AN ABSTRACT OF THE THESIS OF

Morgan P. Bancroft for the degree of Master of Science in Marine Resource Management presented on February 4, 2015

Title: An Experimental Investigation of the Effects of Temperature and Dissolved Oxygen on the Growth of Juvenile English Sole and Juvenile Dungeness Crab

Abstract approved:

________________________________________________________________________

Lorenzo Ciannelli

In the past two decades, occurrences of summertime upwelling-driven low dissolved oxygen (DO) events, or hypoxia, have increased along the northeast Pacific coast. If hypoxic events are severe enough to cause marine species mortality, the areas affected are often called “dead zones.” In 2002 and again in 2006, the events were lethal, resulting in mass invertebrate and fish kills. Many organisms are impacted by these events, but less mobile juvenile fishes and crabs are potentially more vulnerable to the long-term effects of these stressful conditions than adults. Little is known about how low DO impacts coastal fishes and invertebrates, especially during the juvenile phase. Nursery habitats in the nearshore open-coast and enclosed estuaries may be differentially affected by hypoxia by prevailing summertime oceanographic conditions. Both ecosystems are critical for the early development of many ecologically and commercially important species. While one habitat may be compromised by stressful environmental conditions, the other may provide refuge.

In upwelling driven areas, low dissolved oxygen is typically coupled with colder water, while in estuarine or semi-enclosed systems, dissolved oxygen diminishes with increasing temperatures. Temperature and dissolved oxygen are known to have
interactive effects on fish behavior and physiology, which underscores the importance of studying hypoxia within different systems and in relation to other co-occurring environmental variables. In order to isolate the effects of these parameters on growth, I conducted three experiments over the course of two years on juvenile Dungeness crab (*Cancer magister*) and English sole (*Parophrys vetulus*). During each experiment, I held English sole in the lab in fixed oxygen and temperature conditions for at least thirty days, and Dungeness crab for approximately fifty. I tested a total of 15 oxygen and temperature treatment combinations, with at least two replicates of each treatment. These treatments simulated summertime oceanographic conditions that are typically found in two nursery habitats used by these species: the Yaquina Bay estuary and Moolack Beach, Oregon USA.

English sole specific growth (g d^{-1}) increased with higher temperatures (13 and 15°C) and oxygen concentrations (2.0 and 6.0 mL L^{-1}) and was up to four times lower in all low temperature and oxygen treatments (5 and 7°C; < 1.4 mL L^{-1}). Dungeness crab intermolt period decreased with temperature from 48 days at 5°C to 12 days at 15°C. Crab specific growth (mm d^{-1}) increased with temperature, with crabs in the highest temperatures growing nearly five times as much as crabs in the coldest temperatures.

Finally, if people have heard of hypoxia, it is commonly in relation to the condition found in the Gulf of Mexico, which is nutrient driven and can be traced to anthropogenic input. Instead, Oregon hypoxia is more difficult to trace directly to human-related activity, making West Coast hypoxia a relatively lesser understood occurrence. In order to share the results of my research and improve general public understanding of hypoxia,
I designed a ~3:00 minute video exhibit that was displayed at the Hatfield Marine Science Center Visitor Center. Our intent was to create a video that could be distributed to different Visitor Centers and museums that are interested in educating their visitors about the occurrence, causes and frequency of hypoxia on the Oregon Coast.
An Experimental Investigation of the Effects of Temperature and Dissolved Oxygen on the Growth of Juvenile English Sole and Juvenile Dungeness Crab

by
Morgan P. Bancroft

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APPROVED:

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

_____________________________________________
Morgan P. Bancroft, Author
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CHAPTER 1: GENERAL INTRODUCTION

Factors that influence the distribution, growth and survival of juvenile English sole (*Parophrys vetulus*) and Dungeness crab (*Cancer magister*)

1.1 Thesis outline and overview

The frequency of low dissolved oxygen events on the Oregon inner continental-shelf is increasing (Pierce et al. 2012), but consequences for juvenile organisms in these habitats remain largely unevaluated. The ultimate effects of extremely low and prolonged dissolved oxygen events on adult organisms are well documented, often characterized by mass mortality of invertebrates and fishes along the coast (Chan et al. 2008). On the other hand, the long-term effects of severely low dissolved oxygen on the growth of juvenile invertebrates and fishes are especially unstudied. In order to gain an understanding of the effects of these conditions on juvenile species in nursery grounds, I conducted three temperature/oxygen experiments, which simulated the range of conditions that may be encountered in Oregon estuaries and nearshore habitats.

Chapter 1 of this thesis is a general introduction to my research, surveying information about regional hypoxia, the life histories of the two species I studied, and the seasonal oceanographic patterns that impact the long-term growth of these ecologically and commercially valuable species. Chapter 2 describes the materials and methods used in this study, presents results of the temperature/oxygen experiments on English sole and Dungeness crab, and provides a discussion of those results and their implications. Chapter 3 is a report and slideshow presentation of a video that I designed in order to make hypoxia science accessible to a range of audiences. The video, along with two
aquaria that contained live juvenile Dungeness crab and English sole specimens, was on exhibit at the Hatfield Marine Science Center Visitor Center. Finally, Chapter 4 provides further overarching discussion and closing remarks.

1.2 Species of interest

Many commercially valuable fishes and invertebrates utilize Oregon estuaries and shallow nearshore habitats as nurseries during their early life history stages. This study examined the effects of temperature and dissolved oxygen on juveniles of two of the most prevalent species in both habitats: English sole, (*Parophrys vetulus*), and Dungeness crab (*Cancer magister*). Despite obvious taxonomic and morphological differences, the species share similar life history characteristics.

English sole are distributed from the Bering Sea to central Baja California. Most are commercially harvested in waters shallower than 130 m depth, but can be found as deep as 550 m (Eschmeyer & Herald 1999, Rooper et al. 2003). Commercial trawl harvests of English sole between Eureka, CA and Vancouver, B.C. reached a peak between 1940 and 1960 (Stewart 2007) but have declined in recent years. Despite reductions in harvested biomass, they were part of a multispecies groundfish fishery in Oregon that generated $42.7 million in revenue in 2013 (PacFIN 2013). Marketable English sole are primarily female: adults are highly sexually dimorphic with females growing to > 50 cm, nearly twice as large as males. Males are generally undersized and discarded when caught. Females attain maturity as early as 6 years, while males mature after 2 – 3 years (Sampson & Al-Jufaily 1999, Stewart 2007).
The commercial Dungeness crab trap fishery is the most important single-species fishery on the Oregon coast, valued at $71 million in 2013 (PacFIN 2013). On average, most adult crabs are harvested from depths of 10 – 45 m. The Oregon Department of Fish and Wildlife (ODFW) maintains a no-harvest refuge for Dungeness crab below 130 m. Other state and federal regulations help to maintain a healthy fishery: all female crabs must be released upon capture, and only adult male crabs above a minimum carapace width are legal to harvest. Restrictions for females and smaller males help to support a cyclic level of annual reproduction by allowing fecund adult crabs to breed for at least two years before recruiting to the fishery (Kaiser 2002). Commercial harvest is open from December through August, with a significantly reduced total allowable catch from May through August, to moderate the amount of capture stress and mortality on post-molt soft-shell crabs (Kaiser 2002).

Throughout each life-stage, both English sole and Dungeness crab are strongly dependent on their respective habitats. Benthic adults rely on offshore habitats where they feed and reproduce. Pelagic eggs and larvae are carried and moved by seasonal currents, and recently settled juveniles utilize nearshore nursery habitats before migrating offshore. All life history stages – adults, eggs, larvae, and juveniles – are subject to local oceanographic conditions and may be unable to escape stressful events. Severe anoxic events, such as those in 2002 (Grantham et al. 2004b) and 2006 (Chan et al. 2008), may eradicate or cripple populations of breeding age adults, but will also negatively impact young-of-the-year (YOY) age classes that support future fisheries. For each fishery, stock viability may be compromised by unfavorable conditions during some life history stage.
Therefore, an understanding of their entire life histories and how they are influenced by their environment is crucial for successful management of these important fisheries.

1.3 Life histories

Adult English sole spawn in the nearshore during the winter (Laroche et al. 1982), when, compared to the summer, average ocean temperatures tend to be warmer (Huyer 1977). Timing of spawning appears to be correlated with hydrographic conditions, as egg and larval stages are temperature sensitive (Alderdice & Forrester 1968). Consequently, pelagic larval duration and transport may be prolonged during periods of colder than average sea temperatures (Ketchen 1956). The spawning season is highly protracted and variable, occurring between December and April, with a peak during January or February (Barss 1976). Fecundity increases with age in females. Older, larger English sole produce more eggs; a single mature female may produce 0.15 to 2.1 million eggs during a single spawning event (Love 2011). Adults appear to prefer sandy or mixed sand-mud substrate in 35 – 90 m to spawn. Eggs are very buoyant and float to the surface (Budd 1940, Barss 1976). Eggs hatch after ~ 90 hours and the resultant larvae are more mobile, but still buoyant and at the mercy of oceanographic conditions for approximately another 80 days (Budd 1940). Juvenile English sole spend up to one year in coastal and estuarine nursery habitats before migrating offshore (Barss 1976) (See Figure 1.1).

Dungeness crab are distributed from Prince William Sound, Alaska to Magdalena Bay, Mexico (Morris et al. 1980, ADF&G 1994). Mating occurs between April and September, during the time of their annual molt cycle. A premolt female crab is held by a male in a premating embrace up to 7 days prior to her molting. Shortly after the female
crab molts, the hard-shell male and soft-shell female mate. A few months after
copulation, eggs are extruded and attached to the pleopods under the female abdominal
flap (Rasmuson 2013). Like female English sole, older female Dungeness crab also
produce more eggs, between 0.9 and 2.5 million per clutch (Hankin et al. 1989). Females
carry eggs for 60 – 120 days from October to December, and the eggs hatch through
March. Upon hatching, larvae progress through five successive zoal stages for a total
duration of about 80 – 95 days. During this time, molting events, growth, development,
and migration to areas of settlement are heavily influenced by oceanographic conditions
(Rasmuson 2013). Zoea exhibit strong diel vertical migration to feed at the surface during
the night and to the depths to avoid predation during the day. Throughout this phase,
most horizontal migratory transport is driven by prevailing currents. The last and most
advanced pelagic larval stage is the megalopae. Crabs spend 25 – 30 days during April
and May as megalopae before settling into nearshore or estuarine nursery grounds
(Pauley et al. 1989). During the ~1.5 years residence in the estuary or nearshore nursery
habitats, juvenile Dungeness crab molt up to eleven times before reaching sexual
maturity (Morris et al. 1980) (See Figure 1.1).

1.4 Juvenile transport and habitat use

Seasonal changes in wind patterns and ocean conditions heavily influence the
success of year classes of both study species (See Figure 1.2). The onset of winter
downwelling may serve as an environmental cue for adults to begin migrating to
spawning grounds (Kruse & Tyler 1989). Alderdice and Forrester (1968) found a strong
relationship between temperature and hatching success in English sole: winter surface
temperatures and the corresponding prevailing currents influence length, total hatch and viable hatch success of pelagic eggs and larvae. Warmer surface temperatures potentially reduce pelagic larval duration (Ketchen 1956), and strength of prevailing currents determines the distribution of larvae (Kruse & Tyler 1989).

For decades, it has been common knowledge that English sole utilized estuaries during the first year of life. Research in the mid-1980s documented juvenile English sole use of 13 estuaries on the Oregon coast; the species was only absent from three Oregon estuaries on the southern coast (Krygier & Pearcy 1986). Juvenile sole (20 – 180 mm) are regularly found to be one of the most abundant juvenile species in the Yaquina Bay estuary (Pearcy & Myers 1974, DeBen et al. 1990). Yet, larval stages of sole were one of the least abundant during Pearcy and Myers’ (1974) plankton tows, indicating that the flatfish must migrate into the estuary after the larval stage, but the study gave no indication of the mechanism.

How larvae recruit to their respective nursery habitats has been well studied. During several years in the 1980s, Gunderson et al. (1990) found that first instar juvenile crabs were uncommon in April, but substantial settlement began in May, after spring transition and switch to summer upwelling conditions. Without seasonal currents, the authors suggested that settlement success in appropriate nursery areas would be much lower. Boehlert and Mundy (1987) observed that English sole recruitment was linked with temperature and salinity. They suggested that pelagic larval fish enter the estuary by two primary methods: onshore wintertime downwelling via Ekman transport and lunar-phase driven tidal currents. Shanks and Eckert (2005) similarly found that pelagic larvae
settle in their respective nurseries using three mechanisms: 1) by riding onshore currents from offshore during downwelling in the winter/spring months; 2) migrating to depth to catch onshore currents during upwelling favorable conditions in the late Spring; and 3) by riding tides and internal waves to enter estuaries or settle nearshore.

Until the mid-1970s, recently settled English sole were reported mostly in estuaries and a few small coastal bays and inlets on the Oregon coast. Some studies even suggested that the flatfish relied exclusively on estuarine nursery areas during the first year of life (Olson & Pratt 1973). However, in 1979, Laroche and Holton documented newly settled English sole at an area of unprotected open coast 10 km north of Newport, Oregon called Moolack Beach. Juvenile English sole presence on the open coast was previously undocumented. Shortly after this publication, Rosenberg (1982) measured the differences in growth of sole between Moolack Beach and the Yaquina Bay estuary, and determined that the mean growth rates from the two areas were similar, but that there was higher variability in the size-at-age in the estuary than in the open coast. The author suggested that this may be accounted for by oceanographic conditions (greater range of temperature and salinity in the estuary/more consistency in the open coast), predation (fewer predators in the estuary), and intraspecific competition (food limitation in the open coast selects for larger individuals). These findings introduced new focus on the significance of nearshore nursery areas.

Estuaries and nearshore habitat conditions may differentially affect juvenile species growth and survival through a variety of processes. It has been suggested that estuaries may provide English sole refuge from intra-specific competition for food with butter sole
(Isopsetta isolepis), which share a similar diet, but are more abundant in the nearshore (Hogue & Carey Jr. 1982). Food must be ample in the estuary; Meyers (1980) found that juvenile English sole were present year-round, and could be collected as far as 16.1 km up the Yaquina River. In warm years, production and biomass of macroinvertebrates, specifically Crangon spp., is likely to be higher in the estuary than the coast, providing food for many species, but most importantly, for juvenile Dungeness crab (Gunderson et al. 1990). Accordingly, the authors observed that in California and Washington, Dungeness crabs grow nearly twice as fast in estuaries as in surrounding open coastal areas, due to abundant food sources and warmer temperatures.

Rosenberg (1982) suggested that predation on juvenile species is lower in the estuary than in the nearshore because fewer large fish are found in the estuary. Gunderson et al. (1990) later argued that predation is actually higher in the estuary than the nearshore: during the pelagic larval and recently settled juvenile stages, English sole and Dungeness crab are important food sources for a host of predators, contributing to the growth of other commercially valuable finfish species. Common predators that juvenile flatfish may encounter in nearshore and estuarine nursery habitats include ling cod (Ophiodon elongatus), rockfish (Sebastes spp.), age 1+ Dungeness crab, wading birds, seabirds, and marine mammals (Lassuy 1989). Common predators of pelagic larval crabs include Chinook (Oncorhynchus tshawytscha) and Coho salmon (O. kisutch), rockfish (Sebastes spp.), and herring (Clupea pallasi) (Morris et al. 1980, Emiliani & Baxter 1983). After they settle, juvenile crabs are still subject to heavy predation by a range of predators, including starry flounder (Platichthys stellatus), English sole, rock sole
(Lepidopsetta bilineata), ling cod, Cabezon (Scorpaenichthys marmoratus), wolf-eel (Anarrhichthys ocellatus), rockfish (Sebastes spp.), octopus (Octopus dofleini), and white sturgeon (Acipenser transmontanus) (Pauley et al. 1989). Given their small size and limited mobility, predation is a significant factor that regulates survival during early life stages of these organisms.

**1.5 Hypoxia and local oceanography**

Recruitment is often heavily influenced by seasonal oceanographic conditions (Miller & Shanks 2004). Not only do currents drive distribution, environmental variables that are coupled with currents such as temperature, salinity, nutrient availability and – of growing concern recently – dissolved oxygen also influence growth. Fluctuating concentrations of dissolved oxygen appear to be related to global climate change. Climate models predict decreasing trends in levels of dissolved oxygen in the ocean in the coming century (Keeling et al. 2010, Pierce et al. 2012). In the past 50 years, significant de-oxygenation has already occurred on the Oregon inner shelf to an extent that hypoxic events have occurred regularly during summers in the past fifteen years (Keeling et al. 2010, Pierce et al. 2012, Adams et al. 2013).

Normal levels of oxygen in marine environments, known as oxic or normoxic conditions, occur when waters are well-aerated; oxygen concentrations in normoxic waters are often ~ 6 mL oxygen per L water (8.6 mg L\(^{-1}\) or 80% oxygen). Hypoxia is defined as levels of dissolved oxygen below 1.4 mL L\(^{-1}\) (2.0 mg L\(^{-1}\) or 30% oxygen). Complete oxygen depletion is called anoxia (0.0 mg L\(^{-1}\) or 0% oxygen) (Adams et al. 2013). In many warm, photic ecosystems, hypoxia is driven by eutrophication: nutrients
of anthropogenic origin, often from nonpoint source and agricultural operations, leach into enclosed systems. Sustained nutrient loading promotes photosynthetic hyper-productivity and increased biological oxygen demand, which often leads to mass fish and invertebrate mortality (Diaz 2001). Along the northeast Pacific coast however, eutrophication and agricultural nutrient loading is not the primary cause of low dissolved oxygen; rather, nearshore hypoxia is driven by summertime upwelling conditions in the Northern California Current (Grantham et al. 2004b).

In Oregon, low dissolved oxygen events are restricted mostly to summertime upwelling, and can be traced to a naturally occurring deep-ocean oxygen minimum zone (OMZ). The transition from winter to summer conditions generally begins during April on the Oregon Coast. In May, prevailing north winds blow steadily and continue through October. Uniform winds stimulate Ekman transport, currents that are perpendicular to the wind-forcing, push surface waters offshore. Thus, coastal surface water is replaced by cold, saline, nutrient-rich water that originates from the deep ocean (Gan & Allen 2005). Coincidently, this water may also be depleted of dissolved oxygen (DO) as it comes from depths that correspond with the location of the OMZ.

Oxygen minima are not recent phenomena: the term “oxygen minimum zone” was first used by Cline and Richards in 1972. OMZs are the result of two major processes: poor long-term lateral ocean basin ventilation and microbial respiration (Karstensen et al. 2008). They are found at bathyal depths below the photic zone, with average upper boundaries below 200 m and lower boundaries above 1000 m. Minima are strongly correlated with eastern boundary currents, where upwelling is consistent and productivity
is rich: high nutrient consumption, respiration and turnover perpetuates cycles of low dissolved oxygen. They appear along continental margins in much of the eastern Pacific Ocean, southeast Atlantic Ocean off West Africa, and northern Indian Ocean (Helly & Levin 2004). Oxygen depletion is prevalent in the eastern Pacific, with extensive OMZs found off Central and South America (Helly & Levin 2004, Paulmier & Ruiz-Pino 2009), but Washington, Oregon and California also experience seasonal hypoxia originating from an OMZ off the US West Coast (Chan et al. 2008, Connolly et al. 2010, McClatchie et al. 2010). In the past two decades, there have been several nearshore hypoxic events that may be linked to an expansion of this OMZ (Pierce et al. 2012, Wright et al. 2012).

While summer conditions influence growth patterns in many species, winter conditions, in which surface wind drift is generally directed toward the coast, influence pelagic egg and larval distribution and recruitment patterns (Parrish et al. 1981). Summertime hypoxia typically wanes after the fall transition, which generally begins in September in Oregon. Northerly summer winds subside and southerly winter winds begin to blow steadily from about October through February; transition currents are variable north- and southward. Winter winds draw the prevailing coastal current, the Davidson Current (DC), to the surface. The DC is an inshore surface countercurrent that flows from south to north. It flows weakly at depth against the stronger California Current System during the summer and resumes as the prevailing coastal current in the winter (Reid & Schwartzlose 1962). Northward winds also cause onshore Ekman transport, resulting in transfer of well-oxygenated inshore surface waters to depth, or downwelling (Wyatt et al. 1972). Dissolved oxygen conditions in benthic habitats along the shelf return to normoxic
conditions during the winter months (Peterson et al. 2013, Adams et al. 2013). Both
downwelling and the Davidson Current promote surface transport and recruitment to
nursery areas (Shanks & Eckert 2005).

Open coast habitats in the Pacific Northwest are clearly impacted by hypoxia, but
regional estuaries are also subject to low DO by way of tidal inundation. Brown and
Power (2011) undertook a comprehensive review detailing present and historic mixing
patterns of the Yaquina Bay Estuary. Similar to the coastal ocean, seasonal variability is a
major driver of oceanographic conditions within the estuary. From November through
April, the Oregon Coast Range experiences high rates of rainfall, whereby the watershed
becomes saturated and the estuary is riverine dominated. Ingress of coastal water into the
estuary is minimal; in fact, wintertime output of freshwater from the estuary actually
drives down coastal salinity. As a result, during the wet season, hypoxia is unlikely to
occur in the estuary.

Rainfall and river flow decline from May to October, and the estuary switches to
marine dominance; estuarine conditions are therefore similar to those in the nearshore
during the summer. During dry summer conditions, the residence time of water in the
estuary varies, from one day at the mouth to nine days farther up river. With minimal
freshwater flows, saltwater and corresponding coastal conditions (including, but as yet
unrecorded, coastal hypoxia) may occur up to 35 km into the estuary. From the 1930s to
the 1960s, hypoxia in the Yaquina River was eutrophication-driven, associated with
warm river temperatures, and was initiated by upstream inputs of decaying organic matter
from timber mills and nutrients from inadequate wastewater treatment facilities.
Historically, hypoxia resulting from eutrophication occurred about 20 km from the mouth of the estuary and may not have impacted juvenile growth (which may be found up to 16.1 km upriver; Meyers 1980). Recently, hypoxic conditions have occurred near the mouth of the estuary and extend about 10 km into the estuary (Brown & Power 2011), and are therefore likely attributable to upwelling and tidal inundation.

There is a clear dichotomy between nearshore coast and estuarine nursery habitats. Temperature in the nearshore coast is typically colder than in the estuary during the summer, and may be coupled with low concentrations of dissolved oxygen, as seen in the plot of DO and temperature from the oceanographic station five nautical miles from shore (NH-5) in 2012 (Figure 1.4). Colder water may protect juvenile species from the effects of hypoxia in the nearshore. On the other hand, when the water temperature is high, as in the shallower water of the estuary, juvenile tolerance to low DO may be significantly reduced. Contemporaneously, dissolved oxygen levels are generally higher in the estuary, as seen in the plot of DO and temperature from the Yaquina Bay (Figure 1.4), and may provide better habitat during years of severe nearshore hypoxia. Because temperature and dissolved oxygen are strongly correlated during upwelling conditions in nearshore coastal habitats, it is difficult to disentangle the individual effects of these two variables on growth from field sampling alone.

1.6 Recent hypoxic events

An unprecedented occurrence of severely hypoxic water in the California Current Ecosystem was recorded in 2002 from July through September, during months that experience the strongest upwelling (Grantham et al. 2004b). Oxygen levels from the
Strawberry Hill Line off Heceta Head to the Newport Hydrographic Line off Newport were recorded to be between 0.21 – 1.57 mL L\(^{-1}\), in contrast with typical upwelled water DO concentrations of 1.8 – 3.6 mL L\(^{-1}\). The summer of 2006 experienced oxygen conditions that were similar in severity, but of longer duration, observed from June through October, and the vertical extent was greater, occupying up to 80 percent of the water column in 60 m (Chan et al. 2008). During both events, ODFW remotely operated vehicle (ROV) surveys documented mass mortalities of sessile invertebrates and fishes, and an apparent lack of more mobile species that were reported in other habitats with higher oxygen concentrations (Grantham et al. 2004b, Chan et al. 2008).

Mass mortalities of marine organisms in Oregon attributable to low oxygen were not well documented before 2002, but this may be due to limited monitoring (Toole et al. 2011). The anoxic event in 2002 may have been the first extreme case of mass mortality recorded on the Oregon Coast, but the instance of low oxygen was not the first, according to Peterson et al. (2013). Along the US Pacific Coast, brief low oxygen events have been detected off the Oregon coast since 2000. Data show a general decline from 1998–2007, an increase from 2007–2010, and a plateau or potential decline again in 2011–2012 (Peterson et al. 2013).

In the future, low dissolved oxygen may not be uncommon. In fact, a significant amount of research suggests that the frequency, duration and severity of low DO will increase (Matear & Hirst 2003, Diaz & Rosenberg 2008, Keeling et al. 2010, Stramma et al. 2010, Falkowski et al. 2011, Pierce et al. 2012, Peterson et al. 2013). The trend is already apparent, according to Pierce et al. (2012). The authors compiled data from
several studies on the Oregon Coast spanning fifty years. Along the Newport Hydrographic Line station NH-5 during 1960–71, only 9.7 percent of DO measurements fell below the DO concentration considered hypoxic (<1.4 mL L$^{-1}$). However, from 1998 to 2009, 36.6 percent of the measurements showed evidence of hypoxia. The occurrence of hypoxia is highly variable, and with anticipated increases in the frequency of these events, it is important to understand how organisms that use the affected habitats may be impacted.

1.7 Effect of DO and temperature on species survival and growth

While Dungeness crab and English sole spawning is timed to correspond with winter conditions that influence larval distribution, summer oceanographic conditions in the open coast and estuaries influence early juvenile growth. YOY organisms that are spawned in winter should be actively feeding and growing during summertime upwelling conditions; however, extreme environmental variability may inhibit growth. During hypoxic events, adult organisms, those capable of making annual spawning migrations, may be able to seek areas of higher dissolved oxygen (Keller et al. 2010). On the other hand, during any range of stressful conditions, juvenile organisms that are limited by body size, swimming speed, and low tolerance of stressors may not be capable of seeking refugia.

On the Oregon Coast, upwelling delivers the coldest and highest salinity water to the nearshore at this time (Huyer 1977, Rosenberg 1982). Juvenile Dungeness crabs appear to have a narrow range of thermal tolerance: under experimental conditions, survival was found to be highest at coldest temperatures (0°C) and lowest at warmest
temperatures (20°C). They ceased feeding at 0°C. At 20°C, they were very active initially and fed frequently, while over time, became lethargic and stressed. As a result, crab at lower temperatures molt less or not at all, and crab at higher temperatures have a greater potential for molting, but also an increased risk of temperature-related mortality (Kondzela & Shirley 1993). Brown and Terwilliger (1999) found that first instar juveniles exhibited greater sensitivity to temperature than fifth instar juveniles, indicating that between settlement and the end of the first summer young crabs become more tolerant of changes in temperature. They suggest that first instar juveniles are very near the limit of respiratory and circulatory capacities as a result of changes in salinity and temperature during tidal exchange in the estuary.

Based on average annual growth increments from scale readings, English sole appear to be well adapted to colder temperatures. Kreuz et al. (1982) noted that colder than normal summers generally led to better than normal growth. Laboratory research conducted by Williams and Caldwell (1978) on English sole at temperatures between 9.5 and 21°C was consistent with the Kreuz et al. finding: at colder temperatures, 9.5°C, young English sole were more efficient at food conversion (i.e. growth) than those at 21°C, which had negative growth. They also suggested that the maximum potential for growth would occur at 7 – 12°C, which generally corresponds with upwelling temperatures.

Temperature is only one physical factor that may regulate YOY growth; dissolved oxygen is also critical. Eerkes-Medrano et al. (2013) found that larval crabs (Hemigrapsus oregonensis, H. nudus, and Cancer antennarius) exhibited mortality
within 12 hours of exposure to hypoxia (0.5 – 1.4 mL L⁻¹). Larval crabs were less tolerant of low dissolved oxygen than all other crustaceans in the study. Given their ability to swim up to 8.5 cm s⁻¹ (Fernandez et al. 1994), Vaquer-Sunyer and Duarte (2008) suggested that larval crabs might depend on avoidance behavior more than low oxygen tolerance for survival. After settling, juvenile instar mobility becomes limited to peripatetic benthic movement. For a juvenile crab of lower mobility and potentially reduced avoidance capacity, low oxygen may lead to growth limitations. Bernatis et al. (2006) found that when coupled with short-term hypoxia (measured in hours, not days), Dungeness crab reduced both the amount of food eaten and the amount of time spent feeding. Under experimental conditions, crabs were also less likely to enter hypoxic conditions for food, and those that did retrieved and retreated back to areas of higher oxygen. Crabs that are lethargic and starved may be susceptible to higher rates of predation by other organisms that are more tolerant of low DO.

In similarity to Dungeness crab, Boese (1988) observed English sole lethargy in low-oxygen. The author found that age 0+ English sole respond to short term (15 – 20 hours) hypoxia with an increased ventilation rate and volume, and a reduction in oxygen extraction efficiency. At DO concentrations of 3 – 4 mL L⁻¹, sole ceased swimming. At around 1.0 mL L⁻¹ oxygen, opercular movements ceased, but the fishes survived. Compared to round-bodied fish, English sole have a low rate of oxygen consumption, which may be associated with their smaller gill surface area, reflected in their limited ability to sustain pursuit of mobile prey. English sole share similar weight-oxygen consumption ratios with other flatfish, such as winter flounder (Pseudopleuronectes
americanus), plaice (*Pleuronectes platessa*) and starry flounder (*Platicthys stellatus*) that have characteristic energetically low, cryptic life-styles (Boese 1988). Stierhoff et al. (2006) conducted a similar, but longer term (7 – 14 day) oxygen/temperature experiment on summer flounder (*Paralichthys dentatus*) and winter flounder (*Pseudopleuronectes americanus*). They found that growth rates of both flatfishes were generally reduced as DO decreased and as temperature increased.

During seasonal hypoxia in the Hood Canal, Froehlich et al. (2014) reported that contrary to their expectations, adult English sole and Dungeness crab exhibited limited regional-scale response to low oxygen conditions. These species, which were perfectly capable of migrating away from stressful conditions, remained in hypoxic waters rather than moving to areas of higher DO. During the 2007 NOAA National Marine Fisheries Service (NMFS) West Coast Groundfish Bottom Trawl Survey, Keller et al. (2010) found that total catch per unit effort (CPUE) data appeared to have a variable, but significant relationship with bottom DO concentrations: volume of catch increased with increasing levels of oxygen. Interestingly, they observed no significant effect of DO on CPUE for seven species: slender sole (*Lyopsetta exilis*), starry flounder (*Platicthys stellatus*), Dover sole (*Microstomus pacificus*), rex sole (*Glyptocephalus zachirus*), Pacific sanddab (*Citharichthys sordidus*), speckled sanddab (*Citharichthys stigmaeus*) and English sole. Similar to the Froehlich observation, and in accordance with other studies, all of those species were flatfish. During the trawl survey, Keller et al. (2010) also recorded condition factors for several species, which were primarily adults, given the scope of the survey. They found a positive correlation between condition and DO level: species exposed to
low DO were compromised when compared to organisms that were not. This observation underlines the importance of understanding how these conditions affect juveniles of the same species.

1.8 Research objectives and experiments

While DO in the open coast may reach lower concentrations compared to the estuary, lower water temperature may protect organisms against the severity of such events. In contrast, when the water temperature is high, as in the shallower water of the estuary, juvenile tolerance to low DO may be significantly reduced. Thus, in years with severe low oxygen events similar to those in 2002 and 2006, estuaries may be more suitable nursery habitats. On the other hand, in extremely warm years, coastal nurseries may be more suitable for juvenile English sole and Dungeness crab growth. Because temperature and dissolved oxygen are strongly correlated during upwelling conditions in nearshore coastal habitats, it is difficult to disentangle the individual effects of these two variables on growth from field sampling alone. I therefore sought to identify the incidence of an interaction between dissolved oxygen and temperature in experimental conditions, and to quantify the effects on the growth of both juvenile English sole and Dungeness crab.

In order to isolate the differences in growth between dissolved oxygen and temperature and their interactions, I conducted three experiments over the course of two years on juvenile Dungeness crab (*Cancer magister*) and English sole (*Parophrys vetulus*). For each experiment, I held English sole in the lab under a range of oxygen and temperature conditions for at least thirty days, and Dungeness crab for approximately
fifty. I tested three oxygen concentrations (<1.4, ~2.0, and 6.0 ml L\(^{-1}\)) and five temperatures (5, 7, 10, 13, and 15°C) for a total of 15 treatment combinations, with at least two replicates of each treatment. These treatments simulate summertime oceanographic conditions that are typically found in two critical nursery habitats used by these species: the Yaquina Bay estuary and Moolack Beach, Oregon USA (Figure 1.3).

For both Dungeness crab and English sole, I hypothesized that under high temperatures/low oxygen concentrations, growth would be poor; under low temperatures/any oxygen concentration, growth would be equally reduced; and that growth would be maximal under high oxygen concentrations at higher temperatures. Collectively, these results will help develop a better understanding of the role coastal and estuarine nursery habitats provide for juvenile English sole and Dungeness crab. As a result, identifying a particular habitat type as more successful at seeding adult populations under specific environmental conditions may influence management and conservation efforts for protection of critical nursery habitats.

1.9 Outreach and engagement

As part of our commitment to sharing my research with the general public, I also produced a video presentation that was displayed to guests, students and families at the Hatfield Marine Science Center Visitor Center. The semi-interactive exhibit showcased hypoxia, and the cause and source of low oxygen, and a brief synopsis of my experiments and results. The exhibit was displayed on a digital picture frame style screen, and included photos and video of my fish and crab specimens, diagrams and video about how the experimental setup works, and other relevant information (specimen early life history,
oceanographic conditions, an ODFW ROV video segment, etc.) that tied a brief but informative, two to three minute video presentation together. We also displayed live examples of juvenile English sole and Dungeness crab. Based on average annual numbers of guests at the Visitor center, we expected that tens of thousands of people would be exposed to the exhibit.
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Figure 1.1: Life cycles of Dungeness crab and English sole. Both begin life as pelagic eggs. After hatching, Dungeness crab progress through five zoeal stages and one megalopae stage before settling. English sole go through five larval stages before settling (Diagram adapted from: (Wild & Tasto 1983, Matarese et al. 2014; Juvenile and adult sole by Bancroft, 2015)).
Figure 1.2: Influence of seasonal weather patterns on early life history. Timing of oceanographic conditions matched with life histories of Dungeness crab and English sole.

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Figure 1.3: Habitat areas of interest. Coastal and estuarine nursery habitats around Newport, Oregon USA. Moolack Beach to the north of Yaquina Head Outstanding Natural Area. Yaquina Bay 10 km south.
Figure 1.4: 2.2: Temperature and DO field measurements, 2012. Above data collected from Sea-bird Coastal LOBOviz buoy moored in Yaquina Bay. Data below collected at depth (~50 m) from CTD casts along the Newport Hydrographic line station NH-5.
CHAPTER 2: EXPERIMENTAL TREATMENTS

An experimental investigation of the effects of temperature and dissolved oxygen on the growth of juvenile English sole (*Parophrys vetulus*) and juvenile Dungeness crab (*Cancer magister*)

2.1 Abstract

Despite recent increases of summertime coastal hypoxia (DO ~ 0.5 mL L\(^{-1}\)) on the Oregon inner continental shelf, little is known about how low DO affects coastal fishes and invertebrates, especially during the juvenile phase. Because temperature and dissolved oxygen are strongly correlated during upwelling conditions, it is difficult to disentangle the individual effects of these two variables on growth from field sampling alone. In order to isolate the effects of these variables on growth, I conducted three experiments over the course of two years on juvenile Dungeness crab (*Cancer magister*) and English sole (*Parophrys vetulus*). For each experiment, both species were held in the lab at three oxygen concentrations (<1.4, ~2.0, and 6.0 ml L\(^{-1}\)) and five temperatures (5, 7, 10, 13, and 15°C) for a total of 15 treatment combinations, with at least two replicates of each treatment. These treatments simulate summertime oceanographic conditions that are typically found in two critical nursery habitats used by these species: the Yaquina Bay estuary and Moolack Beach, Oregon USA. English sole specific growth (g d\(^{-1}\)) increased with higher temperatures (13 and 15°C) and oxygen concentrations (2.0 and 6.0 mL L\(^{-1}\)) and was four times lower in all low temperature and oxygen treatments (5 and 7°C; < 1.4 mL L\(^{-1}\)). Dungeness crab intermolt period decreased with temperature from 48 days at 5°C to 12 days at 15°C. Crab specific growth (mm d\(^{-1}\)) increased with temperature, with crabs in the highest temperatures growing nearly five times as much as crabs in the
coldest temperatures, with only one crab molting in the normoxic, 5°C treatment. Based on these growth results, it is possible that juvenile English sole are less restricted by habitat requirements than juvenile Dungeness crab. While English sole appear to grow as well in low-oxygen conditions (2.0 mL L⁻¹) across temperatures, Dungeness crab have a lower tolerance for all temperature and low oxygen conditions.

2.2 Introduction

Each summer since 2000, hypoxia, or low dissolved oxygen (DO), has been detected to some degree along the Oregon inner continental shelf (Chan et al. 2008, Peterson et al. 2013). In 2002 and 2006 oxygen levels dropped so precipitously that some marine benthic organisms were unable to escape and perished as a result, leading to the use of the popular term “dead zones” for those areas impacted by low DO (Gewin 2010). Hypoxia can impact many important marine habitats along the Oregon Coast, including soft, sandy bottom substrate (Keller et al. 2010), nearshore rocky reefs (Grantham et al. 2004a), rocky intertidal zones (Eerkes-Medrano et al. 2013), and, to some extent, estuaries (Brown & Power 2011). In estuaries the impacts of hypoxia are dampened by the high rate of tidal exchange and mixing, riverine input, and primary productivity; as a result, estuarine oxygen levels commonly stay above 2.0 mL L⁻¹ (yaquina.loboviz.com). This effect may result in higher quality habitats being found in estuaries rather than the inner shelf during years of severe hypoxia. Oregon estuaries and coastal areas are critical nursery environments for recently settled organisms, many of which are either ecologically or commercially valuable (or both; Laroche & Holton 1979, Krygier & Pearcy 1986, DeBen et al. 1990). Low dissolved oxygen concentrations have potentially
negative implications for the long-term growth of these species, yet the impacts of hypoxia are relatively unknown in this region.

Hypoxia, generally defined as oxygen concentrations less than 1.4 mL oxygen per L water, has become more prevalent in the last two decades along the Pacific Northwest coast (Peterson et al. 2013). During summertime upwelling conditions, hypoxic waters originate from depths below 200 m that are naturally low in dissolved oxygen (Adams et al. 2013). These areas, or oxygen minimum zones (OMZ), occur as a result of nutrient turnover, consumption, and deposition, and are typically found in regions of strong upwelling (Mullins et al. 1985). Until recently, OMZ influences were mostly restricted to depths deeper than those of Oregon coastal habitats. Typically, upwelling draws nutrient rich, cold, saline water from depth, which creates and sustains viable ecosystems and fisheries in Oregon (Huyer 1977). However, in the past two decades, abnormally low dissolved oxygen levels have occurred on the Oregon shelf, possibly as a response to anomalously strong flows of subarctic water into the California Current System (Grantham et al. 2004a). Furthermore, climate models predict a continued expansion of global OMZ due to climate change (Keeling et al. 2010, Pierce et al. 2012).

Understanding the prolonged, coupled effects of summertime hypoxia and temperature on juvenile organisms may be valuable in predicting how species will be affected by climate change. Juvenile recruitment, growth, and survival regulate harvestable fishery biomass. Numerous commercially valuable fishes and invertebrates are dependent on Oregon estuaries and shallow nearshore habitats as nurseries during their early life history stages (Gunderson et al. 1990, Miller & Shanks 2004). I selected
juveniles of two of the most prevalent species found in both habitats, English sole
(*Parophrys vetulus*), and Dungeness crab (*Cancer magister*), to examine the impacts and
interactions of temperature and hypoxia on growth and mortality. These species exhibit
both estuarine and near-coastal habitat usage and may differentially respond to
environmental stresses such as changes in oxygen concentration or temperature.

Previous studies have examined habitat use of these two species on the West Coast
of the U.S. After establishing that English sole use coastal habitats as nurseries to the
same degree as estuaries (Laroche & Holton 1979), several studies endeavored to
elucidate differences between estuarine and coastal nurseries. Rosenberg (1982) found
that the mean growth rates of English sole were similar in both habitats, but variability in
the size-at-age in the estuary was greater than in the open coast. He suggested that this
difference may be a result of oceanographic conditions (the fish experience a greater
range of seasonal temperatures and salinities in the estuary and more stable temperatures
and salinities in the open coast), predation (the fish encounter fewer predators in the
estuary), and intraspecific competition (food limitation in the open coast selects for larger
individuals). Gunderson et al. (1990) noted that along the U.S. West Coast, Dungeness
crab grow nearly twice as fast in estuaries as in surrounding open coastal areas. Krygier
and Pearcy (1986) determined that English sole mortality rates were similar in both
habitats, but found catch rates were higher in the estuary. Catch rates reflected seasonal
variability in estuarine out-migration, with increasing abundance in the nearshore in the
late fall. They proposed that the combined use of the two habitats could strongly increase
the survival and total population size of year-class cohorts. Additionally, they suggested
that utilization of both estuary and nearshore habitats could improve chances of survival in at least one habitat when adverse conditions affected the other.

In addition to impacting dissolved oxygen levels, summertime upwelling typically causes temperature to be very low in the nearshore coast (Figure 2.1). Temperature tends to be higher in estuaries than coastal habitats because solar irradiance warms shallow water in the estuary more quickly than in the nearshore (Figure 2.2). Because DO decreases with decreasing water temperature, DO and temperature are highly correlated in the nearshore coast, making understanding the individual effects of each of these factors from field sampling alone very difficult. Improved knowledge of these effects would help to elucidate which nursery habitats are optimal under certain conditions. For example, in years with severely low oxygen events similar to those in 2002 and 2006, estuaries may be more suitable nursery habitats. On the other hand, in extremely warm years, coastal nurseries may be more suitable for growth.

In order to disentangle the effects of dissolved oxygen from temperature on juvenile growth of these two important species, I conducted three experiments over the course of two years. My experimental setup exposed test organisms to temperatures ranging from 5°C - 15°C, and dissolved oxygen concentrations of <1.4 to 6.0 mL L⁻¹. Across all three experiments, I tested fifteen temperature/DO combinations, which were selected in order to simulate summertime oceanographic conditions typically found in two well-studied nursery habitats along the central Oregon Coast: the Yaquina Bay estuary and Moolack Beach, Oregon, USA (Figure 2.1). I hypothesized that 1) under low temperatures, at any oxygen concentration, growth would be suppressed; 2) growth
would be maximal under high oxygen concentrations at higher temperatures; and 3) under high temperatures/low oxygen concentrations, growth would be poor. I expect that the results of these analyses will be useful for making inferences about habitat quality.

2.3 Methods

2.3.1 Fish collection and pre-treatment maintenance

I conducted three experiments, one in summer 2012 and two in summer 2013. Experimental specimens were collected from Yaquina Bay, Newport, Oregon (44° 37' 21" N, 124° 02' 13" W). Age-0 English sole (< 70 mm standard length) were caught using a small otter trawl (3 m wide, lined with 3 mm mesh) or a 15-m beach seine. The otter trawl was towed at 1 – 2 knots in 4 – 5 m water depth during May and June 2012 and May and June 2013. When I sampled for English sole for the third experiment in July 2013, the area trawled earlier in the summer yielded few fish, so I used the beach seine and sampled east of the Yaquina Bay Bridge (44° 37' 07" N, 124° 03' 24" W). I collected the specimens necessary to complete each trial in approximately six collection trips per experiment.

All fish were immediately transferred to 1 x 2 x 1 m holding tanks in the Alaska Fisheries Science Center at the Hatfield Marine Science Center in Newport, Oregon. Holding tanks were maintained at 9°C (± 1°C) with flow-through seawater circulation. Lab light conditions were diurnal, consisting of a 12-hour light (0700 to 1900) and 12-hour dark (1900 to 0700) photoperiod. A thin layer of sand, into which fish could bury, covered the bottom of each holding tank. Fish were allowed to acclimate to lab conditions for three to five days before they were subjected to a once-per-day, one-hour
formalin dip on three consecutive days to remove parasitic trematodes (Moore 1991). Because all specimens were collected at the beginning of the summer, some were kept for a prolonged period (~50 days) before exposure to treatment. As my experimental interest was in newly settled juveniles, I restricted feeding in holding tanks to one or two times per week to maintain smaller sized fish. Fish were fed on finely grated gel food composed of squid, herring, krill, amino acid supplements, and vitamins (Ryer et al. 2012). Holding tanks were gravel-vacuumed and raked once per week. Before the 2012 experiment and the first of the two experiments conducted in 2013, fish were tagged with Northwest Marine Technology Visual Implant Elastomer. Fish were allowed approximately five days to recover from tagging in their respective temperatures before oxygen levels were experimentally manipulated.

2.3.2 Crab collection and pre-treatment maintenance

For the first experiment, age-0 Dungeness crab (C1 to C3 molt stages) were collected from Yaquina Bay, Newport, Oregon (44° 37' 21” N, 124° 02' 13” W) using a camera sled (Spencer et al. 2005) with a 3 mm mesh cod-end during May and June, 2012. Crab were collected in the same estuarine areas as English sole, during the same collection trips when sole were collected with the otter trawl. Sufficient crab specimens to complete the 2012 experiment were collected in approximately six trips in the early summer. Crabs for the subsequent two experiments were collected in the megalopae stage from various marina docks in Newport using a light trap as described by Miller and Shanks (2004). The trap was deployed for up to 10 hours over the course of several
nights in May and June 2013 for the second experiment, and in August 2013 for the third experiment.

All crabs were immediately transferred into individual 530 mL Solo clear plastic cups, which floated at the surface of the same 1 x 2 x 1 m holding tanks that were used to hold juvenile English sole. Cups were perforated to allow consistent exchange of water. One individual was housed per cup to prevent cannibalism. Lab light conditions were 12-hour light, 12-hour dark. Juvenile Dungeness crab molt between 6 and 8 times in the first year of life (Orensanz & Gallucci 1988), with less frequency as they grow larger. In order to observe at least one molt cycle while in treatment, I attempted to initiate experiments while crabs were at their smallest post-settlement size, stage C1 juveniles. Thus, collection of pre-settlement megalopae before each experiment was required. While in holding, crab feeding was restricted to krill, one time per week. Before exposure to treatment, juvenile C1 stage crabs were held for 3 – 4 days after molting in order to allow the carapace to harden.

2.3.3 Experimental treatments

My experimental setup consisted of a total of eighteen treatment tanks. Water temperature treatments were controlled at three head tanks. Each head tank fed temperature-treated water to six tanks, consisting of two replicates of each of three DO levels (Figure 2.3). The same DO levels were tested in all three experiments: hypoxic (< 1.4 mL L⁻¹), low-oxic (~2.0 mL L⁻¹) and a normoxic control (~ 6.0 mL L⁻¹). In experiment 1, a pilot trial in 2012, the target temperatures were 7, 10 and 13°C. The remaining two experiments were conducted in 2013. Experiment 2 repeated the
temperature treatments of experiment 1, and the target temperatures in experiment 3 were 5, 10, and 15°C. Overall, I tested a total of 15 temperature and dissolved oxygen concentration combinations in the three experiments (5, 7, 10, 13, and 15°C; 1.4, 2.0, and 6.0 mL L⁻¹). This range of parameters was selected in order to simulate summertime oceanographic conditions found in the Yaquina Bay estuary and coastal Oregon nearshore nursery habitats (Grantham et al. 2004a, Chan et al. 2008), areas critical to both species’ early juvenile stages (Laroche & Holton 1979).

Treatment tanks were made from half of a recycled 200 L, low-density polyethylene drum. I cleaned and conditioned them with saltwater for two weeks prior to adding specimens. Final tank size was approximately 44 cm tall, 56 cm in diameter and contained about 90 L. Water flow rate was maintained at approximately 35 mL/s; with a residence time of roughly 42 minutes, or a turnover rate of roughly 34 times per day. Each tank was insulated with 1.3 cm closed cell foam pads to maintain treatment temperatures.

Hypoxic conditions were simulated using a counter-current mixing chamber: a 1-m tower composed of 77 mm (3") inside-diameter PVC pipe was filled with Bio-Balls. At the bottom of the tower, under the Bio-Balls, was a 77 x 38 mm (3 x 1.5") Sweetwater ® Air Diffuser airstone. The airstone was connected via Tygon® tubing to a flowmeter manifold that supplied gas from a 160 L liquid nitrogen Dewar. Slight adjustments to DO levels could be made by changing nitrogen gas flow to a Dakota Instruments flowmeter at each tower. At each head tank, cold temperatures were controlled by mixing chilled (~ 4°C) with ambient seawater (~ 9°C); warmer temperatures were generated with an inline
HTI-4-220 Elecro Titanium Heater. Temperature-treated seawater was gravity-fed into the top of each counter-current tower across Bio-Balls, where nitrogen gas stripped water of oxygen (Figure 2.4). Excess nitrogen was piped through the lab ceiling to prevent ambient air hypoxia. Oxygen concentrations were monitored using a YSI Professional Optical Dissolved Oxygen Meter (ProODO™), which was calibrated in water-saturated air once daily, at the recommendation of the manufacturer.

Dungeness crab and English sole were exposed to experimental conditions concurrently, in the same treatment tanks, but they occupied different regions of the tank. Crabs were transferred in the same individual plastic cups from the holding tank to each treatment tank, where the container floated at the surface. A thin layer of sand lined the bottom of each treatment tank, allowing the sole to occupy the sediment-water interface.

English sole were exposed to at least thirty days of treatment during each experiment. Ten fish were randomly assigned to every tank, for a total of 180 fish per experiment. Dead fish were replaced in an attempt to maintain the same population density in treatment at all times. Fish in experiments 1 and 2 were tagged before entry into treatment, as described above. Fish in experiment 3 were photographed at each biweekly measurement and identified by eyed-side spot pattern and standard length. During the first experiment, in 2012, fish were fed every other day; food and wastes were cleaned from tanks every other day. I compared fish growth from the first experiment to other similar growth experiments with English sole (Ryer et al. 2012) and found that growth rates of fish in these experiments were lower, indicating that food was a potentially limiting factor. Thus, I decided to increase feeding and tank-cleaning
frequency to daily for the second and third experiments. Fish were fed to satiation on the same finely grated gel food to which they were acclimated while in holding.

Fish length and weight were measured before experimental treatment, after two weeks, and at the end of the trial at four weeks. On the day of measurement, fish were collected from each tank with a dip net and put into a separate container filled with treatment water. From the container, each fish was transferred by hand onto a measuring board to record length. I then patted the fish with a damp cloth to remove excess water and placed them into a tared beaker of water on a scale to record weight. At no time were fish out of treatment water for more than 30 seconds. Individual English sole were exposed to only one treatment regime.

Ten Dungeness crab were also randomly assigned to each treatment tank, for a total of 180 crab per experiment. They were exposed to treatment for no less than 50 days in order to observe at least one molt in all replicates. Crab specimens were also fed every other day during the first experiment; food and wastes were cleaned from tanks every other day. As with fish, I increased the frequency of feeding and cleaning to daily for the second and third experiments. All crabs were fed to satiation on an alternating diet of krill, finely chopped capelin or clam every day. I did not know the initial molt stage or length of intermolt period in the first experiment because crabs were collected in the early, post-settlement stages (from C1 to C3). Crabs were collected in the megalopae stage for the second and third experiments, which allowed us to keep track of individual intermolt period as well as to calculate a daily growth rate. Every crab was photographed before treatment and at least five days after molting to allow for the carapace to harden.
before handling. I used ImageJ, a Java based image processing program (Rasband 2014), to measure growth across the widest part of the carapace in front of the ninth carapace spine. All crabs from one tank were removed from treatment and placed into labeled petri dishes; after all were photographed, they were placed back into treatment. At no time was any crab out of treatment water for more than five minutes.

2.4 Statistical Methods

2.4.1 English sole

Fish that were exposed to experimental conditions and survived for at least thirty days were selected for growth comparison. Fish that died shortly after treatment began, or fish that were replaced with only two weeks of treatment remaining, were excluded from the analysis. Specific growth rates in fish (SGR_F) were calculated according to the following formula (Cook et al. 2000, Ryer et al. 2012):

$$ SGR_F = \frac{\ln(W2) - \ln(W1)}{(t2 - t1)} * 100 $$

where W2 and W1 were the last and first measurements of fish wet weight, respectively, and (t2 – t1) was the time interval between measurements. This formula assumes that the weight of fish increased exponentially over time.

In experiment 1 and 2, the dissolved oxygen and temperature conditions were replicated, so we combined the data for analysis. The effect of dissolved oxygen, temperature, experiment number, and all associated interactions on the specific growth rate of sole was determined using a three-way nested analysis of variance (ANOVA),
including individuals in tanks as a nested factor. I compared specific growth in experiment 3 using a two-way ANOVA between dissolved oxygen, temperature and their interaction. Pairwise differences for growth between treatments were determined using a Tukey Honestly Significant Difference (HSD) test.

English sole mortality was compared using ratios, calculated by dividing the number mortalities by the total number of fish (10 individuals) per tank. The fractions were then logit transformed and compared using a two-way ANOVA with individual tank as replicate. The overall sample size at each treatment level was two, or the number of replicate tanks. Pairwise differences for mortality between treatments were determined using Tukey HSD. A significance level of $\alpha = 0.05$ was used in all analyses.

2.4.2 Dungeness crab

Crabs in experiment 1 entered treatment at different stages of ecdysis and because I did not know the time since last molt, analysis was only conducted on the crabs in experiments 2 and 3, where entry into treatment after ecdysis was controlled. The metrics used to measure crab growth and survival were intermolt period, the percentage of mortality/molting, percent increase in carapace width, and specific growth. Specific growth rates in crab ($SGR_C$) were calculated according to the following formula:

$$SGR_C = \frac{\ln(L2) - \ln(L1)}{(t2 - t1)} \times 100$$
where $L2$ and $L1$ were the last and first measurements of crab carapace width, respectively, and $(t2 – t1)$ was the time interval between measurements. Crab growth is episodic, but this formula assumes an exponential, daily increase in carapace width.

As with sole, the effects of dissolved oxygen and temperature and their interaction on the $SGRc$, percent increase in carapace width, and intermolt period (average number of days between molts) were determined using a two-way ANOVA, with individuals in tanks as a nested factor. Pairwise differences were also determined using a Tukey HSD. Further, molt success and mortality were compared as ratios, calculated by dividing the number of molted, non-molted and mortalities by the total number of crabs (10 individuals) per tank. The fractions were then logit transformed and compared using a two-way ANOVA with individual tank as replicate. A significance level of $\alpha = 0.05$ was used in all analyses.

2.5 Results

2.5.1 DO and Temperature parameters

Measurements of dissolved oxygen and temperature recorded twice daily throughout the duration of the experiments indicated that I successfully maintained temperature and DO concentrations near the target levels of each experiment (Table 2.1).

2.5.2 English sole

The effect of hypoxia upon juvenile English sole growth was highly dependent on temperature in the combined analysis of experiments 1 and 2 (Figure 2.5). This was demonstrated by a significant interactive effect between temperature and the level of
dissolved oxygen ($F_{[4, 18]} = 3.50, p < 0.03$). In the lower 7°C temperature, there was no effect of dissolved oxygen on growth (Tukey, $p > 0.05$), whereas at the two higher temperatures, growth was reduced at hypoxic oxygen levels, but did not differ between low-oxic and normoxic conditions (Tukey, $p > 0.05$). The interactive effect of experiment number was also significant ($F_{[1, 17]} = 48.65, p < 0.001$), which may be explained by differences among fish length between experiments.

Initial fish length was smaller at the beginning of experiment 2 than at the beginning of experiment 1 ($F_{[1, 21]} = 47.78, p < 0.001$) (Figure 2.6). The average initial length of fish in experiment 1 was 53.4 mm, while fish in experiment 2 were 46.8 mm. During the first experiment, fish were collected later in the season, and hence, were larger than fish in experiment 2. However, with respect to treatment, initial standard length did not differ according to dissolved oxygen ($F_{[2, 17]} = 0.38, p = 0.69$), temperature ($F_{[2, 17]} = 0.62, p = 0.55$), or their interaction ($F_{[4, 17]} = 0.93, p = 0.47$). Furthermore, there was no three-way interaction between dissolved oxygen, temperature and experiment number ($F_{[4, 17]} = 0.08, p = 0.99$), involving those variables with respect to initial fish length. This pattern of equality in initial fish size was also demonstrated by the lack of an interaction between experiment number and any other independent variables ($F_{[1, 21]} = 47.78, p < 0.001$).

For the third experiment, I decided to expand the range of temperatures examined (Figure 2.7). While I continued to expose English sole to a 10°C treatment, I decreased the lower temperature from 7°C to 5°C, and increased the upper temperature from 13°C to 15°C. Unlike experiments 1 and 2, temperature did not moderate the effect of hypoxia
upon English sole growth. In fact, there was no significant effect of dissolved oxygen on
growth ($F_{[2, 9]} = 1.11, p = 0.37$). There was however, a strong independent effect of
temperature, with more rapid growth at higher temperatures ($F_{[2, 9]} = 58.68, p < 0.001$).
The interaction between dissolved oxygen and temperature was also not significant ($F_{[2, 9]}$
$= 1.78, p = 0.22$).

Unlike in experiments 1 and 2, initial fish length was not similar between
treatments in experiment 3 (Figure 2.7). In fact, at the beginning of the experiment, fish
length was found to be significantly different across temperature treatments ($F_{[2, 9]} =$
$14.53, p < 0.001$). The interactive effects of temperature and dissolved oxygen was also
evident ($F_{[4, 9]} = 3.74, p < 0.05$). There was no difference in initial fish length between 5
and $10^\circ$C (Tukey, $p < 0.05$), however, fish length was smaller in the $15^\circ$C hypoxic and
low-oxic treatments.

Since all three experiments contained a $10^\circ$C treatment, I combined these data for
analysis (Figure 2.8). There was an interactive effect of dissolved oxygen and experiment
number on growth ($F_{[4, 6]} = 3.0, p = 0.02$). The difference between growth in the low-oxic
and normoxic conditions in both experiments 2 and 3 was not significant (Tukey, $p <$
$0.05$). However, fish in these treatments exhibited greater growth than fish in hypoxic
conditions.

As with growth metrics, both temperature and dissolved oxygen influenced English
sole survival (Figure 2.9). In the first and second experiments, there were independent
effects of both temperature ($F_{[2, 18]} = 11.03, p < 0.02$), and dissolved oxygen ($F_{[2, 18]} =$
5.82, p = 0.01) on mortality. In both experiments, mortality was highest in the 13°C treatments (Tukey, p < 0.05), and similar between the 7 and 10°C treatments (Tukey, p > 0.05). Hypoxic and low-oxic treatments differed from each other (Tukey, p < 0.05), while normoxic conditions did not differ from either lower oxygen treatment (Tukey, p > 0.05). Mortality was not influenced by experiment number (F[1, 18] = 0.22, p = 0.65), or any of the associated interactions between temperature and dissolved oxygen and experiment.

In the third experiment, the effect of temperature on mortality depended upon hypoxia. The interactive effects between dissolved oxygen and temperature on mortality was also significant (F[4, 9] = 4.85, p = 0.02). However, the only difference was higher mortality in the low-oxic and hypoxic conditions in 5°C than in the 10°C low-oxic treatments (Tukey, p < 0.05).

2.5.3 Dungeness crab

There were similarities between the survival and growth of English sole and the survival and growth of Dungeness crab. As with English sole, temperature and hypoxia both influenced juvenile Dungeness crab mortality and growth in experiments 2 and 3 (Figures 2.10 and 2.11), but unlike English sole, the interactive effects on these metrics were not apparent.

In experiment 2 (Figure 2.10), dissolved oxygen had a strong independent effect on the percentage of crab mortality (F[2, 9] = 27.50, p < 0.001). There was no difference between the rate of mortality in the low-oxic and hypoxic treatments (Tukey, p > 0.05), although mortality tended to be higher in hypoxic conditions. The effect of temperature
(F_{[2, 9]} = 2.69, p = 0.12) and the interaction between oxygen and temperature (F_{[4, 9]} = 1.94, p = 0.19) on mortality was not significant.

Nearly all crabs that survived the 50 days of treatment during the second experiment molted from the C1 to C2 stage. As with mortality in this experiment, dissolved oxygen had a significant influence on the percentage of crabs that molted (F_{[2, 9]} = 19.04, p < 0.001). The number of successful molts per tank tended to increase from hypoxic to normoxic treatments, however, there was no difference between low-oxic and normoxic treatments (Tukey, p > 0.05). A small number of crabs did not molt or die during treatments, instead, they survived without molting in the 7°C, hypoxic and low-oxic conditions.

Intermolt period was influenced by a strong, independent effect of both dissolved oxygen (F_{[2, 9]} = 9.5, p < 0.006) and temperature (F_{[2, 9]} = 244.4, p < 0.0001). All temperature treatments were significantly different from each other (Tukey, p < 0.05), as demonstrated by a longer intermolt period with decreasing temperature. Intermolt period was also not significantly different between the low-oxic and normoxic conditions (Tukey, p < 0.05); both had a shorter intermolt period than hypoxic treatments.

Similarly to intermolt period, carapace width increase was independently influenced by a strong effect of dissolved oxygen (F_{[2, 9]} = 22.90, p < 0.0001), and temperature (F_{[2, 9]} = 4.85, p = 0.04). Carapace width tended to increase more in normoxic conditions, however, there was no difference between normoxic and low-oxic treatments (Tukey, p < 0.05). Increases in carapace width were consistently lower in hypoxic
conditions. The greatest differences were between 7 and 10°C, although carapace width increase was only marginally greater in 7°C (Tukey, p = 0.05).

Specific growth, the rate of carapace width increase given the length of intermolt period, was independently influenced by dissolved oxygen and temperature during experiment 2. Both dissolved oxygen and temperature had a positive effect on growth \( F_{[2, 9]} = 7.0, \ p = 0.02, \) and \( F_{[2, 9]} = 48.0, \ p < 0.001, \) respectively, with almost twice as much SGR\(_C\) in 13°C, when compared with 7°C (Tukey, \( p < 0.05 \)). Specific growth was significantly different between oxygen treatments, except at 13°C (Tukey, \( p > 0.05 \)), where crabs in low-oxic conditions grew the most.

The results of experiment 3 (Figure 2.11) were similar to experiment 2, although, temperature had a strong influence on the overall outcome of growth in the analysis. In experiment 3, Dungeness crab mortality was influenced by dissolved oxygen \( F_{[2, 9]} = 7.80, \ p = 0.01 \). There was no difference between the rate of mortality in the low-oxic and normoxic treatments (Tukey, \( p > 0.05 \)), with clearly higher mortality in hypoxic conditions. There was a decreasing trend of mortality with increasing temperature \( F_{[2, 9]} = 3.41, \ p = 0.08 \), but it was not significant.

In the two higher temperatures of experiment 3, most crabs molted from the C1 to C2 stage during the 50 days of treatment. In the colder 5°C treatments, only one crab molted in the normoxic conditions, hence, I removed it from the remainder of the analysis of intermolt period, percent carapace width and specific growth. As a consequence, there was no effect of dissolved oxygen on molting \( F_{[2, 9]} = 2.21, \ p = 0.20 \). Molting tended to
increase with increasing temperature, however, temperature was not significant ($F_{[2, 9]} = 5.04, p = 0.07$), nor were there interactive effects of temperature and dissolved oxygen ($F_{[4, 9]} = 0.42, p = 0.72$).

Juvenile Dungeness crab intermolt period was significantly influenced only by temperature in experiment 3 ($F_{[1, 6]} = 175.19, p < 0.001$), but unlike experiment 2, dissolved oxygen did not have a strong effect on intermolt period ($F_{[2, 6]} = 1.58, p < 0.28$). As a result, intermolt period was greater in $10^\circ C$ than $15^\circ C$ (Tukey, $p < 0.05$), but did not differ between dissolved oxygen treatments (Tukey, $p > 0.05$).

In contrast with intermolt period, the increase in carapace width was influenced by a strong independent effect of both dissolved oxygen ($F_{[2, 9]} = 9.50, p < 0.006$), and temperature ($F_{[2, 9]} = 244.44, p < 0.001$). Increases in carapace width were consistently lower in hypoxia, and appeared to be marginally higher in normoxic than low-oxic conditions, however, there was no difference between the two treatments (Tukey, $p > 0.05$). In this experiment, the highest growth occurred in the $10^\circ C$ low-oxic and normoxic conditions (Tukey, $p < 0.05$).

Specific growth was influenced by dissolved oxygen and temperature during experiment 3 ($F_{[2, 6]} = 6.69, p = 0.03$, and $F_{[1, 6]} = 9.04, p < 0.02$, respectively), but their interactive effect was not apparent ($F_{[2, 6]} = 0.69, p = 0.54$). Growth in low-oxic and normoxic $15^\circ C$ treatments was significantly greater than in the $10^\circ C$ hypoxic treatment (Tukey, $p > 0.05$).
Across the 10°C oxygen treatments, the patterns of survival and growth were similar between the second and third experiment (Figure 2.12). Mortality was strongly influenced by dissolved oxygen in both experiments ($F_{[2, 6]} = 12.57, p < 0.007$), and there was no apparent difference between experiment number ($F_{[1, 6]} = 3.84, p < 0.1$). There was a graded effect of oxygen on mortality, with no difference between hypoxic and low-oxic conditions (Tukey, $p > 0.05$), a notable, but not significant difference between low-oxic and normoxic (Tukey, $p = 0.05$), and significantly higher mortality in hypoxic than normoxic conditions (Tukey, $p < 0.05$). Intermolt period was significantly different between experiments ($p < 0.001$), with an average of 25.6 days between molts in experiment 2, and an average of 19.9 days between molts in experiment 3. Dissolved oxygen did not have an effect on intermolt period ($F_{[2, 6]} = 3.05, p < 0.12$) and the interaction was also not significant ($F_{[2, 6]} = 0.38, p < 0.07$). Carapace width increase was also only different between experiment number ($F_{[1, 6]} = 90.59, p < 0.001$), with greater overall increase in carapace width in experiment 3 (Tukey, $p < 0.05$). There were strong independent effects of DO ($F_{[2, 6]} = 19.21, p = 0.003$) and experiment number ($F_{[1, 6]} = 112.72, p < 0.0001$), with crabs growing nearly twice as much in all oxygen concentrations in experiment 3. The interaction between experiment number and dissolved oxygen did not influence growth rates ($F_{[2, 6]} = 3.69, p = 0.09$). Growth was not different between low-oxic and normoxic conditions (Tukey, $p > 0.05$), but were both greater than growth rates in hypoxic conditions (Tukey, $p > 0.05$).
2.6 Discussion

2.6.1 English sole

The results from the combined analysis of experiments 1 and 2 with English sole largely support my initial hypotheses. Those were 1) under low temperatures at any oxygen concentration, growth would be suppressed; 2) growth would be maximal under high oxygen concentrations at higher temperatures; and 3) under high temperatures/low oxygen concentrations, growth would be poor. More specifically, I expected that there would be a strong interactive effect of dissolved oxygen and temperature on the growth of juvenile English sole. Indeed, in experiments 1 and 2, SGR$_F$ was highest in warmer temperatures (10 and 13°C) and oxygen concentrations (2.0 and 6.0 mL L$^{-1}$) and lower, but not significantly different across low temperature/low oxygen treatments (7°C; < 1.4, 2.0 and 6.0 mL L$^{-1}$). Experiment 3, however, did not conform to the same patterns of growth. SGR$_F$ increased with increasing temperature, but fish growth did not respond to oxygen similarly to the two previous experiments.

The highest growth rates of English sole in this study were $> 1.3$ g d$^{-1}$ in both low-oxic and normoxic treatments above 10°C. When compared to English sole growth reported in Ryer et al. (2012), my results were most similar to fish in their 5°C growth treatments. English sole in their experiments began treatments between 30 and 50 mm. Fish in their higher temperatures (13 and 16°C) grew roughly twice the size of fish in this study at the same temperatures and experimental duration. The disparity may be the result of the density of fish and size of experimental tanks. Only 7 fish were held in 1 m diameter tanks in their study, compared to 10 fish in the 54 cm tanks in this study.
Based on unquantified, but perceptible opercular respiration throughout the experiments, I expected that English sole would exhibit a graded growth response to oxygen, with reduced growth in low-oxic conditions (2.0 mL L\(^{-1}\)), especially in higher temperature treatments. However, English sole growth rates were as high in several of the low-oxic conditions when compared with normoxic conditions. High growth under stressful conditions may possibly be attributed to an increase in the basal metabolic rate: flatfish may require more food to subsidize energy lost to constant, heavy respiration.

In relation to specific growth, English sole appear to have an increased tolerance for low oxygen at high temperatures. This apparent degree of resilience in English sole to stressful environmental conditions is evident in a variety of studies. In an experimental study of English sole behavior and oxygen consumption over short term time scales (15 – 20 hours), Boese (1988) found that young-of-the-year English sole was relatively tolerant to low-oxygen. In those experiments, sole oxygen consumption did not change significantly between 6.4 and 1.3 mL L\(^{-1}\), and the authors found no apparent critical oxygen concentration. They noted that the oxygen extraction efficiency of English sole at lower oxygen was similar to that of the hypoxia tolerant species, common carp (Cyprinus carpio).

In contrast with English sole, Stierhoff (2006) found that young-of-the-year summer flounder (Paralichthys dentatus) and winter flounder (Pseudopleuronectes americanus) responded to temperature and hypoxia variations by exhibiting severe growth limitation under moderately low (2.45 mL L\(^{-1}\)) oxygen conditions. Taylor and Miller (2001) observed that juvenile southern flounder (Paralichthys lethostigma),
another flatfish, responded with a similarly suppressed growth response under nearly hypoxic (~1.90 mL L\(^{-1}\)) conditions. In normal oxygen conditions, the overall growth of the three species appears to be similar to English sole in this study (~2% d\(^{-1}\)), however, at low-oxygen (2.0 mL L\(^{-1}\)) conditions, English sole often grew nearly as well as in normoxic conditions (6.0 mL L\(^{-1}\)).

In addition to low-oxygen tolerance, English sole have the tendency to be audacious, often to their detriment. In a study of behavioral patterns in the presence of foraging predators, Ryer et al. (2012) found that of three North Pacific flatfish species tested, English sole was the most vulnerable to predation because they were inherently conspicuous. English sole fed earliest and longest, and spent most time on top of the substrate instead of buried, regardless of the presence of actively foraging predators. The investigators also found it difficult to determine whether the flatfish exhibited predation-induced growth suppression because all of their specimens were consumed during trials. This led them to suggest that English sole are incapable of adopting more conservative anti-predator behaviors, and that this flatfish is a risk-prone species.

2.6.2 Dungeness crab

Juvenile Dungeness crab survival and growth demonstrated a dichotomous response to variations in temperature and dissolved oxygen. I expected that there would be interactive effects on at least some of these metrics, however, temperature and dissolved oxygen only acted independently. For example, the overall rate of molting success was optimal in the higher oxygen treatments (with only one crab molting in the coldest, 5°C temperature), while mortality was highest at lowest oxygen concentrations.
In these experiments, juvenile crab molting and survival was mostly unaffected by temperature. This observation draws parallel with the findings of Kondzela & Shirley (1993), who observed that Dungeness crab exhibited increased molting or survival responses to extreme conditions, much like crabs in my experiments. Juvenile crab in their lowest 5°C temperature molted less, if at all, and crab at higher temperatures had a greater potential for molting, but also an increased risk of temperature-related mortality. In their experiments, most mortality occurred at 20°C, while in 5, 10 and 15°C treatments, mortality was equal. One crab in my 5°C treatment molted, while molting and mortality was equal in the rest of the treatments.

Quantitative growth metrics, intermolt period, carapace width increase and specific growth rate were influenced strongly, but independently by both DO and temperature. Again, the overall outcome of the analysis may have been different if more crabs molted in 5°C. Intermolt period, which is strongly associated with SGR_C, was longer in the colder and hypoxic treatments than normoxic warm temperatures in experiment 2. Temperature may not have been statistically significant because I removed the single crab that molted from the analysis, but temperature obviously had a notable influence on the outcome of experiment 3. The effect of temperature was not apparent in carapace width increase, with maximal growth occurring within the two oxygenated (2.0 and 6.0 mL L⁻¹) treatments.

Dungeness crab in this study responded to experimental conditions similarly to Dungeness crab in other experiments. Intermolt period in all treatments, and mortality in normoxic treatments in this study were very similar to those found by Diehl and Ryer
Brown and Terwilliger (1999) suggested that first stage juvenile Dungeness crabs may be very near the limit of their respiratory and circulatory capacities; the high rate of mortality in hypoxic treatments during this study seems to complement this theory. Rates of molting and growth in this study are also supported by field observations made by Gunderson et al. (1990). The authors found that Dungeness crab growth rates in warmer estuaries were nearly three times greater than those measured simultaneously along the open coast of Washington.

Crabs in the 10°C treatments in experiments 2 and 3 allowed for an interesting comparison (Figure 2.12). In both experiments, crabs began treatment shortly after they molted from the megalopae stage into stage C1 juveniles. Crabs in experiment 2 were collected in late May and exposed to treatment in mid-June; crabs in experiment 3 were collected in late July and exposed to treatment in August. Both sets of crabs were caught using the same light trap, from the same locations. Even though they both began treatments in the C1 stage, the earlier season crabs in experiment 2 were larger than late season experiment 3 crabs (Figure 2.13). Overall, crabs in experiment 3 had a higher SGR\textsubscript{C} and molting rate and a lower rate of mortality than crabs in experiment 2. This discrepancy could reflect different settlement strategies. Crabs that settle earliest in the season progress through several molt stages before crabs that settle later in the season; they therefore represent the largest individuals of the yearly cohort. Larger initial size and early growth could diminish the potential for cannibalism by other crabs, which has been found to strongly influence the survival of other cohorts (Fernandez et al. 1993). On the other hand, late season settlement with rapid growth and reduced mortality may be
advantageous because it could reduce long-term exposure to hypoxic conditions in both coastal and estuarine nursery environments. It is possible that crabs which have already been exposed to and compromised by prolonged hypoxic conditions may be more susceptible to high mortality and poor growth than those that have recently settled.

The enclosures in which Dungeness crabs were kept may present a confounding factor in the experimental procedures. Transparent cups did not provide much refuge for crabs floating just below the surface of each treatment tank. The crab in each cup was entirely visible to the curious approaches and potentially predatory cues from English sole below. Diehl and Ryer (unpublished data 2014) conducted an experiment on the effect of temperature on the rate of growth and molting in C1 stage juvenile Dungeness from 0 to 20°C. Each crab was similarly held in individual containers, but were also provided with a thin layer of sand into which they could bury. The investigators witnessed 100 percent mortality at 0°C, and in 2 and 5°C treatments, crabs ceased feeding altogether. In the colder 2°C treatment, crabs were unable to bury themselves. They proposed that the inability of the crab to bury into sand could render recently molted crabs more susceptible to predation. Across treatments in these experiments, intermolt period and SGR_C measurements were similar, possibly suggesting that visual predatory cues from English sole in the experiments did not have an effect on the overall success of molting. However, without analogous enclosures and substrate, I cannot speculate whether crabs in lower oxygen concentrations are capable of burying themselves to avoid predation.
2.7 Conclusion

The interactive effects of oxygen and temperature were apparent on the growth of English sole in the first and second experiments. There was a marked reduction of growth rates in cold, severely hypoxic conditions (7°C, 1.4 mL L\(^{-1}\)), and growth rates were equivalent in low-oxic (2.0 mL L\(^{-1}\)) and normoxic conditions (6mL L\(^{-1}\)) at warmer temperatures (10 and 13°C). Despite this apparent resilience to low-oxygen (2.0 mL L\(^{-1}\)), English sole may be more susceptible to predation because of their increased level of respiratory activity. Depending on the severity of oxygen depletion, English sole could grow just as well in a marginally hypoxic coastal nursery habitat as a borderline eutrophic estuarine nursery habitat. In contrast, Dungeness crab appeared to be more susceptible to low oxygen conditions and extreme temperatures. Mortality was very sensitive to dissolved oxygen but less so to temperature, while growth metrics (intermolt period, carapace width increase and SGR\(_C\)) were sensitive to both DO and T. This would suggest that crab may benefit from the conditions that an estuarine nursery habitat provides during years of severe hypoxia and low temperature in coastal habitats.
2.8 References


Huyer A (1977) Seasonal variation in temperature, salinity, and density over the continental shelf off Oregon. Limnol Oceanogr 22:442–453


Moore MM (1991) Host responses of English sole (Parophrys vetulus) to infection by the monogenetic trematode Gyrodactylus stellatus


Rasband WS (2014) ImageJ. U S Natl Institutes Heal Bethesda, Maryland, USA


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Table 2.1: Mean target DO (±SE) and temperature (±SE) in each treatment was calculated from measurements taken twice daily in each tank using a handheld YSI ProODO™ Meter.
Figure 2.1: Habitat areas of interest. Coastal and estuarine nursery habitats around Newport, Oregon USA. Moolack Beach to the north of Yaquina Head Outstanding Natural Area. Yaquina Bay 10 kilometers south.
Figure 2.2: Temperature and DO field measurements, 2012. Average dissolved oxygen and temperature in the open coast and Yaquina Bay. Data above collected from Sea-bird Coastal LOBOviz buoy moored in Yaquina Bay. Data below collected at depth (50 m) from CTD casts along the Newport Hydrographic line station NH-5.
Figure 2.3: Experimental setup. Three temperature treated head tanks at top. Each column of tanks is fed by the head tank. Pairs of tanks represent dissolved oxygen tank replicates. From top; hypoxic, low-oxic, and normoxic. Liquefied nitrogen gas (LN2) in 160 liter dewars, top left, supply nitrogen to strip water of oxygen.
Figure 2.4: Counter-current mixing tower. PVC tower designed to strip oxygen from water. Temperature treated water goes into the top, nitrogen gas bubbled in from the bottom. Water and gas mix across Bio-balls and create hypoxic water.
Figure 2.5: Mean (±SE) specific growth rate (SGR_F) of English sole in experiments 1 and 2. SGR_F is the logged difference of final and initial weight, divided by the duration between measurements, 4 weeks here. Sample size for combined replicate tanks are indicated above or within each bar.
Figure 2.6: Mean (±SE) initial standard length of English sole in experiments 1 and 2.
Figure 2.7: Mean SGR$_F$ (±SE, on left) and initial standard length (±SE, on right) of English sole in experiment 3. SGR$_F$ is the logged difference of final and initial weight, divided by the duration between measurements, 4 weeks here. Sample size for combined replicate tanks are indicated above each bar.
Figure 2.8: Mean $SGR_F$ (±SE) of English sole in $10^\circ$C, experiments 1, 2 and 3 (in grey above). $SGR_F$ is the logged difference of final and initial weight, divided by the duration between measurements, 4 weeks here. Sample size for combined replicate tanks are indicated above each bar.
Figure 2.9: Percent mortality of English sole in experiments 1, 2 and 3. Number of mortalities for combined replicate tanks (N=20) are indicated above each bar.
Figure 2.10: Experiment 2 crab survival and growth metrics. Percent mortality, percent molted, intermolt period, carapace width increase (±SE), and specific growth rate (±SE).
Figure 2.11: Experiment 3 crab survival and growth metrics. Percent mortality, percent molted, intermolt period, carapace width increase (±SE), and specific growth rate (±SE). The single individual that molted in normoxic 5°C conditions was removed for the analysis.
Figure 2.12: Experiment 2 and 3 (in grey above) crab survival and growth metrics: percent mortality, percent molted, intermolt period, carapace width increase (±SE), and specific growth rate $SGR_C$ (±SE).
Figure 2.13: Initial carapace width (±SE) of Dungeness crab for experiments 2 and 3. Experiment 2 (left) was conducted in June 2013 and experiment 3 (right) was conducted in August 2013.
CHAPTER 3: EXHIBIT FOR THE HMSC VISITOR CENTER

An exhibit presented to the public about the preceding research project.

3.1 Synopsis

Most people are not directly impacted by the effects of nearshore hypoxia. People that live on or near the US West Coast may be aware of the phenomenon, especially if their lives and professions are intimately linked to the ocean. Moderately low dissolved oxygen events are fairly regular and typically only publicized in scientific circles. On the other hand, visitors to the coast are unlikely to be familiar with the causes and frequency of hypoxia in Oregon, unless the event is severe and results in mass fish and invertebrate mortality. Then, owing to the sensational nature of “dead zones,” national news media may report on the incident, such as in the 2002 and 2006 severely hypoxic and anoxic events. If people have heard of hypoxia, it is commonly in relation to the condition found in the Gulf of Mexico, which is nutrient driven and can be traced to anthropogenic input and warmer temperatures. Instead, Oregon hypoxia is more difficult to trace directly to human-related activity, making West Coast hypoxia a relatively lesser understood occurrence.

The slides included in this chapter are part of a video exhibit which was displayed at the Hatfield Marine Science Center Visitor Center. The actual video that was presented with the exhibit may be found online as a supplement to this thesis. I created this presentation to introduce hypoxia and some of the science that we conducted to visiting guests, tourists and school groups to the Visitor Center. My goal was to make Oregon hypoxia accessible to a wide public audience.
3.2 Materials and methods

This exhibit of my research was designed to be temporary and easily converted for other future research questions. It was located next to the larger, and very popular “Aquatic Invaders” exhibition. The next iteration of this exhibit will consist of two aquariums with juvenile English sole or Dungeness crabs, a graphic panel and a digital picture frame with a PowerPoint presentation. The ~3 minute video presentation was installed on a Nix Hu-Motion 12” digital picture frame. The Hu-Motion picture frame is motion sensitive and looped the video as long as movement was detected in front of the screen. The frame was mounted above two ~90 liter aquaria. These will display the English sole and Dungeness crabs after the juveniles are captured as they enter the Yaquina Bay estuary to settle.

Before installing the exhibit, I conducted a rapid assessment formative evaluation. I displayed the video and all of the slides on a bulletin board in the HMSC West Wing hallway for two weeks. I asked Visitor Center volunteers and HMSC staff to provide critical feedback on the content, which I integrated and discuss later in this chapter. I mounted and leveled the picture frame in the center of the concave exhibit wall at approximately adult eye-level (1.5 m). The following day, the Visitor Center aquarists filled the singular aquarium on the left with nudibranchs, anemones, other invertebrates and various algae. Juvenile fish and crab were collected in the early spring, quarantined for a month, and then displayed in the exhibit aquaria. There was no background image when I first installed the picture frame; it was installed ~ 7 days later. The exhibit ran for
about two weeks without signage, until the intended background image arrived in the mail and could be installed.

3.3 Evaluation

The afternoon before I shared the video and slides to the Visitor Center volunteers and staff, my mentor, Bill Hanshumaker suggested a few edits to improve the presentation. According to studies conducted by the Visitor Center, the average attention span for video-based exhibits is approximately 2:15 minutes. I assumed that people would stand and watch my entire presentation, and therefore pick up on abbreviations that I used commonly throughout. Bill suggested, knowing the transient nature of visitors, many would not stay long enough to engage in acronyms. A prime example: there is another hypoxia exhibit in the Visitor Center, but the video is 4:34 minutes long. Visitors rarely stay to watch the entire presentation (http://www.piscoweb.org/node/394). Moreover, the content is more sophisticated than mine (Chan et al. 2009). Bill and I agreed that the upwelling animation in this video is informative and attractive, and we considered using it in my presentation. We watched the entire video together and timed the upwelling animation segment to be just under a minute long, too long for my presentation, so the much simpler animation was retained.

During the two weeks that the exhibit was displayed to Visitor Center volunteers and staff, I received positive feedback, with some very valuable input. The comment of greatest concern was that I used a term – normoxia – that the staff and volunteers did not think visitors would understand. They recommended limiting new terminology to one word (hypoxia), so I changed all instances of normoxia to normal oxygen levels.
Someone asked for an explanation of the red lasers within the video of the remotely operated vehicle (ROV – slides 4 and 6), so I included a caption about how the lasers allow scientists to measure fish length. In the first several slides, the map of the Oregon Coast is seen from directly above (the transition between slide 8 and 9), and subsequent slides show a cut-off segment of the Oregon shelf: there was concern among staff and volunteers that visitors would not understand the two perspectives. I included a slide to orient the viewer. Someone pointed out that Ekman currents are at right angles to the wind, which I edited for clarification (slide 11). The flatfish on slide 15 that were crossed out (dead) with red “X’s” were difficult to see; someone pointed out they looked like sea-stars. I removed about half of the fish, increased the size of the remaining fish, and made it more obvious that the fish were “dead” because of severely low oxygen. Finally, I neglected to include an explanation as to why the deep ocean water is naturally hypoxic (slide 16 and 17), so I briefly explained that bacteria and decaying organic matter (dead stuff) was the cause.

On a very rainy Saturday (1/17/2015) afternoon following the installment of the digital picture frame, I spent about half the afternoon observing visitors interacting with the exhibit in its nascent form. Of the roughly 50 people that passed by or near the exhibit, 32 stopped to examine the exhibit. Only five adults spent an average of 5 seconds watching the video. The rest of the visitors stopped to look at the contents of the tank, one person spent time photographing some of the invertebrates. Without signage to inform the visitors of what they were watching, or an attractive background image to
create interest, most people passed by the white concave wall without even looking at the
digital picture frame.

After the publication of this thesis, we will continue conducting evaluations on the
effectiveness of the exhibit, making changes accordingly. The presentation is meant to be
a primer for people who are unfamiliar with Oregon Coast summertime hypoxia, and also
complimentary to the existing and more sophisticated exhibit on hypoxia. Regardless of
whether visitors watch the entire video presentation or several slides, they will learn
about fundamental themes on Oregon hypoxia, upwelling, and/or laboratory-based
hypoxia research.
3.4 Reference

Chapter 3 Figures:

1.)

Oregon Hypoxia
When marine life has trouble breathing

This OSU research made possible by:

Sea Grant
MEM
TAMU

2.)

What is hypoxia?

Areas of low dissolved oxygen (DO) near the bottom of the ocean.
What is hypoxia?

Severe hypoxia can kill fish and invertebrates.

Areas of very low DO are popularly called **dead zones**...

First, take a look at normal oxygen levels

The following video shows thriving Oregon rocky reef ecosystems recorded by remotely operated vehicle (ROV).
Normal levels of dissolved oxygen (DO)

Look for:
- Anemones
- Lots of fish
- Sunflower stars
- Live stuff

Lasers help scientists measure fish length underwater!

Now, look at hypoxia – very low DO levels

This video shows a desolate Oregon rocky reef ecosystem.

Mobile organisms have fled for higher oxygen.
Hypoxia = very low levels of DO

Look for:
- Crab parts
- Dead worms
- Decomposing sunflower star
- No fish anywhere
- Dead stuff

Low DO makes the news

Hypoxia is not always deadly, and has occurred almost every year on the Oregon Coast since 2000.
9.) You are still HERE

This is what the [continental shelf] looks like off the Oregon Coast!

10.) Why does hypoxia happen?

Hypoxia occurs during periods of summertime [upwelling].
11.)

What is upwelling?

During the summer, strong winds blow from the north.

12.)

Winds cause Ekman transport

These currents happen to the right of the wind because of the Earth’s rotation.

Ekman currents draw surface water away from the coast.
Deep ocean water is upwelled

Surface water is replaced by cold, salty and nutrient rich water from the deep ocean.

Nutrients feed the food chain from the bottom up

Upwelling is the reason WA, OR, and CA have such productive fisheries!

*Note: English sole is not a schooling pelagic species.*
But...

Sometimes the upwelled water is severely hypoxic.

Upwelling brings good and bad

Oxygen is naturally low below 400 feet because bacteria uses it while eating organic matter (dead stuff).

This area is called an Oxygen Minimum Zone.
Increased hypoxia since 2000 may mean the Oxygen Minimum Zone is expanding, and low dissolved oxygen may be more common in the future.

Hypoxia only happens in the summer.

During the winter, south winds push surface waters to depth, and oxygen levels return to normal.
So, hypoxia clearly has an impact on adult organisms, what about juveniles?

We wanted to learn how much temperature and hypoxia impact juvenile English sole and Dungeness crab growth.
Juvenile animals begin life in nursery areas that are impacted by hypoxia.
Tends to be colder and more hypoxic

Tends to be warmer and less hypoxic

We conducted experiments to figure out how these species would grow in each nursery.
We ran 3 experiments

With:
- 3 temperatures
- 3 DO levels
  - 2 tanks of each DO

Each tank had:
- 10 English sole
- 10 Dungeness crab

And looked like this in real life
Dungeness crab did not grow well in cold temperatures.

They also grew poorly in hypoxic and low oxygen levels.
English sole growth increased with warmer temperatures.

They also grew almost as well in low oxygen conditions as normal oxygen conditions.

As we expected, neither species grew very well in hypoxic conditions.
Thanks for learning with us!
Chapter 3 Figures before edits:

First, take a look at **normoxia** – normal oxygen levels

The following video shows thriving Oregon rocky reef ecosystems recorded by remotely operated vehicle (ROV).

But...

Sometimes the upwelled water is severely hypoxic.
Upwelling brings good and bad

Oxygen is naturally low below 400 feet.

This area is called an Oxygen Minimum Zone or OMZ.

Increased hypoxia since 2000

This may mean that the Oxygen Minimum Zone is expanding, and low dissolved oxygen may become more common in the future.
CHAPTER 4: GENERAL CONCLUSIONS

A discussion of the experimental results, observations on behavior of juvenile English sole (*Parophrys vetulus*) and Dungeness crab (*Cancer magister*) and suggestions for improvements to experimental methodology

4.1 Introduction

This chapter presents a detailed discussion of the results of the experimental studies described in chapter 2, including general observations on behavior of both species and suggestions for future consideration. In general, for all treatments and both species, growth increased with temperature. Growth was suppressed in colder temperatures regardless of oxygen concentrations, and dissolved oxygen concentrations above hypoxia (> 1.4 mL L\(^{-1}\)) produced the best overall growth. Here, I compare my results to those found by others. Throughout the experimental trials, I also made observations of fish behavior. Unfortunately, my observations were unquantified, so I can only make speculative statements. However, that might provide impetus for further hypoxia research, studying flatfish and crab behavioral responses to hypoxic conditions. Finally, I share what I learned and how that might improve future hypoxia experiments and methodology.

4.2 Discussion

4.2.1 English sole

Despite risky behavior that suggested otherwise, I found English sole growth to be relatively resilient to the low-oxygen conditions (2.0 mL L\(^{-1}\)). Although not quantitatively assessed, fish respiration was more perceptible in the hypoxic and low-oxic than normoxic treatments. Fish in these lower oxygen conditions commonly perched on their
dorsal and ventral fins, with head pointed away from the sand while rapidly moving their opercula to pass more water across the gills. Common sole (*Solea solea*) exhibited similar behavior in experimental hypoxic conditions as observed by Dalla Via et al. (1998). The authors suggested the flatfish may do this to increase the probability of reaching more oxygenated water above a natural micro-gradient of lower-oxygen at the sediment-water interface. They also suggested that while this upright posture may increase predator perceptibility, it could also allow for rapid flight response.

The coloration of the eyed side of the fishes also tended to be darker, as English sole seemed unable to adaptively camouflage to the surrounding sediment. In normoxia, fish appeared to spend most time camouflaged while buried in the sand, using less energy while at rest and requiring less caloric intake. In a laboratory study of the coloration of three north Pacific flatfish species, Ryer et al. (2008) found that darker English sole, fish that did not match the surrounding substrate, were more active than light colored English sole. Dark colored were also less likely to be buried, and therefore more susceptible to predation. Regardless of coloration and respiratory activity, fish in most treatments remained invariably stationary, except when the tanks were approached for feeding, cleaning or observation, when they often swam toward the surface (MPB personal observation).

Ryer et al. (2012) also conducted laboratory experiments assessing behavioral patterns of those three north Pacific flatfish species: English sole, Pacific halibut (*Hippoglossus stenolepis*) and northern rock sole (*Lepidopsetta polyxystra*). They found that of the three flatfish, English sole was the most “risk-prone” and most vulnerable to
predation: they were inherently conspicuous and spent most time unburied, on top of the substrate regardless of the presence of foraging predators. In our experiments, the behavior and coloration of the heavily respiring English sole was likewise, very conspicuous.

While these two studies were simulations conducted under laboratory conditions and may not accurately represent natural conditions, Froehlich et al. (2014) recorded patterns of adult English sole (~ 300 ± 22 mm) movement in the field during two seasons of summertime hypoxia in the Hood Canal. Rather than move into nearby normoxic habitats, English sole remained in hypoxic conditions. In natural conditions where emigrations from hypoxia are not possible or are not undertaken, implications for such behavior include the potential for heavy predation by larger or more hypoxia-tolerant organisms and increased susceptibility to selective fishing pressures.

Given the natural risk-prone behavior, outward coloration and resulting vulnerability of English sole to predation in normal oxygen conditions, it is highly likely more vulnerable to predation during hypoxic conditions. This hypoxia study was not designed to include rigorous behavioral observations, but low oxygen experiments with such capacity would be a valuable addition to hypoxia research.

English sole also seem to be tolerant to a range of oxygen conditions. In our experiments, fish in low-oxic (2.0 mL L\(^{-1}\)) treatments often grew as well as fish in normoxic (6.0 mL L\(^{-1}\)) treatments, with no statistical difference between oxygen treatments. This observation aligns well with the suggestion by Boese (1988) that English
sole have an enhanced tolerance to low oxygen environments. Juvenile English sole feed mostly on benthic invertebrates (polychaetes, amphipods, cumaceans, and clam siphons; Lassuy 1989), and do not generally pursue prey actively like predatory piscivores, and therefore, have a low basal metabolic rate. Flatfish tolerance to stressful conditions was also noted by Wasielesky et al. (1997), who exposed Brazilian flounder (*Paralichthys orbignyanus*) to low pH conditions (5.2) for 96 h with 100 percent survival. Tallqvist et al. (1999) found that European flounder (*Platichthys flesus*) actually increased predatory clam siphon cropping under moderate hypoxia (~2.6 mL L$^{-1}$). This may have been because clam siphons were more exposed in the same hypoxic conditions. Regardless, the flatfish was more active in low-oxic than normoxic conditions.

English sole share similar weight-oxygen consumption ratios with other flatfish, such as winter flounder (*Pseudopleuronectes americanus*), plaice (*Pleuronectes platessa*) and starry flounder (*Platichthys stellatus*) that have characteristic energetically low, cryptic life-styles (Boese 1988). In contrast with our findings of English sole growth, Stierhoff (2006) found that young-of-the-year winter flounder and summer flounder (*Paralichthys dentatus*) responded to temperature and hypoxia variations by exhibiting severe growth limitation under moderately low (2.45 mL L$^{-1}$) oxygen conditions. While I expected that there would be a similar graded effect of dissolved oxygen on English sole, with growth rates highest in normoxic conditions, that was not the case.

I observed differences in English sole growth patterns in the first two weeks compared to the second two weeks that may have been caused by a variety of factors related to the time of year the experiment was conducted, and/or acclimation of fish to lab
conditions and handling. During the first two experiments, fish experienced higher growth during the first two weeks than the second two weeks (Figure 4.1). I speculated that tagging may have had sub-lethal effects on the fish that only manifested after two weeks, and decided against tagging fish for the third experiment. Fish growth increased during the last two weeks of experiment 3: it was the only experiment with higher growth during the second half of the experiment than the first. It seems likely that the overall limited growth of English sole in experiment 1 was resulted because of their larger size at the beginning of the experiment, but also because the fish were tagged and feeding was limited to alternating days. Aside from lab and experimental conditions, all fish used in these experiments were from diverse cohorts. Fish caught in the late summer, as in experiments 1 and 3, may have been better adapted to a wider variety of environmental conditions than those caught in the early summer because they were larger and more developmentally advanced, and because they may have been exposed to wider temperature and DO ranges in the wild.

4.2.2 Dungeness crab

Crabs collected using the camera sled during 2012 were already settled and in various stages of juvenile development (between molt stage C2 and C3). This was problematic for the experiments, because the date of previous ecdysis was unknown, making measurements of growth rate and determination of the effect of dissolved oxygen and/or temperature on the rate of growth impossible. In some temperature/oxygen treatments, several crabs did not molt at all throughout the entire experiment. In later molt stages, ecdysis occurs at greater intervals; with C2, C3 and C4 stage crabs the
intermolt period could have exceeded the length of the entire experiment. Optimally, all specimens would have been collected while they were still megalopae.

The separate containers in which Dungeness crab were held in treatment were unlike any conditions they would encounter in the wild. Experimental separation was necessary to eliminate cannibalism, which is often an important factor in regulating juvenile abundance in the first several months of settlement (Eggleston & Armstrong 1995). Container size was probably not a limiting factor in my experiments. Each cell with one juvenile Dungeness crab was at least 6.1 cm diameter at the bottom, and 9.4 mm at the top, by 15.5 cm tall. Swiney et al. (2013) observed that juvenile red king crab (*Paralithodes camtschaticus*) in 20 mm diameter containers grew an average of 17 percent smaller than those in 77 mm containers. Mortality was also a magnitude of order greater in the smaller than larger containers. Solo® plastic cups for crab containers were somewhat labor intensive to make, but the cost of materials was negligible.

In our early trials holding megalopae and stage C1 juvenile crabs, I found that individuals which were provided with a fine layer of sand buried themselves. In an estuarine or nearshore environment, this amount of refuge in the substrate may improve the success of molting and likely reduces intermolt period, cannibalism, and predation. In our experiments, overall crab molt success may have been compromised because they were not provided with a sandy layer to bury into during the experiments, and the bottoms of the clear cups were exposed to the curious approaches and potentially predatory cues from English sole. Larger English sole (> 55 mm) periodically approached and nipped at crabs inside the transparent containers suspended below the surface.
Juvenile English sole are known to be opportunistic and generalist benthic feeders, not predators of Dungeness crab (Toole 1980), but this behavior was not uncommon of English sole in our treatments.

As with English sole, Dungeness crab did not exhibit any differences in growth rate, intermolt period or carapace width increase between the normoxic and low-oxic conditions, however, there appeared to be a trend of higher growth rates in the normoxic conditions. There was also no difference between the rate of mortality and molting between normoxic and low-oxic conditions experiment 3, but that may have resulted from the limited number of crabs that molted in the 5°C treatments. As I expected, the normoxic and low-oxic conditions were significantly different in experiment 2, with higher rates of molting in normoxic conditions, and higher rates of mortality in low-oxic conditions. Many crabs in the lower temperature treatments only molted once, if at all.

Most literature shared similar results about the sensitivity of YOY crabs to stressful conditions. Eerkes-Medrano et al. (2013) found that larval crabs (*Hemigrapsus oregonensis*, *H. nudus*, and *Cancer antennarius*) exhibited mortality within 12 hours of exposure to hypoxia (0.5 – 1.4 mL L⁻¹). Larval crabs were less tolerant of low dissolved oxygen than all other crustaceans in the study. Given their ability to swim up to 8.5 cm s⁻¹ (Fernandez et al. 1994), Vaquer-Sunyer and Duarte (2008) suggested that larval crabs might depend on avoidance behavior more than low oxygen tolerance for survival. After settling, juvenile instar crab mobility becomes limited to peripatetic benthic movement. For a juvenile crab of lower mobility and potentially reduced avoidance capacity, low oxygen may lead to growth limitations.
Brown and Terwilliger (1999) found that first instar juveniles exhibited greater sensitivity to temperature than fifth instar juveniles, indicating that between settlement and the end of the first summer young crabs become more tolerant of changes in temperature. They suggest that first instar juveniles are very near the limit of respiratory and circulatory capacities as a result of changes in salinity and temperature during tidal exchange in the estuary. They did not specify the consequence of exceeding that limit, but based on nearly 50 percent mortality in most hypoxic treatments in my experiments, one can intimate. The fifth instar juvenile was more mobile and had a greater metabolic range, possibly to exploit habitat on exposed mudflats away from predators, including older crab that are concentrated in channels: a refuge that younger juvenile instars crabs would not be able to tolerate.

Bernatis et al. (2006) found that when coupled with short-term hypoxia (measured in hours, not days), Dungeness crab reduced both the amount of food eaten and the amount of time spent feeding. Crabs that are lethargic and starved may be susceptible to higher rates of predation by other organisms that are more tolerant of low DO. Again, I did not quantitatively assess behavior of juvenile Dungeness crab in my experiments, but there seemed to be patterns of food refusal in certain treatments, most notably in lowest oxygen. Behavioral and/or bioenergetics experiments that account for consumption and waste would be a valuable addition to the field of hypoxia research.

4.3 Experimental methodology

If I was to repeat these experiments, there are a few factors that I would reconsider. Treatment combinations were only replicated twice in each experiment. This was because
oxygen levels were controlled at each treatment tank, and I was limited by the number of
tanks that I could fit into the lab. As a result, my sample size was small, with an n = 2 in
the 5 and 15°C treatments, n = 4 in the 7 and 13°C treatments, and n = 6 for the repeated
10°C treatments. In future experiments designed to examine the effects of temperature
and oxygen on organism growth, I suggest increasing the number of replicate tanks.
Conducting more experiments would also increase sample size. Along the lines of
increasing the number of treatment tanks, I would also suggest reducing the number of
specimens per tank from 10 to between five and seven fish. This could reduce any
potential issues with density dependence, which may have been the cause for the
comparatively limited growth rates (Ryer et al. 2012) our fish specimens experienced.

   Another procedure to be considered carefully in future experiments is the elastomer
tagging of the sole. Elastomer tagging flatfish of this size (~ 35 – 75 mm) is a viable
method of tracking individual growth. While I am unable to make a substantive
comparison of fish growth with and without tags, it is appropriate to note that for fish
entering an already stressful treatment (> 10°C and ± 1.4mL L⁻¹), the procedure of
handling the fish and injecting the polymer is an additional stressor. Moreover, for ease
of comparison across experiments, I recommend that all fish should either be tagged or
left untagged. For untagged English sole, it was possible to make comparison of eyed-
side spot patterns from subsequent measurement photographs. I developed a docking
station made from Sintra® plastic PVC sheets, and took photographs with an Apple iPad
enclosed in a LifeProof™ case. The setup allowed one person to seamlessly capture
hundreds of photos, record metrics, and handle fish and crabs all with wet hands, and it eliminated the need for tagging.

Finally, using liquefied nitrogen gas as the medium for stripping water of oxygen was successful and relatively low in cost, but it proved to be challenging, because the experimental setup was sensitive to slight adjustments or blockages. Creating and maintaining equilibrated flow for maintaining oxygen and temperature conditions in the experimental setup required constant monitoring. On one occasion, I found that water flow was blocked by an in situ algal plug that had grown in the plumbing. Nitrogen gas continued to flow, while water flow was reduced to a trickle, and the affected tank was consequently oversaturated with nitrogen and became severely hypoxic. If budget allows for it, I suggest installing an automated system that controls water and nitrogen flow. If not, flow alarms would at least alert the user to abnormalities in the experimental set-up.

4.4 Concluding Statements

While DO in the open coast may reach lower concentrations compared to the estuary, lower water temperature may protect organisms against the severity of such oxygen levels. Depending on the severity of the oxygen depletion, English sole may grow just as well in a moderately hypoxic (>2.0 mL L⁻¹) coastal nursery habitat as a borderline eutrophic estuarine nursery habitat – as long as equally low-oxygen tolerant predators are not present. On the other hand, in extremely warm years when the estuary is also influenced by hypoxia, coastal nurseries may be more suitable for juvenile English sole and Dungeness crab growth. Dungeness crab mortality was very sensitive to dissolved oxygen but less so to temperature, while growth metrics (intermolt period, carapace width
increase and SGRc) were sensitive to both low dissolved oxygen and temperature. Juvenile Dungeness crab may benefit from the conditions that an estuarine nursery habitat provides during years of severe hypoxia and low temperature in coastal habitats.

Overall, this study seeks to complement the existing body of research describing the use of nearshore habitats like Yaquina Bay and Moolack Beach for these important species, from the discovery of nearshore sandy beach habitat as an important nursery (Laroche & Holton 1979, Krygier & Pearcy 1986), to identifying the recruitment mechanisms into each habitat (Boehlert & Mundy 1987, Armstrong et al. 2003, Miller & Shanks 2004, Shanks & Eckert 2005), assessments of the differences in growth between the two habitats (Rosenberg 1982, Kruse & Tyler 1983, Gunderson et al. 1990). Our lab has also contributed to the greater knowledge of the abundance and distribution of young-of-the-year organisms on the Oregon shelf (Johnson, Ciannelli, Wakefield and Yergey) and we are also currently studying the effects of hypoxia and temperature on flatfish lipid content (Stowell).
4.5 References

Armstrong DA, Rooper CN, Gunderson DR (2003) Estuarine Production of Juvenile Dungeness Crab (Cancer magister) and Contribution to the Oregon-Washington Coastal Fishery. 26:1174–1188


Figure 4.1: Mean SGR$_F$ ($\pm$SE) of English sole in all weeks, from week 0 to 2, and week 2 to 4 in experiments 1, 2 and 3.