mature oocytes is rapidly followed by the second male stage, with developing sperm balls expanding in follicles until mature sperm balls again fill the cavity. Cole believed that such cycling continued regularly, with one female and one male phase being completed on an annual basis in Britain.

We conducted this study primarily to examine the timing and patterns of reproductive development of native Olympia oysters that settled onto planted cultch material in San Francisco Bay, California, USA. To track the initial settlers we examined the largest individuals present in monthly samples. After preliminary studies on California *O. lurida* and reviewing literature on oyster gonad categorization and maturation sequence, we concluded that the terminology and sequence used by Cole (1942) for British *O. edulis* provided excellent agreement with our species, although the timing was expected to be different based on water temperature and perhaps a variety of other factors. To the methods of Cole we added examination of maximum oocyte diameter and noted the presence of brooded larvae when apparent in histological preparations.

Disease has been shown to be one of the most important factors regulating animal densities in various oyster populations worldwide. Developing baseline knowledge of pathogen presence and distribution is essential toward gaining an understanding of their potential impacts on restored populations. Therefore in this study we recorded the presence of all symbionts, including potential pathogens.

MATERIALS AND METHODS

Project location and description.—Crassostrea gigas left and right valves, dried at least two years, were deployed as cultch on tidelands in San Francisco Bay at the Marin Rod and Gun Club in San Rafael, California, USA (Figure 1) on 9 June 2007. Plastic mesh (2.5 cm) bags, approximately 70 cm in length and 25 cm in diameter, were filled with approximately 81 valves. Thirty bags were used to create pyramid-shaped mounds around a PVC pipe inserted into the mud substrate at approximately -0.6 m mean lower low water (MLLW) tidal height. We constructed 26 mounds in four rows (either six or seven mounds per row). Mounds within each row were 3.0 m apart with 3.0 m spacing between rows. About 15 of the lowest bags in each mound rapidly settled into the soft substrate, leaving about 15 upper bags available for colonization. Temperature and salinity data were obtained from instrumentation at the Romberg Tiburon Center near Tiburon, California, approximately 6.7 km south-southeast of the project site (Figure 1).

FIGURE 1.— Project location in San Francisco Bay, California, USA. Star shows cultch outplant location on tidelands at the Marin Rod and Gun Club, San Rafael. Circle indicates the Romberg Tiburon Center, 6.7 km from the project location, where temperature and salinity data were recorded.

Sample collecting and processing.—We collected samples of the deployed cultch on an approximately monthly basis for twelve months (Table 1). For each monthly sample we removed one randomly selected bag of exposed cultch from each of two randomly selected cultch bag mounds. After combining the cultch from both bags we selected 60 individuals in the uppermost size range for processing. This subset was selected in order to follow the individuals that settled soon after cultch was deployed, avoiding more newly-settled individuals. We measured the shell height and shucked each individual. Those with shell heights less than 2 cm were placed whole in a histological cassette. For larger individuals, a single cross-section was taken that contained digestive gland, gonad, gill, kidney, and heart. To reduce costs we placed multiple animals into one cassette when cross-sections were sufficiently small, with up to four animals per cassette. One exception was the first sample in July 2007 that consisted of only 25 very small animals; all were placed whole into one cassette. Cassettes were placed in Davidson's fixative (Shaw and Battle, 1957) for 24 hours followed by the routine production of 5 μm, hematoxylin- and eosin-stained tissue sections that were examined under a microscope.

Histological examination.—Using the classification method of Cole (1942) with minor modifications, we categorized the gonad for the first nine months (July 2007-March 2008) as Indeterminate (I) (in place of Cole's term 'indifferent'), Juvenile Male (JM) (in place of Cole's term 'Young Male'), First Male Stage (M1), First Male Stage to First Female Stage Transition (M1F1), First Female Stage (F1), or Second Male Stage (M2). Characteristics of each stage are described in the Results section. Slides from each sample were read in monthly order with knowledge of the sample date. One departure from the category descriptions provided by Cole (1942) was that we categorized some males as Juvenile Males despite the appearance of a very small number (one to several) of mature-appearing sperm balls in minimally developed gonad. For the monthly samples beyond March (April-June 2008) we found it impossible to confidently assign individuals into these and subsequent stages, and thus gonad stage data was only assessed through March 2008. For all individuals that contained female gonad we used an ocular micrometer to measure a maximum oocyte diameter after identifying the largest spherically-dimensioned oocyte in each section. We also recorded the presence of brooded larvae as well as potential pathogens and all other symbionts.

TABLE 1.—Sampling schedule showing date and number of animals processed for histological examination.

Date	Days After Deployment	Number of Animals
7/8/2007	29	25
8/4/2007	56	62
9/9/2007	92	59
10/9/2007	122	60
11/5/2007	149	61
12/17/2007	191	60
1/14/2008	219	60
2/11/2008	247	60
3/12/2008	277	60
4/17/2008	313	60
5/12/2008	338	60
6/11/2008	368	60

RESULTS

Site description and environmental data.—Oyster shell cultch was deployed on mud-bottom private tidelands in northern San Francisco Bay owned by the Marin Rod and Gun Club (Figure 1). Native oysters are common but distributed in patches on low intertidal riprap throughout the region. Water temperatures and salinities recorded during the study period from sensors located 6.7 km from the deployment site are shown in Figure 2. The patterns are typical of this portion of San Francisco Bay, with nearly-oceanic salinity for most of the year, except during heavy winter and spring rains, and a low temperature in late winter rising to a peak in late summer to fall.

Reproductive development.—To investigate the rate at which the first settlers on the cultch became sexually mature, we histologically examined 60 oysters from the uppermost size range in each monthly sample. Based on our unpublished observations, from the second
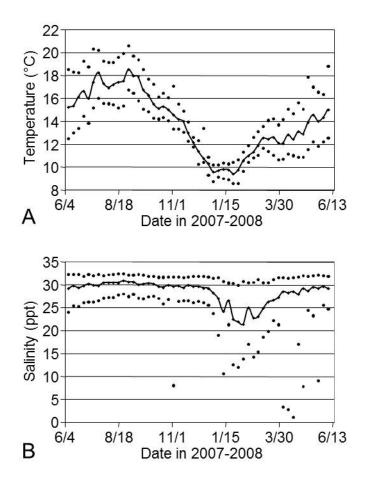

FIGURE 2.—Temperature (A) and salinity (B) in San Francisco Bay, California, USA over the study period, recorded 6.7 km from the project location. Line show weekly means and upper and lower dots show weekly maximum and minimum recordings respectively.

sampling onward, a total of 908 to 2,744 oysters were present on the cultch examined; thus our sample size of 60 resulted in subsets in the uppermost 2-7% of the size range present and should consist of the larger, fast-growing animals among those that settled soon after cultch deployment. The oysters grew steadily throughout the summer, then at a slower but fairly consistent rate from fall through late spring (Figure 3). Characteristics of the sequential stages of reproductive development are shown below.

FIGURE 3.—Mean shell height (± standard error, barely visible) of Olympia oysters in each monthly sample. Dashes show minimum and maximum values in each sample.

Indeterminate (I). No gonad follicles present or follicles contain ciliated epithelium and/or gonad precursors of indeterminate sex (Figure 4A).

Juvenile Male (JM). Initial follicle is a mostly empty, arced, thin duct parallel to the anterior/posterior axis of the animal, with spermatogonia along walls and developing sperm balls in lumen. Oogonia appear along the perimeters. Ducts may expand into the body at right angles to the primary ducts as the number of sperm balls present increases. One to several mature sperm balls per follicle may be present but nearly all are immature (Figure 4B).

First Male Stage (M1). In earliest stages, a single layer of developing oogonia line follicle perimeters, followed by layers of developing sperm balls that surround mature sperm balls in the centers of follicle lumina. In later stages the proportion of mature sperm balls increases and they fill the follicle lumina, while the female gonad continues to develop along perimeters. In latest stages multiple layers of fully mature sperm balls are present in lumina and crowding gonoducts (Figure 4C).

First Male to First Female Transition (M1F1). Some mature and developing sperm balls are still present in follicle lumina and gonoducts. Greatly expanded oocytes line the perimeters one or more layers deep. In latest stages, the gonad is dominated by female tissue but mature sperm balls are still common (Figure 4D).

First Female Stage (F1). Multiple layers of developing or fully mature oocytes are present in follicle lumina, increasing in number and size as the stage progresses (Figure 4E-F). Residual spermatogonia, residual spermatocytes and mature or degraded sperm balls may be present in lumina and along duct perimeters. Early brooded larvae may be present in the mantle.

Second Male Stage (M2). In early examples, sperm ball precursors rapidly divide in follicle lumina while residual oocytes of variable size remain attached to walls of follicles that may be partially empty due to release of oocytes (Figure 4G-H). Degraded oocyte material and phagocytes are usually present and brooded larvae may also be present. Later stage follicles are dominated by mature sperm balls. Gonad volume is many times larger than that of the M1 stage.
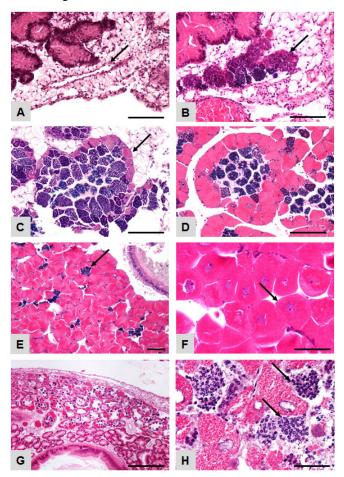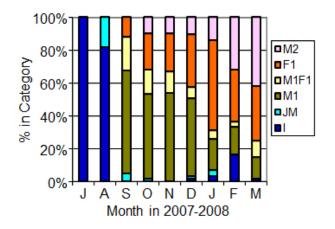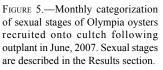

FIGURE 4.—Histology of Olympia oyster gonad development. A: Indeterminate (I) oyster from August 2007 sampling. Arrow points to undeveloped gonad duct. B: Juvenile male (JM), August 2007 sample. Small follicle (arrow) contains a few maturing sperm balls surrounded by loosely-organized developing female gonad. C: Male stage 1 (M1), September 2007 sample. Follicle dominated by mature and developing sperm balls with well-organized female gonad on the periphery (arrow). D: Male to female transition (M1F1), September 2007. Mature sperm balls present but most of the follicle volume contains maturing oocytes lining all or nearly all of the follicle walls. E: Mature female gonad (F1), September 2007 sample, with scattered pockets of residual spermatocytes (arrow). F: High magnification of female gonad, September 2007, showing uniformly fully mature oocytes (arrow). G: Early example of Male stage 2 (M2), November 2007 sample. The animal was brooding abundant larvae. H: Male stage 2, February 2008 sample. Rapidly-developing sperm balls (arrows) are replacing degraded oocytes. Scale bars are 100 μm in A, C, D, E, and F, 50 μm in B and H, 500 μm in G.

The emergence and presence of sequential stages of initial sexual development are shown in Figure 5. In August 2007, 56 days after the cultch deployment, 18% of the oysters were developing as juvenile males (JM) (Figure 5). By September 2007, 92 days after cultch deployment, all showed reproductive development, and oysters both with mature sperm and with mature oocytes were present, with 12% of the oysters in the F1 stage. In October the proportion in F1 increased to 22% and those in M2 appeared, comprising 10% of the oysters sampled. Throughout winter to early spring (November 2007-March 2008) the proportion of individuals in the F1 and M2 stages increased in preparation for spring spawning. In samples from beyond March 2008, it was not possible to confidently distinguish the M2 stage from potential M3 stages and the F1 stage from potential F2 stages with further complexity being present each month. However we did measure maximum oocyte diameter and noted the presence of brooded larvae and symbionts in the April to June 2008 samples.

FIGURE 5.—Monthly categorization of sexual stages of Olympia oysters recruited onto cultch following outplant in June, 2007. Sexual stages are described in the Results section.

The maximum oocyte diameter in the oysters containing female gonad rose steadily from August through September 2007 and then remained fairly stable (Figure 6). These data agree with the first histological appearance of mature oocytes in September 2007.
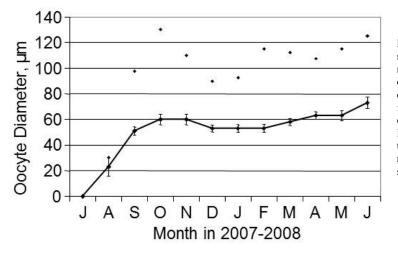
FIGURE 6.—Mean (± standard error) of the maximum diameter of oocytes in Olympia oysters that had female gonad tissue in each monthly sample. Dots indicate size of the largest oocyte measured in each sample.

Most larvae that were being brooded by oysters sampled in this study were probably lost during histological processing, yet brooded larvae were observed at time points when they would be expected to be present based on patterns of gonad status. Brooded larvae were first seen in five individuals in October 2007, following the first observations of mature sperm, mature oocytes, and post-spawn females in September 2007. We observed six brooders in November 2007, none from December 2007 to March 2008, and between two and four were present from April through June 2008. Brooded larvae were found in association with mantle and gill tissues (Figure 7A-C). In order to examine the temperature at which oocyte release occurs, we examined mean temperatures during the five days preceding sampling events for the five monthly samples that included individuals with brooded larvae (October and November 2007; April, May and June 2008). The mean temperatures ranged from 13.1 °C to 15.8 °C.
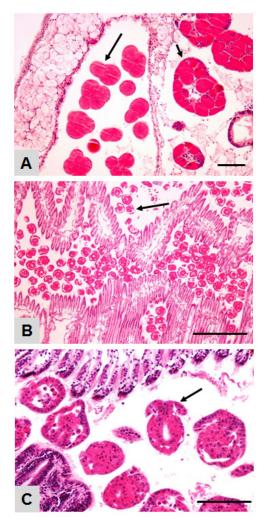

FIGURE 7.—Histology of Olympia oyster brooded larvae. A: Short arrow points to mature oocytes in female gonad and long arrow points to brooded 2-4 cell stages in a fold of the mantle, stage 1 female (F1), November 2007. B: Large numbers of early veliger larvae (arrow) brooded in gill tissue by a stage 2 male (M2), October 2007. C: Higher magnification of the larvae shown in (B). Arrow points to developing larva. Scale bars are 100 μm in A and C, and 500 μm in B.

Symbionts.—Pathological conditions and potential disease agents were rare throughout the study. The most commonly observed symbionts were *Echeneibothrium*-like cestode larvae in the gastrointestinal tract of 12 of the oysters (1.7% overall prevalence, Figure 8A). We first observed them in August 2007. In some instances there were associated localized tissue trauma and host responses at attachment sites to the gut epithelium. Two oysters (0.3% overall prevalence, one in each of the May and June 2008 samples) had a single *Urastoma*-like turbellarian flatworm present in association with gill tissue but no harm to the host was observed (Figure 8B). One oyster in the February 2008 sample had a *Mytilicola*-like copepod in the gastrointestinal tract (Figure 8C). Copepod egg masses were observed in gill tissue of another oyster in the same sample. One individual in the June 2008 sample was infected by a microcell protozoan with characteristics of *Bonamia* sp. (Figure 8D). The parasite was approximately 2.4 µm in diameter with a centrally located, 1.1 µm diameter nucleus. It was present in the cytoplasm of hemocytes and occasionally free in the hemolymph. Infected hemocytes were focally abundant and associated with widespread hemocyte recruitment.
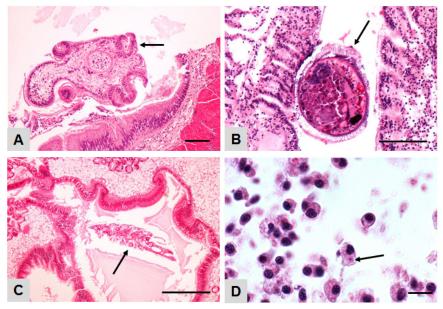

FIGURE 8.—Histology of Olympia oyster symbionts. A: Cestode larva (arrow) in gastrointestinal tract, May 2008. B: Turbellarian flatworm (arrow) between gill filaments, June 2008. C: *Mytilicola*-like copepod in gastrointestinal tract, November 2007. D: Presumptive *Bonamia* sp. microcell in a hemocyte, June 2008. Scale bars are 100 µm in A and B, 500 µm in C and 10 µm in D.

DISCUSSION

The deployment setting.—San Francisco Bay is the largest estuary on the west coast of North America. Human-mediated changes in water flow, filling of wetlands, and non-native species introductions have resulted in drastic alterations in habitat, environmental chemistry, and food webs (Cloern and Jassby 2012). Environmental conditions at our study site during the yearlong study were typical for northern San Francisco Bay, with winter

temperature lows coinciding with periodic, greatly decreased salinity during heavy winter rains. The native oyster *O. lurida* does well under these conditions except when salinities remain low for extended periods, which can result in dramatic mortality events (State Coastal Conservancy 2010, Cheng et al. 2015, Wasson et al. 2015). Our study location, like much of San Francisco Bay, has a fine sediment substrate, and the lower bags in each mound we created settled quickly into the substrate, while the higher bags remained stable. Deployment of the cultch at -0.6 m MLLW resulted in a low intertidal to subtidal setting, somewhat deeper than most natural populations of *O. lurida* in San Francisco Bay. This tidal height was chosen based on our unpublished observations of insignificant difference in recruitment between -0.8 m and -0.3 m MLLW, to minimize competition with non-native mussels *(Mytilus galloprovincialis)* that tend to be more abundant in deeper water, and for ease of access. The timing of our cultch deployment (June) was chosen to minimize colonization of non-oyster sessile invertebrates, particularly barnacles (*Balanus* sp.) that settle earlier in spring.

*Onset of reproduction.—*Coe (1930, 1931a, 1931b, 1932a, 1932b) described sexual development of Olympia oysters of known approximate age. He deployed wood or cement blocks off the Scripps Institution of Oceanography pier in La Jolla, California. Although important studies, his reports are somewhat unclear regarding the number of oysters examined, block type, block depth, and histological methodology. The central findings reported by Coe were that the oysters are hermaphrodites that first release male gametes (protandry) and then cycle between female and male stages, with developmental stasis at temperatures below 16 °C. Oysters that settled in spring could release male gametes as early as five months of age and release female gametes as early as one month later. Our data generally agree with this, with several exceptions. In our study some of the oysters that settled in early June had released gametes by early September (three months) and brooding hermaphrodites were present by early October (four months). Reasons for the discrepancy between our study and Coe's may include our size selection of the animals studied, although Coe also selected for larger animals (Coe 1932b) and/or differences in geographic setting, habitat, genetics, and sample sizes employed.

Cole (1942) provided a very thorough histological description of sexual development in *O. edulis*. Cole's review of previous studies and his own work collectively demonstrated *O. edulis* that settle in spring are capable of sperm production by fall of the same year, and that timing of reproductive stages is heavily influenced by temperature and latitude. He described a reproductive development pattern similar to that reported by Coe for *O. lurida*, i.e., an initial release of male gametes followed by release of female gametes and subsequent cycling. Studying *O. edulis* raised in Spain, da Silva et al. (2009) reported that among cohorts spawned in March to May 2001, all were indeterminate through October of the first year with a very small proportion of males by November. Males and then hermaphrodites and females grew in proportion through the following spring and summer, then over half of the animals became indeterminate again in November before the cycle repeated. This is a much slower and more synchronous sequence of development than reported for *O. lurida* despite reported temperatures (9-18.5 °C) similar to those in central California and Puget Sound, Washington. Millar (1964) and Wilson and Simons (1985) reported similar population synchrony in seasonal cycling of maximum oocyte diameter in *O. edulis* from Scotland and Ireland, respectively. We observed less seasonal synchrony, e.g. maximum oocyte diameters were relatively constant once the first females reached maturity (Figure 5).

Even though some oysters progressed to be producing and releasing oocytes during the first fall, we found that more than half remained in the first male stage through the end of the year (Figure 5). Thus following the initial male phase, significant proportions of the population consists of animals in either mature male or mature female stages at any particular point in time. This lack of synchrony suggests that founder populations would be capable of becoming self-sustaining more quickly than they would if gametogenesis showed a high degree of synchrony. For example, fertilization of newly spawned oocytes could be limited if nearly all of the oysters were simultaneously in the first female phase.

Reproductive categories and terminology.—Many studies on ostreid reproduction categorize each gonad with respect to the proportion of female and male tissue and stage of maturity. Although most of this research includes reasonably detailed definitions of sexes and stages, those definitions differ between studies. Thus Orton (1927), who studied gonad smears, considered *O. edulis* true hermaphrodites to be only those animals containing ripe sperm and mature eggs distributed evenly throughout the gonad, and had seven additional categories for female and male gonads containing various proportions and stages of each type. Most subsequent literature uses the term hermaphrodite to include any animal having female and male gonadal elements. Orton (1927) and later studies that utilized histology vary widely in the use of categories pure female/pure male or solely female/solely male. Orton (1927) considered pure females and pure males to be those individuals with gonads containing only entirely ripe ova and ripe or ripening sperm, respectively. Coe (1932b) did not use such terminology but noted that older *O. lurida* may tend to have one or the other sex dominate the gonad. Cole (1942) described *O. edulis* pure males as having only oogonia (the earliest identifiable female stage) with no later female stages present. Loosanoff (1962) described just three categories of *O. edulis* gonad, all hermaphroditic: ambisexual (with equal female and male representation) and predominantly female or male. Mann (1979) reported the presence of "totally male and totally female individuals" in laboratory-reared *O. edulis* without further description. Siddiqui and Ahmed (2002), studying two populations of *O. nomades* in Pakistan, included unisexual male and female categories in which all follicles contained only male or female tissue, and described the species as 'mostly hermaphroditic'. In his study of *O. lurida* in Coos Bay, Oregon, Oates (2013) included sole female and sole male categories for which follicles contain only female or male gonad material, although a representative micrograph of a female indicates the presence of what appear to be male spermatogonia. In our study, gonads overwhelmingly dominated with male or female tissue were fairly common in older animals, yet precursor cells of the alternate sex were always present. Collectively, these studies suggest that all ostreid oysters are protandric hermaphrodites with at least precursor cells of both sexes always present. We recommend that any use of categorical terms such as 'pure female' be accompanied by a thorough description of the gonad cell types present.

Several researchers observed asynchrony in reproductive stage among different parts of the gonad within individual *O. lurida* and other ostreids, particularly in young animals (Coe 1932b, Cole 1942, Loosanoff 1962). We observed this in a few individuals and in such cases the gonad was assigned a stage based on what was most common in the tissue section. We strongly agree with the conclusions of Coe (1932b) and Cole (1942) that tissue squashes or biopsies through holes drilled in the shell, as used in most studies prior to those of Coe, are inadequate to gain an accurate picture of the state of gametogenesis, particularly in early stages.

Seasonality and temperature-dependence of reproduction.—This study provides evidence of a relatively long reproductive season for Olympia oysters in San Francisco Bay. We identified brooding oysters as early as April and as late as November. Studies on seasonal settlement of *O. lurida* have reported a variety of ranges (Table 2), typically from spring to late fall with a peak in approximately June of each year. More restricted seasons occur at more northerly latitudes, presumably reflecting shorter periods of elevated water temperature. Hopkins (1937) reported the presence of brooded larvae in Puget Sound *O. lurida* beginning when waters reached approximately 13 °C (usually in May), peaking in late May to early June, with small numbers as late as October. Coe (1932a) stated that oysters spawned at La Jolla when waters were at least 16 °C, as early as April and as late as October. Seale and Zacherl (2009) studied *O. lurida* settlement at two southern California estuaries. At Upper Newport Bay, spawning occurred from May until November at temperatures of 16 °C or higher, similar to the results of Coe in La Jolla. However at Aqua Hedionda Lagoon, which is located between Newport Bay and La Jolla, temperatures rose above 16 °C in April, but spawning did not commence until June and continued into February, at temperatures as low as 14 °C. From these studies it is clear that the timing of the initiation, peak, and cessation of spawning show significant variation with latitude, location, and between years, but with a consistent peak in late spring to early summer subsequent to warming water temperatures. It is important for restoration activities that depend on natural spatfall to understand relationships between recruitment events and local environmental conditions, particularly temperature patterns.

By opening oysters and examining for the presence of larvae, Hopkins (1937) found that in a Puget Sound population of *O. lurida,* up to 55 % were brooding during the peak season in early June. The number of brooding oysters we observed by histology was very low; this was not unexpected, since brooded larvae are not physically attached to the mother oyster and most could be lost during histological processing. Our study would have benefited from examination of each opened oyster to determine brood presence before further processing and dissection. Nonetheless, identification of brooders provided unequivocal evidence of successful female gamete release and fertilization at particular points in time.

We calculated mean temperatures during the five days prior to sampling events for which brooded larvae were present. The five-day timeframe was based on Hopkins' (1937) report that brooded Puget Sound *O. lurida* larvae were early veligers at this time and all of the brooded larvae we observed were at this or earlier stages. These data indicated that San Francisco Bay Olympia oysters are capable of spawning at mean temperatures of about 13-16 °C, in accordance with the majority of previous studies on this species (Table 2).

TABLE 2.—Reported spawning season (presence of brooded larvae or settlement) for Ostrea lurida, arranged south to north.

Location	Spawning Season	Temperature	Citation
La Jolla, California	April-October	≥16°C	Coe 1932a
Agua Hedionda Lagoon, California	June-February	≥14°C	Seale and Zacherle 2009
Upper Newport Bay, California	May-November	≥16°C	Seale and Zacherle 2009
San Francisco Bay, California	April-November	≥13°C	This study
Coos Bay, Oregon	July-September	≥15°C	Oates 2013
Puget Sound, Washington	May-October	≥13°C	Hopkins 1937

Symbionts.—We found no significant impact of infectious disease in the population studied, in accordance with previous oyster surveys in San Francisco Bay (Friedman et al. 2005) and other bays in California (Moore et al. 2011). *Echeneibothrium*-like cestode larvae are commonly observed in the digestive tract of oysters in California (Moore et al. 2011) and can be found encysted in various clams (Sparks 1985). They have an elasmobranch as a definitive host; clams appear to be the true intermediate hosts while oysters appear to be accidental intermediate hosts. Turbellarian flatworms are uncommon in California oysters and are typically not associated with significant pathological effects. Although these and other metazoa can be difficult to identify to genus or species in tissue sections, the morphology of the two flatworms observed is consistent with them being members of the genus *Urastoma.* The intestinal copepod we observed in one oyster and copepod egg mass in another could not be identified further in our tissue sections although they are likely to be *Mytilicola orientalis*, which Bradley and Siebert (1978) reported was prevalent during spring to summer at 1-2.7 % in *O. lurida* at the Berkeley Marina in San Francisco Bay. The term "microcell" is used for tiny protozoan parasites that are members of the haplosporidian genus *Bonamia* or the taxonomically uncertain *Mikrocytos.* One oyster in our study was infected by a microcell parasite that had characteristics consistent with the genus *Bonamia*, i.e., several microns in diameter and located within hemocytes, and several types of *Bonamia* have been identified in native oysters and flat oysters *(Ostrea edulis)* from other California embayments (Hill et al. 2014). Friedman et al. (2005) reported a microcell in several *O. lurida* from San Francisco Bay that had characteristics more common to *Mikrocytos*. We saw no cases of the leukemia-like disease known as disseminated neoplasia (Elston et al. 1992); Friedman et al. (2005) reported it to be present in two out of 16 San Francisco Bay *O. lurida* populations they sampled. We also did not see haplosporidian plasmodia that were reported in *O. lurida* from Oregon (Mix 1974), nor a *Hexamita* flagellate described in native oysters from Puget Sound, Washington (Stein and Denison 1959), nor Rickettsia-like bacterial inclusions described in native oysters from British Columbia (Meyer et al. 2010). The young ages of the oysters in our samples likely contributed to the relative paucity of symbionts observed.

The minimal impact of disease on *O. lurida* populations from California to British Columbia contrasts with the situation for native ostreid and crassostreid oyster populations in many other parts of the world. Atlantic and Gulf coast populations of the native oyster *Crassostrea virginica* are significantly impacted by the parasitic dinoflagellate-like organism *Perkinsus marinus* (Smolowitz 2013) and Atlantic populations also are limited by presence of the protozoan parasite *Haplosporidium nelsoni* (Burreson and Ford 2004). European populations of the native flat oyster *Ostrea edulis* have been heavily impacted by the protozoan parasites *Bonamia ostreae* (Engelsma et al. 2014) and *Marteilia refringens* (Berthe et al. 2004).

Conclusions.—The design of effective restoration programs can benefit significantly from knowledge of basic biological processes such as ontogenetic and seasonal reproductive patterns. Oysters recruited onto outplanted cultch in San Francisco Bay in late spring rapidly matured, with significant numbers passing through first male and first female stages by fall of the same year. *O. lurida* appears to be minimally impacted by infectious disease. Collectively, these observations show promise for native oyster recovery in San Francisco Bay.

LITERATURE CITED

BAKER, P. 1995. Review of ecology and fishery of the Olympia oyster, *Ostrea lurida*, with annotated bibliography. Journal of Shellfish Research 14:501-518.

BECK, M. W., R. D. BRUMBAUGH, L. AIROLDI, A. L. CARRANZA, L. D. COEN, C. CRAWFORD, O. DEFEO, G. J. EDGAR, B. HANCOCK, M. C. KAY, H. S. LENIHAN, M. W. LUCKENBACH, C. L. TOROPOVA, G. ZANG, AND X. GUO. 2011. Oyster reefs at risk and recommendations for conservation, restoration and management. Bioscience 61:107-116.

BERTHE, F. C. J., F. LE ROUX, R. D. ADLARD, AND A. FIGUERAS. 2004. Marteiliosis in molluscs: a review. Diseases of Aquatic Organisms 17:433-448.

BRADLEY, W., AND A. E. SIEBERT JR. 1978. Infection of *Ostrea lurida* and *Mytilus edulis* by the parasitic copepod *Mytilicola orientalis* in San Francisco Bay, California. Veliger 21:131-134.

BURRESON, E. M., AND S. E. FORD. 2004. A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelson* (MSX disease). Aquatic Living Resources 17:499-517.

CHENG, B. S., J. M. BIBLE, A. L. CHANG, M. C. FERNER, K. WASSON, C. ZABIN, M. LATTA, A. DECK, A. E. TODGHAM, AND E. D. GROSHOLZ. 2015. Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. Global Change Biology 21:2488-2499.

CLOERN, J. E., AND A. D. JASSBY. 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. Reviews in Geophysics 50, 2012RG000397.

COE, W. R. 1930 (abstract). The life cycle of the California oyster *(Ostrea lurida)*. Anatomical Record 47:359.

COE, W. R. 1931a. Sexual rhythm in the California oyster *(Ostrea lurida)*. Science 74:247-249.

COE, W. R. 1931b. Spermatogenesis in the California oyster *(Ostrea lurida)*. Biological Bulletin 61:309-315.

COE, W. R. 1932a. Season of attachment and rate of growth of sedentary marine organisms at the pier of the Scripps Institution of Oceanography, La Jolla, California. Bulletin of the Scripps Institution of Oceanography 3:37-86.

Coe, W. R. 1932b. Development of the gonads and the sequence of the sexual phases in the California oyster (*Ostrea lurida*). Bulletin of the Scripps Institution of Oceanography 3:119-144.

Coe, W. R. 1934. Alternation of sexuality in oysters. American Naturalist 68:236-251.

Cole, H. A. 1942. Primary sex-phases in *Ostrea edulis*. Quarterly Journal of Microscopical Science 83:317-356

da Silva, P. M., J. Fuentes, and A. Villalba. 2009. Differences in gametogenic cycle among strains of the European flat oyster *Ostrea edulis* and relationship between gametogenesis and bonamiosis. Aquaculture 287:253-265.

Elston, R. A., J. D. Moore, and K. Brooks. 1992. Disseminated neoplasia of bivalve molluscs. Reviews in Aquatic Sciences 6:405-466.

Engelsma, M. Y., S. C. Culloty, S. A. Lynch, I. Arzul, and R. B. Carnegie. 2014. Bonamia parasites: a rapidly changing perspective on a genus of important mollusc pathogens. Diseases of Aquatic Organisms 110:5-23.

Friedman, C. S., H. M. Brown, T.W. Ewing, F.J. Griffin, and G. N. Cherr. 2005. Pilot study of the Olympia oyster *Ostrea conchaphila* in the San Francisco Bay estuary: description and distribution of diseases. Diseases of Aquatic Organisms 65:1-8.

Groth, S., and S. Rumrill. 2009. History of Olympia oysters (*Ostrea lurida* Carpenter 1864) in Oregon estuaries, and a description of a recovering population in Coos Bay. Journal of Shellfish Research 28:51-58.

Hill, K. M., N. A. Stokes, S. C. Webb, P. M. Hine, M. A. Kroeck, J. D. Moore, M. S. Morley, K. S. Reece, E.M. Burreson, and R. B. Carnegie. 2014. Phylogenetics of *Bonamia* parasites based on small subunit and internal transcribed spacer region ribosomal DNA sequence data. Diseases of Aquatic Organisms 110:33-54.

Hopkins, A. E. 1937. Experimental observations on spawning, larval development, and setting in the Olympia oyster *Ostrea lurida.* Bulletin of the Bureau of Fisheries 48:439-503.

Kennedy, V. S., D. L. Breitburg, M. C. Christman, M. W. Luckenbach, K. Paynter, J. Kramer, K. G. Sellner, J. Dew-Baxter, C. Keller, and R. Mann. 2011. Lessons learned from efforts to restore oyster populations in Maryland and Virginia, 1990-2007. Journal of Shellfish Research 30:719-731.

Kirby, M. X. 2004. Fishing down the coast: Historical expansion and collapse of oyster fisheries along continental margins. Proceedings of the National Academy of Sciences 101:13096-13099.

Loosanoff, V. L. 1962. Gametogenesis and spawning of the European oyster, *O. edulis,* in waters of Maine. Biological Bulletin 122:86-94.

Mackie, G. L. 1984. Bivalves. Pages 351-418 *in* A. S. Tompa, N. H. Verdonk and J. A. M. van den Biggelaar, editors. The Mollusca. Volume 7, Reproduction. Academic Press, Orlando Florida, USA.

Mann, R. 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. Journal of the Marine Biological Association of the United Kingdom 59:95-110.

McGraw, K. A. 2009. The Olympia oyster, *Ostrea lurida* Carpenter 1864 along the west coast of North America. Journal of Shellfish Research 28:5-10.

Meyer, G. R., G. J. Lowe, E. Kim, C. L. Abbott, S. C. Johnson, and S. R. Gilmore. 2010. Health status of Olympia oysters (*Ostrea lurida*) in British Columbia, Canada. Journal of Shellfish Research 29:181-185.

MILLAR, R. H. 1964. Breeding and gonadial cycle of oysters in Loch Ryan, Scotland. Journal du Conseil permanent International pour l' Exploration de la Mer 28:432-439.

MIX, M. C. 1974. Occurrence of a haplosporidian in native oysters *(Ostrea lurida)* from Yaquina Bay and Alsea Bay, Oregon. Journal of Invertebrate Pathology 23:252-254.

MOORE, J. D., C. I. JUHASZ, AND T. T. ROBBINS. 2011. A histopathology survey of California oysters. California Fish and Game 97:68-83.

OATES, M. S. 2013. Observations of gonad structure and gametogenic timing in a recovering population of *Ostrea lurida* (Carpenter 1864). MS Thesis, University of Oregon, Eugene, Oregon, USA.

ORTON, J. H. 1927. Observations and experiments on sex-change in the European oyster *(O. edulis)*. Part 1. The change from female to male. Journal of the Marine Biological Association, N.S. 14:967-1045.

POLSON, M. P., AND D. C. ZACHERL. 2009. Geographic distribution and intertidal population status for the Olympia oyster, *Ostrea lurida* Carpenter 1864, from Alaska to Baja. Journal of Shellfish Research 28:69-77.

PRITCHARD, C., A. SHANKS, R. RIMLER, M. OATES, AND S. RUMRILL. 2015. The Olympia oyster *Ostrea lurida:* Recent advances in natural history, ecology and restoration. Journal of Shellfish Research 34:259-271.

SANTOS, J. M., S.L. DOWNING, AND K. K. CHEW. 1993. Studies on the effects of water temperature on the sexual development of adult Olympia oysters, *Ostrea lurida*. World Aquaculture 24:43-46.

SEALE, E. M., AND D. C. ZACHERL. 2009. Seasonal settlement of Olympia oyster larvae, *Ostrea lurida* Carpenter 1864 and its relationship to seawater temperature in two southern California estuaries. Journal of Shellfish Research 28:113-120.

SHAW, B. L., AND H. I. BATTLE. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Canadian Journal of Zoology 35:325-347.

SIDDIQUI, G., AND M. AHMED. 2002. Gametogenic patterns of the larviparous oyster *Ostrea nomades* from Karachi, Pakistan (northern Arabian Sea). Aquaculture Research 33:1049-1058.

SMOLOWITZ, R. 2013. A review of current state of knowledge concerning *Perkinsus marinus* effects on *Crassostrea virginica* (Gmelin) (the eastern oyster). *Veterinary Pathology* 50:404-411.

SPARKS, A. K. 1985. Synopsis of invertebrate pathology, exclusive of insects. Elsevier, Science Publishers, New York, New York, USA.

STAFFORD, J. 1913. The British Columbian oyster. Pages 130-134 *in* J. Stafford, editor. The Canadian oyster. Its Development, environment and culture. Commission of Conservation, Canada. Committee on Fisheries, Game and Fur-Bearing Animals. The Mortimer Company, Ltd. Ottowa, Canada.

STATE COASTAL CONSERVANCY. 2010. San Francisco Bay Subtidal Habitat Goals Report [Internet]. State Coastal Conservancy, Oakland, California. Available from: http://www.sfbaysubtidal.org.

STEIN, J. E., AND J. G. DENISON. 1959. *Hexamita* sp. and an infectious disease in the commercial oyster *Ostrea lurida*. Proceedings of the National Shellfisheries Association 50:67-81.

TRIMBLE, A. C., J. L. RUESINK AND B. R. DUMBAULD. 2009. Factors preventing the recovery of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. Journal of Shellfish Research 28:97-106.

WASSON, K. 2010. Informing Olympia oyster restoration: evaluation of factors that limit populations in a California estuary. Wetlands 30: 449-459.

WASSON, K., C. ZABIN, J. BIBLE, S. BRILEY, E. CEBALLOS, A. CHANG, B. CHENG, A. DECK, T. GROSHOLZ, A. HELMS, M. LATTA, B. YEDNOCK, D. ZACHERL, AND M. FERNER. 2015. A guide to Olympia oyster restoration and conservation: environmental conditions and sites that support sustainable populations. Elkhorn Slough National Estuarine Research Reserve, Watsonville, California, USA.

WHITE, J. M., E. R. BUHLE, J. L. RUESINK, AND A. C. TRIMBLE. 2009. Evaluation of Olympia oyster *(Ostrea lurida* Carpenter 1864) status and restoration techniques in Puget Sound, Washington, United States. Journal of Shellfish Research 28:107-112.

WILSON, J.H., AND J. SIMONS. 1985. Gametogenesis and breeding of *Ostrea edulis* on the west coast of Ireland. Aquaculture 46:307-321.

ZU ERMGASSEN, P. S. E., M. D. SPALDING, B. BLAKE, L. D. COEN, B. DUMBAULD, S.GEIGER, J. H. GRABOWSKI, R. GRIZZLE, M. LUCKENBACH, K. MCGRAW, W. RODNEY, J. L. RUESINK, S. P. POWERS, AND R. BRUMBAUGH. 2012. Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. Proceedings of the Royal Society B 279:3393-3400.