Effects of temperature, salinity and food stress on larval growth and development in the Olympia oyster, *Ostrea lurida*

by

Alicia Rippington
BSc, University of Victoria, 2004

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of the Requirements for the Degree of

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Supervisory Committee

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Abstract

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Ostrea lurida Carpenter 1864 is the only native oyster on the western North American coast, but it is functionally extinct in most of its historic range. Knowledge of environmental tolerances during larval development of *O. lurida* is minimal, which limits recovery strategies for this listed "species of special concern" (Species At Risk Act). The effects of rearing temperature (13, 17, 21°C), salinity (11, 15, 21, 24, 30, 31 psu) and food concentration ($5x10^3$, $1x10^4$ and $5x10^4$ algal cells/ml) on larval growth and development were investigated. Larvae were obtained from laboratory conditioned adults from Ladysmith Harbour, and the Gorge Waterway on Vancouver Island BC. At low temperature (13°C), salinity (15 psu) and food concentration ($5x10^3$ algal cells/ml) larvae did not grow or develop. Higher temperature, salinity and food concentration increased growth and developmental rates. Larvae reared at higher food densities and salinities, but not higher temperature, were larger when eyespots differentiated.

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Dedication

To Andrew and Evan

Introduction

Oysters are of great importance ecologically and economically. Worldwide they provide food, habitat, protection, and many other ecosystem services that are integral to the healthy functioning of coastal ecosystems and communities, particularly within estuaries. The importance of oysters is reflected in the labels they have been given: ecosystem engineers (Gutiérrez et al., 2003), keystone species (Dame, 2012), and foundational species (Dame, 2012; Kimbro and Grosholz, 2006). Oyster reefs have been called the temperate latitude equivalents of tropical coral reefs because of the critical role they play in building, maintaining and supporting coastal marine ecosystems (Beck et al., 2009).

Oyster reefs and beds play an important role in supporting biodiversity. In sandy or muddy estuaries, oyster shells, whether from live or dead organisms, provide otherwise rare hard substrate for colonization by encrusting organisms, as well as bacterial and algal films, which are food for many grazing herbivores. The vertical relief of their shells provide many organisms with microhabitats that afford protection from predation and environmental stress. The structure of oyster reefs and beds attenuate wave action on coastal shores, which provide hospitable nursery grounds for juvenile fish and other organisms and stable sediment substrates for marine plants such as seagrasses (Meyer et al., 1997).

Oysters and other bivalves promote biodiversity not only by providing physical habitat for many other organisms, but also by cycling nutrients between the water column and sediment. Their filtering activity transfers a large amount of organic matter from the water column to the benthos in the form of feces and pseudofeces. This enrichment of the organic content of adjacent benthic substrates supports a diversity of deposit feeding species, which then become prey for other organisms (Newell, 2004). They also transform nutrients into forms that photosynthetic organisms can use (Dame, 2012).

Oysters also support the growth of benthic marine macrophytes by filtering plankton and other particulates from the water column, thereby reducing turbidity and increasing light availability for photosynthetic eelgrass and kelp (Lonsdale et al., 2009; Newell, 2004). This filter feeding action plays a critical role in preventing hypoxic conditions in

estuaries and may reduce the likelihood of harmful algal blooms, both of which have detrimental effects on marine organisms (Cerrato et al., 2004).

Olympia Oysters

Olympia oysters, *Ostrea lurida*, are the only native oyster species on the northwest Pacific Coast. They have been reported to exist from Sitka, Alaska, all the way south to Cabo San Lucas, Baja California Sur (Dall, 1914; Polson and Zacherl, 2009), although, reports of their occurrence in Alaska have not been substantiated. Polson and Zacherl (2009) suggest that the northern limit is more likely northern British Columbia and the southern limit is central Baja California. *Ostrea lurida* Carpenter, 1864 was originally described as a separate species from the Mexican, *Ostrea conchaphila* Carpenter, 1857. However, Harry (1985) synonymized *O. lurida* with the morphologically identical *O. conchaphila*. More recently, comparison of nucleotide sequences from two genetic markers (16S rRNA and cytochrome oxidase III) for members of the North and Central American populations of these oysters suggest they are indeed two distinct species, and hence the original species designations have been re-instated (Polson et al., 2009).

The historical abundance of *Ostrea lurida* is evidenced by its dominance in the fossil record from the Pacific coast (Baker, 1995). Sustenance and commercial fisheries were once supported by *O. lurida*. By the 1930s Olympia oyster populations had been dramatically impacted by harvest pressure and the fishery essentially collapsed (Gillespie, 2009; Quayle, 1969). The decline of Olympia oyster populations and the introduction of *Crassostrea gigas* in the early 1900s, resulted in near-extirpation of *Ostrea lurida* in some areas. Olympia oysters could no longer support commercial interest. The aquaculture industry had shifted its focus to *C. gigas* because it was a larger, faster growing species and was more robust under culture, and *O. lurida* populations were too sparse to support commercial harvest. This led to the Olympia oyster becoming an almost forgotten species (White et al., 2009). To this day many people wrongly believe that *C. gigas*, often referred to as the Pacific oyster, is the native species on the west coast of North America. However, the persistence of *C. gigas* is mainly supported by aquaculture efforts in most areas. Coastal marine water temperatures in British Columbia typically do not reach the required 20 - 23°C for

gamete maturation and spawning by *C. gigas*, though natural spawning does occur in some areas (e.g. Pendrell Sound, Ladysmith Harbour) (Quayle, 1988).

Olympia oyster populations remain functionally extinct in most of their historic range; they no longer play a significant ecological role (Ermgassen et al., 2012). In addition to over-harvest, industrial pollution (e.g. pulp mill effluent), sedimentation and habitat alteration have contributed to significant declines in *O. lurida* populations (Gillespie, 2009; White et al., 2009). Several introduced predator species also pose a threat to Olympia oysters. These include the Japanese oyster drill, *Ocenebra japonica,* and the flatworm, *Pseudostylochus ostreophagus,* which were brought over from Japan in shipments of spat of the Pacific oyster, *Crassostrea gigas* (Couch and Hassler, 1989), as well as the eastern oyster drill, *Urosalpinx cinerrea* (Buhle and Ruesink, 2009) and the European green crab (*Carcinus maenas*). There are no reports of disease that significantly affects *O. lurida* populations. *Bonamia ostreae,* a protozoan parasite that causes significant harm to *Ostrea edulis* populations was suspected in *Ostrea conchaphila* (= *O. lurida*), however, it was found that *O. lurida* was not a natural host for this pathogen and was unaffected when exposed (Arzul et al., 2005). A number of symbiotic pathogens have been identified in *O. lurida* but none of these are fatal (Abbott et al., 2014; Meyer et al., 2010).

The introduction of *Crassostrea gigas* is often suspected as a factor contributing to the lack of recovery of populations of *O. lurida*. However, *C. gigas* may not be a direct competitor of *O. lurida* because they occupy different habitats in the intertidal, *O. lurida* being more subtidal than *C. gigas* (Quayle, 1988; Trimble et al., 2009). A negative association between the two species comes from the fact that *C. gigas* enhances the hard settlement substrate in the upper intertidal habitat that settling *O. lurida* larvae take advantage of (Trimble et al., 2009). Settled larvae of *O. lurida* are not able to survive the intertidal exposure in this region of the intertidal and subsequently die (Trimble et al., 2009).

Biology and Life History

Olympia oysters, *O. lurida*, are small and relatively flat oysters. They have been reported to reach up to 90 mm in length but are more commonly 50-60 mm (Gillespie,

2009; Quayle, 1988). Adults reach their maximum size at approximately four years of age and do not significantly increase in length after this maximum size is reached (Baker, 1995). Maximum age is not known but Baker (1995) reports finding fossil shells with hinge annuli that indicated ages of up to 10 years.

O. lurida have a thin shell with little fluting, and a lower (or left) valve that is not deeply cupped (Baker, 1995; Quayle, 1988). The outer surface of the shell is variable in colour from grey to brown to white, with purple mixed in (Baker, 1995; Quayle, 1988). The nacreous layer of the inner shell surface is usually an iridescent greenish colour (Baker, 1995) but can also be white (Stafford, 1915). The muscle scar on the inside of the shell is clearly outlined but has no colour.

Ostrea are protandric, alternating hermaphrodites. They develop first as males, usually within their first year, and then alternate functional gender, meaning that sperm may still exist within the gonad of an individual when it is functionally female and vice versa as they alternate sexual phases (Coe, 1932). Given this fact, opportunity for self-fertilization exists but it has not been confirmed in *O. lurida*. Individuals may pass through several cycles of alternating sexual phase in one season depending on temperature, food availability and possibly other conditions (Coe, 1932). Low winter temperatures will interrupt sexual phase change and will be completed the following season when the temperature increases again (Baker, 1995; Coe, 1932).

Ripe gametes are not released until the water temperature reaches 12.5°C (Hopkins, 1936) to 16°C (Coe, 1932; Strathmann, 1987) Males broadcast spawn spermatophores, each containing from 250 – 2000 ripe sperm (Coe, 1932). Spermatophores are drawn into the mantle cavity of functional females via the inhalant pallial water current and fertilization of eggs occurs within the pallial cavity (Coe, 1932). *Ostrea* species brood their larvae on the surface of the ctenidia within the mantle cavity. *O. lurida* females brood larvae for 10-14 days and larvae are released as shelled D-stage veligers when they are about 185 µm in length (Loosanoff et al., 1966). They are planktotrophic and can remain in the plankton up to 6-8 weeks (Baker 1995). Under laboratory conditions, the planktonic phase may be as short as 14 days (Gillespie, 2009; Loosanoff et al., 1966).

Settlement size is about 300 µm in shell length (Loosanoff et al., 1966). Larvae of *O. lurida* settle on a variety of hard surfaces where they cement their left (or lower) valve. Unlike mussels, clams and scallops, oysters lose their larval foot at metamorphosis (Gosling, 2003). Olympia oysters are found on floating docks and wharfs, debris, and rocky beaches as well as muddy beaches where they form loose clusters and can even exist singly (Gillespie, 2009). They are found in quiet, calm inlets and bays in the low intertidal and subtidal zones. Adults are "moderately euryhaline"; they can tolerate a broad range of salinities and as such are found in full salinity sea water in deeper inlets and channels (e.g. in California (Baker, 1995)) as well as in shallow regions at the mouth of freshwater creeks (Gillespie, 2009). Olympia oysters cannot tolerate freezing temperatures (Davis, 1955).

Olympia oysters are bed forming, as opposed to reef forming oysters. They provide major structural habitat on soft-bottomed benthos, but the structure they form is not as extensive in terms of vertical relief or coverage as reef forming *Crassostrea* species (Beck et al., 2009). Like other oysters, the structure created by *O. lurida* shells is very important for a variety of marine species and ecosystem functions. A study done by Kimbro and Grosholz (2006) found 47 species across 11 phyla living in an Olympia oyster community in Tomales Bay in central California, U.S.A.

Research and Conservation Efforts

Until recently, knowledge of Olympia oysters was largely based on work conducted in the early 1900s (Coe, 1932; Elsey, 1933; Hopkins, 1936; Stafford, 1915) and on anecdotal evidence (Gillespie, 2009). Within the last ~10 years research interest in this organism has greatly increased. A recent Web of Science search for *Ostrea lurida* brought up 64 results, of which only 16 are dated earlier than 2009. A special issue on Olympia oysters published by the Journal of Shellfish Research in 2009, highlighted the growing interest in this historically abundant species and inspired increased research on these bivalves. The United States is leading the way with research and impressive restoration efforts on Olympia oysters. In 2009 it was reported that over $1 million US dollars had been spent on restoration efforts, despite a lack of basic research and knowledge on the species at the time (McGraw, 2009). Restoration efforts are driven by

several factors, including the desire to promote healthy estuaries by restoring the important ecosystem services provided by oysters (e.g. water filtration, shoreline stabilization and creation of important biogenic structure); a desire to restore a native species at risk of extirpation; and the potential of farming a native species of oyster for commercial harvest (Brumbaugh and Coen, 2009; Dinnel et al., 2009). Citizen interest in restoration is a major driving force for these initiatives. Restoration is currently focused on enhancing habitat limited estuaries by introducing large quantities of oyster shells and artificial reef material to areas where significant losses in hard substrate have resulted from dredging, high sedimentation rates, and shell removal by aquaculture practices – common problems threatening the recovery *O. lurida* in the US (Trimble et al., 2009; Wasson, 2010). Hatchery produced oyster seed is also commonly introduced to supplement and enhance recruitment in restoration sites (Brumbaugh and Coen, 2009). Further research on recruitment dynamics (Seale and Zacherl, 2009) population connectivity (Zacherl et al., 2009) and the impacts of introduced predators (Kimbro et al., 2009) for example, have improved our understanding of population dynamics of *O. lurida* in the U.S. but there are still many knowledge gaps. For instance, very few studies, (except for example Hettinger et al., 2013a and b; Hettinger et al., 2012) have investigated factors affecting the larval phase of *O. lurida* and the possible effects that larval condition may be having on shaping populations. Hettinger et al.'s (2012) research on the effects of ocean acidification suggests that larvae are vulnerable to environmental conditions and these vulnerabilities can influence population structures.

In Canada, there has been much less research and conservation effort directed at Olympia oysters than in the United States. In 2000 Olympia oysters were listed as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and in 2003 under the Species at Risk Act (SARA). This designation affords legal protection and legislates management action and recovery planning. In 2009 a management plan was finalized and, among other actions, population surveys across BC were initiated by Fisheries and Oceans Canada to enumerate and monitor population abundances. Other Canadian research projects by World Fisheries Trust in the Gorge Waterway, Vancouver Island University on the east coast of Vancouver Island and by Habitat Acquisition Trust at Ayum Creek in Sooke indicate that

adult populations of Olympia oysters exist in some of their historic locations in low abundances, but how current abundances compare to historic levels is difficult to quantify. In Nootka Sound, on the west coast of British Columbia, one of the few remaining large, intact Olympia oyster populations exists. Densities as high as 1100 oysters/m^2 were reported in Port Eliza in 2008 (Beck et al., 2009; Gillespie, 2009). This is one of the only examples that provide clues about the historic extent of coverage and density of Olympia oyster populations. This site is important as it can be used to inform targets for conservation and restoration efforts, however, much remains be learned about how to re-establish populations and support existing populations in achieving densities as high as in Port Eliza.

In Canada, recruitment studies in the Gorge Waterway and several locations along the eastern coast of Vancouver Island indicate that Olympia oysters are spawning and settling (World Fisheries Trust unpublished data 2010, Vancouver Island University unpublished data 2011). But beyond the existence of adults and settling juveniles, little is known about the population dynamics in BC and there is little insight into why populations are not recovering despite some of the reduced pressures.

Environmental Influence on Early Growth

A free-swimming, pelagic larval stage is a common stage in the life history of many benthic marine invertebrate organisms. This, however, is the most vulnerable stage of life and success during this time can have significant effects on the population size and structure of adults (Drent, 2002; Rumrill, 1990; Strathmann, 1985). During the pelagic stage, larvae may be dispersed away from favourable habitat occupied by the parental population and where the species has successfully established; they are vulnerable to increased predation in the water column and to chemical, nutritional and physical stress such as changes in temperature, salinity, oxygen concentration and food availability (Pechenik, 1999). Such environmental factors affect the survival and successful development of larvae and subsequently have major impacts on species abundances and species distributions. Therefore, in studying population dynamics of marine organisms it is important to know how environmental factors affect species larval life history stage.

Temperature, salinity and nutrition are the three most often studied environmental parameters affecting larval growth and development (Bayne, 1983). Among these, temperature and nutrition have been identified as the most important (Hoegh-Guldberg and Pearse, 1995; Robert et al., 1988). Development and growth rates have been demonstrated to increase with increasing temperature in many marine invertebrates (Drent, 2002; García de Severeyn et al., 2000; Liu et al., 2010; Zimmerman and Pechenik, 1991) and food availability has significant effects on growth and survival (Bos et al., 2007; Emlet and Sadro, 2006; Millican and Helm, 1994; Pechenik and Rice, 2001; Robert et al., 1988). In a review on molluscan larval development, Bayne (1983) found that the growth rates of veliger larvae showed similar trends – increasing with increased temperature to a maximum and then showing an abrupt decline in growth rate as larvae became competent. This demonstrates a positive relationship between larval growth rate and temperature. In a study on the effects of temperature, salinity and nutrition on larval development of *Ostrea edulis*, Robert et al. (1988) found that nutrition accounted for almost 90% of the variation seen in growth of larvae under different temperature and salinity conditions. Other studies demonstrate that high food availability during larval development leads to larger larvae and juveniles (Phillips, 2002) while low food availability leads to smaller larvae and juveniles (Chiu et al., 2007; Emlet and Sadro, 2006). The effects of salinity on larval growth and development are variable. Some studies found increased larval growth with increasing salinity (Davis and Ansell, 1962; Loosanoff and Davis, 1963; Newkirk et al., 1977), while other studies found no or variable effects of salinity (Pechenik, 1989; Robert et al., 1988).

In general, conditions that favour faster growth lead to larger body size, which is often associated with greater survival, fecundity and mating success (Kingsolver and Huey, 2008). However, achieving and maintaining larger size may be risky and more costly (Kingsolver and Huey, 2008). Larger size may require longer development time resulting in longer exposure to predation and other stresses during vulnerable pelagic developmental stages, ultimately leading to decreased chance of survival and reproduction. In near-shore coastal environments where environmental conditions are highly variable, organisms face antagonistic pressure between achieving a larger body size to achieve better fitness and decreasing development time to reduce the risks

associated with it. Determining which factors exert the greatest pressure is important in understanding population dynamics.

Research on larval ecology of marine invertebrates has been extensive since Thorson's (1950) pioneering studies, but the idea that conditions experienced during larval life, and even during embryogenesis, have effects on juvenile and adult health and fitness are relatively new. For a long time, metamorphosis was thought to be a "new beginning" for marine invertebrates that undergo drastic changes in body form at this major life history transition (Pechenik et al., 1998). This is reflected in how benthic marine organisms and their populations have been studied. Metamorphosis acts as an imaginary divide between larval biology, which focuses on events from fertilization to metamorphosis, and benthic ecology, which focuses on events after metamorphosis although recruitment is also recognized as important. Recruitment studies often assume that if larvae survived the vulnerable planktonic phase of life and successfully completed metamorphosis, then they must have equal chances of post-metamorphic survival, growth and reproduction. Studies show, however, that this is often not the case. Larval experience results in significant differences in larval and juvenile success suggesting that newly settled larvae are a heterogeneous mix of organisms with different fitness levels and performance potential. A review by Pechenik (2006), highlighted carry-over effects in 37 out of 42 studies across many taxa. Understanding what factors influence larval fitness and how variation in larval quality can influence post-metamorphic growth and development are important for understanding population dynamics.

Research Objectives

Despite the elimination of harvest pressure, reductions in toxic inputs into coastal waters, legal species protection (in Canada), and efforts to restore natural populations (in the U.S.), populations of *O. lurida* are not recovering and the reasons remain unclear. Similar to other marine invertebrates, temperature and salinity have been identified as important factors influencing the survival and growth of Olympia oysters (Couch and Hassler, 1989; Strathmann, 1987). However, the existing information on environmental tolerances and the basic requirements of Olympia oysters in all of their developmental stages is very limited and subsequently limits our ability to develop suitable recovery

strategies for this “species of special concern”. This research investigated the influence of temperature, salinity and food concentration on the growth and development of the larvae of *O. lurida*.

It is expected that this estuarine species, in which adults have a wide range of temperature and salinity exposure, will produce larvae that may also demonstrate normal growth and development under different temperature and salinity regimes. As measured by rate of growth and differentiation, the effects of different levels of salinity, temperature, and food availability were examined to determine the influence on larval growth and development. Another goal was to determine if growth and development rate were tightly coupled.

Understanding how species react to changing environmental conditions is of particular importance given the current and predicted future changes in global climatic conditions, for example, rising sea surface temperatures and changes in precipitation (Collins et al., 2013). Understanding how such changes will affect Olympia oysters during their early life history will complement studies on adult populations and contribute to a more comprehensive understanding of factors that are driving current population dynamics of this “species of special concern”.

Methods

Adult conditioning

Experiments were conducted over two years, from 2011 to 2012. In May 2011, 200 adult oysters were collected from Ladysmith Harbour, British Columbia, Canada (N 49° 0.791' W123° 50.466') and transferred to a 200 litre (L) tank in a recirculating sea water system at Vancouver Island University (VIU). Adult oyster broodstocks were continuously drip fed a 1:1 (by volume) mixed diet of live *Isochrysis galbana* and *Chaetoceros muelleri.* In 2012, 40 adult oysters were collected in June from the Gorge Waterway in Victoria, BC (N 48° 27.699' W 123° 24.766') and held in a recirculating sea water system at the University of Victoria in two 19 litre tanks. Oysters were drip fed a daily mixture of three commercial algal paste products in a 2:1:1 ratio (by volume): Shellfish Diet 1800 (a mix of *Isochrysis, Pavlova, Tetraselmis* and *Thalassiosira weissflogii*), *Isoschrysis* 1800 and Pavlova 1800 (Reed Mariculture Inc.). In both years, water temperature was gradually increased over a period of 2 weeks from 12°C to 18°C (±1.0°C) and maintained at 18 °C for three to four months for conditioning prior to spawning. Salinity was constant at 30-31 psu.

Adults were left to spawn and brood naturally. Veliger larvae were collected from the aquaria outflow water on 55 μm catchment screens which were checked daily. Collected veligers were immediately distributed among individual culture beakers for experimentation.

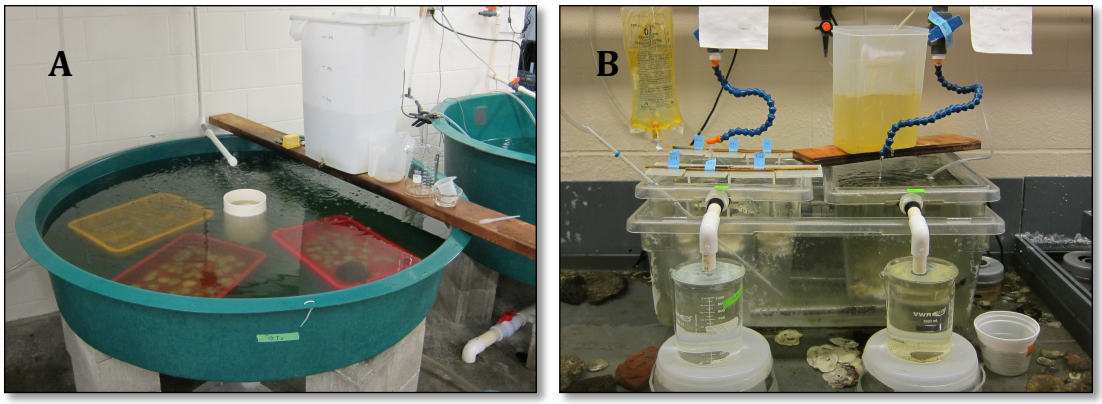

Figure 1**.** Apparatus for maintaining post-metamorphic *Ostrea lurida*. Adults were continuously drip-fed from food reservoirs above tanks. Released larvae were collected for experimentation. **A.** Adults collected from Ladysmith Harbour were held in a two hundred litre tank at Vancouver Island University's (VIU) Centre for Shellfish Research, Nanaimo, BC. **B.** Two 19 litre tanks at the University of Victoria (UVic), Victoria, BC. The tank on the left contains settled juveniles on adult oyster shells. The tank on the right contains adults collected from the Gorge Waterway.

Larval rearing

Larvae released from brooding females were cultured in 1 L glass beakers at an initial density of 1 larva/ml, with a total number of 700 or 800 larvae in each beaker. Culture water was changed every other day by filtration through a 64 µm Nitex screen and then 93 µm when larvae were larger. In 2011, seawater from the recirculating system at VIU was used for larval cultures. This water was filtered to 5 µm and UV sterilized. In 2012, water was collected every other day from the Gorge Waterway and vacuum filtered through a 0.45 µm glass fibre filter. In 2011, light regime was not strictly controlled, but was roughly 12 hours of light and 12 hours of dark. In 2012, experiments were run with a constant light regime of 15 hours of light and 9 hours of darkness.

Experiments were conducted to test the effect of temperature, salinity and food density on larval growth rate and differentiation. Each experiment tested differences in only one of these parameters, while the other two parameters were held constant. Within each experiment, three replicate cultures were set-up for each treatment level.

Except for the experiments in which food concentration was manipulated, a ration of 5x104 cells/ml of live micro algae were added to cultures after each change of culture water. In 2011 the food ration consisted of a 1:1 mix of *Isochrysis galbana* and

Chaetocerus muelleri, and in 2012 it was a 1:1 mix of *Isochrysis galbana* and *Pavlova lutheri*. Except for experiments in which temperature was manipulated, larvae were reared at 18 °C. Except for experiments in which salinity was manipulated, larvae were reared in seawater with salinities of 29 to 31 psu; this variation was due to differences in the salinity of source water. A refractometer was used to measure salinity in 2011 and a handheld, digital YSI was used to monitor salinity in 2012.

For the temperature experiments, larvae were cultured at: 13, 17, or 21°C. For the food level experiments, larvae were fed: $5x10^3$, $1x10^4$, or $5x10^4$ cells/ml. For the salinity experiments, larvae were cultured at: 11, 21, or 31 psu in 2011, and at 15, 24, or 30 psu in 2012. Different levels of salinity were obtained by adding deionized water or Instant Ocean salts to source seawater. Table 1 outlines the conditions for all experiments.

Table 1. Summary of experiments investigating the effects of different environmental conditions on larvae of *Ostrea lurida*.

Year	Treatment	Temp (°C)	Salinity (psu)	Food concen-tration (algal cells/ml)	Water source	Culture size (ml)	Diet	Light regime (hrs L: hrs D)	Facility	Days of Culture	Larval survival measured	Settlement Shells added
2011	Temperature	13, 17, 21	30-31	$5x10^4$	VIU recirc system	700	Live T-Iso & Cm	Not regulated; approx. 12:12	VIU	23	No	
	Salinity	18	11, 21, 31	$5x10^4$	IU recirc system	700	Live T-Iso & Cm	Not regulated; approx. 12:12	VIU	13 for 11psu; 21 for 21 & 31 psu	Yes	
2012	Salinity	18	15, 24, 30	$5x10^4$	Gorge Waterway	800	Live Ig & Pl	15:9	UVic	25	Yes	Day 19 (15 psu); Day 17 (24, 30 psu)
	Food concentration	18	29-30	$5x10^3$, $1x10^4$, $5x10^4$	Gorge Waterway	700	Live Ig & Pl	15:9	UVic	27	Yes	Day 19 (med, high); Day 26 (low)

T-Iso = *Isochrysis galbana*, Tahitian strain
Ig = *Isochrysis galbana*
Cm = *Chaetoceros muelleri*
Pl = *Pavlova lutheri*

Quantifying larval growth and development

Larvae were sampled from cultures every four to six days to measure the length of the larval shell and identify development stage. In 2011, ten larvae were removed from temperature treatment cultures and preserved in approximately 80% undenatured ethanol for measurement of shells at a later date. Larvae were individually pipetted out of culture during culture water changes. Five swimming larvae and five larvae that were settled on the bottom were removed. In 2012, and for the salinity cultures in 2011, larvae were subsampled in three 2 ml aliquots while gently plunging the entire culture that had been concentrated to 350 ml in a graduated cylinder (following Helm et al., 2004). This allowed for more random selection of larvae and enabled an evaluation of culture density over time. Larvae were not returned to cultures. Aliquot volumes of samples were not adjusted over time and with decreasing culture densities; therefore, final culture densities and culture survival (estimated number of live larvae remaining in culture) are underestimated in this study. Preserved (2011) and live (2012) larvae were examined using a Zeiss Axioskop compound microscope and photographed using a QImaging Retiga 2000R microscope camera. Development stage (D-stage, early umbo, late umbo, or eyed veliger) was identified and shell length was measured along the anterior-posterior axis, parallel to the hinge (as in Loosanoff et al., 1966), using ImageJ software ver. 1.48o.

Larval length measurements from the three replicates for each treatment were pooled, as no difference was found between larval lengths in replicate beakers.

The overall increase in larval size, and therefore, growth rate, was underestimated in this study, as it was not calculated from day 1. Size measurements began on the second sampling day.

Larval settlement and growth of juveniles

In 2012, a series of four stacked *Crassostrea gigas* shells were suspended in cultures of each of the food concentration treatments ($5x10^3$, $1x10^4$ and $5x10^4$ algal cells/ml) and each of three salinity treatments (15, 24 and 30 psu) to allow settlement of larvae. Shell stacks were added when the majority (≥ 50%) of larvae had reached the eyed veliger stage: day 17 for the 24 and 30 psu cultures, day 13 for the high food concentration cultures and day 19 for the medium food concentration. Larvae did not develop eyespots

in the 15 psu and low food concentration cultures but shells were added to remain consistent in all treatments. Shells remained in the salinity larval cultures for approximately two weeks. Water changes and feeding continued every other day, but larvae were not sampled during this time.

An average of only 3.2 larvae/shell (± 3.6 s.d.) settled in the food culture treatments. This was insufficient to continue an investigation comparing post-metamorphic survival between treatment levels, therefore, shells were discarded and this experiment did not continue. No settlement occurred in the15 psu cultures and therefore, these shells and cultures were also discarded.

On day 30, all of the shells from the 24 and 30 psu cultures were removed from the culture beakers and suspended in two 19 litre tanks in the recirculating sea water system at UVic, held at a temperature of 18° C. The mean number of settled larvae on these shells was 30.9 larvae/shell (± 16.4 s.d.) at 24 psu and 19.4 larvae/shell (± 18.1 s.d.). The day before being placed in the recirculating system, the salinity of the 24 psu cultures was gradually increased to 30 psu, to match the salinity in the recirculating system.

Juvenile oysters were drip fed a 1:1 (by volume) mixed diet of *Isochrysis* 1800 and *Pavlova* Instant Algae (Reed Mariculture Inc.) daily. Twenty juveniles from the smooth, underside of each shell were randomly selected and examined monthly using a dissecting microscope to monitor survival. On shells with fewer than 20 larvae, all larvae were monitored. Oysters were held in these tanks for 14.5 weeks. In November 2011, ten shells were transferred to the Gorge Waterway and 10 shells remained in the recirculating system in the lab, each with a minimum of ten settled juveniles. Shells remained in the Gorge for 2.5 months and were retrieved in February to compare differences in survival in laboratory versus natural conditions.

Growth Analysis

Larval growth was analysed using a one-wayANOVA to compare overall growth rates to the end of the experiments, as well as growth rates to the eyed veliger stage. The eyed veliger stage was identified when ≥ 50% of the sampled larvae from a culture had reached this developmental stage. A post hoc Tukey HSD (honest significant differences) test followed each ANOVA.

Growth trend lines were fitted to the data using the Weibull growth model (y=a-b*exp(-exp(c)*x^d)). This model was selected over other models (e.g. Von Bertalanffy, Logistic, Gompertz) based on AIC values (Table 2). The Weibull model was clearly the best fit for data from temperature treatment 17°C and salinity treatments 30 and 31 psu (difference of ≥ 6 AIC values). There was no significant difference in AIC values for each of the other models when applied to data from the temperature treatment 13°C, 21°C, and salinity treatments 24, 30 and 31 psu, therefore, the Weibull model was equally as good as the other models for these treatments. There were too few data in all of the food treatments and in the salinity 11 and 15 psu treatment to fit the growth models. The models also could not be applied to salinity 21 psu data as these data showed a very clear linear trend and the non-linear models would not fit the data.

All statistics were done using the R statistical software package (ver. 3.1.1)

Size at Eyed Veliger Stage

The size at which larvae reached the eyed veliger stage within each experiment was compared using a series of one-way ANOVAs for each treatment. A single two-way ANOVA could not be conducted as each developmental stage was not reached in every treatment. A repeated measures ANOVA was not used because it was not the same individuals that were sampled at different developmental stages. Individuals that were removed for measurement were not returned to cultures.

Results

Growth And Development

Temperature

Water temperature had a significant effect on overall average growth rate of larvae (one-way ANOVA $F = 123.75$, $df = 2$, $p < 0.000$, Table 3) and growth rate to the eyed veliger stage (one-way ANOVA $F = 13.26$, $df = 1$, $p = 0.036$, Table 3). Overall, larvae at 17 and 21°C grew four times more quickly than larvae at 13°C (Figure 2). With a mean growth rate of only 1.19µm/day (± 0.27 s.d.), larvae reared at 13°C only showed a 13.0% increase in overall length and no larvae at this temperature developed eyespots. Over the duration of the study, larvae at 17°C and 21°C increased in length by 51.2% and 52.3% respectively and had similar overall growth rates of 4.74µm/day (±0.19 sd) and 4.87µm/day (±0.46 sd). The growth rate to the eyed veliger stage was significantly greater at 21°C (5.38 µm/day, ± 0.33 sd, at 17°C and 7.04 µm/day, ± 0.73 sd, at 21°C; one-way ANOVA $F = 13.26$, $df = 1$, $p = 0.036$, Table 3).

The time at which ≥ 50% of larvae reached the eyed veliger stage, in all treatments where larvae did develop eyespots, coincided with a significant reduction in larval growth rate, illustrated by an asymptote in the growth curves (Figure 2). At 21°C larvae stopped growing after 15 days (51.7% eyed veligers), and at 17°C larvae stopped growing after 19 days (56.7% eyed veligers). There was also no difference in the size at which larvae developed eyespots at these two temperatures (one-way ANOVA $F = 0.618$, $df = 1$, $p = 0.434$) (Figure 3, Table 4).

Table 2. AIC values for Weibull, Logistic, Gompertz and von Bertalanffy growth models applied to growth of larvae of *Ostrea lurida* reared at 13, 17 and 21°C and at 24, 30 and 31 psu. Lower AIC values indicate better model fit.

	AIC value			
Treatment	**Weibull**	**Logistic**	**Gompertz**	**von Bertalanffy**
Temperature (°C)				
13	1631.062	1631.448	1631.620	1631.657
17	1690.712	1700.804	1702.401	1702.857
21	1751.011	1751.363	1751.363	1756.581
Salinity (psu)				
24	1065.204	1065.204	1066.678	1067.971
30	822.113	822.121	876.005	872.571
31	1255.604	1255.233	1262.544	1263.783

Table 3. One-way ANOVA results comparing average larval growth rates for *Ostrea lurida* for the duration of each experiment and to the onset of eyespot differentiation at temperature 13, 17 and 21°C; salinity 11, 15, 21, 24, 30 and 31 psu; and food concentrations of $5x10^3$, $1x10^4$ and $5x10^4$ algal cells/ml.

One-way ANOVA							
		To end of experiment			**To eyed veliger stage**		
		df	**F**	**p**	**df**	**F**	**p**
Temperature	Trt level	2	123.75	<0.000	1	13.26	0.036
	Residuals	6			3		
Salinity (11, 15, 21, 24, 30, 31)	Trt level	5	223.3	<0.000	3	4.52	0.046
	Residuals	12			7		
Salinity (24, 30, 31 only)	Trt level	2	1.389	0.319	2	2.94	0.129
	Residuals	6			6		
Food	Trt level	2	37.93	0.001	1	7.55	0.051
	Residuals	5			4		

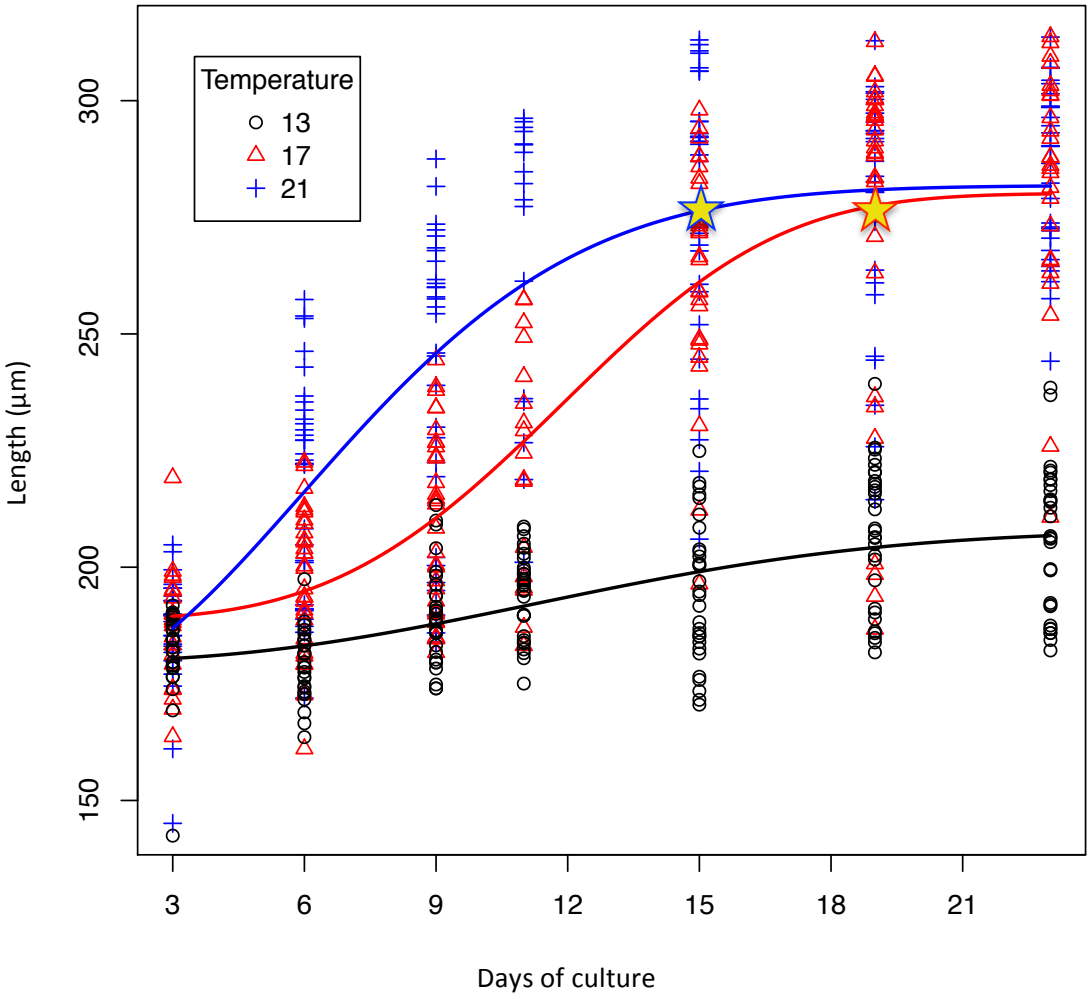

Figure 2. Growth of larval shell of *Ostrea lurida* cultured at 13°C (○), 17°C (△) and 21°C (+). Star indicates when ≥ 50% of larvae had achieved eyespot differentiation. On day 15, 51.7% of larvae had eyespots at 21°C, and on day 19, 56.7% or larvae had eyespots at 17°C. Trend lines applied using Weibull growth equation, y=a-b*exp(-exp(c)*x^d).

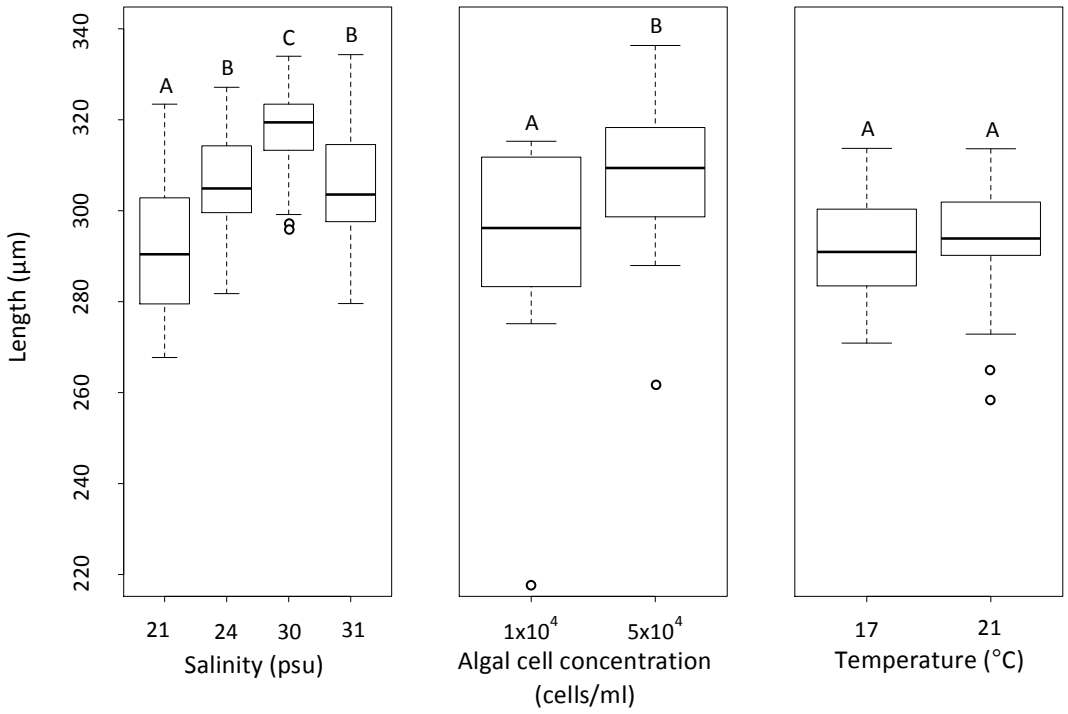

Figure 3. Mean larval length at eyespot differentiation of *Ostrea lurida* reared under different experimental conditions: 21, 24, 30 and 31 psu; $1x10^4$ and $5x10^4$ algal cells/ml; 17 and 21°C. The same letter within experimental treatments indicates no significant difference in larval length. Similar letters between experimental treatments are not related.

Table 4. One-way ANOVA results comparing size of larvae at the time of eyespot differentiation in *Ostrea lurida* given different levels of three experimental parameters: temperature (17 and 21°C), salinity (21, 24, 30 and 31 psu) and food concentration ($1x10^4$ and $5x10^4$ algal cells/ml).

		df	**F**	**p**
Temperature	Trt level	1	0.618	0.434
	Residuals	76		
Salinity	Trt level	3	33.7	< 0.000
	Residuals	140		
Food	Trt level	1	10.34	0.00208
	Residuals	61		

Note: Post hoc Tukey test shows there is no significant difference between 24 psu and 31 psu (p = 0.9996), but significant differences exists between all other treatments.

Salinity

Low salinity had an effect on larval survival, growth and development. At 11 psu all larvae were dead by day 17. Among the other treatments larval survival over the entire study was lowest at 24 psu (14.6%) and highest at 21 psu (43.3%) (Table 5). Survival was also higher at 15 psu (38.9%) compared to 30 and 31 psu (31.6% and 20.0% respectively). Some larvae exhibited irregular swimming behaviour and irregular shell development at both 11 and 15 psu (Figure 4). Larvae did not grow and no larvae developed eyespots in either of these treatments. At 21, 24, 30 and 31 psu larvae grew and developed normally. A one-way ANOVA analysis indicated no difference in the overall average growth rates or in growth rates to the eyed veliger stage at salinities 24, 30 and 31 psu (one-way ANOVA $F = 1.389$, $df = 2$, $p = 0.319$; one-way ANOVA $F = 2.941$, $df = 2$, $p = 0.129$, Table 3). The growth rate of larvae at 21 psu was significantly slower than larvae reared at 30 psu (post hoc Tukey $p = 0.039$, Table 6), but not compared to larvae reared at 24 and 31 psu (post hoc Tukey $p = 0.299$; post hoc Tukey $p = 0.634$ respectively, Table 6).

After 21 days of culture larval length was still increasing steadily in a linear fashion at 21 psu, whereas the growth of larvae at 24, 30 and 31 psu larvae had levelled off (Figure 5). Larvae at 30 psu reached a significantly larger maximum size (318.2 μm) than larvae at 24 (306.4 μm) and 31 psu (302.0 μm) ($p = 0.002$; $p = 0.002$); and the asymptotes on the curves again coincided with the time at which approximately ≥ 50% of larvae had developed eyespots: day 17 for 24 psu (66.7%) and 30 psu (82.6%) and day 21 for 31 psu (70.0%) (Figure 5, Table 7).

Table 5. Percent survival of larval cultures of *Ostrea lurida* reared at different salinities of 11, 15, 21, 24, 30 and 31 psu, and food concentrations of $5x10^3$, $1x10^4$ and $5x10^4$ algal cells/ml. Survival was calculated by comparing the estimated number of live larvae present in cultures at the end of culture period compared to the initial number of larvae in cultures.

Treatment	Level	Days of culture	% Survival
Salinity (psu)	11	17	0
	15	23	38.9
	21	21	43.3
	24	23	14.6
	30	23	31.6
	31	21	20.0
Food Concentration (cells/ml)	$5x10^3$	25	75.0
	$1x10^4$	25	41.7
	$5x10^4$	25	22.2

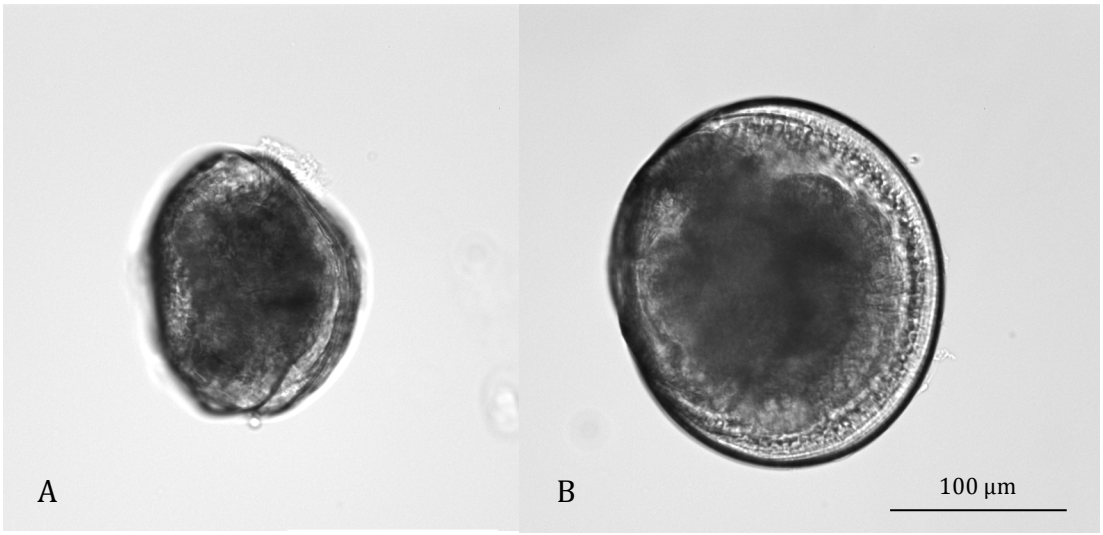

Figure 4. Five day old larvae of *Ostrea lurida* reared at (A) 11 psu and (B) 31 psu. Some larvae reared at 11 psu demonstrated non-normal growth and development exhibited by wavy shell margin.

Table 6. Post hoc Tukey HSD results comparing larval growth rates *Ostrea lurida* for the duration of each experiment and to the onset of eyespot differentiation at temperature 13, 17 and 21°C; salinity 11, 15, 21, 24, 30 and 31 psu; and food concentrations of $5x10^3$, $1x10^4$ and $5x10^4$ algal cells/ml.

Post hoc Tukey HSD

			To end of experiment			To eyed veliger stage		
Treatment	**level**	**level**	**Mean Difference**	**P-value**		**Mean Difference**	**P-value**	
Temperature (°C)	13	17	3.55	0.000	*			
	13	21	3.68	0.000	*			
	17	21	0.123	0.879		1.66	0.036	*
Salinity (psu)	11	15	0.317	0.911				
	11	21	5.90	0.000	*			
	11	24	6.56	0.000	*			
	11	30	7.20	0.000	*			
	11	31	6.83	0.000	*			
	15	21	5.58	0.000	*			
	15	24	6.25	0.000	*			
	15	30	6.89	0.000	*			
	15	31	6.51	0.000	*			
	21	24	0.660	0.361		2.64	0.299	
	21	30	1.30	0.015	*	4.81	0.039	*
	21	31	0.924	0.107		1.68	0.634	
	24	30	0.642	0.389		2.17	0.361	
	24	31	0.264	0.957		-0.966	0.858	
	30	31	-0.378	0.835		-3.13	0.134	
Food (cells/ml)	H	L	-5.03	0.001	*			
	H	M	-1.29	0.123		-2.88	0.052	
	L	M	3.74	0.003	*			

* Mean differences significant at the 0.05 level

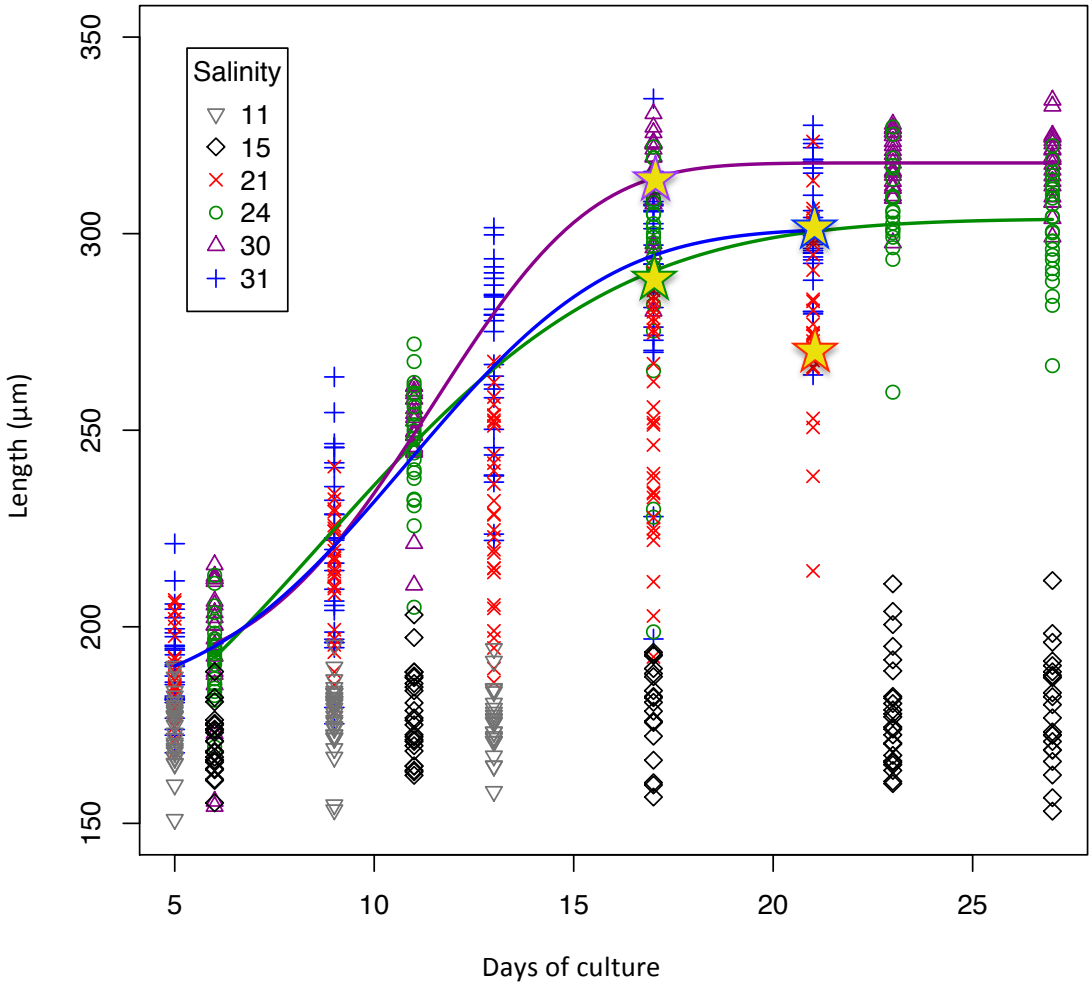

Figure 5. Growth of larval shell of *Ostrea lurida* cultured at different salinities: 11 (▽), 15 (◇), 21 (x), 24 (○), 30 (△) and 31 (+) psu. Star indicates when ≥ 50% of larvae had achieved eyespot differentiation. On day 17, 66.7% and 82.6% of larvae had eyespots at salinities 24 psu and 30 psu respectively, and on day 21, 55.2% and 70.0% of larvae were eyed at salinities 21 psu and 31 psu respectively. Trend lines applied using Weibull growth equation, y=a-b*exp(-exp(c)*x^d). Insufficient data to apply Weibull model to salinity 11, 15 and 21.

Food concentration

Larval length increased almost 30 times more rapidly at the highest algal concentration than at the lowest concentration. At medium and high algal concentrations the size of larvae increased continuously over the course of the study (Figure 6). There was no significant difference in overall growth rates (post hoc Tukey test $p = 0.123$, Table 6) or in growth rates to the eyed veliger stage at medium and high algal concentrations (one-way ANOVA $F=7.55$, $df = 1$, $p = 0.051$, Table 3). There were insufficient data in all of the food treatments to apply the Weibull growth model. Larvae developed eyespots earlier at high food concentration (50.0% eyed veligers on day 13) than at medium algal concentration (52.6% eyed veligers on day 19) (Figure 6, Table 7). Eyed veligers at the high food concentration (307.8 µm) were significantly larger than those reared under medium concentration (293.5 µm) (one-way ANOVA $F = 10.34$, $df = 1$, $p = 0.00208$, Table 4). Larval survival was highest at low food concentration (75.0%) and lowest at high food concentration (22.2%) (Table 5).

Larval Settlement

Larval settlement in the food treatment cultures was less than one percent. Settlement in salinity cultures 24 and 30 psu was 15.5 % (30.9 larvae/shell ± 16.4 s.d.) and 9.7% 19.4 larvae/shell ± 18.1 s.d.) respectively. Settlement density (over the entire shell) was very low: 0.45 larvae/cm^2 at 24 psu and 0.28 larvae/cm^2 at 30 psu. The low settlement in 2012 is in contrast to the settlement density of 12.6 larvae/cm^2 observed previously in 2011.

Out-plant of juvenile Oysters

There were no surviving oysters on settlement shells kept in the laboratory or on shells that were out-planted to the Gorge Waterway.

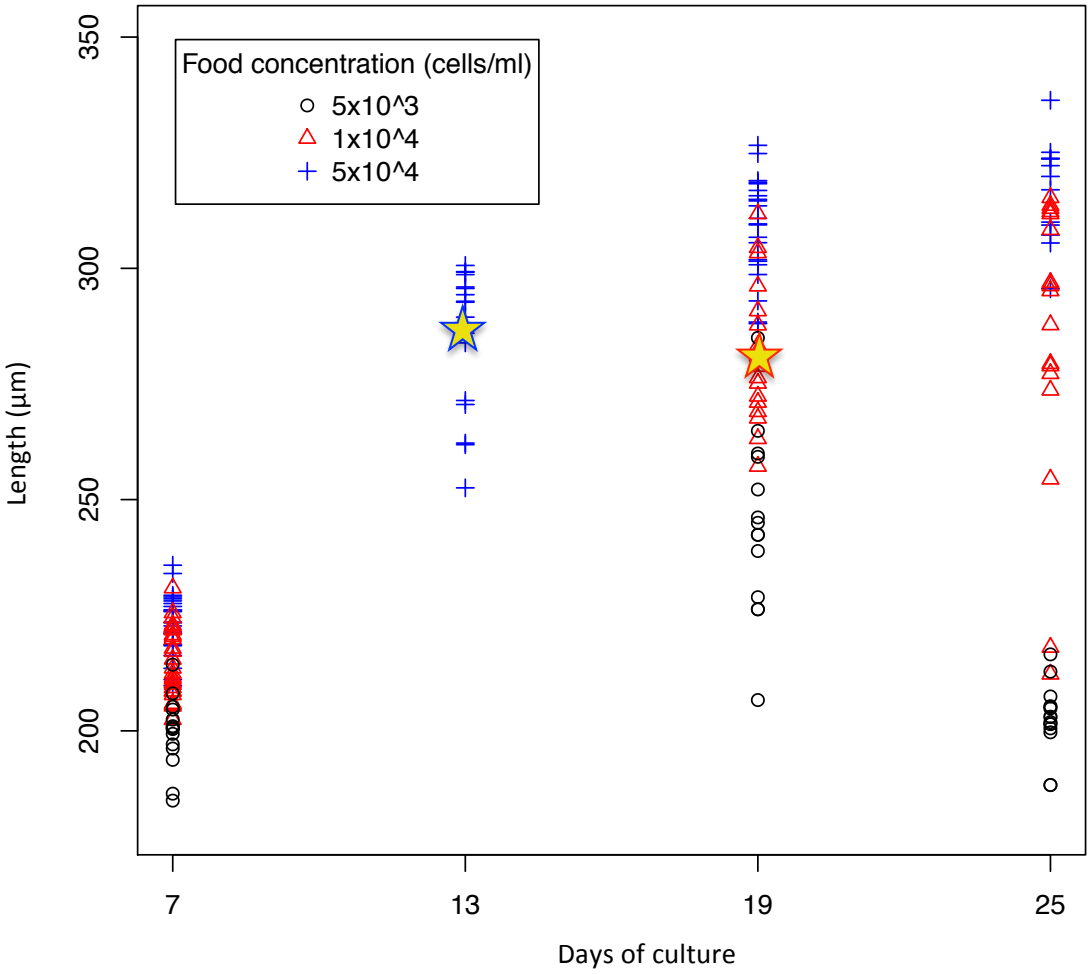

Figure 6. Growth of larval shell of *Ostrea lurida* cultured at different food concentrations: $5x10^3$ (○), $1x10^4$ (△) and $5x10^4$ (+) algal cells/ml. Star indicates when ≥ 50% of larvae reached eyed veliger stage: day 19 for $1x10^4$ cells/ml (medium concentration); day 13 for $5x10^4$ cells/ml (high concentration). Data were insufficient to apply growth model.

Table 7. Size of larvae of *Ostrea lurida* at different developmental stages and the amount of time taken to reach stage of development given different temperature (13, 17 and 21°C), salinity (11, 15, 21, 24, 30 and 31 psu) and food concentration ($5x10^3$, $1x10^4$ and $5x10^4$ algal cells/ml). Larval culture identified as reaching a development stage when ≥ 50% of larvae reached that stage.

	Early Umbo			**Late Umbo**			**Eyed Veliger**		
	Size (µ)	**% at stage**	**Age (days)**	**Size (µ)**	**% at stage**	**Age (days)**	**Size (µ)**	**% at stage**	**Age (days)**
Temperature (°C)									
13	172-239	55.6	6	199-226	-	-	-	-	-
17	161-266	71.4	3	195-308	72.4	15	270-313	56.7	19
21	145-254	88.0	3	208-310	70.0	9	259-314	51.7	15
Salinity (psu)									
11	153-195	76.7	9	-	-	-	-	-	-
15	153-212	84.0	11	165-201	-	-	-	-	-
21	168-254	56.7	5	189-304	63.3	13	268-323	55.2	21
24	169-265	88.9	6	194-322	92.3	11	282-327	66.7	17
30	173-213	75.0	6	193-325	95.0	11	296-334	82.6	17
31	176-289	69.0	5	195-328	60.7	9	280-334	70.0	21
Food (cells/ml)									
$5x10^3$	185-217	93.3	7	205-265	92.9	19	-	-	-
$1x10^4$	202-231	74.1	7	209-282	-	-	218-315	52.6	19
$5x10^4$	210-236	73.9	7	218-327	-	-	262-336	50.0	13

Discussion

This study demonstrated that temperature, salinity and food concentration have significant effects on the larval growth and development of *Ostrea lurida*. At low temperature (13°C), salinity (15 psu) and low food concentration ($5x10^3$ cells/ml) the larvae of *O. lurida* survived but did not grow or develop. With increasing levels of each treatment, growth rate and development increased and were coupled. Increasing levels of salinity and food concentration also resulted in differences in larval size at the time of eyespot differentiation, but changes in temperature did not affect larval size at the onset of eyespot differentiation.

Larval growth and development

Environmental conditions experienced by marine invertebrate larvae can have effects that carry-over and affect the performance and success of organisms at later life stages (see review by Pechenik, 2006). Factors such as food availability, salinity, toxins and elevated pCO_2, have been shown to affect larval size at metamorphic competence which can subsequently affect juvenile growth, fitness and survival (e.g. Emlet and Sadro, 2006; Helm et al., 1973; Marshall and Keough, 2003; Przeslawski and Webb, 2009). The mechanisms responsible for the decrease in post-metamorphic performance are little known (Pechenik, 2006), but some evidence shows, for example, that lipid content of larvae is related to settlement and metamorphic success (Gallager et al., 1986; Helm et al., 1973). Food limitation is therefore an important consideration when investigating the health of larvae. In molluscs, food limitation results in significantly smaller larvae at settlement with lower lipid stores and results in slower growing juveniles, even if larvae are starved for only short periods of time (Emlet and Sadro, 2006; Pechenik et al., 2002, 2002, 1996; Phillips, 2002). Conversely, larger larvae give rise to larger and faster growing juveniles with higher survival. Food availability is also suspected of mitigating the effects of other environmental stresses experienced by larvae. For example, Hettinger et al. (2013a) suspected that larger larval energy reserves, resulting from higher food availability during larval development, resulted in greater

metamorphic success when *Ostrea lurida* were exposed to elevated pCO_2 during larval development. While food availability did not negate the effects of elevated pCO_2 it had a measureable effect.

The effects of salinity on larval growth and development are variable. Some species produce larvae that show normal growth over a wide range of salinities, however, other larvae are less tolerant and have a smaller optimum range of salinity within which they reach their maximum size (e.g. Davis, 1958; Davis and Ansell, 1962). Low salinity levels during larval development have also been shown to reduce post-metamorphic survival (Pechenik et al., 2001; Qiu and Qian, 1999).

Temperature indirectly affects larval size by affecting growth and development rates, but the size at which larvae settle and metamorphose is independent of rearing temperature (Drent, 2002; Loosanoff et al., 1966). In this study, the larvae of *O. lurida* showed no difference in the size at which eyespot differentiation occurred when reared at different temperatures. Larval food concentration and salinity did affect larval size, however. At lower food concentration, larvae were smaller at the time of eyespot differentiation, and from 21 to 30 psu the size of eyed veligers progressively increased with increasing salinity. At 31 psu the size decreased. This is consistent with a study by Robert et al. (1988) who found that the larvae of the European oyster, *Ostrea edulis*, reached a maximum size at 30 psu; but above and below 30 psu maximum larval size decreased. This indicates that the optimum salinity for *O. edulis* and *O. lurida* is 30 psu.

Size of larvae at metamorphosis or settlement is often taken as an indicator of larval quality and hence potential juvenile performance. Larval length and eyespot differentiation have long been used as measures of metamorphic competence in bivalve larval studies and in bivalve hatcheries (Coon et al., 1990; Galtsoff, 1964; Helm et al., 2004; Loosanoff et al., 1966). Similarly, in this study, the size at metamorphosis or settlement was not observed, but, the size of larvae at the onset of eyespot differentiation was measured and taken as a proxy of prospective size at metamorphosis. The growth of bivalve larvae can follow a sigmoidal or asymptotic trend that reflects a period of rapid growth followed by a period in which larvae become metamorphically competent and significantly reduce growth rate (Bayne, 1983, 1965; Coon et al., 1990). Larvae delay metamorphosis during this time as they search for suitable settlement substrate (Bayne,

1965; Coon et al., 1990). This asymptotic trend is observed in the larval growth curve of *O. lurida* (Figures 2 and 5). The growth of larvae at salinities of 24, 30 and 31 psu and 17°C and 21°C clearly reached maxima that coincided with the time at which the majority of larvae developed eyespots. The mean length of eyed veligers, depending on treatment applied, ranged from 291 µm (± 14.9 s.d.) to 318 µm (± 8.1 s.d.), which is consistent with results of a study by Loosanoff et al. (1966) in which the setting size of *O. lurida* larvae was ~300 µm. *Ostrea lurida* larvae will, therefore, presumably become competent and will metamorphose at approximately the size at which eyespot differentiation occurs and larger eyed veligers will settle and metamorphose at larger sizes than smaller eyed veligers. The observed differences in the sizes of eyed veligers in this study, provides evidence that salinity and food conditions experienced during larval life may affect post-metamorphic growth and survival of *O. lurida* juveniles, but further study is required to confirm this. Some marine invertebrate organisms are able to recover from stress, including salinity stress, experienced during larval rearing and subsequently demonstrate no difference in juvenile performance (Diederich et al., 2011; Pechenik, 2006). This suggests that carry-over effects are not universal and should be explored for individual species and individual conditions. Carry over effects have been demonstrated in *O. lurida* as a result of elevated pCO_2 in larval culture water leading to smaller juveniles with slower growth rates (Hettinger et al., 2013b; Sanford et al., 2014). It remains to be shown, however, if carry-over effects arise from larval salinity and food stress, or if *O. lurida* can recover from such stresses.

In addition to the effects of abiotic factors, maternal effects can also contribute to significant differences in larval size and condition and may influence post-metamorphic performance. For example, studies have shown that maternal size, parental diet and origin, and the conditions under which gametogenesis occurs can result in differences in larval growth and development and larval tolerances to environmental conditions (Drent, 2002; Marshall and Morgan, 2011; Millican and Helm, 1994; Newkirk, 1978; Qiu and Qian, 1999). In the current study, larvae likely came from different females so there is a chance that maternal size may have contributed to the observed differences in larval size, as has been demonstrated in other studies (e.g. González-Ortegón and Giménez, 2014), but this would also require further study. Differences in larval growth as a result of

parental origin, as observed by Newkirk (1978) and Drent (2002), can be ruled out in this study, except in the case of the salinity experiments. Salinity experiments were conducted over two years using different adults each year. Larvae for salinity treatments 11, 21 and 31 psu came from adults from Ladysmith Harbour (in 2011); and larvae for treatments 15, 24 and 30 psu came from Gorge adults (in 2012). It may be possible that observed differences in the size and growth rate of larvae could arise from differences in adult adaptations to their environments (Drent, 2002; Newkirk, 1978), however, Ladysmith Harbour and the Gorge appear to have similar summer temperature ranges: 14.4 - 20.7°C in Ladysmith Harbour (Vancouver Island University unpublished data) and ~15 - 21°C in the Gorge (World Fisheries Trust unpublished data). Salinity in these two locations differs however. Vancouver Island University recorded salinities of 22.1-25.0 psu in Ladysmith Harbour from June to August, 2011 (unpublished data), while seawater collected in July and August of 2012 from the Gorge for the current study, was consistently 29-30 psu. Any differences in larval condition arising from parental origin would have to be further investigated with more detailed comparisons of seawater conditions.

From a restoration perspective, differences in larval tolerances and juvenile performance arising from parental origin could have ramifications for stocking restoration areas with juveniles whose tolerances may be mismatched to the environment into which they are introduced. In order to ensure successful restoration, parental and offspring environmental conditions should be matched to optimize the chances of successful growth and reproduction of the introduced organisms.

Summary and future directions

This study illustrates that *O. lurida* larvae tolerate a broad range of temperature, salinity and food density conditions, but survival, growth and development are all affected by each factor. Higher levels among the three parameters that were tested supported faster growth and earlier development, and salinity and food concentration affected the size at which eyespot differentiation occurred. These results are important

because they demonstrate the range of temperature, salinity and food concentrations required to support *O. lurida* larval growth and development, but also highlight that conditions experienced during larval development may influence post-metamorphic survival and performance, which could subsequently influence adult population dynamics. The results also point to many questions that remain to be answered about the influence of environmental conditions on *O. lurida* and how they could help inform future research, monitoring and management of this species.

First is to determine if carry over effects from environmental conditions experienced by larvae exist and to what extent. In this study, research conducted in 2012 did attempt to investigate whether carry-over effects from low salinity and low food stress existed in *O. lurida*, however, larval settlement was very poor that summer and larvae that did settle did not grow. Attempts to rear other invertebrate larvae by others in the laboratory were also unsuccessful, suggesting that there was a larger systemic problem, not just a problem with the oyster cultures. Confirming the existence of larval carry-over effects from these stressors will help inform and guide population monitoring efforts of *O. lurida*.

Currently, the main action from the management plan for *O. lurida* is to enumerate adult populations every one to two years to monitor changes in population size. While this allows the tracking of adult populations, it only provides information on post-metamorphic forces driving changes in population. Larval supply, larval behaviour and larval traits have all been demonstrated to have significant influences on population structure in marine benthic organisms (Giménez, 2004). Further studies on this phase of life for *O. lurida* may provide valuable clues about the lack of population recovery. Further, maternal effects could be investigated as there are several ways in which maternal diet, location and size can affect larval and juvenile performance, and subsequently adult fitness and populations abundances (Drent, 2002; Marshall and Morgan, 2011; Millican and Helm, 1994; Newkirk, 1978).

Studies on the effects of multiple environmental conditions simultaneously are also needed. While it is informative to investigate the effects of environmental conditions on larval development in isolation, it is more ecologically relevant to study the interaction of multiple factors, as this is more reflective of natural, variable conditions

experienced by larvae. Studies demonstrate that the interaction of different environmental factors can have confounding or mitigating effects on one another. For example changes in temperature can affect salinity tolerance and vice versa, and these effects become more significant when larvae approach their tolerance limits (Brenko and Calabrese, 1969; Calabrese, 1969; Verween et al., 2007). When considering the combined effects of temperature, salinity and food availability on *O. edulis* larvae, Robert et al. (1988) found that food availability was responsible for the greatest variation in larval growth and influenced larval tolerance to high temperature and salinity. In his study, salinity was the least influential of the three factors. In contrast, Diedrich et al. (2011) found that salinity stress had a stronger effect on larval survival than did food availability for gastropod larvae.

Finally, monitoring the natural conditions that *O. lurida* larvae and adults encounter in British Columbian waters will also help inform our understanding of possible influences shaping the population dynamics of this species. For example, are larvae already experiencing stressful conditions during development? Understanding how organisms react to changing environmental conditions has never been as important as it is now given the accelerated rate at which global climate change is occurring (Collins et al., 2013). In order to be effective, management strategies must incorporate knowledge of species tolerances at all life stages to ensure actions are matched to species tolerances and future environmental conditions.

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